Experimental Hematology

Experimental Hematology 2019;74:25–32

Lipocalin-2 levels in acute and chronic graft-versus-host disease following allogeneic hematopoietic stem cell transplantation

Alexander Hermann[a], Andreas Winkler[b], Christopher Paschen[a], Zoya Kuzmina[c], Anastasiya Hladik[a], Süphan Icme[a], and Oliver Robak[a]

[a]*Department of Medicine I, Medical University of Vienna, Vienna, Austria;* [b]*Department of Cardiac Surgery, Vascular Surgery, and Endovascular Surgery, Medical University of Salzburg, Salzburg, Austria;* [c]*Department of Haematology and Oncology, Wilhelminenspital, Vienna, Austria*

(Received 16 February 2019; revised 1 May 2019; accepted 4 May 2019)

Lipocalin-2 (LCN2) is an immunomodulatory protein holding major metabolic and immune functions. It is involved in several inflammatory processes and induced by cytokines of the interleukin-1 family known as contributors to the morbidity in graft-versus-host disease (GVHD) following hematopoietic stem cell transplantation (HSCT). The possible role of LCN2 in predicting outcome and course of illness has never been elucidated in patients undergoing HSCT for hematologic malignancies. We conducted a prospective cohort study including 40 patients following autologous or allogeneic HSCT by collecting plasma samples at seven time points with respect to GVHD, relapse, and outcome. LCN2 levels were significantly increased in acute patients with GVHD compared with autologous and healthy controls (125.7 ng/mL vs. 65.9 and 71.4 ng/mL) and correlated with its severity. Similarly, LCN2 levels were significantly elevated in chronic GHVD compared with autologous and healthy controls (295.0 ng/mL vs. 54.9 and 76.5 ng/mL). Moreover, LCN2 correlated with mortality. The suspected role of LCN2 as a predictive parameter for outcome and prognosis needs to be further investigated. © 2019 Published by Elsevier Inc. on behalf of ISEH – Society for Hematology and Stem Cells.

Allogeneic hematopoietic stem cell transplantation (HSCT) is the only therapeutic option for patients with hematological diseases such as acute leukemia [1,2]. However, it is still associated with substantial morbidity partly related to graft-versus-host disease (GVHD). GVHD occurs in around 50% of stem cell recipients, thus remaining a major complication [3–5]. Severe GVHD is associated with reduced survival [6] and impaired quality of life of affected patients [7]. Chronic GVHD (cGVHD) is one of the main causes of nonrelapse mortality (NRM) and prolonged immunodeficiency [3,4]. Its clinical presentation can resemble those seen in various autoimmune disorders such as systemic lupus erythematosus, Sjogren's syndrome, scleroderma, autoimmune thyroiditis, and rheumatoid arthritis [8–10]. The 2014 National Institutes of Health (NIH) Consensus Conference redefined criteria for accurate diagnosis based on the pre-existing guidelines and the widely accepted severity scoring of cGVHD [11,12].

In acute GVHD (aGVHD), transplanted donor T lymphocytes react to host cells and tissues that are regarded as foreign, leading to a distinct inflammatory process [13]. This efferent phase is preceded by an afferent phase when, for example, tissue damage throughout the conditioning regimen occurs, and an induction phase when donor T cells are activated and expand. As a result, host cells are directly and indirectly damaged by activated Tlymphocytes. Pro-inflammatory cytokines such as interleukin-1 (IL1), interleukin-6 (IL6) and tumor necrosis factor (TNF) α are upregulated and contribute to the high morbidity and mortality [14].

Lipocalins are a family of immunomodulatory proteins expressed mainly by neutrophils. They exert various metabolic and immune functions [15]. Lipocalin-2 (LCN2) is a newly discovered molecule that is involved in several inflammatory as well as detoxification processes [16]. Hepatocytes are the major source for LCN2 following bacterial infections. Its production is dependent on the STAT3 signaling pathway upon activation by IL-6 [17]. STAT3 is essential for the differentiation of TH17 cells [18], which have been implicated in the inhibition of regulatory T-cell differentiation [19] and therefore contribute to cGVHD

Corresponding author: Oliver Robak, Department of Internal Medicine I, Intensive Care Unit, Medical University of Vienna, Waehringer Guertel 18-20, A-1090 Vienna, Austria; E-mail: oliver.robak@meduniwien.ac.at

0301-472X/
https://doi.org/10.1016/j.exphem.2019.05.001

[20]. Further data suggest that LCN2 might contribute to recruitment of inflammatory cells [21]. Moreover, it has been reported that LCN2 is strongly induced by the pro-inflammatory cytokine interleukin-1β via nuclear factor κB. Both contribute to the morbidity observed in GVHD [14]. It is furthermore a clinically relevant biomarker for severity of acute kidney injury [22,23]. In urine of patients with HSCT, LCN2 has been considered a predictor of acute kidney injury. However, its actual value in this regard remains controversial [24,25], as current evidence also refers to LCN2 as a growth factor possibly limiting renal injury [26]. Its bacteriostatic effects are explained by its ability to deplete bacterial siderophores.

We conducted a prospective study to investigate the role of LCN2 during HSCT and to assess its influence on occurrence and outcome of GVHD. We decided to investigate autologous transplant recipients in comparison to allogeneic ones to allow for reproducibility of toxicity profiles related to administration of conditioning therapies.

