Von Willebrand Factor Multimeric Assay in Acquired von Willebrand Disease Diagnosis: A Report of Experience from North Estonia Medical Centre

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Abstract

Objective Acquired von Willebrand syndrome (AVWS) is a rare and frequently underdiagnosed bleeding disorder with an unknown prevalence. The diagnosis of AVWS is made based on laboratory investigations and the presence of clinical symptoms. Evaluation and management of affected patients are complex due to the need for multiple laboratory assays.

Materials and Methods Here, we describe the clinical and laboratory data of seven patients with a diagnosis of AVWS. All patients met the criteria for AVWS based on laboratory findings, bleeding symptoms, and the absence of any previous history of a bleeding disorder.

Results In all cases, the laboratory findings, lack of bleeding anamnesis, and family history suggested the presence of AVWS. Von Willebrand factor multimeric analysis showed decreased high-molecular weight (HMW) multimers in six cases. Patients with lower HMW multimers experienced more severe bleeding complications.

Conclusions The diagnosis of AVWS is complex and requires extensive laboratory evaluation. Interdisciplinary collaboration and complex laboratory evaluations are of paramount importance for the early recognition of AVWS and optimal AVWS diagnosis as well as successful clinical management.
Introduction

Acquired von Willebrand syndrome (AVWS) is a rare and frequently underdiagnosed bleeding disorder, mainly due to the broad spectrum of possible clinical and laboratory features affiliated with this condition. The mechanisms behind von Willebrand factor (VWF) abnormalities depend upon the type of underlying disorder and may include increased clearance; enhanced shear stress and subsequent proteolysis; inhibition of VWF functions; adsorption to the platelet surface; or, rarely, decreased synthesis.1

The definition of AVWS was published by the VWF subcommittee in 2000.2 A diagnosis of AVWS can be made based on the following criteria: the existence of a lack of previous lifelong bleeding incidents and relevant family history, clinical picture, and laboratory investigation results,3 for example, VWF levels and factor VIII (FVIII) coagulant activity (FVIII:C) are sometimes decreased, a reduced VWF function/antigen ratio can indicate the existence of functional disorders, even if the absolute activity is within the normal limit, a loss or decrease in high-molecular weight (HMW) multimers may also be observable. The prevalence of AVWS remains unknown and the evaluation and management of affected patients may be complex due to the need for multiple laboratory assays, especially in those in whom the underlying disease (e.g., prosthetic heart valve or essential thrombocythemia [ET]) necessitates antithrombotic therapy. The initial laboratory tests used to assess AVWS include VWF level, VWF activity, and FVIII activity assays. Further tests include VWF multimer analysis, which is a sensitive tool able to detect the structural abnormalities of VWF even in the context of normal VWF activity levels. The frequency of the detection of inhibitors, that is, antibodies against VWF, is low in AVWS. Before 2016, it was not possible to confirm a suspicion of AVWS in Estonia because of a limitation of available laboratory VWF assays, while, since 2016, all VWF-related screening assays have been available to clinicians4 and, recently, a semiautomated VWF multimer assay has been incorporated into routine clinical practice at the North Estonia Medical Centre (NEMC).5,6

Here, we describe the clinical and laboratory data of seven patients diagnosed with AVWS at NEMC.

Materials and Methods

Patients

We included all consequent patients referred to and assessed at NEMC from the January 1, 2016, to December 31, 2017, who met the criteria for an AVWS diagnosis based on laboratory findings and bleeding symptoms together with the absence of any previous history of a bleeding disorder.3

The most common clinical symptoms were easy bruising, epistaxis, menorrhagia, and bleeding complications after tooth extraction. The mean age of the patients was 57.4 years (range: 22–80 years). The study group included five women and two men with various underlying diseases such as non-Hodgkin’s lymphoma (NHL), monoclonal gammopathy of undetermined significance (MGUS), ET, polycythemia vera (PV), secondary polycythemia due to cardiovascular diseases, obstructive sleep apnea syndrome, and autoimmune thyroiditis.

All cases were discussed at interdisciplinary meetings between laboratory and clinical staff. This retrospective study was performed as a collaboration between NEMC and Helsinki University Hospital, HUSLAB laboratory services, Coagulation Disorders Unit in partnership with The Twinning Program of the World Federation of Hemophilia (WFH). The study was performed according to the Declaration of Helsinki and was approved by the Tallinn Medical Research Ethics Committee.

