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Increased von Willebrand factor levels in polycythemia vera and phenotypic differences with essential thrombocythemia

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Abstract

Background: Acquired von Willebrand factor (VWF) deficiency was described in Philadelphia-negative myeloproliferative neoplasms, especially in essential thrombocythemia (ET). VWF phenotype in contemporary patients with polycythemia vera (PV) remains less explored.

Objectives: To characterize the VWF phenotype in PV and to compare VWF phenotype in PV with matched healthy subjects and ET patients.

Patients/Methods: We studied 48 PV patients, treated according to current recommendations (hematocrit ≤ 45%, on low-dose aspirin prophylaxis); 48 healthy and 41 subjects with ET, all sex, age, and blood group matched. We measured VWF antigen, activity, multimeric pattern, ADAMTS-13, and factor VIII (FVIII) antigen.

Results: In patients with PV, VWF antigen and activity were significantly higher than in healthy subjects (antigen: 119[96-137] vs 93[79-107] IU/dL; activity: 114[95-128] vs 90[79-107] IU/dL, respectively, medians and interquartile, $P < 0.01$), with normal multimeric distribution. ADAMTS-13 levels were similar between patients with PV and healthy subjects. FVIII levels were higher in PV than in healthy subjects (141[119-169] versus 98[88-123] IU/dL, respectively, $P < 0.01$). By multivariable analysis, JAK2-p.V617F allelic burden, erythrocyte count, and male sex significantly predicted VWF antigen and activity levels. As compared to patients with ET, patients with PV showed similar VWF antigen levels but approximately 40% higher activity (79[49-104] vs 112[93-125] IU/dL, respectively, $P < 0.01$).

Conclusions: Patients with PV show increased VWF and FVIII levels, predicted by JAK2-p.V617F burden and erythrocyte count. At variance with ET, acquired VWF defect was not observed in PV. High VWF/FVIII levels may sustain the thrombotic diathesis of PV and may be investigated as biomarkers for risk stratification.

KEYWORDS

essential thrombocythemia, factor VIII, myeloproliferative neoplasms, polycythemia vera, von Willebrand factor

Authors Monica Sacco, Paola Ranalli, and Stefano Lancellotti equally contributed to this study.

Essentials

- The von Willebrand factor (VWF) pattern in polycythemia vera (PV) is poorly explored.
- VWF levels in PV were higher than in controls and predicted by JAK2-p.V617 burden and erythrocytes.
- VWF activity in PV was significantly higher than in matched patients with essential thrombocythemia.
- High VWF and factor VIII may favor thrombosis in PV.

1 | INTRODUCTION

Polycythemia vera (PV) is a *BCR/ABL*-negative chronic myeloproliferative neoplasm (MPN), characterized by increased erythrocytes production and mass.[1] The genotypic hallmark of PV is the Janus Kinase (JAK) 2-p.V617F mutation, which is a major diagnostic criterion in the 2008 and 2016 World Health Organization (WHO) classifications, along with increased hemoglobin and hematocrit levels.[2-4] The JAK2-p.V617F is a gain-of-function mutation resulting in a constitutively activated JAK2 tyrosine kinase,[2] which alters the proliferation of bone marrow cells, gives an advantage to the erythrocytes, and suppresses erythropoietin production. This mutation occurs in ≥ 95% of patients with PV and in approximately 50% of patients with another MPN that is essential thrombocythemia (ET).[4] The clinical course of PV is characterized by an elevated incidence of venous and arterial thromboses, higher than in the general population.[5,6] A recent observational study of nearly 10 000 patients with MPN showed that the hazard ratios for arterial thrombosis in patients with PV at 3 months, 1 year, and 5 years from diagnosis were 2.7-fold (95% confidence interval, 2.2-3.3), 1.9-fold (1.6-2.1), and 1.5-fold (1.3-1.7) higher than in the matched general population. Similarly, the hazard ratios for venous thrombosis were 13.1 (8.7-19.6), 5.4 (4.1-7.0), and 3.6 (3.0-4.4) in the same time frame.[7] Due to the increased thrombotic risk associated with PV, antiplatelet prophylaxis with low-dose aspirin is routinely recommended in both primary and secondary cardiovascular prevention, based on the results of the European Collaboration on Low-dose Aspirin in Polycythemia (ECLAP) trial.[6,8] However, the residual risk of major thrombosis in aspirin-treated patients with PV appears still higher than the risk associated with a non-MPN aspirin-treated matched population, that is, >1%/year,[6,9] also in recent observational studies.[10]