Methods

Patients and trial conduct

Plasma samples from patients undergoing either autologous (n = 12; 10 male, 2 female) or allogeneic (n = 28; 11 male, 17 female) HSCT were collected. We obtained blood samples at the following time points: on admission (7 days before HSCT, T − 1), on the day of HSCT (T0), during aplasia (defined by absolute neutrophil counts <0.5 G/L, T + 1), on the day of engraftment (defined by absolute neutrophil counts >0.5 G/L, T + 2), 1 month after HSCT (T + 3), 3 to 6 months after HSCT (T + 4), and 6 to 12 months after HSCT (T + 5), respectively. LCN2 levels were measured at the time point next to maximum GVHD. Time points in control patients were matched accordingly.

Forty-one consecutive patients were included into the study. Because one patient died 2 days after enrolment, she was excluded from further analysis, leaving a total of 40 patients for correlation of plasma LCN2 levels with clinical outcome including occurrence of aGVHD, cGVHD, NRM and overall survival (OS). We enrolled patients prior to the start of myeloablative (n = 26) or reduced-intensity conditioning for HSCT and autologous HSCT patients (n = 12) as controls. Healthy individuals served as controls and were recruited via bulletin. The diagnosis and the severity of aGVHD and cGVHD were determined based on the modified Glucksberg and NIH classification [11,27,28]. All patients received anti-infective prophylaxis as described [29].

Plasma concentrations of LCN2 were measured with an enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions (Thermo Fisher Scientific, Waltham, MA). All patients gave written informed consent in accordance with the Declaration of Helsinki. The study protocol was approved by the local ethical review board according to Austrian law regulations (Registration No. 1344/2017).

Statistical analysis

The study was designed as a prospective cohort study including 40 patients that were followed longitudinally. NRM was defined as any death not related to the underlying malignancy. Cumulative incidence of NRM was estimated using the Kaplan–Meier method, adjusting for relapse as a competing risk event [30]. Relapse was defined as recurrence of malignancy after achievement of complete remission with NRM as a competing risk. Cumulative incidences of aGVHD and cGVHD were estimated considering relapse and death as competing events. OS was calculated from day 0 of HSCT to the day of death from any cause or last follow-up. Patients were censored at the date of last contact.

Statistical comparisons of plasma levels with patient cohorts were made using the unpaired Student t test or Mann–Whitney U test following testing for normality. Fisher's exact test was used to examine the significance of the association between two variables. Differences were considered statistically significant at a two-sided p value <0.05. The data are presented as median and interquartile range. All data were calculated using GraphPad Prism 6.0 (GraphPad Software, San Diego, CA).

Results

Demographics

A total of 40 evaluable patients were included in our study. Apart from gender and disease, no significant differences in patient characteristics between the autologous and allogeneic HSCT patient cohorts were observed (Table 1). With respect to gender, male patients were significantly taller and heavier than female patients (81.6 kg vs. 70.0 kg, $p = 0.009$).

Course of LCN2 during HSCT

Baseline LCN2 plasma levels at T − 1 were 111.3 (±112.6) ng/mL in allogeneic and 76.2 (±65.5) ng/mL in autologous stem cell transplantation, respectively. This difference was not statistically significant. In patients undergoing autologous stem cell transplantation, LCN2 plasma levels peaked at T + 1 with a mean of 9113.6 (±5550.3) ng/mL, in allogeneic stem cell transplantation LCN2 levels peaked at T0 with 5404.3 (±5365.4) ng/mL. Apart from that, the time course of LCN2 did not significantly differ between the allogeneic and autologous groups.

Following HSCT, both donor mismatch and relationship of the donor (related /unrelated) did not show a significant impact on the course of LCN2 levels.

Acute GVHD

In allogeneic HSCT recipients with aGVHD, LCN2 levels were 125.7 (±63.1) ng/mL and, thus, significantly higher compared with levels of patients without aGVHD (65.9 ± 38.0 ng/ml, $p = 0.018$), autologous controls (71.4 ± 40.5 ng/mL, $p = 0.034$), and healthy

Table 1. Demographic data of enrolled subjects

Demographic	*N* (%)			
	All	Allogeneic	Autologous	Healthy controls
Number of patients	40 (100)	28 (70)	12 (30)	15
Median age in years	46	44	48	
(range)	(34–56)	(34–56)	(42–49)	
Gender				
Male	21 (53)	11 (39)	10 (83)	8 (53)
Female	19 (47)	17 (61)	2 (17)	7 (47)
BMI on admission	24.4	22.9	25.8	
BMI	25.2	24.6*	25.8*	
(range)	(17.6–33)	(17.6–33)	(19–31.2)	
Diagnosis				
Acute leukemia	21 (53)	21 (75)	0 (0)	
Chronic leukemia	1 (3)	1 (4)	0 (0)	
Lymphoma	9 (23)	5 (18)	4 (33)	
Myeloma	6 (15)	0 (0)	6 (50)	
Other†	3 (8)	1 (4)	2 (17)	
Disease status at transplantation				
Standard risk†	21 (53)	15 (54)	6 (50)	
High risk†	19 (48)	13 (46)	6 (50)	
Conditioning				
Myeloablative	26 (65)	14 (50)	12 (100)	
RIC	14 (35)	14 (50)	0 (0)	
Stem cell donors				
Related	11 (39)	11 (39)	NA	
Unrelated	17 (61)	17 (61)	NA	
HLA-identical	21 (75)	21 (75)	NA	
HLA-mismatched	7 (25)	7 (25)	NA	
Stem cell source				
Bone marrow	1 (3)	1 (4)	0 (0)	
PBSC	39 (98)	27 (96)	12 (100)	
Post-transplant immunosuppressive prophylaxis				
Cyclosporine only	4 (10)	4 (14)	NA	
Cyclosporine-MTX	14 (35)	14 (50)	NA	
Cyclosporine-MMF	10 (25)	10 (36)	NA	
Follow-up (mo)				
Median	26	24	30.3	
Range	0.1–46	0.1–46	4.3–46	

