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ORIGINAL RESEARCH

Predictive Value of Modified Endothelial Activation and Stress Index (mEASIX) for Ruxolitinib Response in Graft-versus-Host Disease

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ABSTRACT

Graft-versus-host disease (GVHD) continues to be a predominant cause of non-relapse morbidity and mortality following allogeneic hematopoietic stem cell transplantation (allo-HSCT). Ruxolitinib is an established second-line treatment for steroid-refractory or steroid-dependent acute and chronic GVHD; however, predictive biomarkers for treatment response are lacking. The modified Endothelial Activation and Stress Index (mEASIX) is determined by measuring lactate dehydrogenase, C-reactive protein, and platelet count, and it indicates both endothelial damage and systemic inflammation. In this single-center retrospective study, we evaluated the predictive value of mEASIX for ruxolitinib response in 23 adult patients with GVHD. To assess the predictive capability of the mEASIX score, both receiver operating characteristic (ROC) analysis and logistic regression analysis were conducted. The median age was 37 years, and acute myeloid leukemia was the most common indication for transplantation (65.2%). Eleven patients had steroid-refractory/dependent acute GVHD, while 12 had steroid-refractory/dependent chronic GVHD. The overall response rate to ruxolitinib was 65.2%, with the lowest response observed in patients with bronchiolitis obliterans. Patients with ruxolitinib resistance had significantly higher mEASIX scores at treatment initiation compared to responders (37.09 vs. 5.38, p=0.008). Based on the ROC curve analysis optimal mEASIX cut-off value for predicting ruxolitinib resistance was 22.2 (sensitivity: 75%, specificity: 86.7%, AUC: 0.842, p<0.001). In multivariate analysis, mEASIX remained an independent predictor of ruxolitinib response (OR: 0.051, p=0.008). Patients with high mEASIX scores had lower 1-year overall survival compared to those with low scores (50% vs. 92.3%, p=0.078). Our findings suggest that early evaluation of mEASIX can identify patients at risk of ruxolitinib resistance, allowing timely treatment modifications to improve clinical outcomes in GVHD. Prospective multicenter studies are needed to validate these results.

Keywords: Allogeneic hematopoietic stem cell transplantation (allo-HSCT). Graft-versus-host disease (GVHD). Ruxolitinib. Modified Endothelial Activation and Stress Index (mEASIX).

Modifiye Endotelyal Aktivite ve Stres İndeksinin (mEASIX) Graft-Versus-Host-Hastalığında (GVHH) Ruksolitinib Yanıtını Öngördücü Değeri

ÖZET

Allojenik hematopoetik kök hücre nakli (allo-kit) sonrası greft-versus-host hastalığı (GVHH), nüks dışı morbidite ve mortalitenin önemli bir nedenidir. Ruksolitinib steroid refrakter veya bağımlı akut ve kronik GVHH olgularında ikinci basamak tedavi olarak kullanılmaktadır ancak tedavi yanıtını öngörebilecek güvenilir biyobelirteçler hâlâ yetersizdir. Laktat dehidrogenaz, C-reaktif protein ve trombosit sayısını içeren Modifiye Endotelyal Aktivite ve Stres İndeksi (mEASIX), endotel disfonksiyonu ve sistemik inflamasyonu yansıtmaktadır. Bu tek merkezli retrospektif çalışmada, mEASIX skorunun Ruksolitinib yanıtını öngörmedeki değeri 23 yetişkin GVHH hastasında değerlendirildi. Hastaların 11'i steroid-refrakter/bağımlı akut GVHH, 12'si ise kronik GVHH idi. Ruksolitinib'e genel yanıt oranı %65,2 olup, en düşük yanıt bronşiolitis obliterans hastalarında izlendi. Ruksolitinib'e dirençli hastalarda tedavi başlangıcındaki mEASIX skoru, yanıtlı hastalara göre anlamlı düzeyde yüksekti (37,09'a karşı 5,38; p=0,008). ROC analizine göre Ruksolitinib direncini öngörmede en uygun mEASIX eşik değeri 22,2 olarak belirlendi (AUC: 0,842; duyarlılık: %75; özgüllük: %86,7; p<0,001). Çok değişkenli lojistik regresyon analizi sonucunda mEASIX Ruksolitinib yanıtını öngörmede bağımsız bir değişken olarak saptandı (OR: 0,051; p=0,008). Ayrıca, yüksek mEASIX skoruna sahip hastalarda 1 yıllık genel sağkalım daha düşük saptandı (%50'ye karşı %92,3; p=0,078). Bulgularımız, GVHH'de mEASIX'in erken dönemde değerlendirilmesinin, Ruksolitinib direnci açısından riskli hastaların belirlenmesine ve zamanında tedavi değişikliklerile klinik sonuçların iyileştirilmesine katkı sağlayabileceğini göstermektedir. Bulguların doğrulanması için ileriye dönük çok merkezli çalışmalara ihtiyaç vardır.

