A Time-to-Event Model for Acute Kidney Injury after Reduced-Intensity Conditioning Stem Cell Transplantation Using a Tacrolimus- and Sirolimus-based Graft-versus-Host Disease Prophylaxis

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A B S T R A C T

There is a paucity of data evaluating acute kidney injury (AKI) incidence and its relationship with the tacrolimus–sirolimus (Tac-Sir) concentrations in the setting of reduced-intensity conditioning (RIC) after allogeneic stem cell transplantation (allo-HSCT). This multicenter retrospective study evaluated risk factors of AKI defined by 2 classification systems, Kidney Disease Improving Global Outcome (KDIGO) score and "Grade 0-3 staging," in 186 consecutive RIC allo-HSCT recipients with Tac-Sir as graft-versus-host disease prophylaxis. Conditioning regimens consisted of fludarabine and busulfan (n = 53); melphalan (n = 83); or a combination of thiotepa, fludarabine, and busulfan (n = 50). A parametric model, with detailed Tac-Sir consecutive blood levels, describing time to AKI was developed using the NONMEM software version 7.4. Overall, 81 of 186 (44%) RIC allo-HSCT recipients developed AKI with a cumulative incidence of 42% at a median follow-up of 25 months. Time to AKI was best described using a piecewise function. AKI-predicting factors were melphalan-based conditioning regimen (HR, 1.96; $P < .01$), unrelated donor (HR, 1.79; $P = .04$), and tacrolimus concentration: The risk of AKI increased 2.3% per each 1-ng/mL increase in tacrolimus whole blood concentration ($P < .01$). In multivariate analysis, AKI grades 2 and 3 according to KDIGO staging were independent risk factors for 2-year nonrelapse mortality (HR, 2.8; $P = .05$; and HR, 6.6; $P < .0001$, respectively). According to the KDIGO score, overall survival decreased with the increase in severity of AKI: 78% for patients without AKI versus 68%, 50%, and 30% for grades 1, 2, and 3, respectively ($P < .0001$). In conclusion, AKI is frequent after Tac-Sir–based RIC allo-HSCT and has a negative impact on outcome. This study presents the first predictive model describing time to AKI as a function of tacrolimus drug concentration.

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INTRODUCTION

Acute kidney injury (AKI) is a frequent complication after allogeneic stem cell transplantation (allo-HSCT) [1,2], affecting from 10% to 70% of transplant recipients [3-11]. Kidney impairment after allo-HSCT has been shown to negatively influence nonrelapse mortality (NRM) and overall survival (OS) [4,5,7,9,11,12].

Calcineurin inhibitors (CNI), mainly cyclosporine (CsA) and tacrolimus, used alone or in combination with other immunosuppressive agents are the most common drugs for graft-versus-host disease (GVHD) prophylaxis in allo-HSCT. These drugs have well-known nephrotoxic effects, mostly secondary to their potent vasoconstricting properties and their ability to cause endothelial injury. Both have shown similar incidences of AKI after allo-HSCT [13,14]. A tacrolimus and sirolimus (Tac-Sir) combination has emerged as a promising strategy to prevent GVHD, especially in the reduced-intensity conditioning (RIC) allo-HSCT setting [15]. The Tac-Sir combination has been shown to be more effective and less toxic than sirolimus combined with CsA [16-18]. However, the former may increase the risk of transplant-associated microangiopathy (TAM) and sinusoidal obstruction syndrome of the liver [9,19-21], conditions that are closely linked with the development of AKI.

There is limited information on the association between concentration levels of immunosuppressive agents and the occurrence of renal complications after allo-HSCT. Thus, high levels of sirolimus have been associated with the development of TAM [22], whereas CNIs have direct nephrotoxic effects and can also precipitate or contribute to TAM. To establish the potential role of the concentration-over-time of these agents in causing AKI, an analysis of drug exposure through the measurement of the area under the curve would be required, but its logistical complexity and cost make it unfeasible in "real-life" clinical practice. For these type of analyses, recommendations suggest the application of powerful methods that take into account the dependency and association between longitudinal data (ie, through blood concentrations of tacrolimus and sirolimus) and time-to-event data (ie, AKI) [23]. Fully parametric time-to-event analyses can bring these 2 types of data together into a single model so that the evaluation of the dependence and association between the longitudinal marker and time-to-event permit a better assessment of treatment effect and result in a lower bias and less inefficient estimates compared with the classic Cox model method [23-28].

With this background, we assessed renal function by serum creatinine levels and as estimated glomerular filtration rates (GFRs) calculated by the modification of diet in renal disease equation in a large cohort of patients who underwent RIC allo-HSCT with the Tac-Sir combination as GVHD prophylaxis. We examined the pharmacokinetic–pharmacodynamic relationship between Tac-Sir exposure and the development of AKI through a parametric time-to-event model using longitudinal through blood concentrations of both drugs.

METHODS

A completely detailed methods section is provided in an online supplementary methods section.

Patients

From October 2008 to October 2015, 186 consecutive RIC allo-HSCT recipients in 3 Spanish institutions who received the Tac-Sir combination to prevent GVHD were included in the study. The institutional review boards approved the study, and written informed consent was obtained from all patients according to the Declaration of Helsinki. The study was registered by the Spanish Agency of Medicines and Health Products with the reference code PIN-SIR-2016-01.

