Posttransplantation Thrombotic Thrombocytopenic Purpura: A Single-Center Experience and a Contemporary Review

MICHELLE A. ELLIOTT, MD; WILLIAM L. NICHOLS, JR, MD; ELIZABETH A. PLUMHOFF, MD; STEPHEN M. ANSELL, MD, PhD; ANGELA DISPENZIERI, MD; DENNIS A. GASTINEAU, MD; MORIE A. GERTZ, MD; DAVID J. INWARDS, MD; MARTHA Q. LACY, MD; IVANA N. M. MICALLEF, MD; AYALEW TEFFERI, MD; AND MARK R. LITZOW, MD

Objective: To assess the activity of von Willebrand factor–cleaving protease (vWF-CP) in patients with thrombotic thrombocytopenic purpura (TTP) complicating bone marrow transplantation (BMT) and peripheral blood stem cell transplantation (PBSCT).

Patients and Methods: From March 1, 1999, to June 30, 2001, allogeneic and autologous hematopoietic stem cell transplantation was performed in 118 and 400 patients, respectively. We reviewed risk factors for development of posttransplantation TTP and measured vWF-CP activity during active TTP in 10 recipients.

Results: The incidence of TTP after allogeneic and autologous transplantation was 6.8% (8/118) and 0.25% (1/400), respectively. Among the allogeneic transplant recipients, the incidence of TTP after nonmyeloablative (NMA) PBSCT, matched unrelated donor BMT, and sibling BMT or PBSCT was 15.4% (2/13), 11.8% (2/17), and 4.5% (4/88), respectively. Of the 10 patients with TTP, 9 (90%) had received extensive prior therapy, including autologous transplantation in both NMA recipients. Acute graft-vs-host disease (GVHD) prophylaxis consisted of cyclosporine and methotrexate in most affected patients. The vWF antigen level was elevated in all patients, and no patients showed evidence of vWF-CP deficiency. During active TTP, 6 patients had grade II-IV acute GVHD, 1 had extensive chronic GVHD, and 4 had cytomegalovirus viremia. Risk factor analysis for development of TTP showed that transplant type (NMA and matched unrelated donor) and source of stem cells (bone marrow vs peripheral blood stem cell) were significant.

Conclusions: Posttransplantation TTP was not found to be associated with severe vWF-CP deficiency. The elevated levels of vWF antigen are consistent with diffuse endothelial injury likely because of multiple interacting factors such as extensive prior therapy, GVHD, cyclosporine, and reactivation of cytomegalovirus. The disorder appears to be more frequent among patients with, or at risk for, acute GVHD, suggesting a possible role in the pathogenesis. Nonmyeloablative transplantation does not appear to confer a lesser risk, possibly for these reasons.


Thrombotic thrombocytopenic purpura (TTP) is a multisystemic disorder characterized by thrombocytopenia, microangiopathic hemolytic anemia, and ischemic manifestations resulting from platelet agglutination in the arterial microvasculature.1 Thrombotic thrombocytopenic purpura has been hypothesized to be the result of platelet-agglutinating agents in the circulation and/or endothelial cell injury.2 Widespread formation of microthrombi consisting of platelets and von Willebrand factor (vWF) results in consumptive thrombocytopenia and end-organ ischemia with intravascular hemolysis due to fragmentation of red blood cells as they traverse partially occluded arterioles and capillaries.3 Unusually large vWF multimers, similar to those stored in vascular endothelial cells but not present in normal plasma, are demonstrable in plasma samples of some patients with TTP. These highly adhesive forms have been causally implicated in pathologic platelet agglutination.4 Recent evidence suggests that severe deficiency of a specific plasma protease, vWF-cleaving protease (vWF-CP), responsible for the physiological cleavage of vWF, plays a causative role in a large number of patients with congenital and idiopathic TTP.5,7 An inhibiting autoantibody to vWF-CP has been detected in most patients with idiopathic TTP.8,9 Although most cases of TTP are idiopathic, several etiologic associations are well recognized, including infection, pregnancy, drugs, and bone marrow transplantation (BMT).10 Plasma exchange (PE) has...
been effective therapy primarily in idiopathic TTP but not in all variants of TTP.

Thrombotic thrombocytopenic purpura is a recognized, often devastating complication of BMT, and multiple contributing pathogenic factors have been implicated. These include endothelial cell injury due to toxic conditioning regimens (high-dose chemotherapy and total-body irradiation [TBI]), cytomegalovirus (CMV) infection, the use of cyclosporine, and a possible graft-vs-host effect on the endothelium. Because anemia, thrombotic thrombocytopenia, renal impairment, and changes in mental status are common and may have multiple causes in the transplant population, diagnosis can be difficult, possibly contributing to the wide range in reported incidence (Table 1). 

In their often-cited review, Pettitt and Clark estimated the frequency of severe TTP to be 0.5% and 0.13% of allogeneic and autologous recipients, respectively. This varying incidence of TTP after stem cell transplantation among reported series likely reflects the level of physician awareness, the different diagnostic criteria, and the heterogeneity of the transplant populations described.

