Open Access Original Article

Role of Biomarkers in Diagnosing and Post-Treatment Monitoring of Acute Graft-vs-Host Disease

Afsheen Liaqat[1], Nadia Sial[2], Mehwish Gilani[3], Maryam Khan[4], Tariq Ghafoor[5], Raheel Iftikhar[6]

Department of Clinical Hematology and Bone Marrow Transplant, National University of Medical Sciences, Rawalpindi, Pakistan

Address for Correspondence
Dr Afsheen Liaqat
Department of Clinical Hematology and Bone Marrow Transplant, National University of Medical Sciences, Rawalpindi, Pakistan.
afsheenliaqat19@gmail.com

Abstract

Objective: This study aimed to investigate the role of ST2 and Reg3a biomarkers in the diagnosis and post-treatment monitoring of aGVHD.

Methods: A prospective observational study was conducted at the Armed Forces Bone Marrow Transplant Center, CMH Rawalpindi, from July 2023 to December 2024. Patients undergoing Allo-HCT for malignancy and beta thalassemia major were included and monitored for clinical signs of aGVHD. ST2 and Reg3a levels were checked at day 0 and 45 of transplant. For patients developing GVHD, pre and post treatment samples were collected for comparison. Biomarker levels were correlated with clinical outcomes.

Results: A total of number of 15 patients were included, among which 7 patients developed GVHD while 8 patients were considered as controls. At day 0, no significant difference between cases and controls was observed in mean level of ST2 (7504.87 ng/mL vs 3603.41 ng/mL, p=0.19), or in mean level of Reg3a (29.33 ng/mL vs 34.89 ng/mL, p=0.24). However, at day 45, mean level of ST2 was significantly higher in those patients who subsequently developed GVHD (3984.17 ng/mL vs 1742.10 ng/mL, p=0.020) although mean Reg3a level did not show significant difference (29.08 ng/mL vs 32.66 ng/mL, p=0.519).

Comparing levels at onset with post treatment levels in cases, the role of biomarkers in post treatment monitoring was found to be insignificant (ST2: 5900.56 vs 5337.59, p= 0.65, Reg3a: 28.07 vs 23.92, p=0.10).

Conclusion: The ST2 level can be a useful biomarker for predicting acute GVHD. More data with larger cohort is needed to establish clinical utility of ST2 in GVHD prediction and response monitoring.

Key words: Graft-versus-host disease (GVHD), Biomarkers, ST2, Reg3a.

Introduction

Allogeneic hematopoietic stem cell transplant (Allo-HSCT) is the only curative treatment for a variety of malignant and non-malignant hematological disorders.[1,2] Despite advances in Graft versus host disease (GVHD) prophylaxis and management, it remains the most feared complication of Allo-HCT and is the third leading cause of non-relapse mortality after infections and organ failure.[3,4] Historically incidence of acute GVHD (aGVHD) used to be 35−45 % in fully matched while 60−80 % in haplo-matched transplants, however the incidence and severity of GVHD have reduced with newer transplant strategies.[5]

According to the National Institutes of Health (NIH) classification, aGVHD occurs within 100 days of post Allo-HCT and affects skin, liver, and gastrointestinal (GI) tract.[4] High-dose systemic glucocorticoids with optimization of immunosuppressive therapy remain the first line treatment.[1,6] Ruxolitinib, anti-tumor necrosis factor agents, mycophenolate mofetil and mesenchymal stem cells (MSCs) are frequently used for patients with steroid refractory aGVHD.

In clinical practice, transplant physicians often keep a high index of suspicion for aGVHD and use specific clinical presentation combined with histopathological evidence of GVHD for treatment initiation. Waiting for biopsy report can delay timely intervention and lead to suboptimal responses.[1] Recently, using biomarkers for diagnosis and prognostication of aGVHD has gained significant interest among researchers worldwide.[7] In patients receiving Allo-HCT, suppressor of tumorigenesis 2 (ST-2) and regenerating islet-derived 3 alpha (Reg3a) biomarkers are associated with gut damage (8)while tumor necrosis factor 1 (TNFR1), T-cell Ig mucin domain 3 (TIM3), interleukin 6 (IL6) are related with systemic inflammation.[9] Elafin biomarker is related with skin GVHD (1). Other protein biomarkers such as plasma amphiregulin/EGF ratio, plasma IL2Ra, TNFR1, soluble

Authorship Contribution: [1,3] Conceived and planned the idea of the study, [5,6]final approval of the version to be published, Collecting the data, [2,4]drafting the work or revising it critically for important intellectual content.