*Statistically different.

controls (45.9 ± 14.1 ng/ml, $p < 0.0001$), respectively (Figure 1A). Severity of aGVHD correlated with LCN2 levels (Figure 1B).

Chronic GVHD

Characteristics of cGVHD are summarized in Table 2. In allogeneic HSCT recipients with cGVHD, LCN2 levels were 295.0 (±215.7) ng/mL and thus significantly higher than those of patients without cGVHD (54.9 ± 68.2 ng/mL, $p < 0.001$), autologous patients (76.5 ± 68.2 ng/mL, $p < 0.001$), and healthy controls (45.9 ± 14.1 ng/mL, $p < 0.001$), respectively (Figure 2A). Again, severity of cGVHD correlated with LCN2 levels (Figure 2B).

Patients with aGVHD and cGVHD

In patients with aGVHD who also developed cGVHD later ($n = 6$), there were no significant differences compared with patients with either aGVHD (111.3 ± 78.2 ng/mL vs. 135.7 ± 59.1 ng/mL) or cGVHD (322.6 ± 162.9 ng/mL vs. 273.5 ± 193.7 ng/mL) only. Patients with overlapping aGVHD and cGVHD ($n = 4$) also did not show significant differences with respect to LCN2 levels. However, when sampling at the time point during active aGVHD and cGVHD ($n = 2$), LCN2 levels were higher than in patients with aGVHD (275.4 vs. 135.7 ± 59.1 ng/mL) or cGVHD (411.2 vs. 273.5 ± 193.7 ng/mL) only.

Mortality

Overall, 13 patients (32%) died within a mean of 8 months after HSCT. Nine patients (22.5%) died within a mean of 4.7 months after HSCT because of relapse of their primary diseases. Four patients (10%) died within a mean 14.5 months after HSCT from causes that were not related to

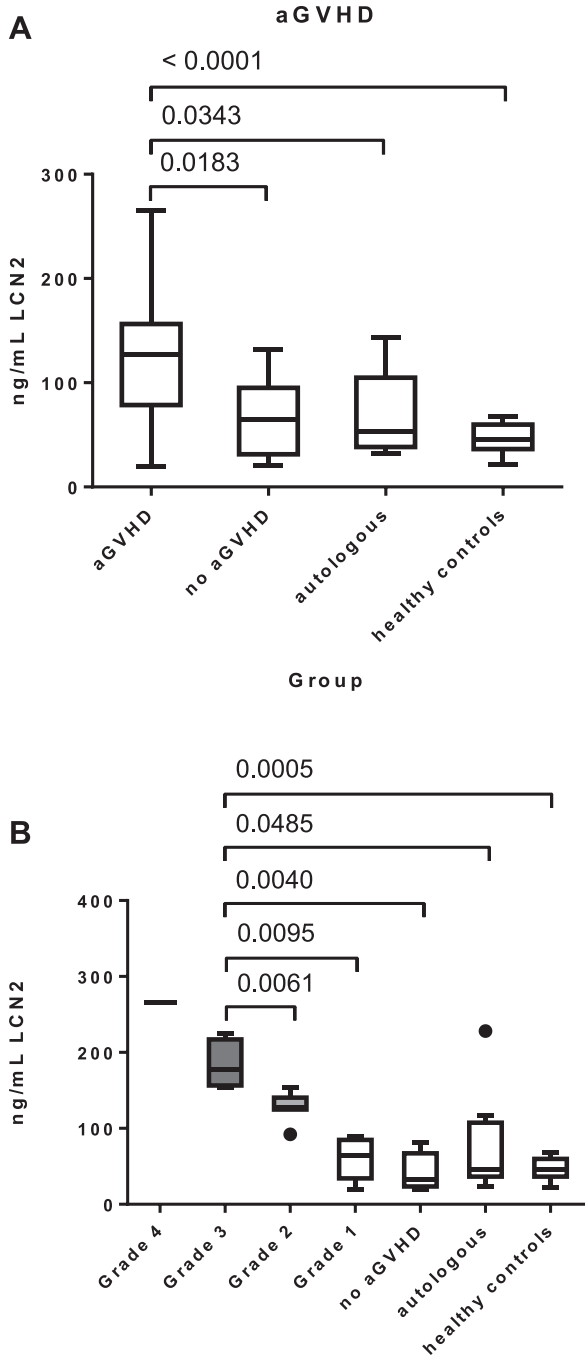


Figure 1. LCN2 levels in aGVHD at disease maximum **(A)** compared with autologous and healthy controls. LCN2 levels correlated with severity of aGVHD **(B)**.

their primary disease (causes of death: sarcoma, infection, cGVHD, aGVHD). We did not observe differences in mortality, relapse, aGVHD, and cGVHD with respect to gender.