Anahtar Kelimeler: Allojeneik hematopoetik kök hücre nakli (Allo-kit). Graft-versus-host-hastalığı (GVHH). Modifiye Endotelyal Aktivite ve Stres İndeksi. Ruksolitinib.

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Graft-versus-host disease (GVHD) is a clinical condition that arises when the donor's immune system mounts an immunologic attack against the recipient following allogeneic hematopoietic stem cell transplantation[1]. Despite advancements in prophylactic strategies and conditioning regimens over the years, GVHD remains one of the leading causes of non-relapse morbidity and mortality following transplantation[2,3]. GVHD is broadly classified into two distinct clinical entities: acute GVHD (aGVHD) and chronic GVHD (cGVHD). While aGVHD occurs in approximately 20–50% of transplant recipients, cGVHD is observed in 30–40% of allogeneic transplant cases [2,4]. In the past, these subtypes were primarily distinguished by their time of onset post-transplantation (<100 days vs. ≥100 days); however, current classification is based on their clinical manifestations and underlying pathophysiology[5]. aGVHD is characterized by intense inflammation and infiltration of inflammatory cells, whereas cGVHD more commonly involves autoimmune-mediated tissue injury followed by fibrosis[2,6].

In cases of aGVHD the skin, gastrointestinal tract, and liver are the organ systems most commonly impacted. Cutaneous involvement typically presents as a widespread morbilliform rash[3]. Initial treatment generally involves systemic or topical corticosteroids, with therapeutic decisions guided by the severity of GVHD. In contrast, the diagnosis, severity grading, and response assessment of cGVHD are organ-specific[7]. Frequently involved sites include the skin and oral mucosa, fascia, visceral organs (such as the lungs and esophagus), eyes, and the musculoskeletal system. Unlike aGVHD, long-term immunosuppressive therapy is often required in cGVHD, and treatment responses are frequently suboptimal[8,9]. For both acute and chronic GVHD patients with steroid-refractory or steroid-dependent disease, ruxolitinib is currently the standard second-line therapy. Although response rates to ruxolitinib approach 50% in acute skin and gastrointestinal GVHD cases, its efficacy is limited in fibrotic manifestations such as bronchiolitis obliterans (BO) and chronic sclerotic skin GVHD (cScGVHD)[9]. In life-threatening lower GI aGVHD, although response rates are acceptable, delayed clinical improvement has led to the early consideration of combination therapies. Predicting non-responders to ruxolitinib is crucial for enabling early implementation of combination therapies, thereby potentially reducing GVHD-related mortality[10]. Similarly, in conditions characterized by fibrosis, such as BO and cScGVHD, predicting treatment response is essential to prevent irreversible functional decline and associated morbidity[11].

