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ORIGINAL ARTICLE – CANCER RESEARCH

Uric acid as a novel biomarker for bone-marrow function and incipient hematopoietic reconstitution after aplasia in patients with hematologic malignancies

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Abstract
Purpose Prolonged aplasia and graft failure (GF) represent life-threatening complications after hematopoietic cell transplantation (HCT) requiring suitable biomarkers for early detection and differentiation between GF and poor graft function (PGF). Uric acid (UA) is a strong immunological danger signal.
Methods Laboratory results were analyzed from patients undergoing either allogeneic or autologous HCT or induction chemotherapy for acute leukemia ($n=50$ per group, $n=150$ total).
Results During therapy, UA levels declined from normal values to hypouricemic values (all $p<0.001$). Alongside hematopoietic recovery, UA serum levels returned to baseline values. During aplasia, UA levels remained low and started steadily increasing (defined as >two consecutive days, median one 2-day increase) at a median of 1 day before rising leukocytes in allogeneic HCT ($p=0.01$) and together with leukocytes in autologous HCT (median one 2-day increase). During induction chemotherapy, a UA increase was also observed alongside rising leukocytes/neutrophils but also several times during aplasia (median 3 increases). Most HCT patients had no detectable leukocytes during aplasia, while some leukocytes remained detectable after induction therapy. No increase in UA levels was observed without concomitant or subsequent rise of leukocytes.
Conclusions Changes in UA serum levels can indicate incipient or remaining immunological activity after HCT or induction therapy. They may, therefore, help to differentiate between PGF and GF.

Keywords Uric acid · Allogeneic hematopoietic cell transplantation · Autologous stem cell transplantation · Leukemia · Danger signal

Abbreviations

ALL	Acute lymphoblastic leukemia
AML	Acute myeloid leukemia
BEAM	Carmustin, etoposide, arabinoside, melphalan
DAMP	Damage-associated molecular patterns
FLAMSA	Fludarabine amsacrine arabinoside
G-CSF	Granulocyte colony stimulating factor
HCT	Hematopoietic cell transplantation
MMRD	Mismatched-related donor
MMUD	Mismatched unrelated donor
MRD	Matched-related donor
MSU	Monosodium urate
MUD	Matched unrelated donor

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n.s.	Not significant
PBSC	Peripheral blood stem cell
SD	Standard deviation
TBI	Total body irradiation
UA	Uric acid

Introduction

Graft failure (GF) after hematopoietic cell transplantation (HCT) represents a major diagnostic and therapeutic challenge. Although primary GF is a rare event (Laughlin et al. 2004; Woolfrey et al. 1999), it is hard to distinguish delayed engraftment from insufficient bone-marrow function. Prolonged aplasia is associated with severe morbidity and mortality due to infections or hemorrhagic complications (Rondon et al. 2008; Schriber et al. 2010). Pathogenesis of GF is multifactorial (Haen et al. 2015) and can comprise drug-related toxicity (Busca et al. 2007), graft versus host disease (GvHD) (Wolff 2002), viral infections (Boeckh et al. 2003; Lagadinou et al. 2010), transplantation from mismatched donors (Petersdorf et al. 2001), or combinations thereof, furthermore, inadequate donor bone-marrow function without any obvious cause. Therefore, the precise diagnosis of the causes and resulting appropriate therapeutic decisions are mandatory. Hence, biomarkers that can detect early or incipient bone-marrow function are needed. Such biomarkers should ideally be easily available with reliable, established technical measurement.

The concept of cellular structures acting as danger signals to the immune system has been widely accepted. These danger signals, including heat shock proteins (HSP) (Mirza et al. 2016a) and uric acid (UA), represent an internal communication system between tissues and the immune surveillance (Matzinger 1994, 2002). UA has been identified to be one of the most important danger signals when released from dying cells (Shi et al. 2003). Besides UA, dying cells release endogenous antigens which can be incorporated by dendritic cells leading to antigen-specific immune responses against antigens to which the host is not tolerant (Shi et al. 2003). When UA was co-injected with antigen, dying cells were able to provide an adjuvant effect in priming T-cell responses in animal models (Shi and Rock 2002; Shi et al. 2000). In patients with trauma or undergoing tumor lysis syndrome, e.g., induced by radiochemotherapy, increased serum levels of UA (sUA) have been observed, which can result in crystalline deposition but do not always induce inflammation (Martinon 2010). These UA crystals have been believed to be pro-inflammatory and to function as danger signal (Shi et al. 2003, 2010). Both stimulating the classical and alternative pathways of the complement system, UA crystals have direct influence on neutrophil migration into tissues, where monosodium urate (MSU) crystals have formed (Ryckman et al. 2003; Tramontini et al. 2004). However, recent studies also identified UA as an important mediator of immune responses when locally released from cells undergoing sterile cell death. In this process, local UA concentrations highly exceed systemic UA levels. Hence, local accumulation of UA can result in immune activation without formation of MSU crystals. In line with these findings, reduction of sUA was described to impair immune responses (Kono et al. 2010).

Allogeneic HCT represents a generalized state of alarm to the immune system indicated by a massive release of cytokines (Holler et al. 1997) and induction of GvHD (Mirza et al. 2016b). In addition, significant tissue damage is induced by the conditioning regimen and complications during subsequent aplasia (Cooke et al. 1998). All these conditions lead to cell damage which could lead to UA release and immune activation. Hence, sUA could also play a role in the hematopoietic reconstitution and development of GvHD.

In this study, we investigated sUA levels as a potential marker for incipient hematologic reconstitution and immunologic activity after aplasia to identify a more sensitive parameter for bone-marrow activity as compared to complete blood counts (CBC) in patients undergoing allogeneic and autologous HCT, as well as after induction chemotherapy (ICT) for acute leukemia.

Patients and methods

Inclusion and exclusion criteria

In each group (allogeneic HCT, autologous HCT, induction chemotherapy), 50 consecutive patients were included. In the induction chemotherapy group, 25 consecutive patients with AML and ALL were included, respectively.

Laboratory parameters [uric acid, complete blood counts (CBC), creatinine, and C-reactive protein (CRP)] were determined daily in routine blood draws. Patients with incomplete sets of laboratory results were excluded. Baseline laboratory parameters are provided in Table 1.

Patient characteristics: allogeneic HCT

Fifty consecutive patients (Tables 1, 2) received a total of 56 HCT (range 1–3 HCT per patient). Three patients received a second transplant due to disease relapse. Two patients were re-transplanted due to graft rejection. Of those, one patient received one, the other two additional grafts from different donors.

