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REGULAR ARTICLE

Von Willebrand Factor and Platelet Levels before Conditioning Chemotherapy Indicate Bone Marrow Regeneration following Autologous Hematopoietic Stem Cell Transplantation

Zita Radnay[1], Árpád Illés[1], Miklós Udvardy[1], Zoltán Prohászka[3], György Sinkovits[3], Mária Csilla Csányi[4], Miklós Kellermayer[4], Attila Kiss[1], Jolán Hársfalvi[2,4,*]

[1] Division of Hematology, Department of Internal Medicine, Faculty of Medicine, University of Debrecen, Debrecen, Hungary
[2] Department of Laboratory Medicine, Faculty of Medicine, University of Debrecen, Hungary
[3] Research Laboratory, 3rd Department of Medicine, Faculty of Medicine, Semmelweis University, Budapest, Hungary
[4] Department of Biophysics and Radiation Biology, Faculty of Medicine, Semmelweis University, Budapest, Hungary

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A B S T R A C T

Autologous hematopoietic stem cell transplantation (HSCT) is often complicated by hemostatic and thrombotic events associated with endothelial cell injury. Thrombotic complications are affected by a disturbed balance between platelets, circulating von Willebrand factor (VWF), and its specific protease, ADAMTS13. HSCT-associated endothelial dysfunction, impaired hemostasis, and inflammation are interrelated processes, and research on the complex interplay of conditioning regimens from engraftment to bone marrow regeneration remains intensive. This prospective observational study comparing lymphoma and multiple myeloma (MM) patients who underwent autologous HSCT explored how platelet count, VWF level, ADAMTS13 activity, and C-reactive protein (CRP) level as potential markers (1) vary in response to therapy, (2) differ between the 2 groups, and (3) correlate with the remission state at 100 days after HSCT. We correlated the quantitative changes in platelet count and levels of VWF, ADAMTS13, and CRP with one another during HSCT and in the remission state in 45 patients with lymphoma and 59 patients with MM who underwent autologous HSCT between 2010 and 2013 at the University of Debrecen. Samples were collected at the start of conditioning chemotherapy, on the day of stem cell transplantation, and at 5, 11, and 100 days following HSCT. CRP levels peaked when platelet counts dropped to a minimum, and these changes were much more pronounced in the lymphoma group. VWF level was the highest, with lower ADAMTS13 activity, at platelet engraftment in both patient groups equally. Diagnostic evidence indicative of thrombotic complications was not found. In the lymphoma group, VWF level prior to conditioning had statistically significant correlations with platelet count, CRP level, and hemoglobin concentration at the time of bone marrow regeneration ($P < .001$) and during the remission state ($P = .034$). In the MM group, platelet count before conditioning was correlated with platelet count ($P < .001$) and white blood cell count ($P = .012$) at the time of bone marrow regeneration. The statistically significant correlation of the markers at the time of bone marrow regeneration with the preconditioning VWF levels in lymphoma and with the preconditioning platelet counts in MM might indicate the clinical significance of the bone marrow niches of arterioles and megakaryocytes, respectively, where the stem cells are located and regulated. Because preconditioning VWF levels are associated with remission after HSCT in lymphoma patients, VWF should be screened before conditioning, along with the markers used in HSCT protocols, to optimize personalized treatment and reduce therapeutic risks.

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INTRODUCTION

Hematopoietic stem cell transplantation (HSCT) is a therapeutic procedure for a broad range of hematologic disorders, including multiple myeloma (MM) and lymphoma [1,2]. Patients undergoing HSCT receive chemotherapy prior to stem cell transplantation. Chemotherapy greatly affects the physiologic state and function of the endothelium and platelets, increasing the risk of bacterial infection, immune reactions, and thrombotic complications [3−5]. Endothelial cells (ECs) can be injured by chemotherapy, profound thrombocytopenia, infections during the engraftment process, and the use of i.v. catheters. Regulation of vascular tone and permeability, platelet activation, coagulation,

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*Correspondence and reprint requests: Jolan Harsfalvi, Department of Biophysics and Radiation Biology, Faculty of Medicine, Semmelweis University, 37-47 Tuzolto St, 1094 Budapest, Hungary

E-mail address: harsfalvi.jolan@med.semmelweis-univ.hu (J. Hársfalvi).

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thromboinflammatory processes, and angiogenesis are connected, leading to consumptive thrombocytopenia and elevated plasma levels of von Willebrand factor (VWF), with HSCT-associated hemostatic complications [6−11].

