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ORIGINAL ARTICLE

Association between low uric acid levels and acute graft-versus-host disease

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Abstract Endogenous danger signals are increasingly recognized in the pathogenesis of graft-versus-host disease (GVHD). Uric acid (UA) is a known danger signal and is released from injured cells during conditioning for allogeneic hematopoietic stem cell transplantation (HSCT), but its role in GVHD is unclear. Here, we retrospectively analyze 228 consecutive patients with malignant diseases undergoing HSCT from 10/10-HLA-matched donors. Low UA levels at the time of HSCT (day 0) were significantly associated with acute GVHD grades II–IV in univariate (HR 0.836, p=0.0072) and multivariate analyses (HR 0.815, p=0.0047). There was no significant association between UA levels and overall survival, non-relapse mortality, and relapse. This is the first report demonstrating a negative association between UA levels and acute GVHD. Due to the easy assessment and established pharmaceutical modification of UA, our findings are potentially clinically relevant. Confirmation in independent cohorts and further investigations into underlying mechanisms, such as reduced antioxidative capacity in hypouricemia, are warranted.

Keywords Graft-versus-host disease · Hematopoietic stem cell transplantation · Inflammation · Uric acid

Introduction

Graft-versus-host disease (GVHD) remains one of the major obstacles in allogeneic hematopoietic stem cell transplantation (HSCT). Endogenous danger signals such as extracellular adenosine-5′-triphosphate have been identified as contributors to the development of GVHD by acting as pro-inflammatory mediators [1–4].

Uric acid (UA) is a known danger signal which has been shown to elicit T cell responses via activation of the NOD-like receptor protein (NLRP)3 inflammasome [5, 6]. During conditioning, UA is released from injured cells [7]. Recently, a pre-clinical study has demonstrated that Nlrp3 inflammasome-mediated IL-1 production regulates GVHD [8]. Additionally, a phase I study reported reduced incidence of acute GVHD in patients undergoing HSCT after depletion of UA using urate oxidase [9]. However, the role of UA in inflammation is ambiguous as several studies have demonstrated an association between low UA levels and inflammatory and degenerative diseases of the central nervous system [10–13]. To date, scarce clinical data exist on the association between UA levels and the incidence of acute GVHD.

Here, we retrospectively assess the association between serum UA levels and transplant outcome in 228 patients undergoing HSCT.

Methods

Patients

Two hundred and ninety consecutive patients transplanted at Charité Campus Benjamin Franklin between 2005 and 2011 were included for retrospective data analysis. Data were analyzed as of January 21, 2013. Final analyses included 228 patients after exclusion of patients with missing UA levels at

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the day of HSCT (day 0) (n=21), bone marrow as stem cell source (n=2), patients with lower than 10/10-HLA-matched donors (n=32), and patients with benign underlying diseases (n=7). All patients gave their informed consent to scientific analysis of their data in accordance with the declaration of Helsinki.

Transplantation procedures

HSCT was performed according to standard transplantation procedures. Myeloablative conditioning (MAC) consisted of cyclophosphamide/total body irradiation (TBI) or cyclophosphamide/busulfan. Reduced intensity conditioning (RIC) consisted of fludarabine/busulfan, fludarabine/treosulfan [14], or fludarabine/2-Gray-TBI. For GVHD prophylaxis, patients received cyclosporine and methotrexate. Patients that were conditioned with fludarabine/2-Gy-TBI received GVHD prophylaxis with cyclosporine and mycophenolate mofetil. Patients with a matched unrelated donor additionally received anti-T cell globulin (ATG; thymoglobulin or ATG-Fresenius). No ATG was given in all patients receiving conditioning with fludarabine/2-Gy-TBI.

Definition of transplant-related variables and statistics

For transplantation-associated risk stratification patients with acute leukemia in first or second complete remission (CR), patients with myelodysplastic syndrome (MDS) or myeloproliferative disease without leukemic transformation and patients with chronic lymphocytic leukemia (CLL) were considered to be at standard risk [15, 16]. All other patients were graded as being at high risk. Gender mismatch between donor and host was graded as high risk in the case of female donors with male recipients, and all others were considered standard risk [17]. GVHD was graded according to standard clinical criteria [18].

