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Research Article

Elevated Galectin-3 Plasma Concentrations in Recipients of Allogeneic Hematopoietic Cell Transplantation

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ABSTRACT

Galectin-3 is a beta-galactoside-binding lectin with an established association to inflammatory and fibrotic conditions. We investigated galectin-3 levels in 68 recipients of allogeneic hematopoietic cell transplantation (HCT) to look for associations with chronic graft-versus-host disease (cGVHD). Plasma galectin-3 concentrations were measured at 1 year post-HCT and correlated with clinical data collected from individual medical records. The median serum galectin-3 level at that time point was 14.9 ng/mL (range, 5.5–61.6), which was significantly higher than that among healthy controls (14.9 versus 6.2, $p < 0.001$). Furthermore, patients with active cGVHD at the time of sample collection had higher median levels as compared to those without cGVHD (16.9 versus 13, $p = 0.03$). In a multivariable logistic model, there was no significant association between the presence of cGVHD at the date of sample collection and elevated galectin-3 levels (>14.9 ng/mL) (odds ratio [OR]: 2.03 (0.60, 6.88), $p = 0.26$). However, among patients with cGVHD at the date of sample collection, active systemic corticosteroid therapy was associated with elevated galectin-3 levels (OR: 20.32 (1.66, 249.39), $p = 0.02$). Furthermore, in a competing risk regression model, elevated galectin-3 levels at 1 year post-HCT were not associated with future development of moderate or severe cGVHD (OR: 1.24 (0.21, 7.45), $p = 0.81$). In conclusion, plasma galectin-3 concentrations are elevated in recipients of allo-HCT, especially among patients with cGVHD. Further investigation will be required to determine whether galectin-3 has a pathophysiologic role in cGVHD or serves as a marker of ongoing inflammation following allogeneic HCT.

1. INTRODUCTION

Chronic graft-versus-host disease (cGVHD) remains a major complication following allogeneic hematopoietic cell transplantation (HCT), and is known to be a leading cause of morbidity, mortality, and impairment in quality of life among long-term survivors [1,2]. Biomarkers that can either predict or prognosticate cGVHD would be of great clinical utility, possibly allowing for preemptive or risk-stratified interventions to improve outcomes. A number of potential biomarkers for cGVHD have been investigated but have not yet been adopted into clinical practice [3].

Galectin-3 is a beta-galactoside-binding lectin which is thought to play a multifunctional role in a number of biologic processes, including cell proliferation, adhesion, differentiation, angiogenesis, and apoptosis [4]. Furthermore, galectin-3 activates a variety of profibrotic factors, promotes fibroblast proliferation and transformation, and mediates collagen production, supporting its role in inflammatory and fibrotic conditions [4]. In the general population, increased levels of galectin-3 have been associated with risk for all-cause mortality, independent of classical risk factors of cardiovascular disease [5]. In established inflammatory and fibrotic conditions, galectin-3 has been identified as a marker of disease activity in rheumatoid arthritis, and has been independently associated with inferior outcomes in patients with heart failure and systemic sclerosis [6–9].

It is now established that donor-derived effector T-cells, B-cell signaling, and fibrosis-promoting factors all contribute to the pathophysiology of cGVHD [10]. These complex immunologic mechanisms result in clinical manifestations of cGVHD that share many features of chronic tissue damage as seen in classical autoimmune and inflammatory conditions [11]. The aim of this study was

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to assess the potential association of serum galectin-3 levels and clinical characteristics in a cohort of allogeneic HCT recipients, with a specific focus on cGVHD.

2. METHODS

The current study was approved by the Institutional Review Boards at the Dana-Farber/Harvard Cancer Center and Massachusetts General Hospital. Adult patients undergoing allogeneic HCT at the Dana-Farber Cancer Institute and Brigham and Women's Hospital had blood samples collected and cryopreserved for research purposes on a bio-specimen protocol. Sixty-eight patients with available cryopreserved plasma samples at 1 year (± 1 month) were selected based on the evidence of presence or absence of cGVHD, but regardless of other individual transplant characteristics or clinical outcomes. Additionally, samples from 21 healthy subjects were collected and analyzed as a non-HCT control. All samples were tested for galectin-3 concentrations in a blinded fashion. Analysis of galectin-3 levels were performed using BGM Galectin-3 ELISA kit developed by BG Medicine (Waltham, MA, USA). The measuring range of the assay is 1.4–94.8 ng/mL. The average intra-assay coefficient of variation (CV) is approximately 3.4%, and the average inter-assay CV is approximately 8.5%. Clinical data were extracted from the institutional database and individual medical records. Patient characteristics were reported descriptively. cGVHD severity was graded according to the NIH consensus criteria [12].