Conditioning Regimen and GVHD Prophylaxis

Three RIC regimens were used in this study. Briefly, fludarabine was combined with busulfan for myeloid neoplasm (Flu-Bu, n = 53) or with melphalan for lymphoid neoplasm (Flu-Mel, n = 83) as detailed elsewhere [29]. The third RIC regimen consisted of fludarabine in combination with busulfan and thiotepa (TBF regimen, which was used for both myeloid and lymphoid

malignancies, n = 50). The GVHD prophylaxis consisted of Tac-Sir. The planned taper schedule has been described elsewhere [15].

AKI and TAM Definitions

GFR was calculated by the modification of diet in renal disease equation as follows: (GFR [mL/min per 1.73 m^2] = 186 × serum creatinine (mg/L) – 1.154 × age (in years) – .203 (× .742 if female)) [30]. AKI was defined as a decrease of at least 25% of baseline GFR or when creatinine levels rose above the standard values and reached ≥1.5 times the baseline value. AKI was classified on the basis of the new Kidney Disease Improving Global Outcome (KDIGO) classification system proposed in 2012 based on serum creatinine and urine output [31] and also by the "Grade 0-3 staging" definition, based on serum creatinine and estimated GFR, as detailed elsewhere [1] (see Supplementary Figures S1 and S2). TAM was classified as confirmed or probable according to previously defined international criteria [32-34].

Sir-Tac Blood Level Monitoring, Management, and Technical Considerations

Tac-Sir trough blood levels were monitored at least 2 times per week during the first 4 weeks after transplantation or until discharge, weekly until day +100, and thereafter on each outpatient visit. All blood samples were trough concentrations collected before receiving a scheduled dose. Doses were adjusted for target blood levels of 5 to 12 ng/mL for sirolimus and 5 to 10 ng/mL for tacrolimus.

Statistical Data Analysis

The primary objective of the study was the identification of risk factors for the development of AKI. Secondary objectives were the effect of AKI (analyzed as a time-dependent covariate) on OS and NRM according to the 2 classification systems, KDIGO and Grade 0-3 staging.

Nonparametric and Semiparametric Analyses

AKI, NRM, GVHD, TAM, and relapse were estimated by the cumulative incidence method [35,36]. Univariate analyses of the association of clinical risk factors with these transplantation outcomes were calculated using the Gray test. Time-dependent covariates were analyzed by univariate Cox regression models. When any time-dependent covariate was included in the final models, multivariate analyses were performed by Cox proportional hazards regression; otherwise, the Fine and Gray test was used. The probability of OS was estimated from the time of transplantation using Kaplan-Meier curves [37], and univariate comparisons were done with the log-rank test [38,39]. If AKI was found to have an impact on OS in the univariate analysis, a semi-landmark plot was constructed to illustrate visually the effect [40]. Tests of significance were 2-sided, with statistical significance considered as $P \leq .05$. All statistical analyses were performed using SPSS version 20 (SPSS, Chicago, IL) and R version 2.12.2 (The CRAN project: https://www.rstudio.com) with the packages survival v2.36-10, Design 2.3-0, prodlim v1.2.1, and cmprsk v2.2-221.

Parametric Analysis

A parametric survival model describing time to AKI, with emphasis on the potential effect of tacrolimus and sirolimus exposure, after RIC allo-HSCT was developed by means of nonlinear mixed-effects modeling using the NONMEM software version 7.4. The model was developed in 2 steps: (1) a baseline model without any explanatory factors, and (2) thereafter the impact of the study variables was explored and included in the baseline model. To describe the time to AKI, a parametric survival function according to the following equation was used:

$$S(t) = e^{-\int_0^t h(t)dt}$$

The final model was then used to simulate new treatment schedules to explore treatment outcomes with different drug exposure levels in terms of time until AKI.

RESULTS
Patient Characteristics

Patient and transplant characteristics and outcomes are summarized in Table 1. Overall, 186 RIC allo-HSCT recipients with a median age of 58 years (range, 23 to 72) and with hematologic malignancies, mostly with acute leukemia and myelodysplastic syndrome (46%), were included in this study. One hundred fourteen patients (61%) were in complete remission at transplantation. Most recipients (73%) were allografted from an adult unrelated donor (URD) and 37 (20%)

Table 1
Patients Characteristics (N = 186)