The Endothelial Activation and Stress Index (EASIX) is a metric derived from fundamental laboratory parameters, serving as a marker for endothelial dysfunction and damage. It is calculated using lactate dehydrogenase (LDH), serum creatinine levels, and platelet count. The modified EASIX (mEASIX), which incorporates C-reactive protein (CRP) into the formula, reflects not only endothelial injury but also the extent of systemic inflammation. Several studies have demonstrated that elevated EASIX and its variant mEASIX are associated with poorer relapse-free and overall survival, as well as a higher incidence of GVHD in allogeneic transplant recipients[12–15]. Studies evaluating dynamic EASIX monitoring both before and after transplantation have demonstrated that higher EASIX scores are correlated with an increased incidence of GVHD and higher non-relapse mortality[16]. Owing to its ability to reflect the severity of endothelial damage and systemic inflammation, EASIX serves as a predictive scoring system for the development and outcomes of acute GVHD[17].

This study aimed to assess the influence of the pre-treatment mEASIX score on treatment outcomes by examining the application of ruxolitinib in both acute and chronic GVHD cases.

Material and Method

This study included 23 allogeneic hematopoietic stem cell transplant recipients over the age of 18 who were followed by the Hematology Department between January 2013 and December 2024. Only patients diagnosed with acute or chronic GVHD who received ruxolitinib treatment were included. Patients who did not develop GVHD after allogeneic transplantation, those with GVHD who did not receive ruxolitinib, and recipients of autologous transplants were excluded. Demographic data, comorbidities, clinicopathological characteristics, laboratory parameters, imaging and pathology results, as well as donor-related information were retrospectively gathered from patient records and the hospital's electronic medical record system. The EASIX score and variants were calculated using the following formulas:

"EASIX = (LDH [U/L] × Creatinine [mg/dL]) / Platelet count [10^9/L];

mEASIX = (LDH [U/L] × CRP [mg/L]) / Platelet count [10^9/L];

sEASIX = LDH [U/L] / Platelet count [10^9/L]"[18].

Myeloablative conditioning regimens included Busulfan-Cyclophosphamide (BU-CY), Etoposide-Cyclophosphamide combined with total body irradiation (TBI), and Fludarabine-TBI protocols. A reduced-intensity conditioning regimen was used in one patient, consisting of Fludarabine and Melphalan[19]. For GVHD prophylaxis, patients receiving grafts from HLA-matched sibling donors were administered a combination of Cyclosporine (starting on day –1 and continued until month 6 in the absence of GVHD) and Methotrexate (given on days +1, +3, and +6). For unrelated donor transplants, prophylaxis included post-transplant Cyclophosphamide (50 mg/kg on days +3 and +5), Cyclosporine (starting on day +6 and continued through month 6 if GVHD was absent), and Mycophenolate Mofetil (administered from day +6 to day +35).

In cases of acute GVHD, diagnosis was established clinically and corroborated by skin biopsy when applicable, with disease severity assessed according to the MAGIC criteria[20]. For chronic GVHD, diagnosis was based on clinical findings and, when necessary, supported by skin biopsy, pulmonary function tests, or chest computed tomography. Severity assessment was performed using the NIH 2015 criteria[1]. In patients requiring systemic treatment for aGVHD, the standard initial therapy was Methylprednisolone at a dose of 2 mg/kg/day. Ruxolitinib was initiated in cases of clinical progression by day +3 or lack of response by day +7 following the initiation of steroid therapy. For cGVHD, systemic therapy began with corticosteroids at 1 mg/kg/day, and ruxolitinib was introduced in cases of inadequate response or progression. Ruxolitinib resistance was defined as failure to achieve clinical response after 14 days of treatment in aGVHD and after 3 months in cGVHD. Overall survival was determined from the commencement of ruxolitinib therapy to the date of death from any cause or the most recent follow-up appointment.

