ADVANCES IN HEMATOLOGY

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Etiology and Diagnosis of Acquired von Willebrand Syndrome

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H&O What are the known causes for acquired von Willebrand syndrome?

Acquired von Willebrand syndrome (aVWS) is thought to be a rare bleeding disorder that largely mimics congenital von Willebrand disease (VWD) in terms of laboratory findings and clinical presentation. Although less than 300 cases have been reported so far, these numbers probably reflect the tip of an iceberg, since most cases go undetected or unreported due to the lack of widespread knowledge of this syndrome and the difficult "differential" diagnosis. The first report of this hemorrhagic disorder came from Simone and colleagues in 1968, who described a case of atypical bleeding in a 12-year-old boy with systemic lupus erythematosus (SLE). The patient was previously asymptomatic (having undergone circumcision, tonsillectomy, adenoidectomy, and open repair of a fractured humerus before that age without overt bleeding episodes) when he suddenly began to experience frequent epistasis, bleeding from an abrasion of the hard palate, and massive bleeding from a dental extraction in spite of vigorous dental therapy. Laboratory studies at that time revealed a normal clotting time, prothrombin time (PT), platelet count, and clot retraction, but a markedly prolonged activated partial thromboplastin time (APTT). Clinical and laboratory abnormalities disappeared after administration of corticosteroid therapy.[1]

The syndrome is believed to mostly occur from 2 leading mechanisms: namely reduced synthesis and/or increased clearance of von Willebrand factor (VWF). In the latter case, the accelerated removal of VWF from plasma has been attributed to the inhibition of VWF function and/or increased clearance of the VWF-factor VIII (FVIII) complex due to the presence of auto-antibodies to either VWF or FVIII; increased clearance of the VWF-FVIII complex due to the adsorption of VWF onto malignant cells; exposure of the bond between VWF amino acids 842 and 843 under high shear stress conditions, making the VWF molecule vulnerable to the activity of a specific von Willebrand protease and thereby causing loss of high molecular weight multimers (HMWM); and increased proteolytic cleavage of VWF by circulating proteases.[2,3] However, it can be hypothesized that none of the proposed mechanisms fully explain the pathogenesis of aVWS, and identical mechanisms might be responsible for the clinical and laboratory findings even when associated with different underlying conditions.

Most cases of aVWS—up to 50–60%—have been diagnosed in association with clonal hematoproliferative diseases, namely monoclonal gammopathy, monoclonal gammopathies of undetermined significance (MGUS), myeloma, Waldenström macroglobulinemia, and other lymphoproliferative disorders such as chronic lymphocytic leukemia, hairy cell leukemia, and non-Hodgkin lymphoma. aVWS has also been occasionally reported in patients with myeloproliferative disorders, including essential thrombocythemia and, less frequently, polycythemia vera and chronic granulocytic leukemia. Other disorders associated with aVWS include Wilms' tumor; adenocarcinomas/adrenal cell carcinomas; immunologic diseases, including SLE; and thyroid disorders, especially hypothyroidism. Finally, occasional reports of aVWS include patients with rare immunologic disorders, gastrointestinal angiodysplasia, mesenchymal dysplasias, uremia, cardiac disorders (eg, aortic stenosis, congenital cardiac defects, mitral valve prolapse), rare congenital

syndromes (eg, Turner syndrome, Ehlers Danlos syndrome, lactoferrin deficiency, hemoglobin E-beta thalassemia), viral and parasitic infections—as seen in patients receiving therapy with antibiotics—anticonvulsants, or plasma expanders.[2-4]

H&O How is acquired von Willebrand syndrome diagnosed? What markers do we know of and what assays are important?

aVWS is largely identified using the same tests used to identify congenital VWD,[5] albeit supplemented with additional strategies (Table 1). Several specific ("diagnostic") tests of VWF level and function are assessed to identify a loss of VWF and/or its functional activity. Classically, the main tests utilized are those for VWF antigen (VWF:Ag) and VWF binding to its platelet receptor (glycoprotein Ib), typically using the ristocetin cofactor (VWF:RCo) assay. Also important is an assessment of FVIII coagulant (FVIII:C). Additional functional assays should also be evaluated and may include collagen binding (VWF:CB). Like VWD, aVWS may present as a quantitative defect (ie, Type 1) or a qualitative defect (ie, Type 2), depending on the mechanism involved (ie, decreased production, increased clearance, increased proteolysis, absorption, or auto-antibody). For example, aVWS in hypothyroidism is typically a Type 1-like disorder, and thought to reflect a lowered production of VWF.[6] In contrast, patients with auto-antibodies or aortic stenosis or showing increased VWF proteolysis may yield a Type 2-like disorder. Accordingly, supplementary tests such as VWF multimers, ristocetin-induced platelet agglutination (RIPA), and/or the VWF-factor VIII binding assay (VWF:FVIIIB) may be useful in select cases to further characterize any defect initially identified with the previously mentioned diagnostic assays.

