

Endothelial and Complement Activation As Predictors of Survival in Adult Allogeneic Hematopoietic Cell Transplantation

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Transplant-associated thrombotic microangiopathy (TA-TMA) and graft-versus-host disease (GVHD) are complications of allogeneic hematopoietic cell transplantation (HCT) that share several common characteristics.¹ Both affect multiple organs and lead to significant morbidity and mortality despite optimal management. Despite a distinct clinical phenotype, both manifest in the early post-transplant period and have a common denominator: endothelial damage.²

Endothelial injury has been long recognized as a contributor to the pathogenesis of TA-TMA. Various underlying processes, including GVHD, contribute to a prothrombotic state, which may eventually lead to microvasculature thrombosis.³ Similar to other thrombotic disorders,⁴ complement activation has been recently recognized as a key event in the pathogenesis of TA-TMA.⁵ Gene mutations related to the alternative pathway of complement seem to predispose both children and adults to its development.^{6,7} Elevated levels of soluble C5b-9 (sC5b-9) have been introduced as a marker of complement activation and poor prognosis in patients with TA-TMA. These data led to the effective use of the first-in-class terminal complement inhibitor, eculizumab, in high-risk TA-TMA.⁸

Despite eculizumab's success, accumulating evidence emphasizes on the necessity to find additional targets against the endothelial injury.⁶ Recent studies have introduced the Endothelial Activation and Stress Index (EASIX) as a prognostic tool.⁹ Given the crosstalk between endothelium and complement,^{7,10}

we hypothesized that EASIX would be associated with complement activation and survival in patients with endothelial dysfunction syndromes.

We enrolled in a 1:1:1 ratio consecutive adult patients diagnosed with TA-TMA, acute and/or chronic GVHD, and control HCT recipients without GVHD or TA-TMA (January 2015 to June 2018). We chose this study design to detect possible differences between patients with GVHD that develop TA-TMA and those that do not, limiting patient selection bias. The three groups were matched according to the day of the first diagnosis of TA-TMA or GVHD accordingly so that samples for each group reflect changes from a similar transplant period. Samples were collected at the first day of confirmed TA-TMA or GVHD diagnosis before initiation of specific treatment and at a similar posttransplant period in control recipients. Patients were then followed-up for a minimum of 6 months. In accordance with the Helsinki Declaration, all patients have given written informed consent. The institutional review board of G. Papanicolaou Hospital approved our study (protocol number: 187/2016).

All patients underwent allogeneic HCT at our Joint Accreditation Committee of the International Society for Cellular Therapy (ISCT) and European Society for Blood and Marrow Transplantation (EBMT) (JACIE) accredited unit. Data were collected retrospectively from our prospectively acquired database. The prophylactic regimens for GVHD in myeloablative conditioning regimens were a calcineurin inhibitor and methotrexate, while in reduced-intensity conditioning regimens cyclosporine plus mycophenolate mofetil were used. In haploidentical HCTs, posttransplant cyclophosphamide (day +3,+4) along with cyclosporine plus mycophenolate mofetil (day +5) was administered as a prophylactic regimen. Anti-thymocyte globulin (2.5-5 mg/kg) was part of the conditioning for all unrelated and haploidentical transplant recipients, as previously described. International Working Group criteria were used for the diagnosis of TA-TMA. Infectious prophylaxis, treatment, supportive care, and GVHD management were performed according to our standard operating procedures, as previously described.¹¹

The EASIX was calculated based on the proposed formula: lactate dehydrogenase (U/L) × creatinine (mg/dL)/thrombocytes (10⁹ cells per L). We calculated EASIX for every patient on days 0, 30, 100, and at last follow-up.

Ethylenediaminetetraacetic acid plasma was collected and stored immediately at -80°C. Aliquots were thawed only once

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The study was approved by the G. Papanicolaou's institutional review board (protocol number: 187/2016) and conducted according to the Helsinki declaration.