Blood Sampling

During this study, peripheral venous blood specimens were collected into K2-EDTA tubes (BD Vacutainer; BD Diagnostics, Plymouth, UK) for a complete blood count, 3.2% sodium citrate tubes (BD Vacutainer; BD Diagnostics) for coagulation assays, and hirudin blood tubes (Roche Diagnostics, Switzerland) for platelet aggregation evaluation.

Laboratory Investigations

Based on the laboratory assays available in Estonia, the diagnostic algorithm for von Willebrand disease (VWD)/syndrome was adopted in this study.4 Initial laboratory evaluations included complete blood count (Sysmex XE-5000; Roche Diagnostics); prothrombin time (PT) (Neoplastine Cl Plus: Diagnostica Stago, Asnières-sur-Seine, France); partial thromboplastin time (APTT) (PTT-A; Diagnostica Stago), VWF antigen (VWF:Ag) (Liatest-VWF:Ag; Diagnostica Stago); FVIII:C determined by a one-stage, clot-based assay (Diagnostica Stago, France); and VWF activity measured as VWF binding to the glycoprotein Ib (GPIb) receptor on the platelet surface (VWF:GPIbM) (Innovance VWF Ac kit; Siemens Healthcare Diagnostics, Marburg, Germany). All parameters were measured on the STA-R Evolution analyzer (Diagnostica Stago) using commercial kits.

Mixing studies were conducted to determine the etiology of prolonged APTT; the APTT test was repeated on a mixture of the patient’s plasma with normal plasma immediately and after incubation for two hours at 37°C. Depending on correction, FVIII, FIX, FXI, FXII, or lupus anticoagulant tests were performed.

Platelet aggregation was measured in whole blood by an impedance multiple aggregometer (Roche Diagnostics) using the RISTOhigh test (final concentration of ristocetin: 0.77 mg/mL) and RISTOlow test (final concentration of ristocetin: 0.2 mg/mL). For both, the measurements were performed within 180 minutes after venipuncture.

The multimeric pattern of VWF was evaluated using the new Hydragel 5 von Willebrand multimers assay (Sebia, Lisses, France).6,10-11 The detailed protocol has previously been described.12 In May 2019, the VWF multimer analysis with 5VWF was accredited in the NEMC laboratory according to the ISO15189:2012 standard. Both the visual evaluation of the gels and densitometric analysis were performed. VWF multimers were classified as low-molecular weight, intermediate-molecular weight, or HMW multimers with densitometry.
The main characteristics of the study participants are shown in Table 1. All patients had other bleeding episodes and no family history for bleeding disorders. The International Society on Thrombosis and Hemostasis–Bleeding Assessment Tool was used to score the risk of bleeding (data not presented).

