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# Monitoring of von Willebrand Factor Inhibitors in Patients with Type 3 von Willebrand Disease Using a Quantitative Assay

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## Summary

**Background:** Antibodies inhibiting von Willebrand factor (VWF) develop in a subset of patients with Type 3 von Willebrand disease (VWD3) and may be detected by their inhibition of ristocetin cofactor activity (VWF:RCo). Some also inhibit factor VIII activity (VIII:C).

**Aim:** To describe monitoring of ten VWD3 patients for VWF inhibitors using a quantitative assay.

**Methods:** VWF inhibitor was measured by comparing VWF:RCo activity of a mix of patient and pooled normal plasma (PNP) with a mix of buffer and PNP, using agglutination of fixed normal platelets in microtiter plates or lyophilized platelets in an aggregometer. VIII:C inhibitor was measured by Bethesda assay. Preanalytical heat treatment of patient plasma was used during treatment episodes.

**Results:** Four of 10 patients monitored developed VWF inhibitors, 2 detected during bleeding episodes refractory to treatment and 2 on routine screening. Data from the first 5 patients were used to establish an arbitrary unit, VWU, defined as the amount of inhibitor per mL of patient plasma inactivating 25% of the activity of 1 mL of PNP. In 3 of 4 patients, both VWF:RCo and VIIII:C were inhibited at some time points, although VIII:C inhibition sometimes disappeared. In one patient, no VIII:C inhibition was seen. Two patients remained inhibitor positive more than 15 years after inhibitor detection, one became negative following immune tolerance induction, and one was deceased.

**Conclusions:** VWF inhibitors can be quantitatively monitored in VWD3 patients. Preanalytical heat treatment may be required for their detection post infusion.

### Keywords

von Willebrand factor; inhibitor; von Willebrand disease; factor VIII

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Author Contributions

CH Miller designed the research, directed performance of the research, analyzed data, and wrote the paper.

Disclosures

The author states that she has no interests which might be perceived as posing a conflict or bias. The findings and conclusions in this report are those of the author and do not necessarily represent the views of the Centers for Disease Control and Prevention.

## Introduction

Antibodies to von Willebrand factor (VWF) are a rare complication of Type 3 von Willebrand disease (VWD3) and have been reported in fewer than 40 cases.[1] VWD3, the most severe form of von Willebrand disease (VWD), is defined by the absence of VWF as demonstrated by undetectable levels of VWF antigen (VWF:Ag) and VWF activity measured in various functional assays.[2] Factor VIII coagulant activity (VIII:C) is secondarily reduced to <10 units per deciliter (U/dL) due to the lack of VWF to stabilize it in the circulation. VWD3 is much less common than other forms of VWD, because it requires homozygosity or compound heterozygosity for VWD alleles.[3] Patients with VWD3 have severe muco-cutaneous bleeding and may have hemarthrosis similar to that seen in hemophilia A patients with equivalent levels of VIII:C.

VWF inhibitors occurring in VWD3 patients have usually been detected by their inhibition of ristocetin cofactor activity (VWF:RCo), a measurement of VWF binding to normal platelets in the presence of the non-physiologic agent ristocetin.[1,4] Some anti-VWF antibodies have also been demonstrated to inhibit VIII:C and to precipitate with VWF in gels, resembling heterologous antibodies.[5] Those studied have been immunoglobulin G and polyclonal,[6] recognizing multiple epitopes on VWF.[7,8] Treatment with VWF-containing products has often resulted in anaphylaxis, with the suggestion that circulating immune complexes may be present.[9,10] Such complex formation has been shown to mask the presence of inhibitors in hemophilia A[11] and hemophilia B.[11,12]

Recent reviews have noted the lack of standardization of VWF inhibitor assays and their inability to detect some antibodies.[1,4] We describe a modified assay for VWF inhibitors with preanalytical heat treatment and present data collected on a series of ten VWD3 patients monitored with quantitative assays for VWF and VIII:C inhibitor.

## Materials and Methods

### Subjects

Ten patients with Type 3 VWD were tested during clinical care at two institutions, New York Hospital-Cornell University Medical College, New York, NY, and Children's Memorial Hospital, Chicago, IL. Monitoring time varied from 1–15 years. Subjects or parents of minor children gave informed consent for use of data.

