Reassessment of ABO Blood Group, Sex, and Age on Laboratory Parameters Used to Diagnose von Willebrand Disorder

Potential Influence on the Diagnosis vs the Potential Association With Risk of Thrombosis

Emmanuel J. Favaloro, PhD, Soma Soltani, Jane McDonald, Ella Grezchnik, Leanne Easton, and James W.C. Favaloro

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Abstract

We reassessed the influence of ABO blood group, sex, and age on plasma levels of von Willebrand factor (vWF) antigen, vWF:ristocetin cofactor, vWF:collagen binding assay, and factor VIII coagulant (FVIII:C). Data show that levels of vWF and FVIII:C increase with increasing age (P < .001 for all parameters) and that the ABO blood group influences plasma levels such that O group levels are significantly less than non–O group levels. There was no significant association with sex and Rh status. The selection of normal ranges based on ABO blood groups may influence the clinical diagnosis of von Willebrand disease (vWD) but might not be clinically relevant or help identify people at increased risk of bleeding. Differences in ABO-related ranges were more extensive at the high end of the ranges. This is of particular interest because high levels of vWF and FVIII are associated with thrombosis risk, and an ABO relationship also has been described. O group individuals may or may not be at greater risk for bleeding (they have lower levels of vWF and FVIII:C) and are more likely to be diagnosed with vWD. It also is possible that O group status may be protective for thrombosis.

von Willebrand disease (or disorder; vWD) is the most common inherited bleeding ailment, estimated to affect up to 1% of the population. Patients with vWD have defects in or reduced levels of von Willebrand factor (vWF), an adhesive plasma protein essential for primary haemostasis. vWD is a heterogeneous disorder subtyped using clinical and laboratory criteria, and the diagnostic process typically requires a panel of tests. The minimal test panel would comprise factor VIII coagulant (FVIII:C) activity, vWF antigen (vWF:Ag), and assessment of functional vWF activity using the ristocetin cofactor (vWF:RCo) and/or the collagen binding activity (vWF:CB) assays.

However, despite the presence of many extensive reviews and diagnostic guidelines, the appropriate diagnosis of vWD remains problematic because of a variety of reasons, including preanalytic variables, analytic problems and test limitations, and postanalytic errors. Most of these problems may be overcome by strict adherence to appropriate protocols, by repeated testing for confirmation, and by more extensive test follow-up procedures. Given appropriate testing processes, all laboratories should be able to detect type 3 vWD (no vWF detectable), moderate or severe type 1 vWD (vWF levels <15%), and most type 2 vWD (functional discordance).

So-called mild type 1 vWD (vWF levels, ~25%-50%) provides special problems and has been the subject of special recent focus. In part, the problems relate to the overlap between normal reference ranges and the levels that one might otherwise accept as being low in “mild vWD.” Also, although it is well known that plasma vWF levels vary according to ABO blood group and race, the value of using normal reference ranges adjusted for ABO group and race remains unresolved and is a matter of ongoing debate.
one hand, it is scientifically logical; on the other, does it actually help to stratify patients at risk of bleeding or really assist in the identification of true vWD? Compounding the issue is the knowledge that the ABO blood group influences platelet function using the PFA-100 (Dade Behring, Newark, NJ), most probably through a vWF-dependent mechanism, and that the ABO blood group might influence the rate of proteolysis of vWF by ADAMTS13. Recent interest has also centered on the relationship between ABO blood group and thrombotic or coronary risk. Finally, that there exists a genetic basis for lower levels of vWF in O group individuals and, therefore, a potential linkage to a definable type of vWD has been demonstrated only recently.

Despite these reports, and although it is generally accepted that normal values fall between approximately 50% and 200%, a review of the literature reveals an extraordinarily wide variation in reported reference ranges for vWF and FVIII:C. Selection of a particular reference range will significantly influence whether a clinical diagnosis of vWD is made (perhaps even irrespective of the clinical history if the clinician is somewhat inexperienced). Accordingly, we reevaluated the influence of ABO blood group, sex, and age on the establishment of the normal reference range using a large number of normal samples, compared our findings with those of previous reports, and attempted to assess the possible effect on the identification of vWD and thrombotic risk.

Materials and Methods

Laboratory Test Procedures

FVIII:C activity was assessed in a 1-stage clot-based assay as previously reported using an ACL-300R instrument (IL-Couler, Sydney, Australia) and factor-deficient plasma (Dade Behring, Sydney, Australia). In-house–derived pooled normal plasma (PNP; containing >40 individual normal plasma samples) and a commercial reference plasma (Verify Assayed Reference Plasma, BioMerieux, via Vital Diagnostics, Yeerongpilly, Brisbane, Australia), and an in-house–derived cryosupernatant sample. For this study, the PNP was defined to contain 100% of all coagulation factors and 100% of vWF.

