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Conjunctival inflammation and dry eye symptoms at day 100 post-transplantation do not predict risk for chronic graft-versus-host disease

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Abstract:

PURPOSE: Chronic graft-versus-host disease (cGVHD) is a significant cause of morbidity and mortality among patients after allogeneic hematopoietic stem cell transplant (HCT). The objective of our study was to determine if early assessment of matrix metalloproteinase-9 (MMP-9) and dry eye (DE) symptoms (via the DE Questionnaire-5 [DEQ-5]) had prognostic utility for the development of cGVHD and/or severe DE symptoms after HCT.

MATERIALS AND METHODS: This was a retrospective study of 25 individuals who underwent HCT and had MMP-9 (InflammaDry) and DEQ-5 performed on day 100 post-HCT (D + 100). Patients also completed the DEQ-5 at 6, 9, and 12 months post-HCT. The development of cGVHD was determined by chart review.

RESULTS: Overall, 28% of patients developed cGVHD over a median follow-up of 229 days. At D + 100, 32% of patients had a positive MMP-9 in at least one eye and 20% had a DEQ-5 ≥6. However, neither the presence of a positive MMP-9 nor a DEQ-5 score ≥6 at D + 100 predicted the development of cGVHD (MMP-9: hazard ratio [HR]: 1.53, 95% confidence interval [CI]: 0.34-6.85, $P = 0.58$; DEQ-5 ≥6: HR: 1.00, 95% CI: 0.12-8.32, $P = 1.00$). In addition, neither of these measures predicted the development of severe DE symptoms (DEQ-5 ≥12) over time (MMP-9: HR: 1.77, 95% CI: 0.24-12.89, $P = 0.58$; DEQ-5 >6: HR: 0.03, 95% CI: 0.00-889.93, $P = 0.49$).

CONCLUSION: Within our small cohort, DEQ-5 and MMP-9 assessment at D + 100 did not predict the development of cGVHD or severe DE symptoms.

Keywords:

Dry eye, graft-versus-host disease, stem cell transplant

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Introduction

Chronic graft-versus-host disease (GVHD) is the leading cause of morbidity and mortality in survivors after allogeneic hematopoietic cell transplantation (HCT) and is challenging to diagnose in its early stages.[1] Approximately 30%-50% of post-HCT patients develop chronic GVHD (cGVHD),[2,3] with associated long-term nonrelapse mortality rates as high as 40%.[4,5] The pathophysiology of cGVHD involves an exaggerated immune response during tissue damage which subsequently activates adaptive immune responses that lead to tissue macrophage activation and tissue fibrosis.[6]

Chronic GVHD can involve almost any organ, with the skin, fascia, eyes, and mouth commonly affected. Per the 2014

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NIH Consensus Criteria,[7] the presence of certain clinical features is considered pathognomonic to diagnose cGVHD. These diagnostic features include lichen planus-like findings of the skin and mucosa, bronchiolitis obliterans syndrome of the lung, fasciitis, and esophageal webs. Nondiagnostic signs are also commonly observed such as nail dystrophy, loss of body hair, mucosal ulcerations, and significant ocular surface disease. Overall, the diagnosis of cGVHD "requires the presence of at least 1 diagnostic clinical sign of cGVHD or the presence of at least 1 distinctive manifestation (e.g., keratoconjunctivitis sicca) confirmed by pertinent biopsy or other relevant tests (e.g., Schirmer's test) in the same or another organ."[7]

Ocular surface symptoms and signs are among the most common initial manifestations of cGVHD, with a majority of post-HCT patients experiencing some level of discomfort.[7-10] These ocular surface changes may include "new-onset dry, gritty, or painful eyes, cicatricial conjunctivitis, keratoconjunctivitis sicca, and confluent areas of punctate keratopathy."[7] Ocular chronic GVHD (oGVHD) is diagnosed, per the 2014 NIH Criteria, by a combination of symptoms and signs in conjunction with slit-lamp examination to evaluate for keratoconjunctivitis sicca.[7,9]

