Current Status of Leukocyte and Platelet Administration in Cancer Therapy

ERNEST M. WALKER JR, M.D., P.H.D., ALBERT CANNON, M.D. and ERNESTINE N. MITCHUM, R.N.

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

ABSTRACT

Leukapheresis and plateletpheresis are rather commonly performed in order to obtain single donor concentrates of granulocytes and platelets. These procedures, although relatively safe, present occasional risks to donors and recipients. Some of the occasional adverse problems experienced by donors include citrate toxicity or acute hypocalcemia, hypotension, hypervolemia, venospasm or vein occlusion, chills, anaphylactoid reactions, hemorrhage, abdominal pain or complications related to equipment failure and related technical problems. Potential risks to donors include those related to the receiving of six percent hydroxyethyl starch (HES), dextrans, or corticosteroids, lymphocyte depletion or immunosuppression, and effects on the complement system.

Prophylactic granulocyte transfusions to prevent the occurrence of infections and associated complications in neutropenic patients have not proven to be efficacious; therapeutic granulocyte transfusions appear to be more effective. Indications for therapeutic granulocyte transfusions include those patients with known infections unresponsive to appropriately aggressive antibiotic chemotherapy over a two or three day period combined with findings of a peripheral granulocyte count less than 500 mm$^3$ and especially those with counts below 100 mm$^3$ and/or prolonged fever greater than 38°C (100.4°F) for 24 to 48 hours. In addition, the patient should have a reasonable chance for bone marrow recovery. Hazards or complications associated with granulocyte transfusions include: (a) immediate transfusion reactions, (b) hypersensitivity reactions, (c) pulmonary infiltrates, (d) alloimmunization, (e) transmission of infections, and (f) the possibility of Graft vs. Host (GVH) disease.

The current best use of apheresis platelets is to provide therapeutic doses of single donor matched platelets for patients refractory to pooled random donor platelets. Alloimmunization represents the major complication of therapeutic platelet transfusion and is characterized clinically by the failure to achieve expected platelet count increments after transfusion.

Future developments which might greatly improve the effectiveness of therapeutic and possibly prophylactic leukapheresis and plateletpheresis include the development of effective sedimenting agents with shorter bi-
ological half-lives, more efficient and less expensive methods of procurement of granulocytes and platelets, improved methods of cryopreservation of granulocytes and platelets, better methods for detecting and quantitating antigranulocyte and/or antiplatelet antibodies, and more efficient evaluation of possible synergism of granulocyte transfusions with antibiotic therapy and residual host defenses. These improvements may be of great value in the effective utilization of granulocyte and platelet products and in determining which patients are most likely to receive the maximum benefit from granulocyte and platelet support.

Introduction

Granulocyte concentrates, one of the newest of the transfused blood components, can be obtained currently by methods including: sedimentation of solutions of red blood cells by hydroxyethyl starch (HES) or other macromolecules followed by centrifugation of the cell-rich plasma to isolate the granulocytes; adhesion filtration leukapheresis using nylon fibers; apheresis techniques using discontinuous-flow centrifugation* or continuous-flow centrifugation†. The transfusion of granulocytes is considered, most often, in cases of infection in patients demonstrating neutropenia and bone marrow aplasia as a result of chemotherapy.28

Platelet concentrates can be obtained by the pooling of individual bags of platelets obtained from multiple donors or as a single donor apheresis platelet concentrate. These platelet concentrates are administered to help prevent bleeding complications in patients demonstrating neutropenia and bone marrow aplasia as a result of chemotherapy.28

Concentrates containing appreciable numbers of platelets and granulocytes can be obtained by using the apheresis techniques of discontinuous-flow centrifugation* or continuous-flow centrifugation.† These products can, therefore, provide therapeutic benefit in a single thrombocytopenic and neutropenic patient.112

Donor Considerations

Only 0.27 percent of a total of 18,288 leuka- and leukaplateletphereses performed in American Red Cross regional blood centers during a 12-month period were discontinued due to acute donor reactions.92 None of the reactions had serious or long-term donor consequences. However, it is obvious that additional data is needed to assess the effects of repeated leukapheresis procedures on donors and to establish rational guidelines for donor recruitment.

Common Acute Adverse Clinical Reactions Associated with Apheresis

Citrate Toxicity

One of the most common adverse reactions is citrate toxicity or the acute hypocalcemia which may result from rapid reinfusion of citrated donor blood.92 Symptoms usually include tingling around the mouth and in the fingers and occasionally, donor hyperventilation. The reaction is usually promptly reversed by slowing down the reinfusion rate of citrate blood and/or by giving the patient calcium in the form of milk or calcium gluconate. However, over-administration of i.v. calcium involves the risk of iatrogenic acute hypercalcemia to the donor.92

Hypotension

Hypotensive symptoms may be seen in situations of donor hypovolemia, especially in discontinuous-flow procedures,
when as much as 550 ml or more whole blood may be in the extracorporeal circulation. The signs of syncope or other hypotensive reactions generally respond to rapid infusion of i.v. solutions or return of the extracorporeal blood to the donor. Occasionally, persistent hypotension, weakness or diaphoresis may occur despite adequate correction of hypovolemia; this prolonged reaction may be due to anxiety or a vasovagal reaction.92

**Hypervolemia**

This complication may be associated with the infusing of six percent hydroxyethyl starch (HES) solution in normal leukapheresis donors. Clinical symptoms include headache, swollen fingers, peripheral edema, and other signs of acute plasma volume expansion. In one study, donors were given daily infusions of 500 ml six percent HES over a two-hour period for a total of four days. These donors demonstrated an average increase of 37 percent in their plasma volume and an average total volume accumulation of 850 ml.87 Volume expansions of this dimension could pose a danger to donors with borderline cardiac function.92

**Venospasm or Vein Occlusion**

This complication is seen most often in continuous-flow procedures in which whole blood is drawn from the donor by suction through an i.v. line. Owing to the great individual variation regarding veins, the complication of venospasm may be seen at different flow rates in different individuals at a point at which the forces of suction place too much stress on the vein being utilized for the procedure.

