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Clinical and Laboratory Characterization of Acquired Von Willebrand Syndrome

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ABSTRACT

Acquired von Willebrand Syndrome (AVWS) is a rare bleeding disorder characterized by quantitative or qualitative defects of von Willebrand factor (VWF) in patients without a personal or family history of bleeding. It is frequently associated with systemic diseases, particularly lymphoproliferative disorders (LPDs) and myeloproliferative neoplasms (MPNs). In this single-center, retrospective cross-sectional study, we included patients diagnosed with AVWS at the Angelo Bianchi Bonomi Hemophilia and Thrombosis Center between April 2014 and March 2025. Bleeding severity was assessed using the ISTH-BAT score. Laboratory tests included FVIII:C, VWF:Ag, VWF:GPIbR, VWF:RCo, VWF:CB, VWFpp, and multimer analysis. Among 140 patients, 106 (76%) had MPNs and 26 (19%) LPDs. At least one bleeding symptom was observed in 70% of patients, with clinically significant bleeding occurring in 24% of the cohort. Clinically relevant bleeding (mainly mucocutaneous and gastrointestinal) was more frequent and severe in LPDs (58%) than in MPNs (13%). LPDs showed severe VWF functional defects, marked HMWM loss, and elevated VWFpp/VWF:Ag ratios (median 6.7), consistent with accelerated clearance. MPNs displayed mild HMWM reduction, normal clearance (median VWFpp/VWF:Ag ratio 1.0), and an inverse correlation between platelet count and the degree of HMWM depletion ($\rho = -0.48$, $p < 0.001$). Bleeding severity correlated inversely with VWF:GPIbR in LPDs ($\rho = -0.50$, $p = 0.02$) and with VWF:RCo in MPNs. Anti-VWF antibodies were found in 30% of tested LPDs or autoimmune cases. The two main phenotypes presented in AVWS were immune-mediated in LPDs and platelet-mediated in MPNs. Understanding the underlying mechanism is crucial for accurate diagnosis and targeted treatment to reduce bleeding risk and improve outcomes.

1 | Introduction

Acquired von Willebrand Syndrome (AVWS) is a rare yet clinically significant hemorrhagic disorder characterized by a deficiency or dysfunction of von Willebrand factor (VWF) arising in patients without a personal or familial history of bleeding diathesis [1]. Despite being classified as a rare bleeding condition, its true prevalence is likely underestimated due to diagnostic complexity and frequent under recognition in clinical practice. Epidemiological data are scarce, primarily derived from case series and registry-based studies [2–4]. Estimates suggest an incidence of approximately 1–3 cases per million individuals per year [5], though this number may not reflect the true frequency, especially among patients with comorbid conditions [6].

Unlike VWD, AVWS arises in association with a broad spectrum of systemic disorders. The most common are lymphoproliferative disorders (LPDs), myeloproliferative neoplasms (MPNs), cardiovascular diseases, and autoimmune conditions. In a large multicenter analysis, LPDs accounted for approximately 45%–50% of cases, cardiovascular diseases for 20%–25%, and MPNs for 15%–20%, autoimmune diseases contributed to 5%–10%, while miscellaneous causes, including hypothyroidism, solid tumors, and drug-induced forms, represented the remaining 10%–15% [3, 7]. Across this broad spectrum of underlying disorders, AVWS results from secondary, disease-specific mechanisms that alter VWF synthesis, multimeric structure, proteolysis or clearance [8]. Although these processes vary according to the primary condition, they typically converge on the depletion of high-molecular-weight multimers (HMWM), the most functionally active forms of VWF [7].

In the following clinical settings, distinct pathophysiological mechanisms of AVWS have been described. (1) Lymphoproliferative and autoimmune disorders (LPDs, MGUS, multiple myeloma, Waldenström macroglobulinemia). In such cases, AVWS is mainly mediated by nonneutralizing monoclonal IgG or IgM antibodies that form immune complexes with circulating VWF, leading to accelerated clearance via the reticuloendothelial system or hepatic Fcγ receptors [9–11]. Less commonly, neutralizing antibodies directly inhibit VWF function, detectable by Bethesda assays [7], whereas nonneutralizing antibodies require ELISA or immunoblotting [12]. An elevated VWF propeptide (VWFpp)/VWF:Ag ratio indicates increased VWF clearance [13, 14]. (2) MPNs (essential thrombocythemia, polycythemia vera, myelofibrosis). The predominant mechanism involves the high number of platelets leading to pathological interaction between VWF A1 domain and platelet Glycoprotein Ib (GPIb), eventually enhancing ADAMTS13-mediated proteolysis [15–17]. This results in selective reduction or loss of HMWM, particularly in cases of thrombocytosis $>1000\times10^9$/L [17, 18]. Functionally, circulating VWF activity is reduced, whereas VWF:Ag and the VWFpp/VWF:Ag ratio remain normal, suggesting proteolytic degradation rather than increased clearance [19]. (3) Cardiovascular and other mechanisms. AVWS may result from shear stress-induced proteolysis in cardiovascular disorders (e.g., aortic stenosis, LVADs) [20–23] or from altered synthesis, clearance, or regulation of VWF in endocrine, hepatic, or drug-induced conditions [24–26].

From a clinical point of view, AVWS typically presents later in adulthood without previous clinical symptoms, most often with mucocutaneous bleeding (bruising, epistaxis, gingival bleeding, menorrhagia) [5, 15]. Gastrointestinal bleeding often occurs in AVWS secondary to cardiovascular disease, most frequently associated with angiodysplasia (Heyde's syndrome) [21]. Severe cases may involve deep hematomas or hemarthroses [27, 28], while some patients remain asymptomatic despite marked laboratory abnormalities [29, 30].

