Prophylactic Platelet Administration During Massive Transfusion

A Prospective, Randomized, Double-Blind Clinical Study

R. LAWRENCE REED II, M.D.
DAVID CIAVARELLA, M.D.
DAVID M. HEIMBACH, M.D.
LOIS BARON, B.S.N.
EDWARD PAVLIN, M.D.
RICHARD B. COUNTS, M.D.
C. JAMES CARRICO, M.D.

Prior studies at Harborview Medical Center have suggested that dilutional thrombocytopenia is a major etiology of microvascular, nonmechanical bleeding (MVB). We undertook a prospective randomized double-blind clinical study to compare the prophylactic effects of 6 units of platelet concentrates (PLT) *versus* 2 units of fresh frozen plasma (FFP) administered with every 12 units of modified whole blood in patients undergoing massive transfusion (12 or more units in 12 hours). After exclusions, three of 17 patients who received PLT and three of 16 patients who received FFP developed MVB, an incidence no different from our previous findings. Regression lines of platelet counts during transfusion were no different between groups, and both groups had higher platelet counts than predicted from a standard washout equation. Only one patient had evidence of dilutional thrombocytopenia as a cause for MVB. Prophylactic platelet administration is not warranted as a routine measure to prevent MVB.

From the Departments of Surgery, Medicine, and Anesthesiology, Harborview Medical Center, and the Puget Sound Blood Center, Seattle, Washington

MASSIVE TRANSFUSION of stored blood may result in diffuse microvascular bleeding (MVB), a syndrome characterized by the onset of oozing from mucosa, raw wounds, and puncture sites.[1,2] A primary disorder of hemostasis is suggested, as standard surgical techniques do not effectively arrest the process. Multiple etiologies have been implicated. However, several studies have shown that dilutional thrombocytopenia is a major contributing factor.[3-5]

We documented a strong correlation between platelet counts and the amount of blood transfused in a prior study of hemostasis in massively transfused trauma patients.[3] This study revealed the likelihood of thrombocytopenia in patients receiving blood replacement greater than 1.5 times their blood volume. The modified whole blood (MWB) transfused (from which platelets and/or cryoprecipitate are salvaged before storage)[6] provided hemostatic levels of all clotting factors in the majority of patients without the need for supplementation with fresh blood or fresh frozen plasma (FFP). Although the levels of factors V and VIII are diminished in MWB, hemostatic levels were maintained *in vivo,* except in patients whose combined deficiencies of platelets and fibrinogen suggested a consumptive process. Microvascular bleeding was corrected by platelet concentrate (PLT) transfusion.[3]

Because of the considerable evidence that dilutional thrombocytopenia is the major cause of the diffuse microvascular bleeding seen during massive transfusion, we sought to determine whether prophylactic administration of platelets to patients undergoing massive transfusion would prevent this complication. Since 6 units of PLT (a standard dose) contain the equivalent of 1.5 to 2 units of FFP, any effectiveness of platelet transfusions may not be solely the result of platelets but also of the plasma constituents. Therefore, we also examined the hypothesis that prophylactic PLT transfusions were more efficacious than prophylactic FFP infusion in the prevention of the microvascular bleeding associated with the massive transfusion of stored blood.

Presented in part as the Winning Paper, American College of Surgeons Committee on Trauma Residents' Papers Competition, Dallas, Texas, March 7, 1985.

Supported by NIGMS Grant 5-RO1-GM31150-03 and NIH Grant 5-T32-GM07037.

Reprint requests: R. Lawrence Reed II, M.D., Department of Surgery, University of Texas Health Sciences Center at Houston, 6431 Fannin, Texas Medical Center, Houston, TX 77030.

Submitted for publication: July 1, 1985.

Methods

Protocol

The study protocol was reviewed and approved by the Human Subjects Review Committee of the University of Washington. All patients 18 years of age or older admitted to the emergency room of Harborview Medical Center who were likely to require massive transfusion (defined as receiving 12 or more units of blood within a 12-hour period) were considered for study. The following were excluded from entry into the study: patients with known severe pre-existing liver disease or portal hypertension; patients with demonstrated pre-existing thrombocytopenia or coagulopathy; patients with penetrating injuries to the brain[7,8]; patients whose major bleeding source was the gastrointestinal tract; patients with burns covering more than 10% of body surface area[9]; and patients receiving low molecular weight dextran (Table 1). Whenever possible, informed consent was obtained prior to entry into the study; however, because of the sudden and serious nature of the patients' conditions, randomization was often undertaken before consent was obtained.

Upon entry into the study, blood samples for hematocrit, platelet count, prothrombin time (PT), partial thromboplastin time (PTT), thrombin time (TT), and assays of coagulation factors I (fibrinogen), II, V, VII, VIII, IX, X, and XI were obtained. After 12 units of blood had been transfused, blood samples were again collected and the patient was randomized to receive either 6 units of PLT (containing approximately 420 ml of plasma) or 2 units of FFP (containing about 440 ml). Either blood component was packaged in a single transfusion pack of identical appearance. The true nature of the blood component being infused was known by the blood bank personnel but was not revealed to either the research staff or the physicians caring for the patient at the time of administration. This sequence was repeated with each 12 units of transfused blood, with the patient receiving the same component after 24 units, 36 units, and so on, as received at the initial 12 units. In most cases, blood samples for the studies mentioned above were also obtained 30 minutes after the component was given.