Comparisons between groups were calculated using the Kruskal-Wallis test for continuous variables and Fisher's exact test for categorical variables. Overall survival (OS) was measured as the time from HSCT to death from any cause. The Kaplan-Meier method was used to calculate the probability of OS, and univariate and multivariate analyses for OS were performed using Cox's proportional hazard regression model. Non-relapse mortality (NRM) was measured as the time from HSCT to death without relapse. Gray's test was used for the estimation of the cumulative incidences of NRM, relapse, and GVHD. Relapse, death without relapse, and death without GVHD were treated as competing events, respectively. For univariate and multivariate analyses of these outcomes, Fine and Gray's proportional hazard model was used. All variables with borderline significance ($p \leq 0.15$) were included in multivariate analyses and deleted stepwisely.

All statistical analyses were performed using R statistical software (The R Foundation for Statistical Computing, Vienna, Austria). For competing risk analysis, the cmprsk package was used. The level of significance was 0.05.

Results

Patient characteristics

There were 228 patients with a median age of 52 years (range 18–77) (Table 1). Sixty-one percent was male and most patients were transplanted for acute leukemia (68 %), with fewer patients transplanted for myelodysplastic syndrome (8 %) or other diseases (24 %). Most patients received RIC (64 %).

Table 1 Patient characteristics

	Total (n=228)
Age	
Median in years (range)	52 (18–77)
Sex	
Male	139 (61 %)
Female	89 (39 %)
Diagnosis	
Acute leukemia	154 (68 %)
MDS	19 (8 %)
Other	55 (24 %)
Risk group	
Standard risk	150 (66 %)
High risk	78 (34 %)
Conditioning regimen	
Myeloablative	81 (36 %)
Reduced intensity	147 (64 %)
Donor	
Matched related	66 (29 %)
Matched unrelated	162 (71 %)
Sex mismatch	
Standard risk	176 (77 %)
High risk	52 (23 %)
Antithymocyte globulin	
No	83 (36 %)
Yes	145 (64 %)
Cyclosporin A	
Median serum level at HSCT in μg/l (range)	245 (42–640)
Median glomerular filtration rate at HSCT in ml/min (range)	100 (18–355)
Uric acid at HSCT	
Median in mg/dl (range)	3.25 (0.6–8.9)

Twenty-nine percent were transplanted from 10/10-HLA-matched-related donors while 71 % had an unrelated 10/10-HLA-matched donor. The majority of patients received in vivo T cell depletion using ATG (63 %) and 34 % were considered at high transplantation-associated risk.

The median serum UA level at the time of HSCT was 3.25 mg/dl. For graphical data representation, patients were grouped in quarters according to UA levels at transplantation (minimum UA level 0.6 mg/dl, first quartile 2.275 mg/dl, mean 3.25 mg/dl, third quartile 4.225 mg/dl, maximum 8.9 mg/dl). Expectedly, patients with higher UA levels were significantly older ($p<0.001$), had lower glomerular filtration rates as calculated using the modification of diet in renal disease (MDRD) formula ($p<0.001$), were more likely to be male ($p=0.0085$), and a higher share of patients was transplanted for diseases other than acute leukemia or MDS ($p=0.0384$) (Supplementary Table 1). In accordance with the difference in age, the share of patients receiving reduced intensity conditioning was also higher in patients with high UA levels ($p=0.0172$). Importantly, UA quarters did not differ significantly regarding CSA levels at transplantation ($p=0.137$) and disease status ($p=0.167$).

Association of low UA levels at transplantation with acute GVHD II–IV

The cumulative incidence of clinically significant acute GVHD (grades II–IV) was 37 % on day +100. In univariate analysis, the development of acute GVHD grades II–IV was significantly associated with a low UA level at transplantation (HR 0.836, $p=0.0072$) and female sex (HR 1.58, $p=0.034$) (Fig. 1a and Table 2; Supplementary Figure 1 shows UA levels and GVHD grading in individual patients). When subjected to multivariate analysis, only UA level remained a significant predictor for GVHD II–IV (HR highest vs. lowest quarter 0.815, $p=0.0047$). Of note, UA was also the only significant predictor of acute GVHD II–IV when including all variables on multivariate analysis that significantly differed between UA quarters.

