

# Laboratory Diagnosis and Management of Von Willebrand Disease in South Africa

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## ABSTRACT

Patients with von Willebrand disease (VWD) in South Africa are cared for in 17 Hemophilia Treatment Centers. The exact prevalence of the disease is uncertain, but 539 patients are annotated in registries. VWD patients are mostly diagnosed in the five largest academic centers, and the classification of the subtypes is performed by one of these, the VWD testing facility. An algorithm is used for the diagnosis of VWD. The distribution of subtypes diagnosed by the VWD reference center is 38%, 58%, and 4% for type 1, 2, and 3, respectively, and ~15% of plasma samples received are rejected due to poor storage and transport conditions. A novel single nucleotide polymorphism has been found in an African patient with type 2B VWD. From the type 1 VWD patients who were diagnosed by the VWD testing facility, 45% seem to have an increased VWF clearance phenotype with a propeptide-to-antigen ratio of  $1.9 \pm 0.3$ . VWD patients are treated with desmopressin, factor (F)VIII/VWF concentrate (Haemosolvate FVIII; National Bioproducts Institute, Durban, South Africa), and tranexamic acid. Haemosolvate FVIII contains a VWF antigen concentration of  $167 \pm 27$  IU/mL, a ristocetin cofactor activity of  $100 \pm 29$  IU/mL, a collagen binding activity of  $99 \pm 29$  IU/mL, normal VWF multimers, and a FVIII concentration of 50 IU/mL. Not all patients with VWD are currently classified, and many VWD patients in South Africa are probably undiagnosed.

**KEYWORDS:** von Willebrand disease, classification, diagnosis, hemophilia treatment centers, South Africa

South Africa has a total land area of slightly more than 1.2-million km<sup>2</sup>, making it roughly the same size as Nigeria, Angola, Mali, or Colombia. It measures some 1600 km from north to south, roughly the same from east to west, and has a population of 49 million.

Patients with bleeding diatheses in South Africa are cared for in 17 Hemophilia Treatment Centers (HTCs) distributed all over the country. The HTCs function in collaboration with the South African National Department of Health, the South African

Haemophilia Foundation (the national members' organization), the Medical and Scientific Council of South Africa, and the National Haemophilia Nurses Committee to ensure optimal management of patients with bleeding disease, including von Willebrand disease (VWD).<sup>1</sup>

Hemophilia care data collected from 2004 to 2007 shows that > 2200 patients with bleeding diatheses were cared for in this period by 79 professionals in 17 HTCs. Of these patients, 59% had hemophilia A, 21%

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von Willebrand Disease: Local Diagnosis and Management

of a Globally Distributed Bleeding Disorder; Guest Editor, Emmanuel J. Favaloro, Ph.D., F.F.Sc. (RCPA).

Semin Thromb Hemost 2011;37:576–580. Copyright © 2011 by Thieme Medical Publishers, Inc., 333 Seventh Avenue, New York, NY 10001, USA. Tel: +1(212) 584-4662.

DOI: <http://dx.doi.org/10.1055/s-0031-1281045>.

ISSN 0094-6176.

VWD, and 12% hemophilia B; the remainder had rare bleeding diatheses and thrombocytopathies.<sup>1</sup>

The exact prevalence of VWD in South Africa is uncertain. The central plateau of South Africa is relatively dry. Epistaxis in the general population is therefore frequent, and there is not a high index of suspicion of bleeding disorders. Because of lack of emphasis during training, menorrhagia is termed *functional* if there is no obvious gynecologic cause. The woman is then offered oral contraceptives if she is younger and a hysterectomy if she is older. The 2008 Global Survey of the World Federation of Hemophilia estimated there are 522 diagnosed patients with VWD in South Africa. The access-based Hemophilia Registry mentions 539 patients in South Africa.<sup>2</sup> African patients might be grossly underdiagnosed because only 3.9% of patients mentioned in the registry are of African origin. Women form 63.8% of patients identified with VWD in South Africa.<sup>3</sup>

VWD patients are mostly diagnosed in the five largest academic centers, and the classification of the subtypes is done by only one of these five centers, the VWD testing facility. This article concentrates on the VWD testing facility's findings regarding the statistics, diagnosis, and challenges in the laboratory diagnosis of VWD in South Africa.