A final blood sample for coagulation studies was obtained 12 to 24 hours after the last component was administered. However, if a patient developed laboratory evidence of disseminated intravascular coagulation (defined as a platelet count less than 80,000/μl and fibrinogen less than 80 mg/dl) or microvascular bleeding, an exit blood sample for coagulation studies was obtained, the patient was removed from the study, and specific therapy was given as indicated. Microvascular bleeding (MVB) was determined by the attending physician based on the following criteria: bleeding from mucous membranes; oozing from catheter sites that persisted after application of pressure; continuous oozing from surgical wound and raw tissue surfaces; and generalized petechiae or increased size of ecchymoses. No other coagulation product was administered during the time period of the study; if such were thought necessary by the attending physician, an exit blood sample was obtained for analysis, and the patient was removed from the study.

TABLE 1. *Criteria for Exclusion from Study*

1. Patients with severe, pre-existing liver disease or portal hypertension. Liver disease was assessed by one of the following:
   a. Biopsy-proven cirrhosis;
   b. Serum bilirubin ≥ 2 mg/dl in the absence of hematologic disease;
   c. Serum albumin ≤ 2.5 g/dl in the absence of nephrosis or malnutrition;
   d. Baseline PT 4 seconds above control (with commercial PT reagents).
2. Patients with pre-existing thrombocytopenia (<100,000/μl) or coagulopathy.
3. Patients with penetrating wounds to the head, with concurrent brain injury.
4. Patients whose major source of bleeding was the gastrointestinal tract.
5. Patients with burns greater than 10% of body surface area.
6. Use of low molecular weight dextran.

Laboratory Methods

Whole blood was drawn from healthy random donors at the Puget Sound Blood Center into CPD anticoagulant and stored at 2–6° according to standard procedures. Platelet concentrates (PLT) were produced according to the method of Slichter and Harker.[10] Cryoprecipitated factor VIII concentrates were prepared by the method reported by Slichter et al.,[6] using a refrigerated freezing-thawing bath that allows the cryoprecipitate to be removed and modified whole blood to be available for transfusion within 8 hours of the time the blood was collected. FFP was prepared by standard methods. Platelets were stored at 22 C for ≤72 hours,[11] each unit containing 60–70 ml of plasma. Clotting factor levels in this plasma have been shown to contain >80% of all factors except factors V and VIII, which are at ≥50% levels. Hematocrits were calculated from the erythrocyte count and the mean corpuscular volume using an electronic particle counter. Platelet counts were also performed using an electronic particle counter.[12,13] Blood for coagulation screening tests and clotting factor assays was collected into plastic tubes containing 1/10 volume of 3.8% sodium citrate, appropriately adjusted for hematocrit.[14] Samples were spun at 15,000 × g for 20 minutes and frozen within 2 hours of collection at −70 C until assayed. The PT was performed by the method of Quick using human brain thromboplastin,[15] the PTT by the method of Proctor and Rapaport

TABLE 2. *Pre-entry Exclusions*

Reason for Exclusion	Number of Patients
Delayed notification	23
Liver disease	3
Penetrating brain injury	2
Burn	2
Dextran use	3
Rapid demise	3
Only packed cells available	1
Suspected platelet antibodies	1
DIC	1
Surgeon's choice	4
Total	43

using human brain cephalin,[16] and the TT by the method of Brodsky.[17] Fibrinogen was assayed by a kinetic method.[14,18] Values < 100 mg/dl were repeated using a total clottable method.[19,20] Factors II, V, VII, and X were assayed by a one-stage test based on the PT. Factors VIII, IX, and XI were assayed by a one-stage test based on the PTT. Normal plasma for coagulation standards was a pool of plasmas from 25 healthy donors. Plasma deficient in Factor II was prepared by mixing equal volumes of aged normal serum and $AL(OH)_3$ (Amphojel®) absorbed plasma. Plasma deficient in Factor V was aged, oxalated plasma. Plasmas deficient in factors VIII and IX were obtained by plasmapheresis of patients with severe hemophilia A or B. Plasma deficient in Factors VII, X, and XI were purchased from Helena Laboratories, Beaumont, Texas.

Data Analysis

Standard statistical methods were used for data analysis, with grouped data presented as the mean ± 1 SEM. A one-way analysis of variance was first performed to test significance of differences between several groups of samples. If this test indicated that a difference existed, then a two-sample t-test was performed to assess the differences between individual groups. Bonferroni's method was used for correction of critical values in the case of simultaneous multiple comparisons.[21] Differences in proportions were analyzed by chi square analysis and, when expected values were less than 5, by Fisher's exact test. Regression lines were calculated by the least-squares method and correlations estimated by calculation of the correlation coefficient, r, and the coefficient of determination, R^2. Analysis of variance was used to calculate variance ratios, distributed as F, to test the significance of the regression. Differences between correlation coefficients were analyzed by Fisher's z-test.[22]

TABLE 3. *Post-entry Exclusions*

Patient #	Component Group*	Diagnosis	Reason for Exclusion
1	FFP	Trauma	Laboratory†
12	PLT	Trauma	Protocol break
17	PLT	Trauma	Blood samples clotted
28	PLT	Trauma	Blood samples lost
32	PLT	Trauma	Protocol break
33	PLT	Trauma	Protocol break
37	FFP	Aneurysm	Laboratory†
38	PLT	Aneurysm	Laboratory†

* FFP = fresh frozen plasma (2 units/dose); Plt or Platelets = platelet concentrates (6 units/dose).