The management for moderate-to-severe-grade cGVHD commonly includes the use of systemic immunosuppressants such as prednisone, sirolimus, and tacrolimus[11] which are well-known for increased rates of infection and secondary organ failure.[5] However, the role of systemic immunosuppression for treatment of oGVHD is not well established, as the predominantly localized symptoms are commonly treated with topical therapies such as artificial or autologous tears for surface lubrication,[12] calcineurin inhibitors[13,14] and topical corticosteroids[15] to reduce inflammation, and supportive measures such as warm compresses to address meibomian gland dysfunction. Despite the use of multifaceted therapies, clinical treatment responses are inconsistent, and most patients do not achieve complete resolution of ocular symptoms and signs.[12-16]

Due to the long-term clinical impact of oGVHD and inconsistent response to treatment, early identification of at-risk patients may allow prompt and targeted intervention to prevent morbidity and mortality.[4,17] Given the overlap between ocular surface disease and other organ involvement of cGVHD,[18] investigation of the ocular surface may reveal potential prognostic biomarkers for both cGVHD and oGVHD.

Matrix metalloproteinase-9 (MMP-9) is a zinc-dependent proteolytic enzyme that interacts with proinflammatory cytokines, signal transduction molecules, and transcription factors.[19] It is an established marker of ocular surface inflammation that is available as a point-of-care test (InflammaDry, Quidel, San Diego, United States).[20,21] Inflammatory stimuli cause the release of MMP-9 into the extracellular matrix and subsequent ocular surface damage.[22,23] A recent study demonstrated that detection of conjunctival MMP-9 (using InflammaDry) was significantly more frequent in a cohort of 45 individuals with oGVHD compared to 40 individuals with non-GVHD-related dry eye (DE) disease (84.4% vs. 33.0%; $P \leq 0.01$).[23] Given the need for prognostic markers in cGVHD, the objective of our study was to determine if two easily accessible tests, point-of-care MMP-9 and patient-reported DE symptom assessment, carried prognostic utility for the development of cGVHD and/or severe DE symptoms in a cohort of post-HCT survivors.

Materials and Methods

Study population

We conducted a retrospective study of 25 individuals who were screened with the DE Questionnaire-5 (DEQ-5, a validated survey that assesses the intensity and frequency of dryness and discomfort over a 1-month recall, range: 0 - 22)[24] and underwent bilateral MMP-9 (InflammaDry, Quidel, San Diego, United States) testing at a GVHD clinic approximately 100 days after HCT (D + 100). The University of Miami Institutional Review Board allowed the retrospective evaluation of charts (20200357), and the research was conducted in accordance with the Declaration of Helsinki.

Data collected

Demographics, comorbidities, and transplant history were collected via chart review. After D + 100, patient charts were reviewed for the development of cGVHD as determined by their transplant physician based on the extent of organ involvement (GI tract, lungs, skin, eyes, etc.), treatment, and performance status in accordance with current guidelines. The date of first cGVHD diagnosis and DEQ-5 responses at 6-, 9-, and 12-month follow-up post-HCT were recorded.

Statistical analysis

All data analysis was performed using SPSS 26.0 (IBM statistics, Armonk, NY, USA). Descriptive statistics were used to present demographic and clinical data. A DEQ-5 ≥ 6 was considered indicative of mild or greater DE symptoms and a DEQ-5 ≥ 12 was considered indicative of severe symptoms.[24] An increase in DEQ-5 scores >3 from baseline was considered a clinically significant increase in DE symptoms. Univariable Cox regression analysis was used to examine whether MMP-9 positivity or a DEQ-5 ≥ 6 at D + 100 predicted the

development of cGVHD and/or severe DE symptoms. $P < 0.05$ was considered statistically significant.

