Acquired von Willebrand’s Disease Following Bone Marrow Transplantation*

JOHN LAZARCHICK, M.D. and CHRISTINE GREEN, M.D.

Department of Pathology/Laboratory Medicine, Medical University of South Carolina, Charleston, SC 29425

ABSTRACT

A 41-year-old male underwent allogeneic bone marrow transplantation for the treatment of acute myelogenous leukemia. Six months later, he was admitted to a hospital with signs and symptoms consistent with worsening chronic graft-vs-host disease. Despite a negative past history for a bleeding diathesis, the patient was found to have absent factor VIII procoagulant and ristocetin cofactor activities with markedly reduced von Willebrand factor antigen, all consistent with a diagnosis of acquired von Willebrand’s disease. Successful treatment of this disorder with aggressive apheresis and von Willebrand factor replacement therapy is noted.

Introduction

It is now a generally accepted practice to consider patients with leukemia who are not cured by conventional chemotherapy as suitable candidates for bone marrow transplantation. A major complication of allogeneic bone marrow transplantation, however, is the development of both acute and chronic graft-versus-host disease (GVHD) which is the major cause of morbidity and mortality following this procedure.1,2 In addition to the temporary immune deficiency state which exists following transplantation, several mechanisms have been proposed to contribute to the pathogenesis of this syndrome, including the development of circulating auto-antibodies. The latter have included rheumatoid factor, antinuclear, antismooth muscle, and antimitochondrial antibodies.3 Hematologic complications in the post transplant GVHD setting attributed to auto-antibody formation have included the development of immune thrombocytopenia with increased levels of platelet associated immunoglobulin, auto-immune hemolytic anemia and immune neutropenia.4,5

The first report is presented of acquired von Willebrand’s disease developing in a patient with leukemia following allogeneic bone marrow transplantation as another immune manifestation of GVHD.

Case Report

A 41-year-old black male was diagnosed with acute myelogenous leukemia on 06/09/91 and sub-
sequently underwent an allogeneic bone marrow transplantation on 10/09/91. Post transplantation he was felt to have low grade chronic graft-versus-host disease (GVHD) with complaints of chronic cough and rhinorrhea. He was readmitted on 04/03/92 with increasing hypoxia and interstitial changes on chest x-ray. Bronchoalveolar lavage was positive for acid-fast bacillus organisms, but cultures were negative at six weeks. Despite this, he was started on triple antibiotic coverage for tuberculosis and agangcyclovir for possible viral pneumonitis. His prothrombin time and activated partial thromboplastin time were within normal reference range. His hospital course was complicated by the development of abdominal pain, daily temperature spikes and a falling hematocrit.

On 04/15/92, blood cultures were positive for gram positive organisms identified as corynebacteria JK and Staphylococcus epidermitis. He continued to complain of abdominal discomfort and was noted to have deteriorating liver function tests, rising creatinine, and decreasing urine output, all of which were felt to be secondary to GVHD. A coagulation profile done at this time showed a prothrombin time of 11 secs but a prolonged activated partial thromboplastin time of 48.7 secs. His fibrinogen was 716 mg/dl, and his platelet count was 179 K/ul. The patient underwent a rectal biopsy on 04/12/92 which was complicated by brisk bleeding requiring three units of packed red cells, fresh frozen plasma, and platelets to achieve hemostasis. A coagulation study was performed which revealed a prothrombin time of 11 secs but a prolonged activated partial thromboplastin time of 46.1 secs with a mixing study showing immediate correction but prolongation at two hour incubation. His factor VIII activity was <1 percent with evidence of factor VIII inhibitor at 1.5 Bethesda units/ml. His bleeding time was >15 minutes. The rectal biopsy was interpreted as showing early GVHD. He was placed on steroids and factor VIII concentrate, cryoprecipitate, and platelets.