Various abnormalities of circulating von Willebrand factor (VWF) have been described in patients with MPNs, usually associated with severe thrombocytosis.[11-13] The main abnormalities consisted of loss of larger VWF multimers and high proteolysis of VWF, all associated with thrombocytosis, mostly resulting in bleeding.[11,14,15] In a previous study on ET, we observed a deficiency of VWF activity and selectively of large VWF multimers over a wide range of platelet counts, rather than in patients with severe thrombocytosis only.[16] This acquired VWF deficiency was likely a continuous phenomenon, driven by high ongoing in vivo platelet activation, which is the hallmark of ET, with consequent platelet-dependent VWF consumption.[17]

Defects of VWF multimers have been less frequently reported in PV with thrombocytosis,[18] and considered also responsible for bleeding events. More recently, an acquired but asymptomatic VWF decrease was observed in ≈12% of 142 patients with PV.[19] Interestingly, platelet counts in these patients were still within the normal range and only slightly higher than in patients with normal VWF (390 000 vs 285 000 platelets/μL, respectively).[19] This recent finding appears inconsistent with older studies.[12]

We investigated the VWF phenotype and its determinants in a contemporary PV cohort with optimal hematocrit control and ongoing aspirin prophylaxis. Furthermore, we compared the VWF phenotype in 2 matched PV and ET cohorts, to assess whether the VWF pattern is similar or rather disease specific.

2 | METHODS

2.1 | Populations under study

This is an observational, cross-sectional study that included 48 patients with PV diagnosed according to the WHO 2008 criteria for MPNs,[3] and when applicable, criteria were reassessed according to the subsequent WHO 2016 revision.[4] The patients were treated according to current recommendations with phlebotomy ± hydroxyurea to keep the hematocrit ≤ 45%,[20] and all patients were on low-dose aspirin prophylaxis (100 mg once daily). Exclusion criteria were cigarette smoking; clinically significant hepatic, renal, cardiac, or pulmonary insufficiency; history of malignant neoplasms other than MPN; poorly controlled hypertension or hypercholesterolemia; pregnancy or lactation; patients requiring chronic treatment with nonsteroidal anti-inflammatory drugs; use of anticoagulants or antiplatelet agents other than aspirin; a recent (<6 months) major vascular event (myocardial infarction or stroke); a recent bleeding (<6 months) or congenital bleeding disorder; obesity (body mass index ≥ 30 kg/m^2). The study was approved by the Ethics Committee of the Santo Spirito Hospital of Pescara, all patients gave their written informed consent.

VWF and factor VIII (FVIII) levels from 48 anonymized healthy subjects, sex, age, and blood group matched with patients with PV (32 M, 16 F; age 67 [57-75] years, median and interquartile range (IQR); 33% blood group O, all *P* > 0.05 vs patients with PV) were retrieved from the anonymized database of the Servizio Malattie Emorragiche e Trombotiche, Fondazione Policlinico Universitario "A. Gemelli" IRCCS, Rome.

To compare PV and ET, we included data from 41 patients with ET of a previously published cohort by our group, who were selected to match patients with PV based on age, sex, and blood group.[16]

2.2 | VWF studies

Patients underwent routine hematochemistry tests, other than VWF and FVIII studies, in the central laboratory of the Hospital of Pescara. For VWF and FVIII studies, patient blood samples were collected by using clean venipuncture with citrate anticoagulant 1:9 (v/v, sodium citrate/blood, final concentration 0.109 M). Plasma was centrifuged at 2000 g for 5 minutes at 20°C. VWF antigen (VWF:Ag) was measured by an automatic instrument (ACL AcuStar; Instrumentation Laboratory, Milan, Italy) using a chemiluminescence assay (Instrumentation Laboratory). VWF activity (VWF:Act) was as measured by a collagen-binding assay using the Asserachrom ELISA kit (Diagnostica Stago, Parsippany, NJ, USA), which explores the collagen-binding capacity of the high-molecular-weight multimers of VWF. The latter were studied with SDS–agarose gel electrophoresis (0.8% agarose, stacking gel –1.5% agarose, running gel), as previously detailed.[16] The deficiency of high-molecular-weight multimers of VWF was defined by the VWF:Act/VWF:Ag ratio < 0.6, that has been shown to reflect a loss of high-molecular-weight multimers,[16] as in the case of type 2A or 2B von Willebrand disease.[21] FVIII levels were measured by an ELISA method (Diagnostica Stago).

