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Acquired von Willebrand Syndrome During the Course of Myelofibrosis: Analysis of 32 Cases

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A – research concept and design; **B** – collection and/or assembly of data; **C** – data analysis and interpretation; **D** – writing the article; **E** – critical revision of the article; **F** – final approval of article

Abstract

Background. Identification of patients with myelofibrosis being at increased risk of acquired von Willebrand syndrome (avWS) would likely facilitate individualization of treatment and improve its outcomes.
Objectives. To determine the prevalence of avWS in patients with myelofibrosis, and to verify if individuals with and without this bleeding disorder differ in terms of their baseline clinical parameters.
Material and Methods. The study included 32 consecutive patients with myelofibrosis. avWS was diagnosed on the basis of abnormally low levels of von Willebrand factor and other routine tests. Patients with and without concomitant avWS were compared in terms of their demographic characteristics, present and past medical histories and laboratory parameters.
Results. Concomitant avWS was found in 5 patients (15.6%). In 1/5 patients with avWS and in 8/27 persons without this bleeding disorder, myelofibrosis developed secondarily to polycythemia vera (n = 7) or essential thrombocytopenia (n = 2). As many as 4/5 individuals with avWS presented with clinical evidence of a bleeding disorder. The subjects with avWS differed from the remaining patients with myelofibrosis in terms of significantly lower activity of von Willebrand factor (vWF) and lower vWF to vWF antigen ratio.
Conclusions. All patients with myelofibrosis should be routinely evaluated for avWS with the panel of specific tests. Further, avWS should be the primary suspicion in each patient with myelofibrosis in whom clinical evidence of a bleeding disorder has emerged (**Adv Clin Exp Med 2015, 24, 6, 1001–1006**).

Key words: coagulology, bleeding disorders, hemorrhage, von Willebrand factor.

Acquired von Willebrand syndrome (avWS) is a rare disorder with the spectrum of clinical and laboratory findings resembling that of the hereditary form of von Willebrand disease, but with no evidence of prior bleeding abnormalities, older age at diagnosis and negative family history [1, 2]. Although avWS frequently develops secondarily to myeloproliferative neoplasms [3], its true incidence in patients with these conditions and clinical implications thereof are still not fully estimated. We have recently confirmed that avWS may be present in a considerable proportion of patients with two common types of myeloproliferative neoplasms, polycythemia vera (PV) [4] and essential thrombocythemia (ET) [5]. Myelofibrosis, a rare chronic myeloproliferative neoplasm which frequently develops secondarily to ET or PV, was also shown to be associated with avWS [6]. However, this evidence originates mostly from isolated case reports and small series [7, 8]. Having access to a relatively large subset of patients with myelofibrosis who have been tested for avWS, we undertook this study to determine the true prevalence of these two conditions and its clinical implications.

Patients and Methods

The study included 32 consecutive patients treated for myelofibrosis at the University Clinical Center, Medical University of Gdańsk between

2004 and 2013. Mean age of the patients was 66.1 ± 11.3 years (range 39–88 years). The study group included 13 (40.6%) women and 19 (59.4%) men. The protocol of the study was approved by the Local Bioethics Committee at the Medical University of Gdansk, and written informed consent was sought from all the participants to use their clinical data for research purposes.

Myelofibrosis was diagnosed on the basis of WHO criteria from 2008 [9]. avWS was diagnosed on the basis of abnormally low levels of von Willebrand factor (vWF, reference limit: 60–150%), von Willebrand antigen (vWF:Ag, reference limit: 50––160%), vWF ristocetin cofactor activity (vWF:Rco, reference limit: 60–170%) and vWF collagen binding activity (vWF:CB, reference limit: 40–250%) or abnormal (< 0.6) values of vWF:Rco/vWF:Ag and vWF:CB/vWF:Ag ratios. avWS was distinguished from the hereditary form of von Willebrand disease on the basis of a lack of personal and family history of bleeding disorders.

Patients with and without concomitant avWS were compared in terms of their demographic characteristics (sex distribution, age at diagnosis of myelofibrosis and age at the time of testing for avWS) and past medical histories. Moreover, we have compared their characteristics at the time of testing for avWS: clinical activity of myelofibrosis, prevalence of typical clinical symptoms (including signs of a bleeding disorder) and laboratory parameters (complete blood count, especially platelet count, coagulation profile, concentration of C-reactive protein, activity of lactate dehydrogenase, prevalence of JAK2 mutations and results of specific tests for avWS: activity of vWF, factor VIII and factor IX, level of vWF:Ag, vWF/vWF:Ag ratio and vWF:CB).

