The Effect of ABO Blood Group on the Diagnosis of von Willebrand Disease

By Joan Cox Gill, Janet Endres-Brooks, Patricia J. Bauer, William J. Marks, Jr. and Robert R. Montgomery

In order to firmly establish a normal range for von Willebrand factor antigen (vWF:Ag), we determined plasma vWF:Ag concentrations in 1,117 volunteer blood donors by quantitative immunoelectrophoresis. The presence of the ABO blood group had a significant influence on vWF:Ag values; individuals with blood group O had the lowest mean vWF:Ag level (74.8 U/dL), followed by group A (105.9 U/dL), then group B (116.9 U/dL), and finally group AB (123.3 U/dL). Multiple regression analysis revealed that age significantly correlated with vWF:Ag levels in each blood group. We then performed reverse ABO typing on stored plasma from 142 patients with the diagnosis of von Willebrand disease (vWD). Of 114 patients with type I vWD, blood group O was found in 88 (77%), group A in 21 (18%), group B in 5 (4%), and group AB in none (0%), whereas the frequency of these blood groups in the normal population is significantly different (45%, 45%, 7% and 3%, respectively) (P < .001). Patients with type II or III vWD had ABO blood group frequencies that were not different from the expected distribution. There may be a subset of symptomatic vWD patients with decreased vWF:Ag levels of structurally normal vWF (vWD, type I) on the basis of blood group O. Some individuals of blood group AB with a genetic defect of vWF may have the diagnosis overlooked because vWF levels are elevated due to blood type.

THE DIAGNOSIS of type I von Willebrand disease (vWD) is often based on the demonstration of a decreased quantity of structurally intact plasma von Willebrand factor (vWF) in a patient with a history of mild mucocutaneous or surgically induced hemorrhage. The diagnosis can be difficult in mildly affected individuals because vWF levels tend to fluctuate, often rising into the normal range during times of stress, inflammation, pregnancy, and ingestion of oral contraceptives. Furthermore, firmly established normal ranges based on von Willebrand factor antigen (vWF:Ag) determinations in a large cross section of normal individuals are lacking.

The quantity of vWF:Ag assayed, as for most coagulation factors, is expressed in relation to a standard derived from a pool of plasma from normal donors, with the pool representing 100 U/dL. Assuming a normal distribution, if the number of individuals in a particular pool is large enough, then high normals should be offset by low normals, and the concentration of a particular plasma constituent should approach the mean. Traditionally, the mean is defined as 100 U/dL, and a vWF:Ag value below 40 to 50 U/dL is used as a criterion for the diagnosis of vWD.

In preliminary studies in our laboratory, five of 100 standard pool members were found to have plasma vWF:Ag values less than 50 U/dL. This finding prompted us to study a much larger group of normal individuals. To firmly establish a normal range for vWF:Ag and to further investigate factors that might influence plasma vWF levels, we studied vWF:Ag in 1,117 blood donors.

MATERIALS AND METHODS

Collection of blood samples. Plasma was obtained from venous blood at the time of volunteer blood donation. After the unit of whole blood had been collected in the usual manner, the plastic tubing between the needle and the bag was sealed. The tubing was then cut between the seal and the needle and venous blood collected into polystyrene tubes prefilled with sodium citrate (3.2%) so as to result in a 9:1 ratio of whole blood to anticoagulant. Plasma was obtained by centrifugation at 4,800 g for 20 minutes in a refrigerated centrifuge. Samples were stored at -80°C until assayed.

vWF:Ag. Measurement of vWF (vWF:Ag) was performed by using quantitative immunoelectrophoresis in 0.9% agarose and rabbit antihuman vWF as previously described. The reference standard for vWF:Ag was pooled plasma from 50 normal donors (stored at -80°C). All reference standards derived from pooled plasmas were assayed against the World Health Organization standard.

Determination of vWD type. Patients referred for evaluation of clinically significant bleeding had the following laboratory studies performed to determine vWD type: vWF:Ag as described earlier, vWF activity by a ristocetin cofactor assay utilizing formalin-fixed human platelets, factor VIII (F VIII) procoagulant activity by a one-stage method utilizing F VIII-deficient plasma as substrate, and multimeric analysis of vWF by electrophoresis of plasma in 0.65% Seakem high gelling temperature ultra pure (HGT-(P)) agarose gels (FMC Corp, Rockland, ME) in the presence of 0.1% sodium dodecyl sulfate (SDS) and visualized with radiolabeled polyclonal rabbit antihuman vWF and autoradiography as previously described. The diagnosis of type I vWD was based on the finding of proportionally decreased plasma vWF:Ag and ristocetin cofactor activity below 45 U/dL and a normal multimeric pattern of vWF:Ag. Type II vWD was diagnosed by the findings of ristocetin cofactor activity below 45 U/dL, vWF:Ag disproportionately higher than ristocetin cofactor activity, and an abnormal multimeric pattern of vWF characterized by an absence of the high-molecular weight multimers. Patients with undetectable vWF were classified as having type III vWD.

