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Desmopressin therapy to assist the functional identification and characterisation of von Willebrand disease: Differential utility from combining two (VWF:CB and VWF:RCo) von Willebrand factor activity assays?

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ABSTRACT

We performed a retrospective audit of desmopressin (DDAVP) usage to assist in the functional characterisation of von Willebrand disease (VWD). Data was evaluated for 208 patients, comprising those with VWD (Type 1 [n = 160], Type 2A [n = 19], Type 2M [n = 10]), plus 19 individuals with haemophilia or carriers of haemophilia. Laboratory testing comprised pre- and post-DDAVP evaluation of factor VIII (FVIII:C), von Willebrand factor (VWF) antigen (VWF:Ag), VWF ristocetin cofactor (VWF:RCo) activity, VWF collagen binding (VWF:CB) activity, and in one laboratory an alternate VWF activity assay. In brief, combined usage of VWF:RCo and VWF:CB appears to provide improved functional characterisation and/or 'classification' of VWD types, in particular better differentiation of Type 2A and 2M VWD, and clearer validation of a Type 1 VWD diagnosis. Thus, (i) Type 1 VWD displayed generally good absolute and relative rises in all test parameters, although relative rises were greatest for FVIII:C and VWF:CB, and CB/Ag ratio increases overshadowed those for RCo/Ag; (ii) Type 2A VWD patients showed good absolute and relative rises in both FVIII:C and VWF:Ag, but poor absolute rises in both VWF:CB and VWF:RCo; although small rises in both CB/Ag and RCo/Ag were also observed, both ratios tended to remain below 0.7; (iii) finally, Type 2 M VWD patients generally showed good absolute and relative rises in FVIII:C, VWF:Ag *and* VWF:CB, but a poor absolute and relative rise in VWF:RCo; thus, there were good rises in CB/Ag ratios but little change in RCo/Ag, which tended to remain below 0.7. Future multi-centre prospective investigations are warranted to validate these findings and to investigate their therapeutic implications.

Introduction

Von Willebrand disease (VWD) is the most common inherited bleeding disorder and is characterised by low levels of, or abnormal function in, the plasma protein von Willebrand factor (VWF). Laboratory investigation typically entails initial plasma testing of factor VIII coagulant (FVIII:C), VWF protein ('antigen'; VWF:Ag) and VWF activity, with this classically assessed using the ristocetin cofactor (VWF:RCo) assay [1–3]. Recent international attention has begun to focus on the collagen binding assay (VWF:CB) and other putative VWF 'activity' assays, as possible replacements to VWF:RCo, or as supplementary tests of VWF activity [3,4]. Depending on local availability, supplementary laboratory testing may also include VWF multimers, ristocetin induced platelet agglutination (RIPA), VWF-factor VIII binding (VWF:FVIIIB), and in some cases genetic analysis [1–3].

Six types of VWD have been defined, namely Types 1, 2A, 2B, 2M, 2N, and 3 [1–3]. The laboratory identification and characterisation of VWD is problematic due to its heterogeneity and current test limitations [1–5]. Of note, many investigators [4–15] have recently reported on diagnostic inconsistencies. As reviewed elsewhere [16], a series of genetic/phenotypic studies using samples derived from 'expert' VWD laboratories [6–9], identified that between 15-33% of cases originally identified as Type 1 VWD could be reclassified as Type

Abbreviations: ELISA, Enzyme Linked Immuno-sorbant Assay; FVIII:C, Factor VIII coagulant (assay); HMW, High molecular weight (VWF); VWD, von Willebrand disease; VWF, von Willebrand Factor; VWF:Act, von Willebrand Factor 'Activity' (assay); VWF:Ag, von Willebrand Factor Antigen (assay); VWF:CB, von Willebrand Factor collagen binding (assay); VWF:RCo, von Willebrand Factor Ristocetin Cofactor (assay); Act/Ag, ratio of VWF:Act/VWF:Ag; CB/Ag, ratio of VWF:CB/VWF:Ag; RCo/Ag, ratio of VWF:RCo/VWF:Ag.

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2 following study reanalysis (generally using multimers and/or VWF:RCo/VWF:Ag ratios). Such misdiagnosis can have both therapeutic implications and psychological effects on affected patients. Although misclassifications have multiple causes, errors are in part likely due to the recognised limitations in the otherwise standard panel of VWF:Ag, VWF:RCo and FVIII:C [2–5] used by most laboratories for most investigations, including the relative assay lower limit of sensitivity [17]. We have previously identified that addition of VWF:CB testing to such a test panel will consistently reduce error rates in VWD diagnosis [4,5].

In the current study, we evaluated whether Desmopressin (DDAVP) therapy, a standard treatment for many forms of VWD [18–21], could be utilised to assist the better identification and characterisation of VWD, and to identify whether differential patterns of test results, in particular, those representative of Types 1, 2A and 2M VWD, could be identified in a cohort of patients with VWD by measuring VWF activity differentially using two distinct assays (viz: VWF:CB and VWF:RCo), and in the absence of multimer testing, given that this last test is largely unavailable to most pathology laboratories.

Materials and Methods

Study Aims

The main aim of this study was to assess whether combined activity-based VWF testing (ie VWF:CB and VWF:RCo) would provide potential improvement in the identification and functional characterisation of VWD compared to use of VWF:RCo alone as the VWF activity assay. Furthermore, whether we could identify differential test pattern results representative of types 1, 2A and 2M VWD in the absence of additional testing (specifically VWF:multimer and genetic analysis, which are generally unavailable within our geographic locality and to most 'real-world' pathology laboratories).

