Diagnostic Problems of von Willebrand’s Disease in a General Hospital Laboratory*

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ABSTRACT

The expression of variant types of von Willebrand’s disease can present an elusive diagnostic problem. Bleeding history of patients can vary greatly, as can the results of tests for the components of the Factor VIII complex. Recent advances in characterizing and measuring the factor VIII complex have greatly improved the diagnosis of the variant forms of von Willebrand’s disease. However, some of the less sophisticated procedures, which are more readily available, can still be utilized by the routine general hospital laboratory to identify or suspect the occasional case of von Willebrand’s disease. These points are exemplified in this report of seven family members who were studied.

Introduction

Classical von Willebrand’s disease (vWd) and its variants are inherited quantitative and/or qualitative derangements of the bipartite factor VIII complex associated with abnormal hemostasis. This heterogeneous group of disorders is characterized by highly variable clinical manifestations and laboratory findings. Severe vWd is rarely a diagnostic problem and is usually diagnosed in infancy as a consequence of profound hemorrhage. Mild vWd for which mild mucocutaneous bleeds and menorrhagia are typical is often not subjected to the appropriate diagnostic tests and may go largely unrecognized until a traumatic or surgical challenge of sufficient magnitude is sustained to unmask the disorder and warrant work-up.

The variety of vWd variants and their often associated mild bleeding tendency make interpretation of results and diagnosis difficult. Further complicating the diagnosis of vWd is the fluctuation in bleeding and laboratory indices known to occur in individual patients. The reason(s) for the cyclic modulation of the components of the factor VIII complex is unknown. Pregnancy and liver disease are associated with increases in factor VIII and may obscure or make the diagnosis of vWd impossible. Because of the...
variant forms, frequent mild bleeding presentation, and cyclic nature of the disorder, vWD can be a difficult diagnosis to establish and may require serial laboratory testing. As a consequence of its heterogeneity and subtleties, vWD may, in fact, be the most common congenital bleeding disorder.  

On the basis of laboratory testing for the components of the factor VIII complex, vWD is classified into four subtypes (types I, IIA, IIB, and III). There are many fine reviews which delineate the classification schema. All congenital varieties of vWD with the exception of type III are transmitted as autosomal dominants with variable penetrance. Most patients are heterozygous and tend to be mildly affected. The most severe variety of vWD and the least common is type III which is an autosomal recessive disorder. The genetic heterogeneity of this disorder makes family studies a valuable tool in the diagnosis of vWD.

von Willebrand's disease is due to decreased activity in one or more of the components of the factor VIII molecule which may be either quantitative (absolute deficiency) or qualitative (defective molecule). The factor VIII complex can be considered to have two components with distinct functional, biochemical and immunologic properties and genetic control. These two components are known as VIII:C or factor VIII procoagulant protein and VIII:R or factor VIII related protein.

The factor VIII procoagulant protein or antihemophilic factor, VIII:C, functions in the intrinsic coagulation cascade by participating as a cofactor in the enzymatic conversion of factor X. VIII:C is under X-chromosomal control and an isolated deficiency of VIII:C is characteristic of hemophilia A. A deficiency in this moiety may be seen in any variant of vWD, but is especially characteristic of types I and III. Despite much research, the site of synthesis of VIII:C remains uncertain. The mechanism by which VIII:C is deficient in vWD, an autosomal disorder, is unknown. It is suggested that VIII:R acts as a transport molecule for and stabilizes VIII:C diminishing its catabolism. The functional or procoagulant activity of VIII:C may be determined by a one- or two-stage assay based on the activated partial thromboplastin time (APTT), the results of which are expressed as a percentage of normal plasma procoagulant activity. Recently, immunoradiometric assays for VIII:CAg, utilizing purified human anti-VIII:C, have become available which will detect qualitative defects of VIII:C.

Factor VIII related protein or von Willebrand factor protein, VIII:R, appears to function as a binding agent for the adherence of platelets to surfaces (subendothelium and platelets), and may act to stabilize factor VIII:C. There is evidence that VIII:C and VIII:R circulate in plasma as two distinct proteins that are non-covalently complexed, rather than being separate activities of a single molecule. The VIII:R molecule is further segregable into properties referred to as ristocetin cofactor activity (VIII:RC0) and antigen (VIII:RAg). All of these terms are used when referring to the different functions or methods of testing this protein. The factor VIII related protein, VIII:R, in promoting adhesion leads to primary hemostasis. Genetic control of this protein is by an autosomal gene and either a quantitatively deficient or immunologically abnormal molecule may be found in vWD. Immunofluorescent studies have identified VIII:R in platelets, megakaryocytes, and endothelial cells throughout the body. Direct evidence of VIII:R synthesis by endothelial cells has been obtained in cell culture.