2.3 | ADAMTS-13 measurements

The level of ADAMTS-13 antigen was determined using an ELISA kit developed by American Diagnostica Inc (IMUBIND® ADAMTS13 ELISA, Sekisui Diagnostics, Lexington, MA, USA).[22]

2.4 | JAK2-p.V617F allelic burden

Quantitative real time PCR was used to detect the JAK2-p.V617F mutation. Briefly, genomic DNA obtained from peripheral blood mononuclear cells isolated from whole blood was amplified with ipsogen JAK2 MutaScreen RS kit (QIAGEN GmbH, Hilden, Germany) and analyzed by ABI PRISM 7900HT instruments (Applied Biosystems, Austin, TX, USA).

2.5 | Statistical analysis

Based on our previous study,[7] where VWF:Act levels in patients with ET was reduced by ~ 20% compared to healthy subjects, we assumed a difference of at least 20% between the mean VWF (VWF:Ag and VWF:Act) levels also in patients with PV as compared with healthy subjects. With a standard error < 30% and a power of 0.90, we calculated that 48 PV and 48 control subjects were needed (type I error, 0.05).

The sample size was calculated by using the Power and Sample Size Program. Bonferroni's correction of data was applied to avoid biases from unequal variance and type I error. Spearman analysis was performed to correlate VWF-related parameters with other hematological parameters. The statistically significant values (P < 0.05) were used as independent variables in multivariable regression analysis (2-sided P < 0.05). Analyses were performed using SPSS software (version 21; IBM, Armonk, NY, USA), and Prism software (GraphPad Software, Inc, La Jolla, CA, USA). Data are expressed as median and [interquartile range], or means ± SD, as indicated.

3 | RESULTS

3.1 | Phenotypic features of VWF in patients with PV as compared to matched healthy subjects

Routine hematological and biochemical characteristics of 48 enrolled patients with PV are listed in Table 1; 13 patients had a previous thrombotic event, none had a previous bleeding.
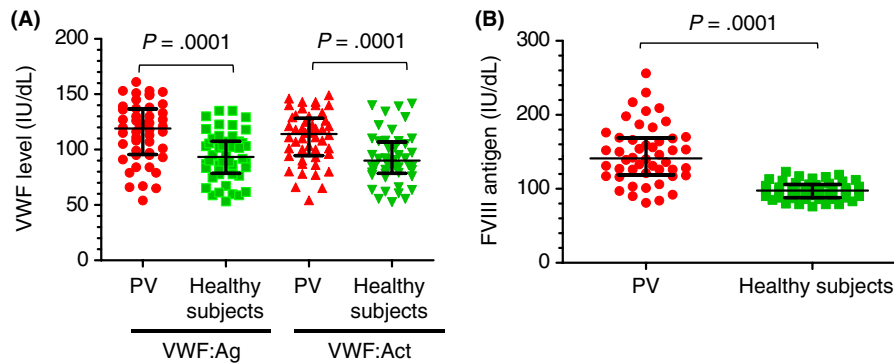
VWF:Ag and VWF:Act levels in patients with PV were significantly higher as compared to VWF:Ag and VWF:Act levels in 48 healthy subjects who were selected to be age, sex, and blood group matched to patients with PV (Figure 1A). By contrast, VWF:Act/VWF:Ag ratios were similar in subjects with PV and healthy subjects (0.97 ± 0.1 vs 0.96 ± 0.1, mean ± SD, respectively, P = 0.27). SDS–agarose gel electrophoresis analyses also showed a normal multimeric pattern of VWF in patients with PV, which included the larger multimers (Figure 2). Consistently with increased VWF levels, also FVIII levels were higher in PV than in healthy subjects (Figure 1B). Moreover, ADAMTS-13 levels were comparable

**TABLE 1** Characteristics of 48 patients with PV

Parameters	Values
Age, y	67.5 [58.0-74.5]
Females, n (%)	16 (33)
Hydroxyurea, n (%)	36 (75)
Blood group type O, n (%)	16 (33)
Phlebotomy, n (%)	35 (73)
JAK2-p.V617F allelic burden, %	58.0 [34.0-82.3]
LDL, units/mL^{-1}	428 [351-511]
Hematocrit, %	41.7 [34.5-44.9]
Hemoglobin, g/dL	13.9 [13.0-14.9]
Erythrocytes, ×10^3/μL	5.0 [4.3-6.5]
Leukocytes, ×10^3/μL	8.1 [6.5-11.3]
Neutrophils, %	68.6 [62.7-77.6]
Platelets, ×10^3/μL	349 [248-458]
Immature platelets, ×10^3/μL	9.3 [6.5-15.7]
Previous thrombosis (arterial and venous), n (%)	14 (29)
Disease duration, y	5.6 ± 5.3

Note: Data are expressed as median and [interquartile range], mean ± SD or as frequency and percentage, as specified.

