Von Willebrand factor and Factor VIII as potential biomarkers for diagnosis and disease monitoring in chronic Graft-versus-Host Disease

Antonela Lelas [1], Lana Desnica [1], Ivan Sabol [2], Desiree Coen Herak [1, 3], Marija Milos [1, 4], Ana Zelic Kerep [1], Ante Vulic [1], Nadira Durakovic [1, 5], Ranka Serventi-Seiwerth [1], Zinaida Peric [6, 7], Radovan Vrhovac [1, 5], Steven Z. Pavletic [8], Drazen Pulanic [1, 5]

1 University Hospital Centre Zagreb, Zagreb, Croatia
2 Rudjer Boskovic Institute, Zagreb, Croatia
3 University of Zagreb, Faculty of Pharmacy and Biochemistry, Zagreb, Croatia
4 University of Mostar, Faculty of Pharmacy, Mostar, Bosnia and Herzegovina
5 University of Zagreb, School of Medicine, Zagreb, Croatia
6 University Hospital Centre Rijeka, Rijeka, Croatia
7 University of Rijeka, Faculty of Medicine, Rijeka, Croatia
8 National Cancer Institute, Center for Cancer Research, Bethesda, Maryland, USA

Corresponding author:

Prof. Dražen Pulanić, MD, PhD

Division of Hematology, Department of Internal Medicine

University Hospital Center Zagreb

Medical School University of Zagreb, Zagreb

Kišpatićeva 12, 10000 Zagreb, Croatia

E-mail: drazen.pulanic@kbc-zagreb.hr, dpulanic@yahoo.com

ORCID number: 0009-0004-3755-4477

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Abstract:

The identification of biomarkers of chronic Graft-versus-Host Disease (cGvHD) remains an unmet clinical need. Elevated von Willebrand factor (VWF) and factor VIII (FVIII) reflect inflammation and endothelial activation and might be interesting candidates for biomarkers of cGvHD.

This prospective study evaluated 83 cGvHD patients and 39 allogeneic hematopoietic stem cell transplants recipients without cGvHD. VWF antigen (VWF:Ag), VWF activity (VWF:Ac), and FVIII activity were significantly elevated in cGvHD patients ($p < 0.001$), with the highest levels observed in active disease. Higher VWF:Ag and VWF:Ac were associated with liver and oral cGvHD. In multivariate analysis, lower albumin was the strongest predictor of both higher VWF:Ag ($R^2 = 0.576$) and higher VWF:Ac ($R^2 = 0.540$), followed by older age, higher LDH, and number of affected organs. For higher FVIII, systemic immunosuppressive therapy emerged as the main predictor ($R^2 = 0.247$). Longitudinal analysis showed declining levels of these factors with cGvHD remission and persistent elevation in active disease. ROC analysis demonstrated diagnostic potential for early cGvHD of VWF:Ag >246.3% (AUC = 0.733) and VWF:Ac >271.4% (AUC = 0.728).

These results show the potential of VWF and FVIII as novel biomarkers for diagnosis and disease activity in cGvHD. Additional validation in independent cohorts is warranted.

Keywords: chronic Graft-versus-Host Disease; biomarkers; von Willebrand factor; factor VIII; endothelial activation; inflammation

1. Introduction:

Despite significant advances in understanding the pathophysiology and better treatments, chronic Graft-versus-Host Disease (cGvHD) remains the most serious late complication of allogeneic hematopoietic stem cell transplantation (alloHSCT) (1). Its clinical manifestations often involve multiple organ systems and substantially impair both survival and quality of life (2).

The 2020 National Institutes of Health (NIH) Chronic GvHD Consensus Conference emphasized the need for developing reliable biomarkers to enable early diagnosis, monitor cGvHD activity, and guide preventive and personalized treatment strategies (3). The identification of reproducible biomarkers could facilitate timely intervention and improve outcomes by preventing progression to severe, treatment-refractory disease.

Chronic GvHD is an immune-mediated disorder that evolves from initial inflammation to chronic immune dysregulation and, ultimately, tissue fibrosis. Endothelial injury plays an important role in this process, serving not only as a target of immune-mediated damage but also as an active contributor to ongoing immune activation and inflammatory signalling (4).

Von Willebrand factor (VWF) and Factor VIII (FVIII), beyond their established roles in hemostasis, are recognized acute-phase reactants that reflect systemic inflammation and endothelial activation (5). Elevated levels of these factors have been described in various panvascular inflammatory conditions (5-18). Notably, in autoimmune diseases that share clinical and pathophysiological features with cGvHD, VWF and FVIII have shown a strong correlation with disease activity, supporting their use as dynamic biomarkers for disease monitoring (10-15). In addition, Biedermann et al. reported elevated VWF levels in patients with cGvHD (19).

VWF is synthesized by vascular endothelial cells and stored in Weibel-Palade bodies within the endothelium, from which it is rapidly released in response to inflammatory stimuli (20). Preclinical studies have demonstrated a heterogeneous distribution of Weibel-Palade bodies along the vascular tree (21) and immunohistochemical analyses have confirmed tissue-specific expression patterns of VWF in human organs (22). On the other hand, FVIII is primarily synthesized by sinusoidal endothelial cells in the liver (23). Given their distinct endothelial origin and responsiveness to inflammatory signals,

elevated VWF and FVIII may reflect not only systemic disease activity but also provide insight into organ-specific involvement.

Based on this rationale, we aimed to evaluate VWF and FVIII as potential biomarkers in cGvHD, focusing on their association with disease onset, organ involvement, severity, activity and longitudinal dynamics.