At transplantation (detailed characteristics in Table 2), the 24 men (48%) and 26 women (52%) had a median age of 53 years (range 19–73 years) and were treated for

Table 1 Patient characteristics

	Allogeneic transplantation		Autologous transplantation		ALL		AML	
Patients	$n=50$		$n=50$		$n=25$		$n=25$	
Male	$n=24$	48%	$n=34$	68%	$n=12$	48%	$n=14$	56%
Female	$n=26$	52%	$n=16$	32%	$n=13$	52%	$n=11$	44%
Median age (range)	53	(19–73)	50 $p=0.42$	(20–69)	28 $p<0.001$	(19–61)	59 $p=0.45$	(18–74)
Disease								
ALL	$n=7$	14%			$n=25$	100%		
AML	$n=29$	58%					$n=25$	100%
CLL			$n=2$	4%				
CML	$n=1$	2%						
EWS			$n=2$	4%				
GCT			$n=9$	18%				
HD			$n=3$	6%				
MDS	$n=7$	14%						
MM			$n=12$	24%				
MPS	$n=2$	4%						
NHL	$n=3$	6%	$n=21$	42%				
NP			$n=1$	2%				
SAA	$n=1$	2%						
Concomitant diseases[a]								
AIHA			$n=1$	2%				
Diabetes	$n=6$	12%	$n=2$	4%	$n=1$	4%	$n=4$	16%
Gout	$n=1$	2%						
Polymyalgia rheumatica	$n=1$	2%						
Renal insufficiency	$n=3$	6%	$n=2$	4%	$n=1$	4%		
Toxic liver injury	$n=1$	2%						
# of interventions (range)	$n=56$	(1–3)	$n=61$	(1–3)	$n=49$	(1–2)	$n=39$	(1–2)
Baseline laboratory results								
Leukocytes[b] (/µl) (mean, range)	4574	(<50–51,380)	4845	(1670–35,210)	5699	(130–43,940)	10,544	(160–99,010)
Uric acid[c] (mg/dl) (mean, range)	4.9	(1.0–10.0)	5.4	(3.0–8.8)	3.7	(1.2–9.0)	4.0	(1.4–10.3)
Creatinin[d] (mg/dl) (mean, range)	0.6	(0.2–1.1)	0.8	(0.4–1.8)	0.8	(0.4–1.4)	0.8	(0.4–1.4)
Bilirubin[e] (mg/dl) (mean, range)	0.6	(0.2–2.0)	0.4	(0.2–1.5)	1.0	(0.3–4.8)	0.7	(0.3–3.7)
Uric acid reducing drugs								
Allopurinol	$n=56$	100%	$n=10$	16%	$n=18$	37%	$n=21$	54%
Raspuricase	$n=1$	2%	$n=1$	2%	$n=1$	2%	$n=1$	3%
None	$n=0$	0%	$n=46$	75%	$n=19$	39%	$n=15$	38%
Unknown	$n=0$	0%	$n=4$	7%	$n=10$	20%	$n=2$	5%
Uric acid influencing medication								
Electrolyte solutions	$n=56$	100%	$n=61$	100%	$n=49$	100%	$n=39$	100%
G-CSF	$n=9$	16%	$n=59$	97%	$n=38$	78%	$n=2$	5%
Parenteral nutrition	$n=53$	95%	$n=13$	21%	$n=2$	4%	$n=10$	26%
Thiazides	$n=23$	41%	$n=9$	15%	$n=3$	6%	$n=7$	18%
Unknown	$n=0$	0%	$n=3$	6%	$n=10$	20%	$n=3$	8%
During intervention								
Infections[f]	$n=56$	100%	$n=48$	79%	$n=34$	69%	$n=36$	92%
Liver toxicity	$n=20$	36%	$n=3$	6%	$n=14$	29%	$n=6$	15%
Renal toxicity	$n=22$	39%	$n=2$	4%	$n=3$	6%	$n=0$	0%
Tumor lysis	$n=6$	11%	$n=2$	4%	$n=9$	18%	$n=3$	8%

Table 1 (continued)

	Allogeneic transplantation		Autologous transplantation		ALL		AML	
Unknown	$n=0$	0%	$n=0$	0%	$n=0$	0%	$n=3$	8%

AIHA autoimmune hemolytic anemia, *ALL* acute lymphoblastic leukemia, *AML* acute myeloid leukemia, *CLL* chronic lymphocytic leukemia, *CML* chronic myeloid leukemia, *EWS* Ewing sarcoma, *G-CSF* granulocyte colony stimulating factor, *GCT* germinal cell tumor, *HD* Hodgkin disease, *MDS* myelodysplastic syndrome, *MM* multiple myeloma, *MPS* myeloproliferative syndrome, *NHL* non-Hodkin lymphoma, *NP* nodular paragranuloma, *SAA* severe aplastic anemia

[a]Comcomitant diseases were assessed for all patients. Only diseases with known influence on uric acid serum levels are denoted here

[b]Leukocytes normal range: 4000–9500/µl (as denoted by the central laboratory of the university hospital Tübingen at the time of analysis)

[c]Uric acid normal range: 2.4–5.7 mg/dl (as denoted by the central laboratory of the university hospital Tübingen at the time of analysis)

[d]Creatinine normal range: 0.5–0.8 mg/dl (as denoted by the central laboratory of the university hospital Tübingen at the time of analysis)

[e]Bilirubin normal range: max. 1.1 mg/dl (as denoted by the central laboratory of the university hospital Tübingen at the time of analysis)

[f]Infections comprise conditions reflected by increased inflammatory parameters and/or fever treated with antibiotic agents. Infections were not necessarily attributable to a specific infectious focus

acute ($n=36$) or chronic leukemia ($n=1$), myelodysplastic ($n=7$) or myeloproliferative syndromes ($n=2$) and non-Hodgkin lymphomas (NHL; $n=3$), as well as for severe aplastic anemia ($n=1$).

All patients received allopurinol (300 mg/day orally) for 5 days starting concomitantly with the conditioning regimen. In all cases, allopurinol was discontinued afterwards. One patient received a single dose of raspuricase on the second day of conditioning therapy.

Patient characteristics: autologous HCT

Laboratory parameters of 50 patients (Table 1/**2**) undergoing a total of 61 autologous HCT (range 1–3 HCT per patient) were analyzed. At transplantation (Table 2), the patient's age (median 50 years; range 20–69 years; $p=0.42$) was comparable to the allogeneic HCT group. The group comprised 34 men (68%) and 16 women (32%). Male predominance was mainly due to patients treated for testicular cancer ($n=9$). Further indications for autologous transplant were resistant or relapsed NHL ($n=21$) and multiple myeloma ($n=12$), therapy resistant or relapsed Hodgkin's disease ($n=3$), nodular paragranuloma ($n=1$), Richter Transformation of CLL ($n=2$), and Ewing sarcoma ($n=2$).

During ten (16%) and 1 (2%) transplantations, allopurinol and raspuricase were applied, respectively. During 46 autologous HCT (75%), no sUA reducing medication was applied. sUA reducing medication could not be evaluated during 4 HCT (7%).