Results

The median age of the cohort was 54 years, with a range of 29-65 years; 36% (n = 9) of patients were male and 68% (n = 17) were Hispanic. The underlying diagnoses leading to HCT included acute lymphoblastic leukemia, chronic lymphocytic leukemia, acute myeloid leukemia, follicular lymphoma, myelodysplastic syndrome, and aplastic anemia. Sixty-eight percent (n = 17) had human leukocyte antigen (HLA)-matched donor HCT and 32% (n = 8) had HLA-mismatched donor HCT. At D + 100, 44% (n = 11) of patients were on prednisone for treatment of acute GVHD, ranging from 5 to 60 mg per day, and all patients were on either tacrolimus or sirolimus as primary immune suppression GVHD prophylaxis [Table 1]. Two patients had a relapse of their original malignancy after D + 100, and neither were diagnosed with cGVHD.

Overall, 28% (n = 7) of patients developed cGVHD and 12% (n = 3) developed severe DE symptoms over a median follow-up of 229 days (range: 45 - 433 days) since the D + 100 visit.

The median time from HCT to cGVHD diagnosis was 9.2 months. Two of the seven patients who developed cGVHD had prior acute GVHD (Grade I and Grade III). None of the patients diagnosed with cGVHD had eye involvement at the time of diagnosis, and only one developed ocular symptoms over time but never received a formal oGVHD diagnosis. At the time of cGVHD diagnosis, 57% (n = 4) had liver involvement, 43% (n = 3) had skin involvement, 28% (n = 2) had lung involvement, and 28% (n = 2) had oral cavity involvement. Overall, 57% (n = 4) had multi-organ involvement at initial cGVHD diagnosis.

At D + 100, 32% (n = 8) of patients had a positive MMP-9 in at least one eye and 20% (n = 5) had a DEQ-5 ≥6. However, neither the presence of a positive MMP-9 nor a DEQ-5 score ≥6 at D + 100 predicted the development of cGVHD (MMP-9: hazard ratio [HR]: 1.53, 95% confidence interval [CI]: 0.34 - 6.85, P = 0.58; DEQ-5 ≥6: HR: 1.00, 95% CI: 0.12 - 8.32, P = 1.00). In a similar manner, neither of these measures predicted the development of severe DE symptoms (DEQ-5 ≥12) over time (MMP-9: HR: 1.77, 95% CI: 0.24 - 12.89, P = 0.58; DEQ-5 >6: HR: 0.03, 95% CI: 0.00 - 889.93, P = 0.49).

Overall, DEQ-5 scores tended to increase over time [Figure 1], irrespective of cGVHD development. At 6 months post-HCT, 35% (n = 5) individuals had a ≥3 increase in their DEQ-5 score from baseline. At 9 months post-HCT, an additional 3 individuals had a ≥3 increase in their DEQ-5 score from baseline. However, most patients (86%, n = 6) who developed cGVHD did not have a ≥3 increase in DE symptoms.

Table 1: Characteristics of our study population

Variable	Values (n=25), frequency (%)
Age, median (range)	54 (29-65)
Female	16 (64)
Cancer type	
Chronic lymphocytic leukemia	2 (8)
Acute myeloid leukemia	6 (24)
Acute lymphoblastic leukemia	5 (20)
Myelodysplastic syndrome	5 (20)
Follicular lymphoma	3 (12)
T-cell lymphoma	2 (8)
T-cell prolymphocytic leukemia	1 (4)
Aplastic anemia	1 (4)
Conditioning regimen	
Myeloablative	9 (36)
Reduced intensity	16 (64)
Total body irradiation	5 (20)
HLA match	
Matched	17 (68)
Mismatched (≤7/8)	8 (32)
Systemic immunosuppressants at D+100	
Tacrolimus	21 (84)
Sirolimus	4 (16)
Prednisone	11 (44)
Eye drops at D+100	
Yes	8 (32)
No	17 (68)
Maximum grade acute GVHD	
None	13 (52)
I	4 (16)
II	7 (28)
III	1 (4)
IV	0
Development of chronic GVHD	
None	18 (64)
Limited	4 (16)
Extensive	3 (12)

D+100=Day 100 post-HCT. HCT=Hematopoietic stem cell transplant, GVHD=Graft-versus-host disease, HLA=Human leukocyte antigen

