Abstract. Background: The aim of the present study was to assess the efficacy of vincristine-laden platelet transfusion for patients with refractory thrombocytopenia. Patients and Methods: Twenty evaluable patients who received vincristine-laden platelets for refractory thrombocytopenia were included in this retrospective study. Vincristine (1 mg) was added to the platelets and incubated for one hour prior to transfusion. Serial platelet counts following vincristine-laden platelet transfusion and units of platelets transfused in the week prior to and the week after transfusion of vincristine-laden platelets were evaluated. Results: The underlying diseases of the patients were lung cancer (n=4), breast cancer following autologous hematopoietic stem cell transplantation and acute myeloid leukemia (n=3 each), myelodyplastic syndrome (n=2), acute lymphoid leukemia, chronic lymphoid leukemia, chronic myeloid leukemia, multiple myeloma, ovarian cancer, aspergillosis, cytomegalovirus infection and systemic lupus erythematosus (n=1 each). The median rate of change of platelet count after transfusion of vincristine-laden platelets was 550/µL/day (range, –1,000 to 12,800/µL/day; p=0.003). The median change in the number of units of platelets transfused in the week following vincristine-laden platelet transfusion was –1.5 as compared to the week prior to the transfusion (p=0.031). Patients with a primary marrow disorder exhibited no difference in either the rate of change in platelet count or in the difference in the units of platelets transfused compared to those without a primary bone marrow disorder. Conclusion: Vincristine-laden platelet transfusion was associated with significantly increased platelet counts and a subsequent decrease in platelet transfusion.

Prophylactic platelet transfusion is the mainstay of therapy for patients with severe thrombocytopenia. Although most patients respond appropriately to platelet transfusion with increased platelet counts, some patients fail to respond (1). Alloimmune platelet refractoriness results from the development of antibodies to foreign HLA class I antigens on the platelet surface (2), which is in turn dependant on the HLA class II antigens present on foreign leukocytes (antigen-bearing cells). Exposure to foreign HLA class II antigen-bearing cells usually results from multiple transfusions or fetal-maternal bleeding during pregnancy (2, 3). The mainstay of management of platelet refractoriness due to alloimmunization involves the transfusion of HLA-matched platelets (4) and/or cross-match compatible platelets (5). However, matched/compatible platelets may not always be available and other modalities such as corticosteroids, intravenous immune globulin and splenectomy have not been proven to be effective.

Vinca alkaloids can be concentrated by platelets that when transfused, are engulfed by macrophages responding to the antibodies responsible for alloimmune directed platelet destruction. Macrophage function is impaired and the rate of platelet destruction decreases. The clinical utility of the ability of platelets to concentrate vinca alkaloids has been successfully tested in patients with idiopathic thrombocytopenic purpura (6, 7). The same principle has also been utilized in the management of other immune conditions such as familial erythrophagocytic lymphohistiocytosis (8), sinu histiocytosis, massive splenomegaly (9) and autoimmune hemolytic anemia (10, 11).

An uncontrolled retrospective study was conducted to assess the efficacy of vincristine-laden platelet transfusion in patients with refractory thrombocytopenia.

Patients and Methods

Patients who received vincristine-laden platelets for the treatment of refractory thrombocytopenia between 1991 and 2003 were included in this retrospective analysis after approval by the
hospital's Institutional Review Board. For the purpose of this study, refractory thrombocytopenia was defined as persistent platelet counts $<20,000/mm^3$, despite daily transfusion of platelets.

Data collected included: age and gender of the patient, the underlying disease, baseline hepatic and renal function, any previous therapy the patient received for thrombocytopenia, platelet counts on days 0, 1, 3, 7, 15, 30 and 90 following administration of vincristine-laden platelets (whenever available), the number of units of platelets transfused in the week before and the week after vincristine-laden platelet transfusion and any medications the patient was receiving at that time.

Patients were given platelet transfusions for either a platelet count of $<20,000/\mu l$ or of $<50,000/\mu l$ and evidence of bleeding. Platelet refractoriness was defined as the continuing need for platelet transfusion, despite transfusions. Vincristine-laden platelets were administered to refractory patients at the discretion of the treating physician. One hour platelet counts following transfusion were not measured.

Vincristine-laden platelets were prepared as follows: one milligram of vincristine was added to the bag containing donor platelets and the bag was then agitated for 1 hour to ensure incorporation of vincristine with platelets. The platelets were then transfused to the patient.

