Isolated Thrombocytopenia After Allogeneic Bone Marrow Transplantation: Existence of Transient and Chronic Thrombocytopenic Syndromes

By Lewis R. First, Brian R. Smith, Jeffrey Lipton, David G. Nathan, Robertson Parkman, and Joel M. Rappeport

Isolated thrombocytopenia after bone marrow transplantation was investigated in 65 fully engrafted patients surviving at least 60 days posttransplant. Twenty-four patients (37%) developed this complication, which occurred most frequently in patients receiving pretransplantation preparation with total body irradiation or busulfan. Two distinct thrombocytopenic syndromes were identified: (1) transient thrombocytopenia (nine patients), in which a normal platelet count (>10,000/uL) was initially established by day +40 but then diminished to <10,000 or 45,000/uL on day +40 to +70, with subsequent resolution of the thrombocytopenia by day +90; (2) chronic thrombocytopenia (15 patients), in which a platelet count >100,000/uL was not achieved at any time during the first four months posttransplant, despite the simultaneous presence of normal granulocyte and reticulocyte counts. Although the transient syndrome did not adversely affect prognosis, the chronic syndrome carried a high mortality (21% actuarial survival at 1,000 days posttransplant compared with 67% survival for all patients, P < .01) and had a high association with both severe (grades 3 to 4) acute graft-versus-host disease (GVHD) and chronic GVHD. In three of nine patients with transient thrombocytopenia, a temporal association with trimethoprim-sulfamethoxazole administration was observed, whereas in all other patients, no drug association could be found. Bone marrow biopsies in those patients with drug-associated thrombocytopenia showed decreased numbers of megakaryocytes, whereas biopsies in the remainder of the transiently thrombocytopenic patients demonstrated adequate numbers of platelet precursors, suggesting peripheral platelet destruction or ineffective thrombopoiesis. Biopsies in the chronic thrombocytopenic patients included those with and without adequate numbers of platelet precursors, although the association with chronic GVHD was strongest in patients demonstrating normal numbers of megakaryocytes. We conclude that isolated thrombocytopenia represents a significant complication of bone marrow transplantation, particularly in patients receiving hematopoietic ablative preparatory regimens, and that it is the chronic, not the transient, thrombocytopenic syndrome that is associated with an adverse patient prognosis.

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with no radiation or buffy coat administration. Preparation for leukemic patients included cytosine arabinoside 500 mg/m²/d continuous intravenous infusion from day −11 to day −4, cyclophosphamide 60 mg/kg days −3 and −2, and total body irradiation (1,000 or 850 rad midline dose at 5 rad/min) on day −1. Patients with congenital disorders were prepared as previously described. Briefly, patients with WAS, Kostmann’s syndrome, CGD, and undefined immunodeficiencies received either total body irradiation (850 rad as above) or hematopoietic ablative doses of busulfan (3 mg/kg/day for four days), whereas the patient with SCID received no pretransplant preparation. Posttransplant patients received methotrexate prophylaxis for graft-versus-host disease (GVHD) given either by the procedure of Thomas et al (15 mg/m² on day +1; 10 mg/m² on days +3, +6, +11; and weekly thereafter up to +102 days posttransplant) or as four doses of methotrexate (10 mg/m²) on days +1, +3, +6, +11. Furthermore, all patients received prophylactic trimethoprim-sulfamethoxazole (TMP-SMX) at a dose of 4 mg of trimethoprim/kg/day seven to ten days before transplantation, stopping on day −1 and beginning again posttransplant when the granulocyte count reached 1,200/μL and the platelet count, 100,000/μL. Posttransplant patients who received TMP-SMX also received folinic acid 5 mg/day. Patients were all transplanted in laminar air flow isolation rooms.

Definition of Thrombocytopenic Syndromes

Engraftment was established in all patients in this study by day +21, and all patients had obtained granulocyte counts of greater than 1,000/μL and reticulocyte counts of greater than 2% by day +40. Isolated thrombocytopenia in the posttransplant patients was defined as a platelet count of less than 100,000/μL when the granulocyte count was greater than 1,000/μL and the reticulocyte count greater than 2%.