Platelets sense shear forces and react to them by activation, which exacerbates inflammation and promotes further endothelial damage and thrombosis as injured ECs locally interact with other platelets and megakaryocytes via VWF [12−14]. A cofactor of platelet adhesion, VWF is one of the best-known markers of EC injury. It circulates in the blood as a multimeric glycoprotein with a half-life of 12.4 ± 2.5 hours.

VWF mediates platelet adhesion and aggregation on vascular injury at sites of high shear rate. It is synthesized in megakaryocytes and ECs and stored in alpha granules of the platelets and Weibel-Palade bodies of the ECs. Stimulation of ECs or platelets induces the release of particularly active, large VWF multimers [15]. On secretion, ADAMTS13 (a disintegrin-like and metalloproteinase with thrombospondin type-1 motifs 13) proteolytically cleaves high molecular weight VWF multimers. The balance of ADAMTS13 and VWF activities prevents the accumulation of large multimers and subsequent platelet aggregation and thrombus formation [16].

De novo hepatic synthesis of C-reactive protein (CRP) is accelerated following an acute-phase stimulus and plasma levels may increase 1000-fold from a normal baseline of <5 mg/L. The synthesis rate determines the circulating CRP level, thus directly reflecting the intensity of the pathologic process(es). The circulating CRP concentration falls rapidly when the stimulus for increased production ends. The plasma half-life of CRP is approximately 19 hours [17].

The aims of our present prospective observational study comparing lymphoma and MM patient groups after autologous HSCT were (1) to determine how platelet count, VWF level along with ADAMTS13 activity, and CRP level change as a consequence of the therapy at 5 representative time points and (2) to reveal whether these parameters correlate with one another and with recovery at the time of bone marrow regeneration. Although numerous previous studies have explored the role of these analytes, none of them examined their relationship at multiple time points of treatment or separated or compared MM and lymphoma groups undergoing autologous HSCT specifically.

METHODS

Patients

One hundred and four patients (45 with lymphoma and 59 with MM) treated with autologous HSCT were selected for this study at Transplantation Unit CIC 648 of the University of Debrecen, Hungary, between 2010 and 2013 (Table 1). The protocol was approved by the local Ethics Committee (DE OEC RKEB/IKEB 3633-2012). Written informed consent was obtained from each patient. Data included in this article have been deposited on the server of the corresponding author's department and are available on request. The HSCT procedure was adapted from the guidelines of the European Society for Blood and Marrow Transplantation [7]. Stem cells were collected from peripheral blood and stored at -70 °C.

Conditioning was provided according to the BEAM protocol for lymphoma patients. This protocol includes 100 mg/m^2 each of etoposide and Ara-C twice daily i.v. for 4 consecutive days, a single 300 mg/m^2 i.v. dose of BICNU (carmustine) on the second day, and a single 140 mg/m^2 dose of melphalan on the sixth day. Patients with follicular lymphoma (n = 8) also received ibritumomab tiuxetan (Zevalin, Z-BEAM; CIS Bio International, Saclay, France). In MM patients, conditioning consisted of a single 200 mg/m^2 dose of melphalan (n = 53), which was reduced to 140 mg/m^2 in patients with renal insufficiency (n = 6). All MM patients received antiangiogenetic treatment before conditioning (Supplementary Table S1) [18]. Patients received only i.v. hydration prior to HSCT, to reduce toxic exposure. Prophylactic low molecular weight heparin was administered to all patients at a dose that depended on kidney function and daily platelet count, which was closely monitored after HSCT. Once platelet count decreased to 40 G/L, low molecular weight heparin was replaced by continuous Na-heparin infusion until engraftment (see definition below). Na-heparin was stopped when the platelet count dropped below 20 G/L and bleeding or fever appeared, or if the platelet count dropped to 10 G/L and platelet transfusion was indicated. Ursodeoxycholic acid was added continuously from day 1 post-HSCT until engraftment. Prophylactic antibiotic, antiviral, and antifungal treatment were administered to all patients during the neutropenic period. The onset of engraftment was established when either the absolute neutrophil count or the platelet count returned to .5 or 20 G/L, respectively, without the need for platelet transfusion in 2 days or when the platelet count was >20 G/L for at least 3 consecutive days. Patients were discharged from the hospital after engraftment following HSCT (at a median of 12 days; interquartile range, 11 to 14 days) and returned for monitoring over the subsequent 3 weeks. At 100 days after HSCT, at complete remission (CR) or partial remission (PR), a complete disease state evaluation was performed, based on a positron emission tomography/computed tomography scan for lymphoma patients (Cheson criteria); bone marrow examination and electrophoretic analysis of serum proteins for MM patients (International Myeloma Working Group criteria); and tests of laboratory markers of bone marrow regeneration (blood counts, CRP) in both groups. For statistical comparisons, we combined the very good PR and PR cases into the PR group of MM patients [1,2].