Statistical Analysis

Data analysis was conducted using SPSS version 29.0 (IBM, NY, USA). The Shapiro-Wilk test was employed to evaluate the normality of the data distribution. Continuous variables were represented by median values along with their minimum and maximum ranges, whereas categorical variables were shown as counts and percentages (n, %). Based on the distribution characteristics, the Mann–Whitney U test was used for comparisons between two groups for continuous variables. Categorical variables were compared using the Chi-square test. The optimal cutoff value of the mEASIX score for predicting treatment response was determined through receiver operating characteristic (ROC) curve analysis, with the Youden index used to identify the most discriminative threshold. Logistic regression analysis was conducted using the backward likelihood ratio (Backward LR) method. Variables with a p-value <0.20 in univariate analysis were included in the multivariate model. A p-value <0.05 in the multivariate analysis was considered statistically significant. To assess differences in overall survival, The Kaplan–Meier approach was applied to conduct the survival analysis, and the survival curves were contrasted using the log-rank test. A p-value <0.05 was considered statistically significant for all analyses.

Results

Table I provides a summary of the clinical features of the 23 patients who participated in the study. The participants had a median age of 37 years, and the majority were male, accounting for 73.9% of the group. The majority of cases were diagnosed with acute leukemia, with acute myeloid leukemia (AML) being the most common subtype, accounting for 65.2% of patients. Matched sibling donors (MSD) were the most frequently used donor source (73.9%), and myeloablative conditioning regimens were the most commonly applied preparative protocols. Cyclosporine and Methotrexate–based GVHD prophylaxis was administered in 73.9% of cases, while post-transplant Cyclophosphamide (Post-Cy)–based prophylaxis was used in 26.1% of patients.

The indications for initiating ruxolitinib in the study cohort were steroid-refractory or steroid-dependent GVHD, with 11 patients classified under acute GVHD and 12 under chronic GVHD. Among the aGVHD cases, the most common clinical presentation was gastrointestinal involvement, whereas in cGVHD, cScGVHD was the predominant subtype. The median duration of ruxolitinib use was 6.4 months. Notably, 34.8% of patients were classified as non-responders to ruxolitinib treatment. Regarding treatment-related adverse events, cytomegalovirus (CMV) reactivation was observed in 9 patients and 14 patients experienced infectious episodes requiring hospitalization. The most frequently encountered hematologic toxicity was drug-induced anemia, observed in 34.7% of cases.

Ruxolitinib response rates according to GVHD subtypes are presented in Table II. Among the 11 patients with acute GVHD, only 2 were classified as non-responders to ruxolitinib. In contrast, the non-response rate in patients with cScGVHD was 50%. Notably, both patients with BO failed to derive clinical benefit from ruxolitinib therapy.

A comparative analysis of ruxolitinib responders and non-responders is presented in Table III. No statistically significant differences were identified between the two groups concerning age, sex, donor

type, or conditioning regimen. All non-responders had received GVHD prophylaxis with Methotrexate and Cyclosporine, while none of the patients in the Post-Cy group were classified as non-responders. However, this difference did not reach statistical significance (p=0.058). The median baseline mEASIX score was significantly lower in the responder group compared to the non-responder group (5.38 vs. 37.09, p=0.008). In contrast, no significant differences were found between the groups regarding baseline EASIX, sEASIX, or pre-transplant ferritin levels.

Table I. Baseline Characteristics of the Study Population

	n:23	%
Sex		
Male	17	73.9
Female	6	26.1
Age, years (median)	37 (20-63)	
Diagnosis		
AML	15	65.2
ALL	7	30.4
PMF	1	4.4
Donor Type		
MSD	17	73.9
MUD	5	21.8
Haploidentical	1	4.3
Donor-Recipient Sex Compatibility		
Compatible	15	65.2
Incompatible	8	34.8
ABO Compatibility		
Compatible	14	60.9
Minor Incompatible	2	8.7
Major Incompatible	7	30.4
Conditioning Regimen		
Myeloablative	22	95.6
Reduced-Intensity	1	4.4
TBI-Based Regimen	13	56.5
GVHD Prophylaxis		
Cyclosporine + MTX	17	73.9
Post- Cy	6	26.1
Indication for Ruxolitinib		
Acute GI GVHD	8	34.8
Acute Skin GVHD	1	4.3
Combined Acute GI + Skin GVHD	2	8.7
Chronic Sclerotic Skin GVHD	9	39.2
Chronic GI GVHD	1	4.3
Bronchiolitis Obliterans	2	8.7
Ruxolitinib Duration, months (median)	6.4 (0.4-25.6)	
Response to Ruxolitinib		
Complete Response	10	43.5
Partial Response	5	21.7
No Response	8	34.8
Adverse Event		
CMV Reactivation	9	39.1
Infection Requiring Hospitalization	14	60.9
Anemia (<10 g/dL)	8	34.7
Thrombocytopenia (<50×10⁹/L)	5	21.8
Relapse-related mortality	3	13.1
Non- relapse mortality	4	17.3