Thrombocytopenic patients were further subdivided into two characteristic groups: those with persistent chronic thrombocytopenia and those with transient acute thrombocytopenia. Transient thrombocytopenia was established when the platelet count that had risen to greater than 100,000/μL by day +40 posttransplant (without platelet transfusion support) subsequently fell to less than 100,000/μL at any time after that and returned to normal level before day +120; hence the term "transient acute" thrombocytopenia. Chronic or persistent thrombocytopenia was established when the platelet count remained below 100,000/μL on day +40 posttransplant at a time when the granulocyte count was greater than 1,000/μL and the reticulocyte count greater than 2%. Platelet counts in all of these patients remained below 100,000/μL from time of transplant through day +120.

Miscellaneous

Bone marrow aspirates and biopsies were performed on 21 patients in the thrombocytopenic categories. Acute graft-versus-host disease (AGVHD) was graded by the criteria of Thomas et al. Skin biopsies were used to confirm the diagnosis of both AGVHD and chronic graft-versus-host disease (CGVHD).

All patients were routinely screened for cytomegalovirus (CMV) infections by biweekly CMV antibody titers and by bimonthly assessment of blastogenic response to CMV. Specific viral cultures were obtained when clinically indicated.

Measurement of both indirect and direct antiplatelet antibodies was as previously described. Platelet survival studies were performed by standard methods using platelets obtained by apheresis from each patient’s bone marrow donor.

Statistical methods used include Student’s t test with Yates’ correction, chi-squared analysis, and life table logrank analysis.

RESULTS

Incidence and Clinical Characteristics of Posttransplant Thrombocytopenic Patients

Of 65 evaluable bone marrow transplant patients who survived at least 60 days posttransplant and who had full engraftment of all hematopoietic lines, nine (14%) developed transient thrombocytopenia and 15 (23%) developed chronic thrombocytopenia, for an overall incidence of 37%.

The course of a typical patient with transient thrombocytopenia is shown in the upper panel of Fig 1. Although the necessity for periodic platelet transfusions during the thrombocytopenic period makes the mean platelet count during nadir difficult to assess, the unsupported platelet count in these transiently thrombocytopenic patients was approximately 35,000/μL (range: <10,000 to 45,000). No changes in WBC or reticulocyte count were observed concomitant with the drop in platelet count. All nine patients had a return of their platelet count to greater than 100,000/μL by day +90 (mean day of return of platelet count was +70).

The course of a typical patient with chronic persistent thrombocytopenia is shown in the lower panel of Fig 1. Patients who were designated as having chronic thrombocytopenia had all failed to have a platelet count rise to greater than 100,000/μL by day +40.
(mean platelet count in this group was less than 30,000/µL). All but two of these patients never obtained a platelet count greater than 100,000/µL. The two patients whose platelet count did recover developed the rise in count at day +150 and day +365 posttransplant.

Pretransplant characteristics of the three groups of patients (transient thrombocytopenia, chronic thrombocytopenia, and no thrombocytopenia) are shown in Table 1. Patients who developed the transient syndrome were characterized by younger age (mean age 10 years, median age 5 years) as compared with both the non-thrombocytopenic (mean and median age 18 years, \( P < .05 \)) and the chronic thrombocytopenic groups (mean and median age 21 years, \( P < .05 \)). Sex distribution and bone marrow dose were comparable among the three groups. The incidence of developing some form of postransplant thrombocytopenia in leukemic patients was 14/26 (54%), in aplastics 8/30 (27%), and in patients with congenital disorders 2/9 (22%). There was a 48% incidence of thrombocytopenia among patients treated with ablative preparative regimens (total body irradiation or busulfan) versus 25% among those receiving nonablative therapy. This difference was statistically significant (\( \chi^2 = 3.84, P < .05 \)) and independent of age of the patients.