2. Subjects and Methods:

This prospective longitudinal study was conducted between March 2017 and March 2021 at the University Hospital Center (UHC) Zagreb, Croatia, in collaboration with the University of Zagreb School of Medicine, as part of the research project “New Biomarkers of Chronic Graft-versus-Host Disease” (IP-2016-06-8046), funded by the Croatian Science Foundation. The study enrolled 83 patients diagnosed with cGvHD, as well as 39 alloHSCT recipients without cGvHD who served as controls. Control participants were at least 100 days post-transplant, off systemic immunosuppressive therapy, and clinically stable at the time of enrollment. Exclusion criteria included active acute GvHD, ongoing systemic antimicrobial therapy, and pregnancy. The study was approved by the institutional ethics committees of both UHC Zagreb and the University of Zagreb School of Medicine, and written informed consent was obtained from all participants.

All participants were evaluated by a specialized multidisciplinary cGvHD team at UHC Zagreb. Patients with cGvHD underwent standardized assessments every 6-8 months, whereas controls were evaluated once at study entry. Biomarker results were not available to clinicians at the time of assessment. The diagnosis and severity of cGvHD were determined according to NIH 2005 criteria (24), supplemented by patient- and clinician-reported severity scores on a 0-10 scale. The activity of cGvHD was defined according to the framework of Grkovic et al. (25), integrating clinician’s global assessment, recent disease dynamics, and the intensity of systemic immunosuppression at the time of evaluation. Patients with cGvHD were classified at each assessment as “active” if systemic therapy was initiated, escalated, or substituted for progression or non-response, or as “inactive” if therapy was tapered due to improvement, maintained due to stability, or modified solely for toxicity. Patients with cGvHD off systemic therapy and without clinical signs of cGvHD at the time of evaluation were also considered as “inactive”.

Collected data included demographic characteristics, cGvHD manifestations, and treatment details. Laboratory assessments focused on von Willebrand factor antigen (VWF:Ag, %), von Willebrand factor activity (VWF:Ac, %), and factor VIII activity (FVIII, kIU/L). VWF:Ag and VWF:Ac were measured using latex immunoturbidimetric assays, and FVIII activity by a one-stage clotting assay, all performed on a coagulation analyzer (Siemens Healthineers, Marburg, Germany). All analyses were conducted in the institutional core laboratory using standardized procedures. In addition, the Endothelial Activation and Stress Index (EASIX) score, an established marker of endothelial stress after alloHSCT, was calculated using lactate dehydrogenase (LDH), creatinine, and platelet count, according to the originally published formula (26). The longitudinal re-assessments of cGvHD patients were postponed in cases of active infection requiring systemic antimicrobial therapy.

Statistical analyses were conducted using MedCalc v19.1. Normality was assessed with the Kolmogorov-Smirnov test. Group comparisons were conducted using parametric or non-parametric tests as appropriate, and categorical variables were analyzed using chi-square or Fisher's exact test. Associations were examined using Kendall's τb; variables with τb≥ 0.2 were entered in forward stepwise multivariate regression. Diagnostic performance was assessed using ROC curve analysis and AUCs. Statistical significance was defined as $p < 0.05$.

3. Results:

Participant characteristics

A total of 122 participants were included in the study, comprising 83 patients with cGvHD and 39 allo-HSCT recipients without cGvHD who served as controls. As detailed in **Table 1**, demographic and transplant-related characteristics were largely comparable between groups. However, patients who developed cGvHD more frequently received peripheral blood stem cell (PBSC) grafts and had a higher frequency of prior acute GvHD, particularly with skin and gastrointestinal involvement (Table 1).

Plasma levels of VWF:Ag, VWF:RCo, and FVIII at enrollment

At enrollment, patients with cGvHD had significantly higher plasma levels of VWF:Ag, VWF:RCo, and FVIII compared with controls. They also showed elevated values of several laboratory parameters, including eosinophils, platelets, CRP, LDH, and β2-

microglobulin, whereas albumin levels were significantly lower. Interestingly, EASIX scores did not differ significantly between groups at enrollment (p=0.772) (**Table 2**).

Plasma levels of VWF:Ag, VWF:Ac, and FVIII among cGvHD patients were significantly higher with older age, PBSC graft source, worse ECOG performance status, more intensive systemic immunosuppressive therapy, higher EASIX scores, and higher total and liver NIH cGvHD scores. In addition, they correlated with elevated ferritin, LDH, triglycerides, AST, ALT, and GGT. Elevated VWF:Ag and VWF:Ac were associated with shorter time since alloHSCT and since cGvHD diagnosis to enrollment, higher cGvHD activity, greater number of organs affected with cGvHD, higher NIH oral cGvHD score, increased eosinophil count, D-dimer, β2-microglobulin, total protein, lower albumin, and higher high-density lipoprotein (HDL). FVIII levels, on the other hand, correlated with higher total cholesterol, white blood cell and neutrophil counts, corticosteroid use, and a greater number of donor lymphocyte infusions (DLI). Detailed correlation data are provided in **Supplementary Table S1**.

Multivariate analysis at enrollment

Multivariate linear regression analysis confirmed that several clinical and laboratory variables were independently associated with higher VWF and FVIII levels in cGvHD patients. The model for VWF:Ag explained 57.6% of the variance; significant predictors included older age ($p = 0.021$), number of affected organs ($p = 0.030$), white blood cell count ($p = 0.006$), higher LDH ($p = 0.006$), and lower albumin ($p = 0.001$), the latter exerting the strongest effect (partial $R = 0.410$). For VWF:Ac the model explained 54.0% of the variance; significant predictors were older age ($p = 0.001$), number of affected organs ($p = 0.001$), higher LDH ($p = 0.002$), and lower albumin ($p < 0.001$); again, with low albumin showing the strongest association (partial $R = 0.446$). The FVIII model explained 24.7% of the variance; significant predictors were older age ($p = 0.015$), systemic immunosuppressive therapy ($p = 0.004$), and white blood cell count ($p = 0.018$), with immunosuppressive therapy showing the strongest association (partial $R = 0.323$). All three models were statistically significant ($p < 0.001$).