Setting

We report a cross-laboratory, retrospective audit of DDAVP usage and effect. The original study population comprised over 300 individuals treated with intravenous DDAVP for treatment of various bleeding disorders. The current report is restricted to an analysis of data from 208 patients, comprising 189 with VWD (Type 1 [n = 160], Type 2A [n = 19], Type 2M [n = 10]), plus 19 with haemophilia or carriers of haemophilia (Table 1). Laboratory testing comprised pre- and post-DDAVP evaluation of factor VIII (FVIII:C), VWF antigen (VWF:Ag), VWF ristocetin cofactor (VWF:RCo) activity, VWF collagen binding (VWF:CB) activity, and in one laboratory an alternate VWF activity assay (VWF:Act). Data derived from seven diagnostic and treatment centers broadly distributed within our geographic region (ie New Zealand, plus differential east, west and north parts of Australia; Table 1). Data collection was from local hospital databases and included the period since 1991 (Westmead Hospital), 2003 (Royal Perth Hospital), 2002 (Canterbury Health), or more recently (other sites). Patients were initially diagnosed by the respective diagnostic/treatment centre, although the specific type of VWD was occasionally revised following a re-review of overall data, inclusive of multiple data points and the DDAVP response (see below).

Table 1
Summary of study participants and patient samples assessed in this study.

Site/Centre	Geographic locality	Total samples	1	2A	2M	Haemophilia A/carrier
ICPMR, Westmead Hospital (WMD)	NSW, Australia	95	68	14	4	9
Royal Perth Hospital (RPH)	WA, Australia	74	59	2	3	10
Royal Brisbane Hospital (RBH)	Qld, Australia	16	16	0	0	0
Canturbury Health (CH)	New Zealand	15	9	3	3	0
Pathwest, Fremantle Hospital (FH)	WA, Australia	6	6	0	0	0
Others (x2)	NSW, Australia	2	2	0	0	0
Totals:		**208**	**160**	**19**	**10**	**19**

Table 2
VWF method summary.

Site / Assay	VWF:Ag	VWF:CB	VWF:RCo	VWF:Act
ICPMR, Westmead Hospital (WMD)	in-house ELISA	in-house ELISA	currently automated agglutination assay; previously performed using aggregometry	not performed
Royal Perth Hospital (RPH)	Automated immuno-turbidimetric assay	commercial ELISA	automated agglutination assay	not performed
Royal Brisbane Hospital (RBH)	Automated immuno-turbidimetric assay	commercial ELISA	aggregometry	Automated immuno-turbidimetric assay
Canturbury Health (CH)	in-house ELISA	in-house ELISA	aggregometry	not performed
Pathwest, Fremantle Hospital	Automated immuno-turbidimetric assay	commercial ELISA	automated agglutination assay	not performed

Patient typing

For the purpose of this report, Type 1 VWD was that which showed consistently low but concordant test values for all VWF tests performed and evident on initial and repeat testing. In practice, concordance is evidence of VWF 'activity'/VWF:Ag ratios above 0.7 [1–5]. Alternatively, Type 2 VWD was that which showed low levels of VWF and consistent *discordance* in VWF test results (ie VWF 'activity'/VWF:Ag ratios of $\leq$0.7), with the type of discordance helping to identify the probable type of VWD. Thus, a reduced VWF:CB/VWF:Ag (CB/Ag) *plus* reduced VWF:RCo/VWF:Ag (RCo/Ag) would suggest either a Type 2A ('VWD-2A') or Type 2B VWD, whereas reduced RCo/Ag *but normal* CB/Ag would suggest a Type 2M VWD (ie platelet function discordant type [1–3]; 'VWD-2M'). This pattern is both consistent with the current classification scheme [1] and with previous observations that optimised VWF:CB assays are better able to identify high molecular weight (HMW) VWF [3] than broadly applied VWF:RCo assays. Thus, the VWF:CB was used within the current study as a surrogate marker of HMW VWF. Type 2B VWD cases identified by heightened responsiveness to ristocetin in a ristocetin-induced platelet agglutination assay were excluded from data analysis. Accordingly, for the current study, a test pattern comprising reduced RCo/Ag *but normal* CB/Ag would by definition comprise platelet function discordant type 2M VWD and exclude a type 2A VWD phenotype, and a test pattern comprising reduced RCo/Ag *and reduced* CB/Ag would most likely reflect a specific loss of HMW VWF and thus a type 2A VWD phenotype. Type 1 VWD was further separated according to 'severity', with severe Type 1 VWD ('VWD-1s') defined in those with VWF:Ag $\leq$15 U/dL (n = 17), moderate/mild Type 1 VWD ('VWD-1m') in those with VWF:Ag between 16-35 U/dL (n = 30), and 'possible mild' VWD ('VWD-1p') in those with VWF:Ag between 36-65 U/dL (n = 113).

Laboratory test methods

All laboratories tested for FVIII:C using a standard automated one stage clot-based assay and for VWF:Ag using either a standard in-house enzyme-linked-immunosorbant assay (ELISA) or a commercial immuno-turbidimetric ('latex-immuno-assay') procedure (Table 2).