## LABORATORY DIAGNOSIS OF VON WILLEBRAND DISEASE

The following diagnostic tests are performed by the VWD testing facility: von Willebrand factor antigen (VWF:Ag), ristocetin cofactor (VWF:RCo), collagen binding activity of VWF (VWF:CB), VWF propeptide levels (VWF:pp), multimeric analysis of VWF, the factor (F)VIII binding assay of VWF, and mixing studies to identify platelet-type VWD (PT-VWD).

The VWD testing facility adopted and modified the guidelines for diagnosis and treatment of VWD in Italy according to the algorithm outlined in Fig. 1.<sup>4,5</sup>

A proportional reduction of both VWF:Ag and VWF:RCo with a RCo:Ag ratio  $>0.7$  as well as a proportional reduction of both VWF:Ag and VWF:CB with a CB:Ag ratio  $>0.7$  suggest type 1 VWD if the VWF:Ag level is low ( $<45\%$ ). If type 1 VWD is diagnosed, it is important to determine the clearance rate of VWF. The VWF:pp is then performed. If the ratio between the VWF:pp and the VWF:Ag is  $>2$ , an increased clearance rate of VWF is suspected for that patient.

If the RCo:Ag ratio and/or the CB:Ag ratio is  $<0.7$ , type 2 VWD is diagnosed. Type 2B VWD can be identified with an enhanced ristocetin-induced platelet agglutination (RIPA) (response with  $<0.8$  mg/mL). Type 2B VWD is distinguished from a PT-VWD (pseudo-VWD) by performing the RIPA mixing studies. Type 2A and 2M typically have reduced RIPA

(response only at  $>1.2$  mg/mL). Multimeric analysis in plasma is necessary to distinguish between type 2A VWD (lack of largest and intermediate multimers) and type 2M VWD (all the multimers are present). The VWF:CB is usually normal in type 2M VWD due to the presence of high molecular weight multimers, except where a collagen binding defect is diagnosed in patients with type 2M VWD. In type 1 VWD the ratio between factor VIII and VWF:Ag is always concordant. When this ratio is discordant with a FVIII level  $<20\%$ , type 2N VWD is suspected, and this type of VWD can be confirmed by performing a factor VIII binding assay.

Genotypic data are only obtained for patients with a functional abnormality of VWD. In an ongoing study we have searched for mutations in exon 28 of the VWF gene in five patients with functional defects of VWF to set up the method for genetic analysis of VWD. We used two patients with type 2M, two with type 2B, and one with type 2A VWD in this study. The whole exon 28 was analyzed in four specific fragments, using polymerase chain reaction with primers that mismatch the pseudogene. The mutations were identified by automatic sequencing of the different fragments. The following polymorphisms were detected. A silent single nucleotide polymorphism (SNP) 4641T/C in all five patients, the SNP 4141A/G in three patients, a silent SNP 3795G/A in one patient, and a new silent SNP 4923G/A in a patient from the African population. It is important to note that no polymorphisms in exon 28 were previously reported from African populations.