Limitations of our study include that the number of samples differed somewhat, because we could not reach all patients at appropriate time points, and that our diagnostic results did not provide evidence of thrombotic complications. Although abdominal pain due to hepatomegaly, edema, and weight gain caused by fluid retention occurred, these cases were not analyzed separately.

Sample Handling

Blood samples were drawn into 3.2% trisodium citrate, K2 EDTA, and native tubes (BD Vacutainer Systems, Plymouth, UK). Following centrifugation (1500 × g, room temperature, 15 minutes), plasma and serum were stored in aliquots at -70 °C until measurements. The sampling time points were the start of conditioning therapy (preconditioning [D_{pre}], which is day 7 [D-7] before HSCT for lymphoma patients and day 2 [D-2] before HSCT for MM patients), day of HSCT (D0), and days 5 ± 1 (D5), 11 ± 1 (D11 or engraftment), and 100 ± 5 (D100 or bone marrow regeneration) post-HSCT.

Routine Laboratory, VWF, and ADAMTS13 Tests

Blood counts, hemostatic parameters, and kidney and liver enzymes were measured at the Department of Laboratory Medicine, University of Debrecen. Hematology parameters, including RBC, WBC, and platelet counts, were measured with an hematology analyzer (XE-2100D; Sysmex, Kobe, Japan). Hemostatic parameters were determined with an automated coagulometer (BCS XP; Siemens, Munich, Germany). Biochemical and immunochemical parameters were determined with an automated analyzer system (Integra 700; Roche, Basel, Switzerland).

VWF antigen level (VWF:Ag) and VWF collagen-binding activity (VWF:CB) were determined by ELISA, using rabbit primary and secondary antibodies from DakoCytomation (Glostrup, Denmark). Human collagen type III was used for coating in VWF:CB ELISA [19]. ADAMTS13 activity was measured by a fluorescence resonance energy transfer (FRET) assay according to Kokame et al. [20] using a synthetic 73-residue peptide (FRETS-VWF73; Peptides International, Louisville, KY). VWF propeptide was not tested owing to insufficient sample quantity. Both VWF:CB and ADAMTS13 activity were normalized to VWF:Ag and recorded as VWF:CB/Ag and AD13/VWF, respectively.

Assay quality was determined as the percentage of variance under routine conditions at normal and high pathologic levels, which ranged between 2% and 12%. Reference intervals were obtained from our parallel study or from the manufacturers of the routine laboratory tests [21].

Statistical Analysis

Statistical analysis and graphical presentation were performed with Microsoft Excel 16.16.24 (https://office.microsoft.com/excel) and GraphPad Prism 9.0.0 (https://www.graphpad.com/), respectively. Variables were tested for normality and lognormality and analyzed accordingly. Because outliers reflected the patients' disease states, none were removed. Variables were analyzed by one-way ANOVA with Bonferroni correction for differences, Pearson correlation and the Benjamini-Hochberg method for associations between variables, and linear and logistic regressions for functional relationships and predictive roles.

RESULTS

Clinical Parameters of Patients

Data on patient characteristics, treatments, basic laboratory tests, and HSCT-related early complications are summarized in Table 1. Among the 104 patients, 55 females and 49 males were diagnosed with hematologic malignancies, including 34 with non-Hodgkin lymphoma, 11 with Hodgkin lymphoma, and 59 with MM. Blood group distribution and basic laboratory results (eg, hematology, kidney and liver function, coagulation, CRP) were