The supplemental diagnostic approach to identifying aVWS and differentiating this from congenital VWD is somewhat analogous (but also different) to that undertaken to differentially diagnose acquired hemophilia A (aHA) versus congenital HA. Thus, in differentiating aHA and HA, one also needs to evaluate the presence of auto-antibodies to FVIII, as well as the clinical history for both the patient and family members. The evaluation of auto-antibodies (or inhibitors) to FVIII is typically achieved using mixing studies and standard Bethesda assays or Nijmegen modifications, or using enzyme linked immunosorbent assay (ELISA), with the former processes detecting functionally inhibiting antibodies, and the latter assay potentially detecting these, as well as nonfunctionally inhibiting antibodies (eg, those associated with increased clearance of FVIII).[7] In regard to the clinical history, aHA is typically late in onset, without prior evidence of significant personal and family history of bleeding.

Similarly, cases of aVWS should also be evaluated for the presence of auto-antibodies and clinical and family histories. However, this process is much more complex than that used for identifying aHA. Firstly, it needs to be noted that, compared to FVIII, VWF is a much larger protein and has many more functions and functional binding sites (eg, binding to platelets, FVIII, and collagen to name a few). Accordingly, no single laboratory assay can enable the identification of all possible auto-antibodies. This was recently highlighted in a small series of patients, where it was identified that a panel of assays might be needed to permit more complete identification of these antibodies, including VWF:RCo, VWF:CB, RIPA, and ELISA.[8,9] Analogous to aHA and the Bethesda assay, the first 3 methodologies would identify functionally inhibiting antibodies, although because VWF has so many functions, these antibodies could be directed to any of a number of different functional sites, potentially yielding different inhibitor patterns in different patients. Detection of functional auto-antibodies to VWF, like the Bethesda assay or the Nijmegen modification for FVIII antibodies, involves serial dilutions of patient plasma mixed with normal plasma and then assessment in different functional VWF assays to identify the level of inhibition. Also like the case for aHA, the ELISA procedure may identify these functionally inhibitory antibodies as well as noninhibitory antibodies. Importantly, whereas a positive finding using any assay will generally identify a positive auto-antibody in an aVWS, a negative finding cannot be used to conclusively exclude such an event. Furthermore, false positive inhibitors may be observed using the ELISA assay.

Again analogous to aHA is the evaluation of personal and family history. However, this is again more complex with aVWS than aHA, which is more typically late onset (>60 years). In aVWS, the large range of associated disorders means that onset can be at an early age, as highlighted in the opening paragraph, with the original case identified in a 12-year-old boy. Nevertheless, late onset of symptoms and absent family history is certainly supportive of a diagnosis. More important than late onset per se, is "recent onset" or lack of earlier history, irrespective of age of onset.

Genetic testing of the VWF gene is not useful in aVWS, as one would expect to see a normal finding. Also, lack of a VWF gene mutation will provide only limited evidence of an acquired event, since mutations are also sometimes not found in a substantial number of cases of congenital VWD.[10]

Finally, pharmacokinetic studies of VWF are also of value in identifying patients with aVWS. In brief, one may predict a faster clearance of VWF in many patients with aVWS. This can be assessed in many ways. One

Table 1. Diagnostic Considerations When Assessing the Possibility of Acquired von Willebrand Syndrome

