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Abstract
To identify the clinical and pharmacological risk factors associated with tacrolimus pharmacodynamics for acute graft-versus-host disease (aGVHD) in pediatric patients receiving allogeneic hematopoietic stem cell transplantation (HSCT) from a matched related donor. A retrospective cohort single center chart review study was conducted with pediatric patients who received tacrolimus prophylaxis after allogeneic HSCT between January 1, 2017, and December 31, 2019. Potential risk factors were tested separately between aGVHD and non-aGVHD patients (p < 0.005). Pharmacological factors associated with tacrolimus treatment failed to demonstrate a significant impact on patient’s risk of aGVHD. Using a best fit logistic regression model that tested all the variables together, donor age was the only significant variable predicting patient’s risk of aGVHD (p < 0.01). Donor relationship and donor age were unable to be evaluated separately and are therefore confounding variables. Among pediatric patients receiving allogeneic HSCT, aGVHD risk is significantly decreased by either sibling donor and/or younger donors. Although no conclusions were drawn on the effect of tacrolimus therapy (p = 0.08), results warrant additional research with a larger sample size to evaluate the accuracy of monitoring tacrolimus serum trough levels.

Abbreviations: aGVHD, acute graft-versus-host disease; AUC, area under the curve; CI, confidence interval; GVHD, graft-versus-host disease; DF, degrees of freedom; HLA, human leukocyte antigen; HSCT, hematopoietic stem cell transplant; IV, intravenous; NIOSH, National Institute for Occupational Safety and Health; PK, pharmacokinetic; PO, oral; RR, relative risk.

Phan and Chavan equally contributed to this study.

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INTRODUCTION

Hematopoietic stem cell transplantation (HSCT) is typically reserved for life-threatening diseases, such as relapsed acute lymphoblastic leukemia and acute myeloblastic leukemia, due to severe and life-threatening complications associated with the procedure. Patients who undergo allogeneic HSCT are at significant risk of graft-versus-host-disease (GVHD), a debilitating condition where the patient’s immune system reacts to the donor’s cells and triggers an inflammatory cascade that ultimately results in tissue damage and organ injury. GVHD can present acutely or chronically and incidences of GVHD in pediatric patients have been reported to be between 30% and 50%, varying by the type of transplant and the recipient’s relationship to the donor. Conditioning the patient to have a successful engraftment while minimizing the risk of developing GVHD is a delicate balance that remains challenging in current clinical practice.

Tacrolimus is a calcineurin inhibitor commonly used for the prevention of acute GVHD (aGVHD), defined as GVHD that occurs within 100 days post-transplant. To date, there are very limited studies examining the pharmacokinetic (PK) profile of tacrolimus in children post solid organ transplant. These PK studies have demonstrated significant interpatient variability in drug exposure. The oral bioavailability of tacrolimus in children ranges even more widely from 5% to 93%, with a mean bioavailability of 25% in patients who have undergone solid organ transplantation. Of note, pediatric patients who undergo transplantation appear to eliminate tacrolimus more rapidly than adult patients who undergo transplantation. In patients undergoing HSCT, the nonlinear PK model of tacrolimus is further complicated by altered hepatic and renal function, and other severe complications, such as sinusoidal obstructive syndrome, that occur after transplantation.

Tacrolimus has a narrow therapeutic index requiring close monitoring of its trough levels. In adult patients who undergo transplant, tacrolimus levels are typically maintained between 10 and 20 ng/ml. However, the target levels of tacrolimus in pediatric patients are not conclusive and vary in practice among different institutions. Given the limited information collected from patient studies, finding a reliable predictive model to guide patient care and clinical practice has been challenging. Additional challenges include being able to swiftly reach a safe therapeutic serum level using an intravenous formulation and maintaining the target serum levels when switching to oral administration, both of which are impacted by the metabolic variability among patients because tacrolimus is extensively metabolized primarily by the hepatic P450 enzymes, such as CYP3A. Identifying an appropriate dosing method, optimal target serum levels, and time to reach those levels may improve patient outcomes, as suggested by Offer et al. Ultimately, more studies are warranted to evaluate the use of tacrolimus in pediatric patients with HSCT and to determine its clinical outcomes, such as patient’s risk of developing GVHD post-transplantation.

Previous studies examining risk factors for developing aGVHD have determined that one of the most important factors is human leukocyte antigen (HLA) disparity, where matched grafts have lower rates of aGVHD than HLA-mismatched grafts. Other factors that may increase the likelihood of developing GVHD—acute and chronic—include

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

WHAT QUESTION DID THIS STUDY ADDRESS?

Donor-related clinical factors play a large role in acute graft-versus-host disease (aGVHD) development. Accounting for these factors while examining the pharmacological properties of tacrolimus is understudied in the pediatric setting.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

This study investigated clinical and pharmacological risk factors that may affect a pediatric hematopoietic transplant patient’s risk of aGVHD while on tacrolimus therapy.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

This study (1) is consistent with other studies in that sibling donors have a lower risk of aGVHD than parent donors, and (2) provides exploratory data to guide future studies that examine the pediatric patient population. Given the significant interpatient pharmacokinetic (PK) variability, our results suggest that monitoring other PK parameters, such as the area under the curve (AUC) in addition to measuring trough levels reduce aGVHD risk in children.
donor age, sex mismatch, and the myeloablative regimen used. Although these risk factors have been largely examined in the adult setting, only a few studies examined these trends in children. Identifying the interaction between these risk factors and tacrolimus use on the likelihood of developing aGVHD in pediatric patients with HSCT is understudied and prompts additional research. Therefore, the purpose of this study is to examine the effect of tacrolimus levels on developing aGVHD in pediatric patients with HSCT. The dose of tacrolimus, the number of days it takes to reach target levels, and other patient clinical and demographic features have been evaluated as potential risk factors to determine their associations with the likelihood of developing aGVHD.