### Methods

FVIII activity (VIII:C) was measured by one-stage assay. FVIII inhibitor was measured by the Bethesda method, with one Bethesda unit (BU) defined as the amount of inhibitor which inactivates 50% of the VIII:C in 1 milliliter (mL) of pooled normal plasma (PNP) during 2 hours at 37° C.[13] Von Willebrand factor antigen (VWF:Ag) was measured by latex-bead-immunoassay (LIATest, Diagnostica Stago, Parsippany, NJ, USA). The limit of detection for VWF:Ag was 3%. Von Willebrand factor activity was measured as ristocetin cofactor (VWF:RCo) using two methods. Method 1 used timed macroscopic aggregation of formalin-fixed platelets by ristocetin (American Biochemical and Pharmaceutical, Marlton, NJ, USA) in microtiter plates.[14] Method 2 used lyophilized platelets (Biopool International, Ventura,

CA, USA) and ristocetin (American Biochemical and Pharmaceutical) in an aggregometer (ChronoLog Corp, Havertown, PA, USA), as previously described.[15] For both methods the limit of detection was 3%. Coefficient of variation (CV) was 7.5% for a normal specimen and 14.2% for an abnormal specimen for Method 2.

VWF inhibitor was measured by a modification of the method of Sarji et al,[16] which is analogous to the Bethesda method for FVIII inhibitors. VWF:RC of a 1:1 mixture of test plasma and pooled normal plasma (PNP) was measured and compared with that of a 1:1 mixture of PNP and assay buffer. The ratio of the two, multiplied by 100, the % residual activity (%RA), was converted to an arbitrary unit, VWU. VWU was defined as the amount of inhibitor in one mL of patient plasma inactivating 25% of the VWF:RC activity in 1 mL of PNP and was read from a graph of log %RA plotted against VWU (Figure 1) or using the equation $VWU = (2\text{-}\log\ \%RA)(0.125)^{-1}$. In the standard assay, no incubation was used. To measure high-titer inhibitors, the patient plasma was diluted in buffer before mixing with PNP and the resulting VWU was multiplied by the dilution factor. For post-infusion specimens, preanalytical heat treatment of patient plasma to 56°C for 1 hour followed by centrifugation was performed.

For blocking studies, antibody was adsorbed onto staphylococcal protein A (SPA) as described by Fricke et al;[17] 250 microliters (μL) of SPA suspension (Pansorbin® Cells, Sigma-Aldrich, St. Louis, MO, USA) were diluted with 1 mL of imidazole-buffered saline (IBS), pH 7.3, and centrifuged at 1620 X g for 8 minutes. The supernatant was discarded and the inside of the tube dried. The pellet was mixed with 125 μL of inhibitor plasma and mixed by vortex. The mixture was incubated at room temperature for 25 minutes, centrifuged as above, and decanted. A wash of 1 mL of IBS was added to the pellet, mixed, centrifuged, and the supernatant discarded. The antibody-bearing SPA was mixed 1:1 with plasma from a hemophilia A patient previously shown to lack FVIII coagulant antigen (VIII:Ag; kindly tested by Dr. Leon Hoyer, University of Connecticut) as a surrogate for purified VWF or with IBS and incubated at 37°C for one hour followed by centrifugation and removal of the supernatant. The pellet was then washed with IBS and the supernatant discarded. The pellet was then mixed with PNP. After incubation for 1 hour at 37°C, the mixture was centrifuged, and the PNP was removed and tested for residual VWF:RCo and VIII:C.

Immunodiffusion in agarose gels was performed using patient plasma and either PNP or cryoprecipitate.

## Results

Characteristics of the ten patients with Type 3 VWD studied and their peak inhibitor titers are shown in Table 1. Patients 1–4 developed VWF inhibitors. Inhibitors in Patients 1 and 4 were detected during acute bleeding episodes refractory to treatment. Those in Patients 2 and 3 were detected by routine screening. In Patients 1–3, inhibition of both VIIII:C and VWF:RCo could be demonstrated at some time points, while in Patient 4 only VWF:RCo inhibition was detected. No evidence of inhibitor development was found in Patients 5–10.