Table 1  Demographic and laboratory characteristics of the study participants

|                     | Reference ranges | Case 1 | Case 2 | Case 3 | Case 4 | Case 5 | Case 6 | Case 7 |
|---------------------|------------------|--------|--------|--------|--------|--------|--------|--------|
| Diagnosis           |                  | Non-Hodgkin’s lymphoma | ET, JAK2 (V617F) | ET, JAK2 (V617F) | PV     | MGUS   | Secondary erythrocytosis due to cardiovascular diseases and obstructive sleep apnea syndrome | Autoimmune thyroiditis |
| Clinical symptoms   |                  | Epistaxis, bleeding complications after tooth extraction | Menorrhagia | Bleeding complications after tooth extraction | Bleeding complications after tooth extraction | Epistaxis | Epistaxis | Spontaneous hematoma |
| Age, gender         |                  | 67 F   | 33 F   | 61 F   | 60 F   | 78 M   | 80 M   | 22 F   |
| PT (sec)            |                  | 11.5–14.5 | 13.3 | 13.2 | 13.0 | 12.6 | 13.0 | 12.9 | 12.6 |
| APTT (sec)          |                  | 29–38 | 44 | 48 | 41 | 48 | 46 | 34.4 | 33.6 |
| APTTmix1:1 (0°, 120° correction) |                  | Correction | Correction | Correction | Correction | Correction | NA | NA |
| VWF:Ag (%)          |      50–160 | 25 | 61 | 83 | 102 | 29 | 269 | 35 |
| VWF:GPIbM (%)       | 46–146 (0 group) | 14 | 34 | 29 | 62 | 11 | 174 | 41 |
| VWF:GPIbM/Ag ratio | > 0.7 | 0.56 | 0.55 | 0.35 | 0.61 | 0.38 | 0.65 | 1.25 |
| FVIII:C %           | 60–150 | 42 | 37 | 48 | 118 | 21 | 253 | 65 |
| RISTOhigh (U)       | 98–180 | 12 | ND | ND | 151 | 38 | ND | 112 |
| WBC count 10⁹/L     | 4–10 | 5.6 | 14.9 | 12.5 | 15.2 | 4.2 | 8.1 | 7.7 |
| RBC count 10¹²/L    | M 4.5–6.0; N 4.0–5.5 | 4.6 | 5.4 | 8.5 | 5.7 | 5.0 | 6.2 | 4.1 |
| Hematocrit (%)      | M 40–52; N 36–47 | 40 | 46 | 50 | 47 | 46 | 57 | 38 |
| Platelet count 10⁹/L | 150–400 | 245 | 1391 | 1120 | 785 | 224 | 142 | 326 |
| VWF multimers       | Persons without VWD (21): Normal distribution | Loss of HMWM | Decrease of HMWM | Loss of HMWM | Decrease of HMWM | Decrease of HMWM | Decrease of HMWM | Normal distribution |
| LMWM (%)            | 15.3 (11–23) | 50.9 | 33.1 | 58.3 | 32.5 | 49.1 | 35.1 | 13.8 |
| IMWM (%)            | 30.2 (23.1–35.8) | 38.3 | 39.4 | 33.5 | 39.1 | 19.1 | 35.9 | 25.0 |
| HMWM (%)            | 54.8 (45.1–65.9) | 10.8 | 27.5 | 8.3 | 28.4 | 31.8 | 29.0 | 61.2 |

Abbreviations: HMWM, high-molecular-weight multimers; IMWM, intermediate-molecular-weight multimers; LMWM, low-molecular-weight multimers; NA, nonapplicable; ND, not determined; VWF: Ag, von Willebrand factor antigen; VWF: GPIbM, VWF activity assays using recombinant gain-of-function mutant GPIb fragments allowing for the spontaneous binding of VWF to the mutant GPIb without ristocetin.
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department due to recurrent bleeding after tooth extraction requiring tamponade and bleeding from the right nasal cavity requiring electrocautereization. The patient was treated with tranexamic acid during all bleeding events and continued to be followed-up in the hematology clinic.

Case 2. A 33-year-old female patient with heavy menorrhagia and high platelet count was investigated. She had no antithrombotic treatment. A diagnosis of ET with a positive finding for a JAK2 (V617F) mutation was made. Menorrhagia was caused by secondary von Willebrand syndrome, and treatment with tranexamic acid was prescribed for use during menstrual bleeding.

Case 3. A 61-year-old female patient was investigated after experiencing bleeding after tooth extraction lasting 2 days. A high blood platelet count suggested the possibility of chronic myeloproliferative disease together with secondary von Willebrand syndrome. Further investigations confirmed JAK2 (V617F)-positive ET. Cessation of bleeding symptoms was achieved after platelet count normalization with hydroxyurea treatment.

Case 4. A 60-year-old female patient with PV from 2000 onward was referred for additional examination and consultation before planned tooth extraction. She experienced bleeding complications 2 years earlier after the tooth extraction. She was treated with hydroxyurea, blood exclusion, and low-dose aspirin. She was advised to stop aspirin 5 days before her next planned tooth extraction. Prophylactic treatment with 10 mg/kg of tranexamic acid given intravenously (IV) was prescribed three times daily on the procedure day and also one day before and after the procedure.

Case 5. A 78-year-old male patient was consulted because of recurrent epistaxis, with a need for cauterization throughout 2 previous years. His complete blood count was normal. Biochemical investigation showed a monoclonal peak (3.1 g/L) in the γ-globulin region. Immunoglobulin G kappa monoclonal protein was confirmed by immunofixation. The kappa/lambda free light-chain ratio was 5.2 (reference range: 0.26–1.65), compatible with a diagnosis of MGUS. Tranexamic acid was prescribed in the case of a bleeding episode and the patient remains under close follow-up observation by the hematology clinic.