† Platelet count <80,000/μl *and* fibrinogen < 80 mg/dl before receiving component.

Results

Patient Entry and Exclusions

During the period from September 1, 1982, through November 7, 1983, 84 patients were admitted to Harborview Medical Center who were at least 18 years old and required at least 12 units of blood transfused within 12 hours. Of these, 23 were omitted from the study because their eligibility was not recognized early enough and/or nonrandomized components were administered. Three others expired before adequate blood samples could be obtained. Thirteen other patients were excluded from study for various reasons: three for severe pre-existing liver disease, two for penetrating injuries to the brain, three for intraoperative use of low-molecular weight dextran, two for severe burns and thrombocytopenia, one in whom it was determined prospectively that only packed red cells would be available, one patient with very early development of disseminated intravascular coagulation (DIC), and one (who had previously received massive platelet transfusions) who was suspected of having antiplatelet antibodies. Four patients were excluded from the study at the request of the attending physicians; all had severe bleeding at the sites of injury. One of these patients was felt to have diffuse microvascular bleeding after only 8 units of transfused blood with thrombocytopenia (platelet count = 45,000/μl) as his only significant clotting abnormality. A summary of these pre-entry exclusions is provided in Table 2.

Thus, 41 patients were eligible nonexcluded candidates for study. These patients were prospectively randomized; 22 received platelet concentrates (PLT) and 19 received fresh frozen plasma (FFP). Eight patients were later excluded because of breaks in protocol or because a reason for exclusion was not realized until after entry (Table 3). There were nine patients with ruptured abdominal aortic aneurysm (AAA) and 32 patients with trauma, most as a result of motor vehicle accidents. Of the nine patients with AAA, two were excluded from the final analysis because of presentation with massive hemostatic breakdown (*i.e.*, a laboratory exclusion). The trauma patients included

TABLE 4. *General Characteristics*

Group	Platelets	Fresh Frozen Plasma
Number	17	16
Age (years ± 1 SD)	58 ± 19	43 ± 22
Range	19–88	20–82
Male/female	11/6	13/3
Mortality	10/17 (59%)	8/16 (50%)
Diagnosis		
Ruptured aneurysm	4	3
Trauma	13	13
Penetrating	2	1
Blunt	11	12
Liver laceration	1	5
Pelvic fracture	3	2
Hours of hypotension*	0.7 ± 0.8	1.4 ± 1.4
Hours of hypoxia†	0.6 ± 1.6	1.6 ± 3.0
Liters of crystalloid	10.5 ± 3.8	13.5 ± 5.5

* Hypotension = systolic BP < 80 mmHg.
† Hypoxia = arterial PO_2 < 60 mmHg.

nine patients with liver trauma (3 PLT, 6 FFP), two of whom were excluded from final analysis for breaks in protocol, and six patients with pelvic fractures (3 PLT, 3 FFP), of whom one was excluded for a break in protocol.

General Characteristics

Of 41 patients entered, 24 expired (59.9% mortality). After exclusions, the mortality rate was 55% (18/33) (Table 4). While age, duration of hypoxia, and diagnoses were relatively well-matched between the groups, the patients receiving fresh frozen plasma did have a longer period of hypotension and slightly more crystalloid solution infused on average than the patients receiving platelet concentrates, although this does not achieve statistical significance ($0.05 < p < 0.10$ by two-tailed t-test).

The transfusions were relatively rapid, with 80% of the total transfusion accomplished within the first 6 hours. Four out of five units of the blood administered were in the form of modified whole blood. These same relationships held when the patients were broken down by treatment group (Fig. 1). An average of 20.6 units of blood was transfused per patient with ranges of 12 to 39 units for the PLT group and 14 to 41 units for the FFP group.

Hematocrits initially taken in the field were relatively normal (Fig. 2). However, by the time the patients arrived at the hospital, these values had dropped by 25% ($p < 0.05$), indicative of the rapidity of hemorrhage, transcapillary refilling, and exogenous fluid administration occurring in these patients. By the time 12 units of blood had been infused, the decline in hematocrit had been arrested.

Incidence of MVB

After exclusions, 33 patients were included in the statistical analysis. Of these, 17 patients were randomized to receive PLT and 16 patients received FFP. Six patients exhibited abnormal microvascular bleeding, three from each component group. This was an incidence of approximately 18%, a rate no different from that seen in our previous study of patients with similar injuries who received no prophylactic therapy whatsoever (Table 5).