**Chills**

Chills or shaking chills may be caused in individuals undergoing apheresis procedures by the rapid returning or infusion of cooled blood or fluids. This complication can be reduced or controlled by covering the donor with a blanket, increasing the donor room temperature, or by adding in-line warming units to the return tubing.92

**Anaphylactoid Reaction**

This complication is an acute cutaneous reaction characterized by urticaria, pruritus, flush, and occasionally angioedema. The cause is not fully understood. Attempts have been made to associate the reaction with the administration of six percent HES solution. However, the reaction has been observed in leukocyte donors receiving six percent HES as well as in platelet donors not receiving six percent HES.92 In addition, antibodies to HES have not been demonstrated convincingly in humans84 and recipients of HES-containing granulocytes do not become sensitized to HES.92

**Infections**

The possibility of infections from contaminated solutions used in apheresis procedures remains, but this complication is rarely seen.92 In one case, gram negative bacteremic shock developed in a healthy donor minutes after termination of a discontinuous-flow leukapheresis procedure with six percent HES and trisodium citrate. It was found that an unobserved hairline crack in the HES-trisodium citrate infusion bottle allowed contamination by Enterobacter cloacae and that this solution was subsequently given to the donor.52

**Other**

Other adverse reactions sometimes seen in apheresis donors include occasional hemorrhage, especially in those receiving heparin or excessive six per-
cent HES during the procedure; abdominal pain,\textsuperscript{133} and complications related to equipment failure or related technical problems.\textsuperscript{92} One recent report suggests a relationship between the administration of six percent HES to a donor and the subsequent development of lichen planus in the antecubital fossa near the infusion site a few days after completion of a leukapheresis procedure.\textsuperscript{5}

### Potential Risks to Donors

#### Potential Toxicity or Hazards of Six Percent HES

Hydroxyethyl starch is a branching glucose polymer derived from amylopectin which is treated with ethylene oxide to convert some of the hydroxyl groups of individual glucose units to hydroxyethyl ether groups.\textsuperscript{92} Hydrolysis of the resulting polymer yields a mixture of molecules with a molecular weight range of 10,000 to 2,500,000 daltons. The average M.W. of commercially available products are 70,000 and 450,000, respectively.\textsuperscript{67} The hydroxyethyl groups are attached most frequently to carbon-2 of the glucose units, which retards the action of serum amylases, preventing hydrolysis of HES molecules and permitting longer intravascular survival.\textsuperscript{25,26} These agents were originally developed to serve as volume expanders.\textsuperscript{92} Virtually all of an injected dose of six percent HES is recovered in urine and feces.\textsuperscript{56,67,86} Approximately 50 percent of an i.v. administered dose of six percent HES is cleared from the blood and excreted via the kidneys with 72 hours.\textsuperscript{67} The smaller HES molecules (<50,000 daltons) are rapidly cleared by the kidneys; the portion of larger molecules which are susceptible to hydrolysis by alpha amylase are hydrolyzed before excretion.\textsuperscript{92}

One study showed that the serum HES concentration did fall to the one percent level (of the initial level after administration) after 17 weeks of observation.\textsuperscript{8} The M.W. of HES molecules remaining 28, 56, and 108 days following an intermittent-flow centrifugation procedure dropped to and remained at 95,000 ± 5,000 so that the vast majority of the remaining molecules present were of a size that would not pass through renal glomeruli. These same molecules apparently contained sufficient numbers of hydroxyethyl groups to greatly retard enzymatic hydrolysis by plasma amylases.\textsuperscript{67} Concern has recently increased regarding multiple infusions of six percent HES into donors. Serial infusions of HES may result in intravascular accumulation of HES and HES has been detected, transiently, in both parenchymal and reticuloendothelial system cells of several organs.\textsuperscript{45,75} Complete elimination of HES from the circulation may require several months or longer after multiple injections.\textsuperscript{66,86}

In one study, a group of eight volunteers underwent four intermittent-flow centrifugation leukapheresis procedures spaced four days apart and were given a total of 500 ml of six percent HES in normal saline solution over a period of about three hours per procedure. Each donor received a total of 120 grams of HES. Serum concentrations of HES dropped to ten percent of prepheresis levels 78 days after the first procedure.\textsuperscript{67} Forward projection of the final portion of the elimination curve (assuming that the rate of elimination remains constant) allowed for the estimation that about 78 weeks of time are required for complete return of serum HES concentrations to baseline levels.\textsuperscript{67} Another concern is the effect of massive doses of HES on the donor.

Hydroxyethyl starch appears to affect the coagulation system in a dose-related fashion.\textsuperscript{49,109,117,119} Moderate amounts of HES (not exceeding 1,500 ml total volume, 20 ml per kg, or 800 ml per m\textsuperscript{2} during a 24-hour period) may cause mild
to moderate effects on the coagulation system including prolonging of prothrombin time (PT) and activated partial thromboplastin time (PTT), and decreased activity of factors V and VIII and fibrinogen, and decreased tensile strength of clots. These changes are statistically significant but are slight and transient and not considered to pose a real threat to effective hemostasis or to produce a tendency toward bleeding or thrombosis. However, massive doses of HES (>25 percent of the recipient’s blood volume) may be more hazardous.

Most studies have been done in dogs and show that massive doses of HES (>25 percent of blood volume) cause laboratory abnormalities in essentially all aspects of hemostasis, detectable almost immediately after HES infusion. Although the parameters of hemostasis began to improve within 48 hours after HES infusion, as long as seven days were required for hemostasis to approach normal values. The mechanism of the effects was not determined. There is an extreme paucity of information regarding the effects of massive doses of HES in humans.

