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Towards improved diagnosis of von Willebrand disease: Comparative evaluations of several automated von Willebrand factor antigen and activity assays

Emmanuel J. Favaloro *, Soma Mohammed

Department of Haematology, Institute of Clinical Pathology and Medical Research (ICPMR), Pathology West, Westmead Hospital, Westmead, NSW, Australia

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ABSTRACT

Introduction: von Willebrand disease (VWD) is reportedly the most common bleeding disorder and arises from deficiency and/or defects of von Willebrand factor (VWF). Laboratory diagnosis and typing has important management implications and requires a wide range of tests, including VWF activity and antigen, and involves differential identification of qualitative *vs* quantitative defects.

Methods: We have assessed several VWF antigen and activity assays (collagen binding [VWF:CB], ristocetin cofactor [VWF:RCo] and the new Siemens INNOVANCE assay [VWF:Ac], employing latex particles and gain of function recombinant glycoprotein Ib to facilitate VWF binding and agglutination without need for ristocetin) using different instrumentation, including the new Sysmex CS-5100, with a large sample test set (n = 600). We included retrospective plus prospective study designs, and also evaluated desmopressin responsiveness plus differential sensitivity to high molecular weight VWF.

Results: VWF:Ag and VWF:RCo results from different methods were respectively largely comparable, although some notable differences were evident, including one high false normal VWF:Ag value (105 U/dL) on a type 3 VWD sample, possibly due to heterophile antibody interference in the latex-based CS-5100 methodology. VWF:Ac was largely comparable to VWF:RCo, but VWF:CB showed discrepant findings to both VWF:RCo and VWF:Ac with some patients, most notably patients with type 2M VWD.

Conclusions: (a) VWF:Ag on different platforms are largely interchangeable, as are VWF:RCo on different platforms, except for occasional (some potentially important) differences, and manufacturer recommended methods may otherwise require some assay optimization; (b) VWF:RCo and VWF:Ac are largely interchangeable, except for occasional differences that may also relate to assay design (differing optimizations); (c) VWF:CB provides an additional activity to supplement VWF:RCo or VWF:Ac activity assays, and is not interchangeable with either.

Introduction

von Willebrand disease (VWD) is reportedly the most common congenital bleeding disorder and arises from deficiency and/or defects of von Willebrand factor (VWF), an adhesive plasma protein essential for effective primary haemostasis [1,2]. Clinical identification, diagnosis and sub-typing of VWD are aided by laboratory testing but remain problematic for a variety of reasons. First, VWD is very heterogeneous, as mirrored by the fact that VWF possesses many functional properties, including binding to platelets via several receptors, most notable glycoprotein Ib (GPIb), binding to sub-endothelial matrix components (most notably collagen), and binding and protection of factor VIII (FVIII) function [3]. Thus, defects may occur anywhere within the *VWF* gene, and lead to a wide variety of phenotypes on a case-by-case basis. Second, the laboratory tests used to assist identification, diagnosis and sub-typing of VWD are themselves heterogeneous and imperfect, being reflective of varied sensitivity to VWF level and activity, as well as often processing considerable inherent variability [4–7].

The most recent classification scheme from the International Society on Thrombosis and Haemostasis (ISTH) recognizes six different types of VWD [8]. Type 1 represents a partial quantitative VWF deficiency, with VWF essentially functionally normal, but produced in lowered quantity. Type 3 VWD represents 'complete' deficiency of VWF. Type 2 VWD represents a heterogeneous group of qualitative VWF defects that comprise (i) 2A VWD (loss of high molecular weight (HMW) VWF), (ii) 2B VWD (enhanced functional binding of VWF that leads to loss of HMW VWF

Abbreviations: AVWS, acquired von Willebrand syndrome; ELISA, enzyme linked immunosorbent assay; EQA, external quality assessment; FVIII:C, FVIII coagulant; GPIb, glycoprotein Ib; HMW, high molecular weight (VWF); LIA, latex-particle immuno-assay; VWD, von Willebrand disease; VWF, von Willebrand factor; VWF:Ac, VWF INNOVANCE activity (assay); VWF:Ag, VWF antigen (assay); VWF:CB, VWF collagen binding (assay); VWF:RCo, VWF ristocetin cofactor (assay).