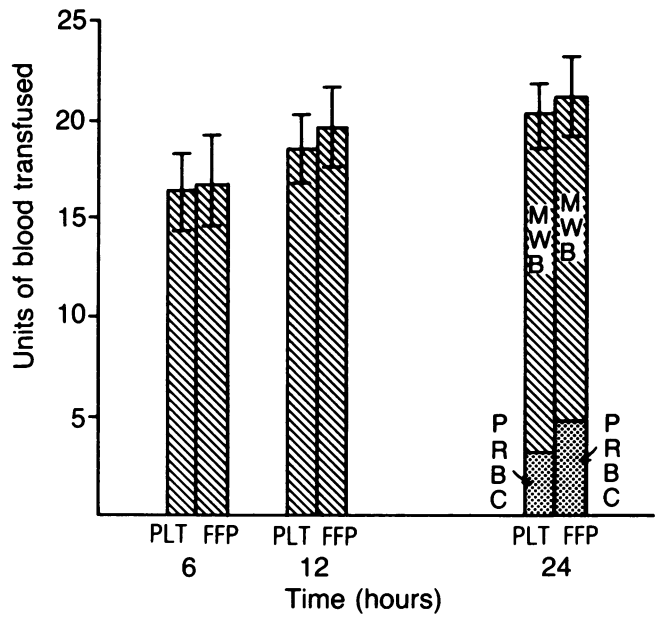

FIG. 1. Cumulative volume of blood (in units) transfused at three different time points. Top of each vertical bar represents group mean with crossbars indicating ±1 SEM. PLT = 17 patients who received prophylactic platelet component therapy; FFP = 16 patients who received prophylactic fresh frozen plasma component therapy. MWB = modified whole blood; PRBC = packed red blood cells.

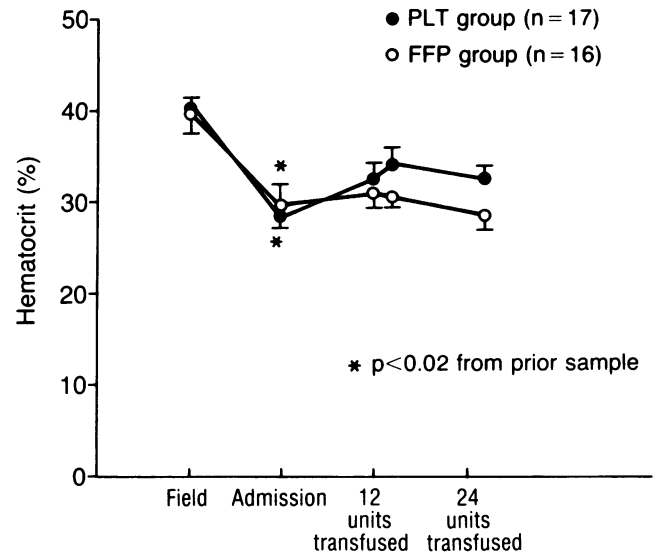

FIG. 2. Alterations in group mean hematocrit values (±1 SEM) at four sample time points. PLT = patients receiving prophylactic platelet component therapy; FFP = patients receiving prophylactic fresh frozen plasma therapy. No significant differences between the two study groups exist.

TABLE 5. *Incidence of Microvascular Bleeding*

	Platelet Group	FFP Group	Prior Study[3] (No Treatment)
Number of patients	17	16	24*
Number with MVB†	3	3	5
(Per cent)	(18)	(19)	(21)

* Excluding patients with disseminated intravascular coagulation.
† MVB = microvascular bleeding.

Six patients had been ingesting alcohol just prior to injury; none of these patients exhibited microvascular bleeding. One patient (#38) who was excluded for laboratory reasons was taking warfarin and dipyridamole at the time of admission. Importantly, patient #31 (PLT group), who developed MVB after 16 units of blood, was taking aspirin at the time of admission for a ruptured AAA. This may have played a role in the development of his abnormal bleeding. There was no other significant history of anticoagulant drug ingestion, bone marrow failure, thrombocytopenia, coagulation factor deficiency, or vasculitis in any study patient.

A clinical description of the patients with MVB is found in Table 6. Only one patient (#19) showed evidence that dilutional thrombocytopenia was responsible for the MVB. This patient was randomized to receive FFP prophylactically, developed thrombocytopenia with MVB after 20 units of blood were transfused, and was effectively treated with a single dose of 6 units of platelet concentrates. Two patients who received PLT (#23 and #31) and one patient who received FFP (#29) required multiple doses of platelet concentrates (6 units per dose) over several days for persistent thrombocytopenia and diffuse oozing. In these cases, infusion of platelets raised the platelet counts and controlled the oozing transiently, but subsequent reductions in platelet counts occurred with recurrent oozing. This phenomenon continued for 2 to 4 days following the termination of the massive transfusion of modified whole blood. The other two patients with MVB (#22 and #40), one from each treatment group, died from other causes with their coagulopathy uncorrected.

TABLE 6. *Patients with Microvascular Bleeding: Response to Treatment*

Patient Number	Study Group*	Platelet Count	Treatment	Result
19	FFP	40,000	Platelets* × 1	Bleeding stopped
22	FFP	43,000	Blood	Died on table
29	FFP	69,000	Platelets × 3	Bleeding > 24 h
23	Plt	—†	Platelets × 3	Bleeding > 24 h
31	Plt	95,000	Platelets × 4	Bleeding > 24 h
40	Plt	117,000	None	Died, still oozing

* FFP = Fresh frozen plasma (2 units/dose); Plt or Platelets = Platelet concentrates (6 units/dose).
† Platelet count after platelet concentrate therapy was 87,000.