Moderate difficulties have been encountered due to rouleaux formation in blood samples containing large quantities of HES (>30 percent) regarding the interpretation of typing and antibody screening. However, the rouleaux formation is readily dispersed with saline and can easily be distinguished from true agglutination by microscopic examination. The difficulty should be easily resolvable by experienced technologists, especially if they are aware that a blood specimen contains HES.

There has been a noted absence of reported complications in the tens of thousands of leukapheresis donors that have received six percent HES, so that the potential risks to HES appear to be minimal or absent. Several suggestions have been made regarding future use of HES. For example, if frequent leukapheresis of individual donors is necessary, then it may be possible to use lower doses of HES in each procedure. However, the effects of lower doses of HES on neutrophil collection have not been reported in clinical trials. Finally, preparations of HES that are more rapidly eliminated from the bloodstream should be available for general use in the future.

**Dextrans**

The dextrans have been tried as sedimenting agents in place of six percent HES with varying degrees of success. Dextran-150 appears to be the most promising of these agents (dextrans and gelatin) used as sedimenting agents and in a total of 700 procedures in which it has been utilized, no significant adverse reactions have been reported. No anaphylactoid reactions and only one episode of mild conjunctivitis has been noted. Granulocyte yields compare favorably with those using six percent HES. Dextran-150 may indeed be a safe and effective macromolecular sedimenting agent for use with the intermittent-flow cell separators. Too few studies have been done to fully evaluate its usefulness in continuous-flow leukapheresis.

**Corticosteroids**

Dexamethasone and prednisone are commonly used corticosteroids given to leukapheresis donors to demarginate leukocytes into the circulation and thus improve granulocyte yields in subsequent granulocyte collections. Dexamethasone is used at most centers because it has the following advantages: (a) it is potent and long-acting, (b) it may be given orally, (c) it has a minimum of mineralocorticoid activity, and (d) its use does not appear to impair the accumulation of neutrophils at inflammatory sites as may occur when prednisone is used. In ad-
dition, dexamethasone in single high doses (10 mg) probably does not cause any clinically important effect on platelet function. Dexamethasone is given orally at an average dose of four mg per m² body surface area, ten to 12 hours prior to the granulocyte donation at most centers, while prednisone, at 1.7 mg per kg body weight, is usually given orally, eight to 12 hours prior to leukapheresis at some centers. Single doses of corticosteroids have been reported to rarely evoke episodes of headache, fever, tiredness, sweating and changes in mood and performance. Other rare complications include: exacerbation of diabetes mellitus, peptic ulcers, tuberculosis and hypertension. The theoretical risk of suppression of adrenal function is considered to be improbable.

**Lymphocyte Depletion**

The total body pool of lymphocytes has been estimated to be at least $1 \times 10^{12}$ cells. The total number of lymphocytes removed during a single centrifugation leukapheresis has been estimated to be 2 to $4 \times 10^9$ cells or about twice that taken in an ordinary whole blood donation. Theoretically, as judged from these figures, a single leukapheresis might remove approximately 0.1 percent to 0.4 percent of the donor’s lymphocyte pool so that lymphocyte depletion and measurable immunosuppression would appear to be unlikely. Theoretically, 60 daily leukapheresis procedures would be needed to remove 10 to $200 \times 10^9$ lymphocytes which reportedly will cause lymphopenia and immunosuppression. However, several recent studies have failed to confirm this optimism.

In one study, 21 healthy donors underwent an average of nine cytaphereses (range six to 17) and were compared to a matched group of whole blood donors who served as controls. An average of six passes per procedure was done using the Haemonetics-30 discontinuous-flow equipment. The following changes were observed in the donors when compared to the parallel control whole blood donors and included: (a) 23 percent lower mean absolute lymphocyte count ($p < 0.01$), (b) 25 percent lower mean T-lymphocyte cell count ($p < 0.01$), (c) 46 percent lower mean B-lymphocyte cell count ($p < 0.001$), (d) serum protein changes including a 27 percent decrease in mean gamma globulin level ($p < 0.01$) and a 14 percent decreased IgG level ($p < 0.05$). Changes in IgA and IgM levels were not statistically significant. The loss of immunocompetent cells in each donation was about four to five times that of a standard whole blood unit. The alarming part of the study is that all but two of the apheresis donors were retested eight months after the first observation with no cytapheresis procedures done on these donors during the interval. There was no significant improvement in the total peripheral lymphocyte count, T-lymphocyte cell count, or immunoglobulin levels. There was a slight but not statistically significant increase in the number of circulating B-lymphocyte cells.

A short-term study of plateletpheresis donors revealed a 20 percent decrease in total lymphocyte count and a statistically significant decrease in the number of B cells. Another group observed no differences in IgG levels on a short-term protocol but noted appreciable differences in a long-term study of apheresis donors. A third group noted significant alterations in serum immunoglobulins in long-term plasmapheresis donors.