Characteristics	Value
Recipients median age, yr (range)	58 (23-72)
Male sex recipient, n (%)	111 (60)
Number of prior therapy lines (range)	2 (0-8)
Prior HSCT, n (%)	66 (35)
HCT-CI, n (%)	
0	38 (20)
1-2	77 (42)
≥ 3	71 (38)
Diagnostic, n (%)	
AL/MDS	63/22 (46)
NHL/MM/HD/CLL	49 (26)/10 (5)/ 18 (10)/10 (5)
Others	14 (8)
Disease status at transplant, n (%)	
CR	114 (61)
PR	44 (24)
SD/PROG	28 (15)
Female donor to male recipient, n (%)	41 (22)
URD, n (%)	136 (73)
HLA match 8/8, n (%)	149 (80)
Conditioning regimen, n (%)	
Flu-Bu	53 (28)
Flu-Mel	83 (45)
Flu-Bu-TT	50 (27)
ATG as a part of conditioning, n (%)	36 (19)
Peripheral blood source, n (%)	180 (97)
CD34$^+$, ×10^6/kg, median (range)	6.06 (.5-13.8)
Pretransplant creatinine, mmol/dL, median (range)	.78 (.33-1.55)
Baseline GFR, mL/min/1.73 m^2, median (range)	102 (44-240)
Transplant outcome	
Median days to myeloid recovery (range)	
neutrophil > .5 × 10^9/L	15 (8-37)
Platelet > 20 × 10^9/L	12 (0-380)
Acute GVHD	
Cumulative incidence of acute GVHD grades II-IV, % (95% CI)	38 (31-45)
Median onset, days (range)	65 (5-275)
Cumulative incidence of acute GVHD grades III-IV, % (95% CI)	18 (13-24)
ARF	
Cumulative incidence of AKI, % (95% CI)	42 (35-49)
Median onset, days (range)	28 (1-392)
Grade 0-3 staging AKI, n (%) (n = 81)	
Grade 1	17 (21)
Grade 2	62 (77)
Grade 3	2 (2)
KDIGO staging AKI, n (%) (n = 81)	
Grade 1	22 (27)
Grade 2	28 (35)
Grade 3	31 (38)
TAM	
Cumulative incidence of TAM, % (95% CI)	19 (13-25)
Median onset, days (range)	40 (8-476)
Chronic GVHD, n/assessable patients	99/166
Cumulative incidence of chronic GHVD at 2 years, % (95% CI)	74 (65-83)
Median onset in days (range)	186 (77-915)
Cumulative incidence of chronic GHVD extended, % (95% CI)	40 (31-49)
NRM, % (95% CI)	
At day +100	9 (5-14)
At day +180	13 (8-18)
At 2 years	21 (16-28)
Relapse at 2 years, % (95% CI)	19 (13-25)
DFS at 2 years, % (95% CI)	60 (58-64)
OS at 2 years, % (95% CI)	68 (64-72)
Median follow-up for survivors, mo (range)	25 (4-85)

HSCT means hematopoietic stem cells transplantation; CR, first complete remission; PR, partial remission; HCT-CI, hematopoietic cell transplantation–specific comorbidity index; TT, thiotepa; ATG, antithymogloubuline; Mel, melphalan; PROG, nonresponders or progression before allo-RIC; DFS, disease-free survival.

had an HLA mismatch. RIC regimens were mainly based on Flu-Bu or Flu-Mel (73%).

Incidence, Etiology, and Risk Factors for AKI

After a median follow-up of 25 months (range, 4 to 85) for survivors, 81 of 186 patients (44%) developed AKI at a median of 28 days (range, 0 to 392) after stem cell infusion, with a 2-year cumulative incidence of 42% (95% confidence interval [CI], 35 to 49). Notably, 69 of 81 cases (84%) of AKI occurred before day +100. Twenty-four patients (29%) developed AKI during the initial hospital admission and 45 cases (55%) occurred after discharge but before day +100. The remaining 12 patients (16%) developed AKI from days +101 to +365.

The KDIGO classification system allowed a better group separation of AKI severity compared with Grade 0-3 staging. As a result, the severity of AKI according to the KDIGO staging was grade 1 in 27% of the recipients, grade 2 in 35%, and grade 3 in 38%, whereas according to Grade 0-3 staging AKI severity was grade 1 in 21%, grade 2 in 77%, and grade 3 in 2% of recipients, respectively. The causes of AKI as considered by the treating SCT physicians are detailed in Supplementary Table S1. Univariate and multivariate analyses of risk factors for AKI are shown in Table 2.

Tacrolimus and Sirolimus Exposure and AKI

For the parametric time-to-event model, we retrospectively included 5977 blood level samples of tacrolimus and sirolimus throughout a median of 266 days (range, 1 to 2550) after transplant. Detailed characteristics of sirolimus and tacrolimus blood levels are shown in Supplementary Table S2.

The objective function value for the main structural models tested in the nonlinear model building procedure are shown in Supplementary Table S3. The hazard function giving the best result with regard to objective function value and Kaplan-Meier visual-predictive-check plots was a piecewise-function, defining 3 time intervals with 1 constant hazard each. The times at which hazards changed were at 50 and 150 days after transplantation. The resulting hazard function had a high hazard for the first 50 days after transplantation, followed by a progressive decrease in hazard to the minimal value after 150.

The results of the univariate testing of the covariate relationships are shown in Table 3. Based on these results, covariates to test in the stepwise covariate modeling were chosen. Tacrolimus exposure as a continuous covariate, URD, and melphalan-based conditioning regimen were identified as significant covariates during the stepwise covariate modeling procedure. Sirolimus exposure was not identified as a significant covariate for the development of AKI in the model. Patients allografted from URD proved to have a higher risk for AKI than those who did not (hazard ratio [HR], 1.79; $P = .04$). In the same manner, melphalan-based conditioning regimen administration was also correlated to a higher risk of AKI (HR, 1.96; $P < .01$). Regarding exposure to tacrolimus, a linear relationship was identified between drug exposure (obtained from therapeutic drug monitoring of trough drug concentration) and AKI hazard ($P < .01$). The influence of tacrolimus exposure and conditioning/donor subgroups on the probability of patients not suffering an AKI are represented through deterministic simulations in Figure 1A and B, respectively.