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Table 1**Patients' Characteristics.**

Patient characteristics	TA-TMA (n = 20)	GVHD (n = 20)	Controls (n = 20)	P
Age (y)	36 (17-56)	42 (19-52)	39 (18-49)	0.212
Disease type (n)				
AML	4	5	6	0.228
ALL	12	9	10	
Lymphoma	3	5	3	
Multiple myeloma	1	1	1	
Disease phase (n)				
Early CR	12	14	13	0.421
Late CR	4	3	4	
Relapsed/Refractory	4	3	3	
Myeloablative conditioning (n)	16	8	16	0.892
Donor (n)				
Sibling	8	10	9	0.732
Unrelated	8	5	6	
Haploidentical	4	5	4	
HLA matched donor (n)	16	17	18	0.343
Follow-up (mo)	8.5 (2.7-102.1)	12.0 (2.9-32.2)	14.2 (4.5-79.1)	0.745
Infections (n)				
Bacterial	12	12	10	0.431
Viral	13	11	9	0.373
Fungal	10	7	12	0.653
GVHD (n)				
Severe acute	12	18	0	<0.001
Extensive chronic	17	19	0	<0.001
EASIX at day 0	1.57 (0.3-18.9)	1.37 (0.3-4.4)	1.4 (0.2-6.2)	0.565
Platelets ($\times 10^9/L$) at day 0	52 (16-198)	80 (21-145)	62 (10-183)	0.528
LDH (mg/dL) at day 0	169 (113-445)	178 (94-365)	161 (91-211)	0.639
Creatinine (mg/dL) at day 0	0.72 (0.32-0.93)	0.60 (0.33-0.98)	0.49 (0.24-0.94)	0.465
EASIX at day 30	2.5 (0.8-16.3)	1.9 (0.1-8.5)	1.4 (0.4-8.5)	0.091
Platelets ($\times 10^9/L$) at day 30	107 (10-167)	99 (31-262)	135 (54-203)	0.462
LDH (mg/dL) at day 30	278 (144-465)	257 (161-698)	264 (151-698)	0.887
Creatinine (mg/dL) at day 30	0.93 (0.32-2.20)	0.82 (0.42-1.50)	0.71 (0.47-1.29)	0.225
EASIX at day 100	7.2 (1.1-118.2)	3.3 (1.8-12.1)	1.41 (0.2-30.8)	0.014
Platelets ($\times 10^9/L$) at day 100	55 (8-168)	61 (29-247)	127 (21-226)	0.012
LDH (mg/dL) at day 100	330 (152-679)	261 (135-666)	203 (113-564)	0.425
Creatinine (mg/dL) at day 100	0.83 (0.41-2.5)	0.77 (1.22-2.34)	0.65 (0.36-0.89)	0.187
EASIX at last follow-up	22.7 (0.3-604.3)	7.8 (0.6-210.1)	0.89 (0.1-62.7)	0.001
Platelets ($\times 10^9/L$) at last follow-up	44 (2-359)	70 (14-270)	154 (90-171)	0.002
LDH (mg/dL) at last follow-up	593 (182-1515)	455 (153-1815)	199 (133-421)	0.001
Creatinine (mg/dL) at last follow-up	0.95 (0.41-4.82)	1.04 (0.62-4.17)	0.75 (0.49-1.43)	0.074
Soluble C5b-9 (ng/mL)	325 (184-902)	243 (175-454)	227 (53-281)	0.001

Continuous variables are presented as median (range). The *P* value represents differences between the three groups, performed with the χ^2 test for categorical and Kruskal-Wallis test for continuous variables.

ALL = acute lymphocytic leukemia, AML = acute myeloid leukemia, CR = complete remission, EASIX = Endothelial Activation and Stress Index, GVHD = graft-versus-host-disease, HLA = human leukocyte antigen, LDH = lactate dehydrogenase, TA-TMA = transplant-associated thrombotic microangiopathy.

to avoid multiple freeze-thaw cycles. Complement activation was detected by sC5b-9 or membrane attack complex. The latter was measured using a robust commercially available enzyme-linked immunosorbent assay kit (Quidel, San Diego, California) in ethylenediaminetetraacetic acid plasma.