AML: Acute Myeloid Leukemia, ALL: Acute Lymphoblastic Leukemia, PMF: Primary Myelofibrosis, MSD: Matched Sibling Donor, MUD: Matched Unrelated Donor, TBI: Total Body Irradiation, GVHD: Graft-versus-Host Disease, MTX: Methotrexate, Post-Cy: Post-transplant Cyclophosphamide, GI: Gastrointestinal, CMV: Cytomegalovirus.

Table II. Evaluation of Ruxolitinib Response According to GVHD Subtypes

	Ruxolitinib Responsive n (%)	Ruxolitinib Non-Responsive n (%)
Acute GVHD		
Acute GI GVHD	6 (75)	2 (25)
Grade III	4 (66.6)	
Grade IV	2 (33.4)	2 (100)
Acute Skin GVHD (Grade IV)	1 (100)	-
Combined Acute GI + Skin GVHD (Grade IV)	2 (100)	-
Chronic GVHD		
Chronic Sclerotic Skin GVHD	5 (20)	4 (50)
Moderate	3 (60)	1 (25)
Severe	2 (20)	3 (75)
Chronic GI GVHD (Moderate)	1 (100)	-
Bronchiolitis Obliterans	-	2 (100)
Moderate		1 (50)
Severe		1 (50)

GI: Gastrointestinal, GVHD: Graft-versus-Host Disease.

Table III. Patient Characteristics According to Ruxolitinib Response

	Ruxolitinib Responsive (n:15)	Ruxolitinib Non-Responsive (n:8)	p-value
Age, years (median)	36 (20-63)	46 (21-61)	0.438[m]
Male gender, (%)	10 (66.7)	7 (87.5)	0.369[χ2]
Diagnosis			
AML, (%)	12 (80)	3 (37.5)	
ALL, (%)	3 (20)	4 (50)	0.084[χ2]
PMF, (%)	-	1 (12.5)	
Donor type			
MSD, (%)	9 (60)	8 (100)	
MUD, (%)	5 (33.3)	-	0.171[χ2]
Haploidentical, (%)	1 (6.7)	-	
Donor–Recipient Gender Matching			
Matched, (%)	9 (60)	6 (75)	0.657[χ2]
Mismatched, (%)	6 (40)	2 (25)	
TBI-Based Regimen, (%)	6 (46.2)	2 (25)	0.400[χ2]
GVHD Prophylaxis			
Cyclosporine + MTX, (%)	9 (60)	8 (100)	0.058[χ2]
Post- Cy, (%)	6 (40)	0	
Pre-transplant ferritin, ng/ml (median)	818 (316-6023)	1785 (668-4658)	0.065[m]
EASIX, (median)	1.48 (0.27-14.62)	1.83 (0.68-7.51)	0.302[m]
mEASIX, (median)	5.38 (1.22-55.69)	37.09 (4.7-781.03)	**0.008[m]**
sEASIX, (median)	1.57 (0.61-10.83)	2.10 (1.03-11.43)	0.302[m]

AML: Acute Myeloid Leukemia, ALL: Acute Lymphoblastic Leukemia, PMF: Primary Myelofibrosis, MSD: Matched Sibling Donor, MUD: Matched Unrelated Donor, TBI: Total Body Irradiation, GVHD: Graft-versus-Host Disease, MTX: Methotrexate, Post-Cy: Post-transplant Cyclophosphamide, EASIX: Endothelial Activation and Stress Index, mEASIX: Modified Endothelial Activation and Stress Index, sEASIX: Simplified Endothelial Activation and Stress Index, m: Mann- Whitney U test, $\chi 2$: Chi-square test.