METHODS

Data collection

A retrospective, institutional review board-approved study was conducted in patients who underwent HSCT and were admitted to a children’s hospital, CHOC Children’s, from January 1, 2017, until December 31, 2019. Patients with HSCT who were younger than 26 years old and received tacrolimus treatment for GVHD prophylaxis were included in the study. Each transplant was examined as an individual datapoint, meaning some patients were analyzed as multiple datapoints for each time they used tacrolimus as GVHD prophylaxis for a transplant. Patients were excluded if they died due to a complication separate from aGVHD.

The clinical information was obtained from each patient via chart review, which includes age at time of transplant, gender, weight, diagnosis, starting dose, dose by weight, i.v. to oral (p.o.) conversion, final p.o. to i.v. ratio (before tapering off p.o.), aGVHD status, donor relationship, donor age, liver toxicity (liver function tests and bilirubin levels), renal toxicity (serum creatinine levels), and the HLA allele match in percentage. All trough levels measured within the first 100 days post-transplant were collected and the following metrics were calculated: the range of the tacrolimus trough levels obtained, days to greater than 5 ng/ml on i.v. medication, days to greater than 10 ng/ml on i.v., percent of serum trough levels less than 5 ng/ml, and percent of serum trough levels less than 10 ng/ml. Due to the low representation of diagnoses, the patients were further grouped into 4 categories in the statistical analysis based on clinical relevance: group 1 (acute lymphoblastic leukemia), group 2 (acute myeloid leukemia and GATA2-related deficiencies), group 3 (aplastic anemia, beta thalassemia intermedia, congenital thrombocytopenia, and sickle cell disease), and group 4 (Hodgkin’s lymphoma and hemophagocytic lymphohistiocytosis).

The donor relation was further divided into sibling, parent, or unrelated. Hepatotoxicity was defined by an elevation of serum transaminases greater than 5× normal upper level, or a serum total bilirubin level greater than or equal to 2.0 mg/dL. Nephrotoxicity was defined as a doubling of serum creatinine greater than baseline pretransplant value or a serum creatinine greater than 2 mg/dL, which requires renal dose adjustment.

Statistical analysis

Statistical analysis was done using “The R Project” for scientific computing version 3.6.2. A Power analysis for sample size calculation was conducted using the data collected, followed by additional post hoc t-test power calculations using the sample sizes in this study. To investigate variables associated with aGVHD, each variable was run separately comparing the aGVHD group to the non-aGVHD group. A Welch t-test was used for continuous variables unless normal distribution assumptions were not met, in which case a Wilcoxon Mann–Whitney was substituted. A Fisher’s exact test was used for categorical variables.

Next, the variables were combined in a logistic regression. The aGVHD status was the dependent variable with the above-mentioned clinical and demographic variables as independent. Due to the large number of variables in comparison to the number of subjects, an exhaustive algorithm, which investigated all possible combinations of independent variables, was used to determine the model with the lowest Bayesian Information Criterion and the lowest Akaike Information Criterion, followed by backward elimination to remove any nonsignificant variables from this model. Due to computational limitations, the algorithm is limited to 15 predictor variables. The 15 variables with the strongest relationship to aGVHD status were used in the algorithm, however, all remaining variables were added during the process of backward elimination and tested for inclusion in the final model. Primary results were based on this logistic regression method and, therefore, p values were not adjusted for the use of multiple statistical tests. Instead, confidence intervals were included when possible. In order to limit the influence of any confounding variables, a partial least squares discriminant analysis was conducted. This is a supervised learning method that uses the independent variables to best predict the outcome of the dependent variable, in this case, aGVHD versus non-aGVHD. It is the preferred method useful in cases of multicollinearity and in data sets where there are many independent variables compared with the number of subjects. Confounding variables are often difficult to determine and can distort the results. In our study, we used multiple statistical methods to account for multicollinearity.
RESULTS

Descriptive statistics

This study was a single-center retrospective study conducted at a 334-bed regional academic children’s hospital. Approval for the study was received from the institutional review board after expedited review. Pediatric patients who received HSCT from January 1, 2017, until December 31, 2019, were identified. Records of 34 pediatric HSCT cases were retrospectively reviewed. Thirty-two individual patients were examined. Of the 32 patients, 2 patients underwent 2 transplants with tacrolimus prophylaxis, totaling 34 transplant cases examined in this study. One was excluded due to exclusion criteria and a total of 33 transplant cases were included in the final analysis.