There was no significant difference in the donor population with regard to age, sex, or blood counts before transplant in any of the groups. Moreover, no donor had evidence of thrombocytopenia or of recent exposure to viral infection at the time of transplant.

Table 1. Characteristic of Bone Marrow Transplant Patients Studied

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Transient Thrombocytopenia (n = 9)</th>
<th>Chronic Thrombocytopenia (n = 15)</th>
<th>No Thrombocytopenia (n = 41)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age in years (range)</td>
<td>10 (3–16)</td>
<td>21 (5–31)</td>
<td>18 (1–47)</td>
</tr>
<tr>
<td>Sex (M:F)</td>
<td>6:3</td>
<td>9:6</td>
<td>24:17</td>
</tr>
<tr>
<td>Mean bone marrow dose (× 10⁹ kg)</td>
<td>3.1</td>
<td>2.3</td>
<td>2.6</td>
</tr>
<tr>
<td>Diagnosis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leukemia</td>
<td>5</td>
<td>9</td>
<td>12</td>
</tr>
<tr>
<td>Aplastic anemia</td>
<td>2</td>
<td>6</td>
<td>22</td>
</tr>
<tr>
<td>Congenital disorders*</td>
<td>2</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>Preparation†</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ablative</td>
<td>7</td>
<td>9</td>
<td>17</td>
</tr>
<tr>
<td>Nonablative</td>
<td>2</td>
<td>6</td>
<td>24</td>
</tr>
</tbody>
</table>

Statistically significant differences occur in mean age between the transiently thrombocytopenic group and the chronic and non-thrombocytopenic groups, and in incidence of thrombocytopenia between those receiving ablative conditioning regimens (incidence = 48%) and those receiving nonablative regimens (incidence = 25%).

*Includes SCID, WAS, CGD, Kostman’s syndrome.
†Ablative therapy defined as either total body irradiation (850 or 1,000 rad midline dose) or busulfan 3 mg/kg/d for four days.

<table>
<thead>
<tr>
<th>Table 2. Incidence of GVHD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transient Thrombocytopenia</td>
</tr>
<tr>
<td>Acute GVHD</td>
</tr>
<tr>
<td>Grade 0–2</td>
</tr>
<tr>
<td>Grade 3–4</td>
</tr>
<tr>
<td>Chronic GVHD</td>
</tr>
<tr>
<td>All grades</td>
</tr>
</tbody>
</table>

Statistically significant increases in the incidence of both severe AGVHD and CGVHD occur in those patients developing chronic thrombocytopenia compared with those without thrombocytopenia. No difference is seen between patients with transient thrombocytopenia and those without thrombocytopenia. Note that the "at risk" population for CGVHD is smaller than that for AGVHD in each group because some patients died before being eligible for the diagnosis of CGVHD.

Association of Thrombocytopenia With GVHD

Table 2 presents the occurrence of AGVHD and CGVHD in each of the three groups of patients. When compared with the non-thrombocytopenic patient group, patients with chronic thrombocytopenia demonstrates a significantly higher incidence of grade 3 or 4 AGVHD (53% vs 20%, \( \chi^2 = 6.15, P < .05 \)) and a significantly higher incidence of CGVHD (92% vs 39%, \( \chi^2 = 7.97, P < .01 \)). In contrast, when patients who developed the transient thrombocytopenic syndrome are compared with the non-thrombocytopenic group, there is no difference in the incidence of either high grade AGVHD (22% vs 20%, \( \chi^2 = 0.03, P > .5 \)) or chronic GVHD (56% vs 39%, \( \chi^2 = 0.25, P > .05 \)). In all cases in which both conditions occurred, the AGVHD occurred before the onset of transient thrombocytopenia.

