Loss of the Largest von Willebrand Factor Multimers From the Plasma of Patients With Congenital Cardiac Defects

By Joan C. Gill, Allen D. Wilson, Janet Endres-Brooks, and Robert R. Montgomery

We identified a consecutive series of 12 children with noncyanotic congenital cardiac lesions with loss of the largest plasma von Willebrand factor (vWF) multimers determined by SDS-agarose electrophoresis. Seven had previous histories of mucocutaneous hemorrhage; ten had a prolonged bleeding time. Analysis of the factor VIII molecular complex revealed that six patients had reduced vWF measured both immunologically (vWF:Ag) and by ristocetin cofactor assay (vWF:rist). All had normal or borderline normal factor VIII procoagulant (F VIII) concentrations. Three children had prolonged partial thromboplastin times due to concurrent factor XII deficiency; none had laboratory evidence of intravascular coagulation. Five of the children were restudied after surgical correction of their cardiac lesions. Four had normalization of vWF multimers; the fifth, whose vWF was abnormal postoperatively, had a residual pressure gradient across a previous pulmonary artery banding site. Multimeric abnormalities were not found in the parents of three patients. Thus some patients with noncyanotic congenital heart disease may have an acquired abnormality of vWF that is normalized with correction of the abnormal hemodynamic state.

THE PLASMA FACTOR VIII molecular complex is composed of two proteins: factor VIII (F VIII), the procoagulant protein that functions as a cofactor in factor I Xa activation of factor X in blood clotting; and von Willebrand factor (vWF), the large multimeric protein that supports platelet adhesion. Both proteins can be measured immunologically and functionally; thus both qualitative and quantitative abnormalities of vWF are possible and have been described. Patients with inherited von Willebrand disease (vWD) of type II have qualitative defects of vWF; these defects are manifested by mild to moderate mucous membrane and cutaneous hemorrhages, prolonged bleeding times, decreased vWF-ristocetin cofactor activity (vWF:rist), and absence of the large vWF multimers demonstrated by sodium dodecyl sulfate (SDS)-agarose electrophoresis of plasma. Acquired defects of vWF have been reported in patients with disseminated intravascular coagulation (DIC), the episodic form of hemolytic uraemic syndrome, acute myocardial infarction, and myeloproliferative syndrome.

We recently had the opportunity to study nine patients with ventricular septal defect (VSD), one with atrial septal defect (ASD), and two with aortic stenosis. Each patient was referred for evaluation of hemorrhagic symptoms or a long bleeding time. All 12 patients had apparent acquired absence of the largest vWF multimers by SDS-agarose electrophoresis.

MATERIALS AND METHODS

Patients. The 12 consecutive patients were referred for either evaluation of hemorrhagic symptoms or abnormal preoperative hemostatic screening tests. Laboratory evaluation was performed at Milwaukee Children's Hospital Clinical Laboratory and the Hemostasis Reference Laboratory of the Blood Center of Southeastern Wisconsin, Milwaukee. Careful histories and physical examinations were conducted for all patients.

Collection of blood samples. Plasma was obtained from venous blood sampled by a double-syringe technique and anticoagulated with sodium citrate (3.2%). Platelet-rich plasma was obtained by centrifugation of anticoagulated whole blood at 1,600 g for five minutes and platelet-poor plasma by centrifugation at 4,800 g for 20 minutes in a refrigerated centrifuge.

Standard coagulation assays. Bleeding times were calculated with the Simplate device (General Diagnostics, Morris Plains, NJ) according to the manufacturer's suggestions. Prothrombin (PT) and partial thromboplastin (PTT) times were determined by recalification assays on an MLA Electra-700 (Dade, Mount Vernon, NY). Platelet counts were performed on an ELT-BDS (Ortho, Boston) and confirmed by visualization of Wrights-stained peripheral blood smears by light microscopy. Fibrinogen assays were performed on a fibrometer utilizing Data-Fibrinogen reagents (Dade, Miami) and fibrinogen degradation products by the Thrombo-Wellico Test (Burroughs-Wellcome, Research Triangle Park, NC).

