Carrier Detection in Hemophilia A: A Cooperative International Study.

I. The Carrier Phenotype

By John B. Graham, Charles R. Rizza, Juan Chediak, Piero Mannuccio Mannucci, Ernest Briët, Rolf Ljung, Carol K. Kasper, E.M. Essien, and P.P. Green

Eight laboratories in six countries cooperated to clarify several issues concerning the phenotypes of heterozygous carriers of hemophilia "A." Plasma levels of factor VIII (F.VIII:C, formerly VIII:C) and von Willebrand factor (VWF:Ag, formerly VIIIIR:Ag) of carriers and normal women were determined by various "in-house" methods; a single lyophilized plasma standard was used for all assays. Analysis of the collated data from 336 carriers (296 obligatory carriers and 40 sporadic carriers) and 137 normal women showed that there was no difference in the F.VIII:C levels of "paternal" carriers (women who had obtained the abnormal gene from their fathers) and "maternal" carriers. Neither was there a difference in the VWF:Ag levels of normal women and either type of carrier. Age was found to have a significant effect on both F.VIII:C and VWF:Ag, values being higher at very young and very old ages, the minima occurring in the 25- to 30-year range. ABO blood type had a striking effect. Women of types A, B, and AB (designated non-O in the study), both normals and carriers, had significantly higher levels of both factors than did women of type O. Analysis by laboratories showed that differences in mean levels of both factors between laboratories were highly significant. It was concluded that age, ABO blood type, and laboratory variation should be taken into account in carrier detection.

*The International Committee on Thrombosis and Haemostasis has recommended that the term F.VIII be used when referring generally to this clotting factor, F.VIII:C when referring to its activity, and F.VIII:Ag (formerly F.VIII:Cag) when it is measured immunologically. It has also recommended that VWF be used when referring generally to the clotting factor missing in severe VWD, and VWF:Ag (formerly VIIIIR:Ag) when this factor is measured immunologically.

Identification of heterozygotes for hemophilia "A" (detection of carriers) by laboratory means was first attempted by Merskey and MacFarlane in 1951,1 prior to the introduction of factor VIII (F.VIII) assays for this purpose by Graham and colleagues2 in 1953. Veltkamp and co-workers applied linear discriminant analysis to factor VIII assays in 1966,3 and Zimmerman and colleagues4 showed in 1971 that detection could be improved by using assays for F.VIII together with assays for an antigen missing in severe von Willebrand's disease (VWD).*

Linear discriminant analysis was applied to conjoint F.VIII:C and VWF:Ag assays by Elston and colleagues in 1976.5 Reisner and colleagues6 extended the analysis to three variables by measuring in addition the rate of agglutination of platelets in the presence of ristocetin. Multivariate analysis has been applied to carrier detection by Wahlberg and co-workers7 who used all of the appropriate plasma variables plus screening tests and pedigree data.

It is not widely appreciated that all available information should be used when the result of a diagnostic procedure, such as phenotyping hemophilia carriers, is a probability. Four kinds of information are sought: (a) information about the antecedents of a possible carrier and transmission of the trait through her family; (b) information about her descendants; (c) information about linkage of the hemophilia gene to other X-linked traits in her family, e.g., colorblindness or G6PD types, etc.; and (d) bioassays of her plasma and that of her relatives. The first three are combined mathematically to provide a prior probability, and the fourth provides the likelihood ratio. The final probability is produced by combining these. It is essential that at least one affected male in each kindred be definitively studied to assure that a correct diagnosis has been made and to establish the degree of severity, principles which have been discussed at length elsewhere.8-11 The latest development has been the genotyping of subjects using restriction fragment-length polymorphisms (RFLPs) to characterize X-chromosomal DNA, the advantages and disadvantages of which are discussed elsewhere.12

There have been conflicting reports concerning the effects of age and other variables on the levels of F.VIII:C and VWF:Ag in hemophilia carriers. Some workers have found that age affects F.VIII:C levels,13,14 whereas others have not.15-17 It has been reported that the F.VIII:C levels of carriers who have received the hemophilia gene from their fathers (paternal carriers) are lower than those of carriers who have received the gene from their mothers (maternal carriers).18,19 an observation which has been disputed.20-23 A differential effect between types of carriers would not be trivial, either theoretically or practically. It might be a signal that paternally and maternally derived X-chromosomes have different probabilities of inactivation, which would require that a reference group of carriers for discriminant analysis consist solely of maternal carriers.