Inhibitor to VWF was initially quantitated by measurement of VWF:RCo activity of a 1:1 mix of patient plasma and PNP using used timed macroscopic aggregation of fixed washed platelets by ristocetin (Method 1) in Bethesda units (one unit of inhibitor equal to inhibition of 50% of the VWF:RCo in 1 mL of PNP). During monitoring of the first five patients, three with inhibitors (Patients 1–3) and two without (Patients 5 and 6), it became apparent that the Bethesda unit had limitations for clinical reporting, as VWF inhibitors appeared to be much lower in titer than VIII:C inhibitors measured concurrently. An arbitrary unit, von Willebrand unit (VWU), was therefore adopted as one VWU equal to inhibition of 25% of the VWF:RCo in 1 mL of NPP, or 75% residual activity, as illustrated in Figure 1. This definition was chosen based on 1) the observation that Patient 1 was completely refractory to treatment when her plasma exhibited 25% inhibition of VWF:RCo, 2) correlation of VWU with units of FVIII inhibition, and 3) VWU observed in patients showing typical response to therapy. Among 41 specimens, VWF:RCo inhibitor units correlated significantly better with VIII:C inhibitor units when measured in VWU (r=0.49, $P$=0.001) than when measured analogously to BU (r=0.22, $P$=0.167). Data comparing VWF inhibitor calculated by both methods are provided in Table S1. Peak inhibitor titers measured for each patient studied are shown in Table 1. Patients 5–10 showed no evidence of VWF:RCo or VIII:C inhibitor during monitoring at annual clinic visits, and showed typical response when treated. The highest titer observed among these patients was 0.9 VWU.

In establishing the assay parameters, the time dependency of inhibition and effects of infused treatment products were examined. Time dependency was studied by measurement of the residual VWF:RCo and VIII:C of a mix of patient plasma and PNP at 0, 1, and 2 hours of incubation at 37°C (Figure 2). VWF:RCo inhibition did not change with incubation, while VIII:C showed no inhibition at 0 time and increase in inhibition at 1 and 2 hours, as usually seen with FVIII-specific inhibitors. No incubation period was required for VWF:RCo inhibition, as previously reported,[16] and none was used for the clinical assay. In post-infusion specimens, preanalytical heat treatment of patient plasma to 56°C for 60 minutes followed by centrifugation could be demonstrated to reveal VWF inhibition, as shown for three specimens from Patient 1 (Figure 3A) and one specimen from each of Patients 2 and 4 (Figure 3B). This method was adopted for all post-infusion titers. Precipitation in gels could not be demonstrated using plasma from Patients 1–3 versus PNP or cryoprecipitate by immunodiffusion.

Examples of test performance for each patient are shown in Table 2, including tests performed prior to and after inhibitor development for Patients 1–4 and specimens from patients 2–4 requiring dilution for measurement. Because of changes in clinical test methods, tests for Patients 4, 7–10, and later specimens for patients 2 and 3 were performed using Method 2, which substituted an aggregometry method of VWF:RCo measurement. Results for VWF inhibitor calculated as BU and as VWU are provided for comparison.

The initial appearance of inhibitor in Patients 1–4 followed treatment with products containing both VWF and FVIII. The time course for each is illustrated in Figure S1. The earliest patient, Patient 1, developed an inhibitor at age 36 after treatment since the age of 5, including whole blood, fresh frozen plasma, cryoprecipitate, and intermediate-purity FVIII concentrate. Stored specimens prior to that date were inhibitor-negative. At inhibitor onset,

bleeding from multiple sites, including a pelvic mass, appeared with inhibitor titers of 1.2 BU and 1.4 VWU and was controlled only by treatment with FEIBA®. FEIBA® treatment was also successful for subsequent abdominal bleeding and dental extractions. Her inhibitor remained measurable against VWF:RCo and FVIII. She later succumbed to infection.

Patient 2 was diagnosed and first treated at age 16 months. At age 8 she was found on routine testing to have inhibitor titers of 4.5 BU and 4.3 VWU after previously negative tests. Titers declined during periods with no treatment, then showed anamnesis with Humate P®. She became positive for VWF:RCo inhibitor and negative for VIII:C inhibitor, remaining so for 15 years after inhibitor development, while successfully treated with Humate P®.