Studies of the factor VIII molecular complex. Factor VIII procoagulant activity (F VIII:C) was determined by a one-stage method using F VIII–deficient plasma as a substrate. Quantitative measurement of vWF (vWF:Ag) was performed using a rocket immunoelectrophoretic technique in 9% agarose with rabbit antihuman vWF. Ristocetin cofactor activity of vWF (vWF:rist) was determined using formalin-fixed human platelets. Multimeric analysis of vWF was determined by electrophoresis of plasma in .65% Seakem HGT-(P) agarose gels (FMC Corp, Rockland, Me) in the presence of .1% SDS and visualized by radiolabeled rabbit antihuman vWF and autoradiography as previously described. The reference standard for factor VIII and vWF assays was pooled plasma from 50 normal donors (stored at −80°C).

RESULTS

During the period from June 1982 to December 1984, nine patients with ventricular septal defect (VSD), one with atrial septal defect (ASD), and two with aortic stenosis were referred for evaluation of hemorrhagic symptoms or an abnormal preoperative hemostatic screening test. Seven of the 12 patients had a history of mucocutaneous bleeding (Table 1). Of these, four patients (3, 6, 8, and 11) had lifelong histories of bleeding; the remaining three were studied at the time of presentation of the symptoms listed in Table 1.
ABNORMAL vWF AND CARDIAC DEFECTS

Table 1. Factor VIII Molecular Complex in Congenital Cardiac Defects

<table>
<thead>
<tr>
<th>Patient</th>
<th>Cardiac Defect</th>
<th>Bleeding Symptoms</th>
<th>F VIII:C (U/dL)</th>
<th>vWF:Ag (U/dL)</th>
<th>vW:rist (U/dL)</th>
<th>vWF Subunits</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>VSD</td>
<td>Yes*</td>
<td>73</td>
<td>27</td>
<td>23</td>
<td>Abnormal</td>
</tr>
<tr>
<td>2</td>
<td>VSD</td>
<td>Yes*</td>
<td>132</td>
<td>140</td>
<td>118</td>
<td>Abnormal</td>
</tr>
<tr>
<td>3</td>
<td>VSD</td>
<td>Yes††</td>
<td>82</td>
<td>65</td>
<td>70</td>
<td>Abnormal</td>
</tr>
<tr>
<td>4</td>
<td>VSD</td>
<td>No</td>
<td>83</td>
<td>36</td>
<td>22</td>
<td>Abnormal</td>
</tr>
<tr>
<td>5</td>
<td>VSD (coarct)</td>
<td>Yes§</td>
<td>145</td>
<td>106</td>
<td>98</td>
<td>Abnormal</td>
</tr>
<tr>
<td>6</td>
<td>VSD</td>
<td>Yes††</td>
<td>49</td>
<td>37</td>
<td>22</td>
<td>Abnormal</td>
</tr>
<tr>
<td>7</td>
<td>ASD</td>
<td>No</td>
<td>94</td>
<td>45</td>
<td>47</td>
<td>Abnormal</td>
</tr>
<tr>
<td>8</td>
<td>VSD</td>
<td>Yes††</td>
<td>81</td>
<td>43</td>
<td>41</td>
<td>Abnormal</td>
</tr>
<tr>
<td>9</td>
<td>VSD</td>
<td>No</td>
<td>54</td>
<td>208</td>
<td>170</td>
<td>Abnormal</td>
</tr>
<tr>
<td>10</td>
<td>VSD (coarct)</td>
<td>No</td>
<td>118</td>
<td>82</td>
<td>62</td>
<td>Abnormal</td>
</tr>
<tr>
<td>11</td>
<td>Supravalvular aortic stenosis</td>
<td>Yes††</td>
<td>101</td>
<td>100</td>
<td>42</td>
<td>Abnormal</td>
</tr>
<tr>
<td>12</td>
<td>Aortic stenosis</td>
<td>No</td>
<td>47</td>
<td>42</td>
<td>32</td>
<td>Abnormal</td>
</tr>
</tbody>
</table>

*Excessive bleeding with laceration.
†Easy bruising or petechiae.
‡Frequent epistaxis.
§Bleeding from tooth extraction for three days.
||Hematoma at cardiac catheterization site.
†Gastrointestinal hemorrhage.