Influence of Thrombocytopenia on Survival

Life table analyses are shown in Fig 2 for each of the three groups of patients. Of interest is the significantly
increased mortality in the patient population having chronic thrombocytopenia ($\chi^2$ by logrank analysis = 14.89, $P < .001$). By contrast, there was no difference between the survival of transiently thrombocytopenic patients and non-thrombocytopenic patients. Because the overall survival of patients transplanted for leukemia is lower than for patients transplanted for aplastic anemia, it is possible that the increased number of leukemic patients in the chronically thrombocytopenic group accounted for the poor survival. This is not the case, however, as shown in Fig 3. When the aplastic anemia patients in the non-thrombocytopenic v the chronically thrombocytopenic group are analyzed separately, the aplastic anemia patients with chronic thrombocytopenia still have a markedly poor survival (1,000-day posttransplant actuarial survival of 33% v 78% for the non-thrombocytopenic aplastics, $\chi^2 = 4.11, P < .05$).

The proximate cause of death in the chronically thrombocytopenic patients was most often infectious (accounting for four of 12 deaths in this group) or owing to idiopathic interstitial pneumonitis/respiratory failure (five of 12). Only two patients in this group had bleeding complications as a contributory cause of death, and one of these patients also had concomitant candidal sepsis. One patient died of relapsed leukemia. There were no deaths from known infectious agents associated with transfusion products.

Figure 4 examines the survival of patients with CGVHD influenced by the concomitant problem of thrombocytopenia. As shown, there was a significantly higher mortality among CGVHD patients who also had chronic thrombocytopenia, as compared with CGVHD patients who did not have thrombocytopenia, and as compared with CGVHD patients who have had only transient thrombocytopenia ($P < .01$ by logrank).

**Mechanism of the Thrombocytopenia**

Additional posttransplant factors were analyzed for their possible relationship to the development of thrombocytopenia. The number of doses of methotrexate administered posttransplant did not correlate with the incidence of thrombocytopenia (data not shown). In addition, the presence of splenomegaly was not noted in any of the thrombocytopenic patients. Concomitant drug administration was examined carefully for its possible temporal relationship to the thrombocytopenic syndromes. In three of the nine patients with transient thrombocytopenia, the administration of posttransplant prophylactic TMP-SMX could be temporally related to the onset of the thrombocytopenia. When the drug was discontinued, the thrombocytopenia likewise resolved within seven days. These three patients were therefore considered to have drug-induced thrombocytopenia. The remaining six patients with transient thrombocytopenia (all of whom received TMP-SMX without a change in platelet count), as well as all of the patients in the chronic thrombocytopenia group, did not demonstrate platelet drops that could be associated with any specific pharmacologic intervention. Only one of the patients in the study had evidence of CMV infection, and that patient did not demonstrate thrombocytopenia posttransplant. Similarly, coagulation studies at the time of the platelet nadirs in the thrombocytopenic patients were performed and demonstrated normal prothrombin, activated partial thromboplastin, and thrombin times, as well as normal fibrin split products. Of particular note were five syngeneic transplants, of whom two developed non–drug-related transient thrombocytopenia. Three were in the non-thrombocytopenic group.

In order to further analyze the mechanism of the thrombocytopenic syndromes, bone marrow aspirates
and biopsies were performed in 21 patients during their platelet nadirs. The results are shown in Table 3. Although all of the TMP-SMX–associated transient thrombocytopenics had decreased numbers of megakaryocytes noted in the biopsies, most of the non–drug-associated thrombocytopenic patients had normal numbers and morphology of their megakaryocytes at the time of platelet nadirs. There was no influence of bone marrow biopsy findings on the survival of transient thrombocytopenic patients. Of the drug-associated transiently thrombocytopenic patients, zero of three had high-grade AGVHD, but two of three subsequently had CGVHD. Among the non–drug-related transiently thrombocytopenic patients, two of six had grade 3 or 4 AGVHD and three of six had CGVHD.