Table 1
Patient Characteristics and Parameters

Variable	On Preconditioning or Post-HSCT		On D100	
	Lymphoma	MM	Lymphoma	MM
Characteristics*				
No. of patients	NHL: 34 HL: 11	59	NHL: 31 HL: 9	57
Sex, female/male, %	42/58	61/39		
Blood group, O/non-O, %	29/71	29/71		
Hematology, median (IQR)				
WBC, G/L	5.19 (3.33-7.02)	5.42 (3.57-6.67)	4.55 (3.04-6.22)	5.33 (4.47-7.33)
Hgb, G/L	116 (102-128)	121 (113-131)	118 (104-134)	123 (117-134)
Plt, G/L	215 (172-280)	239 (171-300)	160 (103-196)	180 (139-223)
Kidney function, median (IQR)				
Urea, mmol/L	5.6 (4.4-6.3)	5.0 (4.0-6.1)		
Creatinine, μmol/L	74.0 (55.5-89.7)	59.5 (50.7-79.0)		
GFR, mL/min/1.73 m^2	90 (67-90)	90 (83-90)		
Liver function, median (IQR)				
tBil, μmol/L	8 (5.3-10.2)	7 (5.1-10.6)		
GOT, U/L	19 (15.0-22.5)	19 (16.0-23.7)		
GPT, U/L	16 (13.2-20.5)	19 (13.7-24)		
GGT, U/L	27 (18-44)	23 (15-36)		
AP, U/L	88 (74-121)	79 (63-121)		
LDH, U/L	240 (187-319)	206 (180-263)		
Coagulation, median (IQR)				
PT, s	10.0 (8.8-10.6)	10.3 (10.0-10.9)		
INR	.98 (.94-1.01)	1.00 (.95-1.06)		
APTT, s	29.0 (27.4-34.5)	32.6 (28.9-35.4)		
TT, s	17.7 (16.7-19.4)	17.2 (16.1-18.8)		
CRP, mg/L, median (IQR)	3 (1-14)	3 (1-7)	2 (2-13)	2 (2-3)
Preconditioning, n (%)				
Melphalan	—	59 (100)		
BEAM	37 (82)	—		
Z-BEAM	8 (18)	—		
Stem cell count, $\times 10^6$/kg†	5.8 (4.5-7.4)	6.1 (4.9-7.1)		
Day of critical cell counts, median (IQR)				
ANC <.5‡	8 (7-9)	5 (5-6)		
ANC = .5§	10 (9-11)	11 (11-12)		
Platelets <20‡	9 (7-11)	4 (3-5)		
Platelets = 20§	13 (11-15)	10 (9-11)		
Transplantation-related complications, n (%)				
Infections	10 (22)	5 (8)		
VOD or TMA‖	0	0		
DVT‖	0	0		
CR/PR, n¶	20/25	18/41	26/14	40/17

IQR indicates interquartile range; NHL, non-Hodgkin lymphoma; HL, Hodgkin lymphoma; GFR, glomerular filtration rate; tBil, total bilirubin; GOT, aspartate transaminase; GPT, alanine transaminase; GGT, gamma-glutamyl transpeptidase; AP, alkaline phosphatase; LDH, lactate dehydrogenase; PT, prothrombin time; INR, international normalized ratio; APTT, activated partial thromboplastin time; TT, thrombin time; ANC, absolute neutrophil count; VOD, veno-occlusive disease; TMA, thrombotic microangiopathy; DVT, deep vein thrombosis.

* Basic and determinant clinical characteristics and laboratory parameters (median and IQR).

† Stem cell counts at HSCT (D0), $\times 10^6$/body weight kg.

‡ Days when cell counts were critically low (neutropenia or thrombocytopenia, .5 and 20 G/L, respectively).

§ Days following HSCT until the ANC or platelet count reached the critical cell level (.5- and 20 G/L respectively, indicating engraftment).

‖ Prophylaxis was administered to all patients.

¶ For the statistical comparison, we combined the very good PR and PR cases into the PR group in the MM patients.

statistically indistinguishable between the 2 groups. The most remarkable observation is the longer duration of neutropenia and severe thrombocytopenia in the lymphoma group compared with the MM group. Accordingly, Platelet engraftment took longer in lymphoma patients than in MM patients, even though the time to reach neutrophil engraftment was similar in the 2 patient groups.

Ten lymphoma patients and 5 MM patients developed a bacterial infection, which was fatal in 2 lymphoma patients and 1 MM patient. Diagnostic results provided no evidence of thrombotic complications. CR or PR was achieved by D100 post-HSCT in both patient groups, and enlarged lymph nodes disappeared completely or partially in all the lymphomas.

Descriptive Statistics

Platelet count and ADAMTS13 activity were decreased, whereas VWF:Ag, VWF:CB, and CRP levels were increased following HSCT (Figure 1, Supplementary Figure S1). Before conditioning, the median values of VWF:Ag and VWF:CB were at the upper limit of the respective reference intervals (gray bands on the graphs). In contrast, the median of the AD13/VWF ratio fell below the lower limit of the reference interval. The decrease in Platelet count and increases in VWF and CRP levels were substantial from the first day of chemotherapy, but all returned to within the limits of the respective reference intervals by D100. VWF:CB activity increased in parallel with VWF:Ag, and thus their ratio (VWF:CB/Ag) remained more or less constant throughout (Figure 1D,E); therefore, in what follows, only the VWF:Ag results, referred to as “VWF,” are discussed.