Coagulation Screening Tests and Clotting Factor Assays

The results of the coagulation screening tests (PT, PTT, TT, fibrinogen) obtained on the 33 patients revealed no significant differences between the two study groups at any time point. Furthermore, there appeared to be no trends in these tests for either study group.[23] As an isolated event, the administration of FFP raised levels of fibrinogen significantly ($p < 0.05$) from 147 to 221 mg/dl. However, this change was not significant when corrected for simultaneous multiple comparisons.

The mean values for per cent of normal activity of most of the clotting factors assayed (II, VII, VIII, IX, X, and XI) also showed no significant differences between the two study groups.[23] After the first dose of component, the mean level of factor V activity was significantly higher in the group receiving FFP ($p < 0.05$ after correcting for multiple comparisons) than those receiving PLT, and was also higher than the level existing before the component was administered ($p < 0.01$). However, the levels of factor V activity were seriously low (<25%) in only six patients (3 from each study group), and MVB developed in only two of these patients (1 from each group). Furthermore, factor V levels were identical between the two study groups by the time of exit from the study.

Platelet Counts

Platelet counts were found to drop significantly in all groups during transfusion (Fig. 3). After the component was administered, the counts increased noticeably but insignificantly in the group receiving platelets; however, this was a short-lived effect as the platelet count continued to decline until the patient exited the study. In the group receiving FFP, the platelet counts continued to decline throughout the study period.

An inverse relationship is apparent when platelet counts are plotted against the number of units of blood transfused before the administration of the component (PLT or FFP) (Fig. 4), confirming previous studies.[3-5] These regressions are highly significant ($p < 0.0002$), but as previously found,[4] the variability is high, as indicated by a coefficient of determination (R^2) of 0.37.

However, after the administration of either component, the relationship between the platelet count and the amount of blood transfused disappears ($p > 0.10$ and $R^2 < 0.15$). This flattening of the slope is seen even in the group that received only FFP and no platelets, making it unlikely that the administration of platelets alone is responsible for arresting the drop in platelet counts. Rather, since massive transfusion with platelet-poor stored blood

has a dilutional effect on platelet counts, the relationship between platelet counts and the amount of blood transfused is exponential rather than linear.[3,5,24,25] Thus, the change in slope seen in linear regressions performed before and after 12 units of blood had been transfused is a function of the incremental reduction in slope present in such a washout phenomenon. Any effect of platelet transfusions on halting the decline in platelet counts is minimal when compared to the major effect already produced by dilution. The regression lines for the two groups are nearly parallel to each other and are not different by Fisher's z-test for comparing correlation coefficients ($p > 0.4$).

When exponential regressions are performed on the data in the two treatment groups (Fig. 5), a significant relationship is seen throughout the time course of the study ($p < 0.05$), but again variability is high ($R^2 = 0.24$ for patients receiving platelets and $R^2 = 0.35$ for patients receiving FFP). Again, there is no significant difference between the regression lines by Fisher's z-test for comparing correlation coefficients ($p > 0.6$).

It is possible to calculate theoretical values for the dilutional effects on platelet counts by continuous exchange transfusion from the equation:

$$\text{Platelet count}_t = \text{platelet count}_O \times e^{-bt/v} \quad (1)$$

where O and t refer to the time of origin and the time of interest, respectively, b refers to the blood volume infused in time t, and v is the recipient's blood volume. The results of such calculations are also depicted in Figure 5; the assumption of a normal average blood volume of 65 ml/kg is made for a standard body weight of 70 kg.[26] From this figure, it is apparent that not only were the platelet counts higher than predicted for the group that received prophylactic platelets, but they were also higher than would be anticipated for the group that received only FFP.

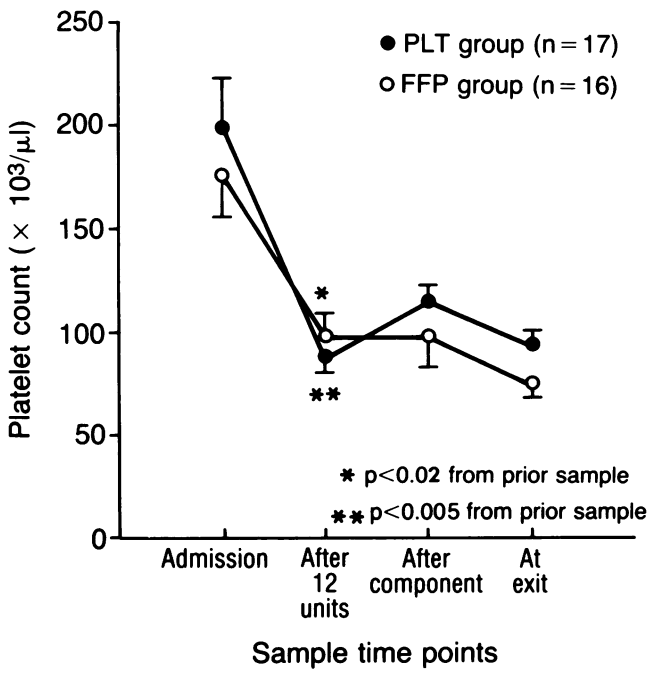

FIG. 3. Alterations in group mean platelet counts (±1 SEM) at four sample time points. PLT = patients receiving prophylactic platelet component therapy; FFP = patients receiving prophylactic fresh frozen plasma therapy. No significant differences between the two study groups exist.