Whereas time points T − 1 until T + 3 did not differ significantly when surviving patients were compared with patients dying during the follow-up period, a significant difference was found at T + 4, when LCN2 levels in non-surviving patients began to rise again, which was not the case in surviving patients (Figure 3). T + 5 showed a trend but failed to reach significance ($p = 0.096$) because by T + 5, a considerable number of patients in this group had died and were censored. This difference also occurred in allogeneic patients with relapse of the underlying disease ($n = 9$; Figure 3) as well as in patients dying from causes other than relapse (such as infection and GVHD, $n = 4$), suggesting a mechanism independent of transplantation or underlying disease.

Table 2. Characteristics of chronic GVHD

Characteristic	*N* (%)
Total	17 (61)
Organ involvement	
Skin	11 (65)
Eyes	11 (65)
Oral mucosa	8 (47)
Liver	10 (59)
Lungs	4 (24)
Gastrointestinal tract	2 (12)
Joints	1 (6)
NIH severity score	
Mild	10 (59)
Moderate	4 (24)
Severe	3 (18)
Onset type of cGVHD	
De novo	6 (35)
Quiescent	6 (35)
Progressive	5 (29)
Median time to first onset of cGVHD	123 (75–222)

Comparison with healthy controls post-GVHD

In patients with resolved aGVHD and no cGVHD ($n = 6$), LCN2 levels did not differ significantly from healthy controls (43.4 ± 23.7 ng/mL vs. 45.2 ± 14.3 ng/mL). Patients with resolved cGVHD ($n = 9$) also did not show differing LCN2 levels compared with healthy controls (54.9 ± 21.4 vs. 45.2 ± 14.3 ng/mL).

Influence of conditioning regimen

Patients undergoing the myeloablative conditioning regimen (MAC) did not have significantly different LCN2 levels compared with those on the reduced-intensity conditioning (RIC) regimen at T0, T + 1, and T + 2.

Patients treated with MAC had higher levels of LCN2 during T0, T + 1, T + 2, and T + 3, whereas patients treated with the RIC regimen had higher LCN2 levels during T + 4 and T + 5 (Supplementary Figure E1 in the Supplementary Material, online only, available at www.exphem.org). However, this difference failed to reach statistical significance.

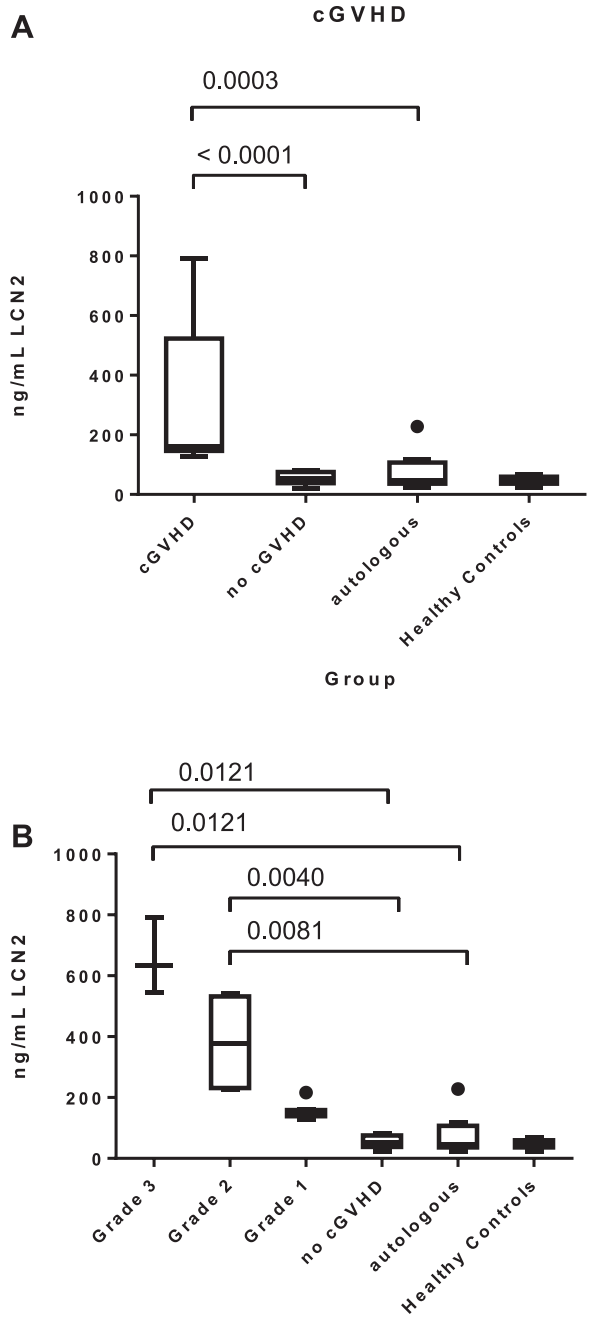


Figure 2. LCN2 levels in cGVHD at disease maximum **(A)** compared with autologous and healthy controls. LCN2 levels correlated with severity of cGVHD **(B)**

Discussion

The main finding of our study is that LCN2 exhibits significant differences between patients experiencing aGVHD or cGVHD and autologous or healthy controls; LCN2 correlated with severity of both forms of GVHD. Also, LCN2 levels were significantly higher in patients post-HSCT without GVHD compared with healthy controls. Furthermore, LCN2 levels correlated with relapse and overall survival. This has been shown for the first time.