To reveal the temporal dynamics of the variables, we normalized them to their preconditioning values (Figure 1C,F,K). In lymphoma patients, the platelet count was reduced to 50% by the time of HSCT and dropped below the critical value of

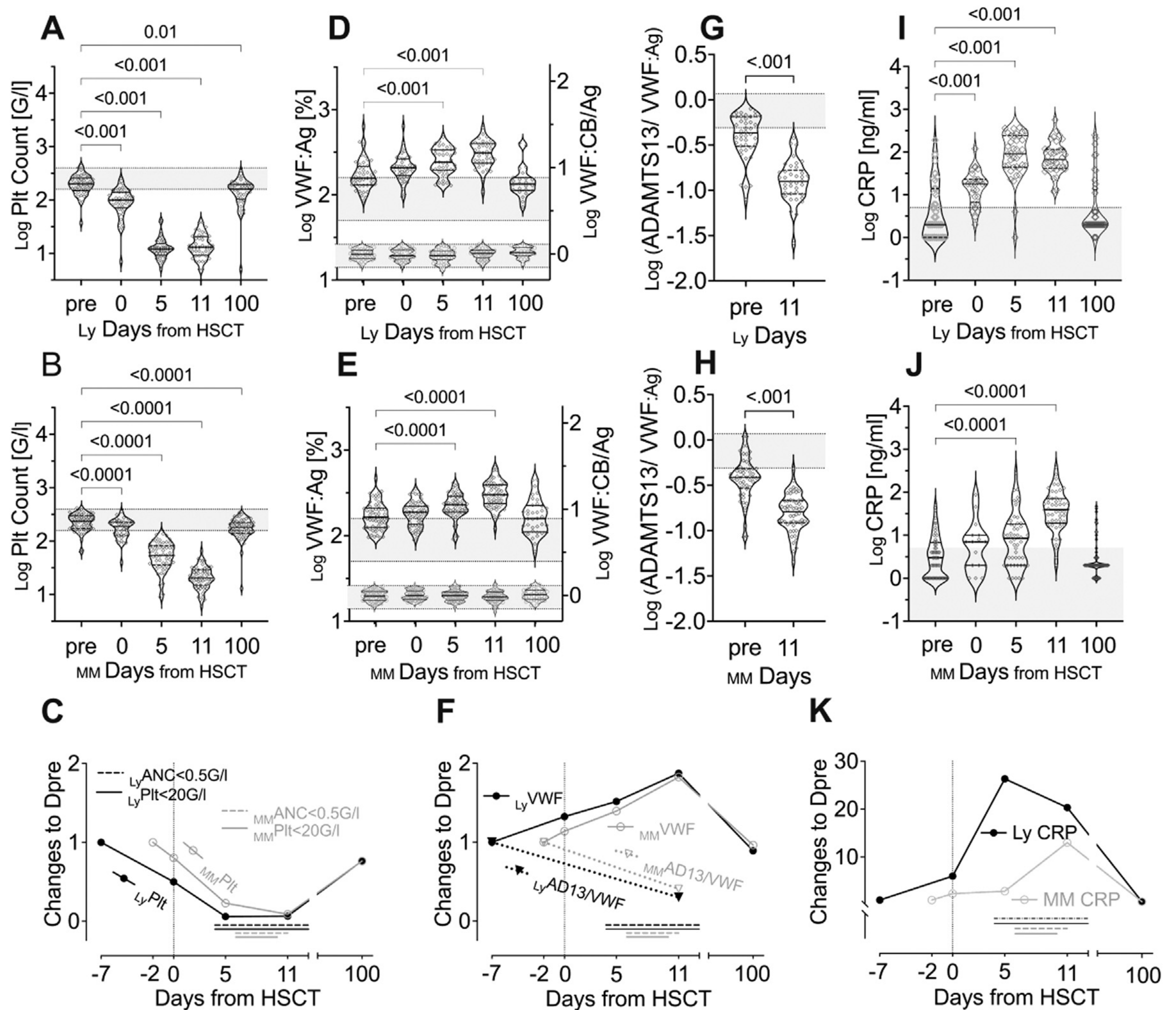

Figure 1. Violin scatterplots of the descriptive statistics and timewise changes in parameters. The scatterplots show median and IQR values of the lognormal platelet, VWF:Ag, AD13/VWF, and CRP levels in the lymphoma (A, D, G, I) and MM (B, E, H, J) patient groups. The VWF:CB to VWF:Ag ratio is shown in the graph of VWF:Ag (right *y*-axis and lower plots). The VWF:CB activity is shown in Supplementary Figure S1A,B, and ADAMT13 activity is shown in Supplementary Figure S1C,D. Reference intervals are shown by the horizontal gray bends on each plot. The level of significance is indicated when the adjusted *P* value was <.05. Panels C, F, and K show the changes in the parameters over time related to their values at preconditioning. Solid black circles, triangles, and lines represent lymphoma patients and gray empty circles, triangles, and lines represent MM patients. Lines running parallel to the *x*-axis indicate the duration of the critical cell counts, with the thrombocytopenic period indicated by solid lines and the neutropenic period by dashed lines. Linking lines between data at different time points are provided as guides for the eye. Ly indicates lymphoma.

.5 G/L between D4 and D13 after HSCT (Figure 1C). In contrast, in MM patients, platelet count was reduced to 80% by the time of HSCT and fell below the critical count by D6 post-HSCT.

VWF increased gradually and doubled by D11 post-HSCT in both patient groups, while AD13/VWF was reduced to 30% to 40% of baseline during the same period (Figure 1F). Remarkably, CRP increased by 26-fold on D5 in the lymphoma group and by 13-fold on D11 in the MM group, which equates to 12 to 13 days in each group after preconditioning, indicating the effect of the conditioning on CRP (Figure 1K). A comparison of the lymphoma and MM groups with one-way ANOVA following Bonferroni correction (Table 2) revealed that the platelet counts at D0, D5, and D11 (Plt_0, Plt_5, and Plt_{11}) decreased and the CRP level increased in parallel, but the changes were significantly more substantial in the lymphoma group compared with the MM group. VWF increased and ADAMTS13 activity decreased equally in the 2 patient groups.

Correlations and Regressions