Funding Source: none
Conflict of Interest: none
Received: Aug 31, 2025
Accepted: Nov 19, 2025

B-cell activation factor (BAFF), chemokine (C-X-C motif) ligand 10 (CXCL10), chemokine (C-X-C motif) ligand 11 (CXCL11), and serum albumin are also reported to correlate with aGVHD. [1]

This study aims to determine the role of two frequently studied biomarkers, Suppression of tumorigenicity 2 (ST2) and Regenerating islet-derived protein 3 alpha (Reg3a), in the diagnosis and post treatment monitoring of aGVHD.

Methodology

This, prospective observational study, is conducted in Armed forces bone marrow transplant center (AFBMTC), combined military hospital (CMH), Rawalpindi. Written ethical approval from the Institutional Review Board and informed consent of the patients were obtained. The study included patients enrolled consecutively undergoing allogeneic stem cell transplantation for beta thalassemia major and hematological malignancies and from July 2023 to December 2024.

The baseline demographic and clinical data were collected on an Excel sheet and then transferred to SPSS software at time of analysis. Considerable variables included date of leukemia/beta thalassemia major diagnosis, history of treatment before going for transplant, HLA matching, patient-donor gender mismatch, ABO mismatch, type of conditioning regimen, use of anti-thymocyte globulin (ATG), source of stem cell collection, stem cell dose and graft versus host disease (GVHD) prophylaxis (combination of cyclosporine, mycophenolate or short course of methotrexate and PT-CY in case of haplo-matched donors only. Follow up information included date of onset of acute GVHD (a-GVHD), type, stage and grade of a-GVHD and treatment of a-GVHD. Assessment for a-GVHD was done till day 100.

Venous samples from all patients were collected on day 0 of transplant and at routine post-transplant follow-up visits in OPD at day 45 of allogenic stem cell transplant. Additionally, samples were collected from those patients who were diagnosed with acute GVHD at the first presentation, prior to initiation of treatment, and who subsequently achieved remission of GVHD following treatment. The clinical diagnosis, staging and grading was made using standardized Mount Sinai Acute GVHD International Consortium (MAGIC) criteria.

Serum was separated and stored at -20°C until the day of analysis for ST2 and Reg3a levels. Levels of acute GVHD markers (ST2 and Reg3a) were measured according to the guidelines of the manufacturer using enzyme-linked immunosorbent assay (ELISA) kit (Human ST2 [IL-33R], Catalog#BMS2231, Invitrogen and Human Reg3a ELISA kit, Catalog#EH390RB, Invitrogen) at stem cell laboratory of AFBMTC, CMH, Rawalpindi. Absorbance of all samples and standards were measured in duplicate using spectrophotometer, MultiskanGo, Thermo scientific. Standard curve was used to determine the concentrations of Reg3A and ST2.

Statistical analysis was conducted using SPSS 25.0 software. Quantitative data were expressed as medians with ranges, and qualitative variables were presented as frequencies and percentages. The Mann-Whitney U test was used to assess statistical differences in biomarker levels between patients with and without aGVHD. A p-value < 0.05 was considered statistically significant. The Kaplan-Meier method was employed for survival analysis, , and group differences were evaluated using the log-rank test. The difference in ST2 levels between patients with and without aGVHD at Day 45 was illustrated using a box plot, showing a higher median level in the aGVHD group.