We reviewed our recent experience of, and risk factors for, development of posttransplantation TTP from March 1, 1999, through June 30, 2001. During this period, a vWF-CP assay was available and allowed us to assess the activity of this protease in this patient population.

**PATIENTS AND METHODS**

This is a retrospective, uncontrolled single-institution analysis of posttransplantation TTP. Thrombotic thrombocytopenic purpura occurring after allogeneic or autologous hematopoietic stem cell transplantation was diagnosed using the clinical and laboratory criteria established for TTP (Table 1). Patients were diagnosed as having TTP if they had thrombocytopenia (platelet count <100,000/mL), microangiopathic hemolytic anemia (Hb <9 g/dL), and evidence of organ dysfunction (e.g., renal impairment) with no other identifiable cause of these findings. Disease severity was graded according to the criteria of James and colleagues: grade I, anemia and thrombocytopenia without organ dysfunction; grade II, anemia and thrombocytopenia with renal impairment; and grade III, anemia and thrombocytopenia with renal impairment and organ dysfunction.

**Table 1. Summary of Recent Publications on Posttransplantation Thrombotic Thrombocytopenic Purpura**

<table>
<thead>
<tr>
<th>Study (date of Tx)</th>
<th>No. (%) of patients</th>
<th>Incidence by Tx type</th>
<th>TTP dx post-Tx, median (range) (d)</th>
<th>Cs Pro</th>
<th>TBI</th>
<th>Acute GVHD</th>
<th>Risk factors</th>
<th>PE, No. and response rate</th>
<th>Mortality (all-cause) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Roy et al11</td>
<td>17/748 (2.3)</td>
<td>17/262 (6.5) Sib, 5/155 (3.2) MUD, 12/107 (11.2)</td>
<td>0/486 (0)</td>
<td>43 (20-99)</td>
<td>17/17</td>
<td>12/17</td>
<td>III-IV, 8/17</td>
<td>Female, MUD, HLA-mismatch, infection, acute GVHD III-IV</td>
<td>17/17; RR=18%</td>
</tr>
<tr>
<td>Paquette et al18</td>
<td>7/409 (1.7)</td>
<td>7/409 (1.7) Sib, 3/341 (0.9) MUD, 4/68 (5.9)</td>
<td>0 (0)</td>
<td>71 (47-105)</td>
<td>7/7</td>
<td>5/7</td>
<td>≥II, 5/7</td>
<td>MUD, Cs/pred/MTX</td>
<td>7/7; RR=0</td>
</tr>
<tr>
<td>Iacopino et al19</td>
<td>12/4334 (0.28)</td>
<td>9/1759 (0.5) Sib, 5/9 MUD, 2/9 HLA-mismatch, 2/9</td>
<td>3/2575 (0.1)</td>
<td>77 (14-240)</td>
<td>8/9</td>
<td>9/12</td>
<td>II, 2/9</td>
<td>MUD, HLA-mismatch</td>
<td>6/12; RR=17%</td>
</tr>
<tr>
<td>Fuge et al10</td>
<td>22/544 (4)</td>
<td>22/456 (4.8) MUD, 20/332 (6) Sib, 2/124 (1.6)</td>
<td>0/8 (0)</td>
<td>58 (3-657)</td>
<td>22/22; T-cell depletion, 19/22</td>
<td>20/22</td>
<td>≥II, 12/22</td>
<td>All: age, female MUD, age, female, acute GVHD</td>
<td>17/22; RR=35%</td>
</tr>
<tr>
<td>Sarode et al11</td>
<td>9/9</td>
<td>9/9 MUD, 6/9</td>
<td>0 (0)</td>
<td>120 (45-365)</td>
<td>9/9</td>
<td>9/9</td>
<td>≥II, 5/9</td>
<td>MUD</td>
<td>8/9; RR=38%</td>
</tr>
<tr>
<td>Elliott et al</td>
<td>9/518 (1.7)</td>
<td>8/118 (6.8) Sib, 4/70 (5.7) MUD, 2/17 (11.8) NMA, 2/13 (15.4)</td>
<td>1/400 (0.3)</td>
<td>44 (16-240)</td>
<td>8/8</td>
<td>6/10</td>
<td>≥II, 6/8</td>
<td>BMT (vs PBSCT) MUD, NMA</td>
<td>10/10; RR=90%</td>
</tr>
</tbody>
</table>

*All values represent number (percentage) unless indicated otherwise. Allo = allogeneic; Auto = autologous; BMT = bone marrow transplantation; Cs/pred/MTX = cyclosporine/prednisone/methotrexate; Cs Pro = cyclosporine prophylaxis; dx = diagnosis; GVHD = graft-vs-host disease; Haplo = haploidentical related; MUD = matched unrelated donor; NMA = nonmyeloablative; PAI = protein A immunoadsorption; PBSCT = peripheral blood stem cell transplantation; PE = plasma exchange; RR = response rate; Sib = HLA-matched sibling; TBI = total-body irradiation; TTP = thrombotic thrombocytopenic purpura; Tx = transplantation.