Regarding model validation, visual predictive check Kaplan-Meier plots (Figure 2) proved an adequate performance of the

Table 2

Analysis of Patient and Transplant Risk Factors for AKI and NRM at Median Follow-up of 25 Months

Variables	AKI				NRM			
	Univariate analysis		Fine and Gray test		Univariate analysis		Cox Regression Hazard Model	
	Cumulative Incidence % (95% CI)	P	HR (95% CI)	P	Cumulative Incidence % (95% CI)	P	HR (95% CI)	P
Donor*								
HLA identical sibling	33 (20-46)	.04			23 (10-36)	.8		
URD	49 (40-57)		1.7 (1-2.9)	.05	21 (14-29)			
Recipient age, yr								
<58	42 (32-53)	.5			18 (10-27)	.3		
≥58	46 (36-56)				26 (16-35)			
TAM†								
Yes	85 (65-100)	.005	NT		23 (15-30)	.02		
No	41 (34-49)				35 (18-52)			
Baseline GFR								
>70 mL/min/1.73 m²	42 (34-50)	.003			21 (14-28)	.4		
≤70 mL/min/1.73 m²	68 (48-89)		2.6 (1.4-4.8)	.002	27 (7-47)			
HCT-CI								
0	46 (30-62)				10 (1-20)			
1-2	38 (27-49)	.3			21 (10-30)	.05		
≥3	50 (39-62)				31 (19-43)			
HLA compatibility,* n (%)								
HLA match 8/8	58 (42-74)	.05	NT		23 (16-31)	.6		
HLA mismatch	41 (33-49)				16 (4-28)			
Conditioning regimen*						.002		
Flu-Bu	27 (15-39)		1		14 (3-24)		1	
Flu-Mel	59 (46-67)	.0007	2.7 (1.5-5)	.02	17 (8-25)		.8 (.3-2.3)	.7
TBF	36 (23-50)		2 (.9-3.9)	.06	40 (25-55)		3.6 (1.4-9)	.008
Acute GVHD grades II-IV‡								
Yes	–				31 (19-42)	.06	NT	
No	–				17 (8-25)			
Acute GVHD grades III-IV‡								
Yes	36 (21-50)	.05	NT		46 (29-63)	.0002	2.6 (1.3-5)	.006
No	47 (39-57)				16 (9-24)			
AKI‡								
Yes	–				33 (22-43)	.005	NT	
No	–				16 (8-25)			
AKI KDIGO staging‡						<.0001		
Grade 0	–				14 (6-21)		1	
Grade 1	–				9 (0-22)		.8 (.2-3.4)	.8
Grade 2	–				24 (7-41)		2.8 (1-6.6)	.05
Grade 3	–				53 (35-70)		6.6 (2.9-15)	<.001
Donor sex mismatch								
Female donor to male recipient	38 (23-53)	.3			38 (23-54)	.005	2.2 (1.1-4.5)	.02
Others	46 (38-55)				17 (10-24)			
Disease status at Transplantation								
CR	40 (30-49)	.1			23 (14-31)	.8		
Active disease	53 (41-64)				21 (11-31)			
Diagnosis*								
Myeloid disease	30 (20-40)	.002	NT		22 (12-32)	.7		
Lymphoid disease	57 (47-67)				22 (14-31)			
No. prior therapies								
<3	35 (25-45)	.005			26 (16-36)	.5		
≥3	55 (42-65)		1.7 (1.1-2.7)	.02	19 (11-28)			
Prior HSCT*								
Yes	60 (48-72)	.004	NT		26 (15-38)	.3		
No	36 (27-45)				20 (12-27)			

HSCT indicates hematopoietic stem cell transplantation; NT, not tested in the multivariate analysis, because grades III-IV acute GVHD is a subcategory of grades II-IV acute GVHD.

* For ARF risk factors analysis we included conditioning regimen into the multivariate model because variables such as ACST and diagnosis showed co-linearity to each other. In the same manner, we only included type of donor because donor–recipient HLA mismatch was strongly associated with unrelated donor (see Methods).

† TAM was not included in the multivariate analysis because we did not consider this condition as a risk factor but rather a cause of ARF (see Supplementary Table S1).