Results

Estimation of Normal Reference Ranges

Reference ranges for all assays were calculated using log-transformed data (to achieve normality) and ± 2 SD to generate 95% confidence intervals, as previously described. Blood samples for testing were obtained with permission from healthy individuals donating to a local Red Cross blood bank and collected into standard (3.2% sodium citrate) anticoagulant tubes (Greiner Vacuette, catalog No. 455322; purchased from Interpath, Sydney, Australia). Following receipt, the sample was centrifuged immediately (2,000g, 15 minutes) and processed; supernatant plasma aliquots were frozen at –80°C until tested.

Normal Donor Characteristics

This study used a total of 452 normal donor samples, although because of technical constraints, not all samples were tested in all assays. Donor age ranged from 16 to 77 years (median, 47.0 years). There were no age-related differences between females (median age, 46.0 years; range, 16-75 years) and males (median age, 48.0 years; range, 17-77 years).

Patient Test Group

We also report some brief findings from patient samples tested in our laboratory following referral from various clinical sources. Although we service the pathology needs of a major university teaching hospital (Westmead, Westmead, Australia), more than 90% of our test material is derived from off-site sources. The present report relates to samples tested between January 1995 and May 2005. During this period, we have evaluated more than 4,000 patient samples for potential vWD.

Effects of ABO Blood Type, Sex, and Age

There was a significant linear correlation between donor age and the presenting plasma level of vWF (vWF:Ag, vWF:RCo, and vWF:CB) and FVIII:C. There was no significant correlation between age and the ratio of vWF:Ag and functional vWF (ie, vWF:RCo [ie, Ag/RCo] or vWF:CB [ie, Ag/CB]), although a trend toward a negative association for Ag/RCo could not be discounted (Figure 1).
There also was a clear association between ABO blood group status and plasma levels of vWF and FVIII:C. Figure 2. There was no significant association between ABO blood group status and Ag/RCo or Ag/CB, although a trend toward an association was suggested for Ag/RCo.

There was no significant association between levels of vWF and FVIII:C and Rh status or sex (data not shown). In addition, there were no age-related differences in our study for sex, Rh status, or ABO group status (data not shown).

Figure 1 Relationship between age and plasma levels of von Willebrand factor (vWF) antigen (Ag; A), vWF:ristocetin cofactor (RCo; B), vWF:collagen binding activity (CB; C), factor VIII coagulant (FVIII:C; D), and the ratios of Ag/RCo (E) and Ag/CB (F). A positive linear correlation was evident for vWF:Ag, vWF:RCo, vWF:CB, and FVIII:C (P < .001 for each), but not for Ag/RCo (P = .0934) or Ag/CB (P = .3976). Lines indicate linear regression lines.

Figure 2 Comparison of non-O vs O blood group data (y-axis, mean ± 95% confidence interval) for von Willebrand factor (vWF) antigen (Ag), vWF:ristocetin cofactor (RCo), vWF:collagen binding assay (CB), and factor VIII coagulant (FVIII:C) and the Ag/RCo and Ag/CB ratios. Statistical comparisons (P values) are identified for each comparison. Numbers at the bottom of the columns are the number of tests performed for each category.
Differential ranges calculated from various blood group types are shown in Figure 3. Levels at the bottom end of all ranges for vWF and FVIII:C essentially increased in the following order: O < A ≤ B ≤ AB. It is worth noting, however, that the major differences between O and non–O group results actually related to the upper (rather than lower) end of the ranges (Figure 3).

Influence of Reference Range Limits on the Potential Determination of Test “Abnormality”

In laboratory practice, it is the lower limit of the normal reference range that determines the cutoff value for identification of abnormal or positive samples. Figure 4 shows the smoothed frequency distribution curves for all normal samples assessed for vWF:Ag, vWF:CB, and FVIII:C and the result of selecting a different lower limit value on the potential identification of abnormal or vWD+ samples. By using the vWF:Ag assay as an example (for illustration only) and a cutoff of 40%, only 2 samples (~0.5%) would be identified as abnormal; with a cutoff of 50%, 13 samples (~3%) would be identified as abnormal; with a cutoff of 60%, 30 samples (~7.5%) would be identified as abnormal.

Representation of vWD Subtypes Identified From Our Center

During the 1995-2004 period, we evaluated more than 4,000 patient samples for potential vWD. From these, we identified laboratory patterns consistent with type 3 vWD in 11 people, probable type 2 vWD in 64 people, and moderate or severe type 1 vWD (vWF levels, 2%-20%) in 58 people. An increasing number of people with vWF levels within the range of 21% to 60% were also identified (n = 732). Again, for illustrative purposes only, by using a cutoff of 40%, 238 (30.1%) of 790 samples would have been identified as type 1 vWD; with a cutoff of 50%, an additional 235 samples (29.7%; total, 473 [59.9%]) would have been identified as type 1 vWD; with a cutoff of 60%, an additional 317 samples (40.1%) would have been identified as type 1 vWD.