1Cs treatment discontinued in all patients after TTP diagnosis except for 1 patient in the series of Paquette et al.18

2Acute GVHD = grade ≥II.

3Three patients also received PAI.

4One patient also received PAI.
nosed in 10 patients and treated with PE at our institution from March 1, 1999, through June 30, 2001. The diagnosis of TTP was made on the basis of characteristic clinical features, including thrombocytopenia (platelet count, <100 × 10^9/L), microangiopathic hemolytic anemia (identified by fragmented red blood cells on peripheral blood smear, absent plasma haptoglobins, and elevated lactate dehydrogenase [LDH]), and the absence of disseminated intravascular coagulation or other causes of such findings.

**Conditioning Regimens**

Conditioning for the allogeneic recipients included cyclophosphamide/TBI (n=5) and BCNU (carmustine), etoposide, cytarabine, cyclophosphamide (BEAC) (n=1). Two nonmyeloablative (NMA) regimens consisted of fludarabine/melphalan and 2-chloro-deoxyadenosine/thiotepa. Autologous conditioning regimens included 1 patient treated with etoposide/thiotepa/cyclophosphamide and 1 patient treated with 166 Ho-DOTMP/melphalan. Autologous conditioning regimens included 1 patient treated with etoposide/thiotepa/cyclophosphamide and 1 patient treated with 166 Ho-DOTMP/melphalan.

**Graft-vs-Host Disease Prophylaxis and Treatment**

Graft-vs-host disease (GVHD) prophylaxis consisted of cyclosporine and short-course methotrexate (MTX) in most patients (7 of 8 allogeneic transplant recipients). Initial oral-dose adjustments were based on subsequent blood levels (therapeutic trough level, 100-300 ng/mL). For GVHD treatment, patients experiencing grade I GVHD with mild involvement of the skin were treated initially with topical corticosteroids. Patients with more extensive skin involvement or with higher grades of GVHD received 1 to 2 mg · kg⁻¹ · d⁻¹ of methylprednisolone in combination with therapeutic doses of cyclosporine.

**Supportive Care**

Standard institutional supportive care guidelines were followed in all patients, which included prophylaxis against bacterial, fungal, Pneumocystis carinii, CMV, and herpes simplex virus infections.

**vWF-CP Assay**

Patient plasma or serum samples were diluted 20-fold in activation buffer (Tris-buffered saline, pH 7.4, containing a serine protease inhibitor, Pefabloc SC [4-(2-aminoethyl)benzenesulfonfyl-fluoride, hydrochloride] and 10 mmol/L barium chloride) and placed in a 37°C water bath for 10 minutes. A patient sample (100 µL) was incubated with 50 µL of protease-free, purified human vWF (27.3 µg/mL). The protease reaction occurred on the surface of a 25-mm filter membrane (VSWP2500, Millipore Corp, Bedford, Mass) floating on 25 mL of denaturing buffer (0.005 mol/L Tris-HCl/1.5 mol/L urea, pH 8) in a small container at 37°C. The protease reaction was allowed to proceed for 24 hours and was then terminated by the addition of EDTA (10 µL 0.2 mol/L, pH 7.4). To assess for inhibition, mixing studies were performed (1:1 patient plasma to normal pool plasma) by using 100 µL of each plasma incubated at 37°C for 15 to 30 minutes and then diluted at 1:20 in activation buffer. Subsequent processing was performed by using the following 2 alternate methods to visualize vWF-CP–mediated vWF cleavage.

**Standard Analytic Method.**—After denaturation, the sample was electrophoresed according to our laboratory’s standard vWF multimer protocol with 2% sodium dodecyl sulfate–agarose gel electrophoresis (Figure 1). This procedure has a turnaround time of 5 to 7 days.

**Modified Analytic Method.**—We adapted previously published methods to assess vWF-CP activity using the Pharmacia PhastSystem system with sodium dodecyl sulfate–polyacrylamide gel electrophoresis of vWF (Figure 2). Samples were denatured and electrophoresed according to the manufacturer’s directions using the PhastSystem and nonreduced 4% to 15% gradient polyacrylamide gels. The gel was press-blotted onto a polyvinylidene difluoride membrane (Millipore Immobilon-P, 9 × 12 cm, Sigma #P2438) overnight and then blocked (membranes/gels soaked on rocking platform in about 50 mL of blocking buffer of 5% nonfat dry milk/0.05% Tween 20/Tris-buffered saline for 10-15 minutes). Gels were then removed from the membranes, which were placed in blocking buffer for 60 minutes, washed, and then probed using diluted (1:22,500) ECL chemiluminescent reagents by autoradiography (Figure 2). This procedure has a turnaround time of 2 days.

For vWF-CP assay controls, normal pooled plasma and samples of patients with idiopathic TTP and a deficiency of vWF-CP activity diagnosed during the study period were examined simultaneously with all study patient plasma assays to ensure proper functioning of the vWF-CP assay with each run (Figures 1 and 2).

**vWF Antigen and Ristocetin Cofactor Activity**

Both assays were performed by using previously published methods.