mEASIX for Ruxolitinib Response in GVHD

The results of the ROC curve analysis are presented in Figure 1. Through ROC curve analysis, the mEASIX score's optimal threshold for predicting a response to ruxolitinib was identified as 22.2, demonstrating a sensitivity of 75%, a specificity of 86.7%, and an AUC of 0.842 (p<0.001). To identify predictors of ruxolitinib response, binary logistic regression analysis was performed, and the findings are summarized in Table IV. In the multivariate analysis, the mEASIX score emerged as the only independent predictor of response to ruxolitinib therapy (OR: 0.051, 95% CI: 0.006–0.456, p=0.008).

Table IV. Logistic Regression Analysis for Predicting Response to Ruxolitinib Treatment in Graft-versus-Host Disease

Factor	Univariate Analysis				Multivariate Analysis			
	OR	95% CI		p-value	OR	95% CI		p-value
		Lower	Upper			Lower	Upper	
Age (Year)	0.966	0.904	1.032	0.309				
Gender (Male [RC] vs Female)	3.500	0.332	36.857	0.297				
Donor Gender (Male [RC] vs Female)	2.500	0.428	14.607	0.309				
Donor Type (MSD [RC] vs Others)	6.700	0.153	67.167	0.999				
ABO Blood Group (Match [RC] vs Mismatch)	2.679	0.521	13.790	0.238				
Conditioning Regimens (BU-CY [RC] vs Others)	2.073	0.674	6.379	0.204				
TBI-Based Regimens (Present [RC] vs Absent)	2.571	0.371	17.831	0.339				
GVHD Type (Acute [RC] vs Chronic)	0.400	0.068	2.337	0.309				
GVHD Prophylaxis (CNIs-Mtx [RC] vs Post-Cy)	0.234	0.053	0.988	0.999				
Leukocyte (10^9/L)	1.109	0.881	1.397	0.378				
Lymphocyte (10^9/L)	2.133	0.718	6.334	0.173				
Platelets (10^9/L)	1.007	0.995	1.018	0.249				
LDH (IU/L)	1.005	0.992	1.018	0.461				
CRP (mg/L)	0.882	0.775	1.003	0.056				
Ferritin (µg/L)	1.000	1.000	1.001	0.581				
EASIX	0.930	0.714	1.212	0.591				
sEASIX	0.859	0.638	1.157	0.318				
mEASIX (Low [RC] vs High)	0.051	0.006	0.456	**0.008**	0.051	0.006	0.456	**0.008**

OR: Odds ratio, CI: Confidential interval, RC: Reference category, BU-CY: Busulfan-cyclophosphamide, TBI: Total body irradiation, GVHD: Graft-versus-host-disease, CNIs-Mtx: Calcineurin inhibitors- methotrexate, Post-Cy: Posttransplant cylophosphamide, LDH: Lactate dehydrogenase, CRP: C-reactive protein, EASIX: Endothelial activation and stress index, sEASIX: Simplified endothelial activation and stress index, mEASIX: Modified endothelial activation and stress index.

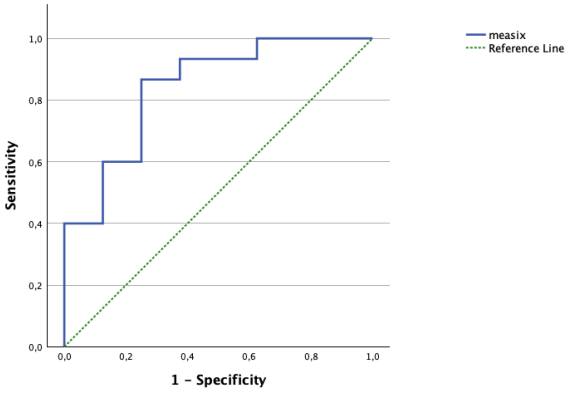

Figure 1:
Roc Curve Analysis of mEASIX Score Predicting Ruxolitinib Response

During the median follow-up period of 15 months, overall survival analysis stratified by mEASIX score at the initiation of ruxolitinib is presented in Figure 2. The one-year overall survival rate was 92.3% in the low mEASIX group, compared to 50% in the high mEASIX group. This difference was near statistical significance (p=0.078). Figure 3 illustrates the non-relapse mortality (NRM) analysis. The one-year NRM rate was 6.7% in the low mEASIX group and 37.5% in the high mEASIX group, demonstrating a difference that nearly reached statistical significance (p=0.053).