Patient 3, the sister of Patient 2, was diagnosed and first treated at age 3. She developed an inhibitor measuring 5.5 BU and 7.0 VWU at age 4 after a total of 21 infusions,. After 4 months, her VIII:C inhibitor fell below 1.0 BU, while her VWU remained elevated. FEIBA® and Humate P® were used for treatment. Fifteen years after inhibitor development, she demonstrated both VWF:RCo and VIII:C inhibition.

Patient 4 developed poor recovery of VWF:RCo and VIII:C during treatment with Humate P® for gastrointestinal bleeding. His history was unknown. He was started on immune tolerance induction (ITI) with 40 U/kg of Humate P® three times per week when his inhibitor had declined to 0 VWU and 0.3 BU. During ITI his inhibitor peaked at 3.8 VWU with consistently negative VIII:C inhibitor. After 6 months of ITI, he was lost to follow up for 7 months with no treatment. His inhibitor titer was then found to be 0 VWU and 0.3 BU. He received Humate P® with no anamnestic response and was subsequently treated with episodic therapy without inhibitor recurrence.

To determine whether the antibodies present reacted only with VWF or with both FVIII and VWF, blocking studies were carried out. Antibody from Patients 1–3 was bound to staphylococcal protein A (SPA). When the antibody-SPA was mixed with PNP, both VWF:RCo and VIII:C were decreased (Table 3). When a separate aliquot of the SPA-bound antibody was first mixed with hemophilia plasma without VIII:Ag as a source of VWF, both VWF:RCo and VIII:C remained in the PNP, suggesting that reaction of the antibody with VWF alone blocked removal of both VWF and VIII:C. Further characterization of these inhibitors was limited by small specimen volumes in the pediatric patients and the low inhibitor titers in the single adult patient, Patient 1, which remained below 2 VWU or BU.

## Discussion

The first inhibitor in a patient with Type 3 von Willebrand disease was reported by Sarji et al in 1974.[16] They adapted a newly described method for measurement of VWF activity in swine[18] to humans. Unlike the porcine assay, the human assay required use of ristocetin for platelet aggregation. Using timed macroscopic aggregation of washed platelets by ristocetin with an endpoint described as the “snowstorm effect,” they demonstrated that the patient’s plasma inhibited the measurement of the VWF activity now called ristocetin cofactor. The patient’s plasma also inhibited ristocetin-induced aggregation of washed normal platelets

in an aggregometer. On first report, this inhibitor interfered only with VWF and not FVIII activity; VIII:C inhibition developed later, and the inhibitor was determined to be an antibody, which showed anamnesis.[19] Other similar cases were rapidly reported,[20–22] including some in which the antibodies precipitated with normal and hemophilic plasma but not with that from other VWD3 patients.[5] Ruggeri et al[6] demonstrated that VWF:RCo was inhibited by dilutions of the patient's plasma in a manner suggesting second-order kinetics, as described for most FVIII inhibitors.[23] They defined 1 unit of inhibitor as the amount inactivating 75% of the VWF:RCo in one mL of PNP. We initially used the method of Sarji et al,[16] modified to use formalin-fixed platelets,[14] for clinical testing. Later, we substituted aggregation of lyophilized platelets by ristocetin in an aggregometer, a method which has been widely used clinically to measure VWF:RCo. We have not performed VWF inhibitor tests with the newer methods now available for measurement of VWF activity.[24] Additional study would be required to determine whether VWF inhibitors can be detected in the newer tests utilizing GP1b fragments instead of whole platelets.

Use of a standard unit for VWF:RCo inhibition facilitates monitoring of VWF inhibitors over time. In our experience, however, neither a quantitative measure equivalent to the BU nor that reported by Ruggeri et al[6] adequately described the clinical course in these patients. Our first patient was completely refractory to treatment when her plasma exhibited 25% or greater inhibition of VWF:RCo. We therefore selected that as the definition of a 1.0 VWU inhibitor. We found good correlation of those results with the levels of VIII:C inhibition in the Bethesda assay early in the course of therapy. All patients showing typical response to therapy maintained inhibitor titers below 1.0 VWU.