Another variable which might affect carrier detection is...
the effect of ABO blood type on factor levels. Levels of F.VIII:C and VWF:Ag appear to be lower in persons of type O than those of types A, B, and AB. This was first reported by Preston and Barr in 1964 and has been confirmed several times since.\(^3,1,14,24-26\)

A possible explanation for the conflicting reports is that they have been based upon studies of small, sometimes nonrepresentative populations. This possibility, particularly as it has concerned the differences between types of carriers, prompted the present study. The phenotypic characteristics of hemophilia A carriers are described in this article, and an analysis of the use of linear discriminants for carrier detection comprises the accompanying report.\(^27\)

**METHODS AND MATERIALS**

**Experimental design.** Each laboratory obtained blood from members of its group of carriers in its particular way. Plasma was prepared and assays were performed in each laboratory by current "in-house" methods. All test samples were collected and assayed between June 1982 and July 1983, and the results were sent to Chapel Hill for analysis. ABO blood types were determined later.

**Participating laboratories.** Participating laboratories were: (a) the Haemophilia Centre, Oxford, England; (b) the University of North Carolina; (c) the Orthopaedic Hospital of the University of Southern California, Los Angeles; (d) the University Hospital, University of Lund, Malmo, Sweden; (e) the A. Bianchi Bonomi Haemophilia and Thrombosis Centre, University of Milan, Italy; (f) the University of Leiden, Netherlands; (g) the Michael Reese Hospital, University of Chicago, Chicago, Illinois; and (h) the University of Ibadan, Nigeria.

**Standard control plasma.** Standard control plasma was produced at the Haemophilia Centre in Oxford by pooling six normal plasmas that had been prepared by centrifugation of blood collected into a buffered citrate anticoagulant (3.8% sodium citrate in 7% HEPES buffer at pH 7.4). Nine parts of blood had been added to one part of anticoagulant. The pooled plasma was freeze-dried in 1-mL aliquots and was stored until use in each laboratory at a temperature below \(-30^\circ C\). It was reconstituted in 1 mL of distilled water. Potency of the reconstituted plasma was maintained for up to two hours at room temperature, and the laboratories were asked not to refreeze and reuse the standard. The potency of the standard was determined to be 0.53 IU of F.VIII:C per ampoule by calibration against the international standard.\(^\dagger\) The VWF:Ag of the lyophilized standard plasma was arbitrarily defined as 100 \(\mu\)U per deciliter, since an international standard was not available at the onset of the study.

**Blood collection and plasma preparation.** Blood was carefully collected in each laboratory to assure that all samples were handled in the same fashion; samples were centrifuged for at least 20 minutes at speeds > 3,000 rpm or forces > 2,000 g, often at room temperature. Some laboratories assayed fresh plasmas; others examined frozen and stored plasmas.

**Assays.** Seven laboratories performed F.VIII:C assays by a one-stage method; a two-stage procedure was used at Oxford. No differences were observed in the distributions of normal values attributable to the type of assay for F.VIII. In four laboratories, F.VIII:Ag was determined by immunoradiometric assays (IRMAs) using polyclonal human antibodies. Assays for VWF:Ag were done in all laboratories by the local adaptation of the electroimmunoassay (EIA) described originally by Laurell.\(^28\) VWF:Ag was also determined by IRMAs in three laboratories. Heterologous antibodies were used in the EIA, and both polyclonal and monoclonal antibodies against VWF:Ag were used in the IRMAs. VWF:Ag was also measured by a commercial enzyme-linked immunosorbent assay (ELISA) in Milan.

**Population studied.** Although the subjects were predominantly of west European ancestry, some subjects of African and Oriental ethnicity were introduced through the American and Nigerian subsamples. Women from a broad age range were examined to assess the effects of age on the variables. Five laboratories collected samples from normal women, and several laboratories collected samples from sporadic carriers. In one laboratory, each carrier was matched by a normal woman of the same age, whereas in four other laboratories a smaller sample of normal women was collected that covered the same age range as the carriers. Table 1 shows the study sample stratified for age and genotype.

**Blood typing.** ABO blood types were determined on as many of the subjects as possible. Unfortunately, it was impossible to ascertain the blood types of the subjects at four laboratories.