T cell depletion has been shown to modify GVHD pathogenesis significantly [19]. In this analysis, low UA levels were significantly associated with GVHD II–IV in patients not receiving ATG (HR 0.738, $p=0.0099$). In contrast, there was no significant association between UA levels and acute GVHD in patients receiving T cell depletion (HR 0.894, $p=0.18$).

Next, we analyzed the association between UA levels at transplantation normalized to the glomerular filtration rate (GFR) and the incidence of acute GVHD II–IV. On analysis of UA/GFR ratio as a continuous variable, there was no significant association with GVHD II–IV while maintaining the same trend (HR <0.001, $p=0.13$). However, after dividing UA values into quarters, univariate analysis revealed both the third and the highest UA quarter to be associated with a lower incidence of GVHD II–IV as compared with the lowest UA quarter (HR 0.501, $p=0.024$ and HR 0.504, $p=0.027$, respectively) (Fig. 1b and Table 2). UA/GFR ratio was also the only variable significantly associated with acute GVHD II–IV in multivariate analysis (HR third and highest UA/GFR quarter vs. lowest quarter 0.529, $p=0.037$ and HR 0.533, $p=0.042$, respectively).

Association of uric acid levels with other transplant outcomes

The probability of survival 2 years after HSCT was 53 %. UA levels at HSCT were not significantly associated with the probability of OS (HR 1.014, $p=0.819$) (Fig. 2a).

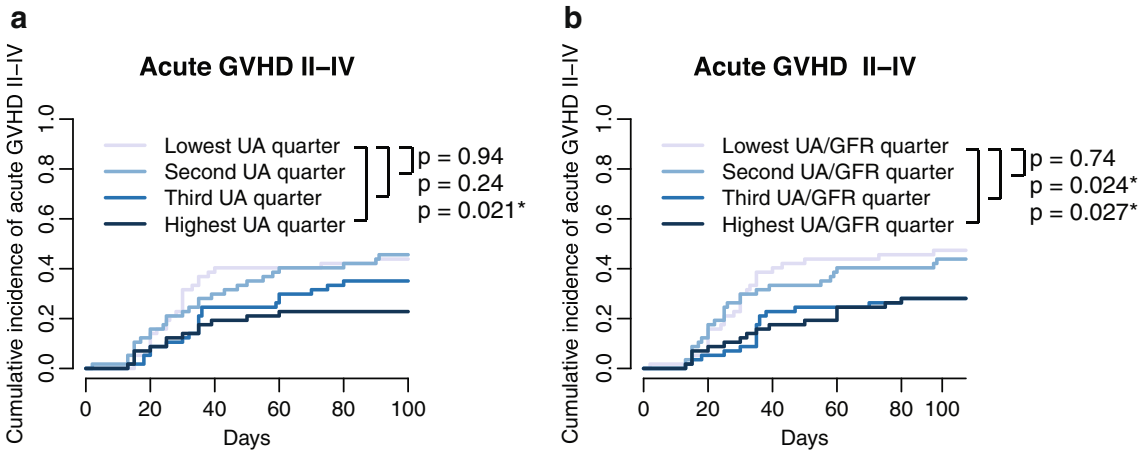

Fig. 1 Association between low uric acid levels and acute GVHD. **a** Low uric acid levels at HSCT were significantly associated with the development of acute GVHD grades II–IV (HR highest vs. lowest UA quarter 0.451, $p=0.021$). **b** UA levels at HSCT normalized to renal function by calculating the UA to glomerular filtration ratio showed a significant association of both the third and fourth UA/GFR quarters to reduced incidence of GVHD II–IV (HR third and fourth vs. lowest UA/GFR quarter 0.501, $p=0.024$ and HR 0.504, $p=0.027$, respectively). *GVHD* graft-versus-host disease; *UA* uric acid