**Therapeutic PE**

Therapeutic PE was performed with cryoprecipitate-poor plasma for a calculated single plasma volume exchange by using a continuous-flow centrifugation apheresis system.
Statistical Analyses

During the study period, allogeneic or autologous hematopoietic stem cell transplantation was performed in 118 and 400 patients, respectively. Data on those patients in whom TTP did not develop after transplantation were analyzed to identify possible risk factors for the development of this syndrome. Because only 1 patient of this cohort developed TTP among the 400 autologous recipients, comparative analysis was restricted to allogeneic transplant recipients. In the other autologous case, transplantation was performed at a hospital elsewhere; therefore, this patient was not eligible for inclusion in this analysis. The 8 allogeneic transplant recipients with TTP were compared with the 110 allogeneic hematopoietic stem cell recipients in whom TTP did not develop. Variables analyzed included age, sex, type of transplant, source of stem cells, conditioning regimen, use of TBI, and the number of transplants. Clinical parameters were statistically compared between the 2 groups by using $\chi^2$ tests and the Fisher exact test. Logistical fit analysis was used for some variables.

RESULTS

Post-BMT and peripheral blood stem cell transplantation (PBSCT) TTP were diagnosed in 10 patients and treated at our institution during the study period. The baseline clinical characteristics of these patients are presented in Table 2. All but 1 patient (who was conditioned with $^{166}$Ho-DOTMP/melphalan and underwent transplantation at another center) underwent transplantation at our institution, where the incidence of TTP after allogeneic and autologous transplantation was 6.8% (8/118) and 0.25% (1/400), respectively. Among allogeneic transplant recipients, the incidence of TTP after related NMA-PBSCT, matched unrelated donor (MUD) BMT, and sibling (BMT and PBSCT) recipients was 15.4% (2/13), 11.8% (2/17), and 4.5% (4/88), respectively. In the sibling group, 18 received haploidentical CD34+ peripheral blood stem cell–selected and T-cell–depleted allografts. If these haploidentical transplant recipients are excluded, the prevalence of TTP in HLA-identical sibling transplants was 5.7% (4/70). Among allogeneic HLA-identical sibling transplant recipients, TTP was diagnosed in 4 patients among 25 BMT recipients (16%) compared with no patients among the contemporary cohort of 45 PBSCT recipients.

The median age of the 10 patients at the time of TTP diagnosis was 39.5 years (range, 14-61 years), and 8 (80%) were male. The indications for transplantation were refractory non-Hodgkin lymphoma (n=4), high-risk myelodysplasia or secondary acute myeloid leukemia (n=4), refractory chronic lymphocytic leukemia (n=1), and multiple myeloma (n=1). Of the 10 patients, 9 (90%) had received extensive prior therapy, including previous autologous transplantation in both NMA transplant recipients; 7 (70%) (5 of 8 allogeneic and both autologous transplant recipients) were at risk for CMV disease (donor or recipient seropositive).
Table 2. Clinical Data for Post-BMT and PBSCT TTP Patients*

<table>
<thead>
<tr>
<th>Patient No./age (y)/sex</th>
<th>Donor sex</th>
<th>Transplant type</th>
<th>Disease</th>
<th>Conditioning</th>
<th>GVHD prophylaxis</th>
<th>Cs (ng/mL)</th>
<th>CMV</th>
<th>Engraftment</th>
<th>Donor</th>
<th>ANC†</th>
<th>PLT‡</th>
<th>Acute GVHD grade</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/44/M M</td>
<td>Sib</td>
<td>BMT</td>
<td>CLL</td>
<td>CY/TBI</td>
<td>Cs/MTX</td>
<td>270</td>
<td>218</td>
<td>Pos</td>
<td>Neg</td>
<td>Day 21</td>
<td>Day 19</td>
<td>0</td>
</tr>
<tr>
<td>2/35/M M</td>
<td>Sib</td>
<td>BMT</td>
<td>CLL</td>
<td>BEAC</td>
<td>Cs/MTX</td>
<td>278</td>
<td>197</td>
<td>Neg</td>
<td>Neg</td>
<td>Day 23</td>
<td>ND</td>
<td>III-IV</td>
</tr>
<tr>
<td>3/55/M F</td>
<td>Sib</td>
<td>BMT</td>
<td>s-AML</td>
<td>CY/TBI</td>
<td>Cs/MTX</td>
<td>332</td>
<td>307</td>
<td>Neg</td>
<td>Neg</td>
<td>Day 19</td>
<td>Day 19</td>
<td>III-IV</td>
</tr>
<tr>
<td>4/29/M F</td>
<td>Sib</td>
<td>BMT</td>
<td>NHL</td>
<td>CY/TBI</td>
<td>Cs/MTX</td>
<td>1467</td>
<td>451</td>
<td>Pos</td>
<td>Pos</td>
<td>Day 21</td>
<td>Day 27</td>
<td>0</td>
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<tr>
<td>5/33/M F</td>
<td>MUD-BMT</td>
<td>NHL</td>
<td>s-AML</td>
<td>CY/TBI</td>
<td>Cs/MTX</td>
<td>311</td>
<td>203</td>
<td>Pos</td>
<td>Pos</td>
<td>Day 14</td>
<td>ND</td>
<td>III-IV</td>
</tr>
<tr>
<td>6/26/M F</td>
<td>MUD-BMT</td>
<td>MDS</td>
<td>CY/TBI</td>
<td>Cs/MTX</td>
<td>MDS</td>
<td>435</td>
<td>212</td>
<td>Neg</td>
<td>Pos</td>
<td>Day 20</td>
<td>Day 21</td>
<td>III-IV</td>
</tr>
<tr>
<td>7/61/M F</td>
<td>NMA-PSCT</td>
<td>t-MDS</td>
<td>Ara-F/M</td>
<td>Cs/TBI</td>
<td>t-MDS</td>
<td>296</td>
<td>255</td>
<td>Pos</td>
<td>Neg</td>
<td>Day 19</td>
<td>Day 18</td>
<td>III-IV</td>
</tr>
<tr>
<td>8/27/F M</td>
<td>NMA-PSCT</td>
<td>NHL</td>
<td>2-CDA, TT</td>
<td>Cs</td>
<td>NMA-PSCT</td>
<td>383</td>
<td>247</td>
<td>Pos</td>
<td>Pos</td>
<td>Day 10</td>
<td>ND</td>
<td>II</td>
</tr>
<tr>
<td>9/52/F NA</td>
<td>APBSCT</td>
<td>MM</td>
<td>166 Ho-DOTMP</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
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<td>Day 20</td>
<td>Day 30</td>
<td>NA</td>
</tr>
<tr>
<td>10/14/M NA</td>
<td>APBSCT</td>
<td>NHL</td>
<td>VP-16/T/T/CY</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>Day 16</td>
<td>ND</td>
<td>NA</td>
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</table>