Diagnosis requires suspicion in adults with new-onset bleeding and an associated systemic condition [31]. Laboratory work-up includes assessment of factor VIII coagulant activity (FVIII:C), VWF antigen (VWF:Ag), and platelet-dependent VWF activity. Traditionally, the latter has been assessed using the VWF ristocetin cofactor assay (VWF:RCo). However, GPIb-based assays are now available, including VWF:GPIbR (ristocetin-dependent binding of VWF to recombinant GPIb) and VWF:GPIbM (ristocetin-independent binding to a gain-of-function GPIb mutant). Measurement of the VWF propeptide (VWFpp) permits calculation of the VWFpp/VWF:Ag ratio, which is useful for evaluating increased VWF clearance. In addition, collagen-binding activity (VWF:CB) and VWF multimer analysis support the identification and characterization of qualitative VWF defects [13, 32–35]. Immune-mediated forms may be confirmed by anti-VWF antibody testing [36]. A comprehensive approach combining bleeding history, functional assays, and specialized testing is essential to establish the final diagnosis.

This study characterizes the clinical and laboratory features of a large cohort of patients with AVWS, emphasizing the relationship between bleeding phenotype, VWF parameters, multimeric patterns, and underlying mechanisms. Particular focus is given to cases linked to hematologic disorders (MPNs and LPDs). By integrating clinical and laboratory data, the study aims to establish a pathophysiology-based classification to enhance diagnosis and guide individualized management.

2 | Methods

2.1 | Study Population

This cross-sectional study included patients diagnosed with AVWS at the Angelo Bianchi Bonomi Hemophilia and Thrombosis Center, Milan, between April 1, 2014, and March 31, 2025. The inclusion was based on VWF activity levels below the normal range (< 50 IU/dL), regardless of the presence of bleeding symptoms. Clinical and laboratory data were retrospectively reviewed to characterize the study population and investigate correlations between phenotypic presentation and underlying pathophysiological mechanisms.

2.2 | Clinical Data

For each patient, clinical data were systematically collected, including age at diagnosis, sex, and the presence of concomitant diseases. Particular attention was given to underlying conditions commonly associated with AVWS, such as hematologic malignancies, cardiovascular disorders, and autoimmune diseases.

A detailed bleeding history was obtained through structured interviews and review of medical records. Bleeding symptoms were quantified using the ISTH-SSC Bleeding Assessment Tool (ISTH-BAT), a standardized scoring system validated for both inherited and acquired bleeding disorders [37]. A bleeding score was considered positive if it was ≥ 4 in adult males or ≥ 6 in adult females, according to established ISTH criteria [38]. Throughout the manuscript, this threshold was used to define a positive bleeding history.

2.3 | Blood Samples Collection and Processing

As part of the diagnostic workup, all patients underwent venous blood sampling for routine coagulation testing and plasma

storage. Blood was collected by standard venipuncture into vacuum tubes containing 1/10 volume of 0.109 M trisodium citrate. Samples were centrifuged within 1 h at 3000 g for 20 min to obtain platelet-poor plasma, which was then frozen and stored at −80°C until analysis. Additional laboratory analyses were integrated to support the investigation of underlying pathophysiological mechanisms.

2.4 | Laboratory Parameters

Laboratory analyses were performed at the Angelo Bianchi Bonomi Hemophilia and Thrombosis Center. The diagnostic evaluation comprised both first- and second-level laboratory assays, as previously described [39, 40]. In brief, FVIII:C was measured by a one-stage clotting assay using FVIII-deficient plasma (Instrumentation Laboratory) on the ACL TOP 700 system (Werfen, Bedford, MA, USA). VWF:Ag was quantified by either enzyme-linked immunosorbent assay (ELISA) or immunoturbidimetric method using the HemosIL VWF:Ag reagent (Werfen) on the ACL TOP 700 analyzer. Platelet-dependent VWF activity was assessed by multiple approaches, including (1) VWF:GPIbR, recombinant GPIb-based assay using HemosIL VWF:GPIbR (Werfen), measuring VWF binding to recombinant GPIb in a ristocetin-dependent manner, and (2) VWF:RCo, automated ristocetin cofactor assay, performed using the BC VWF Reagent (Siemens Healthcare Diagnostics, Marburg, Germany) on the Sysmex CS-2500 system (Sysmex Corporation, Kobe, Japan). For statistical analyses, values below the assay limit of detection (< 6 IU/dL) were not measurable. To allow calculation of summary statistics, these values were imputed with 3 IU/dL, corresponding to half of the lower detection limit. This value is a placeholder used for calculations and does not represent an actual measured factor level.

We further performed the second-level assays. VWF:CB was assessed by ELISA using type I and/or type III collagen substrates (home-made ELISA assay) [41]. VWF multimer analysis was performed using the HYDRAGEL 5 von Willebrand Multimers kit on the semi-automated HYDRASYS 2 SCAN system (Sebia, Lisses, France). Densitometric analysis was performed with the Phoresis 8.6.3 software (Sebia). Multimer fractions were categorized a priori based on densitometric peaks, following the same densitometric approach as previously described [42] and in accordance with the manufacturer's instructions: peaks 1–3 were assigned to low-molecular-weight multimers (LMWM), peaks 4–7 to intermediate-molecular-weight multimers (IMWM), and peaks above 7 to HMWM [42]. This classification was applied consistently across all samples. To quantitatively evaluate the degree of HMWM depletion, the ratio between the percentage of HMWM and the combined percentage of LMWM and IMWM was calculated. This densitometric approach allowed objective grading of multimeric loss and supported visual pattern classification, as previously described [43]. VWFpp antigen was determined with an enzyme-linked immunoassay by using antibodies from Sanquin (Amsterdam, The Netherlands), according to the manufacturer's protocol. We also evaluated anti-VWF antibodies (IgG and IgM), and assessed the presence of inhibitors. Neutralizing antibodies were assessed using the Bethesda assay, whereas nonneutralizing antibodies were detected through ELISA-based methods. Confirmatory assays were performed in-house for patients who tested positive for anti-VWF antibodies, according to the protocol described by Franchi et al. [36]. The Bethesda assay aimed to distinguish between nonneutralizing and neutralizing antibodies by evaluating the capacity of the patients' plasma to reduce exogenous VWF activity in vitro.