Our study showed that 52% (17/33) of transplant cases developed aGVHD while on tacrolimus prophylaxis treatment (Table 1). The average donor age was 14 years old and 15 years old for non-aGVHD and aGVHD patients, respectively ($p < 0.05$). Overall, 64% of transplant cases were with male patients (Table 1). On average, patient weight was 58 kg and 59 kg for aGVHD and non-aGVHD patients, respectively (Table 2). Average patient body weight was also not significant when independently testing for aGVHD risk. Seventy-three percent of the transplants were White patients, with 6% being Asian and 21% identifying as “other.” Transplants also predominantly used tacrolimus in combination with cyclophosphamide on day 3 and 4 post-transplant and mycophenolate on day 5 for aGVHD prophylaxis, with only 9% using a different tacrolimus combination regimen. These patients who received a different regimen were those diagnosed with aplastic anemia (AA), whereas all four AA transplants received different regimens. Two out of the 34 transplants experienced aGVHD, failed tacrolimus therapy, and switched to sirolimus (Table 1).

Thirty of 33 donors were haploidentical. The percentage of HLA allele match between the donor and recipients were 58.3% and 60.8% on average, respectively, in non-aGVHD and aGVHD patients. Of note, the average age of donors in the aGVHD group was 37.5 years old, much higher compared with that of non-aGVHD patients (20.5 years old, $p < 0.005$; Table 2).

Individual variable analysis

The continuous variables were examined independently to test for a significance in difference between the two cohorts— aGVHD and non-aGVHD. Patient age did not reach significance, but donor age was significantly different between these two cohorts, where older donor age was significantly associated with an increased risk of aGVHD ($p < 0.005$; Table 2). Pharmaceutical monitoring factors associated with tacrolimus treatment, such as days to reach target levels (5 or 10 ng/ml), number of readings below target levels, average trough levels, range of trough levels, and initial/final p.o.:i.v. conversion ratios, did not exhibit statistical significance between the aGVHD and non-aGVHD cohorts ($p > 0.05$).

For patients who developed aGVHD, the average starting i.v. dose by weight was 0.02 mg/kg (SD = 0.008), which is a standardized practice with rounding-up to the nearest tenth per protocol. When the patients were stabilized and able to take p.o. medication, i.v. tacrolimus was then transitioned to oral administration. The initial i.v. to p.o. conversion averaged 1:2.9 and 1:2.7, in aGVHD and non-aGVHD patients, respectively ($p > 0.05$ by $t$-test). The oral dose of tacrolimus was then adjusted based on serum trough levels detected. The final oral dosing of tacrolimus for maintenance before tapering off was ~ 2.5-fold the initial i.v. dose on average in patients with aGVHD, whereas such ratio was much higher in non-aGVHD patients (4.7-fold). Given the limited patients, however, this difference was not statistically significant ($p > 0.05$; Table 2).

In addition, the average trough levels of aGVHD and non-aGVHD patients were 7.4 and 7.2 ng/ml, respectively, with notable interpatient variabilities (Table 2). There was a trend for the non-aGVHD cohort to have more subtherapeutic readings, where the average percentage of trough level readings less than 5 ng/ml were 30.7% in the aGVHD cohort and 35.5% in the non-aGVHD cohort. However, such difference did not reach statistical significance ($p > 0.05$). There were 76.8% and 78.8% of trough readings below 10 ng/ml for aGVHD and non-aGVHD cohorts, respectively ($p > 0.05$). For patients who developed aGVHD, it took an average of 2.9 days to reach a target serum level of 5 ng/ml or higher, which was shorter than those who did not develop aGVHD (3.5 days). To reach a level above 10 ng/ml, it took an average of 6.1 days in patients with aGVHD and 8.6 days for the non-aGVHD cohort ($p > 0.05$). Such difference (<3 days) may not exhibit clinical significance in patient care practice.

Likewise, the categorical variables were tested individually for significance. No differences were identified in patient gender, donor/recipient gender matching, presence of liver toxicity, nor clinical diagnosis (Table 3). Among donor relationships, a significant difference was demonstrated between sibling donors compared with parent donors ($p < 0.005$).

Power analysis

Using our collected data, a G-Power analysis for sample size calculation was conducted. For example, in order to detect a statistically significant difference between non-aGVHD and aGVHD patients on the average recorded serum level of tacrolimus (7.188 ng/ml ± 1.556 vs. 7.496 ng/ml ± 1.317, respectively), a sample size of 788 was calculated to be sufficient to attain a power of 80% with a significant level of
| TABLE 1 | Demographic characteristics of pediatric patients who underwent HSCT |
|---------|--------------------------------------------------|
|         | Group 1                                           | Group 2                                           | Group 3                                           | Group 4                                           |
| Diagnosis| Acute lymphoblastic leukemia (n = 12)             | Acute myeloid leukemia (n = 9)                    | Aplastic anemia (n = 4)                           | Hodgkin’s lymphoma (n = 1)                        |
|          | GATA2-related deficiencies (n = 2)                | GATA2-related deficiencies (n = 2)                | Beta thalassemia intermedia (n = 1)               | Hemophagocytic lymphohistiocytosis (n = 2)        |
|          | Congenital thrombocytopenia (n = 1)               | Congenital thrombocytopenia (n = 1)               | Sickle cell disease (n = 1)                       |                                                  |
| Male     | 7                                                 | 6                                                 | 7                                                 | 1                                                 |
| Female   | 5                                                 | 5                                                 | 0                                                 | 2                                                 |
| Non-aGVHD| 6                                                 | 3                                                 | 4                                                 | 3                                                 |
| aGVHD    | 6                                                 | 8                                                 | 3                                                 | 0                                                 |
| Age (mean ± SD) | 14.50 ± 5.75                                      | 15.55 ± 7.35                                      | 9.86 ± 6.39                                       | 17.67 ± 2.08                                      |
| Race     | White                                             | 10                                                | 7                                                 | 2                                                 |
|          | Asian                                             | 2                                                 | 0                                                 | 0                                                 |
|          | Other                                             | 0                                                 | 2                                                 | 5                                                 | 0                                                 |
| Tacrolimus regimen +MTX<sup>a</sup> | 0                                                | 0                                                 | 1                                                 | 0                                                 |
| +MMF<sup>b</sup> | 0                                                | 0                                                 | 0                                                 | 0                                                 |
| +MMF +CPM<sup>c</sup> | 12                                               | 11                                                | 4                                                 | 3                                                 |
| Alone    | 0                                                 | 0                                                 | 1                                                 | 0                                                 |
| Failed tacrolimus therapy | 1                                                | 1                                                 | 0                                                 | 0                                                 |