Reverse ABO blood group typing. Plasma from patients with the diagnosis of vWD, stored at -80°C, was mixed with saline-washed RBC from individuals of blood groups A and B, centrifuged, and observed for hemagglutination.

Statistical analysis. Multiple-regression analysis (FPSS Statistical Package for Social Services), one-way analysis of variance, Student's t test, and the goodness-of-fit test were performed as appropriate.

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Table 1. Influence of ABO Blood Group on vWF:Ag Values in Volunteer Blood Donors

<table>
<thead>
<tr>
<th>ABO Type</th>
<th>n</th>
<th>vWF:Ag Geometric Mean</th>
<th>vWF:Ag Geometric Mean ± 2 SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>O</td>
<td>456</td>
<td>74.8</td>
<td>35.6-157.0</td>
</tr>
<tr>
<td>A</td>
<td>340</td>
<td>105.9</td>
<td>48.0-233.9</td>
</tr>
<tr>
<td>B</td>
<td>196</td>
<td>116.9</td>
<td>56.8-241.0</td>
</tr>
<tr>
<td>AB</td>
<td>109</td>
<td>123.3</td>
<td>63.8-238.2</td>
</tr>
</tbody>
</table>

The groups were statistically significantly different from each other as follows: O v A, B, and AB, P < .01; A v AB, P < .01; B v A, P < .05.

RESULTS

vWF:Ag in 1,101 blood donors. Initially, 1,000 sequential blood donors from the Blood Center of Southeastern Wisconsin had determinations of vWF:Ag levels. To increase the number of individuals of ABO groups B and AB, additional sequential samples from 52 group B and 65 group AB donors were collected for a total of 1,117 samples. Twelve donors were excluded because of vasovagal reactions during the donation and four because their vWF:Ag values were beyond 3 SD above the mean (statistical outliers) for their ABO group after adjusting for age. Statistical analyses were performed with values from the remaining 1,101 donors.

vWF:Ag values were significantly affected by the ABO blood group of donors. As indicated in Table 1, vWF:Ag values in each group were significantly different from the others; group AB individuals had the highest geometric mean vWF:Ag level (123.3 U/dL), followed by group B (116.9 U/dL), then group A (105.9 U/dL), and finally group O individuals with the lowest values (74.8 U/dL).

Multiple-regression analysis of variables other than ABO group including age, gender, Rh type, and the ingestion of medications revealed that in the group as a whole and within each ABO blood group age was the only variable that was significantly predictive of vWF:Ag (Fig 1). Age did not account for the variation in vWF levels among ABO blood groups, since the range and mean age were similar in the groups (Table 2). However, as higher ages were attained, mean vWF:Ag values in persons of blood group A and blood group B approached each other (Fig 2).

Fig 1. Increase in vWF:Ag with age in volunteer blood donors. ABO blood group is indicated by the letters O, A, B, or AB. Mean vWF v age is shown by the solid lines, and 95% confidence intervals are indicated by the broken lines.
In group A individuals only, when age was accounted for, the male gender (P < .02) and ingestion of medications (P < .05) were associated with a higher vWF:Ag concentration. Of the 43 blood group A individuals taking medications, the largest group (n = 18) was using antihypertensive drugs, followed by antibiotics (8), oral contraceptives (6), recent immunizations or desensitization with allergens (5), antihistamines (4), and antidepressants (1).

Since the blood sample for vWF:Ag determination was obtained after the regular blood donation, it was possible that the stress associated with the procedure might bias the results. Therefore, 25 donors agreed to undergo a separate venipuncture for vWF:Ag determination immediately prior to the regular donation, with the second sample collected after the blood donation as done in the other donors. The mean predonation vWF:Ag concentration in these donors was 2.5 U/dL higher than the postdonation samples, not significantly different (P = .80).

To determine whether structurally abnormal vWF might be present in group O donors with low vWF levels, an additional 104 group O donors were evaluated. Ristocetin cofactor assays correlated well with vWF:Ag with a correlation coefficient of 0.80 (P < .01). Multimeric analysis of vWF on SDS agarose gels were performed on the 11 donors from this group of 104 blood group O individuals with a vWF:Ag level <45 U/dL and on three others from this group who had a discrepancy between the ristocetin cofactor and vWF:Ag assays; all 14 patterns were normal.