JAK2-p.V617F: Janus kinase 2-p.V617F; LDL: low-density lipoprotein; PV: polycythemia vera.

FIGURE 1 VWF and FVIII levels in patients with PV and in matched healthy subjects. (A) VWF:Ag and VWF:Act in 48 patients with PV (red circles) and in 48 age-, sex-, and blood group–matched healthy subjects (green squares). The figure shows individual data. Horizontal bars represent the median; vertical bars are the interquartile range. (B) The plot shows individual FVIII levels in patients with PV and healthy subjects. Horizontal bars represent medians; vertical bars are the interquartile range. FVIII, factor VIII; PV, polycythemia vera; VWF:Act, von Willebrand factor activity; VWF:Ag, von Willebrand factor antigen
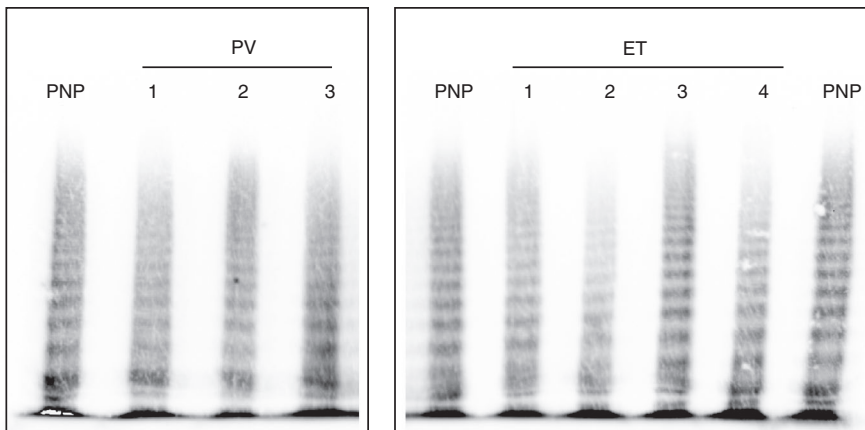
FIGURE 2 VWF multimeric pattern in patients with PV and ET. SDS-agarose gel showing typical patterns of VWF multimers in 3 patients with PV and in 4 patients with ET and in pooled normal plasma (PNP) for reference. In the lower panel, the table indicates the values of VWF:Ag and VWF:Act levels of the corresponding plasma samples. ET, essential thrombocythemia; PV, polycythemia vera; VWF:Act, von Willebrand factor activity; VWF:Ag, von Willebrand factor antigen

Line	VWF:Act (IU/dL)	VWF:Ag (IU/dL)	Line	VWF:Act (IU/dL)	VWF:Ag (IU/dL)
PV1	80	79	ET1	62	102
PV2	115	123	ET2	68	127
PV3	78	84	ET3	42	82
PNP	94	99	ET4	55	133

between PV and healthy subjects: 581 [530-621] ng/mL), and 570 [506-728] ng/mL, respectively (P = 0.83).

Differently from healthy subjects, VWF:Ag and VWF:Act levels in patients with PV showed only a nonsignificant trend toward lower values in O versus non-O blood groups (VWF:Ag: 120 ± 26 vs 105 ± 25 IU/dL; VWF:Act: 114 ± 24 vs 101 ± 17 IU/dL, in non-O vs O blood groups, respectively; both P = 0.08). On univariate analysis (Table 2), VWF:Ag values in patients with PV were positively and significantly correlated with VWF:Act and FVIII levels (Figure 3A,B). Moreover, both VWF:Ag and VWF:Act levels were significantly and directly correlated with age, male sex, hydroxyurea treatment and dose, and JAK2-p.V617F allelic burden (Figure 3C), while they were inversely correlated with erythrocyte (Figure 3D) and platelet counts. The correlation coefficients

and level of significance are reported in Table 2. We did not find any significant correlation of VWF:Ag and VWF:Act with body mass index (normal vs overweight patients), leukocytes (any type), disease or antiplatelet therapy duration. By multivariable analysis, in PV patients VWF:Act level could be significantly predicted by erythrocyte count (inversely), JAK2-p.V617F allelic burden and male sex (adjusted R^2 for the entire model: 0.42, all P < 0.05). Likewise, VWF:Ag was independently predicted by erythrocyte count (inversely), JAK2-p.V617F allelic burden and male sex (adjusted R^2: 0.40, all P < 0.05).