Patient characteristics: ICT

Fifty patients (Table 1) with ICT for acute leukemia (ALL, $n=25$; AML, $n=25$) were analyzed. ALL patients were 12 men (48%) and 13 women (52%) with a median age of 28 years (range 19–61 years). This group was significantly younger than the other patient groups ($p<0.001$). Patients in this group received a total of 49 chemotherapy cycles (range 1–2 per patient) and were mainly treated according to study protocols. sUA reducing medication comprised allopurinol during 18 cycles (37%) and raspuricase during 1 cycle (2%) of induction chemotherapy. During 19 cycles (39%), no sUA reducing medication was applied. If patients received a second induction chemotherapy, no sUA reducing drugs were applied during the second cycle. During 10 cycles (20%), sUA reducing medication could not be assessed.

AML patients were 14 men (56%) and 11 women (44%). Mean age was 59 years (range 18–74 years, $p=0.45$), again comparable to the patients undergoing HCT. Patients received 39 chemotherapy cycles and were mainly treated in or in accordance with study protocols. sUA reducing medication comprised allopurinol and raspuricase during 21 (54%) and 1 cycles (3%), respectively. No sUA reducing drugs were applied during 15 cycles (38%). In two cases (5%), sUA reducing therapy could not be evaluated. Again, in case of application of a second cycle of induction chemotherapy, no sUA reducing drugs were applied during the second cycle.

Laboratory tests

UA and creatinine levels, as well as differential CBC were determined daily from therapy initiation until discharge (median 30 days, range 13–226 days) using clinically validated procedures. In brief, UA (standard range 2.4–5.7 mg/dl) and creatinine (upper limit 1.1 mg/dl) in lithium-heparinized plasma samples (enzymatic method) were measured an on the ADVIA 1800 clinical chemistry analyzer and CBC was determined with the ADVIA 2120 blood analyzer (both from Siemens Healtheneers, Eschborn, Germany). The detection limit of leukocytes was 50/µl and of platelets 10,000/µl. Internal and external quality controls were within the allowed limits at all times of the study.

Table 2 Transplantation characteristics

	Allogeneic HCT		Autologous HCT	
# of transplantations (range)	$n=56$	(1–3)	$n=61$	(1–3)
Conditioning regimen				
BEAM			$n=23$	38%
BEAM + R			$n=1$	2%
Bu Cy	$n=7$	12.5%		
Bu Mel			$n=1$	2%
CE			$n=3$	5%
Cy TBI	$n=5$	9%	$n=1$	2%
FLAMSA	$n=10$	18%		
Flu Bu	$n=7$	12.5%		
Flu Mel	$n=3$	5%		
Flu OKT-3	$n=1$	2%		
Flu TBI	$n=5$	9%		
Flu Thio Mel	$n=6$	11%		
Flu Treo	$n=5$	9%		
Mel			$n=13$	21%
PEI			$n=16$	26%
TBI AraC Mel			$n=2$	3%
TBI Eto	$n=6$	11%		
TLI OKT-3	$n=1$	2%		
Treo Mel			$n=1$	2%
Stem cell source				
PBSC	$n=51$	91%	$n=61$	100%
PBSC & BM	$n=1$	2%		
BM	$n=3$	5%		
cord blood	$n=1$	2%		
Transplantation				
MRD	$n=11$	20%		
MUD	$n=27$	48%		
Mismatch (RD)	$n=1$	2%		
Mismatch (UD)	$n=9$	16%		
Haploidentical	$n=8$	14%		
Autologous			$n=61$	100%
Acute GvHD during transplantation				
Grades ≤ I	$n=48$	86%		
Grades II–IV	$n=8$	14%		

AraC cytarabine, *BEAM* BiCNU etoposide arabinoside melphalan, *BM* bone marrow, *Bu* busulfan, *CE* carboplatin etoposide, *Cy* cyclophosphamide, *Eto* etoposide, *FLAMSA* fludarabine amsacrine AraC, *Flu* fludarabine, *GvHD* graft versus host disease, *Mel* melphalan, *MRD* matched-related donor, *MUD* matched unrelated donor, *OKT* muromonab-CD3, *PEI* cisplatin etoposide ifosfamide, *R* rituximab, *RD* related donor, *TBI* total body irradiation, *Thio* thiotepa, *TLI* total lymph node irradiation, *Treo* treosulfan, *UD* unrelated donor

Evaluation of other factors influencing sUA levels

To evaluate potential other factors which might influence sUA levels, concomitant diseases of the patients were evaluated for their possible influence on sUA levels. Moreover, applied drugs (electrolyte solutions, granulocyte colony stimulating factor (G-CSF), parenteral nutrition, and thiazide diuretics) were evaluated for all patients. The respective data are provided with Table 1.

Definitions

To exclude diurnal variations within sUA levels, an increase of sUA during aplasia was defined as increasing values for at least two subsequent days (while creatinine values remained stable). The same period was required to assume rising leukocytes to exclude detection of leukocytes derived from erythrocyte and platelet transfusions.

sUA levels which increased alongside creatinine during acute kidney failure were not accounted for as increasing values in the sense mentioned above.

Days 0 were defined as the day of stem cell retransfusion (cohorts allogeneic and autologous HCT) or the day of initiation of ICT (cohort ICT).

For the evaluation of kinetics correlation, decreases from the previous day were set to −1, increases to 1 and stable values to 0.

Throughout the article, the following terminology is used: The term 'hematopoietic engraftment' is used for the defined timepoints when >500/µl neutrophils could be detected. The term 'incipient hematopoietic reconstitution' is used to describe an early release of hematopoietic cells irrespective of absolute leukocyte/neutrophil counts.

Statistics

Statistical analyses, including Student's two-tailed paired (for comparison within individual patients) or un-paired (for comparisons between groups) *t* tests, Chi-square tests, and Spearman correlation analyses, were performed using the SPSS software version 22 (2013, IBM Corporation, Armonk, New York, USA). For *t* and Chi-square tests $p<0.05$ and for correlation analyses, $p<0.01$ were considered significant.

Results

Allogeneic HCT

The mean baseline UA level was 4.9 mg/dl (SD 2.0; range 1.0–10 mg/dl). When considering patients with active leukemia only, the mean baseline UA level was 4.2 mg/dl. All patients showed decreasing values during transplantation. The lowest levels were detected in median at day 8 (range 2–22 days; SD 4.3) and ranged from 0.2 to 4.7 mg/dl UA (mean 1.5 mg/dl; SD 0.9 mg/dl; $p<0.0001$). All but nine patients (82%) reached hypouricemic levels

(<2.4 mg/dl). Of note, these patients also presented with decreased sUA levels.