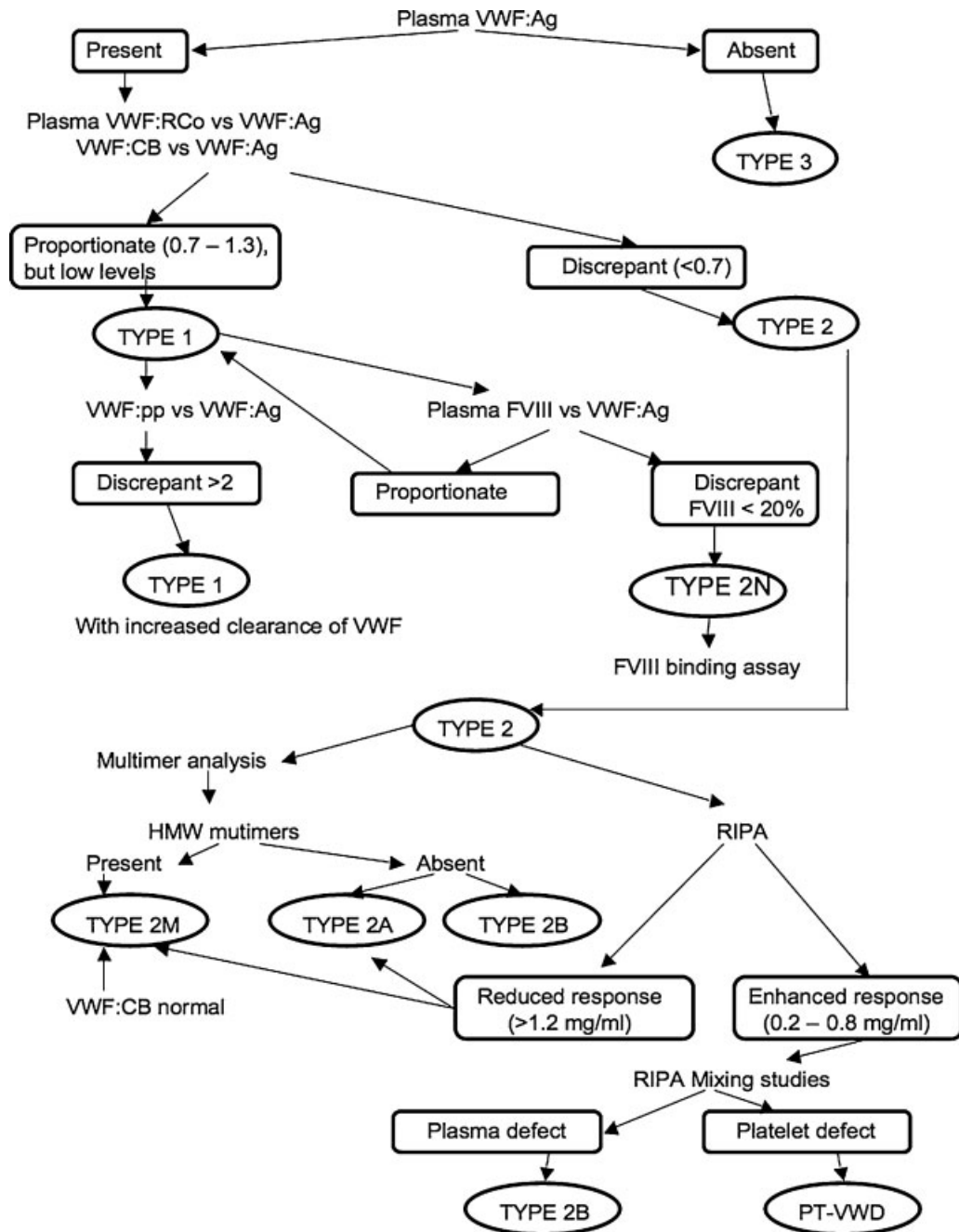
The VWFpp levels are only performed on type 1 VWD patients. We have found that 45% of our type 1 VWD patients seem to have an increased VWF clearance phenotype with a pp:Ag ratio of  $1.9 \pm 0.3$ . Our normal range for the pp:Ag ratio of normal subjects is  $1.3 \pm 0.24$ .

## VON WILLEBRAND DISEASE DIAGNOSTIC STATISTICS

The VWD testing facility is situated in Bloemfontein, the legislative capital of South Africa, in the central part of the country. The academic complex in Bloemfontein serves patients from the Free State and the Northern Cape provinces with a total population of  $\sim 4$  million. The VWD testing facility, however, receives patient samples from all over the country for diagnosis but especially for the classification of VWD.

Table 1 outlines the relative proportion of the various VWD types diagnosed by the VWD reference center. A total of 250 patients were included in this data set.

Because the VWD testing facility receives mostly VWD samples to be classified and not to be diagnosed,



**Figure 1** Algorithm for the laboratory diagnosis of von Willebrand disease (VWD) used in our center. FVIII, factor VIII; PT-VWD, platelet-type VWD; RIPA, ristocetin-induced platelet agglutination; VWF:Ag, von Willebrand factor antigen; VWF:CB, VWF collagen binding; VWFpp, VWF propeptide; VWF:RCo, VWF ristocetin cofactor.

the distribution of subtypes diagnosed is 38%, 58%, and 4% for types 1, 2, and 3, respectively. From the type 2 VWD patients, 23% were diagnosed with type 2A, 22% with type 2B, 13% with type 2M, and none with type 2N. No patients with PT-VWD have so far been diagnosed, probably due to the unavailability of platelets from these patients, because most of the type 2 VWD samples are referrals (i.e., transported plasma) from larger HTC in the country.