3. RESULTS

3.1. Patient and Transplant Characteristics

Patient demographics and transplant characteristics are shown in Table 1. The median age was 56 years (range, 19–73). There were 38 males and 30 females. The majority of patients underwent a matched related (n = 24, 35%) or matched unrelated donor (n = 37, 54%) transplant. The most common graft source was peripheral blood stem cells (n = 63, 93%). Reduced-intensity or non-myeloablative conditioning intensity (n = 61, 90%) was predominantly used. Tacrolimus/sirolimus-based strategies (n = 61, 90%) were primarily used for GVHD prophylaxis. Four patients (6%) received anti-thymocyte globulin as part of their GVHD prophylaxis. Thirteen patients (19%) had previous acute GVHD.

The median follow-up from transplant was 63.5 months (range, 11–203). In those who developed cGVHD, the median onset of cGVHD was 9 months after HCT (range, 5–97). Galectin-3 serum samples were collected at a median of 11 months post-transplant (range, 10–12). At the time of sample collection, 21 patients (30%) had clinical manifestations of cGVHD, of which 13 had mild and 8 had moderate or severe disease. Twenty-six patients (38%) were receiving systemic corticosteroid therapy at the time of sample collection.

Table 1 | Patient demographics and transplant characteristics.

Characteristic	Value
Number of patients	68
Patient demographics	
Median age, range	56 (19–73)
Gender, *n* (%)	
Male	38 (56)
Female	30 (44)
Disease, *n* (%)	
Aplastic anemia	1 (1)
Acute lymphoblastic leukemia	7 (10)
Acute myeloid leukemia	17 (25)
Chronic lymphocytic leukemia	7 (10)
Chronic myeloid leukemia	1 (1)
Hodgkin lymphoma	6 (9)
Multiple myeloma	4 (6)
Myelodysplasia	8 (12)
Myeloproliferative neoplasm	4 (6)
Non-Hodgkin lymphoma	12 (18)
Severe combined immunodeficiency	1 (1)
Transplant characteristics	
Donor type, *n* (%)	
Matched related donor	24 (35)
Matched unrelated donor	37 (54)
Mismatched unrelated donor	6 (9)
Umbilical cord blood	1 (1)
Graft source, *n* (%)	
Peripheral blood	63 (93)
Bone marrow	4 (6)
Umbilical cord	1 (1)
Conditioning intensity, *n* (%)	
Myeloablative	7 (10)
Reduced intensity or non-myeloablative	61 (90)
GVHD prophylaxis, *n* (%)	
Tacrolimus/sirolimus ± other	61 (90)
Calcineurin inhibitor + methotrexate	7 (10)
Year of transplant, *n* (%)	
2005–2009	19 (28)
2010–2015	49 (72)
GVHD characteristics	
Previous acute GVHD, *n* (%)	
Yes	13 (19)
No	55 (81)
cGVHD at time of sample analysis, *n* (%)	
Yes	21 (31)
No	47 (69)
cGVHD severity at time of sample analysis, *n* (%)	
Mild	13 (62)
Moderate or severe	8 (38)
Median time (in months) from transplant to cGVHD onset, range	9 (5–97)
Corticosteroid therapy	
Systemic corticosteroid therapy at time of sample collection, *n* (%)	
Yes	26 (38)
No	42 (62)
Follow-up	
Median follow-up (in months) from transplant, range	63.5 (11–203)

Abbreviations: cGVHD = chronic graft-versus-host disease; GVHD = graft-versus-host disease; NIH = National Institutes of Health.