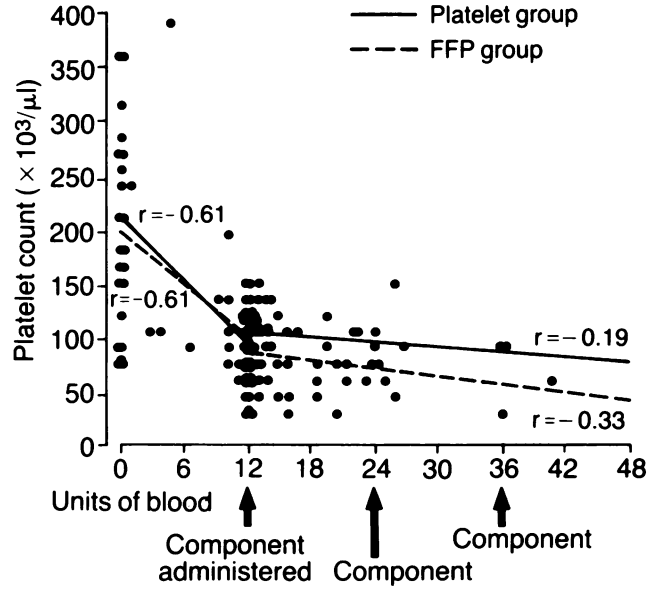

FIG. 4. Plot of platelet counts against the number of units of blood transfused to the patient. Separate linear regression lines are drawn for each treatment group for data taken before prophylactic component therapy was administered (<12 units of blood transfused) and for data taken after prophylactic component therapy was administered (>12 units of blood transfused). Regressions for both treatment groups are statistically significant at <12 units of blood transfused ($p < 0.001$) but are not statistically significant at >12 units of blood transfused ($p > 0.1$).

Discussion

We and others have previously identified dilutional thrombocytopenia as the major cause of diffuse microvascular bleeding that sometimes occurs during the massive transfusion of stored blood.[2-5] Because of this evidence, we conducted a prospective, randomized double-blind clinical study to test the hypothesis that the administration of prophylactic platelet concentrates with every 12 units of blood transfused would effectively prevent thrombocytopenia and diffuse microvascular bleeding, except in cases of disseminated intravascular coagulation. Because platelet concentrates also contain fresh plasma, a control group of patients was also randomly assigned to receive fresh frozen plasma.

Our results indicate that such prophylactic therapy is ineffective in preventing diffuse microvascular bleeding. Three of 17 patients who received PLT (17.6%) and three of 16 patients who received FFP (18.8%) developed diffuse microvascular bleeding that was not a consequence of

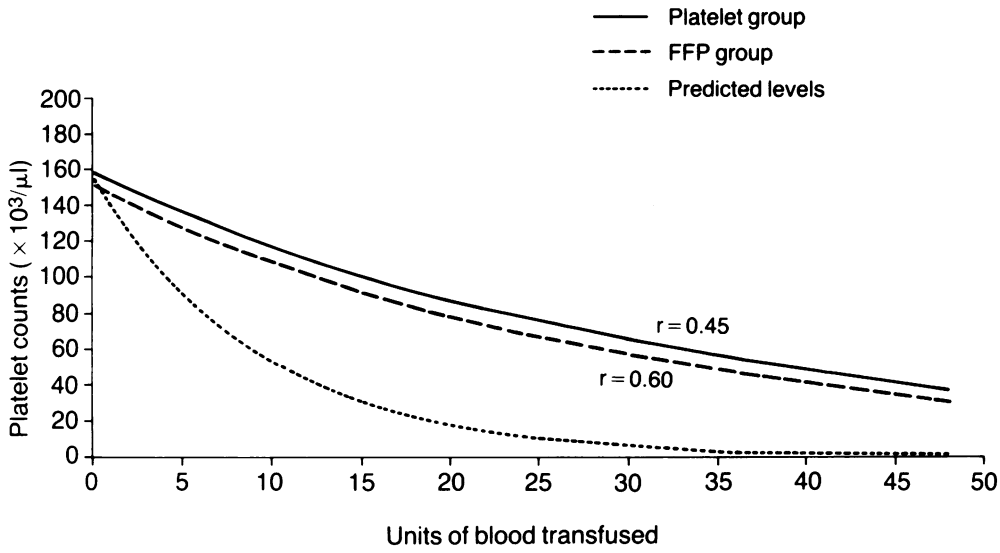

FIG. 5. Exponential regression lines for data points presented in Figure 4. Regression lines for both treatment groups are statistically significant ($p < 0.0001$). Line drawn for predicted levels of platelet counts is based on an exponential equation for continuous exchange transfusion (see text).

DIC as defined by our criteria (platelet count < 80,000/μl *and* fibrinogen < 80 mg/dl). In a previous study,[3] we found that five of 24 patients (20.8%) developed non-DIC diffuse microvascular bleeding, an incidence not significantly different from the current study.