### CHALLENGES IN THE LABORATORY DIAGNOSIS OF VON WILLEBRAND DISEASE

Samples must be stored immediately after centrifugation in polypropylene tubes at  $-70^{\circ}\text{C}$  until analyzed.<sup>5</sup> It is thus important to note that a cryoprecipitate might form if plasma samples are stored at temperatures warmer than  $-70^{\circ}\text{C}$ . Cryoprecipitates contain large quantities of VWF and especially high molecular weight

**Table 1** Relative Proportion of the Various von Willebrand Disease Types

Subtype of VWD	Percentage (Number) of Total Patients Diagnosed*
Mild type 1	30.7% (75)
Moderate to severe type 1	8.2% (20)
Type 2A	22.5% (55)
Type 2B	23.4% (57)
Type 2M	13.1% (32)
Type 2N	0% (0)
Type 3	2.1% (5)
Platelet type	0% (0)
Total	100% (244)

\*Total number of patients = 250.  
VWD, von Willebrand disease.

multimers.<sup>6</sup> All tests therefore must be done on original aliquots that were not previously thawed, and plasma samples should be thawed at 37°C before performing diagnostic tests. Special care should be taken to ensure that no cryoprecipitate is present in the samples. Therefore it must be dissolved before the tests are performed; otherwise it will influence the results.<sup>6</sup>

The VWD testing facility receives plasma samples for subtyping of VWD from most HTC's countrywide. About 15% of samples received are rejected due to poor plasma storage and transport conditions. Samples are now rejected if unfrozen upon arrival or if sent refrigerated only. This decision was made after performing a study where the VWF levels, activity, and multimer distribution were measured on plasma samples following different storage conditions. Normal plasma samples were exposed to different storage conditions and time intervals. We found that the VWF:Ag, VWF:CB, and VWF:RCo results remain normal after storage at -70°C. The multimer patterns also remain normal. However, after storage at -20°C in a household chest freezer (not frost free), both the functional assays showed a decreased activity of VWF, and the multimer analysis showed an absence of the high molecular weight multimers in some samples. The multimer pattern thus stays stable at -70°C but not at -20°C. Table 2 shows the results of one such normal plasma sample, and Fig. 2 shows the associated multimer pattern. Many laboratories in South Africa use household chest freezers. We also found that even when a plasma sample is thawed and frozen up to five times, the multimer pattern stays normal when stored at -70°C. Fig. 3 shows the results of a sample that was frozen five times at -70°C.

### TREATMENT OF VON WILLEBRAND DISEASE IN SOUTH AFRICA

VWD patients are treated with desmopressin (DDAVP), FVIII/VWF concentrate, tranexamic acid, and oral contraceptives. The choice of medication

**Table 2** Test Results of a Normal Plasma Sample Stored at -20°C in a Domestic Chest Freezer with No Frost-Free Facility versus When Stored at -70°C for 3 Weeks before Testing

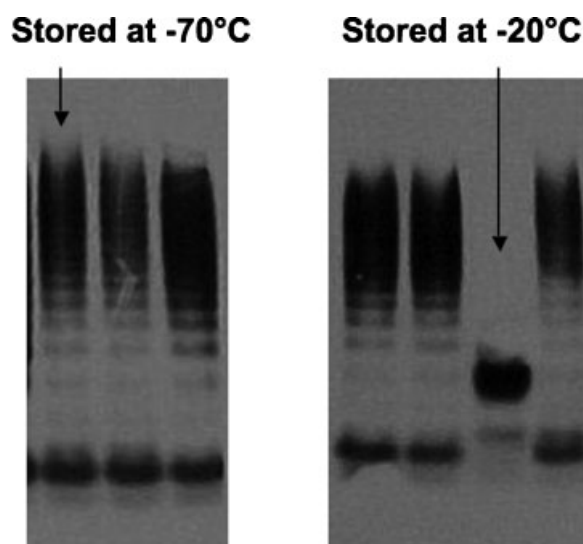
Tests	Stored at -20°C	Stored at -70°C
VWF:Ag	71	74
VWF:CB	0	87
VWF:RCo	0	73
Multimer	LMW multimers patten absent	LMW multimers present

VWF:Ag, von Willebrand factor antigen; VWF:CB, VWF collagen binding; VWF:RCo, VWF ristocetin cofactor; LMW, low molecular weight.

