

Editorial Focus

A better approach to monitoring of therapy in von Willebrand disease?

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Von Willebrand disease (VWD) is the most common inherited bleeding disorder, diagnosed following a clinical and physical review, with personal and familial evidence of (primarily mucocutaneous) bleeding, and characterised by low levels of, or abnormal function in, the plasma protein von Willebrand factor (VWF) (1, 2). Laboratory investigation typically entails initial plasma testing of factor VIII coagulant (FVIII:C), VWF protein ('antigen'; VWF:Ag) and VWF function, with this classically assessed using the ristocetin cofactor (VWF:RCo) assay, although more recent international attention is starting to focus on newer tests of VWF function such as the collagen binding assay (VWF:CB) (3). Supplementary laboratory testing may also be performed and include VWF multimers, ristocetin induced platelet agglutination (RIPA), VWF-factor VIII binding (VWF:FVIIIb), and in some cases genetic analysis (1, 2). The PFA-100® (platelet function analyzer) may also have a role in the identification of VWD (4). Nevertheless, for most laboratories, the core battery of tests remains FVIII:C, VWF:Ag and VWF:RCo.

Broadly, six types of VWD can be defined, comprising type 1, type 2 (with breakdown into 2A, 2B, 2M and 2N), and type 3 (1, 2). Types 1 and 3 are quantitative defects. Type 1 individuals produce low levels of otherwise functionally normal VWF, and VWF is absent in type 3 VWD. In contrast, type 2 VWD represents qualitative defects characterised by the presence of dysfunctional VWF, with the particular defect or dysfunction characterised within the subtype.

Desmopressin (DDAVP) is now considered a standard treatment for some cases of VWD, in particular type 1 (1, 5), although there are some varied viewpoints on the utility of DDAVP for other patients with VWD subtypes, in particular type 2 VWD. The alternative option for therapy in VWD patients unresponsive to DDAVP, or for which DDAVP is contraindicated, or for extended needs, remains VWF concentrate, although the commercially available options vary greatly in relative content (6, 7). Of upmost importance here is the variability in the relative molecular weight species of VWF contained within the concentrate as well as the relative content of factor VIII to VWF. Nevertheless, the significance of this varied content and its effect on thera-

peutic efficacy remains controversial, with some proponents giving preferential weight to the presence of high-molecular-weight (HMW) VWF and others proposing that in-vivo correction of FVIII is more important.

Moreover, there continues to be some contention regarding the best panel of tests with which to identify VWD and to monitor therapy for VWD. The classical approach, using the core test set previously mentioned (FVIII:C, VWF:Ag and VWF:RCo), supplemented where available with multimer analysis, continues to predominate. The main problem with this classical approach in diagnostics, however, are the high diagnostic error rates, identified both within external quality assurance settings (8–11), as well as by expert VWD studies involving genetic/phenotype correlations (reviewed in [3]). Thus, error rates approaching 30% are due to misidentification of VWD or VWD subtype. Our own laboratory's view on this subject has been widely disseminated (2, 3, 8, 9) but perhaps largely overlooked by other experts, as it is somewhat at odds with the prevailing classical view. Indeed, we first reported on the utility of the VWF:CB in diagnostics over 15 years ago (12), and proposed over a decade ago (13) that use of the VWF:CB would provide additional utility within the context of DDAVP therapy monitoring, and then later, in 2001 (14), reported on the combined utility of the VWF:CB and PFA-100 in this setting. The situation was reviewed and updated recently (2–4, 15).

In brief, we could identify striking differential patterns in test results using the panel of FVIII:C, VWF:Ag, VWF:RCo, VWF:CB and PFA-100 closure times (CT), both for the identification of VWD as well as when monitoring DDAVP therapy in VWD. Thus, type 1 VWD was characterised by good absolute and relative rises in all FVIII:C and VWF test parameters, although highest absolute and relative rises were consistently observed for FVIII:C and VWF:CB, and CB/Ag ratio increases in these patients overshadowed increases in RCo/Ag ratios. This is consistent with the view that increases in VWF:CB reflect the initial release of (ultra-) HMW VWF post-DDAVP, together with the higher comparative sensitivity of VWF:CB (ie compared to other VWF assays including VWF:Ag and VWF:RCo) for detection of these forms of VWF (3), but this concurrently challenges

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the prevailing dominant view that VWF:RCo best reflects the functional activity of VWF. Furthermore, in type 1 VWD, prolonged PFA-100 CT tended to correct in concert with elevation in VWF, but the relationship was most striking for VWF:CB. This provided some cross validity of utility between VWF:CB and PFA-100 CT, but also suggested that the PFA-100 was identifying the most functional forms of VWF.

In contrast, type 2A VWD individuals showed a distinctive pattern, with good absolute and relative rises in both FVIII:C and VWF:Ag, but poor absolute rises in both VWF:CB and VWF:RCo, and consequently, both CB/Ag and RCo/Ag ratios remained low. Prolonged PFA-100 CT tended not to correct in these patients, even though VWF:Ag and VWF:RCo sometimes did. Our working hypothesis became that for the PFA-100 CT to correct, the VWF:CB also had to be corrected.