‡ Analyzed as time-dependent covariates.

model given that the observed survival curve was situated inside the simulation prediction intervals. Additionally, bootstrap results reinforced the robustness of the model because final model parameter estimates were inside the CIs obtained from bootstrap re-estimations. Furthermore, the statistical significance of the tacrolimus-exposure effect on AKI-free survival was reinforced because the 95% CI of the re-estimated values for this parameter did not include the 0 value (.00813 to .0484). This was also true for parameters representing other covariates influence.
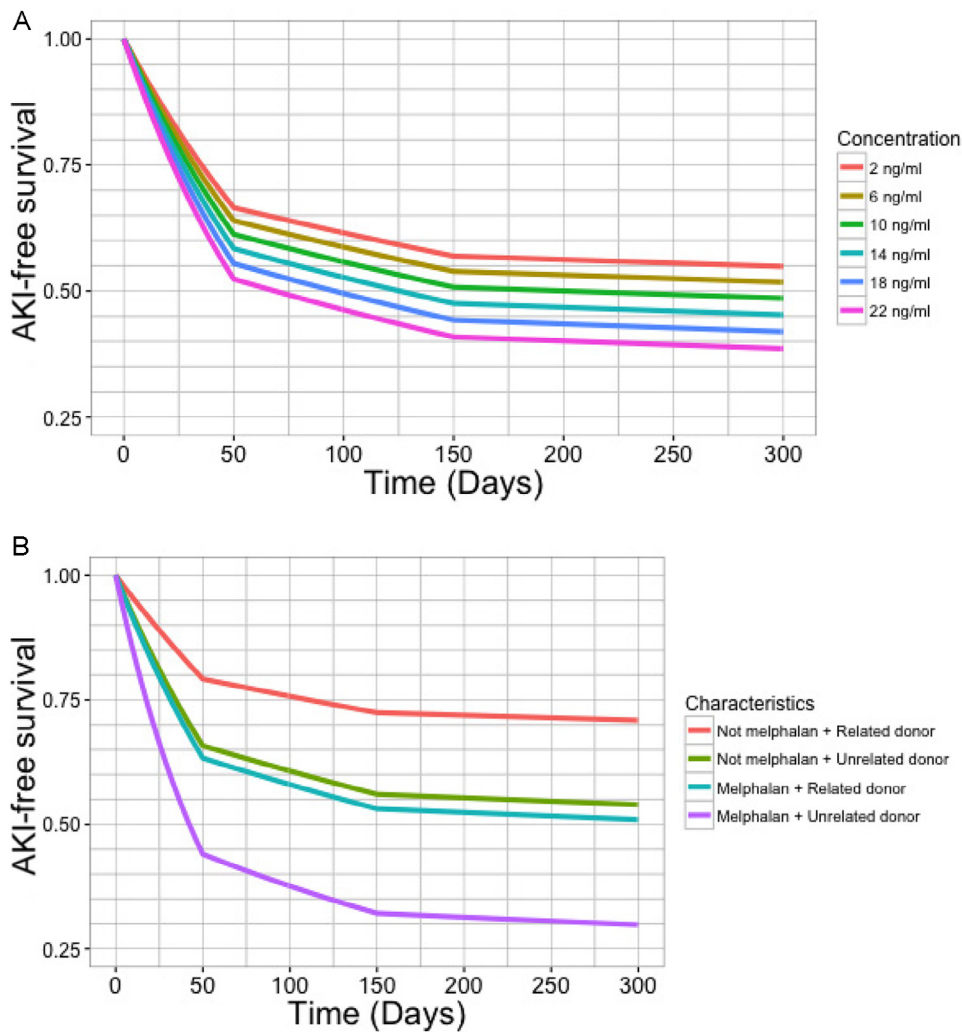
Figure 1. (A) Simulated AKI-free survival curves at different exposure levels. Simulations were based on patients receiving a melphalan-based conditioning regimen, with a related donor and exposed to tacrolimus trough concentrations ranging between 2 and 22 ng/mL. (B) Simulated AKI-free survival curves for different subgroups of patients. Simulations were based on patients receiving/not receiving a melphalan-based conditioning regimen, with a related/unrelated donor and exposed to tacrolimus trough concentrations of 7 ng/mL.

NRM, Relapse, and OS

Thirty-seven patients (20%) died due to NRM at a median of 102 days (range, 13 to 639). The day +100 and +180 and 2-year cumulative incidence of NRM for the whole group was 9% (95% CI, 5% to 14%), 13% (95% CI, 8% to 18%), and 21% (95%

Table 3
Final Model Parameter Estimates and Bootstrap Results

Parameters	Values	RSE (%)	Bootstrap Results	
			Median	95% CI (percentiles)
λ_1 (days^{-1})	$3.97 \cdot 10^{-3}$	22.9	$3.84 \cdot 10^{-3}$	$2.23 \cdot 10^{-3}$-$6.08 \cdot 10^{-3}$
λ_2 (days^{-1})	$7.60 \cdot 10^{-3}$	29.3	$7.46 \cdot 10^{-4}$	$3.78 \cdot 10^{-4}$-$1.37 \cdot 10^{-3}$
λ_3 (days^{-1})	$1.20 \cdot 10^{-4}$	54.0	$1.17 \cdot 10^{-4}$	$2.80 \cdot 10^{-5}$-$2.85 \cdot 10^{-4}$
θ_{MELF}	.673	34.6	.670	.229-1.17
θ_{URD}	.585	42.9	.584	.0337-1.09
$\theta_{Tacrolimus}$	.0231	26.2	.0231	.00813-.0484

RSE indicates relative estimation error; λ_1, hazard estimate for days 0-50; λ_2, hazard estimate for days 50-150; λ_1, hazard estimate for days 150 onward; θ_{URD}, URD regression coefficient; θ_{MELF}, melphalan regression coefficient; $\theta_{Tacrolimus}$, regression coefficient for tacrolimus concentration effect.