**Criteria for inclusion in the study.** Only carriers of severe hemophilia were accepted into the study, i.e., women having a male relative with < 1% F.VIII:C. Each woman was classified as either a "paternal carrier," "maternal carrier," or "normal," according to the following definitions: (a) **paternal carrier:** A woman whose putative father had been definitely established as having hemophilia A; (b) **maternal carrier:** A woman whose putative father was normal and who had produced hemophilic male(s) and/or carrier female(s) and was in the main line of descent of a hemophilia A pedigree; (c) **sporadic carrier:** A woman whose putative father was normal and who has had at least two hemophilic sons, or a hemophilic son and a carrier daughter, or two carrier daughters but no other hemophilic male relatives; or (d) **normal:** A woman with no hemophilic relatives.

**Statistical treatment.** To render laboratories comparable for the pertinent analyses and to assess interlaboratory variation, only the first assay result was used in cases in which multiple assays were performed. A "second" assay was one based upon a separate aliquot from a single plasma sample or on a second sample. The F.VIII:C and VWF:Ag results were logarithmically transformed to reduce

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\(^\dagger\)The International Unit (IU) of F.VIII:C at the time of the study was based on a concentrate \(1.2 \times 0.53\) to produce figures similar to those which are usually obtained in laboratories which use pooled normal plasma as the working standard.
Although both linear and quadratic, but not cubic, terms effect of age on F.VIII:C and VWF:Ag levels was examined.

RESULTS

The distributions by laboratory of normal women, paternal carriers, maternal carriers, and sporadic carriers are shown in Table 2. Approximately equal numbers of normal subjects, paternal carriers, and maternal carriers were examined.

**F.VIII:C levels.** The data obtained by comparison with the lyophilized standard and adjusted as described in the Methods section are shown in Table 3. The mean level of F.VIII:C in 137 normal women (column 1) was 106 U per deciliter; the means of the different laboratories ranged from 84 to 117. The differences of the means of normal women between laboratories was significant ($P < .01$).

The averages of the means for the paternal and maternal carriers, not adjusted for age, (columns 2 and 3) were the same, 54 U per deciliter. When adjusted to age 25, using the regression curves described below, the means were 48.0 (paternal) and 45.0 (maternal) U per deciliter. When the data were adjusted for the effects of both age and institution there was not a significant difference between types of carriers ($P = .29$). Because there was not a difference between the F.VIII:C's of the different types of carriers, all the carrier data, including the sporadic carriers, were pooled for the age effect calculations and the discriminant analyses.

**VWF:Ag levels.** The values for VWF:Ag as assessed by the Laurell method are shown in Table 4. The mean for the 137 normal women (first column) was 106 U per deciliter. As with F.VIII:C, there was a significant effect of age and institution on the level of VWF:Ag. When the means were adjusted for age and institution, there was not a significant difference between the types of carriers ($P = .83$) or between pooled carriers and normals ($P = .16$). The VWF:Ag values of all carriers were pooled for subsequent calculations.

**Effect of age on F.VIII:C and VWF:Ag levels.** The effect of age on F.VIII:C and VWF:Ag levels was examined in both normal women and carriers. Logarithmically transformed values were regressed on a cubic function of age. Although both linear and quadratic, but not cubic, terms were required to obtain a satisfactory fit over the range 10 to 60 years of age, the range from 18 to 50 years of age could be fitted by a linear function. The best fitting quadratic functions of age for the normals and the carriers were the following: F.VIII:C for normal subjects was $\ln(F.VIII:C) = .000360(age)^2 - .0209(age) + 4.78$; for carriers, it was $\ln(F.VIII:C) = .000317(age)^2 - .0193(age) + 4.15$. VWF:Ag for normal subjects was $\ln(VWF:Ag) = .000422(age)^2 - .0225(age) + 4.65$; for carriers, it was $\ln(VWF:Ag) = .000277(age)^2 - .0170(age) + 4.76$.

Although the means for F.VIII:C in normal subjects and carriers were significantly different at all ages as expected ($P < .0001$), the age regression coefficients were not significantly different ($P > .3$). The mean VWF:Ag values were not different between normal subjects and carriers, although both showed an effect of age. Neither were the age regression coefficients on the transformed data different for normal subjects or carriers ($P > .05$).