Discussion

Chronic GVHD is diagnosed in approximately 30%-50% of post-HCT patients and is the leading cause of morbidity and mortality in long-term HCT survivors.[2-5] In the literature, oGVHD is found in a high frequency of individuals diagnosed with cGVHD.[25] Development of prognostic biomarkers for cGVHD and oGVHD would ease the identification of at-risk patients and allow for early intervention to prevent and reduce complications or organ damage and treatment-related toxicities. Studies of prognostic cGVHD biomarkers have

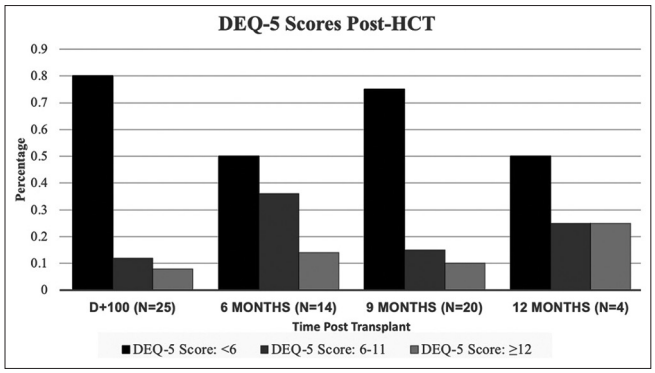

Figure 1: The percentage of individuals with DEQ-5 scores <6, 6-11, and ≥12 at different time points post-HCT. DEQ-5 = Dry Eye Questionnaire-5, HCT = Hematopoietic stem cell transplant

focused on soluble proteins derived from peripheral blood.[26] In a U. S. retrospective study of 172 individuals, elevated serum level of chemokine ligand 9 (CXCL9) at D + 100 was prognostic for cGVHD development within 3 months (HR: 3.10, 95% CI: 1.5 - 6.3, $P < 0.01$), 6 months (HR: 1.47, 95% CI: 1.0 - 2.2, $P = 0.05$), and 12 months (HR: 1.49, 95% CI: 1.1 - 2.1, $P = 0.02$). The same study also found that elevated serum level of suppression of tumorigenicity-2 (ST2) was prognostic for cGVHD development within 3 months (HR: 3.91, 95% CI: 1.3 - 11.6, $P = 0.01$).[27] In another retrospective study of 127 patients, elevated plasma levels of CD163 (≥765 ng/mL vs. <765 ng/mL) at D + 80 correlated with cGVHD development (75% vs. 40%; $P = 0.02$; median months from D + 80 to cGVHD diagnosis: 4.9 months).[28] Peripheral blood cellular biomarkers have also been studied, as a prospective Austrian study of 227 post-HCT individuals found that the percentage of $CD19^{+}CD21^{low}$ B-cells at D + 100 was higher in those who did versus did not develop cGVHD at a later date (23.5% vs. 15.2%, $P < 0.01$; HR: 3.31, 95% CI: 1.53 - 7.17, $P < 0.01$).[29]

A limitation of using peripheral blood biomarkers is that venipuncture is required and testing does not offer immediate results. Given the overlap between cGVHD and oGVHD, we examined whether noninvasive tests that return immediate results, specifically the presence of MMP-9 on the ocular surface (via InflammaDry) and DE symptom assessment at D + 100, could identify patients at risk for cGVHD or development of severe DE symptoms (a component of oGVHD). Biological plausibility for this approach was the finding that several inflammatory cytokines (including MMP-9) were elevated in tears of individuals with oGVHD. For example, a Spanish study found that tear levels of IL (interleukin)-1 receptor antagonist (IL-1Ra), CXC motif chemokine ligand-8 (IL-8/CXCL8), and IL-10 were significantly increased in the oGVHD population ($n = 22$) versus controls ($n = 21$). Not surprisingly, individuals with oGVHD also had significantly higher OSDI scores, a survey of DE symptoms and their impact on patient quality of life, compared to healthy controls (44.58 ± 21.76 vs. 4.29 ± 5.11, $P < 0.01$).[30] Similarly, in an Indian study, individuals with oGVHD ($n = 8$) had elevated levels of MMP-9 in tears, along with elevated interferon-γ (IFN-γ), IL-6, IL-8, IL-10, IL-12P70, IL-17A, and vascular endothelial growth factor, compared to both post-HCT patients without oGVHD ($n = 12$) and controls ($n = 12$).[31] The advantage of using InflammaDry to detect MMP-9 is that it is a commercially available test that gives results in ~10 min. Unfortunately, neither InflammaDry nor DEQ-5 assessments at D + 100 predicted the development of cGVHD or severe DE symptoms in our small cohort.