Data were analyzed using the statistical program SPSS 23.0 (IBM SPSS Statistics for Windows, Version 23.0. Armonk, New York: IBM Corp). Descriptive statistics were performed using median and range for continuous variables and frequency for categorical variables. EASIX cutoff values (high and low EASIX) were determined using optimal binning. We used the χ^2 test to assess the association between categorical variables. Continuous variables were assessed for normality and compared using one-way analysis of variance with Bonferroni's correction or Kruskal-Wallis test. Follow-up was measured from the date of transplantation until the date of the last follow-up or death. For survival analysis, the Kaplan-Meier method was used and survival

curves were compared using a log-rank test. Variables with a significant correlation with overall survival were included in the multivariate analysis. Multivariate analysis was performed using a Cox regression model and the "enter" method. Optimal binning was used to determine the optimal EASIX cutoff for statistical differences in overall survival. The level of statistical significance was defined at 0.05.

We studied 20 TA-TMA, 20 GVHD, and 20 control patients. TA-TMA developed at a median + 125 posttransplant day (range 9-2931) in patients that had already been diagnosed with acute and/or chronic GVHD. The first day of confirmed GVHD diagnosis was at a median of +78 posttransplant day (range 16-145). Therefore, the majority of GVHD patients were recruited while diagnosed with acute GVHD and later developed chronic GVHD. In accordance with sampling for TA-TMA and GVHD, samples from patients in the control group were collected at a median of +94 day (range 31-132). There was

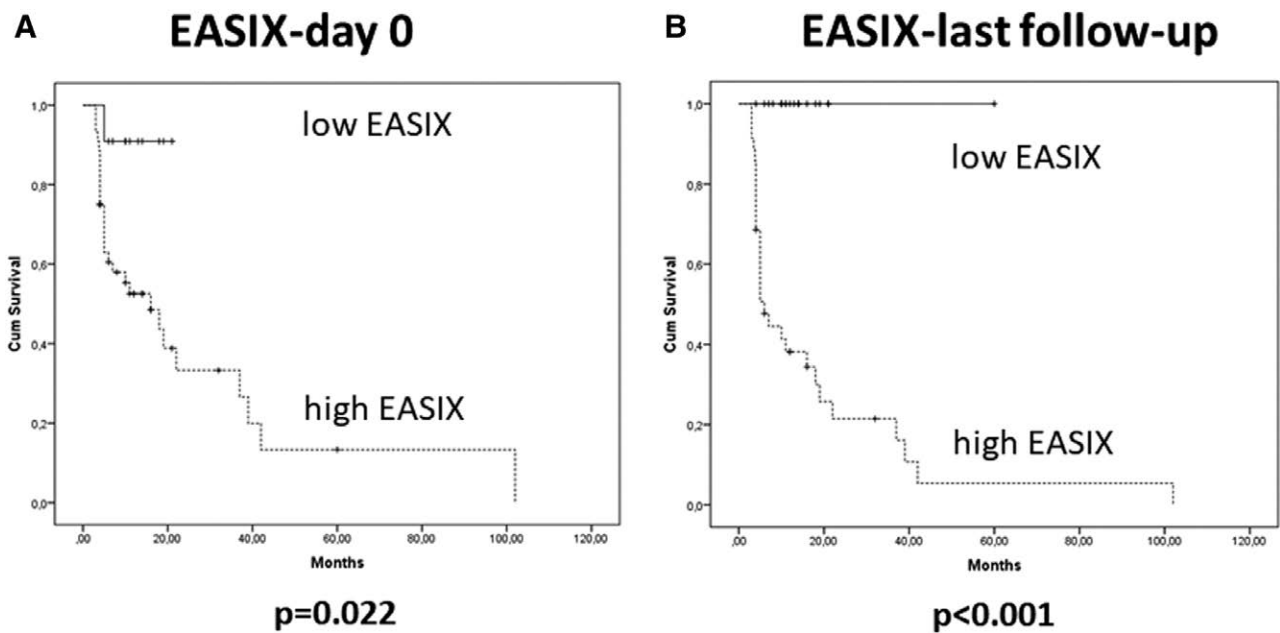


Figure 1. Survival outcomes according to EASIX values. A, EASIX measured at day 0 and overall survival in all patients. Higher EASIX values (dotted line) at day 0 result to significantly lower overall survival. B, EASIX measured at last follow-up and overall survival in all patients. Higher EASIX values (dotted line) at last follow-up result to significantly lower overall survival. EASIX = Endothelial Activation and Stress Index.

no difference in age, HCT indication, use of myeloablative conditioning regimen, or percentage of human leukocyte antigen matched donors among groups (Table 1).