Organ-specific manifestations correlate with VWF or FVIII levels

Further analysis focused on organ-specific cGvHD manifestations that demonstrated significant correlations with VWF or FVIII levels in the univariate analysis. In patients

with liver cGvHD, both VWF:Ag and VWF:Ac levels increased progressively across NIH liver cGvHD severity grades (0-3), with statistically significant differences between groups ($p = 0.002$ and $p = 0.013$, respectively) while FVIII levels did not show significant differentiation ($p = 0.153$). A similar pattern was observed in patients with oral cGvHD, where both VWF:Ag and VWF:Ac levels differed significantly across NIH oral cGvHD severity groups ($p = 0.002$ and $p = 0.003$, respectively). No clear trends or statistically significant differences were found across NIH severity grades for gastrointestinal cGvHD. These associations are illustrated in **Figure 1** using VWF:Ac as a representative marker. Post hoc analyses indicated that, for liver involvement, the overall significance for VWF:Ag was primarily driven by differences between patients without liver cGVHD (NIH grade 0) and those with moderate-to-severe involvement (NIH grades 2-3), whereas VWF:Ac did not retain statistical significance in pairwise comparisons. In oral cGVHD, differences between NIH grade 0 and grades 2-3 were observed for both VWF:Ag and VWF:Ac (data not shown).

Longitudinal results

Patients with cGvHD were followed for a median of 47 months, with a median interval of 9 months between re-assessments. Over this period, 281 re-assessments were conducted: 14% ($n = 40$) within 30 days of cGvHD diagnosis (cGvHD≤30), 21% ($n = 56$) between 30 and 365 days (cGvHD≤365), and 59% ($n = 168$) more than 365 days after diagnosis of cGvHD (cGvHD>365). An additional 6% ($n = 17$) of re-assessments showed complete resolution of cGvHD and were analyzed as a separate category (cGvHD0). At follow-up, mild, moderate, and severe cGvHD were recorded in 17%, 36.3%, and 40.6% of re-assessments, respectively. Ten patients (12%) died during follow-up, with a median time of 318 days from the initial evaluation. Most deaths were due to infectious complications (50%) or the main disease relapse (30%).

Longitudinal analysis demonstrated that VWF:Ag and VWF:Ac levels peaked at cGvHD onset and declined over time after cGvHD diagnosis, approaching control values by one-year post-diagnosis. FVIII levels exhibited a delayed and more modest peak, followed by a less distinct downward trend (**Figure 2**).

VWF:Ag, VWF:Ac, and FVIII as measures of disease activity

We further explored these factors in relation to cGvHD activity and immunosuppressive treatment. VWF:Ag, VWF:Ac, and FVIII levels were significantly higher in patients with active disease compared to those with inactive disease off systemic therapy or controls (VWF:Ag: 270.0% vs 191.9% vs 163.9%; VWF:Ac: 236.2% vs 178.2% vs 174.1%; FVIII: 2.4 vs 1.82 vs 1.84 kIU/L; all post hoc Dunn's test $p < 0.05$), but not significantly different from cGvHD patients with inactive disease while receiving systemic therapy (all $p > 0.05$) (**Figure 3**).

Results of receiver operating characteristic (ROC) analysis confirmed that VWF:Ag and VWF:Ac could distinguish between cGvHD patients and controls (AUC=0.665 and AUC=0.656, respectively), as well as between active and inactive cGvHD (AUC=0.698 and AUC=0.634, respectively). The strongest diagnostic performance was observed within 30 days of cGvHD diagnosis, with optimal cut-offs of VWF:Ag>246.3% (AUC = 0.733) and VWF:Ac>271.4% (AUC = 0.728). VWF:Ag showed greater sensitivity (67.5%), whereas VWF:Ac demonstrated higher specificity (92.3%) (**Figure 4**). Interestingly, EASIX score showed less discrimination between active and inactive disease (AUC=0.584), as well as between cGvHD patients and controls in the early disease phase (AUC = 0.584) (data not shown).

Temporal dynamics were further assessed through an extended longitudinal analysis of VWF:Ag, VWF:Ac, FVIII, and routinely measured laboratory parameters in cGvHD patients stratified by time since diagnosis of cGvHD (**Supplementary Table S2**). In the early phase of cGVHD (<30 days from diagnosis), total protein, albumin, β2-microglobulin, ferritin, LDH, and eosinophils were significantly altered compared with post-alloHSCT patients who did not develop cGVHD. Over time, these parameters approached levels observed in patients without cGVHD, paralleling the dynamics of VWF markers. Among them, albumin and β2-microglobulin showed the strongest association with disease activity and demonstrated significant discriminatory capacity between active disease and disease resolution (data not shown).

4. Discussion:

Our results demonstrated that both VWF and FVIII levels were significantly elevated in patients with cGvHD compared with alloHSCT recipients without cGvHD and remained high in those with ongoing active cGvHD. In this clinically representative and heterogeneus cohort, which included many patients previously exposed to multiple

lines of systemic immunosuppressive therapy, VWF and FVIII levels were the highest in active cGvHD, the lowest in patients in clinical remission off therapy, and intermediate in those in remission while still receiving treatment. This gradient may indicate that persistently elevated factors may signal residual subclinical activity of GvHD not captured by conventional clinical assessment. The dynamic nature of these factors was further underscored by longitudinal analyses, which demonstrated that changes in their levels paralleled those of established acute-phase reactants. To further contextualize endothelial activation, EASIX was included in analysis; however, in this cohort, VWF appeared more closely aligned with clinical disease activity. Taken together, these observations support the potential utility of VWF and FVIII as dynamic, readily measurable biomarkers for disease monitoring in cGvHD.