Various methods are available which
assess the functional parameters of VIII:R activity. These procedures include the bleeding time, aspirin bleeding time, platelet retention in glass bead columns, ristocetin-induced agglutination in patient platelet rich plasma, and ristocetin cofactor assays utilizing washed or formalin-fixed normal platelets and dilutions of patient platelet poor plasma. The quantity and antigenic quality of VIII:R expressed as VIII:RAg can be determined by rocket immunoelectrophoresis or radioimmunoassay techniques. These methods utilize specific heterologous antibodies against factor VIII. Studies of VIII:R utilizing crossed immunoelectrophoresis and SDS agarose gel electrophoresis have demonstrated that the molecule exists in plasma as a series of multimers of varying molecular weight.

The classification of vWD into subtypes is based on the mode of inheritance and laboratory testing for the components of the factor VIII complex. Zimmerman and Ruggeri have proposed a classification of vWD based on inheritance and crossed immunoelectrophoresis or SDS-agarose electrophoresis of VIII:R. This classification divides vWD into four sub-types. The results of the other tests for the properties or activities of the VIII:R protein, while often abnormal, are quite variable, and although they are helpful in the diagnosis of vWD do not lend themselves to a consistent classification scheme.

When evaluating a patient for vWD, a thorough enquiry into the patient’s bleeding history including a drug history and a detailed family history should be obtained routinely. Any history of aspirin or other anti-platelet drug ingestion should be vigorously discerned prior to laboratory testing as aspirin ingestion within nine to 12 days of testing will interfere with results and confuse interpretation. The initial laboratory testing of any individual with a bleeding disorder should include as a screen determination of prothrombin time (PT), APTT, fibrinogen, platelet count, and bleeding time, and possibly the aspirin bleeding time. Laboratory tests which evaluate all aspects of VIII:R should be then performed where indicated. These include in addition to the bleeding time ascertaining platelet retention, ristocetin-induced platelet agglutination as a part of a platelet aggregation panel, ristocetin cofactor assay, measurement of VIII:RAg, and crossed immunoelectrophoresis or SDS-agarose electrophoresis. An APTT and VIII:C assay are necessary to evaluate the procoagulant portion of the factor VIII complex. Platelet count and platelet aggregation with collagen, adenosine diphosphate (ADP), epinephrine and arachidonic acid tend to be normal in vWD, whereas abnormalities of these tests suggest other qualitative platelet disorders. One of the characteristics of vWD is the variability of the test results for VIII:R and VIII:C. Presently, the most consistent results are obtained by determining the plasma or platelet multimeric composition of VIII:R. The manifestations of the bleeding like the laboratory findings tend to vary cyclically in vWD often necessitating serial determinations in suspected cases. It is important to realize that the symptoms decrease in severity with age and may remit during pregnancy.

In a general hospital laboratory, one is occasionally called upon to evaluate a patient suspected of having a qualitative platelet disorder. Because of the infrequent indication, it is often not feasible or cost effective to set up crossed immunoelectrophoresis, SDS-agarose electrophoresis, or even VIII:RAg measurements. Therefore, smaller labs must rely on procedures which can be utilized in the evaluation of other types of bleeding disorders. These include the screening
procedures enumerated above and platelet retention, platelet aggregation and the ristocetin cofactor assay which is available in kit form.* By utilizing these methods, it is usually not possible to identify a specific variant of vWD although marked combined decreases in VIII:C and VIII:R activities in a subject with an appropriate family pedigree may distinguish types I and III.

Case Studies

Seven family members were evaluated in a kindred of 17 (figure 1). The severity of bleeding symptoms in the affected family members varied from absence to severe involvement. Of those family members studied, III-2 had had various episodes of bleeding since age one, III-6 required transfusions following a T&A and miscarried her first pregnancy. The five other family members had a negative bleeding history. History available on those family members not examined indicates the following: I-1 and I-2 had bleeding episodes, but the severity and frequency is not known. II-1 had bleeding episodes following dental work, menorrhagia, and six early miscarriages. II-2 and II-3 were also reported to have significant bleeding problems. III-4 died at age 18 owing to excessive bleeding following surgery.