He then underwent a skin biopsy which resulted in profuse bleeding despite the replacement therapy. The skin biopsy was consistent with acute GVHD. His coagulation abnormality was evaluated further with findings of factor VIII activity of <1 percent, von Willebrand factor antigen of 1.6 percent, and a ristocetin cofactor activity of a < 6 percent, all consistent with a diagnosis of acquired von Willebrand disease. He received a two-day cycle of IVIg and was placed on 10 units of cryoprecipitate every six hours. This was changed to alternating doses of Humate P™ and cryoprecipitate which resulted in a ristocetin cofactor activity level of 49 percent. Nevertheless, the patient continued to bleed. Plasma exchange was initiated and repeated x2 over the next four days. Humate P™ replacement was tapered and eventually discontinued 05/13/92 with cessation of bleeding. Despite these efforts the patient showed evidence of progressive renal and hepatic failure, the latter being attributed to accelerated GVHD. He subsequently died on 05/15/92 with autopsy finding showing sequelae of multi-system organ failure.

Methods

Prothrombin time (PT), activated partial thromboplastin time (aPTT), and platelet count were performed using standard techniques. Factor VIII activity was measured in a one-stage method using factor VIII-deficient plasma as substrate. Ristocetin cofactor activity was measured by the method employing agglutination of lyophilized platelets in the presence of ristocetin. Von Willebrand factor antigen (vWF:Ag) was measured by an enzyme-linked immunabsorbant assay (ELISA). The von Willebrand factor multimer analyses was performed by sodium dodecyl sulfate electrophoresis with 1.5 percent agarose gel according to the method of Ruggeri and Zimmerman. All factor VIII parameters were expressed relative to pooled normal plasma (100 U/dl) obtained from 16 healthy donors. To test for an inhibitor to any of the factor VIII parameters, mixtures of the patient's plasma and normal pool plasma were prepared and incubated at 37° for two hours, and each parameter then assayed as previously described. Antibody titers to both factor VIII and ristocetin cofactor activity were expressed in Bethesda units.

Results

The coagulation profile over the course of this patient's hospitalization is outlined in table I. It should be noted that his PT and aPTT, determined shortly after admission, were within normal reference range. The first indication that he had developed a coagulopathy was noted following his rectal biopsy, at which time his aPTT was 48.7 secs. He had no measurable factor VIII activity, and a mixing study was compatible with the presence

* Behringwerke AG, distributed by Armour Pharmaceutical Company.
of an inhibitor. Unfortunately, since von Willebrand factor antigen and ristocetin cofactor activity were not measured at this time, it was assumed the patient had developed an anti-factor VIII antibody.

Despite attempts at correction with fresh frozen plasma, cryoprecipitate, and commercial factor VIII concentrate, his aPTT remained prolonged. Reevaluation of the abnormalities several days later noted not only absent factor VIII activity but markedly diminished von Willebrand factor antigen and ristocetin cofactor activity. In addition, it was noted his bleeding time was prolonged. A diagnosis of acquired von Willebrand’s disease was made with an antibody titer of 2 Bethesda units. He was then treated with IVIg, prednisone, cryoprecipitate, and Humate P™ with transient improvement but incomplete correction of his aPTT. A combination of plasmapheresis followed by Humate P™ therapy over a five day course resulted in a complete correction of his aPTT and normalization of his factor VIII parameters. His dose of Humate P™ was then tapered over the next several days and finally stopped with his ability to maintain a measurable factor VIII level without additional therapy.

The probability that the patient had developed acquired von Willebrand’s disease syndrome was confirmed with the mixing studies using pool normal plasma and the patient’s plasma and then measuring residual factor VIII parameters on the mixture. The results of these experiments are shown in table II. There was a marked inhibition of both factor VIII and ristocetin cofactor activity with essentially no residual activity of either parameter measurable on a 1:1 dilution of the patient’s plasma and pool normal plasma. In contrast, von Willebrand factor antigen was 100 percent recoverable in this mixture. Similar inhibitory activity was noted when the patient serum was used in this type of experiment.