Despite both VWF and FVIII being elevated in active cGvHD, their diagnostic performance and clinical associations differed substantially. VWF correlated more consistently with cGvHD activity and showed greater sensitivity to longitudinal changes, whereas FVIII declined less over time and did not significantly reflect organ-specific NIH cGvHD severity scores. Several mechanisms may account for this divergence. FVIII levels are physiologically linked to VWF, which acts as a carrier protein stabilizing FVIII and protecting it from proteolytic degradation (27). Prior studies estimate that each 1% increase in circulating VWF corresponds to an approximately 0.54% rise in FVIII (28). This interdependence likely explains part of the parallel elevation observed in our cohort. However, FVIII was also influenced by additional systemic factors. Unlike VWF, FVIII levels were associated with leukocytosis and corticosteroid therapy, suggesting a stronger relationship with general immune activation rather than localized endothelial stress. Corticosteroid-related FVIII elevation has been reported previously (29), although the underlying mechanisms remain incompletely understood. Together, these data indicate that while VWF and FVIII are biologically interconnected, they are modulated by partly distinct regulatory pathways.

Multivariate analysis highlighted lower serum albumin as the strongest determinant of higher VWF levels, making this, to our knowledge, the first report of such an association in cGvHD. Although the mechanistic basis remains unclear, inverse correlations between albumin and VWF have been documented in other endothelial disorders. In acute thrombotic thrombocytopenic purpura, a condition characterized by markedly elevated VWF, hypoalbuminemia predicted adverse outcomes (30). In

addition, both the number of organs affected with cGvHD and advancing patient age independently predicted VWF levels. The association with age is consistent with prior evidence linking endothelial dysfunction and elevated VWF to aging (31).

In subsequent organ-specific analyses, the liver cGvHD emerged as the most clinically relevant site of association. Notably, only VWF:Ag and VWF:Ac, but not FVIII, showed a significant correlation with hepatic cGvHD severity, despite FVIII being primarily synthesized in the liver. This likely reflects that VWF more directly captures acute endothelial activation, whereas FVIII levels are also influenced by clearance dynamics and extrahepatic sources. However, this association should be interpreted cautiously, as other causes of hepatic inflammation or cholestasis were not specifically evaluated. Compounding this complexity, none of the standard liver enzymes demonstrated a consistent or specific correlation with VWF levels. These observations raise concerns regarding the sensitivity and specificity of NIH liver cGvHD scoring, which primarily relies on alanine aminotransferase (ALT) (32). These findings align with a recent study by Yang et al. (33), which identified alkaline phosphatase (ALP) and total cholesterol, but not ALT, as predictors of histologically confirmed liver cGvHD. Interestingly, in the present study, total cholesterol was significantly associated with FVIII levels, suggesting a possible link between lipid metabolism and hepatic regulation of inflammatory protein synthesis.

From a pathophysiological perspective, findings presented here support the evolving view of cGvHD as a condition driven by endothelial involvement. Recent evidence indicates that the endothelium can act as a direct alloimmune target - Umino et al. demonstrated aberrant DBY/HLA class II expression on endothelial cells in patients with cGvHD (34, 35). This concept is supported by mechanistic data showing that inflammatory stimuli can induce endothelial cells to express MHC class II and co-stimulatory molecules, thereby acquiring antigen-presenting properties that sustain T-cell activation (36). In line with this, experimental work has demonstrated that alloimmune stress increases endothelial susceptibility to cytotoxic T-cell injury increased susceptibility of endothelial cells to cytotoxic T-cell–mediated injury under alloimmune stress (37). Taken together, these insights support an important role for the endothelium in cGvHD and highlight it as a potential therapeutic target, where endothelial-stabilizing interventions could complement conventional immunosuppression while preserving graft-versus-leukemia effects.

The present study extends observations from the Basel group (19), who first suggested a direct mechanistic involvement of endothelial dysfunction in cGvHD and further substantiates its contribution to disease pathogenesis.

Several limitations of this current work should be acknowledged. This was a single-center study, and validation in independent cohorts is required. Although patients with active infections were excluded, residual confounding from cardiovascular risk factors or genetic predisposition cannot be fully ruled out. Histopathological correlation at the tissue level was not systematically available, and longitudinal follow-up was incomplete in a subset of patients due to disruptions during the COVID-19 pandemic. Nevertheless, demographic and transplant-related characteristics were consistent with previous reports, and nearly half of patients were evaluated within 30 days of diagnosis, supporting the robustness of findings.

In summary, this study provides evidence that VWF:Ag, VWF:Ac, and FVIII may serve as clinically informative biomarkers for the diagnosis and assessment of cGvHD activity. Importantly, VWF and FVIII are intended to complement established clinical assessment rather than function as standalone diagnostic tests. These markers may be particularly useful at initial clinical presentation, early after diagnosis to support evaluation of disease activity, and during longitudinal follow-up to monitor treatment response and disease dynamics. In addition, they may offer added value in clinically challenging settings, such as suspected hepatic cGvHD, where non-invasive assessment tools are limited. Given their widespread availability, reproducibility, non-invasiveness, and fully automated performance in routine tertiary-care laboratories without the need for additional sample processing and with modest associated costs, these markers offer realistic potential for integration into clinical practice. Altogether, these findings indicate that VWF and FVIII are novel biomarker candidates for cGvHD with direct translational relevance.