CI, 16% to 29%), respectively. Causes of NRM were GVHD and/or infections (n = 30; infection without GVHD [n = 16], GVHD without infection [n = 8]), sinusoid obstruction syndromes (n = 2), TAM (n = 1), central nervous system bleeding (n = 1), sudden death (n = 1), and from unknown causes (n = 2).

Factors associated with NRM in univariate and multivariate analyses are shown in Table 2. The variables associated with NRM were TBF (HR, 3.6; P = .008), female donor to male recipient (HR, 2.2; P = .02), acute GVHD grades III to IV (HR, 2.6; P = .006), and grade 2 KDIGO staging (HR, 2.8; P = .05) and grade 3 KDIGO staging (HR, 6.6; P < .001). The cumulative incidence of relapse was 19% (95% CI, 13% to 25%) for the entire group. Again, KDIGO staging led to a more homogeneous risk stratification for OS probabilities than Grade 0-3 staging. As a result, recipients without AKI, according to the KDIGO staging, showed an OS probability of 78% versus 68%, 50%, and 30% for grades 1, 2, and 3, respectively (log-rank, 31; P < .0001). According to Grade 0-3 staging the probability of OS for recipients without AKI was 78% versus 78% for grade 1, 44% for grade 2, and 0% for the 2 recipients who developed grade 3 (log-rank, 28; P < .0001) (Figure 3A,B).
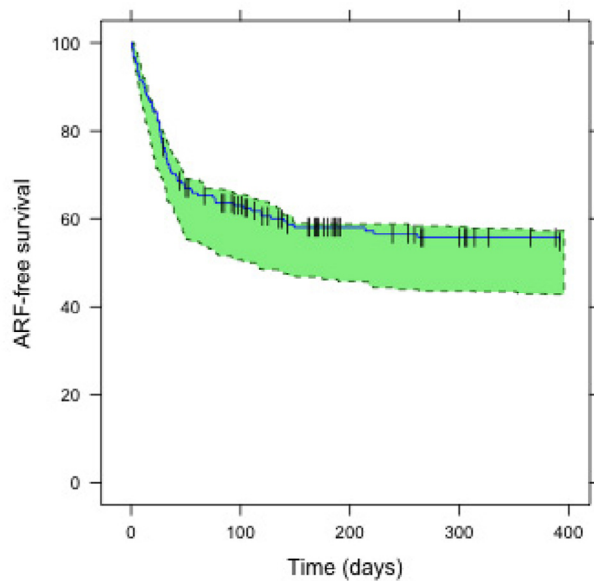
Figure 2. Kaplan-Meier visual predictive check plot. Blue line represents observed AKI-free survival data; green area represents 95% confidence interval of 200 simulated AKI-free survival curves based on the final model. (This figure is available in color online at www.bbmt.org.)

DISCUSSION

This study showed a 2-year AKI incidence of 42% in a cohort of patients who received Tac-Sir combination as GVHD prophylaxis after RIC allo-HSCT, in line with that observed in previous reports [4,5,10-12]. By means of a fully parametric survival data analysis, using a detailed drug concentration dataset, we report a significant linear relationship between tacrolimus concentrations over time and the development of AKI, even when targeting a narrow therapeutic range level (5 to 10 ng/mL) of tacrolimus.

The current series represents the larger study performed in allo-HSCT that evaluates AKI according to the new classification suggested by the KDIGO expert panel [31]. We assessed the usefulness of classifying AKI severity grades by KDIGO staging in comparison with Grade 0-3 staging, which has been previously used by us and others in this setting [1,10,12]. Both classification significantly predicted NRM and OS. However, the KDIGO score allowed a more homogenous risk stratification for both outcomes compared with Grade 0-3 staging, suggesting a better discrimination profile of the former. Thus, the KDIGO score seems to be a useful tool in the allo-HSCT setting and should be explored prospectively.

However, the inclusion of Grade 0-3 staging allowed us to compare the characteristics of AKI in the current cohort with our prior AKI experience [12]. The overall incidence of AKI was similar to our previous study of 188 patients who received a CsA-based GVHD prophylaxis in a similar RIC allo-HSCT setting. However, with the Tac-Sir combination, AKI was more severe than with the CsA-based prophylaxis (Grade 0-3 staging AKI grade > 1, 79% versus 14%, respectively). Although only 1 study was performed in 1998 where tacrolimus was related to severe kidney toxicity compared with CsA [41], several other studies did not confirm this observation [14,42,43]. In fact, a cell culture model showed that tacrolimus had a lesser deleterious effect on the endothelium than CsA. However, the addition of sirolimus to tacrolimus synergistically increased endothelium injury by increasing the

expression of intercellular adhesion molecule-1 [44]. Also, sirolimus increased chronic nephrotoxicity by CNIs, both in rats [45] and in humans [46,47]. Additionally, sirolimus is known to cause proteinuria. In a randomized study the Tac-Sir combination showed more common elevation of creatinine levels than tacrolimus plus methotrexate [48]. Also, the authors observed a trend toward increased rates of the endothelial injury syndromes, veno-occlusive disease, and TAM within 100 days of transplantation in the Tac-Sir arm compared with the tacrolimus plus methotrexate arm [48]. There are no reasons to believe these differences are only related to tacrolimus exposure, because this study targeted tacrolimus serum levels from 5 to 10 ng/mL in both arms. All these data together suggest that it is not only tacrolimus by itself but rather the addition of sirolimus to tacrolimus that may synergistically increase the AKI incidence and/or its severity. Although our data suggest that the combination of sirolimus to tacrolimus may increase AKI severity compared with CsA in combination with other agents, prospective comparative studies are required to confirm this hypothesis.