The LCN2 levels reported here are comparable to those of other studies [31]. Maier et al. reported a median of 255 ng/mL on day 1 post-kidney transplantation, decreasing to 134 ng/mL on day 7, which is a bit higher than the data in our patients without GVHD and might be a consequence of the fact that Maier reported data only up to the seventh postoperative day [31]. LCN2 is an acute phase protein and, as such, is typically elevated in postoperative conditions [32]. It must be taken into account that the immunosuppressive treatment, as a cornerstone of post-HSCT therapy, may alter LCN2 levels in these patients. However, studies on whether or not immunosuppression influences LCN2 are lacking.

We found that LCN2 levels are higher in patients with both aGVHD and cGVHD. This is of interest because LCN2 influences the function of regulatory T helper cells [33], which play a prominent role in the development of cGVHD [34,35]. It has been shown that this T-cell subtype can ameliorate GVHD in a mouse model [20,36]. On the other hand, injection of LCN2 exacerbates psoriasiform skin inflammation via Th-17 cells [37]. In this case, LCN2 would causally contribute to deterioration of GVHD. Further studies have to elucidate this correlation. Another important observation by Wang et al. reported that IL-17 enhanced LCN2 mRNA expression in human neutrophils [38], which also play a major role in the development of aGVHD [39]. Because LCN2 also correlated with the severity of both forms of GVHD, it may be used as a disease activity marker, similar to inflammatory bowel disease [40].

LCN2 plasma concentrations in patients with aGVHD were significantly higher than those in patients following autologous HSCT. Baseline LCN2 levels originally differed between allogeneic and autologous groups. However, it is highly unlikely that this fact was responsible for the observed effect in aGVHD, as the onset and course of aGVHD depend mainly on the tissue damage caused by the respective conditioning regimen [14]. Conditioning itself, however, did not influence LCN2 levels in our study.

GVHD is a major contributor to mortality following HSCT [41,42]. LCN2 was higher in patients dying from relapse or other causes; this is in accordance with Srinivasan et al., who reported that LCN2 deficiency exacerbates sepsis [43]. Elevated LCN2 has been described as a risk factor for all-cause mortality in the general population [44]; the authors attribute this to the fact that LCN2 acts as a scavenger protein for a variety of chemotactic peptides, thereby preventing directed migration of inflammatory cells into the tissue. In our case, this might also contribute to autoimmune-associated damage in cGVHD, which might explain the higher