We found that EASIX at day 100 and last follow-up was significantly higher in TA-TMA and GVHD compared with controls (Table 1). EASIX was not significantly different between TA-TMA and GVHD patients at any time point and, therefore, was not associated with TA-TMA per se. In contrast, sC5b-9 levels were significantly higher in TA-TMA compared with GVHD ($P=0.008$) and control patients ($P<0.001$, Bonferroni's correction).

Interestingly, sC5b-9 levels were strongly associated with EASIX at day 100 and last follow-up ($r=0.318$, $P=0.018$ and $r=0.321$, $P=0.020$). Among laboratory values used to calculate EASIX (lactate dehydrogenase, creatinine, platelets), sC5b-9 was only associated with creatinine levels at day 100 ($r=0.316$, $P=0.023$).

EASIX at day 0 and last follow-up was significantly associated with overall survival ($P=0.013$ and $P=0.046$). Patients with high EASIX at day 0 and last follow-up had markedly inferior overall survival compared with patients with low EASIX (Figure 1). In particular, 1-year overall survival was 52.6% in patients with high EASIX at day 0, compared with 91% in low EASIX ($P=0.022$). Similarly, 1-year overall survival was 38.2% in high EASIX at last follow-up compared with 100% in the low EASIX group ($P<0.001$). Interestingly, sC5b-9 values were not associated with overall survival. Among other transplant variables (type of donor, disease, and conditioning), EASIX at day 0 was an independent predictor of overall survival ($\beta=2.627$, $P=0.029$) in the multivariate analysis.

Therefore, our study highlights the association between endothelial and complement activation in patients with endothelial dysfunction syndromes and control allogeneic HCT recipients. More importantly, we confirm that EASIX at day 0 independently predicts survival in this population.

Luft et al⁹ have successfully used EASIX to predict mortality in GVHD patients who had received reduced-intensity conditioning. EASIX-pre (calculated prior to conditioning)

also predicted mortality and TA-TMA.^{12,13} A recent study also showed that EASIX at admission predicts fluid overload, implicating endothelial damage in this toxicity of multifactorial origin post allogeneic HCT.¹⁴

In TA-TMA, high EASIX and sC5b-9 levels are explained by several parameters. First, all three parameters of EASIX are used for the diagnosis of TA-TMA, irrespectively of diagnostic criteria. Second, complement activation has emerged as an important component of TA-TMA pathogenesis. Higher levels of sC5b-9 have been previously detected in TA-TMA compared with control patients. Furthermore, our group has also proposed a cutoff value for distinguishing complement activation in TA-TMA from GVHD by the degree of complement activation.¹⁵ In line with our previous findings, the present study confirms for the first time that EASIX is associated with sC5b-9.

Our study has several limitations. First, we conducted a single-center study with a small number of patients, and thus, our results need confirmation in larger cohorts. Given the rather low number of patients included in our study, we were not able to assess the effect of different conditioning regimens or specific disease groups on endothelial function or complement activation, although these factors were included in our analysis. We limited bias by enrolling consecutive 20 patients from each group. We did not enroll patients with other endothelial dysfunction syndromes, such as sinusoidal obstructive syndrome/veno-occlusive disease, since this complication is only rarely seen in our patients. Furthermore, we calculated EASIX at different time points than those set by other investigators, and therefore, our results are not directly comparable with previous studies.

In conclusion, our study shows for the first time that EASIX is associated with complement activation in patients with endothelial dysfunction syndromes and control allogeneic HCT recipients and supports evidence that EASIX can be used as a predictor of overall survival. Our findings suggest that EASIX may be a useful dynamic marker reflecting the course of endothelial dysfunction in these patients.

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