A study in which rheumatoid arthritis patients underwent removal of about $3.5 \times 10^9$ lymphocytes, twice or three times per week for five to seven weeks revealed an absolute lymphocyte depletion in the patients comparable to that reported for chronic thoracic duct drainage. The greatest changes in the peripheral lymphocyte count occurred...
after only three or four leukaphereses. There was a selective depletion of T-lymphocytes. In addition, there was a loss of PHA mitogenic responsiveness, and decreases in immunoglobulin (IgG, IgA and IgM) levels. In follow-up evaluations of these patients, the lymphocyte counts returned to at least 50 percent of normal in some in a period of days to weeks, but remained less than 50 percent in some patients for over a year.136

Lymphocytopenia and selective depletion of B-lymphocytes have been noted in plateletpheresis donors undergoing frequent donations.50

Several questions have developed from these studies.88 First, do frequent cytaphereses offer long-term hazards to the immune status of donors? Second, could HES cause reticuloendothelial blockage which might interfere with antigen processing by macrophages? Third, does the failure of donors to demonstrate a return of peripheral lymphocyte count and immunoglobulin levels to normal after a series of cytapheresis procedures represent a need for concern? Fourth, does long-term cytapheresis produce an imbalance in “helper” vs. “suppressor” T-lymphocytes? Further studies are definitely needed to determine the effects of cytapheresis on the ratios of helper T-cells to suppressor T-cells in these individuals. Fifth, what is the nature of the observed decreases in numbers of B-lymphocytes and immunoglobulin (IgG) levels? Is the decrease in IgG due to the decreased numbers of B-lymphocytes or due to a decrease in T-cell helper function?998

**Effects on the Complement System**

Profound changes in the complement system appear to occur during intermittent-flow centrifugal leukapheresis (IFCL) and may be due to the adsorption of complement proteins onto the surfaces of IFCL software.116 There is some evidence that there is complement activation involving both the classic and alternate pathways at the filter site as blood flows through the filters and returns to the donor in continuous-flow filtration leukapheresis (CFFL) procedures.116 In addition, changes in donor venous blood are a new finding that may indicate *in vivo* activation of the alternative pathway.115 Certainly, additional studies are needed to firmly establish the safety or hazards of cytapheresis to individuals regarding the complement system.

**Granulocyte Progenitor Cells (CFU-C)**

During leukapheresis, the donor undergoes cellular depletion to yield a cellular suspension containing a certain proportion of granulocyte progenitors that form colonies in agar (CFU-C).68 The effects of cytapheresis on the number of CFU-C cells was investigated in a total of 25 donors.68 Blood samples and evaluation were done on the day of donation and at further fixed dates for a period of six months after donation. Corticosteroids were given to granulocyte donors but not to platelet donors. In most cases there was no postdonation effect, and in those donors demonstrating minor changes, all values returned to normal (0-250 CFU-C per ml) within three months after donations. Several observations and conclusions were apparent in the study. First, the CFU-C count is very low in normal individuals. There is much individual variation, but the count remains relatively constant in a given individual.91 Second, the cell suspensions obtained by cytapheresis contained CFU-C numbers proportional to the CFU-C count of the donor peripheral blood. Third, the findings appeared to apply in cases of collection of granulocytes and immunocompetent cells.73,128
Suggested Guidelines for Allowable Frequency of Cyta- and/or Plateletpheresis

Suggested limitations have recently been made regarding donors undergoing cytapheresis procedures. Some of these suggestions are: (a) no more than eight leukocytapheresis procedures within a 12-month period of time should be performed on the same donor. (b) a donor should not generally undergo a combined total of more than 12 plateletpheresis and/or leukocytapheresis procedures during a calendar year, (c) a donor should usually not undergo more than two procedures within a week and six procedures within a month with at least 48 hours between procedures. The same general criteria should be used to select donors for apheresis procedures as is used to select donors for whole blood donation. In addition, and especially in cases of multiple frequent donations, the donors should have satisfactory white blood cell counts including differential and platelet counts prior to each procedure. Most centers require the platelet count to be greater than 150,000 per μL and the white blood cell count to be greater than 4,000 per μL. Other centers impose additional criteria for donor selection other than those already mentioned.

Donor Selection of Donors for Plateletpheresis (Leukapheresis)

Two main systems have been considered in donors to help prevent alloimmunization in recipients of platelets. These are: (a) ABH and (b) HLA, which is an extremely immunogenic and polymorphic system of antigens carried by platelets. Antibodies to platelet-specific antigens (PLA, Ko, PLE, and BAKa systems) are less commonly encountered (only one case of anti-BAKa) but can cause serious problems whenever they occur. Anti-PLA1 is probably the most frequently encountered platelet-specific antibody. Platelet transfusions matched for HLA antigens (A and B loci) are usually effective in preventing hemorrhage in some thrombocytopenic patients refractory to random-donor platelet transfusions. However, recent observations suggest that HLA matching does not reliably predict platelet transfusion responses. For example, poor posttransfusion increments are occasionally observed following HLA-matched platelet transfusion and alternatively, excellent posttransfusion increments have resulted after HLA-mismatched platelet transfusions. It has been suggested that the platelet migration inhibition assay is predictive of platelet response in both HLA-compatible and HLA-incompatible donor-recipient pairs. These same authors suggest that lymphocytotoxicity tests are predictive only in HLA-incompatible donor-recipient pairs, and that granulocytotoxicity, microleukoagglutination, and capillary leukoagglutination are of almost no value in predicting platelet transfusion increments, either in HLA-compatible or HLA-incompatible donor-recipient pairs. The presence of HLA-A2 antigen in alloimmunized recipients may be of significance in locating therapeutically suitable platelets. Recent data suggests that adequate platelet support of alloimmunized recipients may be more difficult in those who possess the HLA-A2 antigen.