3.2. Serum Galectin-3 Levels

The median serum galectin-3 level among allogeneic HCT recipients collected around 1 year after transplant was 14.9 ng/mL (range, 5.5–61.6) (Fig. 1A). This was significantly higher than the median level from healthy controls (14.9 versus 8.2, p = 0.006) (Fig. 1B). When analyzing median levels of galectin-3 based on the presence of cGVHD, we found higher median levels among patients with cGVHD, as compared to those without cGVHD (16.9 versus 13, p = 0.03). However, there was no significant difference in galectin-3 levels based on the presence of moderate or severe cGVHD, as compared to mild or no cGVHD (18.5 versus 14.3, p = 0.27). The

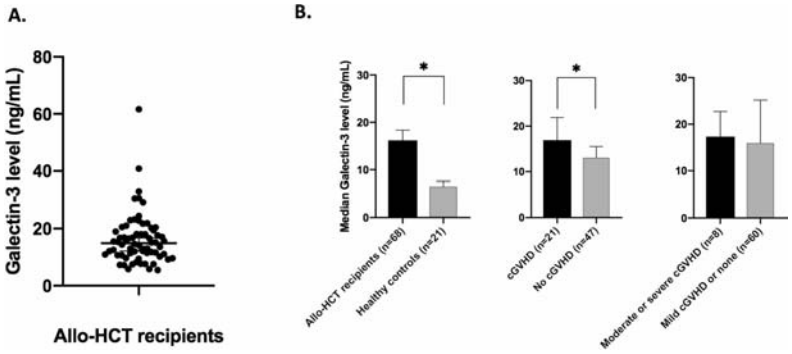

Figure 1 | (A) Column scatter graph depicting serum galectin-3 levels collected at 1 year after allo-HCT. (B) Median serum galectin-3 levels comparing allo-HCT recipients to healthy controls, patients with cGVHD to those without cGVHD, and patients with moderate or severe cGVHD to those with mild or no cGVHD. *Indicated p-value < 0.05. cGVHD = chronic graft-versus-host disease; HCT = hematopoietic cell transplantation.

median serum galectin-3 levels were higher among patients receiving systemic corticosteroids, as compared to those not thus treated (17.6 versus 12.4, $p = 0.0006$).

3.3. Multivariate Analysis of Clinical Factors and 1-Year Galectin-3 Levels

We examined the relationship between donor type, a clinical history of GVHD, cGVHD at the date of sample collection, systemic corticosteroid therapy at the time of sample collection, and serum galectin-3 levels. In a multivariate logistic model, neither cGVHD at the date of sample collection (odds ratio (OR): 2.04 (0.60, 6.88), $p = 0.26$) nor systemic corticosteroid use (OR: 2.55 (0.69, 9.48), $p = 0.16$) were significantly associated with elevated galectin-3 levels (Table 2). However, in the 21 patients with cGVHD at the date of sample collection, treatment with systemic corticosteroids was associated with elevated galectin-3 levels (OR: 20.32 (1.66, 249.39), $p = 0.02$) (Table 2).

3.4. Association of 1-Year Galectin-3 Levels With Future Moderate or Severe CGVHD

We examined the relationship between serum galectin-3 levels around 1 year after HCT and future development of moderate or severe cGVHD for patients without a diagnosis of cGVHD at the time of sample collection. In univariate analysis, patients with elevated galectin-3 levels had a higher cumulative incidence of moderate or severe cGVHD at 3 years post sampling, although not statistically significant (14.3% versus 7.4%, $p = 0.42$). In a competing risk regression model accounting for donor type, history of prior GVHD (acute or chronic) and systemic corticosteroid therapy, elevated galectin-3 levels were not associated with future moderate or severe cGVHD (OR: 1.24 (0.21, 7.45), $p = 0.81$) (Table 2).

Table 2 | Multivariate analysis.