ABO blood groups in patients with a diagnosis of vWd. Reverse ABO typing of 142 plasma samples from patients carrying a diagnosis of vWd was performed (Table 3). The frequency distribution of ABO blood groups in type I vWd (quantitative deficiency of structurally normal vWF) was significantly different from the expected distribution (personal communication, J.E. Menitove, Blood Center of Southeastern Wisconsin) when analyzed by the goodness-of-fit test (P < .001). Of 114 patients with a diagnosis of type I vWd, 88 (77%) had blood group O, whereas the expected frequency of blood group O in the normal population was 45%. In contrast, blood groups A, B, and AB were under-represented in type I vWd patients when compared with the expected pattern of distribution. When the ABO frequency distribution of type I vWd patients was adjusted by excluding the 23 individuals with vWF values greater than 37, of the 91 total, 65 (71%) had group O, 21 (23%) had group A, 5 (5%) had group B, and none had group AB. This distribution was also significantly (P < .001) different from the expected. The 22 patients with a diagnosis of type II vWd (structurally abnormal vWF) had an expected frequency of ABO groups, and the six with type III vWd (undetectable vWF) were too few for a meaningful analysis.

**DISCUSSION**

This study confirms previous reports that plasma vWF:Ag concentration is significantly affected by the ABO blood group. As in this study, all but one investigator found that blood group O was associated with the lowest vWF:Ag values; the single exception was a small study conducted by Mohanty and coworkers, who found the lowest vWF:Ag values in blood group B individuals and suggested that this difference was due to ethnic variation. In the studies by McCallum and coworkers of female blood donors and Stormorken and Erikssen of middle-aged men, no significant differences in vWF:Ag values were found between individuals of blood groups A, B, or AB, whereas this study demonstrated that the vWF:Ag concentration in group AB was highest, lower in group B, and lower still in group A. The smaller sample size (136) of McCallum and colleagues' study and the restriction of the sample to middle-aged men in Stormorken and Erikssen’s study may account for these differences.

In a study of monozygotic and dizygotic twins, Orstavik and coworkers determined that F VIII:C was dependent on vWF:Ag levels and that 30% of the genetic variance of vWF:Ag was due to the effect of ABO blood group. The twins were all greater than 33 years of age, and those with blood group A1 had higher vWF:Ag levels than those with group B in whom levels were higher than group A2. These findings differ from our investigation but could be explained by the differences in age in the two samples and our finding...
that vWF:Ag values in group A and group B individuals approach each other as age increases; furthermore, we did not differentiate between groups A1 and A2.

Genetic linkage analyses previously suggested that the loci for vWF and ABO blood groups are not linked.16,17 This conclusion has been substantiated by the recent location of the gene for vWF on chromosome 12,18 whereas the gene for ABO blood group determination is on chromosome 9.19-21

Thus, a direct linkage could not explain the significant variation in plasma vWF concentration among individuals of different ABO blood groups.

vWF is a highly glycosylated protein22,23; its platelet-agglutinating activity in the presence of ristocetin,24-28 susceptibility to proteolytic degradation,29 and survival in the normal rabbit circulation30 have been shown to be affected by the removal of sialic acid or galactose residues, which suggests that carbohydrate is important in the structure/function of vWF. Furthermore, blood group A, B, and H oligosaccharide structures have been identified on human vWF.23 It is possible that the blood group determinants may affect the processing, release, or catabolism of vWF, thus influencing the plasma concentration of the protein.

Since ABO blood group was found to be a significant determinant of plasma vWF concentration, we wondered whether the diagnosis of vWD was influenced by the patient’s ABO status and found that significantly more type I vWD patients than expected had blood group O. The additional finding of increased numbers of group O individuals when the diagnosis was based on ABO-adjusted normal ranges suggests that there may be a subset of type I vWD patients with decreased concentrations of structurally normal vWF on the basis of blood group rather than specific inherited abnormalities of vWF production or release. Similarly, individuals of blood group AB with a vWF genetic defect may have the diagnosis overlooked because the vWF is elevated due to blood type.

We do not intend to imply that symptomatic individuals with vWF in the range of 37 to 45 U/dL and blood group O do not have a form of vWD and should not be treated as such. The treatment of such individuals, as in any clinical situation, should not be altered because of this theoretical consideration. However, the inheritance of vWD in such an individual may be different than in an individual with a specific vWF gene abnormality. Thus, the mechanism of decreased concentrations of plasma vWF in various patients may be heterogeneous; further clarification of this issue may evolve as specific chromosomal abnormalities resulting in type I vWD are defined.

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