The mean of the lowest sUA level was significantly [mean −0.9 mg/dl; range (−2.2)−0.6 mg/dl] below the lower standard value ($p < 0.0001$). The median interval between last dose of allopurinol and the detected lowest sUA levels was 16 days (range 2–88 days). Assuming a serum half-value period of 180 min for allopurinol and 26 h for its active metabolite oxipurinol, this period constitutes about 15 serum half-value periods. Hence, only about 0.003% of the active substance should be present at the sUA nadir and, therefore, was considered neglectable.

During and after hematopoietic recovery from aplasia, sUA increased to pre-treatment levels (mean 5.0 mg/dl; range 0.7–10.6 mg/dl; SD 2.2 mg/dl) without significant differences between pre- and post-treatment values ($p = 0.87$). Highest values were reached in median at day 25.5 (range days 11–49) after HCT (Fig. 1a, c).

Autologous HCT

The mean baseline sUA level was 5.4 mg/dl (SD 1.5; range 3.0–8.8 mg/dl). All patients showed a decrease during the transplantation. The lowest levels were detected on a median of day 6 (range 2–11 days; SD 1.8) and ranged from 0.7 to 7.4 mg/dl (mean 2.1 mg/dl; SD 1.0 mg/dl; $p < 0.0001$). All but three patients (94%) reached hypouricemic levels (<3.4 mg/dl). The mean of the lowest sUA level was significantly (mean −0.6 mg/dl; range −2.5–5 mg/dl) below the lower standard value ($p < 0.0001$). Of note, the patients without hypourikemia also presented with a decrease in sUA levels (−0.2, −2.5, and −2.6 mg/dl, respectively).

In contrast to patients undergoing allogeneic HCT, sUA values did not increase to pre-treatment levels (mean 4.2 mg/dl; range 1.3–9.4 mg/dl; SD 1.7 mg/dl; $p < 0.001$). The highest values were reached at a median of day 12 (range days 8–25) (Fig. 1a, d). This might be partly due to the fact that sUA reducing drugs (in 18% of patients) were discontinued at a median of 3 days after detection of the lowest sUA values (range −3 to 14 days). Hence, allopurinol could also hamper an sUA re-increase in these patients. Of note, during 46 autologous HCT (75%), no sUA reducing drugs were applied.

ICT

As controls, we evaluated laboratory values of patients undergoing ICT for AML and ALL.

Regarding AML patients (Fig. 1a, e), the mean baseline sUA level was 4.0 mg/dl (SD 1.7; range 1.4–10.3 mg/dl). These values were significantly lower as compared to the ones observed in patients undergoing allogeneic HCT ($p = 0.03$) but comparable with the ones observed in patients with active leukemia before allogeneic HCT ($p = 0.72$). Only one patient did not show a decrease of sUA. The lowest sUA was detected in median at day 15 (range 5–32 days; SD 5.6) and ranged from 0.7 to 3.2 mg/dl (mean 1.7 mg/dl; SD 0.8 mg/dl; $p < 0.0001$). After engraftment, sUA values returned to pre-treatment levels (mean 3.9 mg/dl; range 1.9–8.4 mg/dl; SD 1.5 mg/dl; $p = 0.71$). The highest values were reached in median at day 27 (range days 17–38; SD 5.4). sUA reducing drugs were discontinued latest before the second cycle of induction chemotherapy. With regard to the first and second ICT cycles, sUA reducing were discontinued on median day −1 [range days (−15)−(+20)] and median day −27 [range days (−47)−(−10)], respectively. Therefore, the mean time between the lowest sUA values and the last dose of allopurinol was applied at a median of -7 days before the lowest sUA levels (range −25 to +12 days) during the first cycle ICT. During the second ICT cycle, no allopurinol was applied. Therefore, the drug was discontinued at a median of −33 days (range −50 to −19 days) before the detected lowest sUA levels during the second ICT cycle.

Considering ALL patients (Fig. 1a, f), mean baseline sUA was 3.7 mg/dl (SD 1.6; range 1.2–9.0 mg/dl). Again, these values were significantly lower as compared to the ones observed in patients undergoing allogeneic HCT ($p = 0.001$) but also comparable with the ones observed in patients with active leukemia before allogeneic HCT ($p = 0.30$). All but four patients showed stable sUA levels. The sUA nadir was detected in median at day 13 (range 1–23 days; SD 5.6) and ranged from 0.3 to 4.4 mg/dl (mean 1.8 mg/dl; SD 0.9 mg/dl; $p < 0.0001$). After engraftment, sUA returned to pre-treatment levels (mean 4.1 mg/dl; range 1.3–7.6 mg/dl; SD 1.4 mg/dl; $p = 0.27$). The highest values were reached at a median of day 21 (range days 4–55; SD 9.5). Here also, sUA reducing drugs were discontinued latest before the second cycle of induction chemotherapy. With regard to the first and second ICT cycles, sUA reducing were discontinued on median day −7 [range days (−25)−(+12)] and median day −32 [range days (−50)−(−14)], respectively. Therefore, the mean time between the lowest sUA values and the last dose of allopurinol was applied at a median of −1 days before the lowest sUA levels (range −15 to +20 days) during the first cycle ICT. During the second ICT cycle, no allopurinol was applied. Therefore, the drug was discontinued at a median of −27 days (range −42 to −10 days) before the detected lowest sUA levels during the second ICT cycle.