Patient or Recipient Considerations

Indications for Granulocyte Transfusions

The indications for granulocyte transfusion to granulocytopenic patients are usually based on the following criteria: (a) reasonable chance for bone marrow re-
covery, (b) peripheral granulocyte count below 500 per cubic millimeter, and probably more critically a peripheral granulocyte count below 100 per cubic millimeter, (c) fever greater than 38°C (100.4°F) for 24 to 48 hours, and (d) known infection unresponsive to an adequate broad spectrum antibiotic regimen over a period of two or three days.26,38,41,78,113 Once granulocyte therapy has been initiated, it should be continued aggressively with granulocyte transfusions on a daily basis in surviving patients until the infection is under control and the bone marrow has recovered.26,41 Discontinuing the granulocyte transfusions after apparent infection control but before bone marrow recovery may lead to recurrence of the infection, or the appearance of new infections.41

Other influences on the decision to continue or discontinue granulocyte transfusions include: (a) inevitable or impending patient death in spite of granulocyte transfusions, (b) failure of bone marrow response after long-term maintenance with granulocytes and poor prospects of functional bone marrow recovery should granulocyte transfusions be continued, (c) development of antibodies against donor granulocytes, and (d) occurrence of complications associated with granulocyte transfusion such as severe transfusion reactions, fluid overload, sequestration of granulocytes in pulmonary lesions, intravascular aggregation of granulocytes, and possible reactions of transfused granulocytes with circulating endotoxins.36,41 Failure of bone marrow recovery leads eventually to ineffectiveness of subsequent granulocyte transfusions due to sensitization or to infection with resistant organisms.36 It is reasonable to provide therapeutic granulocyte support for at least seven days after initiation before reassessing or changing the therapeutic program.36

Lithium carbonate is a granulopoietic agent90,107 that can limit the degree of neutropenia in patients receiving chemotherapy ordinarily associated with mild to moderate myelotoxicity.32,106 In one study,105 27 patients receiving a standard cytosine arabinoside and daunorubicin regimen as induction or reinduction therapy of acute myelogenous leukemia (AML) were randomly assigned to receive lithium carbonate, 300 mg, three times per day, or no lithium. Lithium did not prevent the onset of severe neutropenia (0.1 x 10^9 granulocytes per liter) but did appear to shorten the duration of granulocytopenia (time during which granulocyte count was 1.0 x 10^9 per liter) from a median duration of 24.6 days for controls to 16.0 days for those receiving lithium (p = 0.013). The shortened duration of neutropenia may reflect an earlier granulocyte recovery in patients receiving lithium. However, the possibility remains that lithium might stimulate the recovery of leukemic cells as well as the recovery of normal granulocytes.105

Similar myeloprotective effects of lithium during chemotherapy have been reported in cases of malignancies other than leukemia, in which lithium would not be expected to increase recovery of the tumor cells.32,63,106 Protracted severe neutropenia may necessitate extensive broad spectrum antibiotic therapy with a risk of superinfection with fungi. Thus, shortening the duration of severe neutropenia during induction therapy of AML should decrease morbidity. Unfortunately, in this study lithium failed to prevent the granulocyte count from decreasing to values 0.1 x 10^9 per liter, did not appear to affect the rate and duration of remissions, and did not affect the incidence of infections so that the potential benefits of lithium appear to be limited.105

**Prophylactic Granulocyte Transfusions**

A number of studies have been conducted to evaluate the ability of prophylactic granulocyte transfusions to prevent infection and to support bone marrow recovery in patients receiving chemotherapy. While the results have been variable, there is some evidence that granulocyte transfusions can provide a measure of protection, particularly in high-risk patients.113,114,120,121 For example, in a study of patients with acute myelogenous leukemia (AML), granulocyte transfusions were associated with a reduced incidence of febrile neutropenia and an improved survival rate.113 In another study, patients receiving high-dose chemotherapy for lymphoma had a lower incidence of severe neutropenia and a higher neutrophil recovery rate when granulocyte transfusions were given prophylactically.120

In summary, the use of prophylactic granulocyte transfusions in cancer therapy appears to be a promising approach for reducing infection risk and supporting bone marrow recovery. However, further research is needed to optimize the timing, dosage, and selection of patients for this approach.114,120
lactic granulocyte transfusions to prevent the occurrence or complications of infections in neutropenic leukemic patients. One study involved prophylactic granulocyte transfusions during initial induction chemotherapy for AML. Of 102 uninfected randomized patients with AML, 54 were given daily granulocyte transfusions for a total of 28 days or until one of the following occurred: bone marrow recovery (granulocyte count > 0.5 × 10^9 per liter for 48 hours), death, severe transfusion reactions, gram-negative septicemia or withdrawal of patient consent and the remaining 48 patients served as controls and were not given granulocytes. Granulocyte transfusions decreased the proportion of patients with bacterial septicemia (nine percent vs. 27 percent in controls; p = 0.01). However, granulocyte transfusions did not reduce the incidence of other infections or improve bone marrow recovery, remission rate and duration of survival. A total of 72 percent of the patients given granulocytes experienced transfusion reactions. Pulmonary infiltrates were more common in granulocyte-treated than in control patients (57 percent vs. 27 percent in controls; p = 0.002). A total of 35 percent of the patients with pulmonary infiltrates died, as compared with five percent of those without infiltrates.

The investigators in this study concluded that prophylactic granulocyte transfusions should not be used during remission-induction chemotherapy in AML because the risks appear to outweigh the benefits. In a similar study, 13 of 24 patients with AML received granulocyte transfusions on alternate days during periods of marrow aplasia caused by initial induction chemotherapy with transfusions started whenever peripheral granulocyte counts became less than 500 per μl. Recipients received from one to 13 granulocyte transfusions with a median number of 1.45 × 10^{10} granulocytes per transfusion (range 0.28 to 3.45 × 10^{10}). Conclusions resulting from the study are that prophylactic granulocyte transfusions offer no significant advantage regarding: (a) deaths owing to infection, (b) reduction in the frequency of febrile episodes, (c) delay in the onset of fever, (d) reduction in the length of febrile episodes, or (e) reduction in the frequency of proven infection.

Of 65 noninfected patients with acute nonlymphocytic leukemia (ANLL), 29 were chosen to receive daily granulocyte transfusions commencing when the peripheral granulocyte count fell below 0.5 × 10^9 per liter and continued daily as long as the count remained below 0.5 × 10^9 per liter. Conclusions of the study are that prophylactic granulocyte transfusions from random donors, given once daily to patients with ANLL during chemotherapy-induced neutropenia: (a) do not decrease the number of septicemic episodes, (b) do not increase the chance of remission-induction, (c) do not prolong the patient’s survival, and (d) have no practical value in the routine care of patients with ANLL.