Methods

The APTT’s and factor VIII:C assays were performed on the Dual Channel Coagamate* using Automated APTT Reagent** containing micronized silica as an activator. Factor VIII procoagulant levels were determined by a one-stage factor assay based on the activated partial thromboplastin time utilizing a commercial factor VIII deficient substrate plasma.6,8

Bleeding times were performed by a modified template Ivy technique utilizing a Simplate II device.16**

Platelet retention (adhesion) studies were based on Hellman’s technique.12 A sample of whole blood is passed through a standardized glass bead column† employing a fixed rate infusion pump‡. All tests were performed in duplicate and the results averaged. Results were expressed as the percentage of platelets retained in the column.

The factor VIII related antigen procedure was not available in our laboratory and was performed in a reference laboratory which utilized the Laurell immunoelectrophoresis technique with rabbit anti-human factor VIII.31

Platelet aggregation studies on citrated platelet-rich plasma adjusted to 250,000 platelets per cubic millimeter with PPP were performed on a Payton dual channel aggregometer with Omnisccribe§ as described by Born and Cross,5 and then modified.§ Collagen, ADP, and epinephrine|| were used in the following final concentrations: collagen (200 mcg per ml), ADP (2 \times 10^{-5}M), and epinephrine (1 \times 10^{-5}M). Ristocetin¶ was used in a final concentration of 1.5 mg/ml.

Ristocetin cofactor assays were also performed on the Payton dual channel aggregometer using the vW Factor Assay Kit¶ as previously described.2,22,23 The kit utilizes lyophilized normal platelets and ristocetin in a final concentration of one mg per ml. A standard curve is constructed from dilutions of normal pooled plasma. Levels of von Willebrand factor are then determined by the ability of a test plasma dilution and ristocetin to induce agglutination of the normal platelet suspension. The slope of the test sample pattern is determined and read off the standard curve as percent activity of von Willebrand factor. The range of the standard curve is 25 percent to 100 percent activity; however, the kit does include instructions for altering the dilutions of the test plasma for values greater than 100 percent activity or less than 25 percent activity.

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* Bio-Data Corporation, Horsham, PA.
** General Diagnostics, Morris Plains, NJ.
† Adelpat S Tube, Diagnostica, Inc., Miami, FL.
‡ Sienco, Inc., Morrison, Co.
|| Dade, Miami, FL.
¶ Bio/Data Corporation, Horsham, PA.
Results

The results of all tests performed on the family studies are illustrated in table I. Two of the family members (III-5 and III-6) had normal test results, however, their children (IV-3 and IV-4) had abnormal results in the tests which evaluate the von Willebrand’s factor. Although III-6 had normal test results at this particular examination, she did have a positive bleeding history. All family members, with two exceptions (III-5 and III-6), had abnormal results in tests for the von Willebrand’s factor activity (bleeding time, platelet adhesion, or ristocetin cofactor assay). Only two family members (III-2 and IV-1) had a decrease in procoagulant Factor VIII activity. One (III-2) had a decrease in Factor VIII related antigen. The bleeding history, as previously mentioned, was variable in the seven family members studied.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Bleeding History</th>
<th>Bleeding Time (Min)</th>
<th>Platelet Adhesiveness (%)</th>
<th>APTT (Sec)</th>
<th>Factor VIII Activity (%)</th>
<th>Factor VIII Related Antigen (%)</th>
<th>Ristocetin Cofactor (%)</th>
<th>Platelet Aggregation *</th>
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<tr>
<td>Normal</td>
<td></td>
<td>4 - 7</td>
<td>48 - 90</td>
<td>26 - 39</td>
<td>50 - 170</td>
<td>45 - 185</td>
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<tr>
<td>I11 - 2</td>
<td>+</td>
<td>3.5</td>
<td>43</td>
<td>65.5</td>
<td>7</td>
<td>32</td>
<td>40</td>
<td>N</td>
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<tr>
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<td>-</td>
<td>7.5</td>
<td>54</td>
<td>35.3</td>
<td>133</td>
<td>48</td>
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<td>N</td>
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<td>+</td>
<td>3.0</td>
<td>66</td>
<td>31</td>
<td>124</td>
<td>64</td>
<td>NT</td>
<td>N</td>
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<tr>
<td>IV - 1</td>
<td>-</td>
<td>8.5</td>
<td>55</td>
<td>49.5</td>
<td>36</td>
<td>46</td>
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<tr>
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<td>10.5</td>
<td>50</td>
<td>35.4</td>
<td>100</td>
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<td>-</td>
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<td>28</td>
<td>32.2</td>
<td>100</td>
<td>68</td>
<td>30</td>
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NT = Not treated
N = Normal response
* = Final Concentration of reagent in PRP sample
Collagen - 200 mcg/ml
Epinephrine - 1 x 10^-5 M
ADP - 2 x 10^-5 M
Ristocetin - 1.5 mg/ml