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Figure Legends:

Figure 1. Association between organ-specific cGvHD severity and levels of VWF:Ac.

Box plots display plasma levels of VWF:Ac stratified by NIH severity scores (0–3) for:

a) liver involvement (top row),

b) oral involvement (middle row),

c) gastrointestinal tract involvement (bottom row).

VWF:Ac levels increased with higher NIH severity grades in liver and oral cGvHD, supporting their association with organ-specific disease burden.

Figure 2. Longitudinal changes in VWF:Ag, VWF:Ac and FVIII levels in cGvHD.

Box plots illustrate plasma levels of VWF:Ag, VWF:Ac and FVIII over time following cGvHD diagnosis. Groups include:

1. Control: alloHSCT recipients without cGvHD
2. cGvHD ≤30: within 30 days of diagnosis
3. cGvHD ≤365: within 1 year of diagnosis
4. cGvHD >365: more than 1 year after diagnosis

VWF:Ag and VWF:Ac levels peaked at cGVHD onset and progressively declined over time, approaching control levels by one year after diagnosis, consistent with restoration of immune homeostasis. In contrast, FVIII showed a delayed and less pronounced peak, followed by a more gradual and less distinct decline.

Figure 3. VWF:Ag, VWF:Ac, and FVIII levels in relation to cGvHD activity and systemic immunosuppressive therapy.

Box plots compare levels of VWF:Ag, VWF:Ac, and FVIII across four groups:

1. controls
2. inactive without IS: patients with inactive cGvHD without systemic immunosuppressive therapy
3. inactive with IS: patients with inactive cGvHD receiving systemic immunosuppressive therapy
4. active cGvHD.

VWF:Ag and VWF:Ac levels were clearly elevated in patients with active disease, while values in inactive patients off therapy approached those of controls. In contrast, patients with inactive disease on therapy exhibited intermediate levels. FVIII followed a similar, but less distinct pattern.

Figure 4. Receiver operating characteristic (ROC) curves for VWF:Ag and VWF:Ac.

Receiver operating characteristic (ROC) curves depict the discriminative ability of VWF:Ag and VWF:Ac to distinguish between:

a) newly diagnosed cGvHD (≤30 days) and controls

b) active versus inactive cGvHD

Optimal thresholds, area under the curve (AUC), sensitivity, and specificity are indicated

In early disease (panel a), VWF:Ag and VWF:Ac demonstrated moderate diagnostic accuracy, with VWF:Ag providing greater sensitivity and VWF:Ac achieving higher specificity. These complementary profiles support their potential utility as clinically meaningful biomarkers.

Table 1. Participant characteristics at enrollment.

		cGvHD (n=83) Median (min - max)		Controls (n=39) Median (min - max)		
Age at alloHSCT (years)		42	25-54	40	32-50	
Age at enrollment (years)		45	28-55	43	33-53	
Time from alloHSCT to enrollment (months)		12	8-31	17	10-32	
Time from alloHSCT to cGVHD diagnosis (months)		9	2-199			
Time from cGVHD diagnosis to enrollment (months)		1	0-192			
No. of affected organs		3	1-6			
NIH score of most affected organ		2	1-3			
Total NIH organ score		4	1-12			
cGVHD severity (clinician-assessed)		5	1-8			
cGVHD severity (patient-assessed)		5	0-10			
		N (%)				**p**
Sex	Male	44	53.0	19	49.7	0.701
	Female	39	47.0	20	51.3	
Primary disease	ALL, AML, MDS, MPN	70	84.4	31	79.5	0.573
	CLL, HL, NHL, MM	7	8.4	6	15.4	
	AA, PNH, immunodeficiencies	6	7.2	2	5.1	
Donor type	HLA-matched related	43	51.8	20	51.3	1
	HLA-matched unrelated	40	48.2	19	48.7	
Stem cell source	Bone marrow	22	26.5	20	51.3	**0.014**
	PBSC	61	73.5	19	48.7	
Prior aGvHD		56	67.5	8	20.5	**<0.001**
	Skin	51	61.4	7	17.9	**<0.001**
	GIT	14	16.9	0	0.0	**0.005**
	Liver	13	15.7	2	5.1	0.14
Blood group after alloHSCT	0	25	30.1	15	38.5	0.467
	A	22	25.5	8	20.5	
	B	6	7.2	3	7.7	
	AB	10	12.1	1	2.6	
	Unknown	20	25.1	12	30.7	
cGvHD onset	de novo	28	33.7			
	progressive	22	26.5			
	quiescent	33	39.8			
cGvHD type	Classic	72	86.7			
	Overlap	11	13.3			
Global cGVHD severity (NIH criteria)	Mild	5	6.0			
	Moderate	43	51.8			
	Severe	35	42.2			
ECOG status	0	28	34.1			
	1	41	50.0			
	2	13	15.9			
	3	0	0.0			

Organ involvement	Mouth	49	59.0
	Skin	45	54.2
	Eyes	45	54.2
	Liver	36	43.4
	Lungs	30	36.6
	Joint and fascia	17	20.5
	GIT	16	19.3
	Genital tract (women)	19	54.3
No. of systemic therapy lines	0	22	31.9
	1	23	33.3
	2	11	15.9
	3	6	8.7
	4	5	7.2
	5	2	2.9
Intensity of current immunosuppressive therapy	None	36	43.4
	Mild	7	8.4
	Moderate	35	42.2
	High	5	6.0
cGvHD activity	Active	49	59.8
	Inactive	33	40.2