The effectiveness of prophylactic platelet administration in preventing thrombocytopenia during massive transfusion is also questionable. While the average platelet counts were higher after the administration of platelet concentrates with every 12 units of blood than in patients who received FFP (Fig. 3), the overall declining trend of platelet counts during massive transfusion was not appreciably altered. Simple linear regression was able to find a relationship between platelet counts and the amount of blood transfused only during the initial phase of transfusion, before any blood component had been administered (Fig. 4). However, there was a great deal of variability in the relationship, as we had seen in our previous study,[3] implying that more than mere dilution was responsible for the platelet counts observed. By using exponential regression for what is presumably an exponential function, it can be seen that the curves for the two treatment groups in our study are essentially parallel and not significantly different from each other (Fig. 5). Approximately 35% of the variability in platelet counts can be accounted for by dilution ($R^2 = 0.35$) in the patients who received only FFP, while dilution accounts for less of the variability seen in the patients who received platelet concentrates ($R^2 = 0.24$), most likely because of the slight positive influence on platelet counts from the administration of platelets. However, it is interesting that in both groups, the regression lines seen are noticeably higher than those predicted to occur if thrombocytopenia were 100% the result of dilution. Miller, et al.,[5] noted similar findings in their study of massive blood transfusion in battle casualties. When the estimated blood volume is calculated from the exponential regression lines observed in the group receiving FFP, one obtains an amount (15.4 liters) that is roughly three times larger than the normal range.[22] This volume refers to the platelet-containing pool that is theoretically being depleted by exchange transfusion with platelet-poor stored blood. The implication is that platelets are being released into the circulation counteracting the effects of dilution. The most likely source for such platelet reserves is the spleen,[27-29] which normally pools about one-third of the circulating platelets.[30] Alternative explanations for higher than expected platelet counts include circulation of nonfunctional platelets (such as may have been transfused in the blood units) and premature release from bone marrow. The prophylactic administration of platelet concentrates adds little to the large endogenous release implied in Figure 5.

It may be argued that it is difficult to prevent a situation that has already occurred. The important feature of an exponential function as given in equation (1) is that the major drop in platelet counts occurs very early, and later changes become progressively smaller. From the estimated curves in Figure 5, it is apparent that platelet counts in an asplenic person might fall below 100,000 from dilution with as little as 6 units of platelet-poor blood. While the observed exponential regression line for the group of patients who received only FFP indicates that endogenous mobilization was able to maintain counts above 100,000 beyond 12 units of transfused blood (Fig. 5), the overall average platelet counts actually observed (Fig. 3) would indicate that platelet counts had already fallen below 100,000 before the first unit of platelet concentrates was due to be given. Indeed, as previously mentioned, there

was one patient excluded from entry into this study because his platelet counts had already fallen below 80,000, and he had developed diffuse microvascular bleeding after only 8 units of transfused blood; his fibrinogen level and coagulation screening tests were not markedly abnormal at the time. Obviously, the platelet count a patient initially presents with is a significant determinant of the levels his platelet count may drop to from dilution alone. However, the large portion of the decline in platelet counts will occur early in the dilution. It is therefore not surprising that our administration of platelet concentrates after 12 units of blood had been transfused had little effect on the platelet counts; at that point, the counts were already markedly depressed and the continuing effects of dilution were minimized.

It should not be concluded, though, that platelet concentrates should be administered earlier in the course of transfusion to prevent dilutional thrombocytopenia. It is difficult to anticipate which patients are necessarily going to require large transfusions initially and even more difficult to anticipate which will develop diffuse microvascular bleeding.[3] Additionally, simple dilutional thrombocytopenia as a cause of diffuse microvascular bleeding may be less common than previously thought. In our current study, we identified only one patient (#19) out of 16 who developed thrombocytopenia while receiving platelet-poor transfusions and whose bleeding promptly ceased following the administration of a single six-pack of platelet concentrates. It would be wasteful to administer platelets prophylactically to all patients for a condition that at most may affect only one out of 16 (6.25%) of those with massive transfusion, and for a condition that is so readily and effectively treated when it occurs. This is especially true if one undertook to prevent the condition by the early administration of platelet concentrates to maintain normal platelet counts; many patients who may initially require rapid blood transfusions do not ultimately require massive amounts.

Additionally, it appears that thrombocytopenia in patients who develop MVB is caused not only by dilution but also by consumption of platelets; indeed, two-thirds of the thrombocytopenia was not accounted for by dilution with blood transfusions ($R^2 = 0.35$). Consumption is indicated by the fact that three of the six patients who developed MVB required repeated doses of platelets to maintain adequate platelet counts and to halt their persistent oozing. Much of this took place after the initial period of massive transfusion, and thus ongoing dilution was not a factor in the loss of platelets. Two of these patients had sustained pelvic fractures and one had suffered a ruptured abdominal aneurysm; these are clinical situations that may well predispose patients to develop platelet consumption.[31] Evidence for the consumption of other clotting factors was present in most of the patients with MVB, although rarely to severe levels unless DIC had developed.[23] Only one patient (#19) had MVB in the presence of thrombocytopenia and adequate levels of other clotting factors.

Our results indicate that thrombocytopenia developing from the rapid, massive transfusion of stored blood starts early, but is counteracted by endogenous platelet release keeping platelet counts to levels higher than predicted by standard washout equations. The subsequent development of diffuse microvascular bleeding in certain patients is most often the result of platelet and/or clotting factor consumption initiated by the patient's injuries. Dilutional thrombocytopenia as a cause of MVB is infrequent enough that the prophylactic administration of platelet concentrates to attempt to prevent such bleeding is unwarranted. If evidence of platelet consumption can be found once diffuse microvascular bleeding has developed, then platelet administration is obviously necessary, but large amounts and repeated doses are often required. Currently, no reliable method exists to identify which patients are likely to develop this complication.