Both clinical and laboratory parameters were presented for the entire cohort and for the subgroups of patients with MPNs and LPDs, which were the most represented.

2.5 | Statistical Analysis

Statistical analyses were performed using R version 4.3.0 (R Foundation for Statistical Computing, Vienna, Austria). Continuous variables were tested for normality using the Shapiro–Wilk test, and given the nonnormal distribution of most parameters, data were summarized as median and interquartile range (IQR). Categorical variables, such as sex, were expressed as absolute frequencies and percentages.

The median age and median values (with IQR) for clinical (e.g., ISTH-BAT) and laboratory parameters were reported for the entire study cohort and the two subgroups, as previously reported. The correlation between the ISTH-BAT score and laboratory parameters was evaluated using Spearman's rank correlation coefficient.

Comparative analysis of VWF multimer distribution was performed between patients with MPNs and those with LPDs using the Mann–Whitney U test. The correlation between platelet count and multimeric distribution was assessed using Spearman's correlation. A two-sided $p < 0.05$ was considered statistically significant for all analyses.

3 | Results

A total of 140 patients with a diagnosis of AVWS were included in the analysis. A considerable number of cases were evaluated following referral from the Hematology Unit, either for investigation of a bleeding diathesis or after reduced VWF levels were incidentally identified during the diagnostic workup of a hematologic condition. Of the entire cohort, 106 patients (75.7%) had a history of MPNs, including 11 with polycythemia vera, 41 with essential thrombocythemia, 30 with myelofibrosis, and 24 with MPNs, unclassifiable. Twenty-six patients (18.6%) had LPDs, comprising two with chronic lymphocytic leukemia, 19 with MGUS, one with multiple myeloma, two with Waldenström macroglobulinemia, and two with systemic amyloidosis. The remaining patients included three individuals with autoimmune diseases (2.1%, one systemic lupus erythematosus and two ulcerative colitis), one with severe aortic stenosis (0.7%), one with prostate cancer (0.7%), and three (2.1%) with no identifiable underlying condition at the time of analysis. Sociodemographic and clinical characteristics of the study population are reported in Table 1.

3.1 | Bleeding Phenotype

At least one bleeding manifestation was observed in 70% of the entire cohort, including 65% of patients with LPDs and 25% of those with MPNs. However, a positive bleeding history, as defined by the ISTH-BAT normal ranges for age and sex, was observed in 24% of the overall cohort, with a substantially higher prevalence among patients with LPDs (58%) compared to those with MPNs (13%). Consistently, patients with MPNs demonstrated a milder bleeding phenotype, as witnessed by a lower median ISTH-BAT score (median 1 vs. 3, $p < 0.001$).

The most common bleeding symptoms were cutaneous bleeding (20%) and epistaxis (16%), followed by minor wound bleeding (9%), oral cavity bleeding (6%), and gastrointestinal bleeding (5%) (Figure 1). The subgroup analysis revealed a markedly different bleeding profile between patients with LPDs and those with MPNs. Among LPDs patients, both epistaxis and cutaneous bleeding were reported in 38% of cases, and gastrointestinal bleeding occurred in 21%, indicating frequent and often mucosal bleeding. In contrast, the MPNs group exhibited a milder bleeding pattern, with cutaneous bleeding in 16%, epistaxis in 11%, and a very low prevalence of gastrointestinal bleeding (1%).

3.2 | Laboratory Parameters

Table 2 shows the laboratory characteristics of the study population. Median FVIII:C in the overall cohort was 85 IU/dL (IQR 62–107), with significantly higher levels in MPNs (93 IU/dL, IQR 73–112) compared with LPDs (21 IU/dL, IQR 13–47, $p < 0.001$). Median VWF:Ag was 84 IU/dL, IQR 59–111 overall and differed between groups, being higher in MPNs than in LPDs (96 IU/dL, IQR 74–116 vs. 21 IU/dL, IQR 6–27; $p < 0.001$). In contrast, VWF:GPIbR activity was reduced in both LPDs and MPNs, with a more pronounced decrease in LPDs than in MPNs ($p < 0.001$). The VWF:GPIbR/VWF:Ag ratio was reduced in both cohorts (0.28, IQR 0.21–0.44 in MPNs; 0.37, IQR 0.17–0.58 in LPDs; $p < 0.001$), indicating a qualitative VWF defect. The median VWF:RCo was 48 IU/dL (IQR 37–65)

TABLE 1 | Sociodemographic and clinical characteristics of the study population.

	Total	MPNs	LPDs	*p*
Number	140	106	26	
Age (median, IQR)	57.6 (45.7, 69.2) years	53.5 (42.4, 65.2) years	68.7 (60.9, 72.0) years	< 0.001
Sex (male), *n*. (%)	62 (44)	42 (40)	15 (58)	0.27
ISTH-BAT (median, IQR)	3 (0, 4)	1 (0, 3)	4 (3, 7.25)	< 0.001
Significant bleeding history (*n*, %)	34 (24)	14 (13)	15 (58)	< 0.001
At least one bleeding (*n*, %)	98 (70)	65 (61)	25 (96)	< 0.001

Abbreviations: IQR, interquartile range; ISTH-BAT, International Society on Thrombosis and Hemostasis—Bleeding Assessment Tool; LPDs, lymphoproliferative disorders; MPNs, myeloproliferative neoplasms.