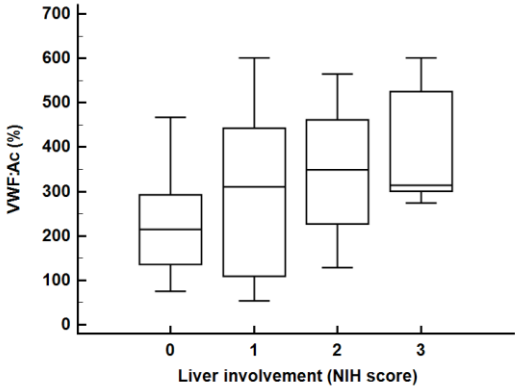
Abbreviations: alloHSCT – allogeneic hematopoietic stem cell transplantation; cGvHD – chronic graft-versus-host disease; aGvHD – acute graft-versus-host disease; NIH – National Institutes of Health; ALL – acute lymphoblastic leukemia; AML – acute myeloid leukemia; MDS – myelodysplastic syndrome; MPN – myeloproliferative neoplasm; CLL – chronic lymphocytic leukemia; HL – Hodgkin lymphoma; NHL – non-Hodgkin lymphoma; MM – multiple myeloma; AA – aplastic anemia; PNH – paroxysmal nocturnal hemoglobinuria; PBSC – peripheral blood stem cells; GIT – gastrointestinal tract; ECOG – Eastern Cooperative Oncology Group.

Table 2. Laboratory parameters at enrollment.

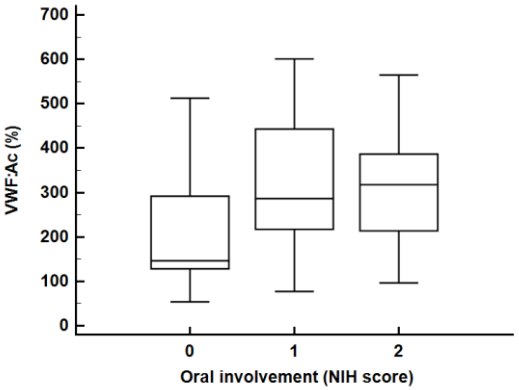
	cGvHD Median (min-max)		Controls Median (min-max)		p
VWF:Ag (%)	268.2	76.60-601.0	191.9	51.9-600.0	**0.003**
VWF:Ac (%)	261.8	54.0-601.0	178.2	48.6-451.9	**0.003**
FVIII (kIU/L)	2.17	0.52-4.53	1.82	0.51-3.41	**0.006**
PLT (×10⁹/L)	211.0	17.0-404.0	183.5	35.0-263.0	**0.027**
EOS (×10⁹/L)	0.20	0.00-3.75	0.15	0.01-0.42	**0.034**
ALP (U/L)	116.0	28.0-505.0	83.00	33.0-365.0	**0.007**
AST (U/L)	35.5	15.0-944.0	29.0	20.0-149.0	**0.026**
GGT (U/L)	72.0	6.0-1536.0	32.5	6.0-197.0	**<0.001**
LDH (U/L)	209.0	116.0-582.0	180.0	131.0-272.0	**0.003**
Anti-cardiolipin IgG	7.0	1.0-36.0	10.5	2.0-39.0	**<0.001**
Anti-cardiolipin IgM	6.0	0.9-36.0	9.5	1.0-60.0	**0.002**
β2M (mg/L)	2.9	1.4-12.9	2.45	1.3-18.4	**0.014**
Ferritin (µg/L)	1178.6	5.0-8001.0	741.8	21.9-4395.0	**0.036**
ALB (g/L)	42.0	22.0-53.1	44.3	24.5-51.9	**0.005**
Total proteins (g/L)	67.0	31.0-87.0	69.5	61.0-88.0	**0.034**
IgA (g/L)	0.69	0.06-5.17	0.99	0.16-4.1	**0.038**

Abbreviations: VWF:Ag – von Willebrand factor antigen; VWF:Ac – von Willebrand factor activity; FVIII – factor VIII activity; PLT – platelets; EOS – eosinophils; ALP – alkaline phosphatase; AST – aspartate aminotransferase; GGT – gamma-glutamyl transferase; LDH – lactate dehydrogenase; β2M – beta-2 microglobulin; ALB – albumin; IgA – immunoglobulin A; aCL – anti-cardiolipin antibody.
Variables not significantly different between groups (p>0.05): WBC, NEU, LYM, ESR, PT, aPTT, fibrinogen, D-dimer, TBIL, ALT, creatinine, EASIX score (p=0.772), total cholesterol, triglycerides, HDL, LDL, CRP, RF, C3, C4, IgG, IgM.

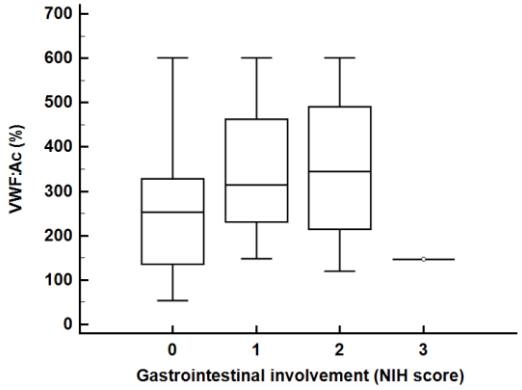
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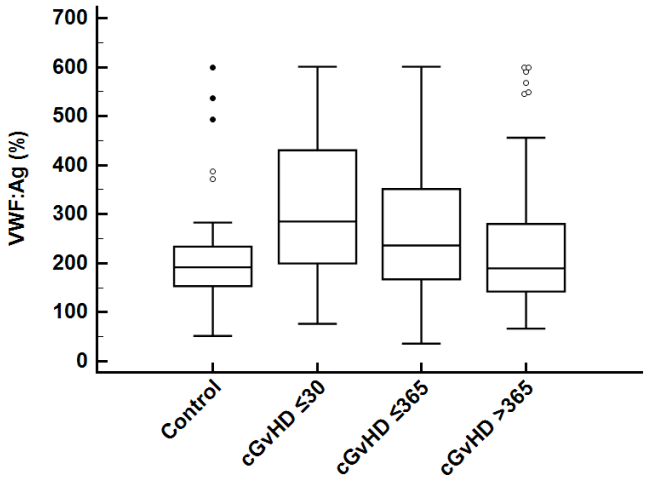