	OR	95% CI	p-value
Association between GVHD-related factors and elevated galectin-3 levels			
MMUD vs other	1.62	0.22–11.84	0.64
MUD vs other	0.75	0.25–2.28	0.61
Prior aGVHD (yes vs no)	1.61	0.35–7.48	0.55
cGVHD at date of sample collection (yes vs no)	2.03	0.60–6.88	0.26
Systemic corticosteroid therapy at date of sample collection (yes vs no)	2.55	0.69–9.48	0.16
Association between GVHD-related factors and elevated galectin-3 levels among patients with cGVHD at date of sample collection (n = 21)			
MUD vs other	0.28	0.02–3.80	0.34
Systemic corticosteroid therapy at date of sample collection (yes vs no)	20.33	1.66–249.39	0.02
Association between GVHD-related factors and elevated galectin-3 levels among patients with cGVHD at date of sample collection (n = 47)			
MMUD vs other	1.30	0.15–11.46	0.82
MUD vs other	0.93	0.27–3.28	0.91
Prior aGVHD (yes vs no)	1.77	0.29–10.82	0.54
Systemic corticosteroid therapy at date of sample collection (yes vs no)	1.13	0.21–6.00	0.89
Association between elevated galectin-3 levels and future moderate/severe cGVHD			
MMUD vs other	1.43	0.08–26.41	0.81
MUD vs other	1.39	0.29–6.72	0.68
Prior GVHD (yes vs no)	1.59	0.28–9.06	0.60
Galectin-3 level (elevated vs not elevated)	1.24	0.21–7.45	0.81
Systemic corticosteroid therapy at date of sample collection (yes vs no)	1.90	0.34–10.52	0.46

Abbreviations: aGVHD = acute graft-versus-host disease; cGVHD = chronic graft-versus-host disease; CI = confidence interval; GVHD = graft-versus-host disease; MMUD = mismatched unrelated donor; MUD = matched unrelated donor; OR = odds ratio.

4. DISCUSSION

In this study, we found galectin-3 concentrations to be elevated in recipients of allogeneic HCT, as compared to healthy controls. In the former population, the median galectin-3 concentrations at 1 year after HCT were higher in patients with cGVHD at the time of sample collection, as compared to those without cGVHD. The strongest association was between cGVHD and elevated galectin-3 levels in patients receiving systemic corticosteroid therapy. However, we did not find associations between galectin-3 concentrations and severity of cGVHD or with the future development of moderate or severe disease.

There are limitations to this analysis, in addition to the retrospective nature of the study and limited number of patients and samples. The plasma samples used for analysis were collected from patients

at 1 year after transplant, which allowed for investigation of correlations with active cGVHD. However, earlier time points would be much more informative to evaluate whether elevated galectin-3 levels could serve as a predictive marker for future cGVHD. We found a strong association between elevated galectin-3 levels and cGVHD in patients receiving systemic corticosteroid therapy. The impact of this therapy on galectin-3 levels is not known, so it remains undetermined if the elevated levels reflect more clinically significant disease (requiring ongoing systemic therapy), or are partially increased as a medication effect. Another limitation is that we do not yet understand the full significance of elevated galectin-3 levels in patients with cGVHD. It is known that galectin-3 is secreted by macrophages and fibroblasts in response to stress and injury, and can subsequently lead to tissue fibrosis by stimulating accumulation of extracellular matrix and fibroblast proliferation [4]. In cGVHD, macrophages can accumulate in fibrotic lesions and promote pathology through activation of transforming growth factor beta [13,14]. However, the mechanistic role of galectin-3 in cGVHD models has not been investigated, so whether galectin-3 is implicated in the causal pathway of cGVHD pathophysiology or serves a secondary marker of inflammation is unknown. This is of particular interest, as animal models have demonstrated that galectin-3 function can be reduced by exposure to modified citrus pectin [15,16], and that galectin-3 can be depleted through therapeutic apheresis [17], suggesting that galectin-3 could be a potential target for therapeutic intervention.

In conclusion, plasma galectin-3 concentrations are elevated in recipients of allo-HCT, especially in those with cGVHD. Further investigations will be required to determine whether galectin-3 has a pathophysiologic role in cGVHD or serves as a marker of ongoing inflammation following allogeneic HCT.

CONFLICT OF INTEREST

ZD receives research support from Incyte, Corp. JR has received research support from Equillium, Kite Pharma, Merck and Neovii Biotech and consulting income from Avrobio, Celgene and TScan Therapeutics. The other authors declare no conflict of interest.

AUTHORS' CONTRIBUTIONS

Z.D., N.N.A., S.L., T.R.S., and C.A.H. designed the study. Y.B.C., V.T.H, J.R., and T.R.S provided data. Z.D., N.N.A., A.R.A., and A.J. gathered the data. Z.D., N.N.A., S.L., T.R.S., and C.A.H. performed the analysis. Z.D. and N.N.A. wrote the manuscript. The final manuscript was read and approved by all authors.

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