A total of 38 uninfected patients undergoing bone marrow transplantation were assigned at random to receive prophylactic granulocyte transfusions and oral nonabsorbable antibiotics (group 1) or oral nonabsorbable antibiotics alone (group 2) when their neutrophil count fell below 0.5 × 10^9 per liter. Both groups contained 19 individuals comparable in terms of age, sex, underlying disease, immunosuppressive therapy, and days of neutropenia. Conclusions made from results of the study of prophylactic granulocyte transfusions include: (a) they may prevent or decrease the number of episodes of septicemia, (b) they have no effect on other infections or survival in patients undergoing bone marrow transplantation, (c) they are associated with a higher incidence of cytomegaloviral (CMV) infections and (d) oral nonabsorb-
able antibiotics alone are equally effective in preventing serious infections in bone marrow transplant recipients.134

Finally, a study has been made recently of the cost-effectiveness of therapeutic and prophylactic leukocyte transfusions. Findings of the study lead to the estimates that prophylactic granulocyte transfusions would add an average of 35.2 percent to the recipient’s hospital bill and cost $37.8 million annually, while therapeutic granulocytic transfusions (in those patients at particular risk of death from infection) would add an average of 10.9 percent to the recipient’s hospital bill and cost $17.7 million annually nationwide.89 Leukocyte transfusion is an extremely expensive technological procedure and should be made as cost-effective as possible. One possibility of improving cost-effectiveness would be granulocyte transfusions to patients at particular risk of death from infection rather than transfusion of all patients with neutropenia or neutropenia and infection, but data on this possibility is not yet available. When such data become available, cost-effectiveness analysis may be helpful in determining which individuals should receive this expensive treatment.89

An evaluation of 67 episodes of gram-negative bacteremia was made.61 Patients had a median absolute granulocyte count of $1 \times 10^9$ per liter at the time of bacteremia. Empiric antibiotic regimens were begun at the first evidence of suspected infection. Granulocyte transfusions were employed only as clinically indicated by inadequate patient response to antibiotic therapy. Among the 29 patients who demonstrated an increase in their granulocyte count of >100 per $\mu l$ over the 14 days subsequent to the initiation of therapy, 27 (93 percent) recovered while among the 38 patients exhibiting no appreciable increase in granulocyte count 21 (55 percent) improved ($p = 0.006$). In the latter group (no appreciable increase in granulocyte count) susceptibility of the pathogen to the initial empiric antibiotic regimen (two different antibiotics) was of major importance. In four cases the pathogenic organism was resistant to both antibiotics and 0/4 of the patients responded. In 14 cases the organism was susceptible to one of the two antibiotics and 6/14 (44 percent) responded. In 20 cases the organism was susceptible to both antibiotics and 15/20 (75 percent) responded ($p = 0.025$).

A number of conclusions were made from results of the study.61 First, patients with gram-negative bacteremia and persistent granulocytopenia will often respond to antimicrobial therapy alone provided the initial choice of empiric antibiotics is appropriate and that their use is instituted promptly. Second, regarding granulocyte transfusions: (a) they are expensive and time-consuming to obtain, (b) there are potential complications for both donor and recipient, (c) it is imperative to carefully identify the patients for whom granulocytes can be most beneficial38,61,78,97,113 and advisable in some patients to obtain a bone marrow aspirate in order to assess the likelihood of prolonged aplasia before reaching a decision regarding granulocyte transfusion,97 (d) granulocyte transfusions can be beneficial in patients with prolonged aplasia, gram-negative bacteremias, and inadequate initial clinical response, and (e) granulocyte transfusions are not necessary for the majority of patients who are anticipated to have evidence of granulocyte or bone marrow recovery within a short time period.61 Knowledge of donor and recipient ABO-Rh blood types and, if possible, HLA types and using donor granulocytes from donors of the same ABO-Rh type and similar or identical HLA type can usually increase survival times of granulocytes (and platelets) and probably delay alloimmunization to the granulocytes and platelets.61

Finally, in making decisions about the
administration of granulocyte transfusions to granulocytopenic individuals, one should be aware of the hazards and contraindications concerning these transfusions. Some of the hazards and complications of granulocyte transfusion include:\(^{110}\) (a) immediate transfusion reactions, (b) hypersensitivity reactions, (c) pulmonary infiltrates, (d) alloimmunization, (e) transmission of infections, especially viral (such as hepatitis or CMV), and (f) the possibility of inducing graft vs. host (GVH) disease. Situations such as extracorporeal perfusion, trauma, sepsis, or acute pancreatitis may chaotically activate a patient’s complement system resulting in production of complement component C5a which may cause granulocyte aggregation, increased granulocyte adherence to endothelium, and leukoembolization.\(^{46}\)

In addition, C5a may induce granulocytes to produce toxic oxygen compounds such as superoxide and hydrogen peroxide which can damage endothelium and, in cases of pulmonary involvement, contribute to the development of the adult respiratory distress syndrome (ARDS). This is characterized by the plugging of the pulmonary microvasculature with granulocytes which may cause pulmonary endothelial damage leading to protein-aceous interstitial and, ultimately, alveolar edema. Granulocyte transfusions may be clinically dangerous under these circumstances and worsen the recipient’s prognosis since the lungs might be the principal organ of granulocyte sequestration.\(^{46}\) Corticosteroids, in high doses, inhibit granulocyte aggregation, adherence to endothelium, and superoxide production, and thus may play an important role in preventing or lessening pulmonary complications such as ARDS in septic individuals receiving granulocytes.\(^{46}\) In addition, amphotericin-B is used in the treatment of fungal infections in cancer and immunosuppressed patients. There have been recent reports claiming serious interactions between amphotericin-B and transfused granulocytes in the recipients.\(^{137}\)