Moreover, VWF:Act values in the 14 patients with PV who had a previous thrombotic event were significantly higher than VWF:Act values of patients with PV without a thrombotic history (121 [109-149] IU/dL vs 110 [93-146] IU/dL, respectively, P = 0.04).

TABLE 2 Univariate analysis of the main parameters in 48 PV patients

| Variable	VWF:Act	JAK2-p.V617F burden	VWF:Ag	Sex (male)	Hydroxyurea treatment	Erythrocytes	Platelets	Age
VWF:Act	1.00	0.26	0.95**	0.42**	0.42**	−0.39**	−0.39**	0.34*
JAK2-p.V617F burden	0.26	1.00	0.34*	0.35*	−0.04	0.13	0.05	−0.01
VWF:Ag	0.95**	0.34*	1.00	0.45**	0.37*	−0.32*	−0.36*	0.34*
Sex (male)	0.42**	0.35*	0.45**	1.00	0.20	0.13	−0.37*	0.11
Hydroxyurea treatment	0.42**	−0.04	0.37*	0.20	1.00	−0.55**	−0.47**	0.59**
Erythrocytes	−0.39**	0.13	−0.32*	0.13	−0.55**	1.00	0.21	−0.38**
Platelets	−0.39**	0.05	−0.36*	−0.37*	−0.47**	0.21	1.00	−0.39**
Age	0.34*	0.01	0.34*	0.11	0.59**	−0.38**	−0.39**	1.00

Note: The table shows the rho coefficients according to Spearman and their level of significance.

JAK2-p.V617F: Janus kinase 2-p.V617F; PV, polycythemia vera; VWF:Act: von Willebrand factor activity; VWF:Ag: von Willebrand factor antigen.

*$P < 0.05$; ** $P < 0.01$.

FIGURE 3 Correlations between VWF:Ag and VWF:Act, FVIII, JAK2-p.V617F, or erythrocytes in 48 patients with PV. (A) Correlation between VWF:Ag and VWF:Act levels. (B) Correlation between VWF:Ag and FVIII levels; (C) Correlation between VWF:Ag and JAK2-p.V617F allelic burden; (D) Correlation between VWF:Ag and erythrocyte count; the rho and P values are indicated in each panel. FVIII, factor VIII; JAK2-p.V617F: Janus kinase 2-p.V617F; PV: polycythemia vera; VWF:Act, von Willebrand factor activity; VWF:Ag, von Willebrand factor antigen
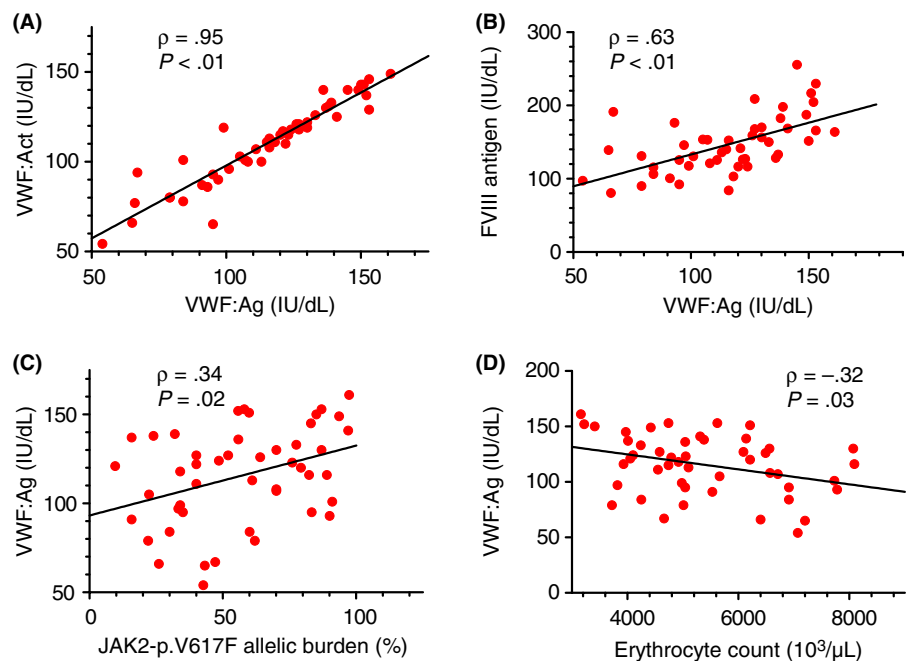
3.2 | Phenotypic features of VWF in PV compared to matched patients with ET