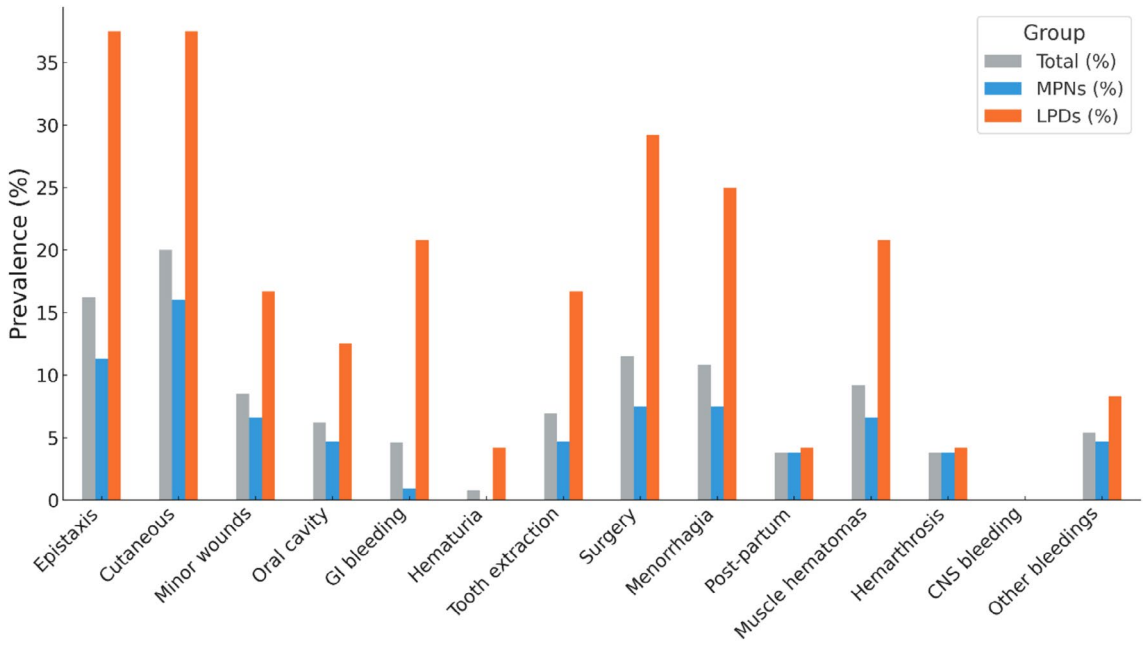

FIGURE 1 | Prevalence of bleeding symptoms in patients with AVWS. Bar chart showing the prevalence (%) of various bleeding symptoms in the total cohort and stratified by diagnostic group (MPNs and LPDs). CNS bleeding, central nervous system bleeding; GI bleeding, gastrointestinal bleeding; LPDs, lymphoproliferative disorders; MPNs, myeloproliferative neoplasms; postpartum, postpartum hemorrhages.

TABLE 2 | Laboratory characteristics of the study population.

	Total	MPNs	LPDs	Normal range	*p*
Number	140	106	26	—	
FVIII:C (IU/dL)	85 (62, 107)	93 (73, 112)	21 (13, 47)	50–147	< 0.001
VWF:Ag (IU/dL)	84 (59, 111)	96 (74, 116)	21 (6, 27)	50–165	< 0.001
VWF:GPIbR (IU/dL)	27 (16, 34)	29 (20.5, 35)	7 (3, 20)	50–168	< 0.001
VWF:GPIbR/VWF:Ag ratio	0.28 (0.21, 0.44)	0.28 (0.22, 0.41)	0.37 (0.17, 0.58)	0.74–1.23	< 0.001
VWF:RCo (IU/dL)	48 (37, 65)	49 (39, 65)	12 (6.5, 17.5)	50–168	0.03
VWF:RCo/VWF:Ag ratio	0.55 (0.42, 0.65)	0.56 (0.46, 0.66)	0.33 (0.19, 0.36)	0.74–1.23	< 0.001
VWF:CB (IU/dL)	64 (47, 68)	65 (53, 82)	7 (3, 21.5)	50–194	< 0.001
VWF:CB/VWF:Ag ratio	0.73 (0.64, 0.83)	0.80 (0.68, 0.85)	0.32 (0.22, 0.33)	—	< 0.001
VWFpp/VWF:Ag ratio	1.1 (0.9, 1.9)	1.0 (0.9, 1.3)	6.7 (3.0, 18.2)	0.5–1.5	< 0.005

Note: Summary of laboratory parameters in the total cohort and stratified by diagnostic group: Myeloproliferative neoplasms (MPNs) and lymphoproliferative disorders (LPDs). Values are reported as medians with interquartile range (IQR).
Abbreviations: FVIII:C, factor VIII coagulant activity; VWF:Ag, von Willebrand factor antigen; VWF:CB, von Willebrand factor collagen binding activity; VWF:CB/VWF:Ag ratio, ratio of VWF collagen binding activity to antigen level; VWF:GPIbR, von Willebrand factor glycoprotein Ib binding activity; VWF:GPIbR/VWF:Ag ratio, ratio of VWF glycoprotein Ib binding activity to antigen level; VWFpp/VWF:Ag ratio, ratio of VWF propeptide to antigen level; VWF:RCo, von Willebrand factor ristocetin cofactor activity; VWF:RCo/VWF:Ag ratio, ratio of VWF ristocetin cofactor activity to antigen level.