Normal distributions of continuous variables were verified with a Kolmogorov-Smirnov test, and their statistical characteristics were presented as arithmetic means, 95% confidence intervals (95% CIs) and ranges. The statistical characteristics of discrete variables were presented as numbers and fractions. A Student *t*-test or Mann-Whitney *U* test were used for intergroup comparisons of continuous variables, and distributions of discrete variables were compared with a Fisher exact test. All calculations were carried out with STATISTICA 10 package (StatSoft, USA), with the threshold of statistical significance set at $p \leq 0.05$.

Results

Demographic Characteristics of the Patients

The group of 32 patients with myelofibrosis included 5 individuals (15.6%) who were diagnosed with avWS, among them 1 woman and 4 men. The distribution of sex among patients with concomitant avWS did not differ significantly as compared to the remaining subjects with myelofibrosis (12/27 women and 15/27 men, p = 0.625).

Moreover, the two groups did not differ significantly in terms of their age at diagnosis of myelofibrosis and age at the time of testing for avWS (Table 1).

In one case, avWS was diagnosed at the same time as myelofibrosis; in the remaining cases, clinical and laboratory evidence of avWS was found 3 years (n = 1), 4 years (n = 2) and 10 years (n = 1) following the diagnosis of the primary condition.

In 1/5 patients with avWS and in 8/27 persons without this bleeding disorder, myelofibrosis developed secondarily to polycythemia vera (n = 7) or essential thrombocytopenia (n = 2). The intergroup difference in the prevalence of secondary myelofibrosis was not statistically significant (p = 0.660).

Clinical Symptoms at the Time of Testing for avWS

None of the patients with myelofibrosis were in remission at the time of testing for avWS. Furthermore, no significant intergroup differences were found in the occurrence of splenomegaly (3/5 vs. 16/27, p = 0.975), hepatomegaly (3/5 vs. 8/27, p = 0.310), signs of bleeding disorders (4/5 vs. 12/27, p = 0.333) and thrombosis (1/5 vs. 0/27, p = 0.156) at the time of testing.

Table 1. Statistical characteristics of age in myelofibrosis patients with/without concomitant avWS

Parameter	avWS (n = 5)		Lack of avWS (n = 27)		p-value
	mean (95% CI)	range	mean (95% CI)	range	
Age at diagnosis of myelofibrosis (years)	61.2 (44.3–78.1)	44–77	58.7 (53.7–63.7)	36–85	0.659
Age at the time of testing for avWS (years)	65.4 (49.8–81.0)	54–81	66.2 (61.8–70.7)	39–88	0.876
Time between diagnosing myelofibrosis and avWS testing (years)	4.2 (0.3–8.7)	0–10	7.5 (5.2–9.9)	0–20	0.308

Laboratory Parameters at the Time of Testing for avWS

Complete Blood Count

Patients with concomitant avWS did not differ from the remaining individuals with myelofibrosis in terms of any of the complete blood count parameters including platelet count, except for a significantly lower absolute basophil count and lower (at the threshold of statistical significance) absolute eosinophil count (Table 2).

Coagulation Profile

Subjects with concomitant avWS did not differ from the remaining individuals with myelofibrosis in terms of any of the analyzed parameters of the coagulation profile (Table 3).

Other Laboratory Parameters

Patients with concomitant avWS did not differ significantly from the remaining persons with myelofibrosis in terms of their lactate dehydrogenase (421.60 [95% CI: 221.53–621.67] vs. 473.15 [359.64–586.66], p = 0.938) and C-reactive protein concentrations (7.98 [1.32–14.64] vs. 11.48 [5.28–17.68], p = 0.421), as well as with regards to the occurrence of JAK-2 mutations (3/5 vs. 18/27, p = 0.773). Detailed analysis showed that the signs of bleeding disorders were present in 12/21 patients with JAK-2 mutation and in 4/11 without (p = 0.458) and thrombosis in 1/21 and 0/11, respectively (p = 0.462).

Specific Tests for avWS

Individuals with concomitant avWS differed from the remaining patients with myelofibrosis solely in terms of significantly lower activity of vWF and lower vWF/vWF:Ag ratio (Table 4).