Similarly, the chronic thrombocytopenics could be broken down into those with increased and those with decreased numbers of megakaryocytes in the marrow. Although the incidence of high-grade AGVHD was similar in these two groups (three of six and six of eight, respectively, \( \chi^2 = .93, P > .10 \)), the incidence of CGVHD was much higher in the patients with normal numbers of megakaryocytes on bone marrow examination (six of six) than in the patients with decreased numbers of megakaryocytes (three of eight, \( \chi^2 = 4.73, P < .05 \)).

Antiplatelet antibodies (both direct and indirect) were found in four of eight thrombocytopenic patients tested (one of two transient, three of six chronic). There was no correlation of antiplatelet antibodies to bone marrow biopsy findings. Six patients underwent platelet survival studies (two transient, four chronic), and all showed a markedly decreased platelet half-life (mean six hours compared with normal platelet survival of 6½ days). There was no evidence obtained for the production of other autoantibodies at the time of these studies.

**Treatment of the Thrombocytopenia**

All three patients whose transient thrombocytopenia was temporally related to TMP-SMX administration had resolution of the thrombocytopenia on discontinuation of the drug. The two patients in the transient thrombocytopenic group who had associated grade 3 or 4 AGVHD and CGVHD but who were not on TMP-SMX, were initially treated with prednisone alone for their GVHD (3 mg/kg/d) with no effect either on their GVHD or thrombocytopenia. However, when azathioprine was added to the regimen (1.5 mg/kg/d), GVHD improved and it was incidentally noted that the platelet count rose to greater than 100,000/µL within ten days of the commencement of azathioprine treatment. The remaining four patients with lesser degrees of AGVHD in the transient non–drug-associated thrombocytopenic group all had spontaneous resolution of the thrombocytopenia without pharmacologic intervention.

Among the patients with chronic thrombocytopenia, six of those who had associated CGVHD were treated with both prednisone and azathioprine with no effect on symptoms or platelet counts. The two patients with chronic thrombocytopenia that eventually resolved (and the only two long-term survivors in that group) had had AGVHD but either no or only limited CGVHD. Both were treated with prednisone alone, with no associated change in platelet count; neither received azathioprine.

**DISCUSSION**

Our results demonstrate an unexpectedly high incidence (37%) of isolated acquired thrombocytopenia developing after allogeneic bone marrow transplantation, particularly in those patients requiring ablative preparatory therapies. In addition, we were able to identify two subpopulations of thrombocytopenic patients: the transient (about one third) and the chronic (about two thirds). The two syndromes are summarized in Table 4. Although both groups of thrombocytopenic patients were similar to the non–thrombocytopenic patients in terms of sex and bone marrow dose, the patients with transient thrombocytopenia were generally younger than those with no thrombocytopenia or those who developed chronic thrombocytopenia.