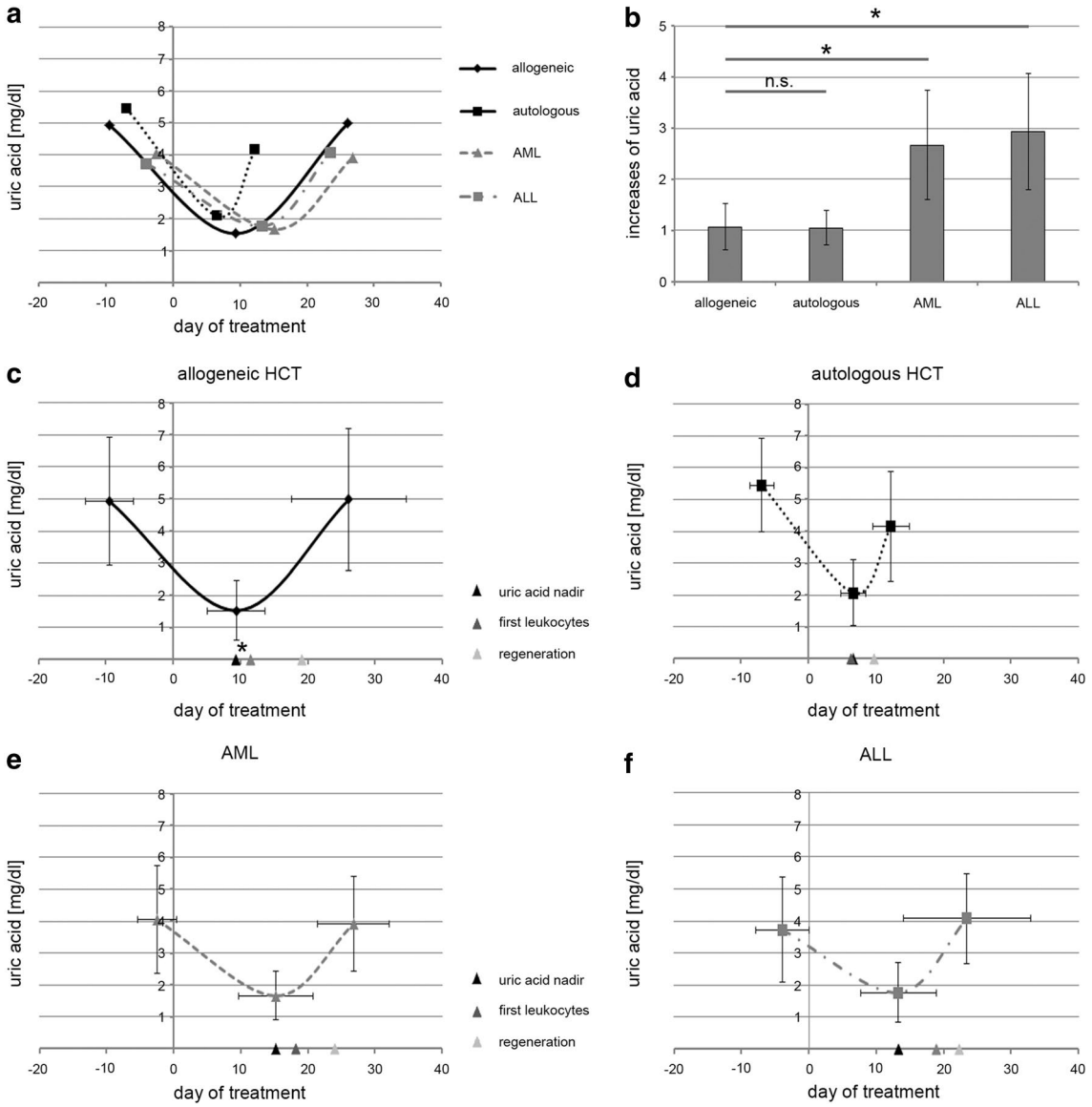
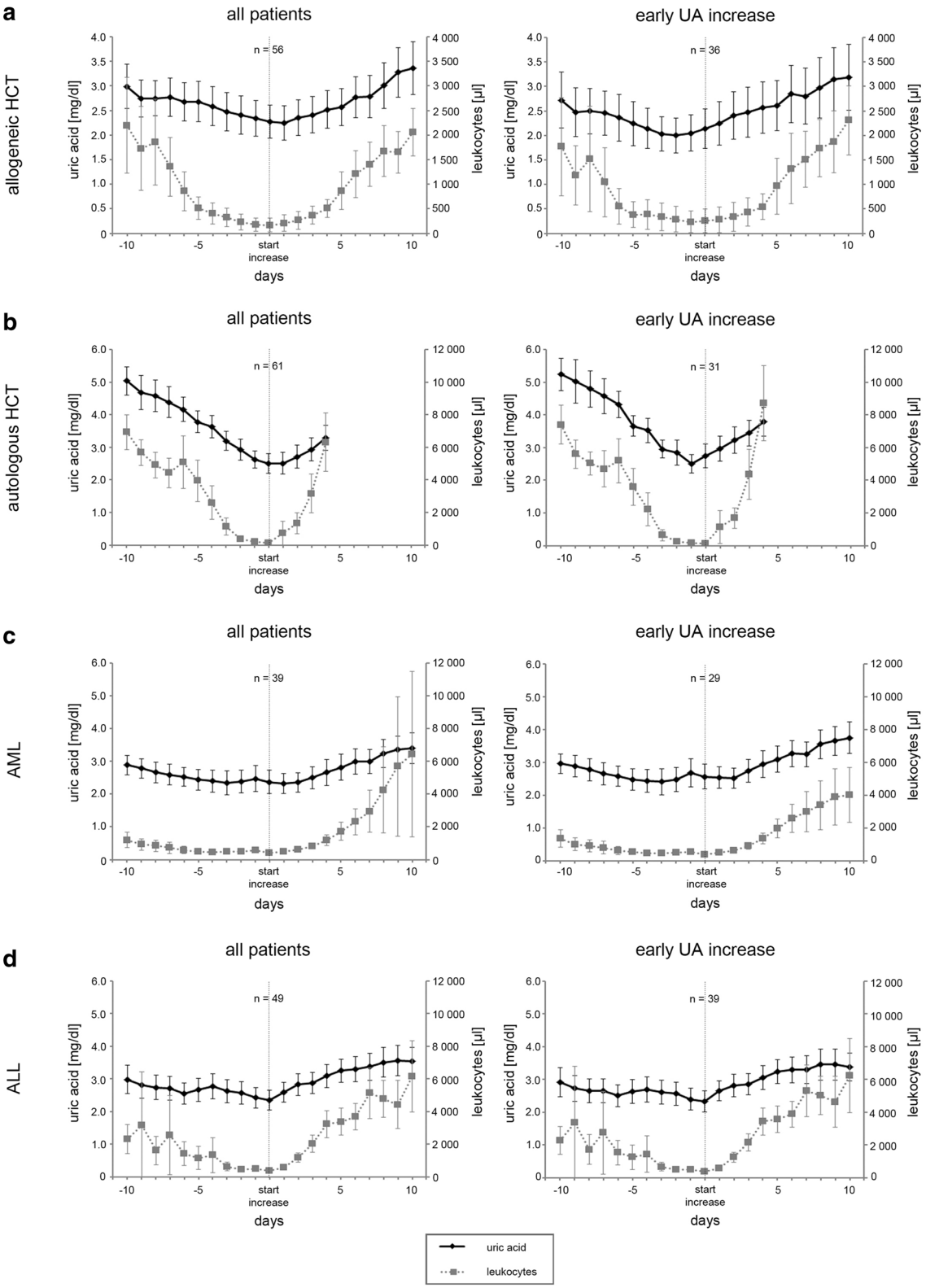