overall, higher in MPNs (49 IU/dL, IQR 39–65) than in LPDs (12 IU/dL, IQR 6.5–17.5; $p = 0.03$), with a significantly lower VWF:RCo/VWF:Ag ratio in LPDs ($p < 0.001$). VWF:CB was markedly reduced in LPDs (7 IU/dL, IQR 3–21.5) compared to MPNs (65 IU/dL, IQR 53–82; $p < 0.001$), and the VWF:CB/VWF:Ag ratio confirmed severe loss of HMWM in LPDs (0.32 vs. 0.80; $p < 0.001$). The VWFpp/VWF:Ag ratio was normal in MPNs (1.0, IQR 0.9–1.3) but significantly elevated in LPDs (6.7, IQR 3.0–18.2; $p < 0.005$), suggesting accelerated VWF clearance in the latter.

3.3 | Multimer Analysis

The comparison of HMWM distribution, expressed as the ratio HMWM/(LMWM + IMWM), revealed a tendency toward variation among patient groups (Figure 2). MPNs exhibited higher multimer ratios, indicating a relatively preserved profile of HMWM. In contrast, LPDs showed consistently lower ratios, reflecting a more pronounced depletion of HMWM (representative multimer patterns are shown in Figure S1). Although this trend suggests a greater impairment of HMWM in LPDs, the difference between the two groups did not reach statistical significance ($p = 0.12$).

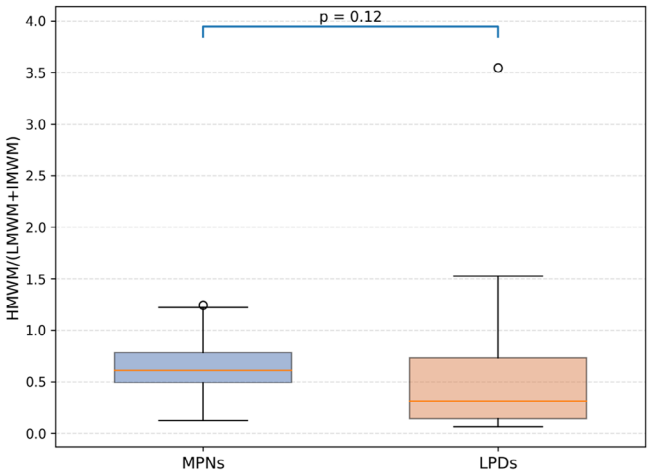

FIGURE 2 | Distribution of VWF multimers in MPNs and LPDs. Boxplot comparing the ratio of high-molecular-weight multimers to the sum of low- and intermediate-molecular-weight multimers (HMWM/[LMWM + IMWM]) in patients with myeloproliferative neoplasms (MPNs) and lymphoproliferative disorders (LPDs). Lower ratios indicate a greater relative depletion of high-molecular-weight multimers, the most functionally active forms of von Willebrand factor, and therefore reflect a more clinically relevant multimeric defect.

Furthermore, in patients with MPNs, we evaluated the relationship between platelet count and VWF multimeric distribution (Figure 3). A statistically significant negative correlation was observed between platelet count and the HMWM/(LMWM + IMWM) ratio ($\rho = -0.48$, $p < 0.001$), suggesting that higher platelet levels are associated with a greater loss of VWF HMWM.

To further address heterogeneity within the MPN cohort, exploratory analyses were performed by disease subtype. The inverse correlation between platelet count and the HMWM/(LMWM + IMWM) ratio remained evident in essential thrombocythemia ($\rho = -0.60$, $p = 0.002$) and myelofibrosis ($\rho = -0.57$, $p = 0.035$), while a borderline trend was observed in unclassified MPNs ($\rho = -0.57$, $p = 0.053$). No significant association was detected in polycythemia vera ($\rho = 0.40$, $p = 0.379$), although the number of evaluable cases was limited.

3.4 | Correlation Between ISTH-BAT and Laboratory Parameters

Table 3 shows the correlation between laboratory markers and the bleeding score (ISTH-BAT) in patients with MPNs and

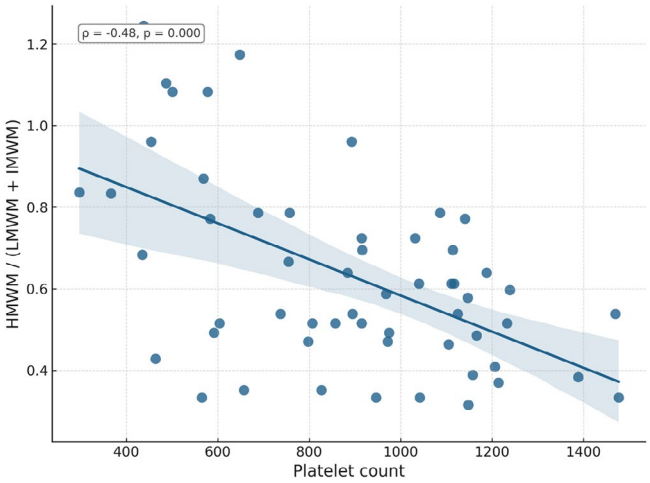

FIGURE 3 | Correlation between platelet count and multimeric distribution in myeloproliferative neoplasms. Scatterplot showing the relationship between platelet count and the ratio between high-molecular-weight multimers (HMWM, %) and the sum of low- and intermediate-molecular-weight multimers (LMWM, %+IMWM, %) of von Willebrand factor in patients with myeloproliferative neoplasms (MPNs).