APTT: Activated partial thromboplastin time
Discussion

The family members were studied on only one occasion, so it was not possible to observe the variation in test results which may occur in von Willebrand’s disease. As discussed in the introduction, it is often necessary to do repeated testing to diagnose definitively von Willebrand’s disease, particularly when utilizing tests which analyze the activity of the von Willebrand’s factor or the total amount of the factor VIII related antigen. In our general hospital population it is often difficult to motivate patients to return for repeat testing. Quite often these people have a long distance to travel or are unable to cover the cost of the laboratory testing and, therefore, do not keep further appointments. The laboratory itself, has no mechanism to absorb the cost of an expensive battery of tests; therefore one must evaluate as well as possible those tests which can be performed. In this particular family study, we could rule out a primary platelet function defect on the basis of the normal platelet aggregation response (Table I) with collagen, ADP, and epinephrine. Two family members (III-2 and IV-1) exhibited low levels of factor VIII procoagulant activity coupled with a low von Willebrand’s factor (ristocetin cofactor) assay in addition to other abnormal tests which measure directly or indirectly factor VIII related activities. Three other family members (IV-2, IV-3, IV-4) had abnormal results of one or more of the tests which evaluate the activity of the von Willebrand’s factor. These tests results and the autosomal mode of transmission suggested by the family pedigree militate against any single factor deficiency and support the diagnosis of a von Willebrand’s disease variant.

All of the test methods that were utilized by our laboratory, with the exception of the ristocetin cofactor assay, are applicable for the evaluation of other bleeding disorders. The modified template bleeding time has been shown to be an effective screen for platelet function. Platelet retention can also be an effective screen for platelet function if performed carefully. Even though the normal range for platelet retention is very wide, definite abnormals can be detected. The APTT is a screening test for clotting factor proteins, while specific factor assays functionally quantitate the amount of the clotting factor. It is important to remember, however, that the screening tests (bleeding time, platelet adhesion, and APTT) are often normal in mild bleeding disorders. It is often necessary to pursue more specific tests when any of the screening tests are abnormal, or when a patient presents with a positive history and negative screening tests.

This family study exemplifies the broad spectrum of a variant form of von Willebrand’s disease. As many previous studies have described, it is necessary to do a complete family study to establish the diagnosis in family members with a negative bleeding history. A comprehensive laboratory study is necessary so that all components of the factor VIII complex are studied. Rocket immunoelectrophoresis is available for measuring the von Willebrand’s factor antigen, however, this method does not evaluate the biologic activity of the protein. Ristocetin cofactor assays identify that portion of the protein that can interact with platelets. One observation that was made during this study was that all family members had normal ristocetin-induced platelet aggregation using a ristocetin concentration of 1.5 mg per ml. However, when a ristocetin cofactor assay utilizing a normal standardized platelet suspension and a ristocetin concentration of 1.0 mg per ml was performed, the four family members tested had clearly abnormal results of 40 percent activity or less. Olson et al reported that patients with mild von Willebrand’s disease may
have normal aggregation in response to ristocetin at high concentrations (1.5 to 2.0 mg per ml) while they do not respond to lower concentrations of ristocetin (1.0 to 1.2 mg per ml). Also, it has been demonstrated that in type IIB von Willebrand's disease, there is a hypersensitivenes to ristocetin. Because of these observations, we now use at least two concentrations of ristocetin, between 1.0 and 1.5 mg per ml, for ristocetin induced platelet agglutination.

Tests to include for a complete von Willebrand's workup are VIII:C assay, VIIIR:RAg, bleeding time, platelet retention and ristocetin cofactor assay. If possible, crossed immunoelectrophoresis or SDS agarose electrophoresis should be performed, as identifying the multimeric structure of the von Willebrand's factor protein will allow definitive subtyping of von Willebrand's disease. Ideally, all testing should be repeated at least once, because of the variable expression of the disease and the widely reported observation that negative test results may be seen on one or more occasions particularly in mild cases. Therefore, it is important to identify these individuals and advise them on the complications which may occur.

References