* Corresponding author at: Department of Haematology, Institute of Clinical Pathology and Medical Research (ICPMR), Pathology West, Westmead Hospital, WESTMEAD, NSW, 2145, Australia. Tel.: +61 612 9845 6618; fax: +61 612 9689 2331.
E-mail address: emmanuel.favaloro@health.nsw.gov.au (E.J. Favaloro).

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and typically mild thrombocytopenia), (iii) 2N VWD (loss of VWF-FVIII binding), and (iv) 2M VWF (VWF dysfunction not associated with loss of HMW VWF). For cases reflecting a minor quantitative deficiency of VWF but without a formal diagnosis of VWD, the concept of 'low VWF' as a risk factor for bleeding has alternatively been proposed [9].

The proper identification of VWD and differentiating its type is important for therapeutic management [6,10]. In normal practice, VWD and VWD type can be determined by laboratory testing that encompasses a broad panel of different tests [1,2,4–9]. Virtually all laboratories perform VWF antigen (VWF:Ag) and FVIII coagulant (FVIII:C) [1,5], respectively measuring the level of VWF protein and FVIII activity. VWF:Ag is most usually assessed using either ELISA (enzyme linked immunosorbent assay) or LIA (latex-immuno-assay) technologies. The most commonly performed activity based test is VWF ristocetin cofactor (VWF:RCo) [1,2,4–9,11], usually performed as a platelet agglutination assay using aggregometry or automated methods with standard coagulation instruments. VWF collagen binding (VWF:CB) is an additional VWF activity assay typically performed by a smaller proportion of laboratories, typically by ELISA [1,2,4–9,11]. Ostensibly, VWF:RCo and VWF:CB represent surrogate laboratory markers for two essential *in vivo* primary haemostasis VWF functions (notably, platelet GPIb and collagen binding respectively). As both assays also have a similar preference for high molecular weight (HMW) VWF, reflective of the most adhesive/functional form of VWF, both can also be used as surrogate markers for HMW VWF [12,13], which may otherwise be assessed using gel-based assays of VWF multimer analysis to determine loss of HMW VWF as well as structural abnormalities [14].

A variety of additional or alternative VWF 'activity' assays may be employed by laboratories, either in addition to, or instead of, VWF:RCo and VWF:CB [1,7]. These assays include newly released commercial assays, many based on LIA technology, but generally representing distinct assays, using distinct reagents, and detecting VWF differently. Some of these assays do not use ristocetin, so cannot truly claim to be VWF:RCo assays, although many laboratories may use these instead of VWF:RCo. Indeed, one of these new assays, the Siemens INNOVANCE VWF activity (VWF:Ac) assay uses recombinant 'mutant/gain of function' GPIb that binds VWF in the absence of ristocetin, and has been proposed to represent a possible replacement to VWF:RCo. Importantly, the perceived problems with classical VWF:RCo have lead to a high proportion of laboratories changing over to the VWF:Ac assay [5], despite the paucity of comparative published data. Nevertheless, the theoretical benefits of the VWF:Ac compared to VWF:RCo include easier performance (including easier automation), better assay precision and accuracy, lack of sensitivity to VWF polymorphisms affecting ristocetin binding, as well as improved detection of VWF at low levels [7].

Accordingly, we have assessed the comparative utility of different VWF activity assays, namely VWF:CB, VWF:RCo as well as the new Siemens INNOVANCE VWF:Ac assay, using a large sample set, and both a retrospective and prospective study design.