TABLE 3 | Spearman correlation between bleeding score (ISTH-BAT) and laboratory parameters.

	MPNs		LPDs	
	Spearman rho	*p*	Spearman rho	*p*
FVIII:C	−0.24	0.05	−0.33	0.14
VWF:GPIbR	−0.02	0.89	**−0.50**	**0.02**
VWF:GPIbR/VWF:Ag ratio	0.10	0.40	−0.34	0.13
VWF:RCo	**−0.25**	**0.07**	−0.50	0.67
VWF:RCo/VWF:Ag ratio	−0.08	0.59	−0.50	0.67
VWF:CB	−0.11	0.44	−0.16	0.71
VWF:CB/VWF:Ag ratio	0.00	0.98	0.08	0.83

Note: Spearman's rank correlation coefficients (ρ) and *p* values for the association between bleeding score and selected laboratory parameters in patients with MPNs and LPDs.
Abbreviations: FVIII:C, factor VIII coagulant activity; LPDs, lymphoproliferative disorders; MPNs, myeloproliferative neoplasms; VWF:CB, von Willebrand factor collagen binding activity; VWF:CB/VWF:Ag ratio, ratio of VWF collagen binding activity to antigen level; VWF:GPIbR, von Willebrand factor glycoprotein Ib binding activity; VWF:GPIbR/VWF:Ag ratio, ratio of VWF glycoprotein Ib binding activity to antigen level; VWF:RCo, von Willebrand factor ristocetin cofactor activity; VWF:RCo/VWF:Ag ratio, ratio of VWF ristocetin cofactor activity to antigen level.

LPDs. In the MPNs group, no statistically significant associations were identified. However, an inverse trend was observed between VWF:RCo and bleeding score ($\rho = -0.25$, $p = 0.07$), suggesting that reduced VWF:RCo levels can be considered in this population a reliable marker of increased bleeding tendency. FVIII:C showed a similar trend. In contrast, a moderate and statistically significant inverse correlation was found in the LPDs group between VWF:GPIbR activity and ISTH-BAT ($\rho = -0.50$, $p = 0.02$), indicating that reduced VWF:GPIbR levels may better reflect clinical bleeding severity as measured by the ISTH-BAT in this population. Other variables, such as FVIII:C and the VWF:GPIbR/VWF:Ag ratio, also showed negative correlations, albeit not reaching statistical significance.

3.5 | Anti-VWF Antibody Testing

Anti-VWF antibodies (IgG and IgM) were investigated in a subset of 20 patients with suspected immune-mediated AVWS, including 15 with LPDs, two with autoimmune diseases, and three with MPNs. Patients who tested positive for anti-VWF IgG and/or IgM were also evaluated for inhibitors using a Bethesda assay. IgG and/or IgM anti-VWF antibodies were identified in six patients (30%), comprising five patients with LPDs and one patient with systemic lupus erythematosus, with no antibody-positive cases observed in the MPNs group.

4 | Discussion

In this large cohort of patients with AVWS, we identified two distinct clinicopathological profiles associated with the underlying hematologic disorder. Patients with LPDs exhibited a laboratory pattern characterized by increased VWF clearance, marked depletion of HMWM, and, in our cohort, a more severe bleeding phenotype. This profile supports an immune-mediated mechanism leading to accelerated VWF clearance and loss of the most functionally active VWF multimers. Conversely, AVWS associated with MPNs was characterized by higher residual VWF activity, normal VWFpp/VWF:Ag ratios, an inverse association between platelet count and VWF multimer integrity, and generally milder bleeding phenotypes despite abnormal functional assays.

Our findings confirm the well-documented heterogeneity in bleeding presentation among AVWS subgroups and align with data from the ISTH registry, which identifies LPDs as one of the most symptomatic forms of AVWS, often associated with severe bleeding due to the loss of functionally active VWF multimers [3, 34, 44]. In contrast, a substantial proportion of patients with MPNs in our cohort had no positive bleeding history according to ISTH-BAT thresholds, despite clear laboratory evidence of AVWS. This dissociation between laboratory phenotype and clinical manifestations has been reported by Song et al. [45] and Sánchez-Luceros et al. [46], who observed that MPNs-associated AVWS is often subclinical, with hemorrhagic events occurring less frequently than in other etiologies such as LPDs or autoimmune diseases. The reasons for this discrepancy may include differences in the degree of functional impairment, the preservation of certain platelet-VWF interactions, or compensatory hemostatic mechanisms in patients with MPNs. However, an important consideration is that MPNs patients may have been more frequently referred for VWF testing as part of the evaluation of thrombocytosis or routine hematologic workup, whereas LPDs patients may have been more likely to undergo testing because of bleeding manifestations. If so, this indication bias may have amplified the apparent difference in bleeding

burden between groups and should be taken into account when interpreting clinical comparisons.

Multimeric analysis was a key discriminator between subgroups. In LPDs-associated AVWS, we observed a pronounced and selective loss of HMWM. This pattern is consistent with immune-mediated clearance and has been previously described in patients with monoclonal gammopathies or B-cell malignancies, where paraproteins or autoantibodies form immune complexes with VWF, accelerating its removal from the circulation [10, 28, 47]. In patients with MPNs, only a slight loss of HMWM was observed, often showing features suggestive of proteolysis secondary to interactions with platelet GPIb. These findings are consistent with the reports of Mital et al. [48] and Kubo et al. [49], who highlighted the role of platelet-VWF binding in promoting multimer degradation in MPNs, particularly in essential thrombocythemia and polycythemia vera. Thus, while the clinical severity of AVWS may have been influenced by referral bias, the laboratory differences between the two groups remained biologically coherent and support distinct underlying mechanisms.