Figure 3 ABO group–related normal reference ranges identified from study data (for O, A, B, AB, and composite non-O blood groups) compared with all study data (All) and data from a previous publication from our laboratory (1997) for plasma von Willebrand factor (vWF):antigen (Ag; A), vWF:ristocetin cofactor (RCo; B), vWF:collagen binding assay (CB; C), factor VIII coagulant (FVIII:C; D), and the Ag/RCo (E) and Ag/CB (F) ratios. Numbers at the bottom of the columns are the number of tests performed for each category. Dashed horizontal lines at 50% or ratio of 1.0 are for illustration only. Bars indicate the lower and upper limits of the normal reference ranges, calculated using ±2 SD of the log-transformed data to capture 95% confidence limits.
Comparison of Normal Reference Ranges in the Present Report With Those of Previous Studies

This comparison is shown for vWF parameters in Figure 6. A wide variation in normal ranges has been reported, although a consistent pattern of ABO blood group–related differences in vWF is observed.

Discussion

We reevaluated the influence of ABO blood group, sex, and age on plasma vWF and FVIII:C levels. We confirmed a linear correlation between age and the levels of vWF:Ag, vWF:RCo, vWF:CB, and FVIII:C. A correlation with vWF:Ag has been described previously.19 We are unaware of any previously published correlation with age and vWF:RCo and vWF:CB, although an association with age and vWF:RCo has been described.28 It is interesting that although there was no significant correlation between age and the ratio of vWF:Ag and functional vWF (ie, vWF:CB [ie, Ag/CB] or vWF:RCo [ie, Ag/RCo]), a trend toward a negative correlation for Ag/RCo could not be discounted (Figure 1).

We also confirmed a clear association between ABO blood group status and levels of vWF and FVIII:C (Figure 2). There was no significant association between ABO blood group status and Ag/RCo or Ag/CB, although a trend toward an association was suggested for Ag/RCo. Haley et al25 noted a small-magnitude rise (but statistically significant) in the Ag/RCo ratio in O group compared with non–O group people (Ag/CB data were not different), and Miller et al27 noted a differential ratio of RCo/Ag based on race. A previous study found a potential direct role of A and B blood group antigens on the adhesive activity of vWF;41 such that removal of A and B antigens reduced vWF:RCo activity but not vWF:CB activity. Although consistent with our findings and those of others, such study data should be confirmed and the significance further explored.

Differential ranges calculated from various blood group types (Figure 3) showed increases at the bottom end of the range in the following order: O < A ≤ B ≤ AB. This pattern was reproducible for each of the vWF assays (ie, vWF:Ag, vWF:RCo, and vWF:CB). That O group plasma vWF levels generally are lower than non-O is similar to the trends previously reported (Figure 6; many reports for vWF:Ag; fewer reports for vWF:RCo and vWF:CB). Nevertheless, the actual ranges quoted by many of the previous publications varied greatly. Selection of a particular reference range will significantly influence whether a clinical diagnosis of vWD is made, as illustrated herein and as reported elsewhere. This might even occur irrespective of the clinical history, especially if the clinician is somewhat inexperienced and is guided largely by the laboratory test result (eg, a clinician may identify the presence of vWD based on a finding of an “abnormal test result” [ie, a result lower than the bottom end of the normal reference range for vWF]).

We used data from our laboratory to illustrate the possible effect of selection of different ranges on the perceived identification of a type 1 vWD pattern in individuals being evaluated for vWD. In our illustration, an additional 40% of individuals could be identified as potentially having an abnormal test result.
(ie, potential type 1 vWD) if a lower limit cutoff of 60% is selected instead of 50%. The lower limit of reported ranges in some published reports is higher than 60%. Indeed, some of the reported ranges are very tight, and it is difficult to identify how these were generated. For vWF, a normal range should be established using 95% confidence intervals, which for a normal distribution would include the mean ± 2 SD. However, the frequency distribution curve for vWF is non-gaussian (eg, Figure 4) and becomes gaussian only after log transformation.27,40 That is, the normal range should be generated using the mean ± 2 SD of log-transformed data.