From the time of our first observation of the significant clinical impact of a relatively low titer VWF inhibitor on Patient 1, we performed routine screening of all of our VWD3 patients for VWF:RCo and VIII:C inhibitors. While the ten patients studied had undetectable levels of VWF by the methods used, it was not possible to confirm that all were completely deficient and thus at risk for inhibitor development. Genotypes were unavailable. Four of the 10 patients followed developed inhibitors. This is a higher frequency than the prevalence estimates of 7.5 and 9.5% based on survey and registry data would predict.[1] It is closer to the 4 of 27 well-studied patients described by Ruggeri et al.[6] The VWD3 patients followed at our hemophilia treatment centers may be more severely affected and not representative of the patient group as a whole. All, however, had negative tests when first seen and were thus not selected for this complication. They may have been monitored more intensively than other groups reported: two of the four inhibitors were first detected on routine screening rather than due to poor response to therapy. The reported failure to identify inhibitors in some patients showing poor response[4] could be due to two factors: first, the amount of *in vitro* inhibition required to produce a clinical effect is much lower than that seen with FVIII inhibitors, and recognition of this fact is required for correct interpretation of inhibitor assay results; and second, antibodies may not be detectable when they are bound to infused VWF. We found preanalytical heat treatment to release bound antibodies to be necessary for demonstration of VWF:RCo inhibition during treatment episodes, similar to our findings for FVIII inhibitors.[11] We are not aware that this method has been previously reported for VWF inhibitors.

All four inhibitor patients had been exposed to products containing both VWF and FVIII, usually cryoprecipitate when first treated. They predate the availability of products containing only VWF. Although their mutations are not known, most VWD3 patients have null mutations, predisposing them to react to VWF as a foreign protein. VWD3 patients produce FVIII, since the *F8* gene is normal, but it is short-lived in the circulation due to lack of VWF to stabilize it, as illustrated by the fact that Infusion of VWF alone raises their FVIII to normal levels; thus, they would be unlikely to produce separate antibodies to FVIII. In 3 out of 4 of our patients, the inhibitors also inhibited VIII:C at some points in their course. The ability of VIII:Ag-negative hemophilic plasma, as a source of VWF, to block removal of both VWF:RCo and VIII:C by three of the antibodies is a crude demonstration that the VIII:C inhibition is not caused by a separate VIII:C inhibitor but by the VWF inhibitor, agreeing with other reports.[19] The VWF and VIII:C inhibition appeared together in three of the four patients; however, VIII:C inhibition was not detectable in Patient 4 and disappeared in Patient 2. The difference was not an artifact of the *in vitro* tests, since Patient 2 showed post infusion levels of 25% VWF:RCo and 156% VIII:C, consistent with her inhibitor levels at the time. This effect may be due to changes over time in the population of antibodies present and their specificities and/or affinities, as has been observed for FVIII inhibitors in hemophilia patients.[25] The intriguing difference between the time-dependent VIII:C inhibition and non-time-dependent VWF inhibition suggests that in some patients the VIII:C inhibition may not be simply due to its concurrent removal with VWF but to interaction of some specific antibodies with sites on VWF that cause steric hindrance influencing the clot-based assay. When VIII:C inhibition was lacking for long periods of time, as in Patient 3, the population of antibodies being produced may have changed. The discrepant VWF and VIII:C inhibitor titers in these patients, with the total absence of VIII:C inhibition in Patient 4, suggests that monitoring of VIII:C inhibitor alone is not sufficient to fully characterize these patients.