References

1. Howland WS, Schweitzer O, Boyan CP, Dotto AC. Physiologic alterations with massive blood replacement. Surg Gynecol Obstet 1955; 101:478–482.
2. Krevans JR, Jackson DP. Hemorrhagic disorder following massive whole blood transfusion. JAMA 1955; 159:171–177.
3. Counts RB, Haisch C, Simon TL, et al. Hemostasis in massively transfused trauma patients. Ann Surg 1979; 190:91–99.
4. Clagett GP, Olsen WR. Non-mechanical hemorrhage in severe liver injury. Ann Surg 1978; 187:369–374.
5. Miller RD, Robbins TO, Tong MJ, Barton SL. Coagulation defects associated with massive blood transfusions. Ann Surg 1971; 174: 794–801.
6. Slichter SJ, Counts RB, Henderson R, Harker LA. Preparation of cryoprecipitated factor VIII concentrates. Transfusion 1976; 16: 616–626.
7. Goodnight SH, Kenoyer G, Rappaport SI. Defibrination after brain tissue destruction: a serious complication of head injury. N Engl J Med 1974; 290:1043–1047.
8. Attar S, Boyd D, Layne E, et al. Alterations in coagulation and fibrinolytic mechanisms in acute trauma. J Trauma 1969; 9:939–965.
9. Davies JWL, Ricketts CR, Bull JP. Studies of plasma protein metabolism. III. Fibrinogen in burned patients. Clin Sci 1966; 30: 305–314.
10. Slichter SJ, Harker LA. Preparation and storage of platelet concentrates. I. Factors influencing the harvest of viable platelets from whole blood. Br J Haematol 1976; 34:395–402.
11. Filip DJ, Aster RH. Relative hemostatic effectiveness of human platelets stored at 4° and 22° C. J Lab Clin Med 1978; 91:618–624.
12. Bull BS, Schneiderman MA, Brecher G. Platelet counts with the Coulter counter. Am J Clin Pathol 1965; 44:678–688.
13. Harker LA, Finch CA. Thrombokinetics in man. J Clin Invest 1969; 48:963–974.
14. Thompson AR, Harker LA. Manual of Hemostasis and Thrombosis, 3rd ed. Philadelphia: FA Davis, 1983.
15. Quick AJ. Hemorrhagic Diseases and Thrombosis, 2nd ed. Philadelphia: Lea & Febiger, 1966.
16. Proctor RR, Rapaport SI. The partial thromboplastin time with kaolin. Am J Clin Pathol 1961; 36:212–219.

17. Brodsky I, Dennis LH. Evaluation of fibrinolysis in hepatic cirrhosis. Relation of serial thrombin time and euglobulin lysis time. Am J Clin Pathol 1966; 45:61–69.
18. Clauss A. Gerinnungs physiologische schell Methode sur Bestimmung des Fibrinogens. Acta Haematol 1957; 17:237–246.
19. Ratnoff OD, Menzie C. A new method for the determination of fibrinogen in small samples of plasma. J Lab Clin Med 1951; 37: 316–320.
20. Jacobsson K. Studies on the determination of fibrinogen in human blood plasma. Scand J Clin Lab Invest 1955; 7(suppl 14):3–54.
21. Wallenstein S, Zucker CL, Fleiss JL. Some statistical methods useful in circulation research. Circ Res 1980; 47:1–9.
22. Rosner B. Fundamentals of Biostatistics. Boston: Duxbury Press, 1982.
23. Ciavarella D, Reed RL, Heimbach DM, Counts RB. Unpublished observations.
24. Collins JA. Problems associated with the massive transfusion of stored blood. Surgery 1974; 75:274–295.
25. Marsaglia G, Thomas ED. Mathematical consideration of cross-circulation in exchange transfusion. Transfusion 1971; 11:216.
26. Diem K, Lentner C, eds. Scientific Tables. Basle: Ciba-Geigy Ltd, 1973; 554–556.
27. Slichter SJ, Funk DD, Leandoer LE, Harker LA. Kinetic evaluation of hemostasis during surgery and wound healing. Br J Haematol 1974; 27:115–125.
28. Keleman E, Lehoczky D, Cserhati I, et al. Demonstrability of a serum factor inducing thrombocytosis prior to acute rises of platelets in mice and men. Acta Haematol 1963; 29:16–26.
29. Slichter SJ. Identification and management of defects in platelet hemostasis in massively transfused patients. *In* Collins JA, Murawski K, Shafer AW, eds. Massive Transfusion in Surgery and Trauma. New York: Alan R. Liss, 1982; 225–258.
30. Shulman NR, Jordan JV. Platelet dynamics. *In* Coleman RW, ed. Hemostasis and Thrombosis. Philadelphia: JB Lippincott, 1982; 250–251.
31. Harker LA, Slichter SJ, Sauvage LR. Platelet consumption by arterial prostheses: the effects of endothelialization and pharmacologic inhibition of platelet function. Ann Surg 1977; 186:594–601.