VWF:Ag and VWF:Act levels in 41 patients with PV were also compared to the levels measured in 41 patients with ET selected from a previously-published cohort to be sex, age, and blood group matched with patients with PV (Table 3).[16] While VWF:Ag levels were similar in patients with PV and ET (Figure 4A), the VWF:Act values were significantly higher in patients with PV as compared to patients with ET (Figure 4A), and VWF:Act/VWF:Ag ratios were also higher in patients with PV as compared to patients with ET (Figure 4B). The electrophoretic VWF pattern confirmed a defect of large VWF multimers in patients with ET only (Figure 2). In the 41 patients with PV included in this comparison, JAK2-p.V617F allelic burden was also directly correlated with

VWF:Ag and VWF:Act (Figure 5A,B). However, in patients with ET, JAK2-p.V617F was not associated with VWF:Ag (140 ± 49 and 132 ± 74 IU/dL in JAK2-p.V617F-positive and -negative patients, respectively, $P = 0.7$) or with VWF:Act (89 ± 43 and 78 ± 60 IU/dL in JAK2-p.V617F-positive and -negative patients, respectively, $P = 0.6$). Moreover, within the JAK2-p.V617F-positive ET patient subgroup, the allelic burden was not associated with VWF:Ag or VWF:Act levels (Figure 5C,D).

4 | DISCUSSION

To our knowledge, this is the first study describing VWF phenotype in a relatively large and contemporary cohort of patients with PV, diagnosed according to revised WHO criteria and treated according

TABLE 3 Characteristics of matched patients with PV and ET for VWF comparison

Parameters	PV (n = 41)	ET (n = 41)
Age, y	65 ± 10	64 ± 12
Females, n (%)	16 (33)	17 (41)
Hydroxyurea, n (%)	30 (73)	25 (61)
Blood group type O, n (%)	14 (34)	13 (32)
Phlebotomy, n (%)	24 (59)	0*
JAK2-p.V617F, n (%)	41 (100)	26 (63)*
Hematocrit, %	43.5 ± 3	41 ± 39
Hemoglobin, g/dL	13.9 ± 1.3	14 ± 1.3
Erythrocytes, ×10³/μL	5.2 ± 1.3	4.4 ± 0.8*
Leukocytes, ×10³/μL	9.2 ± 4.2	6.9 ± 1.9*
Platelets, ×10³/μL	358 ± 168	426 ± 126*

Note: Data are expressed as mean ± SD, or as frequency and percentage, as specified.

ET, essential thrombocythemia; JAK2-p.V617F, Janus kinase 2-p.V617F; LDL, low-density lipoprotein; PV, polycythemia vera.

*$P < 0.05$.

to current recommendations vis-à-vis both healthy subjects and subjects with ET, all matched with patients with PV. We observed (a) significantly higher levels of both VWF:Ag and VWF:Act in patients with PV than in healthy subjects, independently predicted by erythrocyte count, JAK2-p.V617F mutation allelic burden, and male sex; (b) significantly higher FVIII levels than in healthy subjects; and (c) a distinct VWF phenotype in PV as compared to ET in the 2 matched cohorts.

Sozer et al[23] detected the JAK2-p.V617F mutation in hepatic endothelial cells (ECs) of patients with PV with Budd-Chiari syndrome. JAK2-p.V617F in ECs may account for a high release of circulating VWF in humans, as recently shown in mouse models.[24,25] Furthermore, previous studies showed that the JAK2-p.V617F may contribute to a systemic chronic inflammation in MPNs, mediated by cytokines such as pentraxin-3.[26] Systemic inflammation may also stimulate the release of VWF from ECs.[27] It

was also demonstrated that PV erythrocytes with a constitutively activated JAK2 mutant are abnormally adherent to ECs via the erythroid Lutheran/basal cell-adhesion molecule and endothelial laminin.[28,29] All these findings suggest a prevalent activation and degranulation of ECs in PV that may maintain a high release of VWF into the circulation.[30]