Abbreviations: aGVHD, acute graft-versus-host disease; CPM, cyclophosphamide; HSCT, hematopoietic stem cell transplantation; MMF, mycophenolate mofetil; MTX, methotrexate.

<sup>a</sup>T + MTX: tacrolimus + methotrexate.

<sup>b</sup>T + MMF: tacrolimus + mycophenolate.

<sup>c</sup>T + MMF + CPM: tacrolimus + mycophenolate + cyclophosphamide.
For the “percent of readings <5 ng/mL,” our calculated sufficient sample size was 506 in order to detect statistically significance with a power of 80%. However, to conduct a retrospective study with such a big sample size is challenging, particularly for pediatric patients who received HSCT. Further post hoc test power calculations with using the sample sizes in this study indicate with an effect size \(d = 0.80\) power is 0.60.\(^1\) The power is lowered when the distributions are skewed, necessitating the use of the Wilcoxon Mann–Whitney test. Effect sizes are shown in Table 2, indicating areas that may warrant further study. However, in our study, there is sufficient power to obtain significance with large effect sizes (26 subjects are needed to achieve 82% power with an effect size of 1.18 using G-power), so the large effect size and statistical significance of donor age merit attention despite our small sample size. In addition, we also gathered information on the magnitude of effect on these other measures to help with future multi-center and prospective studies.

### Logistic regression model

After the model selection process and backward elimination, the only significant factor remaining in our model analysis was donor age \((p < 0.01; \text{Table 4})\). When donor age was removed from the model, donor relationship remained as a significant variable associated with aGVHD risk \((p < 0.005; \text{Table 4})\). In either of the reduced models, donor age or relationship, donor gender match was the final variable removed from the model. Applying a likelihood ratio test in either of the reduced models demonstrated that donor gender match had \(p\) values of 0.06 and 0.07, when compared with the models without gender match.
GVHD RISK FACTORS IN PEDIATRIC TRANSPLANTATION

To further confirm the importance of potential confounding factors included in our regression model, a partial least squares discriminant analysis was conducted. The highest variance was explained using a two-factor model as shown in Figure 1. The first component explains 13% of the variance and adding the second component only yields an additional 4%. The largest factor loading of the first component is the donor age, indicating that this variable had the highest relative importance in discriminating between patients who develop aGVHD from those that do not. The factor loading for this variable was −0.64, whereas the next closest variable was 0.36. This supports our findings that were demonstrated by using the logistic regression model, suggesting that donor age was associated with significantly higher risk of developing aGVHD in pediatric patients with HSCT. Notably such risk of aGVHD post-HSCT was independent of the allele matching between donor and recipient.

Our main statistical test was a single regression model attempting to predict aGVHD using multiple predictors. Secondary to this approach, each variable was assessed separately in order to examine its relationship to aGVHD and stimulate further research. The developed model is a type 1a prediction model, where the predictive performance was directly evaluated using the same study data. Effect sizes and confidence intervals were included to further assess the cost and feasibility of such a study based on the sample sizes needed. Because this investigation is exploratory in nature, we did not correct for multiple testing. The inclusion of confidence intervals allows other researchers to draw their own conclusions regarding the strength of the findings.

DISCUSSION

Allogeneic HSCT is an important therapeutic option and a potentially curative procedure for a variety of malignant and nonmalignant severe conditions. Despite the advancement in pharmacology and the development of less toxic preconditioning regimens, GVHD remains the most frequent and serious complication following allogeneic HSCT, which remarkably impacts a patient’s survival and quality of life. Previous studies showed that 22% to 44% of patients who underwent HSCT developed grades II to IV aGVHD post-transplantation from HLA-match siblings even with tacrolimus prophylaxis treatment. How to reduce the incidence of GVHD overall—acute and chronic—and its severity remains the biggest challenge for clinical professionals.

This retrospective study aimed to determine the potential confounding risk factors associated with aGVHD in pediatric patients who underwent HSCT using a logistic regression analysis and partial least squares discriminate analysis. Many clinical and pharmacological risk factors have been shown to be associated with development of aGVHD in pediatric transplant patients, such as patient age, total body irradiation, diagnosis at transplantation, donor/recipient gender, and graft source. A study compared potential risk factors associated with aGVHD and chronic GVHD in over 2900 adult and pediatric patients who received allogeneic hematopoietic cell transplantation and found that

### TABLE 3

| Variable                  | RR (95% CI)       | p value |
|---------------------------|-------------------|---------|
| Gender                    | 1.048 (0.522–2.104) | 1.0     |
| Donor                     |                   |         |
| Sibling: parents          | 3.569 (1.264–10.067) | 0.00385 |
| Sibling: unrelated         | 2.333 (0.422–12.911) | 0.450   |
| Parents: unrelated         | 0.654 (0.160–2.680)  | 0.468   |
| Donor/recipient gender matching | 0.643 (0.340–1.214) | 0.282   |
| Hepatic toxicity          | 1.413 (0.610–3.271)  | 0.465   |
| Diagnosis group           | b                 | 0.165   |

Bold indicates the statistical significant values.