	Acquired von Willebrand Syndrome	Congentital von Willebrand Disease
Personal Bleeding History	Recent onset or short-term	Usually life-long or long-term
Family Bleeding History	Weak or not evident	Usually evident
Laboratory testing		
Screening assays	Routine coagulation tests (PT and APTT) plus FBC. APTT may be normal or else will be prolonged due to low FVIII:C. PT should be normal. FBC may show evidence of blood loss (eg, low Hb).	Routine coagulation tests (PT and APTT) plus FBC. APTT may be normal or else will be prolonged due to low FVIII:C. PT should be normal. FBC may show evidence of blood loss (eg, low Hb) and (mild) thrombocytopenia in some cases (eg, Type 2B VWD).
Diagnostic assays	VWF:Ag, VWF:RCo, VWF:CB, FVIII:C. Different test patterns may be evident according to etiology of aVWS. Thus, a quantitative defect (Type 1) will show low and concordant (similar) levels for all test parameters whereas a qualitative defect (Type 2) will show low and discordant (dissimilar) levels for VWF test parameters.	VWF:Ag, VWF:RCo, VWF:CB, FVIII:C. There are 6 distinct types of VWD and each gives a distinct test pattern. For example, a quantitative defect (Type 1) will show low and concordant (similar) levels for all test parameters, whereas a qualitative defect (Type 2) will show low and discordant (dissimilar) levels for selective test parameters, with the test pattern potentially reflective of the subtype (ie, 2A, 2B, 2M, or 2N VWD). Type 3 VWD will present with very low (<5%) to absent VWF test results.
Supplementary assays	VWF multimers, RIPA, and/or VWF:FVIIIB may be performed depending on the results of the above diagnostic assays. vWFpp may be useful to assess VWF clearance. Mixing studies (ie, inhibitor assays) should also be performed using the above diagnostic assays and/or by ELISA to identify potential auto-antibodies. A positive ELISA result is suggestive, and a positive functionally inhibitory antibody test result is proof, of aVWS; however, a negative finding cannot be used to discount aVWS.	VWF multimers, RIPA, and/or VWF:FVIIIB may be performed depending on the results with the above diagnostic assays. vWFpp may be useful to assess VWF clearance.
Supplementary investigations	Pharmacokinetic testing using desmopressin challenge, or concentrate administration to assess VWF clearance and proteolysis. Genetic testing of the VWF gene will be uninformative (ie, will be mutation negative).	Pharmacokinetic testing using desmopressin challenge, or concentrate administration to assess VWF clearance and proteolysis, as well as to assist subtype determination. Genetic testing of VWF gene may be informative in select cases (ie, may be mutation positive).

aVWS=acquired von Willebrand syndrome; APTT=activated partial thromboplastin time; FBC=full blood count; FVIII:C=factor VIII coagulant; Hb=hemoglobin; PT=prothrombin time; RIPA=ristocetin induced platelet agglutination; VWD=von Willebrand disease; VWF=von Willebrand factor; VWF:Ag=von Willebrand factor antigen; VWF:CB=von Willebrand factor collagen binding; VWF:FVIIIB=von Willebrand factor-factor VIII binding assay; VWF:RCo=von Willebrand factor ristocetin cofactor.

classic way in which to assess clearance and proteolysis is to undertake a desmopressin (DDAVP) trial and to assess findings relative to normal expectations. Like the case for diagnosis of VWD, and for identification of auto-antibodies in aVWS, it is important to perform a panel of laboratory tests in this evaluation, as well as to allow a sufficiently long time-course for the evaluation. An increased clearance and proteolysis of VWF is present in some forms of congenital VWD.[11] Nevertheless, an increased clearance or proteolysis, in context with other expected features of aVWS, may alternatively provide suitable evidence of aVWS. Another potentially useful test is the VWF propeptide (VWFpp) assay, which will also help identify normal or elevated clearance of VWF.

H&O What is the significance of von Willebrand factor propeptide as a marker in diagnosis?

Before VWF synthesized de novo leaves the endothelial cell, it undergoes endoproteolytic cleavage of its propeptide (VWFpp). The processed VWF and VWFpp are either released constitutively or, after activation of the endothelium, are released through the regulated pathway. Of interest, the plasma half-life of mature VWF and VWFpp differ several-fold (respectively around 8–12 hours and 2–3 hours). This property can be exploited to evaluate the potential for reduced VWF half-life, or increased VWF clearance, which we identified earlier as a pathogenic mechanism in some forms of aVWS. In this context, it is possible to assess the relative elimination half-life of either or both VWFpp and VWF:Ag post-DDAVP or to calculate the VWFpp/VWF:Ag ratio either at steady state or post-DDAVP. A reduced half-life is present in some forms of congenital VWD. Nevertheless, a reduced half-life, in context with other expected features of aVWS, may alternatively provide suitable evidence of aVWS. Conversely, a normal half-life will still be found in a large number of cases of aVWS, and hence cannot be used to exclude aVWS.[12] Furthermore, some forms of aVWS are more likely to show increased VWF clearance than others. For example, VWF pp/Ag ratios have been shown to be higher in patients with aVWS due to monoclonal gammopathies than in patients with aVWS resulting from cardiovalvular disorders.

H&O What is the aim of therapy in patients with von Willebrand syndrome? What options are available and what do we know of their efficacy?