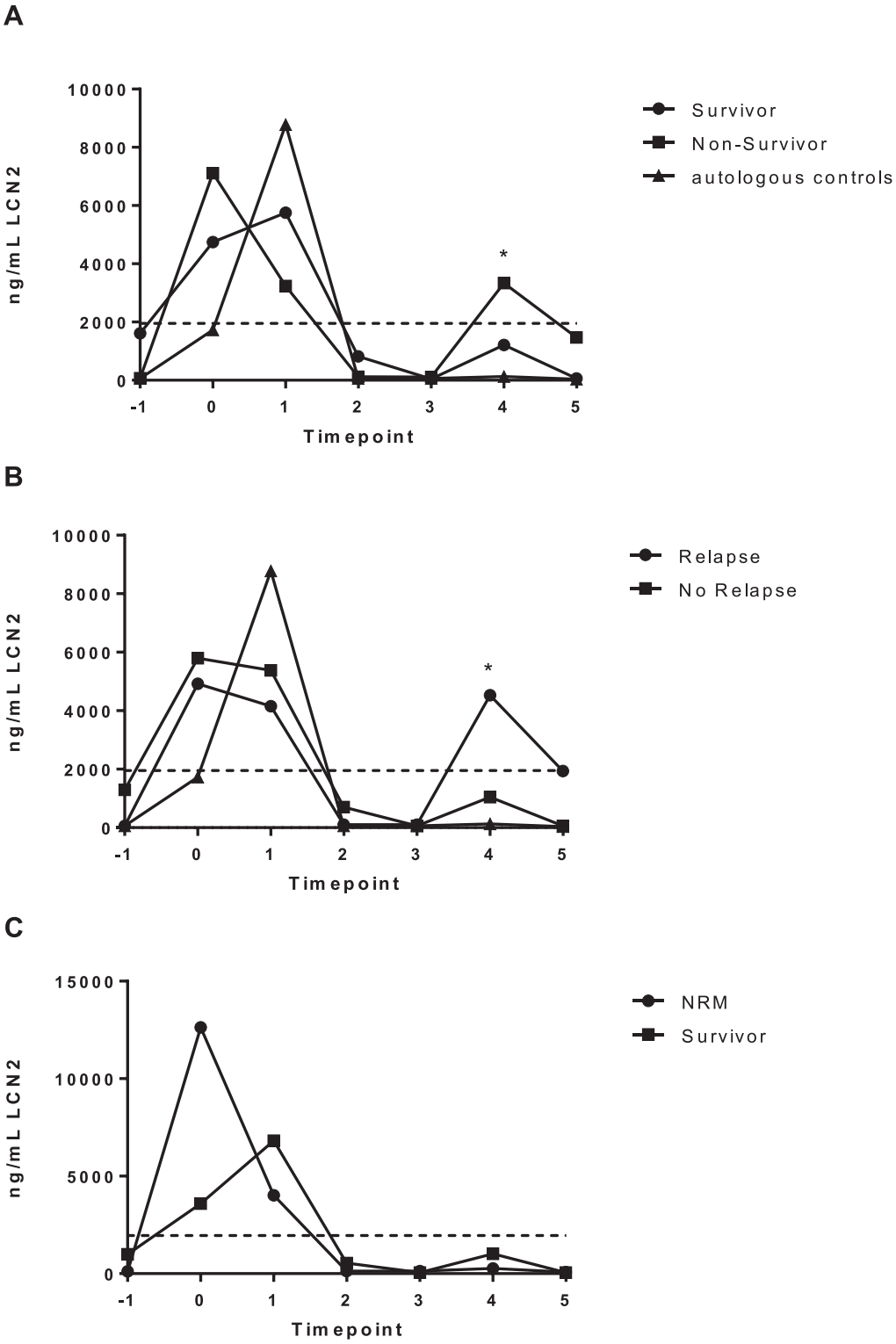


Figure 3. LCN2 levels correlate with overall survival **(A)** and relapse **(B)**, but not non-relapse mortality (NRM) in allogeneic HSCT recipients.

concentrations in cGVHD patients. However, whether this is causal or an epiphenomenon cannot be determined. LCN2 also seems to play a role in autoimmune diseases such as multiple sclerosis [45] and autoimmune encephalomyelitis [46]; cGVHD might mimic such autoimmune processes.

A recent study reported that LCN2 levels are higher during acute kidney injury following HSCT [24].

Interestingly, LCN2 levels are influenced mainly by the gut microbiota in a MyD88-dependent manner [47] which also contributes to GVHD [48]. Whether this had an effect in our cohort can only be speculated, as we did not analyze a possible shift in the microbiome of the patients. This question needs to be addressed in the future.

Whether the occurrence of an active infection influences the expression of LCN2 is of interest. We did not find a significant correlation between bacterial infections and LCN2 levels. However, all patients received a prophylactic antibiotic and antifungal treatment during neutropenia following HSCT, which might hinder proper diagnosis. Furthermore, LCN2 levels did not correlate with other inflammatory markers by means of C-reactive protein and leukocyte count. Hepatocytes are the major source for LCN2 during bacterial infections [17]. In our cohort, LCN2 did not correlate with cGVHD of the liver.

Possible strengths of our study comprise its prospective design, the homogenous cohorts, and the fact that we not only used healthy controls but also autologous patients as a second control group to account for the effect of the conditioning regimen including the chemotherapeutic treatment and whole-body irradiation. Possible weak points are a rather small patient number in each of the respective groups, the heterogenicity of diseases before HSCT, and the fact that therefore we cannot elucidate underlying pathomechanisms.

Our study raises several questions that need to be addressed by follow-up studies. First and foremost, whether LCN2 is somehow causally involved in GVHD development needs to be clarified. Because aGVHD and cGVHD are very different diseases, a common underlying pathomechanism may be suspected. Several mechanisms have been described therefore [1]. Second, if LCN2 knockout ameliorates GVHD and whether or not LCN2 reduction by an intravenous agent (antibody) has similar effects need to be elucidated. We therefore encourage other groups to verify our results.

Conclusively, we found that LCN2 is elevated in aGVHD and cGVHD as well as in patients with adverse outcome and relapse in a patient cohort with hematological malignancies undergoing HSCT.