Notes: The only variable that appears significantly different between the aGVHD groups is the donor relation.

Abbreviations: aGVHD, acute graft-versus-host disease; CI, confidence interval; HSCT, hematopoietic stem cell transplantation; RR, relative risk.

*Fisher’s Exact test.

Individual RR not included due to multiple comparisons and nonsignificant value.

### TABLE 4

| Variable                  | Odds ratio | CI       | Z-value | p value |
|---------------------------|------------|----------|---------|---------|
| Donor age                 | 1.088      | 1.030–1.168 | 2.707   | 0.007   |
| Donor relation: parent-sibling | 0.084      | 0.013–0.412 | −2.859  | 0.004   |
| Donor relation: parent-unrelated | 0.308      | 0.010–8.973 | −0.773  | 0.440   |

Bold indicates the statistical significant values.

Abbreviation: CI, confidence interval.

*Significant when run separately from donor age.
recipient HLA mismatching and the use of unrelated donors exhibited a greater risk of aGVHD. For pediatric patients, limited studies showed that older donor age and female donor sex were particularly associated with increased rate of aGVHD. In our study, only two patients received transplantation from unrelated donors; all the other donors were related, being either parents or siblings of the recipient. Further analysis demonstrated that among the related donors, both the donor relation and the donor’s age were significantly related to the development of aGVHD. These two factors—donor’s age and relation—could not be analyzed separately in our study, because all but two of our patients were either siblings or parents. However, both variables are independent of HLA allele matching status and gender matching. The parent donors, ranging from 25 to 59 years old, exhibited a relatively higher aGVHD risk of 3.5 times compared with that of sibling donors (2–24 years old). Our finding of an older donor’s age being a significant predictive factor of aGVHD in pediatric patients with HSCT with related donor was consistent with an early study reported by Mori’s et al. It is well-documented that tacrolimus exhibits significant pharmacological interpatient and intrapatient variability. Such variability necessitates the collection of serial trough concentrations to ensure that the drug remains within the targeted therapeutic range to minimize the risk of aGVHD. The metabolism of tacrolimus is further complicated by the developmental stages of pediatric patients, CYP3A5 genotype, race/ethnicity, concomitant CYP-inducing medications, such as antifungals, and dosing regimen. The clearance of tacrolimus has shown to be age-dependent, with children younger than 5 years old exhibiting a higher weight-normalized clearance compared with older children and adults.

In current practice, the drug monitoring of tacrolimus is based on its serum trough levels and the dosage is adjusted to maintain the target serum levels between 5 and 15 ng/ml. As previously mentioned, target serum levels are varied among different institutions and guidelines. Interpreting tacrolimus serum levels and the associated risk of toxicities, such as nephrotoxicity, has been studied well. Yet, only a few studies in children have evaluated the relationship between the serum trough levels of tacrolimus and the occurrence of aGVHD, and the relationship remains controversial and inconclusive. There were studies suggesting that a higher target tacrolimus level post-transplant was associated with reduced risk of aGVHD. The cutoff or target trough levels varied among different studies, ranging from 7 to 20 ng/ml in children post-allogeneic bone marrow transplantation. One study demonstrated that every decrease of 1 ng/ml in tacrolimus mean concentrations over weeks 2–3 post-transplant leads to an ~13% increased incidence of aGVHD. On the contrary, certain reports failed in identifying a relationship between the blood concentrations of tacrolimus and the occurrence of aGVHD in children.

Another notable observation of our study is that we failed to demonstrate a significant impact of the target trough levels of tacrolimus on the development of aGVHD, nor was it affected by the time to reach target levels. The treatment and monitoring plans after transplantation were standardized in the study institution and implemented by oncology pharmacists, which minimized the potential discrepancies associated with tacrolimus dosing and monitoring. In our retrospective study, 17 (-%) patients who underwent HSCT on tacrolimus
treatment experienced aGVHD, despite the fact that ~ 70% of trough levels were above the therapeutic target of 5 ng/ml. Neither the mean serum trough level nor the time to reach target level (≥5 ng/ml or ≥10 ng/ml) was shown to be associated with the risk of aGVHD among our studied pediatric patients.

Area under the concentration-time curve (AUC) is generally used as the PK exposure parameter after an oral administration, which is shown to be best associated with clinical outcomes. Notably, PK studies demonstrated that the trough levels of tacrolimus correlates poorly with the AUC0–12, suggesting a calculated AUC based on two measured serum levels may provide a more precise and cost-effective model for tacrolimus monitoring. However, to the best of our knowledge, very limited prospective studies have been conducted in pediatric transplant recipients to determine the potential benefits of AUC0–12 monitoring compared with trough level-guided pharmacotherapy. An earlier study revealed that the AUC0–12 after the first oral dose of tacrolimus was significantly lower in heart transplantation recipients who experienced acute rejection compared with those who did not.