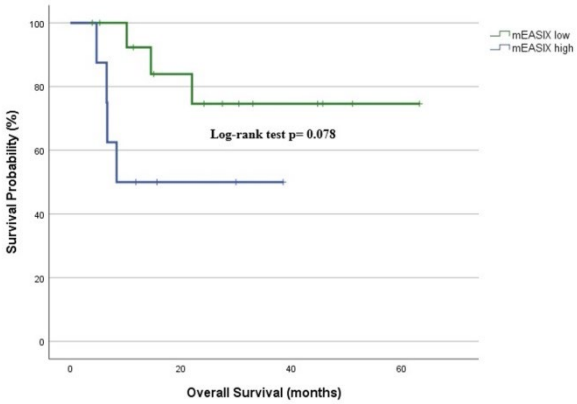

Figure 2:
Kaplan-Meier Analysis for Overall Survival

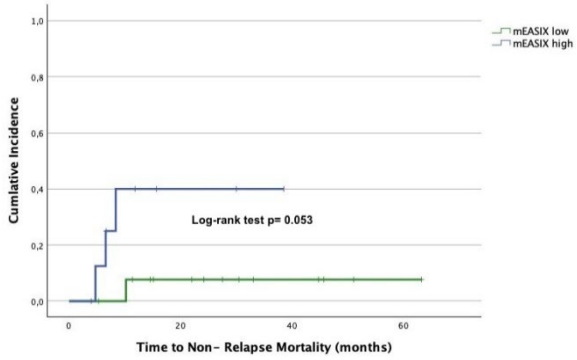

Figure 3.
Cumulative Incidence of Non-Relapse Mortality

Discussion and Conclusion

Ruxolitinib is the established second-line therapy for GVHD that is either dependent on or resistant to steroids. However, in cases unresponsive to ruxolitinib, there is currently no widely established or evidence-based alternative therapy. In such patients, individualized evaluation and timely transition to subsequent treatment options are recommended. Our findings demonstrate that the mEASIX score at the initiation of therapy may serve as a useful predictive marker for response to ruxolitinib in GVHD cases. Early assessment of mEASIX could facilitate clinical preparedness for alternative therapies in patients likely to experience treatment resistance.

Endothelial injury is a key initiating event in both acute and chronic GVHD[21,22]. It can be triggered by factors such as high-dose conditioning regimens, infections during the peri-transplant period, and mucosal damage, all of which contribute to antigenemia and the development of GVHD. In addition, particularly in cGVHD and to some extent in aGVHD, resistance to corticosteroid therapy is associated with an enhanced local and systemic inflammatory response, neovascularization, and the accumulation of chronic inflammatory cells[23–25]. Given these pathogenic mechanisms, biomarkers that reflect both inflammation and endothelial injury—such as the EASIX score—are closely associated with GVHD development and prognosis[16]. In our study, among patients who received ruxolitinib for steroid-refractory or steroid-dependent GVHD, the mEASIX score—incorporating both inflammatory and endothelial markers—was identified as a useful indicator for early identification of individuals less likely to respond to standard therapy.