To reveal associations between the evolution in time of the different variables and the remission status at bone marrow recovery, platelet count, VWF level, ADAMTS13 activity, and CRP level were correlated with one another, with critical cell counts, and with remission state. The correlation is considered significant if the Pearson correlation coefficient (r) is above +.4 or below −.4 and q is less than the threshold P at Q = 5%, as calculated using the Benjamini-Hochberg method (Table 3).

Multivariable regression analysis between different combinations of continuous variables yielded the best result only between univariable pairs shown in Table 3 or Supplementary Table S2. For dichotomous dependent variables, the odds ratios with 95% confidence intervals and *P* values of the significant results are shown in Table 4.

In lymphoma patients, VWF_{pre} showed a negative correlation with Plt_{100} (R = -.627) and hemoglobin $(Hgb)_{100}$ (R = -.512) and a positive correlation with CRP_{100} (R = .497) (Figure 2A). In addition, VWF_{pre} was associated with remission state at the time of bone marrow regeneration (Table 3). Correlations of ADAMTS13 activity with these parameters were not statistically significant. A quantitative relationship was found between Plt_{pre} and WBC_{100} but not between Plt_{pre} and $ADAMTS13_{pre}$, $CRP_{pre,}$ or any other

Table 2
ANOVA

Parameter	Lymphoma Group, median (IQR)	MM Group, median (IQR)	Bonferroni-Adjusted *P* Value
Plt_{pre}, G/L	200 (150-259)	236 (172-298)	.8125
Plt_0, G/L	100 (73-143)	190 (127-226)	<**.0001**
Plt_5, G/L	12 (9-17)	54 (36-82)	<**.0001**
Plt_{11}, G/L	13 (9-20)	21 (15-29)	**.0004**
Plt_{100}, G/L	153 (94-194)	180 (139-223)	.1505
VWF_{pre}, %	157 (131-216)	165 (125-210)	>.9999
VWF_0, %	208 (169-264)	188 (137-221)	.2463
VWF_5, %	238 (185-333)	230 (188-287)	>.9999
VWF_{11}, %	294 (232-393)	301 (238-392)	>.9999
VWF_{100}, %	140 (114-188)	159 (111-225)	.2445
$AD13/VWF_{pre}$	.43 (.31-.63)	.39 (.29-.48)	>.9999
$AD13/VWF_{11}$	.13 (.09-.19)	.16 (.12-.21)	.1040
CRP_{pre}, ng/mL	3 (1-14)	3 (1-7)	.9345
CRP_0, ng/mL	18 (6-23)	7 (2-10)	**.0316**
CRP_5, ng/mL	79 (40-241)	9 (2-18)	<**.0001**
CRP_{11}, ng/mL	61 (40-115)	39 (19-71)	**.0413**
CRP_{100}, ng/mL	2 (2-3)	2 (2-3)	.1397

Platelet count, VWF and CRP levels, and AD13/VWF were compared between the lymphoma and MM patient groups by one-way ANOVA with Bonferroni correction. A 2-sided *P* value <.05 was considered to indicate statistical significance. Subscript numbers of the parameters denote the number of the sample collection days from HSCT. Significant *P* values are in bold type.

variables. In MM patients, the correlations of Plt_{pre} with Plt_{100} (R = .564) and WBC_{100} (R = .388) were significant (Figure 2B).