Table 2 Univariate and multivariate analysis for acute GVHD grades 2–4

	Univariate hazard ratio (95 % CI)	Univariate *p* value	Multivariate hazard ratio (95 % CI)	Multivariate *p* value
Age	0.997 (0.982–1.01)	0.68		
Sex				
Male	1		1	
Female	1.58 (1.04–2.4)	0.034*	1.413 (0.918–2.174)	0.12
Diagnosis				
Acute leukemia	1		1	
MDS	0.454 (0.172–1.2)	0.11	0.395 (0.145–1.078)	0.07
Other	0.822 (0.498–1.36)	0.45	1.045 (0.614–1.78)	0.87
Disease status				
Standard risk	1			
High risk	1.24 (0.806–1.92)	0.33		
Conditioning regimen				
Myeloablative	1			
Reduced intensity	0.924 (0.59–1.45)	0.73		
Donor				
Related matched	1			
Unrelated matched	1.05 (0.673–1.63)	0.83		
Sex mismatch				
Standard risk	1			
High risk	0.754 (0.44–1.29)	0.3		
ATG				
No	1			
Yes	1.17 (0.759–1.81)	0.47		
Uric acid	0.836 (0.734–0.953)	0.0072*	0.815 (0.707–0.939)	0.0047*
Uric acid/GFR ratio	<0.001 (<0.0000001–8.58)	0.13		

ATG Antithymocyte globulin; *CI* confidence interval; *HR* hazard ratio

*$p<0.05$

The cumulative incidences of NRM at day +100 and 2 years after HSCT were 9 and 19 %, respectively. UA levels at HSCT were not significantly associated with NRM (HR 1.09, $p=0.41$) (Fig. 2b).

The cumulative incidence of relapse 2 years after HSCT was 35 %. There was no significant association between UA levels at HSCT and the risk of relapse (HR 0.976, $p=0.71$) (Fig. 2c).

Discussion

In this study, we retrospectively analyze the data of 228 recipients of HLA-matched HSCT for the association between UA levels and major transplant outcomes. We found that the incidence of acute GVHD grades II–IV was significantly higher in patients with low UA levels.

Danger-associated molecular patterns are increasingly recognized to play a role in GVHD pathogenesis (reviewed by Zeiser et al. [1]). UA is a known danger signal and is released from injured cells during conditioning for HSCT. The broad availability of its assessment as well as its established pharmaceutical modification makes UA an attractive candidate as a predictive marker and as a potential target in GVHD prevention. Pre-clinical data and a phase I study have suggested a role of UA as an endogenous mediator of GVHD [9, 20]. However, the role of UA in inflammation and degeneration is complex, as hypouricemia has been linked to several inflammatory and degenerative diseases [10–13, 21]. This association has been attributed to reduced antioxidative capacity in hypouricemia, as UA constitutes the most important natural antioxidant in the peripheral blood, providing up to 60 % of the free radical scavenging capacity [22]. Intriguingly, reduced antioxidative capacity has been linked to GVHD, offering a possible explanation for our findings [23–25]. Indeed, reduced antioxidative capacity and decreased UA levels have been found in the saliva of patients experiencing GVHD [26].

Our results seem to be in conflict with previous publications reporting reduced GVHD after administration of urate oxidase prior to HSCT in pre-clinical models and in a