Fig. 1 Timely variance and changes of uric acid serum levels. In all patients, sUA levels and peripheral CBC were determined daily in clinical routine. Three detection timepoints [baseline values, nadir after therapy, and highest levels after hematopoietic engraftment (neutrophils > 500/µl)] are shown for patients undergoing allogeneic (*solid black line*, panels **a** and **c**) or autologous (*dotted black line*, panels **a** and **d**) HCT, and patients with ICT due to AML (*dashed grey line*, panels **a** and **e**) or ALL (*dashed–dotted grey line*, panels **a** and **f**). For all timepoints, *error bars* are given representing means and SD for the respective days (*horizontal error bars*), as well as means and SD of the extent of sUA levels (*vertical error bars*). Connecting lines do not represent real detected values (for such analyses, refer to Figs. 2, 3). Median days of the lowest sUA values, detection of the first leukocytes (>50/µl), and hematopoietic engraftment (neutrophils > 500/µl) are marked with black, dark grey, and light grey triangles on the *x*-axis, respectively (panels **c**–**f**). Days of treatment are defined as the date of hematopoietic cell retransfusion (groups allogeneic and autologous HCT) or initiation of ICT (groups AML and ALL). Steady increases of sUA levels were defined as increases of detected values on at least two subsequent days during aplasia (<500/µl neutrophils). Median numbers of sUA increases are depicted for patients undergoing allogeneic and autologous HCT (panel **b**, *left columns*) and for patients with ICT (panel **b**, *right columns*). *Error bars* represent means with SD. Statistically significant differences (paired *t* tests) are marked with an *asterisk* (*)

Changes in sUA as a biomarker for bone marrow function

In patients undergoing allogeneic HCT, the first leukocytes (≥50/µl) were detected at a median of day 10 (range day 5–40). Neutrophilic engraftment (>500/µl) was noted at a median of day 20 (range day 10–43), while after four transplantations (8%) no engraftment was noted (see in the following). Hence, the median time span between detection of the first leukocytes and hematopoietic engraftment was



10 days (range 3–15 days). In comparison, the sUA nadir occurred 2 days earlier (median day 8, $p = 0.01$; positive predictive value 100%, 95% CI 90–100%). To closer study the biology of sUA levels and their dependency of defined subsets of leukocytes, patients were stratified between patients with uric acid nadir before and after detection of the first leukocytes. Patients with uric acid nadirs after detection of the first leukocytes are discussed separately

◂**Fig. 2** Uric acid serum levels and leukocyte counts over time in all patients and patients with early uric acid increases. Absolute sUA levels (*solid black lines*) were compared with leukocytes (*dotted grey lines*) in the respective whole patient cohort (*left panels*) or the subgroup of patients with sUA increases before or alongside detection of first leukocytes (*right panels*). Laboratory parameters are normalized to the first day of detectable leukocytes (as marked in the *middle*) and 10 days before and after detectable leukocytes, respectively. In patients undergoing autologous HCT (**b**), the days after detection of first leukocytes had to be truncated due to early discharge from hospital. The numbers of transplantations included in the respective panels are indicated. Leukocytes and sUA correlated directly (Spearman correlation). In patients undergoing allogeneic (**a**) and autologous (**b**) HCT, one increase of leukocytes was observed before or closely connected to leukocyte increases, while in patients undergoing ICT for AML (**c**) or ALL (**d**), several increases (>2 days) of UA serum levels were observed. *Error bars* represent means with 95% confidence intervals

in the section "Evaluation of leukocyte subsets influencing uric acid serum levels" (see in the following).

After exclusion of patients ($n=20$; 36%) with an sUA nadir after detection of the first leukocytes (Fig. 2a, right panel), this nadir was even evidenced 5 days earlier (range 1–34 days) before detection of earliest leukocytes. Importantly, HCT patients exhibited in median only one increase of sUA levels during aplasia (range 0–3 increases, Fig. 1b). This increase occurred in close chronologically proximity to detection of increasing leukocytes and was not dependent on the time span between discontinuation of allopurinol and re-increase of serum uric acid (time span ranging from 2 to 88 days; median day 13; SD 12.9).

After autologous HCT (Fig. 2b), the first leukocytes (≥50/µl) were detectable in median at day 6 (range day 4–9). Neutrophilic engraftment (>500/µl) was notable in median after 9 days (range day 7–15). Compared to allogeneic HCT, the interval between detection of the first leukocytes and hematologic engraftment was shorter (median 3 versus 8 days, respectively; $p<0.0001$). This median interval between detection of the first leukocytes and hematopoietic engraftment was of 2 days (range 0–9 days). The re-increase of sUA occurred mostly together with rising leukocytes in most cases (positive predictive value 100%, 95% CI 88.78–100%). These patients also exhibited only a median of 1 UA increases during aplasia (range 0–2 increases, Fig. 1b).

After ICT, the first steadily increasing leukocytes (increasing leukocytes without further decreases) were detected at a median of day 17 (range day 3–35). Neutrophilic engraftment (>500/µl) was noted in all patients at a median of day 23 (range day 10–39). The median interval between detection of the first leukocytes and hematopoietic engraftment was very variable with 4 days (range 1–23 days; SD 3.3). In contrast to allogeneic or autologous HCT, leukocytes remained regularly detectable (>50/µl) and could just be below detection limit on single days (Fig. 2c, d). This was reflected by significantly higher leukocytes during aplasia after ICT as compared to HCT (mean 458/µl versus 239/µl; $p<0.0001$). Leukocytes were not detectable in 4/1029 measured samples (0.4%) in patients undergoing ICT, but in 25% of measured samples (396/1580; $p<0.0001$) in patients undergoing HCT.

Further supporting the hypothesis that changes in sUA reflect immunologic activity during aplasia, patients undergoing ICT exhibited in median 3 sUA increases (range 1–6 increases, Fig. 1b, variability shown in Fig. 2c, d). The observed re-increases of sUA were not associated with increasing leukocytes.

Overall, leukocyte counts and sUA levels followed similar kinetics with a close temporal proximity of decreases and increases. In correlation analyses, a clear correlation was observed with regard to absolute values (Spearman correlation for sUA/leukocytes 0.441; $p<0.0001$). With regard to kinetics, again, a significant correlation was observed for sUA/leukocytes (Spearman correlation 0.146, $p<0.0001$).

Evaluation of leukocyte subsets influencing uric acid serum levels

Furthermore, we aimed to evaluate whether certain leukocyte subsets could be identified that correlated with sUA. Since in the autologous HCT cohort, the later increases occurred only marginally later and no clear tendencies could be observed in the ICT cohort, these groups were excluded from this analysis.

During 20 allogeneic HCT, sUA started increasing later than leukocytes (Fig. 3a). With regard to leukocyte subsets, both neutrophils and sUA levels started increasing first on median day 8 (range, days 5–21, respectively; Fig. 3b). Hence, sUA serum levels indicated neutrophilic activity (positive predictive value 100%, 95% CI 93.73–100%). Supporting this hypothesis, in one patient, an early increase of undifferentiated white bloods cells was detectable (start increase at day 5; Fig. 3c, dark grey line), while the sUA levels increased later (start increase at day 7; Fig. 3c, black line). The increase of neutrophils began on day 8 (Fig. 3c, light grey line). Here, the sUA level was indicative for incipient neutrophil recovery, since the patient received a high number of $CD34^+$ hematopoietic cells in the graft (14.21×10^6 per kilogram body weight), with detection of an early release of hematopoietic precursors detected as leukocytes in automated CBC.