Previous studies have also not arrived at a consensus regarding ocular surface prognostic biomarkers for GVHD. For example, a prospective Spanish study of 25 individuals post-HCT found that the combined detection of elevated IL-6, IL-1Ra, and fractalkine in pre-HCT tear samples was not prognostic for the development of cGVHD itself (relative risk [RR]: 0.79, 95% CI: 0.51 - 1.22, $P = 0.28$) but was associated with a reduced risk for oGVHD in patients who had developed cGVHD ($n = 16$, RR: 0.30, 95% CI: 0.10 - 0.91, $P = 0.03$).[32] However, this finding was not replicated in a prospective US study of 34 post-HCT patients where various biomarkers assessed at D + 100 (tear and serum levels of IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12, IL-13, IL-17α, MMP-9, IFN-γ, chemokine ligand-19, monocyte chemoattractant protein-1, macrophage inflammatory protein-1α, Regulated upon Activation, Normal T-cell Expressed and Presumably Secreted (RANTES), and tumor necrosis factor-α) were not prognostic for oGVHD development, although certain tear proteins such as MMP-9 and IL-8 trended toward statistical significance.[33]

Limitations of our study include a limited number of patients and specific metrics for MMP-9 and DE symptom assessment. In addition, not all individuals with cGVHD or DE symptoms were seen by an ophthalmologist, so we cannot comment on the presence of other aspects of oGVHD, such as aqueous tear deficiency or epithelial erosions. Despite our negative results, this line of research is important as minimally invasive and point-of-care tests with the ability to prognosticate or diagnose cGVHD and oGVHD would improve patient outcomes after HCT. By promoting targeted follow-up in high-risk individuals to allow early intervention, prognostic and diagnostic biomarkers can also reduce patient and hospital burden in low-risk subjects by decreasing the number of unnecessary provider visits, and invasive testing or procedures. In this study, we provide pilot data on the utility of two such tests to inform the community that our noted magnitude of effect likely does not warrant larger studies to examine their prognostic capabilities for cGVHD at D + 100. However, future studies are needed to examine the utility of other ocular biomarkers, in addition to clinical assessments, at various time points post-HCT

to identify patients most at risk for cGVHD and oGVHD development. Although we did not use an OSDI score in this study, previous studies have demonstrated that OSDI and DEQ-5 scores are significantly correlated. Future studies may utilize either score to evaluate DE symptoms. We are also planning a separate, larger study evaluating ocular surface and serum protein markers beyond MMP-9 to build upon this initial pilot investigation. Systemic immunosuppressive agents may also impact ocular MMP-9 expression, as corticosteroids may decrease inflammatory MMP-9 expression.[34] In addition, our study demonstrates that DE symptom scores generally increase over time, regardless of cGVHD diagnosis. Consistent monitoring with timely intervention or referral to ophthalmology is also necessary for appropriate care of patients after transplantation.

Conclusions

Within our small cohort of patients, MMP-9 (via InflammaDry) and DEQ-5 assessments at D + 100 did not predict the development of cGVHD or severe DE symptoms.

Acknowledgments

Shah, Galor, and Wang designed the research study and analyzed the data.

Shah, Galor, Mones, Jean, Komanduri, and Wang performed the research study.

Shah, Galor, and Wang wrote the paper.

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Conflicts of interest

The authors declare that there are no conflicts of interests of this paper.

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