Discussion

Although the occurrence of avWS in patients with myelofibrosis and other myeloproliferative neoplasms is a well-established phenomenon [9], the true co-prevalence of these conditions is still unknown and the available data in this matter is fairly inconclusive. Furthermore, the vast majority of evidence originates from single case reports or small case series [7, 8, 10–12]. In the case of

Table 2. Complete blood counts in myelofibrosis patients with/without concomitant avWS

Parameter	avWS (n = 5)		Lack of avWS (n = 27)		p-value
	mean (95% CI)	range	mean (95% CI)	range	
Erythrocytes ($\times 10^6$/L)	3.28 (2.23–4.32)	2.49–4.6	3.64 (3.34–3.95)	2.2–5.2	0.350
Hemoglobin concentration (g/dL)	10.36 (7.00–13.72)	7.2–14.0	11.31 (10.46–12.17)	6.6–16	0.421
Hematocrit level (%)	32.08 (21.74–42.42)	21.4–42	35.61 (33.25–37.98)	22–46	0.405
MCV (fl)	97.64 (83.01–112.27)	85.9–116	98.97 (94.90–103.04)	84–119	0.876
MCH (pg)	31.26 (27.62–34.90)	28.9–36	31.60 (30.22–32.98)	26–39	0.897
MCHC (g/dL)	32.58 (31.59–33.57)	32–33.6	31.81 (31.25–32.38)	28.9–35	0.189
Leukocytes ($\times 10^3$/L)	7.25 (1.30–15.79)	1.42–19.2	11.44 (7.22–15.66)	2.41–56.23	0.153
Neutrophils ($\times 10^3$/L)	5.31 (1.85–12.48)	0.6–15.36	8.13 (4.53–11.73)	1.37–49.23	0.153
Neutrophils (%)	65.16 (45.83–84.49)	42.2–80	65.80 (58.68–72.92)	3.60–87.9	0.836
Eosinophils ($\times 10^3$/L)	0.07 (0.10–0.25)	0–0.33	0.18 (0.11–0.26)	0–0.8	0.051
Eosinophils (%)	1.42 (1.99–4.83)	0–6.30	1.88 (1.28–2.49)	0–6.2	0.101
Basophils ($\times 10^3$/L)	0.03 (0.02–0.07)	0–0.09	0.16 (0.06–0.26)	0–0.94	0.039
Basophils (%)	0.74 (0.20–1.68)	0–1.7	1.36 (0.77–1.96)	0–6	0.391
Lymphocytes ($\times 10^3$/L)	1.28 (0.26–2.30)	0.67–2.66	2.05 (1.31–2.80)	0.47–9.9	0.264
Lymphocytes (%)	24.08 (6.62–41.54)	14–47.2	20.13 (16.14–24.11)	6.5–43.2	0.815
Monocytes ($\times 10^3$/L)	0.54 (0.03–1.06)	0.13–1.15	0.63 (0.43–0.84)	0–2.45	0.795
Monocytes (%)	8.60 (3.68–13.52)	4.8–15	6.89 (4.95–8.82)	0–20.3	0.264
Platelets ($\times 10^3$/L)	309.20 (31.60–586.80)	59–669	343.78 (252.19–435.37)	11–902	0.999

Table 3. Coagulation profile in myelofibrosis patients with/without concomitant avWS

Parameter	avWS (n = 5)		Lack of avWS (n = 27)		p-value
	mean (95% CI)	range	mean (95% CI)	range	
Activated partial thromboplastin time (s)	37.40 (30.51–44.29)	33–46	35.00 (33.20–36.80)	23–46	0.619
Prothrombin time (s)	13.20 (11.16–15.24)	12–16	12.48 (12.10–12.87)	11–14	0.480
Prothrombin index (%)	90.00 (78.29–101.71)	74–99	92.81 (89.97–95.66)	82–111	0.835
Thrombin time (s)	18.40 (16.98–19.82)	17–20	18.48 (17.67–19.29)	14–23	0.999
Fibrinogen (g/L)	3.60 (2.18–5.02)	2.53–5.19	3.79 (3.29–4.28)	2.12–6.53	0.775
D-dimer (μg/L)	931.38 (873.51–2736.28)	170–3518	726.12 (435.64–1016.61)	209–2955	0.350
ADP closure time (s)	175.20 (116.82–233.58)	126–251	159.52 (130.42–188.62)	69–300	0.421
EPI closure time (s)	202.00 (138.34–265.66)	143–284	218.67 (195.67–241.66)	89–300	0.466

Table 4. Parameters used in the diagnosis of avWS in myelofibrosis patients with/without concomitant avWS