Response was measured in terms of rate of change in platelet counts and decrease in the transfusion requirements for platelets. The rate of change in platelet count was calculated for each patient as a slope using linear regression. The Wilcoxon rank sum test was used to analyze whether the rate of change in platelet count was significantly greater than zero. The number of units of platelets transfused in the week prior to therapy with vincristine-laden platelets was also compared to the number of units of platelets transfused in the one week after therapy using the Wilcoxon signed rank test. A pool of platelet concentrate was considered equivalent to a unit of apheresis platelets and was counted as one unit. A $p$-value of $<0.05$ was considered statistically significant.

**Results**

Twenty-one consecutive patients (15 females and 6 males) who received vincristine-laden platelets between 1991 and 2003 were included in this retrospective analysis. Three patients who received vincristine-laden platelets twice were included in the dataset twice and only their first treatment was included for further analysis. One patient died on day 0 and was excluded from further analysis. Two patients died in the first week after transfusion of vincristine-laden platelets and were excluded from the comparison of the change in need for platelet transfusion.

The median age of the patients was 49.5 years (range: 33-76 years). Underlying diseases included lung cancer (n=4), acute myeloid leukemia (AML) and breast cancer following high-dose chemotherapy with stem cell support (n=3 each), myelodysplastic syndrome (n=2), acute lymphoid leukemia (ALL), chronic lymphoid leukemia (CLL), chronic myeloid leukemia (CML), multiple myeloma, ovarian cancer, aspergillosis, cytomegalovirus infection and systemic lupus erythematosus (SLE) (n=1 each).

Two patients, one with ALL and the other with SLE had abnormal renal function as evidenced by an elevated creatinine level. Hepatic dysfunction was seen in two patients, one each with ALL and extensive stage small-cell lung cancer. Four patients had received a trial of HLA-matched platelets prior to receiving vincristine-laden platelets. Nine patients received vancomycin and five patients received amphotericin B during the same hospitalization that a vincristine-laden platelet transfusion was given.

The median platelet count on the day the patients received the vincristine-laden platelets was $<10,000/\mu l$ (range: $<10,000/\mu l$ to 40,000/\mu l). Two patients, one with AML and the other with SLE, had evidence of bleeding. Five patients, with multiple myeloma, CLL, SLE, lung cancer or cytomegalovirus infection had received prior treatment for thrombocytopenia with steroids, intravenous immunoglobulin and plasmapheresis. One patient received desmopressin for bleeding prophylaxis as well. One patient developed peripheral neuropathy after receiving a second course of vincristine-laden platelets.

Eleven patients had an increase in the platelet counts, 7 patients had a decrease in their counts, while 5 patients had no change in their counts on day 1, following transfusion of vincristine-laden platelets. Of the 19 patients who had data available at day 7 following transfusion of vincristine-laden platelets, 12 patients had an improvement and 4 had a decrease in their platelet counts, while 3 patients had stable platelet counts.

Following administration of vincristine-laden platelets, the median rate of change in platelet counts was 550/\mu l/day (range: $-1,000/\mu l$/day to 12,800/\mu l/day) (Figure 1). This rate of change was significantly greater than zero ($p=0.003$). The median number of units of platelets transfused in the week following transfusion of vincristine-laden platelets was 6 (range: 0-16) and the median number of units of platelets transfused in the week prior to transfusion was 6.5 (range: 0-17). The median change in the number of units of platelets transfused following the transfusion of vincristine-laden platelets was -1.5 (range: -7 to 6). This difference was statistically significant ($p=0.031$) (Table 1).

**Discussion**

The most common associations with platelet refractoriness include hematopoietic stem cell transplantation, disseminated intravascular coagulation, splenomegaly and HLA antibody grade (12). A recent study demonstrated that an increase in the number of prior platelet transfusions decreased the post-transfusion increments in platelet counts following subsequent transfusions (13). Other implicated associations include severe infection with high fever, drug-mediated antibody production and/or alloimmunization, administration of platelets damaged or activated during
collection or storage (14) and administration of concurrent intravenous antimicrobial agents including amphotericin B, ciprofloxacin and vancomycin (15).

Alloimmune platelet refractoriness results from antibody production to foreign antigens, usually HLA class I antigens, present on the transfused platelet surface (2, 4). Platelet-specific antigens on the other hand, are only rarely implicated in the pathogenesis of platelet refractoriness (16). In fact, the presence of antibodies directed against platelet glycoproteins does not influence the frequency of refractoriness (2).