The decrease in the number of transplanted stem cells correlated significantly with the duration of the thrombocytopenic period, time to reach a critical platelet count, and platelet count at engraftment in both lymphoma patients and MM patients (Supplementary Table S3). On the other hand, no significant relationship was found between stem cell numbers and neutropenia and remission status at the time of bone marrow regeneration. There also was no correlation between liver function parameters and coagulation variables at preconditioning with any of the laboratory markers post-HSCT or the status of bone marrow regeneration.

None of the variables showed a statistically significant regression for the occurrence of infection (data not shown). The correlations of preengraftment and engraftment variables or their regressions are presented in Supplementary Item S1 and Table S2.

DISCUSSION

The aim of this study was to explore quantitative relationships among platelet count, VWF level, ADAMTS13 activity, CRP levels, and remission state of patients in the course of autologous HSCT. New in this work are the comparisons of lymphoma patients with MM patients and of the variables at the preconditioned state and at the time of bone marrow regeneration.

Table 3
Correlations, Continuous Variables

Variables	Lymphoma		MM	
	r*	q†	r*	q†
Plt_{pre}-WBC_{100}	**.411**	**.01088**	**.388**	**.01194**
Plt_{pre}-Plt_{100}	.302	.06135	**.564**	**.00005**
VWF_{pre}-Plt_{100}	**-.627**	**.00001**	-.046	.73750
VWF_{pre}-Hgb_{100}	**-.512**	**.00072**	-.131	.18393
VWF_0-Hgb_{100}	**-.525**	**.00084**	-.113	.48209
VWF_{pre}-CRP_{100}	**.497**	**.00109**	.315	.04484
CRP_{pre}-CRP_{100}	**.569**	**.00019**	.192	.23582

Bold type indicates significant correlation.

* r, Pearson correlation coefficient.

† *q*, adjusted *P* value by the Benjamini-Hochberg method, which resulted in threshold *P* values of <.04285 in the lymphoma group and .02142 in the MM group when *P* = .05 was used for calculation.

The differences in the time-dependent changes in platelet count, VWF, and CRP between the lymphoma and MM patient groups might be related to differences in their neutropenic and thrombocytopenic periods, which were longer in the lymphoma patients by 3 days and 5 days, respectively. The time to reach neutrophil engraftment in our lymphoma patient group was similar to that reported in the Karolinska study [22]. In contrast, the time to reach platelet engraftment was shorter in the present study compared to the study reported by Ungerstedt et al. [6], but in that study, the critical platelet count was 50 G/L without transfusion.

The substantial platelet count reduction elicited by the very different conditioning therapies corroborates previously published results [6,23]. Platelet and WBC counts at the time of bone marrow regeneration were linked to preconditioning platelet counts in MM patients, whereas the remission state was not associated with any variables.

The high preconditioning VWF levels, which increased further following HSCT in lymphoma and MM patients, have been observed previously and are related to diffuse endothelial injury [6,24-29]. However, those previous studies did not separate the hematologic malignancies and transplantation types. An inverse correlation between ADAMTS13 activity and VWF level during autologous HSCT supports earlier findings [30−32] and is explained by endothelial dysfunction associated with chemotherapy-induced toxicity [33].

The kinetics of VWF increase were similar during HSCT in the lymphoma and MM groups, whereas the platelet count fell with different kinetics during this period. Thus, our results indirectly imply that platelet-derived VWF, accounting for approximately 25% of the total VWF in the circulating blood

Table 4
Correlations, Logistic Regression

Variables	Lymphoma Group			MM Group		
	OR*	95% CI	*P* Value†	OR*	95% CI	*P* Value†
VWF_{pre}-CR/PR	.987	.973-.997	**.034**	.447	.01-15.8	.655

Bold type indicates significant correlation.

* OR, odds ratio for logistic regression; equation: $Y \sim Y_{intercept}$ (β0) + slope (β)*X.

† Significance of OR (P < .05).