To our knowledge, this work has only been taken up by one other group of researchers (16–19). Accordingly, the paper from van Vliet et al. (19) in the current issue of *Thrombosis and Haemostasis* provide a timely addition to this debate within the above context. These researchers have analysed the dose-response relationship between PFA-100 CT and the factor VIII and VWF parameters previously noted in a larger number of patients with type 1 and type 2 VWD before and after treatment with DDAVP (n=84) or VWF concentrate (n=38). Like our smaller studies (14, 15), DDAVP treatment of patients with type 1 VWD was found to normalise the PFA CT by increasing VWF levels to normal. In addition, there was a strong inverse relationship between CT and VWF parameters. In contrast, of the 14 patients assessed with type 2 VWD, PFA-100 CT did not normalize in eight of them. In addition, VWF concentrate (Haemate-P®) substitution in patients with type 1 VWD induced a less favourable response compared to DDAVP, because PFA CT did not correct in all patients. Moreover, of 12 patients with type 2 VWD treated with Haemate-P, six showed a correction of PFA CT, which correlated with the normalisation of the CB/Ag ratio. In-vitro studies were also performed by van Vliet et al. (19). Using whole blood from patients with VWD, they added varying amounts of VWF concentrate. Addition of Haemate-P (a concentrate containing relatively normal levels of HMW VWF) induced an increase of the CB/Ag ratio from 0.30 to 0.70 in patients with type 2 VWD and this also corrected PFA-100 CT. In contrast, addition of Immunate (a concentrate relatively deficient in HMW VWF) did neither yield an increase in the CB/Ag ratio in these patients, nor did it correct the PFA-100 CT. Van Vliet et al. (19) conclude that the PFA-100 is useful not only for diagnosis of VWD, but also for monitoring of DDAVP responses and VWF factor concentrate therapy, and that the efficacy of VWF substitution in patients with type 1 and type 2 VWD, and the ability for the PFA-100 to identify this, is dependent upon both the type of VWD and the VWF concentrate used.

Interestingly, whereas our working hypothesis was that correction of the PFA-100 during DDAVP therapy was dependent on the VWF:CB, van Vliet et al. (19) suggest that their findings indicate that correction of both VWF test parameters and the CB/Ag ratio is important, particularly when addressing factor replacement therapy. To ultimately address this issue, further studies are probably needed. It is important, for example, to consider differential patient selection, the limited cases evaluated,

and the slightly different test conditions used in our earlier studies. Thus, factor concentrate therapy was not extensively studied in our earlier report (14), although we have recently assessed this within the context of a pharmacokinetic study (20, 21). In this setting, the PFA-100 was not particularly sensitive to factor concentrate therapy. Whether this relates to the kind of VWF concentrate used (Biostate® and AHF high purity in our study) or whether it relates to the dosing (or amount) of concentrate given to patients is unclear. However, in-vitro comparisons suggest that Biostate is very similar to Haemate-P in terms of composition relating to retention of HMW VWF (22). Other studies have evaluated the PFA-100 within the setting of factor concentrate therapy, but none of these have utilised the VWF:CB, so they cannot contribute to the current debate.

Lastly, PFA-100 CT did not correct in any of our type 2 VWD patients post DDAVP, or the VWF:CB and CB/Ag ratios, although VWF:Ag, VWF:RCo and RCo/Ag ratios did correct in a subset of patients (14, 15). Accordingly, this suggests (for comparative DDAVP studies at least) that we were perhaps assessing a more severe group of patients than van Vliet et al. (19).

In the end, I would agree with van Vliet et al. (19) on the potential combined utility of the VWF:CB and PFA-100 in both the diagnosis of VWD and for monitoring DDAVP therapy, and suggest that others take up this approach and forthwith report on their experience. It is important in such studies to utilise a VWF:CB that is validated sensitive to the presence of HMW VWF (3), otherwise the results will be meaningless. Unfortunately, not all VWF:CB assays as currently used within laboratories actually meet these criteria. Some assay methodologies remain overly sensitive to low molecular weight forms of VWF, and I remain to be convinced that any suitable commercial VWF:CB assay actually exists (3). Standardisation of VWF:CB assays for 'fitness of purpose' is one of my dreams, and I hope it is accomplished soon, before reports using inferior VWF:CB assays flood the journals and do irreparable damage to a potentially great addition to VWD diagnostics. Fortunately, I have no great concerns regarding the assay used by van Vliet et al. (19), which I believe has been suitably validated.

I would also agree with van Vliet et al. (19) that not all factor concentrates are the same in terms of efficacy, but rather that those with retention of HMW VWF (and thus better mimicking normal plasma) would perform better than those in which HMW VWF is deficient, or where there is an exaggerated level of low-molecular-weight VWF species, potentially derived from the manufacturing process and from degradation of HMW VWF forms. This would be reflected in high relative CB/Ag ratios in the former concentrates (including Humate-P and Biostate®), but low relative CB/Ag ratios in the latter concentrates (including Immunate®). Nevertheless, future work will no doubt be forthcoming, and I await with great interest clarification of these issues.

Abbreviations

HMW, high molecular weight (VWF); VWD, von Willebrand disease; VWF, von Willebrand factor; VWF:Ag, von Willebrand factor antigen (assay); VWF:CB, von Willebrand factor collagen binding (assay); VWF:RCo, von Willebrand factor ristocetin cofactor (assay); CB/Ag, ratio of VWF:CB/VWF:Ag; RCo/Ag, ratio of VWF:RCo/VWF:Ag.

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