Patients with VWD3 and VWF antibodies are rarely treated today with VWF-containing products due to their high incidence of anaphylaxis and the availability of other treatment modalities.[4] Treatment with recombinant FVIII alone is not satisfactory due to its instability in the absence of VWF, although large doses given by continuous infusion have been successful for short periods.[4] Newer bypassing agents such as recombinant factor VIIa have been used successfully,[4] and emicizumab use has recently been reported for FVIII-dependent bleeding.[26] Although neither replaces VWF, they have been effective for joint and surgical bleeding. Our experience with Patient 4 shows that inhibitors in VWD3, as in hemophilia A, can be eliminated by ITI, although this may be difficult in patients showing anaphylaxis.[27] Quantitative inhibitor measurement can be particularly useful in this effort, and preanalytical heat treatment may be required to demonstrate true disappearance of the inhibitor.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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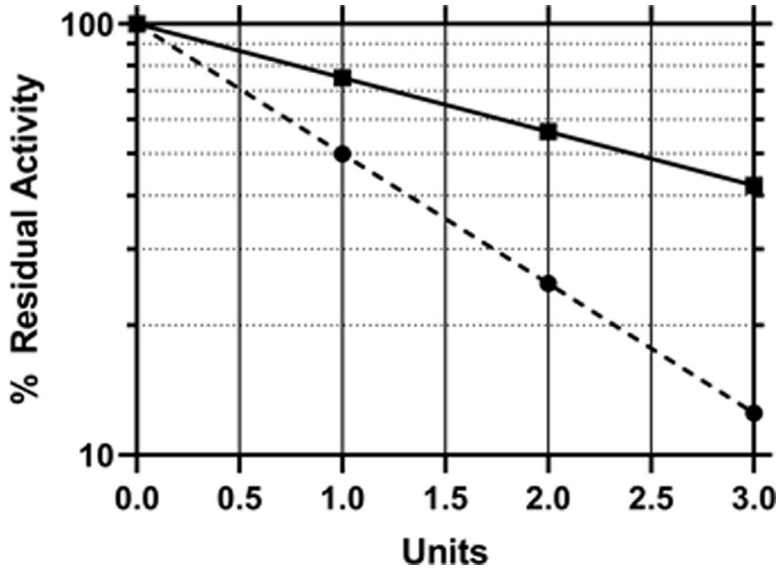


**Figure 1.**

Graph of the relationship between % residual activity of a mix of patient plasma and normal pooled plasma for the newly defined von Willebrand factor inhibitor units (VWU), shown by the solid line, compared with factor VIII inhibitor units (BU) shown by the dashed line

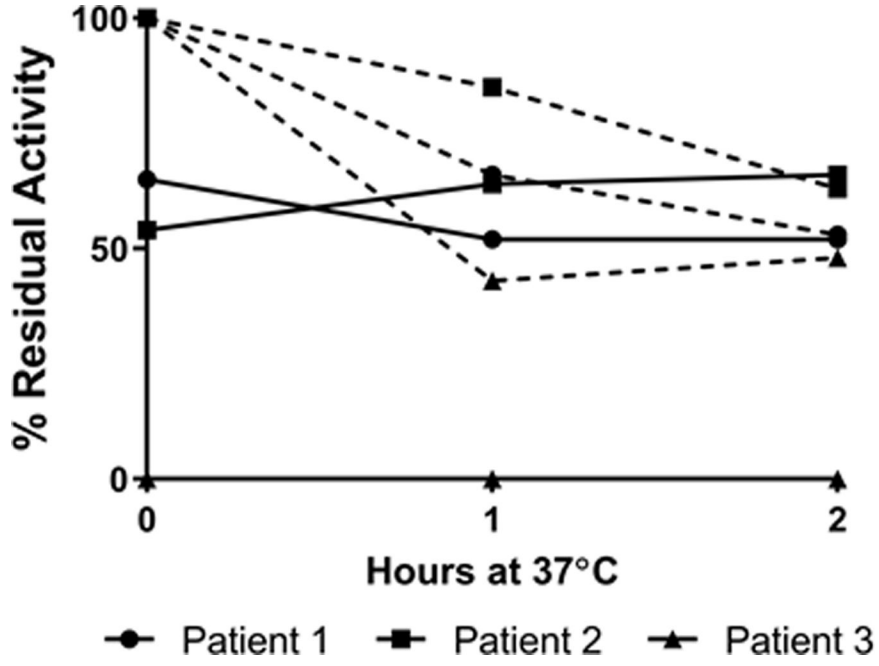


**Figure 2.**

Measurement of ristocetin cofactor (solid lines) and factor VIII activity (dashed lines) after Incubation of patient plasma mixed 1:1 with pooled normal plasma at 37°C for 0, 1, and 2 hours