*APBSCT = autologous peripheral blood stem cell transplant; ANC = absolute neutrophil count; Ara-F/M = fludarabine/melphalan; BEAC = BCNU (carmustine), etoposide, cyclophosphamide; BMT = bone marrow transplantation; 2-CDA = 2-chloro-deoxyadenosine; CLL = chronic lymphocytic leukemia; CMV = cytomegalovirus; Cs = cyclosporine; CY = cyclophosphamide; GVHD = graft-vs-host disease; 166 Ho-DOTMP = 166 Ho-1,4,7,10-tetraazacyclododecane-1,4,7,10-tetramethylene-phosphonic acid; MDS = myelodysplastic syndrome; MM = multiple myeloma; MTX = methotrexate; MUD = matched unrelated donor; NA = not applicable; ND = not demonstrable; NHL = non-Hodgkin lymphoma; Neg = negative; NMA-PSCT = nonmyeloablative sibling PBSCT (peripheral blood stem cell transplantation); PLT = platelet count; Pos = positive; s-AML = secondary acute myeloid leukemia; Sib = sibling; TBI = total-body irradiation; t-MDS = therapy-related myelodysplastic syndrome; TT = thiopeta; TTP = thrombotic thrombocytopenic purpura; VP-16 = etoposide.

† First day after transplantation that the ANC was 0.5 × 10⁹/L for 3 consecutive days.
‡ First day after transplantation that the PLT was >20 × 10⁹/L for 7 days without transfusion.

Clinical Features at Diagnosis of TTP

The clinical and laboratory features at the time of TTP diagnosis are presented in Table 3. Thrombotic thrombocytopenic purpura was diagnosed at a median of 44 days (range, 16-239 days) posttransplantation. One patient, diagnosed as having TTP 134 days after transplantation, received donor lymphocyte infusion 60 days before the diagnosis of TTP for cytogenetic evidence of persistent acute myeloid leukemia that was complicated by severe acute GVHD. All patients met the diagnostic criteria for TTP (thrombocytopenia, microangiopathic hemolytic anemia without disseminated intravascular coagulation, and increased serum LDH). Various isolated and concomitant changes in mental status, including confusion (n=3), grand...
Table 3. Clinical and Laboratory Features at Time of Diagnosis of Thrombotic Thrombocytopenic Purpura*