In correlation analyses, again, a significant correlation was observed with regard to absolute values (Spearman correlation for sUA/neutrophils 0.428, $p<0.0001$).

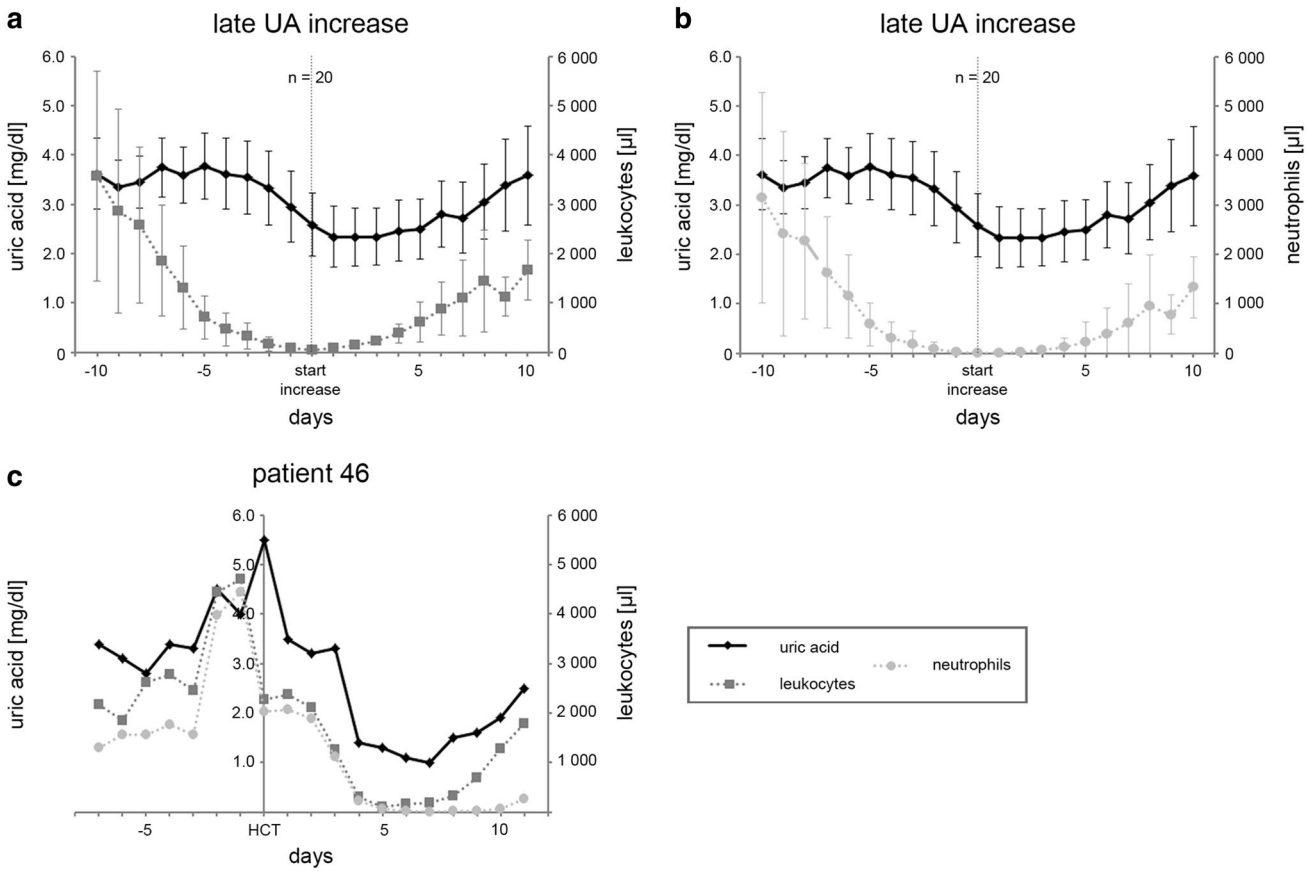


Fig. 3 Association of uric acid serum levels and neutrophil counts in patients undergoing allogeneic HCT with late uric acid increases. Only patients with subsequently increasing sUA levels (*solid black lines*) as compared to detection of leukocytes (*dotted dark grey lines*) were included in this analysis (**a**). The numbers of therapy cycles included into the respective panels are indicated. Correlation of sUA levels and of neutrophils (*dotted light grey lines*) revealed earlier or simultaneous detection of increasing sUA levels (**b**). Data of one exemplified patient (**c**) are shown. This patient received high numbers of $CD34^+$ cells in the graft leading to early release of hematopoietic progenitors (detected as undifferetiable cells in automated differential CBC on day 5) detected in the leukocyte fraction. Note the indicative increase of sUA levels as compared to the later detection of neutrophils. Error bars represent means with 95% confidence intervals

sUA levels during transplantations without engraftment

No hematopoietic engraftment (neutrophils > 500/µl) was noted after four allogeneic HCT. Some limited leukocytes could be detected (Fig. 4a, b). Here, also preceding or concomitant increases of sUA levels could be observed. Graft failure was indicated by a rapid decline of sUA levels (Fig. 4b).

Discussion

Graft failure and PGF after HCT represent a serious cause of transplantation-related morbidity and mortality, being associated with increased risk for infections or hemorrhagic complications due to leukopenia and thrombocytopenia (Lucarelli et al. 2016). In case of missing detectable hematopoietic recovery upon day 25 after HCT, it can become hard to distinguish PGF from full GF resulting in difficult clinical decisions for the very different treatment of both conditions. The treatment of PGF aims at the management of underlying conditions. Treatment with granulocyte colony stimulating factor (G-CSF) or $CD34^+$ selected stem cell boosts (SCB) have proven effective, but effects may be limited (Bittencourt et al. 2005; Haen et al. 2015). However, in case of full GF, also G-CSF application or SCB is ineffective and other treatment options should be prioritized, including a second donation after application of another conditioning regimen (Chewning et al. 2007; Guardiola et al. 2000; Haen et al. 2016; Heinzelmann et al. 2008; Jabbour et al. 2007; Wolff 2002). Second donations and coordination of such therapies can elongate aplasia even more. Therefore, biomarkers that are suitable to distinguish delayed engraftment from GF can be helpful to guide decision making in clinical practice.