High levels of VWF have been shown to be associated with major vascular events in large observational studies of non-MPN patients at high cardiovascular risk,[31-37] which is consistent with its role as mediator of platelet adhesion to the subendothelial matrix and subsequent platelet activation.[38] Increased levels of VWF may then sustain both macro- and microcirculatory disturbances in PV. In fact, histopathological studies in erythromelalgia show platelet-rich microthrombi, endothelial inflammation, intimal proliferation, and VWF deposition in the microcirculation.[39] As VWF is a physiological chaperone for FVIII,[30] FVIII levels increased in parallel to VWF in PV. High FVIII levels per se are associated with increased venous thromboembolism[40-42] and may then contribute to venous thromboses in PV.[40] In fact, FVIII accelerates the formation of activated factor X (FXa) and thrombin.[43] Thus, our study suggests that the thrombotic diathesis of PV may be supported by high VWF and FVIII levels in addition to other known pathogenetic factors such as high hematocrit,[44] elevated blood viscosity,[45] platelet activation,[8,17] and leukocytosis.[46] Notably, patients with previous thrombosis had the highest VWF:Act levels. Therefore, levels of VWF/FVIII should be explored as biomarkers of thrombosis, useful for risk stratification in PV. Further studies are needed to investigate the mechanisms linking JAK2-p.V617F burden, VWF/FVIII, and the thrombotic complications in PV.

Both VWF:Ag and VWF:Act levels in patients with PV were inversely associated with erythrocyte count. A similar association was reported by Mital et al[19] in 142 patients with PV in whom those with VWF:Ag and VWF:Act < 60% and < 50%, respectively, had significantly higher erythrocyte counts. Moreover, PV erythroblasts were found to have increased calreticulin expression on plasma membrane,[47] and calreticulin seems to be involved in the
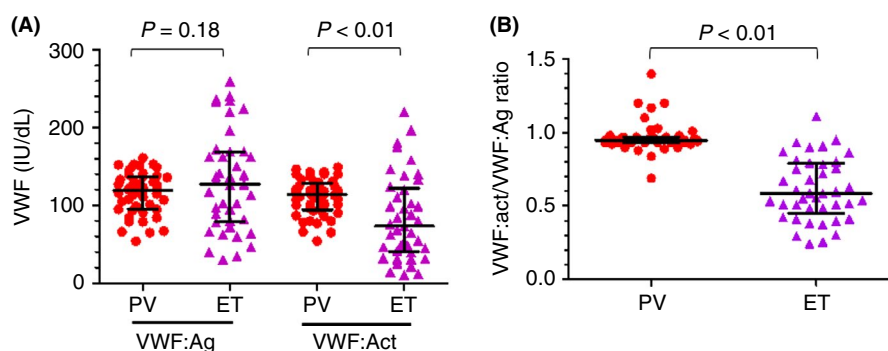
FIGURE 4 VWF levels in PV and in matched patients with ET. (A) VWF:Ag and VWF:Act levels in 41 patients with PV (red circles) and in 41 patients with ET age, sex, and blood group matched (purple triangles). The figure shows individual data. Horizontal bars represent median; vertical bars are interquartile range. (B) VWF:Act/VWF:Ag ratios in patients with PV and ET. The plot shows individual data. Horizontal bars represent the median; vertical bars are the interquartile range. ET, essential thrombocythemia; PV, polycythemia vera; VWF:Act, von Willebrand factor activity; VWF:Ag, von Willebrand factor antigen