A notable finding of our analysis was the correlation between platelet-dependent VWF activity assays and the severity of clinical bleeding manifestations. Among patients with MPNs, VWF:RCo demonstrated a stronger correlation with ISTH-BAT scores than VWF:GPIbR. In this subgroup, VWF:GPIbR frequently produced disproportionately low activity values, suggesting a laboratory overestimation of defect severity compared with the actual clinical picture, a finding consistent with the observations of Noye et al. [50]. In line with this, patients with MPNs showed a more markedly reduced VWF:GPIbR/VWF:Ag ratio than patients with LPDs, despite exhibiting a more preserved VWF multimer pattern. This indicates that the extent of multimer disruption is less pronounced than would be inferred from VWF:GPIbR results alone, suggesting that the assay may overestimate defect severity in MPNs. Moreover, the VWF:CB/VWF:Ag ratios in the two patient groups were more consistent with the observed multimeric pattern and bleeding phenotype, further supporting a discrepancy between VWF:GPIbR results and the underlying biology in MPNs-associated AVWS. Taken together, these findings indicate that, in MPNs-associated AVWS, assay methodology can markedly influence diagnostic interpretation, and VWF:GPIbR results should be interpreted with caution and not relied upon in isolation. One possible explanation for the unexpectedly low VWF:GPIbR values observed in MPNs-associated AVWS is related to assay-specific characteristics. Because VWF:GPIbR is measured in plasma using an artificial ristocetin-dependent GPIb-binding system, the assay may be particularly sensitive to reductions in HMWM, the most functionally active VWF species. In this setting, even partial HMWM depletion may result in disproportionately low VWF:GPIbR values, thereby accentuating the apparent severity of the defect. This interpretation is supported by the closer agreement of multimer pattern, VWF:CB/VWF:Ag ratio, and bleeding phenotype observed in our MPNs patients. Conversely, in LPDs-associated AVWS, the VWF:GPIbR assay appeared more sensitive in detecting functional abnormalities associated with higher ISTH-BAT scores compared to VWF:RCo. This suggests that, in the setting of immune-mediated pathology, VWF:GPIbR may better reflect the underlying pathogenic alterations of VWF than the VWF:RCo assay.

A major strength of this study is the large nature of the cohort and comprehensive laboratory assessment performed in this AVWS population, encompassing both standard VWF parameters and second-level assays which enabled detailed characterization of the different pathophysiological subtypes. The comparative design, with MPNs- and LPDs-associated AVWS analyzed side by side under identical laboratory conditions within the same referral center, also reduced analytical variability and strengthened the interpretation of subgroup-specific differences in assay performance.

Among the limitations of this study, the relatively small sample size of the LPDs cohort should be acknowledged. In addition, selection bias is likely, particularly within the MPNs subgroup, where VWF testing may have been performed more often as part of disease evaluation or because of thrombocytosis rather than because of bleeding symptoms. Conversely, testing in LPDs patients may have been more frequently prompted by clinically evident bleeding. This imbalance in testing indication may have influenced the observed prevalence of bleeding and may have affected the interpretation of the relationship between hemostatic laboratory abnormalities and ISTH-BAT scores. A further methodological consideration concerns the handling of values below the assay limit of detection, which were imputed with a fixed value for statistical purposes. While this approach allowed inclusion of these observations in descriptive analyses, it may have influenced results involving platelet-dependent VWF assays near the lower limit of detection. Also, the pathogenic mechanisms were not systematically characterized across the entire cohort. Although anti-VWF antibodies were detected in several patients with LPDs, systematic testing would be required to determine their overall prevalence and clarify their pathogenic significance.

In conclusion, this study on a large cohort of patients provides clinically relevant insights into AVWS associated with hematologic disorders. Within the limits of a retrospective referral-based cohort, the underlying condition (MPNs versus LPDs) was associated with distinct laboratory and clinical profiles. In our series, MPNs-associated AVWS was characterized by lower bleeding severity, normal VWF clearance, mild HMWM loss, and thrombocytosis, whereas LPDs-associated AVWS showed higher bleeding severity, immune-mediated clearance, and marked multimer depletion. From a diagnostic standpoint, a context-specific interpretation of assays is essential: in MPNs, low VWF:GPIbR values should be confirmed by complementary tests, while in LPDs, VWF:GPIbR may better reflect functional impairment. Overall, these findings support a mechanism-based interpretation of AVWS in hematologic disorders and indicate that management should be tailored to the underlying disease biology, as summarized in Figure 4. In MPN-associated AVWS, the laboratory profile of preserved VWF clearance, mild HMWM loss, and platelet-count dependence suggests that cytoreductive therapy and control of the underlying myeloproliferative clone may be the most biologically coherent strategy, whereas DDAVP or VWF-containing concentrates may be mainly useful for short-term bleeding control or peri-procedural support. In this context, the intrinsic thrombotic risk of MPNs should be taken into account when considering DDAVP. In LPD-associated AVWS, by contrast, the pattern of increased VWF clearance and marked HMWM depletion is more consistent with treatment of