PK studies demonstrated distinct metabolic profiles among patients, which may result in significant difference in drug exposure. Simply monitoring trough levels may limit its capacity to precisely guide and optimize the tacrolimus therapy. Most of the comparison studies of different tacrolimus formulations utilized AUC instead of trough levels to compare the efficacy of tacrolimus. Consistently, the final p.o. to i.v. ratio of tacrolimus was much higher in non-aGVHD patients compared with that of the patients who developed aGVHD (4.9-fold and 2.5-fold of i.v. dosing, respectively), yet, we failed to observe any significant changes of the average trough levels between the two groups. A prospective study to investigate the association between tacrolimus AUC and clinical outcomes, such as incidence of aGVHD and patient’s survival, is greatly warranted.

In general, patients with HSCT were initiated tacrolimus therapy as a continuous i.v. infusion. After the patient reached the target level and was capable of oral intake, tacrolimus was then converted to p.o., which continued until at least day +90 and was tapered off by day +180 post-transplant. The pediatric dosage of tacrolimus is generally individualized based on patient body weight, which is much smaller compared with the adult dose. Current available oral dosage forms are limited to capsule and tablet forms, which are not feasible for pediatric patients, especially younger children. As a general practice, many children’s hospitals are compelled to extemporaneously compound oral solutions using immediate-release capsules. Of note, tacrolimus is a National Institute for Occupational Safety and Health-listed hazardous drug requiring special handling when compounding extemporaneously. As such, there is an urgent need of developing an age-appropriate, safe, and effective formulation of tacrolimus for pediatric patients.

In addition, the prophylaxis efficacy of tacrolimus may be affected by distinct combination regimens, such as sirolimus, mycophenolate, and methotrexate. In our study institution, the most commonly used GVHD prophylaxis regimen was the combination of tacrolimus, mycophenolate, and cyclophosphamide, which minimized the need to examine the potential effects of different regimens on patient’s risk of aGVHD.

Importantly, in earlier studies of patients with HSCT, using tacrolimus, either alone or in combination, cumulatively developed nephrotoxicity during the first 100 days after marrow transplantation. However, in our retrospective study, no significant nephrotoxicity was identified. The overall lower incidence of nephrotoxicity in our study may be related to the lower levels of tacrolimus (5–15 ng/ml) that were maintained in our study population and the low target level of 5 ng/ml as recommended by the institution. One study suggested that the risk of nephrotoxicity was markedly increased when the mean trough level over 14 days was greater than or equal to 20 ng/ml. Our patients rarely exceeded trough levels greater than or equal to 20 ng/ml with pharmacist-led drug therapy monitoring practice (<1% of all the documented levels). In the event of greater than or equal to 20 ng/ml, tacrolimus doses were quickly reduced or placed on hold.

A potential limitation is the nature of a single-institution retrospective cohort study, which restricts the number of eligible patients and reduces the power for statistical analysis. Pediatric cancer incidences are generally lower than adults. Approximately 0.61% of new cancer cases in the United States in 2020 will be pediatric patients aged 0–14 years old, and of that, 28% will be leukemia. There is also an increasing rate of pediatric patients achieving remission; for example, the 5-year survival rate for children with acute lymphoblastic leukemia has now greatly increased to about 90% overall. HSCT is reserved for relapsed or refractory patients who failed other treatments, so the total number of patients meeting our study criteria will be much smaller compared with other similar studies conducted in adult patients. That explains why similar studies with pediatric patients are limited with patient sample size (22–55 patients). Due to the relatively small sample size and the large number of predictors, this study should be considered exploratory. The small sample size creates a higher risk of both type I and type II errors. Type I errors could occur if our population was systematically different from the population. The possibility of type II errors is increased as the power of this study makes it difficult to detect moderate or small effect sizes, which may be clinically significant. Based on the TRIPOD guidelines by Collins et al., this study falls under the type 1a: development only model, which suggests that our results could be improved.
with an enlarged patient sample size and a multicenter study to validate and refine our predictive risk factor model. The data generated from this study is critical for power analysis, which will allow us to better estimate the adequate sample size relative to the study goals and the possible variabilities of the study, in order to optimize the study design.

CONCLUSION

Our single-institution retrospective cohort study demonstrated that the risk of aGVHD was significantly increased with parent donors and/or donors older in age among pediatric patients receiving allogeneic HSCT from matched-related donors. We failed to observe a significant decisive role of pharmacological factors associated with tacrolimus treatment in a patient’s risk of aGVHD, although some studies indicated that these factors may play a role in aGVHD. Further prospective studies in a larger, more racially diverse cohort to investigate these findings are warranted.

CONFLICT OF INTEREST

The authors declared no competing interests for this work.

AUTHOR CONTRIBUTIONS

M.P., S.Y., R.B., R.C., and G.M. wrote the manuscript. S.Y., R.B., M.P., S.Y., R.C., and D.B. performed the research. N.B., M.P., S.Y., R.B., R.C., and G.M. designed the research. N.B., M.P., S.Y., R.B., M.P., S.Y., R.B., R.C., and G.M. wrote the manuscript. S.Y., R.B., M.P., S.Y., R.B., R.C., and G.M. wrote the manuscript.