Indications for Platelet Transfusions

Approximately \(3.5 \times 10^{10}\) platelets are produced daily by the bone marrow, and production rates up to six to eight times normal have been observed.\(^{98}\) About one-third of these are normally pooled in the spleen. Under normal conditions, the platelet has an age-related life span of approximately ten days. However, in thrombocytopenic patients with platelet counts of less than \(70 \times 10^9\) per liter, platelets survive for only about five days,\(^{102}\) possibly related to an ongoing platelet-endothelial cell interaction. Bleeding times remain normal at four and one half ± one and one half minutes, with platelet counts of \(100 \times 10^9\) per liter or greater, and vary inversely with the platelet count in patients with depressed platelet production.\(^{35}\) At levels between 10 and \(20 \times 10^9\) platelets per liter, the bleeding time may exceed 30 minutes.\(^{102}\) Stool blood loss measurement in patients with production-related thrombocytopenia has indicated that bleeding does not exceed the five ml. per day found in normal individuals until platelet counts are less than \(10 \times 10^9\) per liter. Substantial increases in stool blood loss are recorded only in patients with less than \(5 \times 10^9\) platelets per liter.\(^{102}\)

Under optimum conditions, transfused platelets should maintain hemostasis for approximately five days following transfusion.\(^{30}\) A one-hour posttransfusion platelet count is helpful in determining if an adequate level of platelets has been obtained to achieve improved hemostasis. Subsequent platelet counts at four to six hours and 24 hours postinfusion allow a rough calculation of survival and thus an indication of the necessary transfusion frequency.\(^{99}\) Platelet counts much higher than \(10 \times 10^9\) per liter are needed
in situations in which the vascular system is not intact, prior to surgical procedures, and in many cases in which individuals are receiving therapy for cancer or other disorders. In addition, much higher platelet counts are needed in situations producing significant platelet dysfunction. Common mechanisms for induction of platelet dysfunction are drugs such as aspirin or semisynthetic penicillins, uremia, fibrinogen-fibrin degradation products, the events associated with certain types of surgery such as cardiopulmonary bypass surgery, and certain clinical conditions associated with the production of abnormal platelets. Absolute platelet counts cannot be used as the only measure for determining the need to transfuse platelets in these situations, and the need for transfusion has to be evaluated on an individual basis. Platelet transfusions have a role in preventing hemorrhage and thus reducing mortality in thrombocytopenic cancer patients. Platelet transfusions would appear to be indicated, therefore, in any patient with excessive bleeding who has a documented deficiency of either platelet number or function. Effective platelet transfusions should show improvements in hemostasis that directly reflect the increment in functional platelets achieved. Among the problems associated with frequent platelet transfusions are alloimmunization and the transmission of viral infections such as hepatitis and CMV.

The current best use of apheresis platelets is to provide therapeutic doses of single donor matched platelets for patients refractory to pooled random donor platelets. It is interesting that in some series as many as half of the chronically transfused recipients never become immunized to platelet transfusions, whereas exposure to only a few donors may immunize susceptible recipients to these donors as well as a large number of other donors. Fortunately, those individuals who do become immunized to random platelet donations do not show impaired responses, in most cases, to subsequent transfusions obtained from matched donors.

A retrospective analysis of 352 donors who underwent plateletpheresis at least four times resulted in the following observations: (a) restricting plateletpheresis to donors with a prepheresis platelet count of 150,000 per μl would have allowed postpheresis platelet counts of less than 100,000 per μl in only 1.3 percent of the procedures and would have eliminated only 3.7 percent of donations, and (b) plateletpheresis yields were related to the prepheresis platelet count, number of cycles, sex, type of procedure (plateletpheresis or leukoplateletpheresis), and yield recorded during previous collections.

Various instruments are available to obtain single donor platelets (and granulocytes) and include one discontinuous-flow and two continuous-flow models.

Characteristics of Platelets Collected by Plateletpheresis

A number of studies have been made regarding the function and survival of platelets collected by plateletpheresis or leukoplateletpheresis and subsequently administered to recipients. In one study, the effects of intermittent-flow centrifugation pheresis (IFCP) were evaluated by comparing platelets collected from ten donors by combined

* Haemonetics Model 30 Blood Processor.
† IBM-2997.
‡ Fenwall CS3000.
plateletleukapheresis (LP) with HES with those collected from ten donors using plateletpheresis without HES (PP).66 These investigators concluded that combined LP collected greater numbers of platelets than PP, and that HES, at doses currently used, does not adversely affect platelet function. Combined LP can be performed by IFCP using HES to increase neutrophil yields and obtain large numbers of platelets as a by-product during the procedure.66 Investigators in another study agreed that HES does not induce platelet dysfunction after one exposure in previously unpheresed donors65 and pointed out that the results are similar to those seen by using dextran at low doses.130

**Effects of ABO Compatibility on Platelet Transfusions**

Studies have suggested that ABO incompatibility significantly reduces the effectiveness and survival of transfused platelets. The mean 24-hour recovery of platelets from histocompatible donors and from donors selectively mismatched for crossreactive HLA antigens was decreased by approximately 23 percent of the donor types for blood group A and/or B not found in the recipient. However, the reduction in platelet recovery associated with ABO incompatibility is not of a magnitude that would contraindicate transfusion of ABO-mismatched platelets.17

**Alloimmunization**

Alloimmunization represents the major complication of platelet transfusion therapy and is characterized clinically by the failure to achieve expected platelet count increments after transfusion.96 However, recent data from a number of centers indicate that in cancer patients receiving platelet transfusions as well as cytotoxic and perhaps immunosuppressive therapy, alloimmunization develops in only a minority of recipients.55,96 In recent studies of patients treated with standard, intensive induction chemotherapy, only about 40 to 50 percent of patients with acute leukemia20,21,95 and an even smaller number (ten percent) of patients with solid tumors became alloimmunized.39

Another approach to the prevention or management of alloimmunization is to use autologous frozen platelets. Patients with leukemia undergo plateletpheresis during remission, with the platelets frozen using dimethylsulfoxide (DMSO), and these platelets are saved for subsequent transfusion during later courses of chemotherapy.93,94 The results using this technology have been encouraging with transfusion recovery averaging approximately 60 to 65 percent of that expected using fresh platelets. For many patients, autologous frozen platelets have represented the only source of platelet transfusion making administration of further maintenance therapy possible.96 This approach is presently not practical except at large cancer centers.