The treatment of aVWS is focused on 3 directions: to control or prevent bleeding, to eradicate the inhibitor when present, and to treat the underlying disorder.[13] Therapeutic options include the use of DDAVP, plasma-derived concentrates of VWF-FVIII complex and intravenous immunoglobulin (IVIG), the efficacy of which depend on the cause and etiology of aVWS.[14,15] DDAVP, administered intravenously or subcutaneously in doses of 0.3 µg/kg, leads to a transient correction of bleeding time and a short-lived increase in the VWF-FVIII complex in most cases of aVWS (ie, those associated with auto-antibodies to VWF, or increased clearance or proteolysis of VWF). Thus, the effects of DDAVP in aVWS are transient and of lesser magnitude than in congenital VWD, probably due to a more rapid plasma clearance of the FVIII-VWF complex or a removal of the DDAVP-released endogenous VWF by the inhibitory auto-antibody.[16,17] Infusion of FVIII-VWF concentrates may be more effective, but large doses may be required to overwhelm the auto-antibody against exogenous VWF provided by the concentrates or to counterbalance the accelerated clearance of the VWF-FVIII complex.[2] Other treatment options include administration of high-dose IVIG.[18] The mechanism of action of IVIG is thought to be an anti-idiotype effect, blockage of reticulo-endothelial Fc-receptors, or elimination of circulating immune complexes by circulating immunoglobulin.[17] IVIG has been used successfully in patients with aVWS and IgG-type MGUS or lymphoproliferative disorders.[19-22] The therapeutic doses of IVIG for aVWS are 1 g/kg/day for 2 days or 0.4 g/kg/day for 5 days, as proposed for immune thrombocytopenic purpura. IVIG usually requires 24–48 hours to normalize plasma VWF-FVIII activity; DDAVP or VWF-FVIII concentrates can be given together with IVIG to achieve normal hemostasis immediately in cases of emergency bleeding or surgery. Repeated doses of IVIG given every 21 days (long-term therapy) can produce consistent responses in VWF measurements.[18]

The antifibrinolytic agent tranexamic acid has also been successfully used in some cases associated with increased fibrinolysis.[23] Bypassing agents, such as recombinant activated factor VII (rFVIIa), have been used successfully in cases with severe bleeding refractory to standard therapy.[24,25] Other modalities of treatment include plasma exchange, extracorporeal immunoadsorption—especially in patients with high titers of VWF inhibitors—corticosteroids, and immunosuppressive drugs.[2]

On behalf of the Scientific Subcommittee on VWF, the International Registry on aVWS (available at: http://www.intreavws.com) was organized in 1998 with the aim to provide information on diagnostic and therapeutic approaches for these patients. Among the 186 cases collected, which represent the largest case series published so far, the success rate in controlling bleeding was reported to be 32% with the use of DDAVP, 37% with VWF-FVIII concentrates, 33% with IVIG, 19% with plasmapheresis, 19% with corticosteroids, and 35% with immunosuppressive agents. These findings again reflect the heterogeneous nature of aVWS and its etiology.[26]

However, the complete resolution of the acquired coagulopathy is ultimately achieved by controlling the underlying disease that is responsible for the development of aVWS. In fact, the successful correction of the bleeding disorder has been reported after tumor resection, cardiac valve replacement chemotherapy, radiotherapy, or thyroxine replacement.[27-30] Table 2 summarizes the therapeutic options for the management of aVWS.

H&O What are the unmet needs in the diagnosis and treatment of von Willebrand syndrome? What research in this field will be most important?

aVWS is a complex multicausal disorder. Early recognition of aVWS and its primary cause is mandatory to

Table 2. Therapeutic Options for Patients with Acquired von Willebrand Syndrome

1. **Control/prevention of bleeding**
   - Desmopressin
   - Tranexamic acid
   - Factor VIII-von Willebrand factor concentrates
   - Recombinant factor VII activated
2. **Inhibitor eradication**
   - Intravenous immunoglobulin
   - Immunosuppressive agents and corticosteroids
   - Plasma exchange
   - Immunoadsorption
3. **Treatment of underlying disorder**
   - Chemotherapy, radiotherapy
   - Surgery

achieving adequate therapy and cure. Although similar mechanisms may be responsible for aVWS of differing causes, the differing etiology, and therefore varying efficacy of different treatments, create several challenges to diagnosis and effective treatment. VWF is a large and complex molecule, and there are several non-VWF genetic components that control the level and functional activity of VWF.[10]

Thus, despite 40 years of accumulated experience in the diagnosis and management of aVWS, many aspects are still unclear and are evolving. There is a clear need to develop more sensitive and specific assays to better identify and characterize the potential presence of VWF antibodies. Furthermore, large multicenter studies are required to better establish the underlying mechanisms, more comprehensively evaluate pharmacokinetic VWF profiles and VWFpp patterns, and establish guidelines for an appropriate therapeutic strategy in this heterogeneous syndrome.

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