**Effect of ABO blood group on F.VIII:C and VWF:Ag levels.** ABO blood types were available on most of the subjects of four laboratories. The frequencies of the blood types were: (among 54 normal subjects) O = 0.50, A = 0.362, B = 0.12, and AB = 0.017; and (among 198 carriers) O = 0.49, A = 0.38, B = 0.10, and AB = 0.03. These frequencies are typical of West European peoples. Approximately 75% of non-Os are type A, and types B and AB appear to have somewhat larger effects on the variables concerned than does type A. Therefore, the subjects are grouped into two classes: O and non-O. The data relating F.VIII:C and VWF:Ag to ABO blood type are shown in Table 5 as comparisons between O and non-O subjects stratified for carriership and examined separately for F.VIII:C and VWF:Ag. In 13 of the 14 possible comparisons, the levels are higher in the non-O member of a pair. Using the data in Table 5, the differences in weighted averages (non-O > O) were for F.VIII:C, 22.9 U per deciliter in normal subjects and 10 U per deciliter in carriers. Comparable results for VWF:Ag were: 25.8 U/dL in normal subjects and 34.6 U/dL in carriers.

The significance of the difference between O and non-O subjects for F.VIII:C and VWF:Ag was confirmed using a general linear model which included age and laboratory as covariates ($P < .01$).

**Table 3. Factor VIII:C Levels**

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Normal Women</th>
<th>Paternal Carriers</th>
<th>Maternal Carriers</th>
<th>Pooled Carriers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxford</td>
<td>92 ± 16</td>
<td>51 ± 14</td>
<td>53 ± 15</td>
<td>52 ± 14</td>
</tr>
<tr>
<td>Chapel Hill</td>
<td>—</td>
<td>54 ± 22</td>
<td>50 ± 33</td>
<td>52 ± 29</td>
</tr>
<tr>
<td>Los Angeles</td>
<td>110 ± 44</td>
<td>53 ± 23</td>
<td>62 ± 25</td>
<td>57 ± 29</td>
</tr>
<tr>
<td>Milan</td>
<td>117 ± 49</td>
<td>54 ± 25</td>
<td>51 ± 21</td>
<td>52 ± 23</td>
</tr>
<tr>
<td>Chicago</td>
<td>—</td>
<td>62 ± 20</td>
<td>71 ± 30</td>
<td>68 ± 28</td>
</tr>
<tr>
<td>Malmo</td>
<td>109 ± 43</td>
<td>60 ± 30</td>
<td>61 ± 25</td>
<td>64 ± 28</td>
</tr>
<tr>
<td>Leiden</td>
<td>84 ± 24</td>
<td>48 ± 21</td>
<td>47 ± 26</td>
<td>48 ± 24</td>
</tr>
<tr>
<td>Ibadan</td>
<td>—</td>
<td>72 ± 29</td>
<td>72 ± 29</td>
<td></td>
</tr>
<tr>
<td>Average of means</td>
<td>106 ± 42</td>
<td>54 ± 26</td>
<td>54 ± 24</td>
<td>55 ± 27</td>
</tr>
</tbody>
</table>

Levels are standard plasma = 53 U per deciliter. Values in tables are shown in “working” units, ie, values obtained x 0.64. Values are mean ± SD.
LINEAR DISCRIMINANTS IN CARRIER DETECTION

Table 4. VWF:AG Levels

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Normal Women</th>
<th>Paternal Carriers</th>
<th>Maternal Carriers</th>
<th>Pooled Carriers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxford</td>
<td>86 ± 31</td>
<td>95 ± 43</td>
<td>116 ± 34</td>
<td>104 ± 41</td>
</tr>
<tr>
<td>Chapel Hill</td>
<td>—</td>
<td>110 ± 57</td>
<td>107 ± 87</td>
<td>108 ± 76</td>
</tr>
<tr>
<td>Los Angeles</td>
<td>122 ± 44</td>
<td>124 ± 69</td>
<td>125 ± 34</td>
<td>124 ± 53</td>
</tr>
<tr>
<td>Milan</td>
<td>107 ± 61</td>
<td>99 ± 36</td>
<td>103 ± 38</td>
<td>101 ± 36</td>
</tr>
<tr>
<td>Chicago</td>
<td>—</td>
<td>123 ± 54</td>
<td>116 ± 77</td>
<td>119 ± 67</td>
</tr>
<tr>
<td>Malmo</td>
<td>85 ± 35</td>
<td>84 ± 24</td>
<td>105 ± 50</td>
<td>94 ± 40</td>
</tr>
<tr>
<td>Leiden</td>
<td>103 ± 27</td>
<td>110 ± 36</td>
<td>110 ± 41</td>
<td>110 ± 40</td>
</tr>
<tr>
<td>Ibadan</td>
<td>—</td>
<td>—</td>
<td>151 ± 64</td>
<td>151 ± 64</td>
</tr>
<tr>
<td>Average of means</td>
<td>101 ± 47</td>
<td>105 ± 49</td>
<td>112 ± 60</td>
<td>108 ± 47</td>
</tr>
</tbody>
</table>