**Storage of Granulocytes and Platelets**

Granulocytes retain some functional capacity for about 24 hours when preserved in whole blood62 or in concentrates obtained from leukopheresis procedures.27,83 Agitation of the granulocytes during this period is probably undesirable and may cause a reduced chemotactic response.71,72,104 Some investigators suggest storage of granulocytes at 4°C while others suggest 22°C.27,72 The use of dexamethasone to improve storage characteristics has been tried.39 Glucocorticoids have beneficial effects in the storage of certain tissues and organs and are known to stabilize the membranes of lysosomes4,29,43,69,103,131 which conceivably could enhance the storage of neutrophils by lessening autolysis as well as
by decreasing the lysis of adjacent cells by enzymes released into the storage fluid. However, to date, results of this approach have been disappointing. Although storage of granulocytes should be possible, it is technically not clearly possible especially in regards to long-term storage or freezing. A great deal of technical advancement is desperately needed before long-term storage of granulocytes is possible and practical.

The capabilities of platelet storage are well advanced over that of granulocyte storage. Platelets stored at room temperature with gentle agitation can be transfused for up to three days after preparation and still exhibit reasonable survival and function although the numbers of recoverable platelets may gradually decrease progressively during storage. Recently, new platelet container bags have been developed and made available which allow the platelets to “breathe” or undergo limited gaseous exchange with the environment, thus delaying pH changes in the platelet concentrate. Platelets can be transfused as long as five days after collection and maintenance in these new bags with reasonable platelet recovery and function. Five key factors are currently of importance in the short-term storage of platelets at ambient temperature and the maintenance of platelet viability. These are: (a) pH of the suspending plasma, (b) concentration of platelets, (c) continual gentle agitation during storage, (d) temperature of storage, and (e) plastic composition of the storage container. Platelets collected by plateletpheresis are generally administered within 24 hours after collection, largely due to considerations of possible infectious contamination. A study was conducted in which platelet concentrates were examined for evidence of bacterial contamination and subsequent growth from three to 11 days after collection. Four to 126 platelet concentrates examined revealed bacterial growth, and growth patterns indicated contamination during the microbiological manipulations rather than contamination of the units during collection or preparation. These researchers concluded that platelet concentrates prepared can be safely transfused for up to three days after collection if stored at 4 to 6°C.

New formulations, CPDA-1, CPDA-2, and CPDA-3 which contain 34 mg adenine per 63 ml preservative and extra glucose, have made possible the extended storage of whole blood or packed red blood cells. The effects of storing platelets in these new formulations at 22°C for periods up to 72 hours have been studied. Morphology score, pH changes, platelet size, population distribution parameters and electron microscopic ultrastructural considerations did not show any adverse effects which could be ascribed to the presence of adenine or extra glucose or both. Adenine and extra glucose in the storage medium do not appear to be harmful to platelets in vitro.

Cryopreservation of platelets offers the advantages of stockpiling of serocompatible platelets and the preservation of autologous platelets at some of the larger medical or blood centers. Current methodology involves the storage of platelets at −80°C in five percent dimethylsulfoxide (DMSO) or five percent glycerol or in glycerol-glucose (five percent glycerol and four percent glucose) solutions in the vapor phase of liquid nitrogen (approximately −120°C). Recovered platelets with both methods have been reported to perform adequately up to six hours postthaw. One group reported that platelets can be cryopreserved by these methods for greater than three years with satisfactory posttransfusion increments. It is interesting to note that platelets frozen in

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* Fenwal.
† Collected by Haemonetics-30 equipment.
DMSO for periods up to 20 weeks appear to retain full HLA antigenic activity in spite of the combination of freezing, thawing and washing procedures, and the presence of the surface-active cryopreservation. In all cases, the previously frozen platelets were quantitatively equal or superior to the platelets stored at 4°C with regard to their capacity to specifically reduce the HLA antibody activities of selected typing sera against a panel of antigen-positive lymphocytes.

Prospects or Goals for the Future

Several prospects or goals have recently been suggested regarding the collection of granulocytes and platelets by apheresis techniques and subsequent administration to recipients. First, more reliable methods are needed to determine which febrile neutropenic patients are likely to recover without granulocyte support and which patients definitely require this expensive form of therapy. Second, better, more efficient and less expensive methods of procurement are clearly needed. The limiting factor is donor-time and maximizing yields during each procedure would be of great advantage, and probably make the prophylactic use of granulocytes more practical. Third, a more reliable system for measuring antigranulocyte antibodies is needed and would permit better donor-recipitent pairing. Fourth, the possibilities of synergism between granulocyte transfusions and antibiotic therapy and residual host defenses should be investigated thoroughly. Fifth, successful cryopreservation of granulocytes might offer significant advantages especially at the larger medical or cancer centers and blood centers where storage of such products is practical. Finally, the development of additional sedimenting agents for use in leukapheresis is needed. These agents should have shorter biological half-lives within donors and recipients and not present the lingering safety concerns seen with HES.

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