REFERENCES

1. NCCN Guidelines and Clinical Resources [Internet]. 2020 [cited April 3, 2020]. https://www.nccn.org/professionals/physician_gls/default.aspx.
2. Jacobsohn DA. Acute graft-versus-host disease in children. Bone Marrow Transplant. 2008;41:215-221.
3. Przepiorka D, Devine S, Fay J, Uberti J, Wingard J. Practical considerations in the use of tacrolimus for allogeneic marrow transplantation. Bone Marrow Transplant. 1999;24:1053-1056.
4. Andrews LM, Hesselink DA, van Gelder T, et al. A population pharmacokinetic model to predict the individual starting dose of tacrolimus following pediatric renal transplantation. Clin Pharmacokinet. 2018;57:475-489.
5. Rower JE, Stockmann C, Linakis MW, et al. Predicting tacrolimus concentrations in children receiving a heart transplant using a population pharmacokinetic model. BMJ Paediatr Open. 2017;1:e000147.
6. Staatz CE, Tett SE. Clinical pharmacokinetics and pharmacodynamics of tacrolimus in solid organ transplantation. Clin Pharmacokinet. 2004;43:623-653.
7. Torlén J, Ringden O, Garming-Legert K, et al. A prospective randomized trial comparing cyclosporine/methotrexate and tacrolimus/sirolimus as graft-versus-host disease prophylaxis after allogeneic hematopoietic stem cell transplantation. Haematologica. 2016;101:1417-1425.
8. Brunet M, van Gelder T, Åsberg A, et al. Therapeutic drug monitoring of tacrolimus-personalized therapy: second consensus report. Ther Drug Monit. 2019;41:261-307.
9. Skeens M, Pai V, Garee A, et al. Twice daily i.v. bolus tacrolimus infusion for GVHD prophylaxis in children undergoing stem cell transplantation. Bone Marrow Transplant. 2012;47:1415-1418.
10. Offer K, Kolb M, Jin Z, et al. Efficacy of tacrolimus/mycophenolate mofetil as acute graft-versus-host disease prophylaxis and the impact of subtherapeutic tacrolimus levels in children after matched sibling donor allogeneic hematopoietic cell transplantation. Biol Blood Marrow Transplant. 2015;21:496-502.
11. Birdwell KA, Decker B, Barbarino JM, et al. Clinical Pharmacogenetics Implementation Consortium (CPIC) guidelines for CYP3A5 genotype and tacrolimus dosing. Clin Pharmacol Ther. 2015;98:19-24.
12. Yanik G, Levine JE, Ratanatharathorn V, Dunn R, Ferrara J, Hutchinson RJ. Tacrolimus (FK506) and methotrexate as prophylaxis for acute graft-versus-host disease in pediatric allogeneic stem cell transplantation. Bone Marrow Transplant. 2000;26:161-167.
13. Nash RA, Etzioni R, Storb R, et al. Tacrolimus (FK506) alone or in combination with methotrexate or methylprednisolone for the prevention of acute graft-versus-host disease after marrow transplantation from HLA-matched siblings: a single-center study. Blood. 1995;85:3746-3753.
14. R Core Team. R: A Language and Environment for Statistical Computing. Vienna, Austria: R Core Team, 2013.
15. Morgan JA, Tatar JF. Calculation of the residual sum of squares for all possible regressions. Technometrics. 1972;14:317-325.
16. Rohart F, Gautier B, Singh A, Le Cao KA. mixOmics: an R package for ‘omics feature selection and multiple data integration. PLoS Comput Biol. 2017;13:e1005752.
17. Perez-Enciso M, Tenenhaus M. Prediction of clinical outcome with microarray data: a partial least squares discriminant analysis (PLS-DA) approach. Hum Genet. 2003;112:581-592.
18. Faul F, Ederfeld E, Lang AG, Buchner A, G*Power 3: a flexible statistical power analysis program for the social, behavioral, and biomedical sciences. Behav Res Methods. 2007;39:175-191.
19. Collins GS, Reitsma JB, Altman DG, Moons KG. Transparent reporting of a multivariable prediction model for individual prognosis or diagnosis (TRIPOD): the TRIPOD Statement. BMC Med. 2015;13:1.
20. Wingard JR, Nash RA, Przepiorka D, et al. Relationship of tacrolimus (FK506) whole blood concentrations and efficacy and safety after HLA-identical sibling bone marrow transplantation. Biol Blood Marrow Transplant. 1998;4:157-163.
21. Simpson E, Dazzi F. Bone marrow transplantation 1957–2019. BMJ Open. 2017;7:e016124.
22. Flowers ME, Inamoto Y, Carpenter PA, et al. Comparative analysis of risk factors for acute graft-versus-host disease and for chronic graft-versus-host disease according to National Institutes of Health consensus criteria. Blood. 2011;117:3214-3219.
23. Eisdorfer MD, August CS. Impact of donor and recipient characteristics on the development of acute and chronic graft versus-host disease following pediatric bone marrow transplantation. Bone Marrow Transplant. 1995;15:653-668.
24. Martin P, Bleyzac N, Souillet G, et al. Clinical and pharmacological risk factors for acute graft-versus-host disease after pediatric
bone marrow transplantation from matched-sibling or unrelated donors. Bone Marrow Transplant. 2003;32:881-887.

25. Mori T, Kato J, Shimizu T, et al. Effect of early posttransplantation tacrolimus concentration on the development of acute graft-versus-host disease after allogeneic hematopoietic stem cell transplantation from unrelated donors. Biol Blood Marrow Transplant. 2012;18:229-234.