FIGURE 5 Association between VWF levels and JAK2-p.V617F mutation in matched patients with PV and ET. Panels A and B show the correlations between VWF antigen and activity, respectively and JAK2-p.V617F allele burden in 41 patients with PV (red circles); panels C and D show the same correlations in 26 patients with ET (purple triangles) owing the same mutation. ET, essential thrombocythemia; PV, polycythemia vera; VWF, von Willebrand factor
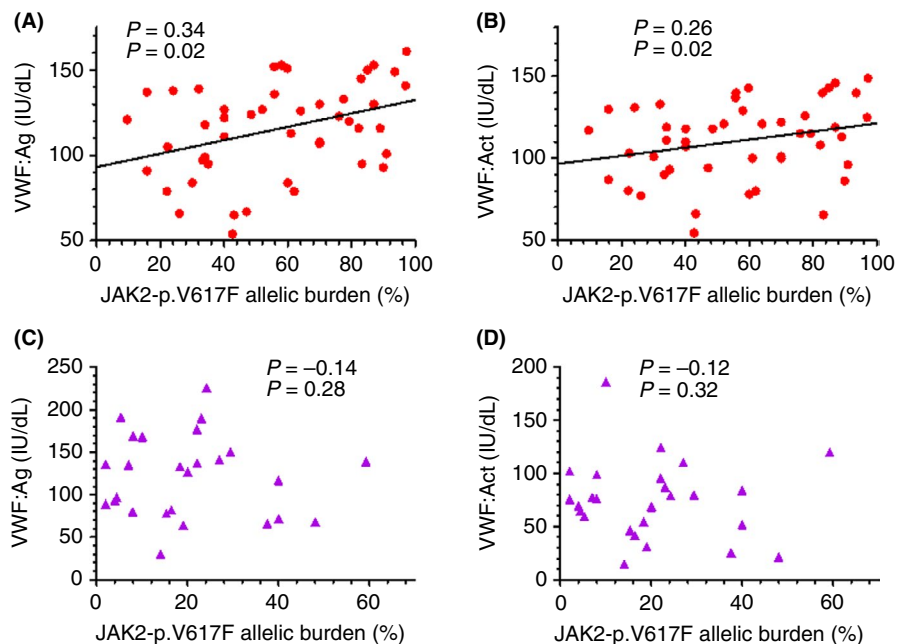
degradation of the circulating VWF/FVIII complex.[48] We also observed a significant association of VWF levels with male sex, not observed in healthy subjects.[49] Interestingly, in a study on over 250 patients with MPN, a significantly higher JAK2-p.V617F allele burden was reported in male versus female patients with PV, even after adjusting for age and disease duration.[50] The direct correlation of JAK2-p.V617F allelic burden with VWF levels (Figure 3C) may possibly contribute to sex-related difference. Interestingly, PV appears to have a sex-related gene (de)regulation and consequent clinical features.[51] However, the mechanism(s) associating erythrocyte counts and/or sex to VWF phenotype deserve further investigations.

Overall, the VWF phenotype substantially differed between patients with PV and matched patients with ET and appeared rather disease specific. To our knowledge, this is the first study that compared 2 matched PV and ET cohorts, since in other studies PV and ET cohorts were never matched. PV showed no VWF acquired defect with normal VWF:Act/Ag ratio and multimeric pattern. Differently from patients with PV, patients with ET showed an acquired VWF:Act defect with a clear unbalance between VWF:Ag and VWF:Act and reduced high-molecular-weight VWF multimers. VWF:Act defect in ET could be inversely predicted only by mature and immature platelet count[1,16] while in PV, platelet count was not confirmed in multivariable analysis, where erythrocytes, JAK2 mutation, and male sex were stronger predictors. Therefore, in ET the VWF proteolysis seems prevalent even at relatively lower platelet counts and appears largely driven by the underlying ET-specific abnormal platelet production and activation.[16] Also among JAK2-positive patients with ET a correlation between the mutation burden and VWF levels could not be observed (Figure 5) at variance with previous data.[52] The cause of these differences among ET and PV is unknown, although some hypotheses may be formulated. JAK2 mutation could be less relevant in ET, likely due to the different clonality

of this mutation with a different expression in ECs and red blood cells. Moreover, high platelet activation and turnover that characterize ET appear to drive the impact on the pathophysiology of VWF.

Current thrombosis-preventive strategies in PV recommend hematocrit < 45% and low-dose aspirin. Based on our study, these interventions, which have proved their efficacy in randomized trials,[6,53] may not be sufficient in PV to target increased VWF/FVIII and possibly thrombin generation through FXa. Thus, a low degree of FXa blockade as achieved by very low-dose rivaroxaban (2.5 mg twice daily) may have a rationale to be tested in patients with PV. Recently, the combination of low-dose aspirin and very low-dose rivaroxaban (2.5 mg twice daily) was superior to aspirin alone in reducing major adverse cardiovascular events of patients with stable cardiovascular disorders.[54] Considering that aspirin-treated patients with PV show a residual thrombotic risk comparable to stable cardiovascular patients, in spite of aspirin (up to 4.4%/yr)[6,55] and that our study suggests a role for FVIII and thrombin generation, the low-dose aspirin and rivaroxaban combination may be beneficial in patients with PV.

Some limitations of our study warrant consideration. Our study is relatively small in size and observational in nature. Another limitation is that mutations other than JAK2-p.V617F[56] were not explored in patients with PV.

In conclusion, VWF and FVIII levels are increased in PV, with no evidence of defects and with a substantially different pattern from ET. VWF and/or FVIII levels may be independent risk factors for thrombosis and may be worth testing as biomarkers of risk stratification of patients with PV. These data provide a rationale for testing combined antithrombotic regimens in PV, which target both platelets and coagulation factor(s).

RELATIONSHIP DISCLOSURE

The authors declare nothing to report.

AUTHOR CONTRIBUTIONS

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