Results

This study includes 15 patients of hematological disorders who underwent related allogeneic hematopoietic stem cell transplants at AFBMTC. Median CD34 positive stem cell dose and MNC were 5.6x106/kg (3-13.2) and6 x108/kg (4.3-9.3), respectively (Table I). Oral GVHD was not found in any of the patient. However, mucositis occurred in 10 patients (66.7%) (Table II). No significant differences were observed in ST2 levels at Day 0 between patients who developed aGVHD and those who did not (Median: 6459.3 vs. 1262.2 pg/mL; Mann-Whitney $U = 14.0$, $p = 0.22$). At Day 45, ST2 levels were significantly raised in patients with aGVHD (Median: 4687.6 vs. 950.3 pg/mL; $U = 6.0$, $p = 0.02$), as depicted in the box plot Figure 1.

REG3 Alpha levels did not reveal the statistical difference between aGVHD and non-aGVHD patients. At Day 0, the median REG3 Alpha level was 37.1 ng/mL in the aGVHD group and 18.6 ng/mL in the non-aGVHD group (Mann-Whitney $U = 15.0$, $p = 0.28$). Similarly, at Day 45, median levels were 36.7 ng/mL vs. 18.5 ng/mL, respectively ($U = 19.0$, $p = 0.57$).

The change in ST2 and REG3 Alpha levels from onset to post-treatment of GVHD were not statistically significant

(Z=-4.47, p=1.00 vs Z = -1.604, p = 0.25). Kaplan-Meier log rank survival analysis showed no significant difference in Day 180 survival times between patients who developed aGVHD and those who did not (χ^2 = 0.01, p = 0.94). In non-aGVHD group the mean survival time was 162.5 days (95% CI: 138.2–186.8) and 171 days (95% CI: 154.9–187.0) in the aGVHD group.

Table I: Patient and Transplant Characteristics (N=15)	
Characteristics	n (%)
Patient Age	
Less than 18	4(26.7%)
More than 18	11(73.3%)
Patient Gender	
Male	9 (60%)
Female	6 (40%)
Donor Gender	
Male	6(40%)
Female	9(60%)
Gender Mismatch	
Yes	7(46.7%)
No	8(53.3%)
ABO Mismatch	
Minor ABO mismach	3(20%)
Bi-directional ABO mismatch	2(13.3%)
No mismatch	10(66.7%)
Diseases	
Malignancy	12(80%)
Thalassaemia	3(20%)
Co-morbidity	
Yes	3 (20%)
No	12(80%)
HLA-Type	
Fully matched	11(73.3)
Haplo matched	4 (26.7%)
Stem Cell Source	
BMH	12(80%)
BMH plus PBSC	2(13.3%)
PBSC	1(6.7%)
Conditioning Regimen	
Bu-Cy	6 (40%)
Flu-Bu	8 (53%)
Flu-Mel-TG	1 (7%)
ATG	
Yes	12(80%)
No	3(20%)
PTCY Administered	
Yes	4(26.7%)
No	11(73.3%)
GVHD Prophylaxis	
CSA-MMF	3 (20%)
CSA-MMF-PTCy	3 (20%)
CSA-MTX(3 doses)	9 (60%)
CD34 Dose x 10^6/kg (Median; Range)	5.6 (3-13.2)
MNC Dose x 10^8/kg (Median; Range)	6 (4.3-9.3)

Restricted mean survival for non-relapse mortality were 162.5 days (95% CI: 132.8–192.2) and 171 days (95% CI: 152.5–189.5), respectively. However, the survival between the groups did not reveal the significant difference (χ^2 = 0.31, p = 0.58). Comparison of survival outcomes between GVHD prophylaxis were insignificant (χ^2 = 0.205, p = 0.650). The mean survival time was 254.3 days (95% CI: 178.4–330.3) for the CSA-MMF group and 337.2 days (95% CI: 265.3–409.2) for the CSA-MTX group.