Evaluation of the factor VIII molecular complex (Table 1) revealed that all but two of the children had normal concentrations of plasma F VIII:C (50 to 150 U/dL); patients 6 and 12 had borderline abnormal values of 49 and 47 U/dL, respectively. However, analysis of vWF showed that six patients had reduced (<50 U/dL) concentrations of vWF measured both immunologically (vW:Ag) and by ristocetin cofactor assay (vW:rist). Patients 2, 4, 6, 9, 10, 11, and 12 had lower vW:rist values compared to vW:Ag, but there was good agreement between the immunologic and ristocetin cofactor assays in the remaining five patients. All 12 patients had loss of the largest mol wt subunits of vWF demonstrated by SDS-agarose electrophoresis (Fig 1). Low-dose (0.6 mg/mL) ristocetin-induced platelet aggregation was absent in two patients (4 and 8); others were not studied. Five patients were studied on two or three occasions over a period of 1 to 18 months prior to surgery. Each patient had repeatedly abnormal vWF multimeric analyses.

The routine coagulation studies were summarized in Table 2. Ten of the 12 patients had a prolonged bleeding time (>10 minutes); in no case was the long bleeding time explained by thrombocytopenia since all twelve patients had a normal or slightly elevated platelet count. With the exception of patient 7, who had a borderline low fibrinogen value, none of the patients had evidence of intravascular consumption of coagulation factors; none had an abnormal PT or thrombin time or elevation of fibrinogen degradation products. Three patients (10, 11, and 12) had a prolonged PTT explained by a decreased factor XII level (14, 44, and 41 U/dL, respectively).

To determine whether the abnormal vWF multimeric pattern was present in association with the cardiac anomaly, five patients were studied after surgical correction of the cardiac defect. Four patients (4, 6, 8, and 9) who had loss of the largest vWF multimers preoperatively had normalization of the multimeric pattern following repair (Fig 2). Although patients 4, 8, and 9 had normal vWF as measured by all methods including vWF multimers and patient 6 had only a mild decrease in both vW:Ag and vW:rist (43 and 45 U/dL, respectively), all four patients still have markedly prolonged bleeding times. Patient 4 had platelet aggregation studies both preoperatively and postoperatively; her platelets aggregated normally when the agonists epinephrine, collagen, arachidonate, and ADP were added to her platelet-rich plasma. The fifth child (patient 5) was studied initially 8 years after repair of her VSD and had abnormal vWF multimers; she also had a residual pressure gradient across a previous pulmonary artery banding site.

Further evidence for the acquired nature of the vWF anomaly was found by family studies. None of the parents available for study had evidence of vWF multimeric abnormalities. Two parents did have mildly decreased concentrations of vW:Ag (36 and 43 U/dL respectively).

To determine if these children were different from others with VSD or ASD who underwent catheterization and surgical repair during the same time period, cardiac catheterization findings were evaluated. Eight of nine patients with VSD had a sealed foramen ovale, and all had high-end
Table 2. Other Coagulation Studies