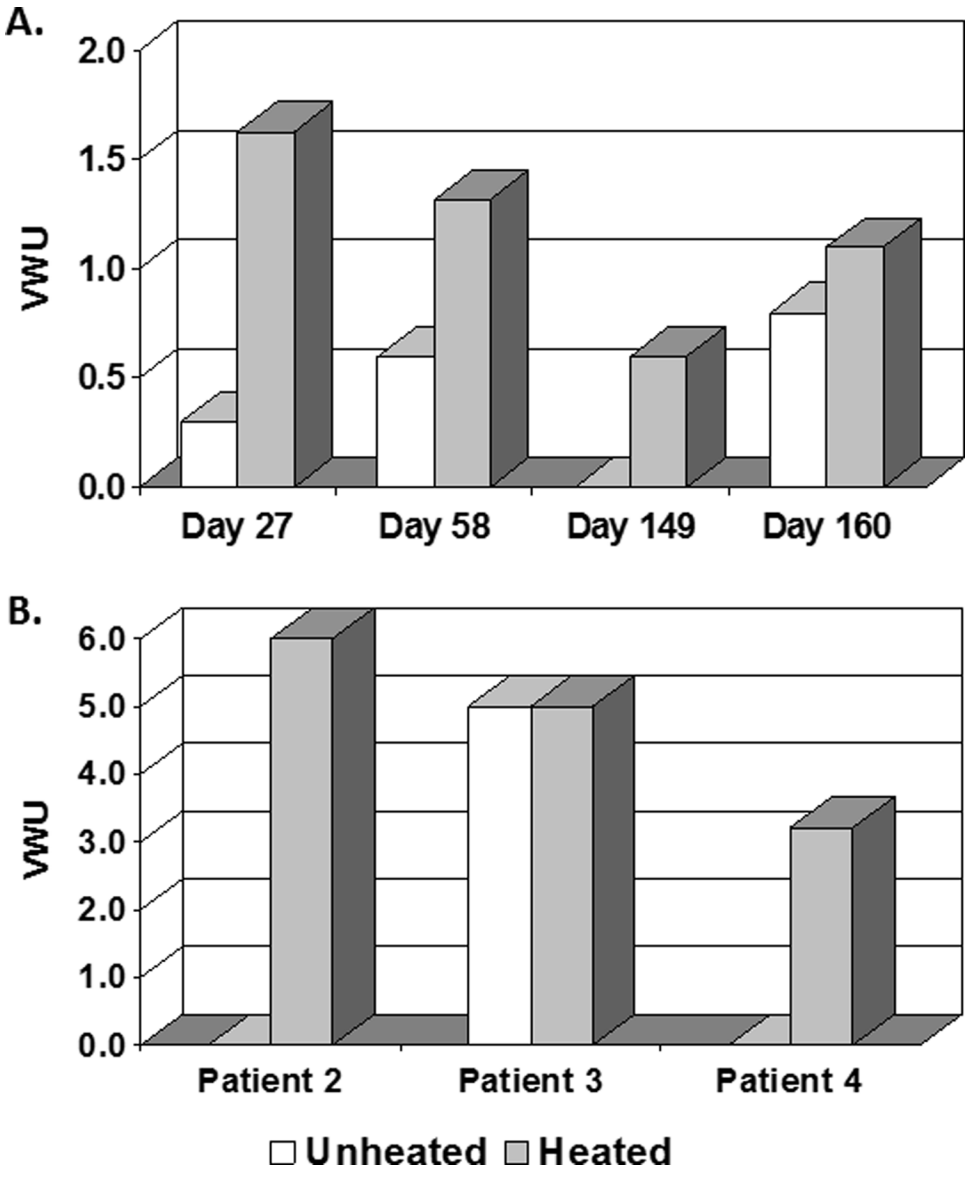


**Figure 3.**

von Willebrand inhibitor units (VWU) measured before (Unheated) and after (Heated) heating of patient plasma to 56°C for 1 hour for A. Patient 1 and B. Patients 2–4

**Table 1.**

Characteristics of patient with von Willebrand disease Type 3 including baseline levels of factor VIII activity (VIII:C), von Willebrand factor antigen (VWF:Ag), and ristocetin cofactor (VWF:RCo) in units per deciliter and highest inhibitor titer reached to VIII:C in Bethesda units (BU) and to VWF:RCo in von Willebrand units (VWU)