Table II: GVHD in Post-Transplant	
Acute GVHD	
Yes	7 (46.7%)
No	8 (53.3%)
Gut GVHD	
Yes	4 (26.7%)
No	11 (73.3%)
Hepatic GVHD	
Yes	1 (6.7%)
No	14 (93.3%)
Skin GVHD	
Yes	5 (33.3%)
No	10 (66.7%)
GVHD Stage	
1	2 (25%)
2	2 (25%)
3	3 (37.5%)
4	1 (12.5%)
GVHD Grade	
Grade I	2 (28.5%)
Grade II	1 (14.5%)
Grade III	2 (28.5%)
Grade IV	2 (28.5%)
GVHD Treatment Response (Yes/No)	
First (Steroids)	1 (6.7%)/6 (40%)
Second (Infliximab, Ruxolitinib)	6 (40%)/1 (6.7%)
Third (Mesenchymal stem cells)	0 (0%)/1 (6.7%)

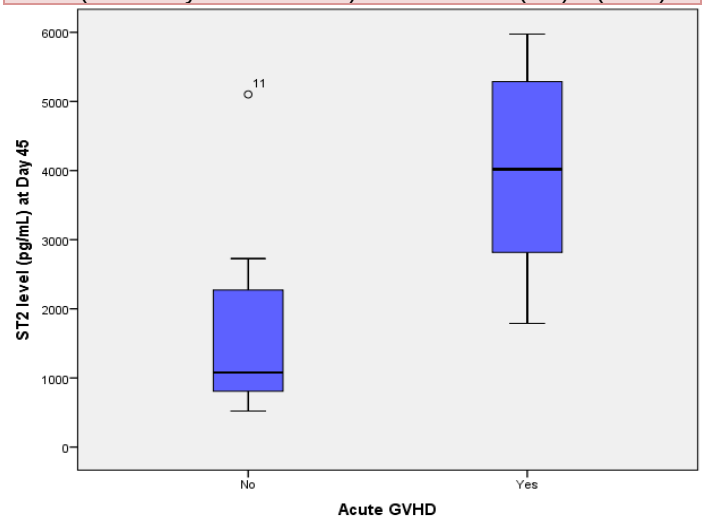

Figure 1: Box plot showing ST2 levels (pg/mL) at Day 45 post-transplantation in patients with and without acute GVHD (aGVHD). The box represents the interquartile range (IQR), the horizontal line within the box indicates the median, and the whiskers denote the minimum and maximum values within 1.5× IQR. An outlier is marked by a small circle.

Discussion

Pre transplant conditioning in allo-HSCT significantly depletes the host immune system to prevent graft rejection. GVHD often develops within the first month, during early immune reconstitution from the donor graft. [10] The use of plasma biomarkers has emerged as a valuable tool in confirming acute GVHD at both systemic and organ-specific levels.[11,12] The two commonly investigated biomarkers, i-e, ST2 and Reg3a, are shown to have elevated blood levels in patients developing aGVHD before the onset of clinical symptoms. [13 -15] It has been reported that these biomarkers can predict the severity of GVHD along with non-relapse mortality. [16]

In the present study, ST2 level at day 45 was found to be higher in patients with acute GVHD compared to control indicating its use as a predictive biomarker. The findings of a study conducted by ALADAĞ et al. are consistent with the present study. The authors have found that mean ST2 levels were significantly raised in patients with aGVHD indicating its potential as a predictive biomarker for this condition. [6] Similar trends have been observed by study performed by Vander Lugt et al. The results revealed that ST2 has a strong link with resistance to GVHD therapy and non-relapse mortality. Patients having elevated ST2 levels had a 2.5 times greater risk of developing treatment-resistant GVHD compared to those with lower ST2 levels. [17]

In aGVHD, tissue damage raises IL-33 levels and upregulates membrane-bound ST2 on activated T cells, enhancing inflammatory cytokines such as IFN-γ and worsening tissue injury. Meanwhile, soluble ST2 binds IL-33, driving a Th1-skewed immune response that contributes to aGVHD progression. [18]

In our study, Reg3a levels did not demonstrate any significance as a diagnostic or predictive biomarker. This contrasts with the findings of Maria, et al. in which the authors incorporated Reg3α and Elafin biomarkers to an established four-biomarker panel comprising IL-2Rα, IL-8, HGF and TNFR1.[19] Their study showed that Reg3α, a gastrointestinal-specific biomarker, was not only elevated in patients with aGVHD but also had potential in predicting response to steroid therapy. Similarly, the study findings of Hartwell et al., revealed elevated levels of both ST2 and Reg3a. These biomarkers were not only associated with higher six-month mortality but also have potential in predicting aGVHD. [20]

There was no statistically significant difference in survival outcomes between cases and controls in this study. A predictive model known as the MAGIC algorithm probability (MAP) was developed using combined levels of ST2 and Reg3α to relate with six month NRM (11) with greater emphasis on day 7 levels of ST2 measured after Allo-HSCT. The results of MAP were tested in a Japanese retrospective cohort (n 112). [21] The study confirmed that early plasma biomarkers can predict 6-month NRM inhigh risk GVHD patients.