Research examining the EASIX score has demonstrated that elevated pre-transplant EASIX values correlate with a poor prognosis and an increased incidence of acute GVHD. Similarly, a high EASIX score at the onset of aGVHD has been linked to increased mortality[26,27]. In cGVHD, elevated EASIX values assessed at diagnosis have also been correlated with worse survival outcomes[28,29]. Recent research suggests that adding CRP to the EASIX formula—resulting in the mEASIX—may provide a more comprehensive representation of both endothelial injury and inflammation[15,30]. In our study, neither the standard EASIX nor the simplified sEASIX scores were found to be significant predictors of ruxolitinib non-response in GVHD patients. However, mEASIX emerged as a predictive parameter. Consistent with existing literature, patients with higher mEASIX scores at the initiation of ruxolitinib had notably lower one-year overall survival. However, the observed difference did not achieve statistical significance, which is likely attributable to the combined analysis of both acute and chronic GVHD cases.

Although acute and chronic GVHD share common pathogenic pathways, they differ in the predominant inflammatory cell types and patterns of the immune response. In acute GVHD, the primary therapeutic objective is to suppress inflammation, cytokine release, and the migration of inflammatory cells. Therefore, systemic corticosteroids are used as first-line therapy, while second-line options for steroid-refractory or severe cases include ruxolitinib, extracorporeal photopheresis, and anti-TNF agents. In contrast, chronic GVHD is characterized by B-cell and fibrotic macrophage activation secondary to disturbed T-cell homeostasis. Although systemic corticosteroids remain the standard initial therapy, second-line treatments may involve ruxolitinib, as well as B-cell–targeted agents such as rituximab and ibrutinib[5]. More recently, novel agents including axatilimab, which inhibits macrophage migration, and belumosudil, which modulates T-cell signaling, have been approved for use in chronic GVHD[31,32]. In a 2021 study by Zeiser et al. involving patients with chronic GVHD, the overall response rate to ruxolitinib was reported as 49.7%, with complete responses observed in 11% and partial responses in 71% of cases. Notably, the lowest response rates in that cohort were observed among patients with bronchiolitis obliterans[33]. Consistently, in our study, at least a partial response to ruxolitinib was achieved in 6 out of 12 patients with chronic GVHD, whereas neither of the two patients diagnosed with bronchiolitis obliterans exhibited clinical benefit.

In a recent study by Namdaroğlu et al. evaluating acute GVHD cases, the rate of achieving at least a partial response to ruxolitinib was reported as 61%[34]. Similar findings have been reported across the literature, with response rates ranging between 60–70% in patients with acute GVHD[35,36]. In our study, only two out of 11 patients with acute GVHD were classified as non-responders to ruxolitinib. Compared to previous reports, the relatively higher response rate observed in our cohort may be attributed to the predominance of matched sibling donors and the absence of a stratified analysis based on GVHD severity.

In GVHD cohorts treated with ruxolitinib, the reported incidence of CMV reactivation ranges between 10% and 30%. This risk is particularly increased in patients with severe GVHD, especially when concomitant systemic corticosteroid therapy is administered. In addition to CMV, reactivation of other viral infections such as EBV and herpes zoster has also been observed[37]. The underlying pathophysiological mechanisms are thought to involve a reduction in inflammatory cytokines—particularly interferon-β—and impaired dendritic cell activation, leading to

altered cytotoxic T-lymphocyte and NK-cell function during ruxolitinib therapy[38,39]. In our study, the overall incidence of CMV reactivation was 39%, which is consistent with previously reported rates in the literature.

Currently available scoring systems are useful for identifying patients with severe GVHD; however, they remain limited in predicting which individuals will fail to respond to standard second-line therapy. The mEASIX score appears to be an effective biomarker for the early prediction of treatment response to ruxolitinib in both steroid-refractory and steroid-dependent acute and chronic GVHD. A baseline mEASIX score ≥22.2 was associated with lower response rates and reduced one-year overall survival. These findings indicate that incorporating mEASIX into baseline assessment may help identify patients at risk of poor response early in the disease course, thereby enabling timely adjustment of therapeutic strategies. Further validation in larger, multicenter prospective studies is warranted.

Study Limitations

The limitations of our study include its single-center design, small sample size, its retrospective nature, the absence of longitudinal data for dynamic monitoring of EASIX and its variants, and the heterogeneity in donor types.

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