Von Willebrand factor multimer analysis was performed on several patient samples obtained pre and post apheresis/Humate P™ therapy. The patient’s initial
### TABLE II

<table>
<thead>
<tr>
<th>Mixture</th>
<th>Percent Recovery</th>
<th></th>
<th>Ristocetin</th>
<th></th>
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<tr>
<td></td>
<td></td>
<td>VIII:C</td>
<td>Cofactor</td>
<td>WF:Ag</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(u/dl)</td>
<td>(u/dl)</td>
<td>(u/dl)</td>
<td></td>
</tr>
<tr>
<td>Patient plasma</td>
<td>&lt;1 (50)</td>
<td>&lt; 6 (50)</td>
<td>52:1 (50.8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(1) + PNP (1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Buffer (1) + PNP (1)</td>
<td>48 (50)</td>
<td>55 (50)</td>
<td>50 (50)</td>
<td></td>
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</tbody>
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The patient plasma used in this experiment had a VIII:C of < 1 u/dl, vWF:Ag of 1.6 u/dl, and ristocetin cofactor activity of < 6 u/dl. The numbers in parentheses reflect expected values for each parameter in the absence of an inhibitor.

Sample (<1 percent factor VIII:C, <6 percent vWF ristocetin cofactor activity, 2.7 percent vWF antigen) showed essentially the complete absence of all multimers consistent with severe von Willebrand’s disease. Samples obtained following apheresis and Humate P™ infusion at times when there was measurable levels of both von Willebrand factor antigen and ristocetin cofactor activity revealed normal multimeric patterns (figure not shown).

**Discussion**

The patient’s acute development of a bleeding diathesis associated with a marked deficiency of all factor VIII parameters is consistent with a diagnosis of acquired von Willebrand’s disease. That this was not acquired hemophilia A as was initially thought based on limited studies reinforces the need to assess all components of the factor VIII complex prior to making diagnostic and, therefore, therapeutic decisions. The demonstration in this patient of marked decrease of von Willebrand factor antigen and ristocetin cofactor activity in association with absent factor VIII procoagulant activity suggests an inhibitor being directed at an epitope on the von Willebrand protein with indirect inhibition of factor VIII procoagulant activity. This distinction is critical, since the effectiveness of therapy for each of these disorders requires specific sources of factor VIII replacement therapy.

In general, the approaches to treatment of patient’s with anti-von Willebrand factor inhibitor include overwhelming the inhibitor with infusion of excess von Willebrand factor, removal of the inhibitor with apheresis, or a combination of both as acute therapy. Immunosuppression of antibody production can be initiated simultaneously, but its utility in acute therapy of this disorder is limited. Initial attempts to overwhelm the inhibitor with infusion of commercial factor VIII concentrate preparations resulted in no detectable factor VIII response.

Commercial factor VIII concentrates are primarily of use as a factor VIII procoagulant replacement source; although some von Willebrand factor activity is present, most of the ristocetin cofactor is lost in the processing techniques. There are, however, two commercial concentrates on the market which retain ristocetin cofactor activity.†‡ The combination of inhibitor removal by aggressive apheresis followed by the infusion of Humate P™ over a five day period resulted in normalization of the patient’s von Willebrand activity and cessation of bleeding. Correction of all factor VIII parameters was evident by the sixth day using this form of therapy. The possible role of the single course infusion of intravenous immunoglobulin (IVIg) in the disappearance of the inhibitor is uncertain, but it is unlikely to have had any ameliorating effect given the time course of response.

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† Humate P™, American Hoechst Corporation, Behring Diagnostics Division, La Jolla, CA 92037.
‡ Koate HP, Miles Diagnostics, Elkhart, IN 46515.
and the limited amount of IVIg infused. Despite the successful treatment of this disorder with normalization of all factor VIII parameters, other manifestations of chronic GVHD resulted in multi-organ failure and death.

The pathogenesis of the von Willebrand factor inhibitor in this patient is speculative. The development of von Willebrand’s disease in association with autoimmune or lymphoproliferative disorders is well documented. Chronic GVHD has many characteristics of an autoimmune disorder, and experimental studies support the autoimmune etiopathogenesis. In addition to a variety of serologic abnormalities, patients with chronic GVHD can develop immune cytopenias, hypergammaglobulinemia, and circulating immune complexes. It is thus not unreasonable to assume the occurrence of an inhibitor to von Willebrand factor in this setting is an additional manifestation of an autoimmune manifestation of chronic GVHD.

References