This study had some limitations. Firstly, the sample size was relatively small. Secondly, the median follow-up duration was short (180 days), which may have affected the ability to detect long-term outcomes. Consequently, a survival analysis revealed no significant difference between patients who developed aGVHD and those who did not. Further researches involving larger patients' cohort and long follow-up periods is needed to establish clinical utility of ST2 in predicting GVHD and monitoring treatment response.

Conclusion

Our findings suggest that ST2 may serve as a promising biomarker for the early prediction of aGVHD. However, further studies with larger patient cohorts are essential to validate its clinical utility in both diagnosis and response monitoring. Additionally, a collaborative multicenter research efforts will be critical in establishing standardized protocols for the integration of ST2 into routine post-transplant care.

ACKNOWLEDGEMENTS: The authors acknowledge the support of the Stem Cell Laboratory, Routine Laboratory, and the phlebotomists for their assistance in sample collection.

References

1. Alexoudi VA, Gavriilaki E, Cheva A, Sakellari I, Papadopoulou S, Paraskevopoulos K, et al. Graft-versus-host disease: can biomarkers assist in differential diagnosis, prognosis, and therapeutic strategy? Pharmaceuticals (Basel). 2024;17(3):298. https://doi.org/10.3390/ph17030298
2. Karaszewski K, Góra J, Ploch W, Jędrzejczak WW. Long-term results of hematopoietic cell transplantation for adults with blood disorders: a systematic review. Stem Cell Rev Rep. 2025;21:1–20. https://doi.org/10.1007/s12015-025-10912-w
3. Etra A, El Jurdi N, Katsivelos N, Kwon D, Gergoudis S, Morales G, et al. Amphiregulin, ST2, and REG3α biomarker risk algorithms as predictors of nonrelapse mortality in patients with acute graft-versus-host disease. Blood Adv. 2024;8(12):3284–3292. https://doi.org/10.1182/bloodadvances.2023011049
4. Kujawska J, Zeiser R, Gil L. Recent advances in acute gastrointestinal graft-versus-host disease: aspects of steroid-resistant disease. Ann Hematol. 2024;103:1–11. https://doi.org/10.1007/s00277-024-05952-0