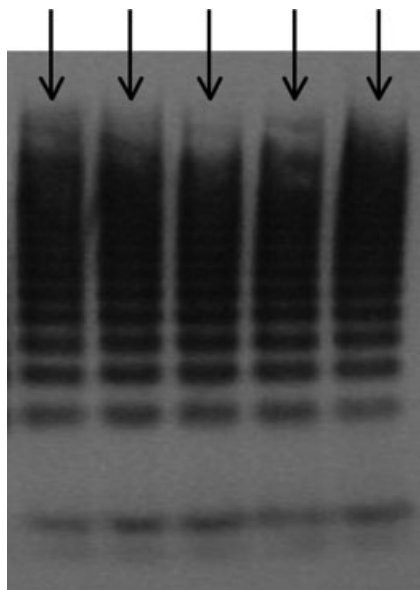
See Fig. 2 for multimer patterns.

depends on the severity of the bleeding or the type of surgical or dental intervention. DDAVP is usually the first product of choice for treatment of new patients with VWD.

The FVIII/VWF concentrate used in South Africa is Haemosolvate FVIII (National Bioproducts Institute, Durban, South Africa). Haemosolvate Factor VIII is an intermediate purity factor VIII concentrate, currently used for the treatment of hemophilia A and VWD. In a previous study we determined the concentration and activity of VWF in Haemosolvate Factor VIII. We received 32 batches of the concentrate from the National Bioproducts Institute in Pinetown, South Africa, and performed the VWF:Ag assay to determine the VWF levels. The functional activity of VWF was determined by performing the VWF:RCo and VWF:CB assays. We also determined the FVIII levels and the multimeric analysis of VWF in these concentrates. For all the tests, we needed to dilute the concentration



**Figure 2** Multimer patterns of a normal plasma sample stored at -70°C versus when stored in a domestic chest freezer at -20°C for 3 weeks.



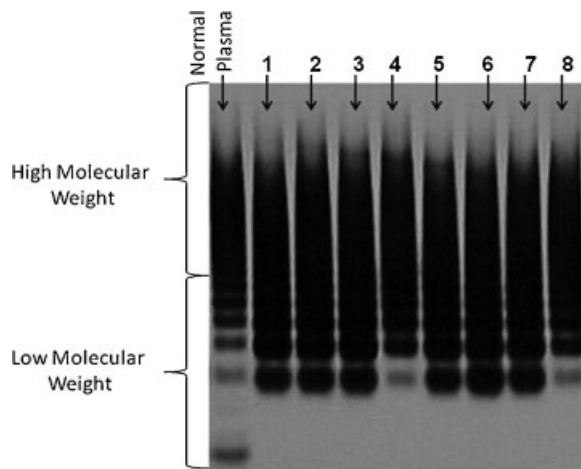
**Figure 3** Multimer patterns of a normal plasma sample that was repeatedly thawed and frozen five times at  $-70^{\circ}\text{C}$ .

extensively. The VWF:Ag concentration of all batches had a mean value of  $167 \pm 27$  IU/mL, a VWF:RCo activity of  $100 \pm 29$  IU/mL, a VWF:CB activity of  $99 \pm 29$  IU/mL, and a factor VIII concentration of 50 IU/mL. The multimeric analysis showed a normal multimer pattern as seen in Fig. 4. We thus found that the VWF levels and activities in Haemosolvate Factor VIII are more than twice that of the FVIII level. This is now taken into account when this product is administered to patients for the treatment of VWD.

The antifibrinolytic drug tranexamic acid is often given as treatment for mucocutaneous bleedings, if necessary in combination with DDAVP or Haemosolvate Factor VIII. It is also given before and after surgical or dental procedures.

## CONCLUSION

Patients with VWD in South Africa are cared for in 17 HTC's distributed throughout the country. These patients are treated according to international guidelines and with the VWF/FVIII concentrate produced and used for VWD in South Africa, Haemosolvate FVIII, a highly active VWF concentrate. The diagnosis of VWD is done mostly in only the five largest academic centers. Except for the VWD testing facility, all these centers diagnose VWD mostly by the VWF:Ag and the VWF:RCo or automated VWF activity assays. A discrepancy between these two tests would indicate type 2 VWD. The further typing of VWD is done only by the VWD testing facility, situated in Bloemfontein. However, only a very limited number of patient samples are referred to the VWD testing facility from the large



**Figure 4** von Willebrand factor multimer patterns of eight batches of Haemosolvate FVIII.

academic centers. This is mostly due to expensive shipping costs. The inherent limitations in sensitivity, reproducibility, and interlaboratory variability of the agglutination-based VWF:RCo and RIPA tests are well known.<sup>7</sup> Thus, given the limited tests applied in most centers, many VWD patients in South Africa might be misdiagnosed or remain undiagnosed.

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