<table>
<thead>
<tr>
<th>Patient</th>
<th>Bleeding Time (mm)</th>
<th>Platelet Count (U/mm³)</th>
<th>PT (s)</th>
<th>PTT (s)</th>
<th>TT (s)</th>
<th>Fibrinogen (mg/dL)</th>
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</thead>
<tbody>
<tr>
<td>Normal</td>
<td>2.5–10.0</td>
<td>150,000–350,000</td>
<td>11.0–12.6</td>
<td>25.0–35.5</td>
<td>12.6–15.2</td>
<td>&lt;10</td>
</tr>
<tr>
<td>1</td>
<td>&gt;20.0</td>
<td>352,000</td>
<td>12.0</td>
<td>30.1</td>
<td>13.0</td>
<td>—</td>
</tr>
<tr>
<td>2</td>
<td>&gt;20.0</td>
<td>602,000</td>
<td>10.8</td>
<td>25.6</td>
<td>13.5</td>
<td>—</td>
</tr>
<tr>
<td>3</td>
<td>&gt;20.0</td>
<td>388,000</td>
<td>11.9</td>
<td>30.4</td>
<td>13.5</td>
<td>&lt;10</td>
</tr>
<tr>
<td>4</td>
<td>&gt;20.0</td>
<td>289,000</td>
<td>11.4</td>
<td>30.6</td>
<td>12.6</td>
<td>&lt;10</td>
</tr>
<tr>
<td>5</td>
<td>8.5</td>
<td>450,000</td>
<td>12.6</td>
<td>31.9</td>
<td>13.5</td>
<td>—</td>
</tr>
<tr>
<td>6</td>
<td>17.0</td>
<td>306,000</td>
<td>11.9</td>
<td>33.5</td>
<td>12.5</td>
<td>&lt;10</td>
</tr>
<tr>
<td>7</td>
<td>16.0</td>
<td>370,000</td>
<td>12.2</td>
<td>27.3</td>
<td>13.5</td>
<td>&lt;10</td>
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<tr>
<td>8</td>
<td>&gt;20.0</td>
<td>366,000</td>
<td>12.5</td>
<td>32.5</td>
<td>13.6</td>
<td>&lt;10</td>
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<td>9</td>
<td>&gt;20.0</td>
<td>312,000</td>
<td>11.9</td>
<td>33.3</td>
<td>13.5</td>
<td>—</td>
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<tr>
<td>&lt;10</td>
<td>19.5</td>
<td>363,000</td>
<td>12.7</td>
<td>40.2</td>
<td>—</td>
<td>&lt;10</td>
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<td>11</td>
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<td>244,000</td>
<td>12.1</td>
<td>36.2</td>
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<td>—</td>
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<tr>
<td>12</td>
<td>9.0</td>
<td>359,000</td>
<td>12.2</td>
<td>35.2*</td>
<td>14.4</td>
<td>&lt;10</td>
</tr>
</tbody>
</table>

*This PTT was outside the normal range established at the time of this study.

diastolic pulmonary artery pressure. Since other patients with cardiac defects may also present these features, it is unknown whether this finding is the only explanation for the vWF abnormalities.

One patient (6) was studied before and after infusion of cryoprecipitate. After administration of 1 donor unit/5 kg body weight, his vW:Ag rose from 37 to 60 U/dL and his vW:rist from 22 to 65 U/dL, and his abnormal multimeric pattern was almost completely corrected (Fig 2). His bleeding time, however, only partially corrected from a preinfusion value of 17 minutes to 12 minutes postinfusion. None of the patients had excessive intraoperative or postoperative bleeding, but all received large amounts of fresh frozen plasma and platelet concentrates during the postoperative period as part of the routine surgical management of open-heart cases. No increased use of such products was noted.