5. Öztürk G, Bayrakoğlu D, Haskoloğlu Ş, Baskın K, Deveci N, İnce E, et al. ST2 and REG3α as predictors of acute graft-versus-host disease, steroid refractoriness, and transplant-related mortality in pediatric patients after hematopoietic stem cell transplantation. Hematol Transfus Cell Ther. 2024. https://doi.org/10.1016/j.htct.2024.02.026
6. Karakulak EA, Demiroğlu H, Malkan ÜY, Akman Ü, Göker H, Büyükaşık Y. Assessment of ST2 and REG3α levels in patients with acute graft-versus-host disease after allogeneic hematopoietic stem cell transplantation. Turk J Med Sci. 2021;51(1):355–358. https://doi.org/10.3906/sag-2007-17
7. Gjærde LK, Ostrowski SR, Schierbeck F, Andersen NS, Friis LS, Kornblit B, et al. Pretransplantation plasma ST2 level as a prognostic biomarker of 1-year nonrelapse mortality in allogeneic hematopoietic cell transplantation. Transplant Cell Ther. 2023;29(2):97.e1–97.e6. https://doi.org/10.1016/j.jtct.2022.11.009
8. Öztürk G, Bayrakoğlu D, Haskoloğlu Ş, Baskın K, Deveci N, İnce E, et al. ST2 and REG3α biomarkers in pediatric allogeneic hematopoietic stem cell transplantation. Hematol Transfus Cell Ther. 2024.
9. Etra A, Gergoudis S, Morales G, Spyrou N, Shah J, Kowalyk S, et al. Assessment of systemic and gastrointestinal tissue damage biomarkers for graft-versus-host disease risk stratification. Blood Adv. 2022;6(12):3707–3715. https://doi.org/10.1182/bloodadvances.2022007296
10. Major-Monfried H, Renteria AS, Pawarode A, Reddy P, Ayuk F, Holler E, et al. MAGIC biomarkers predict long-term outcomes for steroid-resistant acute graft-versus-host disease. Blood. 2018;131(25):2846–2855. https://doi.org/10.1182/blood-2018-01-822957
11. Bidgoli A, DePriest BP, Saatloo MV, Jiang H, Fu D, Paczesny S. Current definitions and clinical implications of biomarkers in graft-versus-host disease. Transplant Cell Ther. 2022;28(10):657–666. https://doi.org/10.1016/j.jtct.2022.07.008
12. Olivieri A, Mancini G. Current approaches for the prevention and treatment of acute and chronic graft-versus-host disease. Cells. 2024;13(18):1524. https://doi.org/10.3390/cells13181524
13. Abu Zaid M, Wu J, Wu C, Logan BR, Yu J, Cutler C, et al. Plasma biomarkers of risk for death in a multicenter phase 3 trial with uniform transplant characteristics post-allogeneic hematopoietic cell transplantation. Blood. 2017;129(2):162–170. https://doi.org/10.1182/blood-2016-08-735324
14. Balakrishnan B, Illangeswaran RSS, Rajamani BM, Pai AA, Raj IX, Paul DZ, et al. Prognostic plasma biomarkers of early complications and graft-versus-host disease in patients undergoing allogeneic hematopoietic stem cell transplantation. EJHaem. 2020;1(1):219–229. https://doi.org/10.1002/jha2.26
15. Garnett C, Apperley JF, Pavlů J. Treatment and management of graft-versus-host disease: improving response and survival. Ther Adv Hematol. 2013;4(6):366–378. https://doi.org/10.1177/2040620713489842
16. McDonald GB, Tabellini L, Storer BE, Lawler RL, Martin PJ, Hansen JA. Plasma biomarkers of acute graft-versus-host disease and nonrelapse mortality: predictive value of measurements before graft-versus-host disease onset and treatment. Blood. 2015;126(1):113–120. https://doi.org/10.1182/blood-2015-03-636753
17. Vander Lugt MT, Braun TM, Hanash S, Ritz J, Ho VT, Antin JH, et al. ST2 as a marker for risk of therapy-resistant graft-versus-host disease and death. N Engl J Med. 2013;369(6):529–539. https://doi.org/10.1056/NEJMoa1213299
18. Nagasawa M. Biomarkers of graft-versus-host disease: understanding and applications for the future. World J Transplant. 2021;11(8):335–346. https://doi.org/10.5500/wjt.v11.i8.335
19. Iacobescu M, Pop C, Uifălean A, Mogoşan C, Cenariu D, Zdrenghea M, et al. Unlocking protein-based biomarker potential for graft-versus-host disease following allogeneic hematopoietic stem cell transplantation. Front Immunol. 2024;15:1327035. https://doi.org/10.3389/fimmu.2024.1327035
20. Hartwell MJ, Özbek U, Holler E, Renteria AS, Major-Monfried H, Reddy P, et al. An early biomarker algorithm predicts lethal graft-versus-host disease and survival. JCI Insight. 2017;2(3):e89798. https://doi.org/10.1172/jci.insight.89798
21. Hotta M, Satake A, Yoshimura H, Fujita S, Katayama Y, Ota S, et al. Elevation of early plasma biomarkers in patients with clinical risk factors predicts increased nonrelapse mortality after allogeneic hematopoietic stem cell transplantation. Transplant Cell Ther. 2021;27(8):660.e1–660.e8. https://doi.org/10.1016/j.jtct.2021.04.025