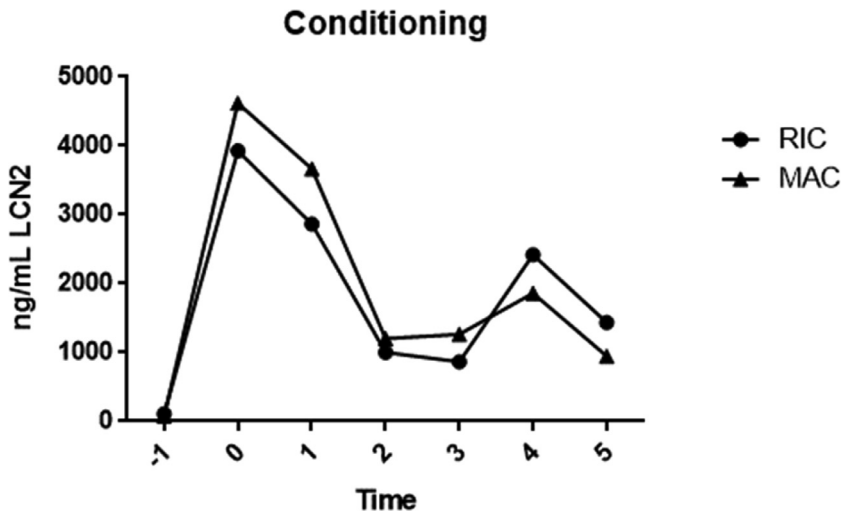
Acknowledgments

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

References

1. Appelbaum FR. Hematopoietic-cell transplantation at 50. N Engl J Med. 2007;357:1472–1475.
2. Passweg JR, Baldomero H, Gratwohl A, et al. The EBMT activity survey: 1990–2010. Bone Marrow Transplant. 2012;47:906–923.
3. Lee SJ, Vogelsang G, Gilman A, et al. A survey of diagnosis, management, and grading of chronic GVHD. Biol Blood Marrow Transplant. 2002;8:32–39.
4. Socie G, Stone JV, Wingard JR, et al. Long-term survival and late deaths after allogeneic bone marrow transplantation. Late Effects Working Committee of the International Bone Marrow Transplant Registry. N Engl J Med.. 1999;341:14–21.
5. Jagasia M, Arora M, Flowers ME, et al. Risk factors for acute GVHD and survival after hematopoietic cell transplantation. Blood. 2012;119:296–307.
6. Pasquini MC. Impact of graft-versus-host disease on survival. Best Pract Res Clin Haematol. 2008;21:193–204.
7. Pidala J, Anasetti C, Jim H. Quality of life after allogeneic hematopoietic cell transplantation. Blood. 2009;114:7–19.
8. Rouquette-Gally AM, Boyeldieu D, Gluckman E, Abuaf N, Combrisson A. Autoimmunity in 28 patients after allogeneic bone marrow transplantation: Comparison with Sjogren syndrome and scleroderma. Br J Haematol. 1987;66:45–47.
9. Cwynarski K, Goulding R, Pocock C, et al. Immune haemolytic anaemia following T cell-depleted allogeneic bone marrow transplantation for chronic myeloid leukaemia: Association with leukaemic relapse and treatment with donor lymphocyte infusions. Bone Marrow Transplant. 2001;28:581–586.
10. Rouquette-Gally AM, Boyeldieu D, Prost AC, Gluckman E. Autoimmunity after allogeneic bone marrow transplantation: A study of 53 long-term-surviving patients. Transplantation. 1988;46:238–240.
11. Filipovich AH, Weisdorf D, Pavletic S, et al. National Institutes of Health consensus development project on criteria for clinical trials in chronic graft-versus-host disease: I. Diagnosis and staging working group report. Biol Blood Marrow Transplant.. 2005;11:945–956.
12. Jagasia MH, Greinix HT, Arora M, et al. National Institutes of Health Consensus Development Project on Criteria for Clinical Trials in Chronic Graft-versus-Host Disease: I. The 2014 Diagnosis and Staging Working Group report. Biol Blood Marrow Transplant. 2015;21. 389–401.e381.
13. Goker H, Haznedaroglu IC, Chao NJ. Acute graft-vs-host disease: Pathobiology and management. Exp Hematol. 2001;29:259–277.
14. Harris AC, Ferrara JL, Levine JE. Advances in predicting acute GVHD. Br J Haematol. 2013;160:288–302.
15. Chakraborty S, Kaur S, Guha S, Batra SK. The multifaceted roles of neutrophil gelatinase associated lipocalin (NGAL) in inflammation and cancer. Biochim Biophys Acta. 2012;1826:129–169.
16. Abella V, Scotece M, Conde J, et al. The potential of lipocalin-2/NGAL as biomarker for inflammatory and metabolic diseases. Biomarkers. 2015;20:565–571.
17. Xu MJ, Feng D, Wu H, et al. Liver is the major source of elevated serum lipocalin-2 levels after bacterial infection or partial hepatectomy: A critical role for IL-6/STAT3. Hepatology. 2015;61:692–702.
18. Yang XO, Panopoulos AD, Nurieva R, et al. STAT3 regulates cytokine-mediated generation of inflammatory helper T cells. J Biol Chem. 2007;282:9358–9363.
19. Hartigan-O'Connor DJ, Hirao LA, McCune JM, Dandekar S. Th17 cells and regulatory T cells in elite control over HIV and SIV. Curr Opin HIV AIDS. 2011;6:221–227.
20. Zheng J, Liu Y, Liu Y, et al. Human CD8+ regulatory T cells inhibit GVHD and preserve general immunity in humanized mice. Sci Transl Med. 2013;5. 168ra169.
21. Borkham-Kamphorst E, Drews F, Weiskirchen R. Induction of lipocalin-2 expression in acute and chronic experimental liver injury moderated by pro-inflammatory cytokines interleukin-1beta through nuclear factor-kappaB activation. Liver Int. 2011;31:656–665.
22. Cowland JB, Borregaard N. Molecular characterization and pattern of tissue expression of the gene for neutrophil gelatinase-associated lipocalin from humans. Genomics. 1997;45:17–23.