Levels of standard plasma activity arbitrarily set at 100 U/dL. Values are means ± SD.

DISCUSSION

Despite the fact that each of the participating laboratories collected blood somewhat differently and used similar though not identical assays, it was believed that the geographically separate groups of carriers could be collated for analysis, since all were based on the same standard. This lyophilized plasma has essentially the properties of plasma #80/511, now adopted by the WHO as the First International Reference Preparation for Factor VIII Related Activities in Plasma, and is very stable at temperatures below –30 °C.

A major variable in the study was the difference in mean levels of F.VIII:C and VWF:Ag between laboratories. Klein and colleagues ascribed similar interlaboratory variation largely to differences in laboratory technique, because the same group of women was examined in a single laboratory setting by different technicians using a single standard. Barrowcliffe and colleagues and we have observed significant differences in the means between laboratories when a single standard was used on separate small populations.

The data of Klein and co-workers clearly document that differences in technique between laboratory workers is an important source of variation in carrier detection. Their study involved a single population and they were, therefore, unable to assess variation between populations. The present study was of separate small populations, and the interlaboratory variation which persisted after correction for age and ABO blood type could reflect population differences as well as differences in technique. There are several obvious genetic reasons why population differences might be encountered: (a) the normal F.VIII locus of each carrier will be occupied by any one of several isoalleles with different effects, and the frequencies of the isoalleles may vary between small populations; (b) the frequencies of the isoalleles of (unspecified) modifying genes at other loci may vary; and (c) the average ratio of lyonization between the normal and abnormal alleles of groups of heterozygotes may not be the same.

The original goals of this study were narrow, ie, to determine whether there is a difference in F.VIII:C levels between "paternal" and "maternal" carriers and whether age has an effect on levels of F.VIII:C and VWF:Ag. Answers were obtained on both. The results show quite clearly that there is no difference between the levels of F.VIII:C and VWF:Ag of "paternal" and "maternal" carriers. The populations that had produced opposite results had been much smaller than that of this study, and it is likely that some of the groups had not been representative of carriers generally. Second, age does affect levels of F.VIII:C and VWF:Ag, although not as much as blood type. Because significant age effects were also found in the twin study of Orstavik and co-workers, age must be regarded as a variable to be taken into account in hemophilia carrier detection.

This study confirms that women of blood type O have lower F.VIII:C and VWF:Ag levels than do women who are

Table 5. ABO Blood Group and Factors VIII:C and VWF:Ag in Normal Women and Carriers of Hemophilia A