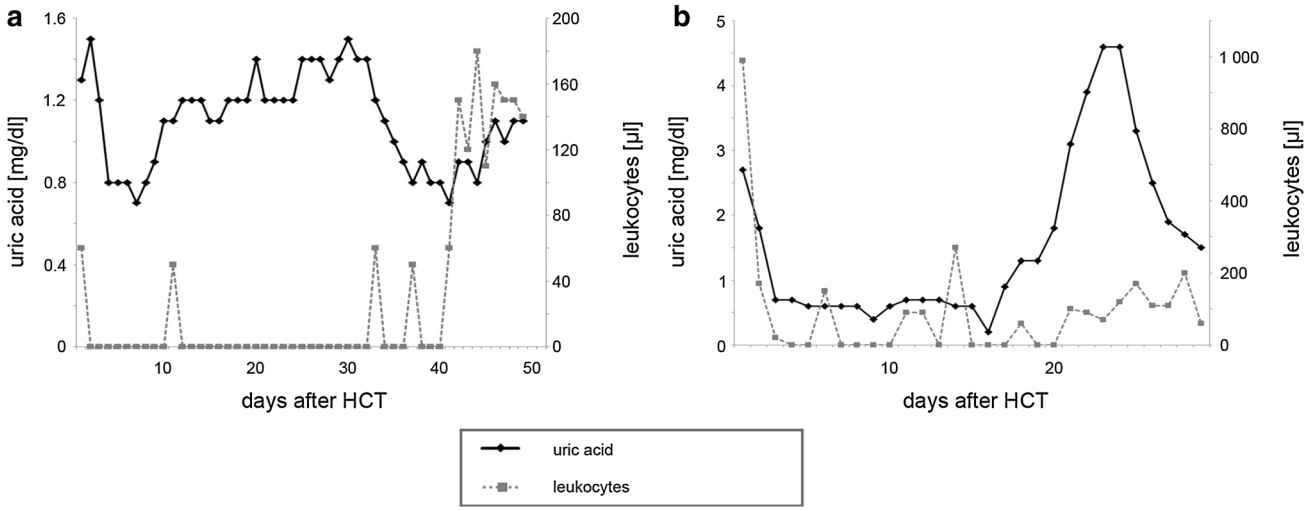


Fig. 4 sUA levels in patients experiencing graft failure. Time-dependent courses of sUA levels (*solid black lines*) and leukocytes (*grey dotted lines*) obtained in two exemplified patients are shown. **a** After prolonged aplasia, only very limited leukocytes (max. 200/µl) could be detected. All detectable leukocytes were accompanied or preceded by an at least 2 day increase of sUA levels. sUA decreased alongside leukocytes. **b** Here also, an increase of sUA levels was noted before detection of first leukocytes. A rapid decline of sUA levels indicated graft failure

Here, we evaluated sUA levels (without concomitant acute renal failure) as potential biomarkers for incipient hematological reconstitution after aplasia in patients undergoing allogeneic and autologous HCT, as well as ICT for leukemia. We observed an increase of sUA levels at a median of 1 day before detection of first leukocytes, which could be indicative for a forthcoming increase of leukocytes (and subsequently neutrophils). This was underlined by the observation that sUA levels only started increasing in case of a subsequent increase of leukocytes. Hence, sUA levels and kinetics may help to interpret persistently low peripheral CBC differently. Of note, sUA levels are not indicative for incipient release of leukocytes in all patients. When comparing sUA levels with leukocyte subsets, we identified neutrophils as the relevant subset which sUA levels are indicative for. However, detection of neutrophils is by far less sensitive as compared to detection of complete leukocytes, since blood count differentiation requires detection of >100/µl leukocytes in our laboratory.

Interestingly, increases of sUA levels occurred only in patients who exhibited an increase of leukocytes later, while patients without later increases of sUA levels also showed no rising leukocytes. Therefore, sUA levels can help to differentiate between PGF (with rising sUA levels) and full GF (without rising sUA levels).

The initial decrease of sUA levels can partly be ascribed to allopurinol administration. However, hypouricaemia has also been reported in patients with AML, resembling an increased urate clearance due to proximal renal tubular dysfunction (Liamis and Elisaf 2000; Mir and Delamore 1974). Whether this impairment of UA resorption was due to kidney injury caused by chemotherapy could not be verified, since increased urate excretion occurred about one week after the therapy (Liamis and Elisaf 2000). Both mechanisms—drug reduction and metabolic changes—have presumably contributed to this decrease of sUA levels. In addition, other factors, including medication, parenteral nutrition, and organ function, could contribute to sUA levels. Since we observed a significant correlation between both neutrophils and leukocytes and sUA levels, the effect of these therapies should be limited but should be further evaluated in the future.

Interestingly, sUA levels remained low until incipient hematopoietic recovery in HCT patients, here indicating a dependency of sUA levels particularly on neutrophil function. Most probably, increasing sUA levels might be caused by consumption of early appearing neutrophils at infected sites or sites damaged other causes, including radiochemotherapy, in the periphery. The hypothesis that sUA levels can serve as biomarkers for bone-marrow function was further underlined by our observation that sUA baseline levels were comparable in patients undergoing ICT and patients with active leukemia before allogeneic HCT. Here, active leukemia might hamper normal neutrophil production and consumption which was also indicated by lower uric acid serum levels as compared to patients undergoing autologous or allogeneic hematopoietic cell transplantation. Moreover, sUA levels could also indicate graft failure.

Through cell damage the innate immune system is activated and cells are mobilized to the site of injury. This leads to the neutralization of injurious agents and the clearance of dead cells as well as induction of cellular repair (Kono and

Rock 2008; Majno and Joris 1995). All cells contain highly concentrated intracellular deposits of UA which is the endpoint of purine catabolism. Thus, it has been proposed that release of UA from dying cells into circulation leads to a local oversaturation of UA in the extracellular fluid (Kono et al. 2010). However, the inflammation induced by cell death does not only have the beneficial effects of tissue repair and protection but can also promote further cellular injury, as shown in the ischemic heart, lung, and hepatitis as well as the development of chronic diseases (Adams et al. 2010; Sawa et al. 1996; Srikrishna and Freeze 2009).

The use of allopurinol during the whole transplantation might represent a tool for reducing the incidence of graft versus host disease. However, this might also prolong aplasia. Therefore, the use of allopurinol would only be an option in patients with the lowest possible risk of infection associated mortality and highest risk to develop graft versus host disease.

Conclusion

In summary, sUA levels can serve as sensitive biomarkers for incipient hematopoietic reconstitution in patients with delayed engraftment and may be a suitable parameter for differentiating between PGF and full GF. It can, furthermore, support the interpretation of detectable leukocytes, which could have also been transfused with erythrocyte or platelet concentrates or being a sign of incipient release from the bone marrow.

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Compliance with ethical standards

These retrospective analyses were reviewed and approved by the institutional ethics committee of the Tuebingen University (reference 820/2015BO2).

Conflict of interest All authors declare that they do not have any conflict of interest to disclose.

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