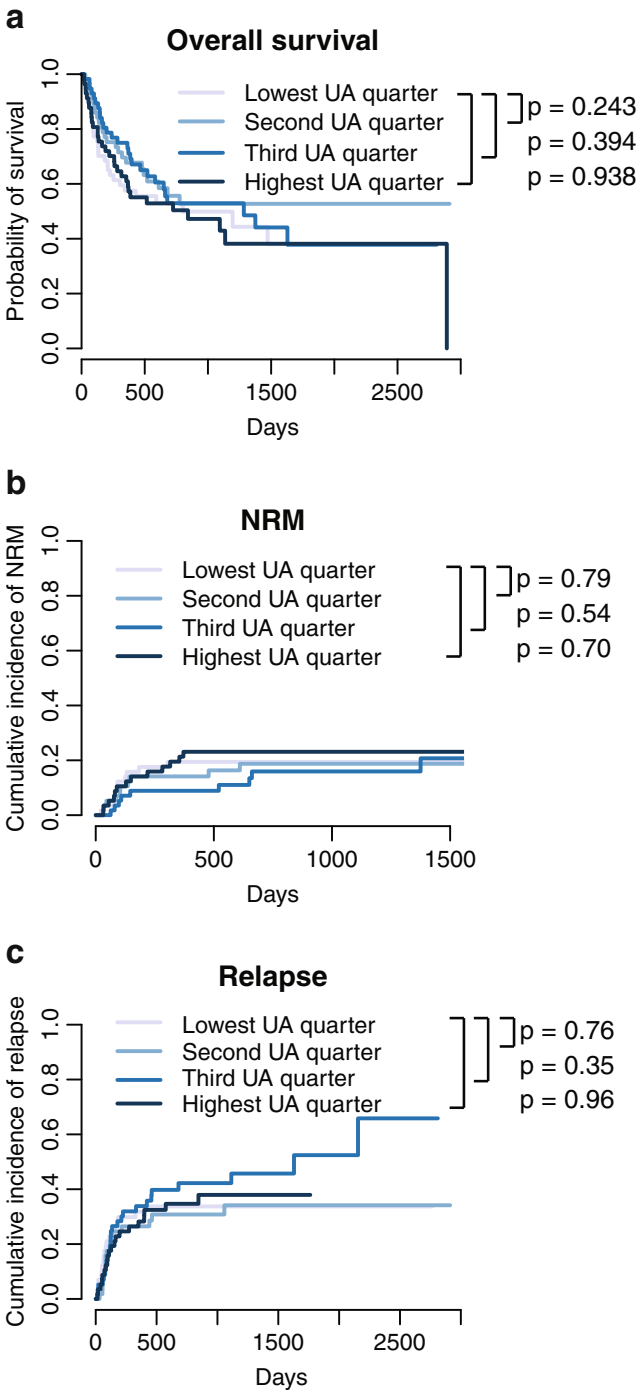

Fig. 2 Outcomes of HSCT stratified by uric acid level at HSCT. a No significant association was observed between UA levels at HSCT and overall survival (HR highest vs. lowest UA quarter 1.0205, p=0.938), b non-relapse mortality (HR 1.173, p=0.7) (c) and relapse (HR 1.015, p=0.96). *NRM* non-relapse mortality; *UA* uric acid

small phase I study [9, 20]. However, these reports assessed the effect of urate oxidase on GVHD as opposed to analyzing the association of serum UA levels with GVHD. Additionally, the clinical influence of urate oxidase on GVHD needs to be confirmed in a larger prospective study because the retrospectively case-matched design and low patient numbers are limitations of the mentioned publication [9].

Our data show that UA levels negatively correlate with acute GVHD in patients without T cell depletion. In contrast, no significant association was seen in patients receiving ATG for in vivo T cell depletion. This is in line with previous reports demonstrating that the administration of T cell-depleting agents alters the impact of danger signals on GVHD [19].

A strength of our study is the size and the homogeneity of the analyzed population, as only patients receiving peripheral blood stem cells from 10/10-HLA-matched donors were included. Additionally, the data are comprehensive, providing more details than registry studies (e.g., CSA levels), thereby excluding potential confounders. It is noteworthy that the conventional GVHD-associated risk factors such as age and donor type (i.e., MUD vs. MRD) did not show significant association with acute GVHD in our study. This finding is explained by the modification of treatment for patients with known risk factors for GVHD: Older patients were more likely to receive RIC, and patients transplanted from unrelated donors more often received ATG.

The main reason for hyperuricemia in non-inflammatory states is renal insufficiency. In order to differentiate between elevated UA levels secondary to decreased renal clearance and hyperuricemia due to cell injury, we analyzed the impact of UA levels normalized to renal function on acute GVHD. Notably, UA/GFR ratio as a continuous variable did not show a significant association with GVHD, but there was a pronounced association after division into quarters with both the third and fourth quarters showing a significantly reduced association with acute GVHD II–IV.

It is unknown which time point is ideal for measuring the impact of UA levels on GVHD occurrence. Pre-clinical data showed that UA depletion peri-transplantation had an impact on GVHD incidence, while depletion on day 5 after HSCT did not alter GVHD severity [8]. These pre-clinical data in conjunction with pre-existing knowledge on significant T cell activation very early after HSCT provided the rationale for us to analyze UA levels on day 0 [1, 2].

In conclusion, this is the first report on a negative association between UA levels at HSCT and acute GVHD. Low UA levels were significantly associated with acute GVHD grades II–IV, demonstrating the need for further investigations into the potential role of UA in HSCT as an antioxidant or modulator of allogeneic immune responses. These results are potentially clinically significant and warrant confirmation in independent cohorts.

Conflict of interest The authors have no conflict of interest to declare.

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