In one third of these transient thrombocytopenic patients, the acute drop in platelets could be associated with pharmacologic manipulation, specifically TMP-SMX. Platelet counts diminished within seven to 16 days of starting TMP-SMX and normalized within two weeks of stopping the drug, an interval consistent with other reports of TMP-SMX–induced cytopenia.15,16 Furthermore, bone marrow biopsies in these patients done at the time of the nadir demonstrated absent megakaryocytes consistent with a mechanism of decreased production. It is worthwhile noting that these bone marrow findings occur despite the use of folinic acid, which has been found to reverse the suppressive effect of the drug.13 It is possible that the dose of folinic acid used could not overcome the effect
of the TMP-SMX combination. In the remaining two thirds of the patients developing transient thrombocytopenia, no drug intervention could be implicated as a causative factor. Bone marrow biopsies showed adequate numbers of precursor cells, implying that the mechanism of the thrombocytopenia is probably either one of peripheral platelet destruction or ineffective thrombopoiesis. The appearance in some patients of both direct and indirect antiplatelet antibodies and the markedly decreased platelet survival time of bone marrow donor platelets in these patients favor the former explanation but, given the complex pathophysiology of the posttransplant state, do not totally establish it. From this mechanistic point of view, it is interesting to note that the two patients with associated severe AGVHD and CGVHD and transient thrombocytopenia had a marked and rapid resolution of the decreased platelets when therapy with azathioprine was instituted. This intervention resulted in improvement in the CGVHD as well as the platelet count. This striking temporal association has now been seen in two additional patients not included in the current series, both of whom had a chronic rather than a transient thrombocytopenic syndrome plus CGVHD and who responded with respect to both these entities when azathioprine was added to their anti-GVHD regimen. We cannot, of course, exclude the possibility that the platelet count would have returned to normal without intervention (especially in those two patients with the transient syndrome). Nonetheless, the rapid resolution after therapy of these patients and the fact that two patients with the chronic syndrome have now been observed to respond in a similar manner suggests that the azathioprine led to the return to normal of the platelet count. Furthermore, it again suggests a close association of thrombocytopenia as an index of severity of GVHD. Recently, Bierling et al. have shown a direct correlation between elevated platelet-associated IgG and CGVHD, although not all of the patients in that study with high platelet-associated IgG also had CGVHD. Glucksberg et al. have also reported rapid platelet destruction in a patient with CGVHD and idiopathic thrombocytopenic purpura. Based on these data and on our anecdotal experience of the difficulty of increasing the platelet count in these patients by transfusion from unrelated HLA-matched platelet donors, or even when using platelets obtained from the bone marrow donors themselves (data not shown), we postulate that the mechanism of the thrombocytopenia seen in this transient syndrome is one of peripheral platelet destruction and, at least in some cases, may be a paraphenomenon of GVHD. It will be important to monitor antiplatelet antibodies and platelet survival studies to better prove or disprove this mechanism prospectively. The fact that four patients have shown a response of the thrombocytopenia to therapy of CGVHD with azathioprine implies that "anti-CGVHD" azathioprine therapy need not be withheld in this group of patients (even though they are significantly thrombocytopenic), but rather they may be given a cautious trial for two to four weeks to assess its effectiveness.

Regardless of the mechanism, we found that the entity of transient thrombocytopenia posttransplant is quite benign—survival for these patients is the same as for patients whose platelet counts remain in the normal range posttransplant. This is in sharp distinction to the other group of thrombocytopenic patients—those with a chronic persistent syndrome.

The chronic thrombocytopenic patients showed a high association with both severe (grade 3 or 4) AGVHD and CGVHD. In none of these patients was the thrombocytopenia associated with any pharmacologic agents or with viral infection. Some of these patients showed markedly decreased numbers of megakaryocytes on bone marrow biopsy and some showed normal numbers. The association of CGVHD with this chronic thrombocytopenic syndrome was strongest in the subgroup of patients whose bone marrows demonstrated adequate megakaryopoiesis. Thus, the mechanism of the thrombocytopenia in the
chronic persistent group may be mixed—either peripheral destruction associated with GVHD syndromes or decreased production perhaps indicative of "slow engraftment."

This latter mechanism may be consistent with the higher incidence of thrombocytopenia in patients having ablative therapy and may also account for why thrombocytopenia has been noted in patients having autologous transplants as well. However, it is important to state that in the absence of similar bone marrow and platelet survival studies in control (non-thrombocytopenic) patients, all mechanisms remain hypothetical and speculative.

Regardless of the mechanism, the prognosis in this group of patients is far from benign, with only a 21% overall survival at three years posttransplant compared with a 67% survival in the non-thrombocytopenic and transiently thrombocytopenic group of patients. There is a particularly foreboding association of CGVHD and chronic thrombocytopenia. In fact, among patients with CGVHD, it is those who are in the subgroup with thrombocytopenia who have the worst overall prognosis (Fig 4). Because these patients do not die of hemorrhagic complications, but predominantly of other GVHD-associated complications (especially infection), we feel that the thrombocytopenia is reflecting a more severe degree of CGVHD and may even represent a useful "grading" and stratification criterion for the purpose of subdividing patients with CGVHD into subgroups of differing prognosis.

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