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>TTP dx (d†)</th>
<th>Hb (g/dL)</th>
<th>Plt (x 10^9/L)</th>
<th>LDH (U/L)</th>
<th>Cr (mg/dL)</th>
<th>vWF-Ag (%)</th>
<th>vWF-CP activity</th>
<th>vWF multimers</th>
<th>Acute GVHD grade</th>
<th>CMV viremia</th>
<th>PE</th>
<th>Survival post-TTP (d)</th>
<th>Status</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>41</td>
<td>9.7</td>
<td>76</td>
<td>276</td>
<td>2.2</td>
<td>108</td>
<td>Normal</td>
<td>Normal</td>
<td>None</td>
<td>Yes</td>
<td>4</td>
<td>Partial</td>
<td>&gt;689</td>
</tr>
<tr>
<td>2</td>
<td>30</td>
<td>9.1</td>
<td>11</td>
<td>1185</td>
<td>1.7</td>
<td>246</td>
<td>Normal</td>
<td>Normal</td>
<td>III-IV</td>
<td>No</td>
<td>30</td>
<td>Complete</td>
<td>&gt;351</td>
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<tr>
<td>3</td>
<td>134‡</td>
<td>7.5</td>
<td>18</td>
<td>368</td>
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<td>ND</td>
<td>III-IV</td>
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<td>6</td>
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<tr>
<td>4</td>
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<td>9.7</td>
<td>18</td>
<td>335</td>
<td>1.3</td>
<td>334</td>
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<td>Normal</td>
<td>Chronic GVHD</td>
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<td>14</td>
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<td>5</td>
<td>24</td>
<td>9.8</td>
<td>8</td>
<td>635</td>
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<td>230</td>
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<td>III-IV</td>
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<td>67</td>
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<td>479</td>
<td>1.4</td>
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<td>Unusually large</td>
<td>III-IV</td>
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<td>7</td>
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<td>11</td>
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<td>47</td>
<td>7.4</td>
<td>52</td>
<td>1025</td>
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<td>136</td>
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<td>Unusually large</td>
<td>Yes</td>
<td>10</td>
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*Ag = antigen; CMV = cytomegalovirus; Cr = creatinine; dx = diagnosis; GVHD = graft-vs-host disease; Hb = hemoglobin; LDH = lactate dehydrogenase (reference range, 91-198 U/L); NA = not applicable; ND = not determined; PE = plasma exchange; Plt = platelets; TTP = thrombotic thrombocytopenic purpura; vWF = von Willebrand factor; vWF-CP = vWF-cleaving protease.
†Number of days after transplantation that TTP was diagnosed.
‡Received donor lymphocyte infusion 60 days before diagnosis of TTP.

Thrombotic Thrombocytopenic Purpura

- Mal seizures (n=2), focal neurologic deficits (n=2), and somnolence (n=4), occurred in 8 patients (80%), and 9 (90%) had renal insufficiency. All patients had achieved engraftment (defined as an absolute neutrophil count >0.5 × 10^9/L for 3 consecutive days) before the diagnosis of TTP was established. Engraftment occurred in all patients at a median of 19 days (range, 10-23 days) after transplantation. Platelet engraftment was not demonstrable in 4 patients in whom the development of TTP preceded platelet engraftment on posttransplantation days 16, 24, 30, and 31.

- The median vWF antigen level was increased at 290% (range, 108%-565%; reference range, 55%-200%); vWF multimeric analysis revealed unusually large vWF multimers in 3 of the 10 patients and normal results in the remainder. Severe vWF-CP deficiency was not found in any of the patients. The vWF-CP activity in samples obtained during active TTP, and before PE was initiated, appeared normal in all patients, as shown by normal vWF proteolysis after incubation of the activated protease with purified vWF substrate (Figures 1 and 2).

- All allogeneic recipients were receiving cyclosporine for GVHD prophylaxis or therapy at the time TTP was diagnosed. The median peak cyclosporine blood level before TTP diagnosis was 321.5 ng/mL (range, 270-1467 ng/mL), and the median of the mean cyclosporine levels during the 2-week period preceding the diagnosis of TTP was 229.5 ng/mL (range, 197-451 ng/mL). These values should be interpreted in the context of therapeutic cyclosporine trough levels of 100 to 300 ng/mL. During active TTP, 6 of 8 allogeneic recipients had grade II-IV acute GVHD (grade III-IV in 5 of 8), and 1 had extensive chronic GVHD. During this time, 4 of the 10 patients, including 1 autologous recipient, had CMV viremia, and 2 allogeneic recipients had severe herpes simplex virus stomatitis. Although most patients were receiving broad-spectrum antimicrobials for prior or suspected infection, bacterial and fungal blood cultures were negative in the immediate period preceding or following the diagnosis of TTP.

Therapy for TTP

Cyclosporine was discontinued in all allogeneic recipients at TTP diagnosis, and alternative immunosuppression was continued with corticosteroids alone (n=1), tacrolimus alone (n=1), or both agents combined (n=6). Tacrolimus was discontinued because of adverse renal effects and neurotoxicity in 2 of 7 patients but did not exacerbate other parameters of thrombotic microangiopathy. Of the 10 patients, 9 (90%) received corticosteroids for various indications, including TTP itself, GVHD, or, in 2 allogeneic patients, as an ongoing taper of high-dose methylprednisolone therapy administered for diffuse alveolar hemorrhage, which had occurred 6 and 14 days before TTP diagnosis. Plasma exchange was performed in all patients for a median of 7 exchange procedures (range, 3-30 exchange procedures). Response to PE (as defined by improvement in mental status and normalization of LDH and haptoglobins) was complete in 1 patient, partial in 8, and absent in 1. Plasma exchange was discontinued often when these re-
response parameters plateaued. In 1 patient, PE had to be discontinued because of hemodynamic instability and gastrointestinal hemorrhage. The platelet count was not a reliable indicator of response because a hypocellular bone marrow (5%-10% cellular with decreased megakaryocytes) was seen in all 5 patients in whom a bone marrow biopsy was obtained during active TTP. As such, the benefit of PE could not be confirmed on the basis of an increasing platelet count, the usual marker of disease response. One patient received additional treatment with protein A immunoabsorption (PAI) alternating with PE, using cryoprecipitate-poor plasma, and eventually achieved a complete response after a total of 30 exchange procedures. This patient had also received concurrently a total of 7 doses of vincristine, 2 mg intravenously, over 7 weeks, and the relative contribution of each of these simultaneously applied treatment strategies could not be determined. Three additional patients received vincristine intravenously (total doses: 2 mg, 2 mg, and 5 mg) without definite evidence of benefit.