Methods

Assays and instrumentation

FVIII:C was assessed for patient samples as part of their original analysis within our standard VWD diagnostic test panel, using a one stage clot-based assay on a Behring BCS analyser (Siemens Healthcare, Marburg, Germany) and Siemens reagents, but has not otherwise been formally assessed in the current report. Our standard VWF:Ag was performed as an in house sandwich ELISA assay, essentially as previously reported [15,16]. The assay is currently performed on a BioKit BEST 2000 ELISA workstation (Werfen, Barcelona, Spain), using polyclonal antibodies from Dako (Glostrup, Denmark; rabbit anti-human VWF; catalogue no. A0082) for coating 96-well plates (Linbro Titertek EIA plate; ICN Biomedicals, Aurora, OH, USA) and Dako horse radish peroxidase labeled rabbit anti-human VWF (catalogue no. P0226), as the detection system. Our current standard VWF:CB was performed in parallel with VWF:Ag on the same ELISA workstation as an in house sandwich ELISA assay, essentially as previously reported [15,16], using bovine collagen from ICN Biomedicals (catalogue no. 193492) for coating 96-well plates (Pierce Maleic Anhydride activated plates; catalogue no. 15110; Thermo Scientific, Rockford, USA) and Dako horse radish peroxidase labeled rabbit anti-human VWF (catalogue no. P0226), as the detection system. Our standard VWF:RCo was performed as an agglutination assay, essentially as previously reported [15–17], on a Behring BCS analyser using Siemens reagents (BC VWF reagent; catalogue no. 10446425). For the purpose of this study, these three assays define the assay 'reference' methods, and they have been thoroughly validated for use in the identification and preliminary typing of VWD cases, as previously extensively reported by our laboratory [7,15–25]. Siemens Standard Human Plasma (catalogue no. 10446238) was used as the calibrator for all three 'reference' assays.

The test systems evaluated in this study comprised additional assays performed on a Siemens CS-5100 instrument, and namely: (a) VWF:Ag using a LIA based Siemens assay (catalogue no. 10445967), (b) VWF:RCo using the Siemens agglutination assay (BC VWF reagent; catalogue no. 10446425; same reagent methodology as employed on the BCS); (c) VWF:Ac using the Siemens INNOVANCE VWF activity assay (catalogue no. 10487040). Each of these tests were performed using standard manufacturer provided protocols for the CS-5100, except for VWF:RCo which was performed using the manufacturer protocol plus an additional ('low curve') protocol based on a published validated method for the BCS instrument [17].

The CS-5100 is the latest addition to the CS family of systems from Sysmex Corporation (Kobe, Japan), and is distributed by Siemens in Australia. The CS-5100 instrument represents advanced instrumentation using a random access multi–wavelength scanning of clotting reactions. Further information is available via the Siemens website (http://www.healthcare.siemens.com/hemostasis/systems/sysmex-cs-5100-system). Of importance to the current report is that the system is optimised for use of Siemens INNOVANCE reagents, including the VWF:Ac assay, and also incorporates active stirring for VWF:RCo agglutination reactions.

The tests evaluated in this study are summarised in Table 1.

Retrospective study and VWD cases

The retrospective study comprised assessment of a large set of samples (n = 97) from patients previously identified to have VWD or possible VWD according to clinical bleeding histories and subsequent assessment with our standard ('reference') test panels, selectively supplemented as required by additional evaluation using desmopressin trials, genetic testing and multimer analysis [5,25–27].

Table 1
Summary of VWF test methods comparatively evaluated in this study.

VWF assay	Description
VWF:Ag	Assessment of VWF protein level using an 'antigen' assay. Performed in this study by automated ELISA using an ELISA workstation (Best 2000) (as 'reference') and by automated LIA using a CS-5100 (as 'comparator')
VWF:RCo	Assessment of VWF activity level utilising ristocetin and an 'agglutination' assay. Performed in this study by automated assays using BCS ('reference') and CS-5100 ('comparator') instruments.
VWF:CB	Assessment of VWF activity level utilising collagen. Performed in this study by automated ELISA using an ELISA workstation (Best 2000).
VWF:Ac	Siemens INNOVANCE 'activity' assay. Assessment of VWF activity level utilising a Glycoprotein Ib binding method. The system employs two gain of function Glycoprotein Ib mutations within a recombinant molecule that facilitates VWF binding. Performed in this study by automated LIA using a CS-5100.

Abbreviations: ELISA, enzyme linked immunosorbant assay; LIA, latex-particle immunoassay; VWF, von Willebrand factor.