Parameter	avWS (n = 5)		Lack of avWS (n = 27)		p-value
	mean (95% CI)	range	mean (95% CI)	range	
vWF (%)	86.00 (16.24–155.76)	40–181	157.67 (136.44–178.89)	80–308	0.019
Factor VIII (%)	131.80 (82.47–181.13)	81–178	162.63 (130.19–195.07)	82–489	0.568
Factor IX (%)	101.50 (53.44–149.56)	69–140	131.94 (105.59–158.29)	84–230	0.420
vWF:Ag (%)	118.80 (51.97–185.63)	50–184	171.85 (150.76–192.95)	82–286	0.087
vWF/vWF:Ag ratio	0.71 (0.48–0.95)	0.49–0.98	0.93 (0.84–1.02)	0.61–1.94	0.033
Collagen-binding assay (vWF:CB) (%)	107.40 (34.38–180.42)	55–200	128.00 (113.72–142.28)	70–200	0.231
vWF:CB/vWF:Ag ratio	1.10 (0.11–2.09)	0.30–2.41	0.78 (0.69–0.86)	0.43–1.47	0.276

myelofibrosis, this is additionally complicated by the fact that this condition may develop secondarily to ET or PV [6]. Therefore, some patients may present with avWS before being diagnosed with myelofibrosis.

To the best of our knowledge, the hereby presented series of 32 patients with myelofibrosis who have been examined for avWS is the largest analyzed to date. Our findings imply that avWS may develop in *ca.* 15% of individuals with myelofibrosis. In only one case, avWS was diagnosed at the same time as myelofibrosis and, importantly, this patient had no history of previous ET or PV. However, our series also included one individual with avWS in whom myelofibrosis developed secondarily to ET. Although probably the largest to date, our sample was still relatively small and the patients were not routinely tested for avWS at the time of myelofibrosis detection but rather when the suspicion of a bleeding disorder emerged. Therefore, it cannot be unequivocally concluded if avWS observed in the course of myelofibrosis is really a direct consequence of this condition. According to the literature, 5–30% of patients with ET also may develop bleeding disorders of various types [13], and we recently showed that even up to 20% of individuals with this myeloproliferative neoplasm may present with concomitant avWS [5]. Furthermore, avWS was shown to develop in more than 10% of patients with PV [4]. Owing to the fact that myelofibrosis may also develop secondarily to ET or PV [6], avWS observed in the course of this condition may also be inherent to one of these myeloproliferative neoplasms.

Both our previous observations of patients with ET or PV [4, 5] and the literature evidence imply that avWS associated with myeloproliferative neoplasms develops due to absorption of vWF on the surface of malignant cells [14–18] and/or due to the loss of the high-molecular-weight multimers of vWF due to their exposure to high shear stress and proteolysis [19–21]. Since myelofibrosis, ET and PV share common etiologies [6], the mechanisms leading to development of avWS

during the course of these conditions are likely also the same.

In 4 out of the 5 patients with concomitant avWS included in our series this condition coexisted with typical signs of a bleeding disorder. However, according to the literature, many patients who develop avWS secondarily to a myeloproliferative neoplasm can be asymptomatic [7, 8]. This is also consistent with the results of our recently published research on the co-occurrence of PV and avWS, in which clinical evidence of this bleeding disorder was found in no more than *ca.* 60% of patients with these two conditions [4]. Nevertheless, the hereby presented data implies that it is avWS which should be primarily excluded as a potential cause of bleeding abnormalities during the course of myelofibrosis. Importantly, our patients with concomitant avWS differed significantly from the remaining subjects with myelofibrosis in terms of some specific tests used routinely in the evaluation for this bleeding disorder, namely vWF activity, vWF:Ag level and vWF/vWF:Ag ratio [22]. This finding implies that all patients with myelofibrosis should be tested for avWS with the commonly available tests – if not routinely, at least whenever the suspicion of a bleeding disorder emerges. Notably, all the hereby reported coagulology tests were conducted at least 7 days after discontinuation of platelet aggregation inhibitors to exclude false positive results, which should be a standard of evaluation for avWS.

Importantly, we also showed that 12 out of 27 myelofibrosis patients without concomitant avWS (*ca.* 44%) presented with bleeding abnormalities. Therefore, aside from avWS, other causes of bleeding disorders, e.g. impaired function of platelets, should also be considered in subjects with myelofibrosis and concomitant bleeding abnormalities. Consequently, platelet aggregation assay seems to be the test of choice in avWS-negative patients with myelofibrosis and clinical evidence of bleeding abnormalities.

Interpreting the hereby presented findings, one should consider the potential methodological limitations of this study which are primarily related to its retrospective character.

Up to 15% of patients with myelofibrosis may develop avWS. The etiopathogenic mechanisms behind avWS in myelofibrosis patients seem to be similar to other myeloproliferative neoplasms. Optimally, all patients with myelofibrosis should be routinely evaluated for avWS with the panel of specific tests. Further, avWS should be the primary suspicion in each patient with myelofibrosis in whom clinical evidence of a bleeding disorder has emerged.

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