| | | | | Baseline Levels | | | | Highest Inhibitor Titer | |
|---|---|---|---|---|---|---|---|---|---|
| **Patient** | **Gender** | **Age at First VWF Inhibitor Test** | **Age at Inhibitor Detection** | **VIII:C** | **VWF:Ag*** | **VWF:RCo*** | **Ethnicity** | **VIII:C (BU)** | **VWF:RCo (VWU)** |
| 1 | F | 36 | 36 | 2–4 | <3.0 | <3.0 | African American | 1.7 | 1.7 |
| 2 | F | 6 | 8 | 4.0 | <3.0 | <3.0 | Armenian** | 7.3 | 4.5 |
| 3 | F | 3 | 4 | 2.0 | <3.0 | <3.0 | Armenian** | 7.0 | 7.4 |
| 4 | M | 15 | 16 | 3.0 | <3.0 | <3.0 | Caucasian | 0.3 | 3.8 |
| 5 | F | 31 | None to age 34 | -- | <3.0 | <3.0 | Caucasian | 0 | 0 |
| 6 | M | 22 | None to age 25 | -- | <3.0 | <3.0 | Caucasian | 0 | 0 |
| 7 | M | 7 | None to age 11 | 2.0 | <3.0 | <3.0 | Caucasian | 0.3 | 0 |
| 8 | M | 5 | None to age 9 | 2.5 | <3.0 | <3.0 | Hispanic | 0.2 | 0.9 |
| 9 | F | 10 | None to age 14 | 1.5 | <3.0 | <3.0 | Iranian** | 0.3 | 0.1 |
| 10 | M | 1 | None to age 3 | 8.0 | <3.0 | <3.0 | Caucasian | 0 | 0 |

*All were undetectable with the methods used.

**known parental consanguinity

**Table 2.**

Measurement of von Willebrand factor inhibitor in von Willebrand units (VWU) and factor VIII inhibitor in Bethesda units (BU) in 10 patients with Type 3 von Willebrand disease. Patients 1–4 show examples of results before and after inhibitor development. In post inhibitor specimens of Patients 2–4, dilution of patient plasma was required to calculate VWU and BU.

| Patient | VWF:RCo Patient | VWF:RCo Patient + PNP | VWF:RCo Buffer + PNP | % Residual Activity | VWU | BU |
|---|---|---|---|---|---|---|
| 1 Before | <3.0 | 51 | 53 | 96 | 0.1* | 0 |
| 1 After | <3.0 | 30 | 50 | 60 | 1.8* | 1.2 |
| 2 Before | <3.0 | 46 | 44 | 100 | 0* | 0.3 |
| 2 After | <3.0 | 0 | 52 | 0 | -- | -- |
| | | 24 (1:2) | 52 | 47 | 5.2* | 4.5 |
| 3 Before | <3.0 | 49 | 44 | 100 | 0* | 0.5 |
| 3 After | <3.0 | 0 | 52 | 0 | -- | -- |
| | | 29 (1:2) | 52 | 56 | 4.0* | 5.5 |
| 4 Before | <3.0 | 48 | 46 | 100 | 0** | -- |
| 4 After | <3.0 | 6 | 40 | 15 | -- | 0.1 |
| | | 25 (1:2) | 40 | 63 | 3.2** | -- |
| 5 | <3.0 | 52 | 50 | 100 | 0* | -- |
| 6 | <3.0 | 50 | 48 | 100 | 0* | -- |
| 7 | <3.0 | 53 | 50 | 100 | 0** | 0.3 |
| 8 | <3.0 | 37 | 48 | 77 | 0.9** | 0.2 |
| 9 | <3.0 | 31 | 32 | 97 | 0.1** | 0.3 |
| 10 | <3.0 | 43 | 34 | 100 | 0** | 0 |

VWF:RCo=ristocetin cofactor in units per deciliter; PNP=pooled normal plasma

*VWF:RCo inhibitor test performed by Method 1

**VWF:RCo inhibitor test performed by Method 2

**Table 3.**

Partial removal of ristocetin cofactor (VWF:RCo) and factor VIII activity (VIIII:C) from pooled normal plasma by patient antibody bound to staphylococcal protein A (A) and blocking of removal by hemophilia A (HA) plasma lacking factor VIII coagulant antigen (B)

| | % Residual Activity | |
|---|---|---|
| | VWF:RCo | VIII:C |
| A. Patient 1 + Buffer | 43 | 79 |
| B. Patient 1 + HA Plasma | 100 | 90 |
| A. Patient 2 + Buffer | 55 | 53 |
| B. Patient 2 + HA Plasma | 100 | 99 |
| A. Patient 3 + Buffer | 62 | 55 |
| B. Patient 3 + HA Plasma | 91 | 99 |