In the current study the Tac-Sir combination showed a high incidence of confirmed TAM (10%; 95% CI, 4% to 15%) according to the Blood and Marrow Transplant Clinical Trials Network Toxicity Committee consensus and the International Working Group criteria. However, when probable-TAM criteria were included, the TAM incidence was even higher (18%) and in line with other reports [19,22,49,50].

Regarding CNI exposure, different targeted serum levels of CNI (low, medium, and high) have been randomly and prospectively compared in the kidney transplantation setting. High CNI targeted levels have been consistently associated with renal allograft nephropathy [51]. However, in the field of allo-HSCT such studies are lacking, and most large studies conducted in the context of single arm, preset, low narrow therapeutic levels failed to show a significant association between CNI blood levels and development of AKI [7,8,52]. These circumstances may have potentially limited the power to find statistical correlations between the risk of AKI and drug exposure in allo-HSCT. Furthermore, if weekly mean drug concentrations are chosen as a surrogate marker of drug exposure to simplify the analysis, a big part of the information is disregarded, and this can potentially lead to bias and poor power of the conventional analyses, such as the Cox model.

Parametric longitudinal and time-to-event data analysis in the area of pharmacometrics has emerged as a powerful tool [23]. The semiparametric Cox model has been the most frequently used regression tool for time-to-event data [24]. However, fully parametric models can offer some advantages such as more efficient parameter estimates than the Cox model [23], reduced bias in the estimates of the overall treatment effect [23-28], and smaller sample size requirements. Furthermore, this approach may provide insights into the shape of the baseline hazard and simulation of survival functions becomes possible. For this reason we performed a parametric modeling analysis to evaluate the effect of tacrolimus and sirolimus exposure with detailed records of drug concentrations in blood in the development of AKI. With the present methodology we observed a significant linear association between tacrolimus whole blood levels and the risk of AKI, even in the low-range concentrations. Earlier reports of studies evaluating a wider range of concentrations (10 to 40 ng/mL) found a cutoff level of 20 ng/mL discriminating between low- and high-risk AKI [14,53]. However, the establishment of a cutoff level or the quantification of drug effect on AKI risk in the setting of narrow
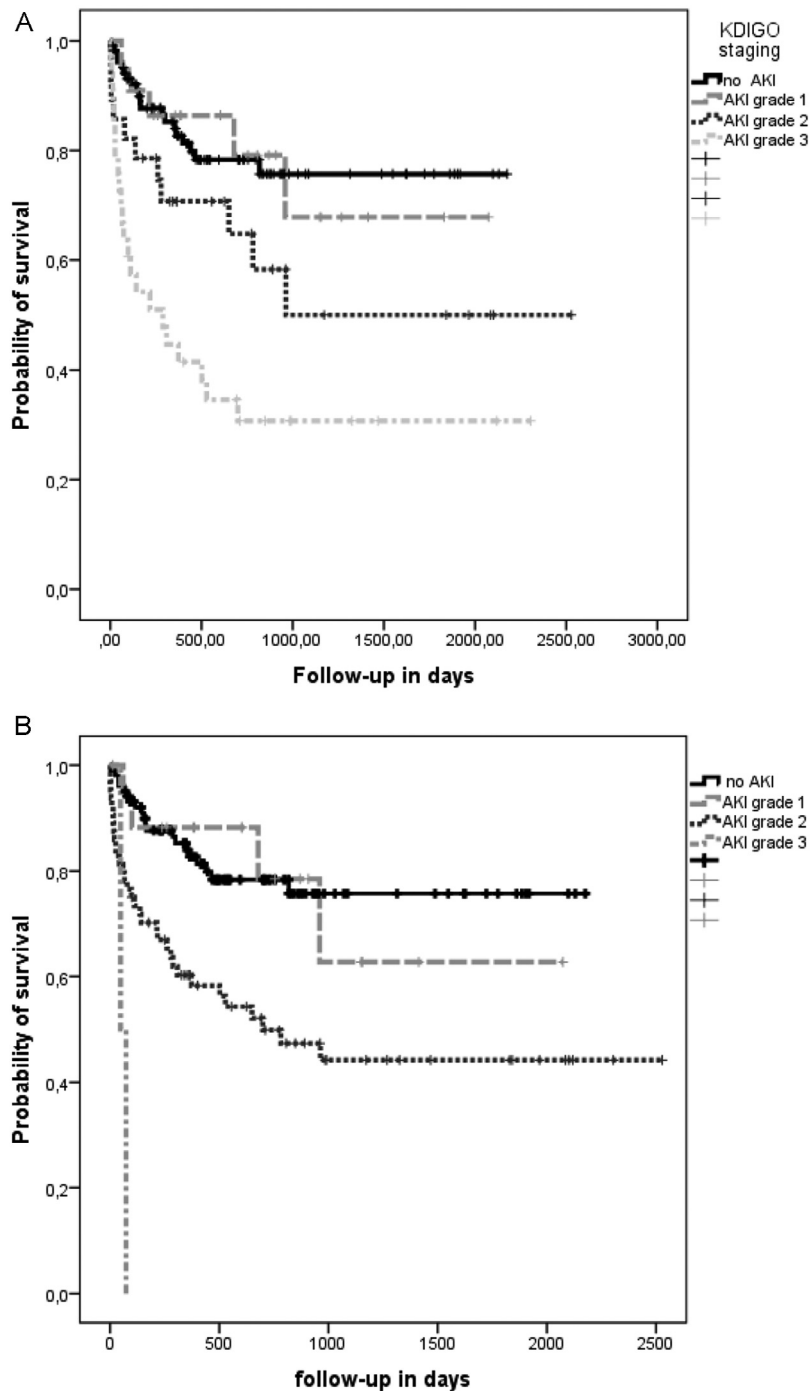
Figure 3. (A) OS according to KDIGO staging severity grade of AKI. No AKI had 78% of OS probability versus 68% for grade 1, 50% for grade 2, and 30% for grade 3 (*P* < .0001). (B) OS according to "Grade 0-3 staging" severity grade of AKI. No AKI 78% of OS versus 62% for grade 1, 42% for grade 2, and 0% for grade 3 (*P* < .0001). (Semi-landmark plot illustrating impact of moderate to severe ARF on overall survival.)