Whether the use of race- or blood group–specific ranges is warranted remains unresolved and is a matter of ongoing debate.1,8,10,14,22,26 On a purely scientific basis, such use would be warranted if the only requirement were simply to identify individuals with levels less than the lower limit of the normal reference range (ie, high likelihood that the result is abnormal using the classic scientific definition). However, does the use of ABO-specific ranges actually better help identify individuals with vWD or stratify those at risk of bleeding? Is an O group individual with a level of vWF of 40% more likely to have vWD or be at risk of bleeding than an AB group individual with a level of 40%? The major problem with having ABO-specific ranges is that we are more likely to infer that such discrimination is clinically important, whereas the appropriate evidence is lacking. Accordingly, we do not currently use ABO-specific ranges at our institution, although we suggest determination of ABO blood group status in individuals being evaluated for vWD, and we keep this information in mind when reviewing overall findings (laboratory and clinical). Nevertheless, recent reports have identified an association of O group–related vWF genetic changes that might provide

![Figure 6](https://example.com/figure6.png) ABO group related normal reference ranges identified from present study data (for O, A, B, AB, and composite non-O blood groups; Westmead) compared with previously published data for plasma von Willebrand factor (vWF):antigen (Ag; A), vWF:ristocetin cofactor (RCo; B), and vWF:collagen binding assay (CB; C). The y-axis indicates the percentage of normal. Present study data represent the mean ± 2 SD using log-transformed data as indicated in the “Materials and Methods” section to capture 95% confidence intervals and for reasons explained elsewhere in the text and as published.27,40 In the case of previous publications, reference range data are as reported in that publication or have been calculated from the publication–provided means and SDs. Unfortunately, it was not always clear how these data had been calculated (eg, log-transformed data or not) or whether SDs are for mean value estimates only or meant to represent the range data. Numbers at the bottom of each figure represent the reference citation number as provided in the text. Dashed horizontal lines at 50% are for illustration only.
evidence for a genetic basis for lower vWF levels in these individuals.35-37 On the other hand, there are valid arguments to suggest that we should not identify type 1 vWD unless vWF levels are very low (eg, <15%).13,14

Also relevant to our findings and those of previous reports is the recent finding that the ABO blood group influences the rate of proteolysis of vWF by ADAMTS13.32 It is interesting that the rate of proteolysis was greater for group O than non–O vWF in the following order: O ≥ B > A ≥ AB, which is the reverse of the pattern observed for the lower end of the ABO-specific reference range. Although this is logical, the clinical significance of this finding is unclear.

In most previous ABO-related studies, the focus on ABO vWF is the effect on the bottom end of the normal range and the influence on vWD diagnosis.15,19,22,23,27 It is, however, worth noting that the major differences in our study data between O and non–O group results related to the upper (rather than lower) end of the ranges. Pertinent to these findings, therefore, is increasing evidence of elevated thrombosis risk with high levels of vWF42-49 and the recent identification of ABO vWF–related thrombosis risk.33,34 Accordingly, not only do non–O group individuals have higher levels of plasma vWF and FVIII:C than O group individuals, but they also are relatively overrepresented in a thrombosis group.34 This finding is complementary to our data and in keeping with the reports linking high levels of vWF and FVIII:C to thrombosis risk.42-49

We reassessed the influence of ABO blood group, sex, and age on plasma levels of vWF (vWF:Ag, vWF:RCo, vWF:CB) and FVIII:C and compared our findings with those of previous studies. Previous data are extensive for vWF:Ag and FVIII:C, but somewhat lacking for vWF:RCo and vWF:CB. There was no significant association for plasma vWF or FVIII:C with sex and Rh-status; however, levels of vWF and FVIII:C increased with increasing age (linear correlation: P < .001 for all parameters), and a significant ABO influence on levels also is evident such that O group levels are significantly less than non–O group levels. It is interesting that a trend toward an association for Ag/RCo for age (Figure 1) and ABO group status (Figure 2) could not be discounted.

We also showed that selection of normal ranges based on the ABO group might influence the clinical diagnosis of vWD, and while the approach of using ABO group ranges is scientifically sound, it might not be clinically useful or really assist in identifying people at increased risk of bleeding. We also noted, however, that the differences in ABO-related ranges were more extensive at the high end (rather than the low end) of the ranges. This finding is of particular interest because high levels of vWF and FVIII are associated with thrombosis risk, and an ABO relationship also has been described. Accordingly, O group individuals may or may not be at greater risk for bleeding (they have lower levels of vWF and FVIII:C) and are more likely to be diagnosed with vWD. However, it also is likely that O group status may be protective for thrombosis.

From the Department of Haematology, Institute of Clinical Pathology and Medical Research, Westmead Hospital, Westmead, Australia.

Address reprint requests to Dr E.J. Favaloro: Dept of Haematology, Institute of Clinical Pathology and Medical Research, WSAHS, Westmead, NSW, 2145, Australia.

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