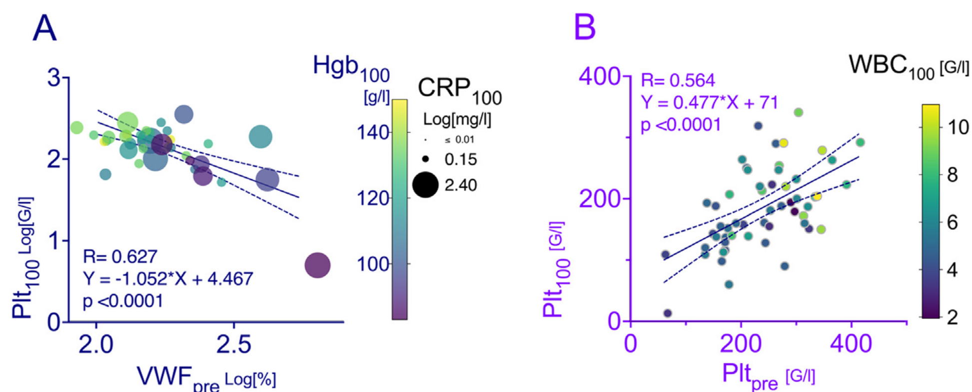

Figure 2. Bubble plots of regressions. (A) Lymphoma patient group, linear regression between VWF_{pre} (X) and Plt_{100} (Y1, location of bubbles), CRP_{100} (Y2, size of bubbles), and Hgb_{100} (Y3, blue-green-yellow fill color of bubbles). Equations: Plt_{100} inserted in the graph, Hgb_{100} = -.1*VWF_{pre}+134, *R* = .497, *P* < .0001; CRP_{100} = 1.88*VWF_{pre}-3.5, *R* = .498, *P* = .001. (B) MM patient group, linear regression between Plt_{pre} (X) and Plt_{100} (Y1) and WBC_{100} (Y2, blue-green-yellow fill color of bubbles). Equations: Plt_{100} inserted in the graph, WBC_{100} = .013*Plt_{pre} + 2.724, *R* = .462, *P* = .0003. Subscript numbers of the parameters denote the number of sample collection days from HSCT.

[34], did not contribute significantly to the changed plasma VWF levels, which must be essentially of endothelial origin.

Importantly, in lymphoma patients, preconditioning VWF levels correlated negatively with platelet count and Hgb level, correlated positively with CRP level at the time of bone marrow regeneration, and were linked with the remission state.

Induction treatment of MM patients contains antiangiogenic compounds that might cause long-term modulation of ECs and hence VWF synthesis [18]. The treatment to reach remission in transplantation candidates leads to normal hemopoiesis prior to launching the HSCT protocol, but the remission state at preconditioning could be influenced by these therapeutic interventions. One particular culprit may be the antiangiogenic treatment in MM patients, which may elicit various disease-specific disturbances in regulatory pathways [35,36].

Infection was the most common HSCT-related morbidity in our study, similar to studies involving both allogeneic and autologous HSCT [28,37,38]. VWF level did not correlate with CRP level, indicating that the VWF increase was not only an acute-phase response, even though the half-life of VWF is somewhat shorter than that of CRP [39].

CRP levels were elevated and peaked 11 days from the start of conditioning in both groups. These results imply a linkage between CRP elevation and the conditioning therapy, but there was no linkage to patient groups in terms of the kinetics of CRP elevation after HSCT. Our results are in accordance with previously reported observations [40,41]. CRP is produced by hepatocytes under the control of proinflammatory cytokine IL-6, which is sensed and responded to by ECs in the course of endothelial dysfunction [35].

Endothelial dysfunction, angiogenesis, and platelet function are associated [35], and thus the production of platelets and VWF is likely to be affected owing to specific bone marrow niches and the commitment of hematopoietic stem cells to their lineage [5].

CONCLUSION

The statistical correlation of preconditioning VWF levels and platelet count with the markers of remission state at the time of bone marrow regeneration might indicate the clinical significance of the stem cells located in and regulated by separate bone marrow niches containing arterioles and megakaryocytes in lymphoma patients and MM patients, respectively. Determination of platelet count, VWF level, and CRP level prior to conditioning is recommended to optimize treatment and decrease the potential risks of HSCT, as these parameters are associated with systemic inflammation, endothelial dysfunction, and activation of the primary hemostasis system. Evaluation of the diagnostic value of VWF level—and more recently activity—in the different protocols during HSCT requires further clinical study.

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SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.jtct.2022.08.028.

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