26. Marfo K, Alshuler J, Lu A. Tacrolimus pharmacokinetic and pharmacogenetic differences between adults and pediatric solid organ transplant recipients. Pharmacuetics. 2010;2:291-299.

27. Abdel Jalil MH, Hawwa AF, McKiernan PJ, Shields MD, McElnay JC. Population pharmacokinetic and pharmacogenetic analysis of tacrolimus in paediatric liver transplant patients. Br J Clin Pharmacol. 2014;77:130-140.

28. Kolb M, Offer K, Jin Z, et al. Risk factors for subtherapeutic tacrolimus levels after conversion from continuous intravenous infusion to oral in children after allogeneic hematopoietic cell transplantation. Biol Blood Marrow Transplant. 2016;22:957-961.

29. Niioka T, Satoh S, Kagaya H, et al. Comparison of pharmacokinetics and pharmacogenetic analysis of tacrolimus in paediatric liver transplant patients. Br J Clin Pharmacol. 2014;77:130-140.

30. Kolb M, Offer K, Jin Z, et al. Risk factors for subtherapeutic tacrolimus levels after conversion from continuous intravenous infusion to oral in children after allogeneic hematopoietic cell transplantation. Biol Blood Marrow Transplant. 2016;22:957-961.

31. Huynh DAH, Freeman T, Marshall V, Markstrom D, Frame D. Evaluation of tacrolimus levels and risk of acute graft-versus-host disease in allogeneic hematopoietic stem cell transplant. Biol Blood Marrow Transplant. 2019;25:S282.

32. Ganetsky A, Shah A, Miano TA, et al. Higher tacrolimus concentrations early after transplant reduce the risk of acute GVHD in reduced-intensity allogeneic stem cell transplantation. Bone Marrow Transplant. 2016;51:568-572.

33. Watanabe N, Matsumoto K, Muramatsu H, et al. Relationship between tacrolimus blood concentrations and clinical outcome during the first 4 weeks after SCT in children. Bone Marrow Transplant. 2010;45:1161-1166.

34. Hagen PA, Adams W, Smith S, Tsai S, Stiff P. Low mean post-transplantation tacrolimus levels in weeks 2–3 correlate with acute graft-versus-host disease in allogeneic hematopoietic stem cell transplantation from related and unrelated donors. Bone Marrow Transplant. 2019;54:155-158.

35. Tsapepas D, Saal S, Benkert S, et al. Sublingual tacrolimus: a pharmacokinetic evaluation pilot study. Pharmacotherapy. 2013;33:31-37.

36. Nasiri-Toosi Z, Dashki-Khavidaki S, Nasiri-Toosi M, et al. Clinical pharmacokinetics of oral versus sublingual administration of tacrolimus in adult liver transplant recipients. Exp Clin Transplant. 2012;10:586-591.

37. Wong KM, Shek CC, Chau KE, Li CS. Abbreviated tacrolimus area-under-the-curve monitoring for renal transplant recipients. Am J Kidney Dis. 2000;35:660-666.

38. Undre NA, Stevenson PJ, The European Tacrolimus Heart Study Group. Pharmacokinetics of tacrolimus in heart transplantation. Transplant Proc. 2002;34:1836-1838.

39. Miura M, Satoh S, Niioka T, et al. Early phase limited sampling strategy characterizing tacrolimus and mycophenolic acid pharmacokinetics adapted to the maintenance phase of renal transplant patients. Ther Drug Monit. 2009;31:467-474.

40. Tholking G, Schutte-Nutgen K, Schmitz J, et al. A low tacrolimus concentration/dose ratio increases the risk for the development of acute calcineurin inhibitor-induced nephrotoxicity. J Clin Med. 2019;8(10):1586.

41. NIOSH. NIOSH List of Antineoplastic and Other Hazardous Drugs in Healthcare Settings, 2016. In: CDC, editor. 2016.

42. Bolanos-Meade J, Reshef R, Fraser R, et al. Three prophylaxis regimens (tacrolimus, mycophenolate mofetil, and cyclophosphamide; tacrolimus, methotrexate, and bortezomib; or tacrolimus, methotrexate, and maraviroc) versus tacrolimus and methotrexate for prevention of graft-versus-host disease with haemopoietic cell transplantation with reduced-intensity conditioning: a randomised phase 2 trial with a non-randomised contemporaneous control group (BMT CTN 1203). Lancet Haematol. 2019;6:e132-e143.

43. Cutler C, Logan B, Nakamura R, et al. Tacrolimus/sirolimus vs tacrolimus/methotrexate as GVHD prophylaxis after matched, related donor allogeneic HCT. Blood. 2014;124:1372-1377.

44. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2020. CA Cancer J Clin. 2020;70:7-30.

45. Wallin JE, Friberg LE, Fasth A, Staatz CE. Population pharmacokinetics of tacrolimus in pediatric hematopoietic stem cell transplant recipients: new initial dosage suggestions and a model-based dosage adjustment tool. Ther Drug Monit. 2009;31:457-466.

46. Durlak JA. How to select, calculate, and interpret effect sizes. J Pediatr Psychol. 2009;34:917-928.

47. Cohen J. Statistical power analysis for the behavioral sciences. 2nd Edition, Hillsdale, NJ: L. Erlbaum Associates; 1988.

48. Mangiafico S. Rcompanion: Functions to Support Extension Education Program Evaluation. R package version 2.3.25. 2020.

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