The development of alloimmunization to HLA class I antigens is dependent on the HLA class II antigens present on leukocytes. Multiple blood transfusions or fetal-maternal bleeding during pregnancy are the most common situations leading to the exposure to foreign HLA class II antigens (2, 3). Anti-HLA antibodies are produced in almost half of all recipients of leukocyte-containing blood products (17) and in up to one-third of women who have been pregnant, without a history of blood transfusion (18). Leukocyte-reduced platelet transfusions are much less immunogenic, since platelets express only HLA class I antigens.

Table I. Response to vincristine-laden platelets.

<table>
<thead>
<tr>
<th></th>
<th>Median</th>
<th>Range</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rate of change of platelet count</td>
<td>550/µl/d</td>
<td>–1000 to 12,800/µl/d</td>
<td>0.003</td>
</tr>
<tr>
<td>Platelet transfusions in 1 week prior to vincristine-laden platelets</td>
<td>6.5 Units</td>
<td>0-17 Units</td>
<td>NA</td>
</tr>
<tr>
<td>Platelet transfusions in 1 week after vincristine-laden platelets</td>
<td>6 Units</td>
<td>0-16 Units</td>
<td></td>
</tr>
<tr>
<td>Change in platelet transfusions after vincristine-laden platelets</td>
<td>–1.5 Units</td>
<td>–7 to 6 Units</td>
<td>0.031</td>
</tr>
</tbody>
</table>
Macrophages which affect both the afferent and the efferent limbs of the immune response play a major role in the destruction of the antibody-coated platelets. They not only aid in antigen recognition and processing, but also potentiate the immune response by interacting with both B- and T-lymphocytes (19-21). Once coated with the antibody, platelets interact with the Fc receptor of the macrophages in the reticulo-endothelial system (22), which then leads to sequestration and subsequent destruction of the platelets thereby leading to thrombocytopenia (23).

Selective delivery of a vinca alkaloid to the macrophages occurs if platelets are incubated with the specific agent for 1 hour at 37°C (24, 25). Ahn et al. incubated platelets with vinblastine and transfused them to 11 patients with refractory idiopathic thrombocytopenic purpura. Six had a complete remission, 3 patients had a partial remission and 2 failed to respond (6). In contrast, Kelton and associates reported only one response in 6 patients with platelet antibody-positive immune thrombocytopenia who received vinblastine-laden platelets and a number of adverse effects, including a sudden drop in platelet counts, infusion reactions, hypotension and hepatitis. Using a rabbit model, they found that vinblastine eluted out of the platelets within 2 h of the infusion, suggesting that the delivery of the vinblastine to the macrophages depended upon platelet phagocytosis within that time period (24).

Gout et al. suggested that vincristine is superior to vinblastine as it does not elute out of the platelets (26). In another study, Agnelli et al. treated 14 patients with autoimmune thrombocytopenic purpura using vincristine-laden platelets and observed five complete remissions lasting more than one year, three good responses, one fair response and five poor responses. No side-effects were reported and they also showed that the binding of vincristine to platelets was more stable than vinblastine binding (27).

The pathophysiology of refractory thrombocytopenia may be related to alloimmunization and vincristine-laden platelets could benefit these patients as well. One possible mechanism is that vincristine-laden platelets are engulfed by the macrophages. Vincristine then impairs the phagocytic ability of these macrophages and the degree of platelet destruction following subsequent platelet transfusion is decreased, thereby increasing the platelet counts and decreasing the need for further platelet transfusions.

In the 20 patients with refractory thrombocytopenia studied here, the administration of vincristine-laden platelets was associated with a significant increase in platelet counts and a decrease in the need for subsequent platelet transfusions. The response to vincristine-laden platelets was similar in patients with a primary bone marrow disorder as compared to those without a marrow disorder.

This was an uncontrolled, retrospective study and the patients were heterogenous with respect to the underlying cause of thrombocytopenia. However, all the patients had refractory thrombocytopenia and had received multiple platelet transfusions without a response. All these patients had received previous blood transfusions, a factor that may have led to refractory thrombocytopenia, presumably due to the development of allo-antibodies. The change in platelet counts was modest but there was a decrease in the need for platelet transfusions. Since the patients were transfused with vincristine-laden platelets only because they did not derive any benefit from untreated platelets, the decreased need for platelet transfusion in the week following vincristine-laden platelet transfusion, probably reflects the effects of the vincristine-laden platelets rather than the natural history of the thrombocytopenia.

The transfusion of vincristine-laden platelets is rarely associated with adverse effects as seen in this and another study (25, 27). The need for platelet transfusions and the inherent risk of transfusion reactions was decreased. This procedure is simple and can be a relatively inexpensive treatment of refractory thrombocytopenia.

References