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Statistic</th>
<th>Factor VIII:C</th>
<th>VWF:Ag</th>
<th>Factor VIII:C</th>
<th>VWF:Ag</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal Subjects</td>
<td>O</td>
<td>Non-O</td>
<td>O</td>
<td>Non-O</td>
</tr>
<tr>
<td>Chapel Hill</td>
<td>Mean</td>
<td>50.6</td>
<td>64.7</td>
<td>79.9</td>
<td>132.2</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>30.7 ± 12.3</td>
<td>36.0 ± 15.8</td>
<td>48.3 ± 17.1</td>
<td>87.9 ± 29.8</td>
</tr>
<tr>
<td></td>
<td>(No.)</td>
<td>(42)</td>
<td>(46)</td>
<td>(42)</td>
<td>(46)</td>
</tr>
<tr>
<td>Oxford</td>
<td>Mean</td>
<td>54.5 ± 20.2</td>
<td>60.8 ± 15.8</td>
<td>86.2 ± 29.8</td>
<td>120.7 ± 33.9</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>18.6 ± 15.8</td>
<td>17.1 ± 15.8</td>
<td>29.8 ± 20.2</td>
<td>40.3 ± 24.6</td>
</tr>
<tr>
<td></td>
<td>(No.)</td>
<td>(27)</td>
<td>(29)</td>
<td>(27)</td>
<td>(29)</td>
</tr>
<tr>
<td>Leiden</td>
<td>Mean</td>
<td>42.6 ± 13.8</td>
<td>53.2 ± 15.8</td>
<td>98.1 ± 29.8</td>
<td>118.5 ± 33.9</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>15.5 ± 13.8</td>
<td>28.4 ± 15.8</td>
<td>33.9 ± 24.6</td>
<td>42.2 ± 24.6</td>
</tr>
<tr>
<td></td>
<td>(No.)</td>
<td>(16)</td>
<td>(22)</td>
<td>(16)</td>
<td>(22)</td>
</tr>
<tr>
<td>Los Angeles</td>
<td>Mean</td>
<td>49.6 ± 13.8</td>
<td>51.5 ± 15.8</td>
<td>143.0 ± 33.9</td>
<td>121.7 ± 33.9</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>17.8 ± 13.8</td>
<td>16.2 ± 15.8</td>
<td>85.0 ± 24.6</td>
<td>27.8 ± 24.6</td>
</tr>
<tr>
<td></td>
<td>(No.)</td>
<td>(9)</td>
<td>(7)</td>
<td>(9)</td>
<td>(7)</td>
</tr>
</tbody>
</table>
not of type O, and that this is a significant variable that affects carrier detection. The physiological relationship between ABO type and the level of these clotting factors is obscure. Highly purified preparations of F.VIII containing ABO blood group substance have been described, but this does not necessarily mean that it is a functionally important part of the F.VIII complex. The genetic information tends to suggest the opposite in fact, ie: (a) the ABO types are present when F.VIII:C and VWF:Ag are absent; (b) the genes for ABO and VWD are not linked in the usual sense; and (c) the gene for F.VIII:C is on the X-chromosome whereas the ABO locus is on chromosome 9. Nevertheless, F.VIII:C and VWF:Ag levels are clearly lower in persons of blood type O, a variable included in the discriminant function published by Winter and co-workers. Orstavik and colleagues concluded that the ABO blood group has a greater genetic effect on levels of F.VIII:Ag and VWF:Ag in normal persons than does any other genetic mechanism, including isoalleles at the F.VIII:C locus. Their data suggested that the primary effect of ABO is on the level of VWF:Ag and that the effect on F.VIII:C is secondary. How these independent genes interact is unknown, but it might occur posttranslationally through interaction of their products. The type of ABO substance on the cell membrane for instance, may influence the rate at which the clotting factors exit from the cell. But there are many other possibilities.

Two other matters that were of concern to the WHO Committee in 1977 were not studied, but it seems worthwhile to note them. They wished to determine whether carriership can be detected accurately in women who are already pregnant when first seen. Subsequent studies by Mibashan and colleagues, Hoyer and colleagues and Barrow and colleagues have demonstrated that the procedures recommended by the WHO are satisfactory for pregnant carriers who can be detected without difficulty at least until the 22nd week of gestation.

They also wished to discover whether the taking of oral contraceptives confounds carrier detection. Stableforth and colleagues and McCallum and colleagues have reported that the use of oral contraceptives does not affect levels of F.VIII:C and VWF:Ag. This was not a matter of importance in the present study, since only 5 of 473 subjects (1.1%) reported using this form of birth control. This may well be an underestimate of the true frequency of contraceptive use by all women, however, since many of our subjects were unmarried, prepubertal, or postmenopausal.

Kobrinsky and co-workers have reported that in plasma samples obtained one hour after the administration of a small dose of DDAVP there is a comparable rise of VWF:Ag in both carriers and normal women, whereas the increase in F VIII:C is less among carriers. The rate of misclassification on single testing after DDAVP administration in their small sample was ~5%, comparable to the rate found earlier with triple testing using the ordinary procedure. If this is confirmed, administration of DDAVP together with corrections for age, ABO blood type, and laboration variation might greatly enhance the likelihood of correctly classifying hemophilia carriers by phenotypic methods.

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