**Outcome and Survival**

The overall survival in this group of patients was 50% (3 allogeneic patients [37.5%] and 2 autologous patients [100%]). Five allogeneic recipients died at a median of 29 days (range, 11-419 days) after diagnosis of TTP. Of these, 3 (including both patients conditioned with NMA regimens) had active TTP at the time of death; death was attributable to intracranial hemorrhage in 1 patient, disseminated aspergillosis in another, and CMV pneumonitis in the third. Two patients died of septic complications of acute and chronic GVHD at 29 and 419 days after TTP diagnosis, respectively, without evidence of active TTP at the time of death. Five patients are alive at a median of 219 days (range, 61-689 days) after diagnosis of TTP. All are in remission both from their underlying disease and from TTP. Both surviving autologous transplant recipients remain hemodialysis-dependent as a result of TTP-related renal injury, and 2 of the 3 surviving allogeneic recipients are receiving therapy for extensive chronic GVHD.

**Analysis of Potential Risk Factors for TTP**

Data were analyzed for the 118 allogeneic hematopoietic stem cell transplant recipients by comparing those patients in whom TTP did and did not develops to identify possible risk factors for the development of this syndrome. The analysis included the following variables: age, sex, type of transplant (HLA-identical sibling, HLA-identical unrelated, related NMA, and haploidentical related), conditioning regimen, use of TBI, number of transplants, and the source of stem cells (BMT and PBSC). Statistically significant risk factors for development of TTP were transplant type (NMA and MUD vs other) (Fisher exact test, P=.01) and source of stem cells (BMT vs PBSC) (Fisher exact test, 2-tail, P=.02). There were no statistically significant associations with age, sex, number of transplant procedures, conditioning regimen, or use of TBI. It is acknowledged that any statistical analysis should be interpreted cautiously, given the small number of cases.

**DISCUSSION**

Thrombotic thrombocytopenic purpura has been recognized increasingly as a complication of BMT; although endothelial injury has been implicated, the exact mechanisms of posttransplantation TTP remain to be defined. Like others, we were unable to show severe vWF-CP deficiency in posttransplantation TTP, consistent with the theory that endothelial injury, rather than inhibited or deficient protease activity, is the primary pathogenic mechanism in these patients.27 Nevertheless, these studies have involved only a relatively small number of cases, and a contributing role of a vWF-related protease in the pathogenesis of posttransplantation TTP should not yet be dismissed.

Endothelial injury results in release of large quantities of vWF. Theoretically, this may temporarily overwhelm the physiological degradation systems, resulting in a relative or transient deficiency of vWF-CP, analogous to that described in cases of autoimmune or familial TTP but which may not be demonstrable in vitro by using the current analytical methods.36 Markers of endothelial injury such as vWF antigen and soluble thrombomodulin (TM) have been studied in the thrombotic microangiopathies.28-30 The vWF antigen is released from endothelium by physiological and damaging stimuli. In contrast, TM appears to be released by damaging insults only.31 One group measured levels of vWF antigen and soluble TM in patients with idiopathic TTP and post-BMT TTP.28 The vWF antigen and TM levels were elevated in both patient groups compared with controls. The TM was significantly higher in post-BMT TTP compared with idiopathic TTP, providing supportive evidence of endothelial damage in the former.28 In our study, all posttransplantation TTP patients had vWF antigen levels that were higher than normal, and 3 of 10 had unusually large vWF multimers, which appeared to correlate with a more severe course and mortality. It has been postulated that the appearance of unusually large vWF multimers in this setting is a more specific marker of large-scale disruption of endothelial cells.30 All patients in this series had normal vWF-CP activity, at least in vitro.

Endothelial injury is likely the result of multiple contributing pathogenic factors, including toxic conditioning regimens, CMV infection, use of cyclosporine, and a possible graft-vs-host effect on the endothelium.12,16 In an analysis of consecutive allogeneic BMT recipients, evi-
dence for thrombotic microangiopathy was found in transplant recipients receiving cyclosporine as GVHD prophylaxis but not in those treated with MTX. Risk factor analysis revealed a highly significant association of microangiopathy only with severe acute GVHD and the use of cyclosporine. Among patients with microangiopathy and GVHD, there was a significant correlation between the time of monocyte engraftment and activation of coagulation. The observed microangiopathic process was postulated to be the result of inflammatory cytokine production in the course of GVHD. Specifically, allo-sensitization of donor T lymphocytes leading to interleukin 2 production, T-cell expansion, and interferon γ release results in the production of interleukin 1 and tumor necrosis factor α by monocytes. These inflammatory cytokines may mediate microangiopathy directly through endothelial injury or through induction of endothelial cell procoagulant activity. Alternatively, through cytokine-induced up-regulation of endothelial HLA class II antigen expression, the endothelium can become a target organ in the GVHD process. These reactions may be aggravated by the vascular toxicity of cyclosporine or prior endothelial damage caused by intensive conditioning regimens. A prospective study of posttransplantation TTP by the same group showed a significant correlation of serum tumor necrosis factor α with elevated levels of vWF antigen and TM and the severity of thrombotic microangiopathy.