**DISCUSSION**

Von Willebrand factor is a plasma protein composed of large multimers of \( .4 \times 10^6 \) to \( 20 \times 10^6 \) daltons mol wt. Both inherited and acquired abnormalities of the protein have been described; patients with a vWF hypoproteinemia (decreased amounts of normal vWF) have von Willebrand disease of type I, and those with a dysproteinemia (abnormal vWF with normal or reduced amount of protein) have type II. Type II vWD is characterized by the absence of the large vWF multimers when analyzed by SDS-agarose electrophoresis. The absence of these hemostatically important multimers in type II vWD results in defective platelet adhesion and a clinically significant bleeding disorder.1,3

Acquired type II–like vWF abnormalities have been described in patients with episodic hemolytic uremic syndrome,4 DIC,5 myeloproliferative syndrome,6 and acute myocardial infarction,7 and we have seen this in one case of acquired type II vWD (Scott and Montgomery, unpublished observation). These patients exhibit an absence of the largest plasma vWF multimers and a discrepancy between vWF measurements by antigenic and functional assays. Our 12 patients with congenital cardiac defects and a discrepancy between vWF measurements by antigenic and functional assays. Our 12 patients with congenital cardiac defects also appear to have a form of acquired vWD; they have loss of the largest vWF multimers, long bleeding times, histories of mild hemorrhagic episodes, and, in one patient studied, a prolonged bleeding time that was partially corrected by the administration of cryoprecipitate. It is uncertain if all symptoms are related to the vWF defect, however, since four patients have residual hemostatic abnormalities (prolonged bleeding times) even though their vWF multimers have become normal following surgical repair of the cardiac defect. The acquired nature of the phenomenon is demonstrated by the
correction of the vWF defect with surgical correction of the cardiac defect in four of the patients studied both preoperatively and postoperatively and the lack of the abnormality in parents of the affected patients. The one patient with persistence of the abnormality after correction of her VSD had an abnormal pressure gradient across a previous pulmonary artery banding site: the vWF abnormality might be explained by persistence of an abnormal hemodynamic state in this patient.

The mechanism of loss of the largest vWF multimers associated with these congenital cardiac defects is unclear. Review of cardiac catheterization data in the 12 patients failed to reveal a specific characteristic that was different from those found in other children with similar cardiac lesions, with the exception that all but one of the patients with VSD had a sealed foramen ovale. Perhaps the abnormal hemodynamic flow is associated with activation of platelets or endothelial cells with resultant adsorption of the largest vWF multimers from the plasma. If adsorption of vWF to platelets is responsible for its absence from plasma, one might expect that the platelets would be agglutinated by low-dose ristocetin as is seen in type IIB vWD;2 this was not present in the two patients tested (patients 4 and 8), although the multimeric pattern on electrophoresis in SDS-agarose is more reminiscent of type IIB vWD than of type IIA, in which all of the high-mol wt multimers are absent, rather than only the largest as seen in these patients. Another possible mechanism for the phenomenon is cleavage of the vWF multimers into smaller forms by proteolytic enzymes (possibly by plasmin13-15 and/or the calcium-activated protease16 similar to that described in patients with DIC4,5. However, there was no evidence of DIC in these patients; all had a normal PT, thrombin time, and platelet count and, in the eight studied (patients 1, 3, 4, 6, 7, 8, 10, and 11), normal concentrations of fibrinogen with lack of fibrin(ogen) degradation products.

The three patients with prolonged PTTs had concurrent factor XII deficiency; none has as yet undergone surgical correction of their cardiac lesion. An association of factor XII deficiency with vWD has been reported previously,17,18 the significance in these patients is unknown.

The variety of hemostatic defects associated with congenital cardiac lesions manifested by thrombocytopenia, platelet dysfunction, and deficiency of clotting factors because of decreased synthesis, fibrinolysis, and DIC appears to be directly related to the hypoxia and hyperviscosity associated with cyanotic congenital heart disease.19 However, as described here, in 7 of 12 children with noncyanotic cardiac lesions, clinically significant bleeding histories were found. All 12 had an associated alteration in von Willebrand factor multimers. However, it is not clear that the von Willebrand factor abnormality contributed to the bleeding histories or prolonged bleeding times since the bleeding times were not corrected with normalization of vWF following corrective cardiac surgery. Further studies to elucidate the mechanism of this phenomenon and to determine its prevalence and clinical significance in VSD and other cardiac lesions are ongoing in our laboratory.

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REFERENCES

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