therapeutic range levels has been commonly frustrating. The linear relationship found in the current series, characterized by a 2.3% increase in AKI probability per each 1-ng/mL increase of tacrolimus levels, could also explain in part the inability of conventional analyses to find a reliable cutoff level and/or a negative effect because the difference between concentrations in the lower and higher limits of the targeted therapeutic range represented only a 11.5% increase of AKI risk. Nevertheless, tacrolimus exposure was the most relevant risk factor of AKI in this study, even when physicians

had the capability of modulating exposure throughout the transplant procedure. These finding represents a first step toward a model-informed exposure risk-to-benefit evaluation. In the present study the exposure-to-risk relation for tacrolimus and AKI has been quantified. These results, in conjunction with the future evaluation of tacrolimus exposure-to-benefit relationship (ie, tacrolimus exposure and GVHD incidence) will provide physicians with valuable tools for evidence-based clinical decision-making when using this immunosuppressant drug.

Other risk factors identified in the parametric time-to-event model analysis were the use of URD and melphalan-based RIC regimen. Both conditions have not been previously reported as risk factors for AKI [2]. However, melphalan has a well-known nephrotoxic effect. In addition, Flu-Mel has been recently related with higher gastrointestinal toxicity, in particular severe mucositis, and acute GVHD compared with Flu-Bu RIC [54]. Persistent gastrointestinal damage characterized by poor oral intake and/or persistent diarrhea could result in a negative hydric imbalance early after discharge. These factors surely influence the development of AKI. In fact, we observed that most cases of AKI (55%) occurred in the outpatient clinic before day +100. Also, the piecewise-function modeling defined the higher risk interval from day 0 until day +50. An optimal outpatient fluid replacement program until day +50, or extended to day +100, may help to reduce the risk of AKI and merits further research. Regarding the identification of URD as an AKI risk factor, several studies reported that the use of an URD was related with higher incidence of infections and GVHD [55]. The common use of nephrotoxic antimicrobial agents and the development of severe forms of GVHD may in part explain this association.

An important issue that deserves special attention is the fact that the multivariate Fine and Gray model identified low baseline GFR and more than 3 prior therapy lines as risk factors for AKI, but these findings were not confirmed in the time-to-event model. It is likely that in the latter model the importance of tacrolimus levels outweighed other risk factors associated with increased tacrolimus exposure. In fact, tacrolimus clearance is significantly reduced with impaired renal function [56]. It is also possible that recipients with a high number of prior therapy lines, considered as heavily pretreated patients, may have had subclinical kidney damage. These findings emphasize the important role of flexible modeling techniques to accurately evaluate the weight of each clinical risk factor in the presence of longitudinal covariate data that could be strongly involved with the event (ie, tacrolimus and AKI).

Finally, our study confirms that the development of AKI has a negative effect on NRM and OS. We also identified other variables as independent risk factors for NRM. Acute GVHD grades III to IV and female donor to male recipient were previously associated with higher NRM in the RIC allo-HSCT setting [12,57], whereas the association of TBF conditioning regimen with higher NRM in this study merits further investigation, currently ongoing.

In conclusion, AKI is common in the setting of RIC allo-HSCT and has a negative effect on the outcomes. The KDIGO staging system is useful in predicting outcomes in RIC allo-HSCT and needs to be further explored. The proof of a linear relationship between tacrolimus exposure and the development of AKI by a parametric time-to-event model could help physicians in routine clinical practice, although prospective studies to validate these findings are required.

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SUPPLEMENTARY DATA

Supplementary data related to this article can be found online at doi:10.1016/j.bbmt.2017.03.035.

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