23. Review Devarajan P. Neutrophil gelatinase-associated lipocalin: A troponin-like biomarker for human acute kidney injury. Nephrology (Carlton). 2010;15:419–428.
24. Taghizadeh-Ghehi M, Sarayani A, Ashouri A, Ataei S, Moslehi A, Hadjibabaie M. Urine neutrophil gelatinase associated lipocalin as an early marker of acute kidney injury in hematopoietic stem cell transplantation patients. Ren Fail. 2015;37:994–998.
25. Deger SM, Erten Y, Suyani E, et al. Early diagnostic markers for detection of acute kidney injury in allogeneic hematopoietic stem cell transplant recipients. Exp Clin Transplant. 2017. https://doi.org/10.6002/ect.2016.0161.
26. Schmidt-Ott KM, Mori K, Li JY, et al. Dual action of neutrophil gelatinase-associated lipocalin. J Am Soc Nephrol. 2007;18:407–413.
27. Przepiorka D, Weisdorf D, Martin P, et al. 1994 Consensus Conference on Acute GVHD Grading. Bone Marrow Transplant.. 1995;15:825–828.
28. Glucksberg H, Storb R, Fefer A, et al. Clinical manifestations of graft-versus-host disease in human recipients of marrow from HL-A-matched sibling donors. Transplantation. 1974;18:295–304.
29. Tomblyn M, Chiller T, Einsele H, et al. Guidelines for preventing infectious complications among hematopoietic cell transplantation recipients: A global perspective. Biol Blood Marrow Transplant. 2009;15:1143–1238.
30. Fine JP, Gray RJ. A proportional hazards model for the subdistribution of a competing risk. J Am Stat Assoc. 1999;94:496–509.
31. Maier HT, Ashraf MI, Denecke C, et al. Prediction of delayed graft function and long-term graft survival by serum and urinary neutrophil gelatinase-associated lipocalin during the early postoperative phase after kidney transplantation. PLoS One. 2018;13:e0189932.
32. Hunsicker O, Feldheiser A, Weimann A, et al. Diagnostic value of plasma NGAL and intraoperative diuresis for AKI after major gynecological surgery in patients treated within an intraoperative goal-directed hemodynamic algorithm: A substudy of a randomized controlled trial. Medicine (Baltimore). 2017;96:e7357.
33. La Manna G, Ghinatti G, Tazzari PL, et al. Neutrophil gelatinase-associated lipocalin increases HLA-G(+)/FoxP3(+) T-regulatory cell population in an in vitro model of PBMC. PLoS One. 2014;9:e89497.
34. Cardenas PA, Huang Y, Ildstad ST. The role of pDC, recipient T (reg) and donor T(reg) in HSC engraftment: Mechanisms of facilitation. Chimerism. 2011;2:65–70.
35. Robb RJ, Lineburg KE, Kuns RD, et al. Identification and expansion of highly suppressive CD8(+)FoxP3(+) regulatory T cells after experimental allogeneic bone marrow transplantation. Blood. 2012;119:5898–5908.
36. Pankratz S, Bittner S, Herrmann AM, et al. Human CD4+ HLA-G+ regulatory T cells are potent suppressors of graft-versus-host disease in vivo. FASEB J. 2014;28:3435–3445.
37. Hau CS, Kanda N, Tada Y, et al. Lipocalin-2 exacerbates psoriasiform skin inflammation by augmenting T-helper 17 response. J Dermatol. 2016;43:785–794.
38. Wang Y, Jia M, Yan X, et al. Increased neutrophil gelatinase-associated lipocalin (NGAL) promotes airway remodelling in chronic obstructive pulmonary disease. Clin Sci (Lond). 2017;131:1147–1159.
39. Schwab L, Goroncy L, Palaniyandi S, et al. Neutrophil granulocytes recruited upon translocation of intestinal bacteria enhance graft-versus-host disease via tissue damage. Nat Med. 2014;20:648–654.
40. Stallhofer J, Friedrich M, Konrad-Zerna A, et al. Lipocalin-2 is a disease activity marker in inflammatory bowel disease regulated by IL-17A, IL-22, and TNF-alpha and modulated by IL23R genotype status. Inflamm Bowel Dis. 2015;21:2327–2340.
41. Cooke KR, Luznik L, Sarantopoulos S, et al. The biology of chronic graft-versus-host disease: A Task Force Report from the National Institutes of Health Consensus Development Project on Criteria for Clinical Trials in Chronic Graft-versus-Host Disease. Biol Blood Marrow Transplant. 2017;23:211–234.
42. Singh AK, McGuirk JP. Allogeneic stem cell transplantation: A historical and scientific overview. Cancer Res. 2016;76:6445–6451.
43. Srinivasan G, Aitken JD, Zhang B, et al. Lipocalin 2 deficiency dysregulates iron homeostasis and exacerbates endotoxin-induced sepsis. J Immunol. 2012;189:1911–1919.
44. Lindberg S, Jensen JS, Mogelvang R, et al. Plasma neutrophil gelatinase-associated lipocalinin in the general population: Association with inflammation and prognosis. Arterioscler Thromb Vasc Biol. 2014;34:2135–2142.
45. Al Nimer F, Elliott C, Bergman J, et al. Lipocalin-2 is increased in progressive multiple sclerosis and inhibits remyelination. Neurol Neuroimmunol Neuroinflamm. 2016;3:e191.
46. Berard JL, Zarruk JG, Arbour N, et al. Lipocalin 2 is a novel immune mediator of experimental autoimmune encephalomyelitis pathogenesis and is modulated in multiple sclerosis. Glia. 2012;60:1145–1159.
47. Singh V, Yeoh BS, Chassaing B, et al. Microbiota-inducible innate immune, siderophore binding protein lipocalin 2 is critical for intestinal homeostasis. Cell Mol Gastroenterol Hepatol. 2016;2. 482–498.e486.
48. Shono Y, Docampo MD, Peled JU, et al. Increased GVHD-related mortality with broad-spectrum antibiotic use after allogeneic hematopoietic stem cell transplantation in human patients and mice. Sci Transl Med. 2016;8. 339ra371.



Supplementary Figure E1. LCN2 levels do not differ between myeloablative conditioning regimen (MAC) and reduced-intensity conditioning regimen (RIC) in allogeneic HSCT recipients.