VWF:Ac (%)
700
600
500
400
300
200
100
0
0
1
2
3
Liver involvement (NIH score)

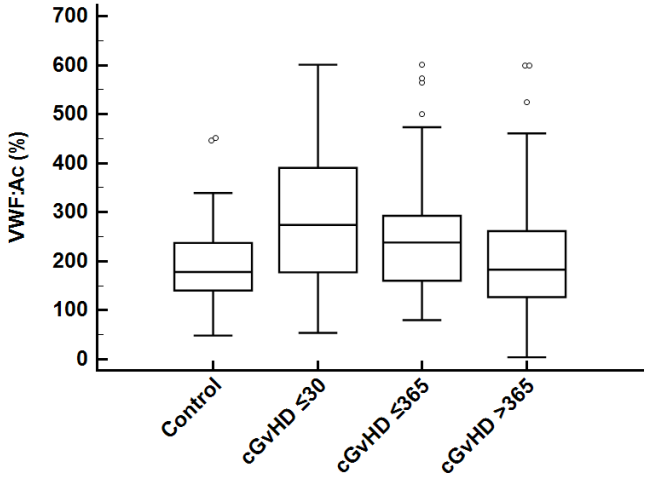
b)

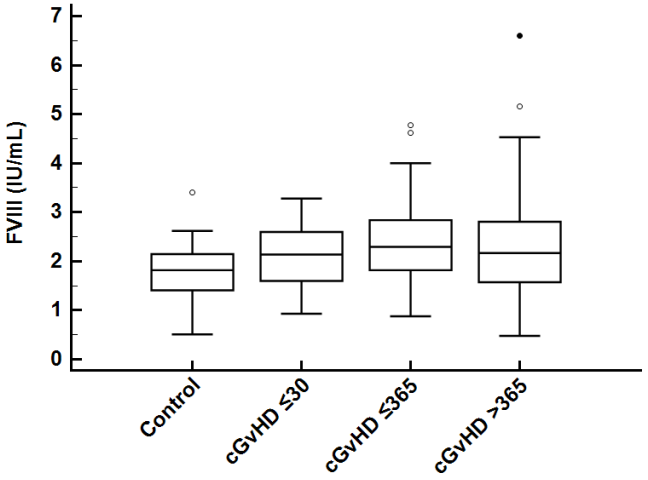

VWF:Ac (%)
700
600
500
400
300
200
100
0
0
1
2
Oral involvement (NIH score)

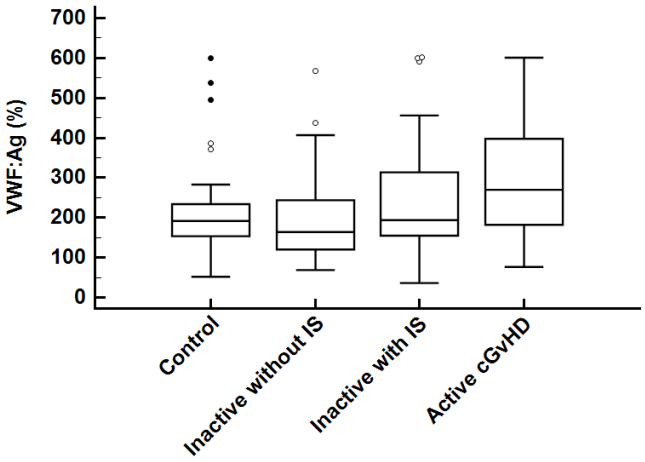
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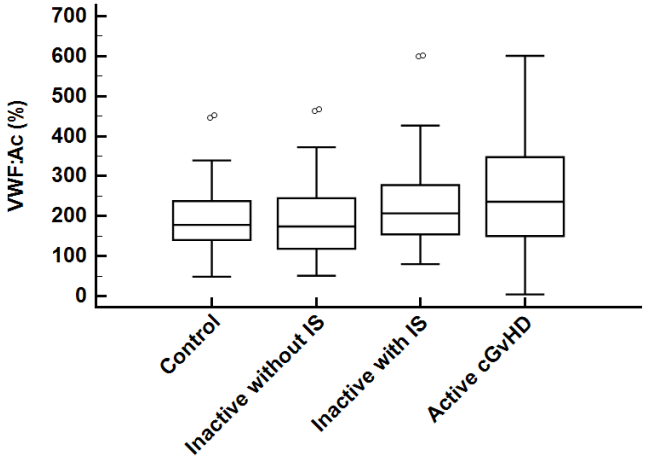

VWF:Ac (%)
700
600
500
400
300
200
100
0
0
1
2
3
Gastrointestinal involvement (NIH score)