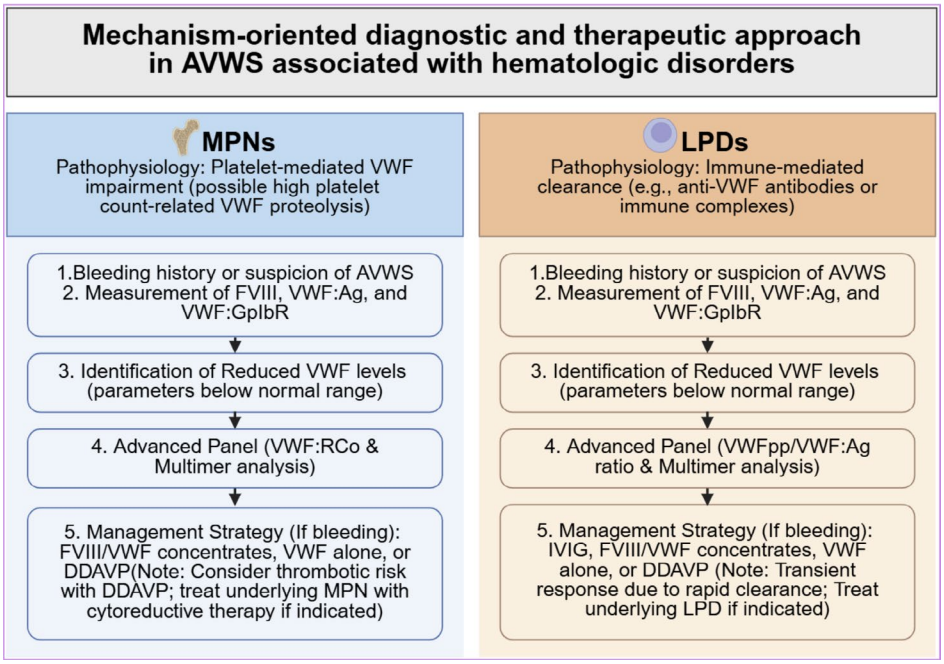

FIGURE 4 | Mechanism-oriented diagnostic and therapeutic approach in AVWS associated with hematologic disorders. Schematic representation of a mechanism-based diagnostic and therapeutic pathway for acquired von Willebrand syndrome (AVWS) in myeloproliferative neoplasms (MPNs) and lymphoproliferative disorders (LPDs). In MPNs, AVWS is mainly driven by platelet-mediated von Willebrand factor (VWF) impairment, whereas in LPDs it is primarily related to immune-mediated clearance. The figure outlines key diagnostic steps and mechanism-oriented management strategies according to the underlying pathophysiology. AVWS, acquired von Willebrand syndrome; DDAVP, desmopressin; IVIG, intravenous immunoglobulins; LPDs, lymphoproliferative disorders; MPNs, myeloproliferative neoplasms; VWF:Ag, von Willebrand factor antigen; VWFpp, von Willebrand factor propeptide; VWF:RCo, von Willebrand factor ristocetin cofactor activity. Created with BioRender.com.

the underlying clone and, in selected cases, immunomodulatory approaches such as intravenous immunoglobulins. In patients with elevated VWFpp/VWF:Ag ratios, suggesting shortened VWF survival, the hemostatic response to DDAVP or replacement therapy may be short-lived, potentially requiring closer monitoring and, in selected situations, repeated dosing or continuous infusion strategies. Taken together, these findings support an individualized, mechanism-oriented approach to the diagnosis and management of AVWS in hematologic disorders.

Author Contributions

A.C., E.I., D.C., A.T., and S.M.S. enrolled the patients and collected clinical data. P.C. and M.T.P. performed laboratory testing. A.C., L.B., O.S., and S.M.S. conceived the study concept and design, and jointly interpreted the results. A.C. and E.I. analyzed the data. A.C., L.B., and O.S. wrote the first draft of the manuscript. A.I., S.M.S., and F.P. critically revised the manuscript and approved the final version. All authors reviewed the manuscript and agreed on its submission.

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Ethics Statement

This retrospective observational study was approved by the Ethics Committee "Comitato Etico Milano Area 2" (Milan, Italy). The study was conducted in accordance with the Declaration of Helsinki and applicable local regulations. Given the retrospective design of the study and the use of anonymized data, the requirement for written informed consent was waived by the Ethics Committee.

Consent

Patient informed consent was waived by the Ethics Committee "Comitato Etico Milano Area 2" because of the retrospective nature of the study and the use of anonymized data.

Conflicts of Interest

F.P. reports participation at educational meetings of Sanofi, Sobi, Biomarin, Kedrion and Bayer, and the advisory board of Sanofi, Sobi, Roche, CSL Behring, Novo Nordisk, Biomarin, Regeneron, Metagenomi, Star-Therapeutics, Bayer, Pfizer, Takeda, TargED. A.C. reports participation at educational meetings of Roche and Novo Nordisk, and the advisory board of Bayer. The other authors declare no conflicts of interest.

Data Availability Statement

All relevant data are provided within the manuscript. Additional information can be requested from the corresponding authors at flora.peyvandi@unimi.it.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Figure S1:** Representative VWF multimer patterns in patients with MPNs and LPDs. Densitometric profiles of VWF multimers from representative patients with myeloproliferative neoplasms (MPNs) and lymphoproliferative disorders (LPDs) compared with normal pooled plasma (NPP). Multimer fractions are divided into low-molecular-weight (LMWM), intermediate-molecular-weight (IMWM), and high-molecular-weight multimers (HMWM). In MPN-associated AVWS, only a partial reduction of HMWM is observed, with relatively preserved high-molecular-weight fractions and modest changes in the HMWM/(LMWM + IMWM) ratio. In contrast, LPD-associated AVWS shows a marked depletion of HMWM, with a shift toward lower-molecular-weight fractions and substantially reduced HMWM/(LMWM + IMWM) ratios. These representative patterns illustrate how lower HMWM/(LMWM + IMWM) values reflect a greater loss of functionally active multimers and a more pronounced multimeric defect. Black lines represent normal pooled plasma (NPP), and colored lines represent patient samples (Pt).