A summary of recent publications on posttransplantation TTP is provided in Table 1. Similar to our experience, most patients were allogeneic transplant recipients receiving cyclosporine as GVHD prophylaxis. A notable feature is the proportion of MUD to related transplant recipients represented in these reports and ours. The finding of a relatively greater representation of patients among MUD vs related transplant recipients further implicates a GVHD effect in the evolution of this syndrome. The higher incidence in the allogeneic setting may reflect the need for GVHD prophylaxis and the higher incidence in the allogeneic setting may reflect the prior extensive therapy received (including an autologous stem cell transplant in both patients) that may have rendered the vascular endothelium vulnerable to any additional injury, including cyclosporine-mediated or immunologic (GVHD) endothelial injury or a combination thereof. Cytokine release results in the induction of altered anti-monocyte engraftment to occur and associated graft-vs-host disease remains a significant factor in the occurrence of posttransplantation TTP. The occurrence of TTP has not been reported specifically in prospective comparative analyses of stem cell sources to date but should be considered in future analyses of complications of PBSCT as this practice increases.

Another noteworthy observation in our analysis is the occurrence of posttransplantation TTP in 2 of 13 patients with NMA transplants performed during this time. Non-myeloablative conditioning regimens are selected often on the basis of decreased potential for regimen-related toxicity yet with sufficient immunosuppression to enable stable donor cell engraftment to occur and associated graft-vs-host disease effects to be realized. Despite the theoretically lower regimen-related toxicity, significant morbidity was observed in both patients in this series who developed TTP. This may reflect the prior extensive therapy received (including an autologous stem cell transplant in both patients) that may have rendered the vascular endothelium vulnerable to any additional injury, including cyclosporine-mediated or immunologic (GVHD) endothelial injury or a combination thereof. Cytomolgavirous has been implicated as a contributing factor in the development of posttransplantation TTP. In this series, 4 of the 10 patients had documented CMV viremia at approximately the time of TTP diagnosis. Both of the NMA-PBSCT recipients, along with 1 HLA-identical sibling BMT and 1 autologous BMT recipient, were affected. Among the unaffected 13 NMA-PBSCT and 24 HLA-identical sibling BMT recipients, CMV viremia did not occur. This is similar to a series reported by Sarode et al in which 45% of patients had evidence of CMV infection at the time of TTP diagnosis. Cytomolgavirous infection may cause endothelial cell injury directly or as a result of its role in GVHD through the induction of altered anti-
gen expression on infected endothelial cells, leading to alloantigen recognition by donor T cells.

Although the higher rate of posttransplantation TTP in autologous compared with autologous transplant recipients (Table 1) raises the possibility of an immunologic mechanism or the use of cyclosporine to prevent GVHD as possible underlying pathogenic mechanisms, TTP is well described after autologous transplantation in which neither is involved.52 The occurrence of TTP after autologous transplantation is consistent with primarily regimen-related toxicity or possibly infection.36,37,53

Unlike the situation in idiopathic TTP, responses to PE are suboptimal, and posttransplantation TTP carries a poor prognosis (Table 1).13,18,20 Most patients treated in our series showed some improvement in clinical and hematologic parameters in response to PE. The usual marker by which response to PE is determined (platelet count) cannot be used typically in posttransplantation TTP because platelet engraftment may not yet have occurred, and the newly engrafted pool of donor stem cells is vulnerable to suppression through multiple drug, immune, and infectious mechanisms. Nevertheless, even with this caveat, the typically incomplete responses and high mortality rates call for better therapeutic approaches. Some data support the role of PE with PAI.52,54 The only patient in this cohort to achieve a complete response with PE also received PAI. The concurrent and prior therapy with standard PE, vincristine, and corticosteroids preclude us from drawing any conclusions with regard to therapeutic efficacy, as is typical for studies of this syndrome.

CONCLUSIONS

Posttransplantation TTP was not found to be associated with severe vWF-CP deficiency or inhibition in this cohort. The elevated levels of vWF antigen are consistent with diffuse endothelial injury likely the result of multiple interacting factors. The greater incidence among populations at highest risk for GVHD and those with concurrent GVHD suggests the possibility that this may represent a “graft-vs-endothelial” process, aggravated by preceding or concomitant endothelial insults such as extensive prior therapy, use of cyclosporine, and reactivation of CMV. Nonmyeloablative transplantation does not appear to confer a lesser risk, possibly for these reasons. The generally poor prognosis of this complication of transplantation calls for further investigation of the pathogenesis and consideration of alternative approaches to improve or replace the current inadequate therapeutic options.

REFERENCES