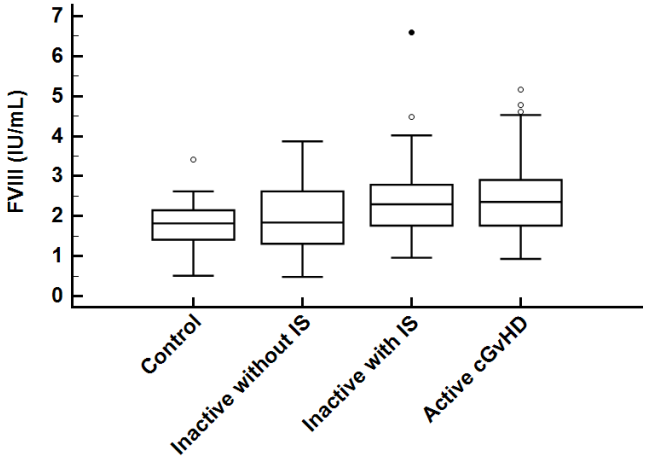

VWF:Ag (%)
0
100
200
300
400
500
600
700
Control
cGvHD ≤30
cGvHD ≤365
cGvHD >365


VWF:Ac (%)
0
100
200
300
400
500
600
700
Control
cGvHD ≤30
cGvHD ≤365
cGvHD >365

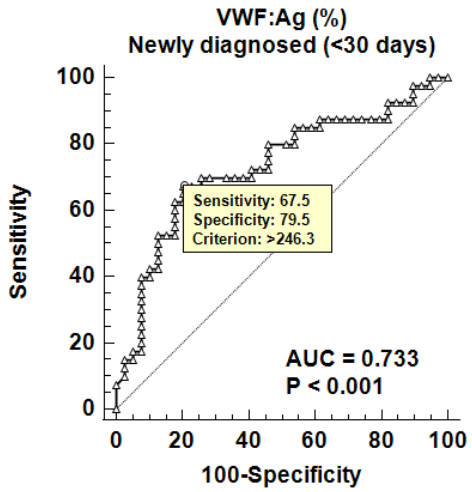

FVIII (IU/mL)
0
1
2
3
4
5
6
7
Control
cGvHD ≤30
cGvHD ≤365
cGvHD >365

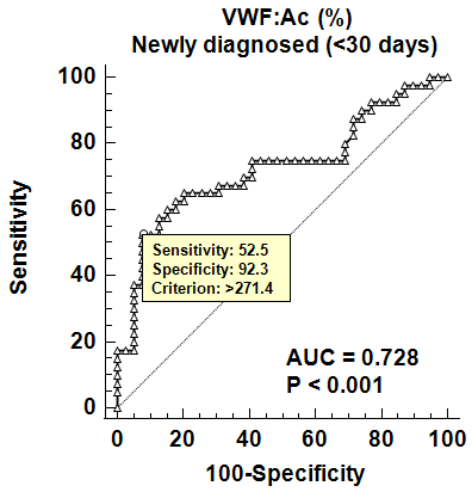

VWF:Ag (%)
700
600
500
400
300
200
100
0
Control
Inactive without IS
Inactive with IS
Active cGvHD


VWF:Ac (%)
700
600
500
400
300
200
100
0
Control
Inactive without IS
Inactive with IS
Active cGvHD

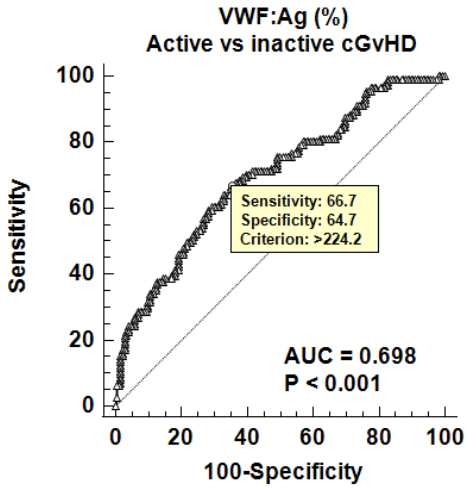

FVIII (IU/mL)
7
6
5
4
3
2
1
0
Control
Inactive without IS
Inactive with IS
Active cGvHD

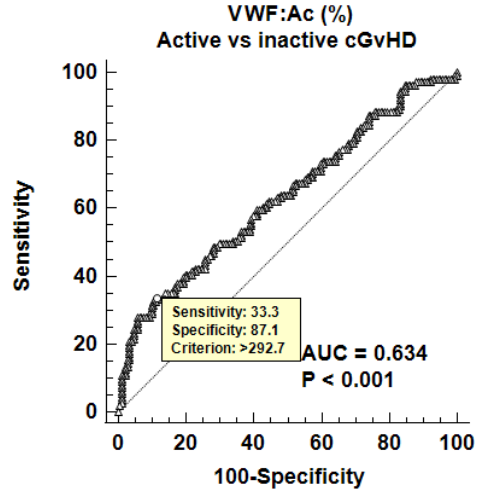
a)


VWF:Ag (%)
Newly diagnosed (<30 days)
Sensitivity
100-Specificity
Sensitivity: 67.5
Specificity: 79.5
Criterion: >246.3
AUC = 0.733
P < 0.001


VWF:Ac (%)
Newly diagnosed (<30 days)
Sensitivity
100-Specificity
Sensitivity: 52.5
Specificity: 92.3
Criterion: >271.4
AUC = 0.728
P < 0.001

b)


VWF:Ag (%)
Active vs inactive cGvHD
Sensitivity
100-Specificity
Sensitivity: 66.7
Specificity: 64.7
Criterion: >224.2
AUC = 0.698
P < 0.001


VWF:Ac (%)
Active vs inactive cGvHD
Sensitivity
100-Specificity
Sensitivity: 33.3
Specificity: 87.1
Criterion: >292.7
AUC = 0.634
P < 0.001