Case 6. An 81-year-old male patient with cardiovascular disease and obstructive sleep apnea syndrome was referred to a hematologist by his general practitioner due to frequent epistaxis (nosebleeds) occurring in the 2 previous years, with the need for nasal tamponade at the emergency department. The complete blood count revealed an increased red blood cell count (6.10×10^12/L), increased hemoglobin level (176 g/L), and increased hematocrit concentration (54.9%), which raised the suspicion for PV. However, further studies on BCR/ABL p210 and JAK2 V617F mutations were normal, supporting the diagnosis of secondary erythrocytosis due to cardiovascular disease, which is one condition that can cause AVWS. The patient was counseled, and instructions were given for handling future bleeding episodes. Tranexamic acid was also prescribed to treat further bleeding episodes.

Case 7. A 22-year-old female patient was referred to the hematologist for bleeding evaluation. She reported the development of apparently spontaneous subcutaneous hematomas, unrelated to trauma or physical activity, during the last 3 years. Additional examination showed increased thyroid-stimulating hormone (TSH) and thyroid peroxidase (> 1000 U/mL) levels, consistent with a diagnosis of autoimmune thyroiditis, and the patient was referred to the endocrinologist. Her hypothyroidism was treated and, 1 year later, normal TSH values were recorded together with normalization of coagulation test findings for VWF:Ag (69%), VWF:GPIIbM (86%), fibrinogen (2.58 g/L), and CRV (< 1 mg/L).

Results

Coagulation Workup for AVWD Diagnosis

In this case series, coagulation studies showed normal PT and prolonged APTT (Cases 1–5). Mixing study revealed corrections for both immediate and incubated APTT tests, indicating a mild deficiency of FVIII in Cases 1, 2, 3, and 5. FIX, FXI, and FXII levels were normal. Follow-up assessments demonstrated severely decreased (< 35%) VWF activity in four of seven patients (Table 1), fulfilling the criteria for VWD diagnosis. Both decreased VWF:Ag and VWF:GPIbM levels in Cases 1 and 5 and normal VWF:Ag levels with low VWF:GPIbM levels in Cases 2 and 3 were observed. In patient 6, the levels of VWF:Ag, VWF:GPIbM, and FVIII:C were increased, while a decreased VWF function/antigen ratio (VWF:GPIbM/VWF:Ag) was recorded. High-dose ristocetin-induced platelet aggregation was decreased in two patients (cases 1 and 5), while low-dose ristocetin-induced platelet aggregation was normal. In Case 7, the levels of VWF:Ag and VWF:GPIbM were both decreased with a normal VWF function/antigen ratio. Complete blood count and platelet aggregation studies were normal.

VWF multimeric analysis (Figs. 1 B–F) revealed decreased HMW multimers, supporting AVWS in all instances (Cases 1–6). In Case 6, during the visual investigation of gel, we did not detect any abnormalities in the VWF pattern, yet densitometric data provided additional information about the VWF multimeric structure. Multimeric analysis (Fig. 1 H) showed a normal distribution pattern, suggesting type 1 AVWS. We noted that patients with lower HMW multimers by densitometric evaluation presented with more severe bleeding complications.

Discussion

We herein describe the clinical and laboratory data of seven patients with AVWS. All cases were discussed in a multidisciplinary meeting involving both clinical and laboratory experts. In all cases, the laboratory findings and lack of previous lifelong bleeding episodes and family history suggested AVWS.

Earlier studies have documented that MGUS, NHL, ET, and autoimmune hypothyroidism are associated with AVWS. The pathogenesis of AVWS is variable but may have an overlapping mechanism among patients with different underlying disorders.
In our series, six patients showed a type 2–like phenotype with decreased VWF activity to the Ag ratio and a loss/decrease of HMW multimers. One patient had a type 1 VWD phenotype. Recently developed diagnostic algorithms, based on standard laboratory assays, may assist clinicians in the diagnostic workup and help differentiate between AVWS.

Fig. 1 Electrophoresis gels and densitograms: A—healthy person, B—Case 1, C—Case 2, D—Case 3, E—Case 4, F—Case 5, G—Case 6, H—Case 7. LMWM, low-molecular-weight multimers; IMWM, intermediate-molecular-weight multimers; HMWM, high-molecular-weight multimers; PNP, pool normal plasma.
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Conclusions

Our data also demonstrate that the diagnosis of AVWS is complex and requires extensive laboratory evaluation.31 Our data support that VWF multimer analysis should be included in the AVWS diagnostic algorithm. Interdisciplinary collaboration and complex laboratory evaluations are of paramount importance for the early recognition of AVWS and the selection of appropriate clinical management protocols.

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Conflicts of Interests

None declared.

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