Reprinted from AMERICAN JOURNAL OF CLINICAL PATHOLOGY, Vol. 68, No. 4, October 1977

Effects of Transfusion on Serum Iron, Serum Lactate Dehydrogenase, and Platelets in Megaloblastic Anemia

GEORGES FILLET, M.D., JEAN-MARIE ANDRIEN, M.D., AND JEAN BURY, M.D.

Fillet, Georges, Andrien, Jean-Marie, and Bury, Jean: Effects of transfusion on serum iron, serum lactate dehydrogenase, and platelets in megaloblastic anemia. Am J Clin Pathol 68: 458–462, 1977. In 11 patients with megaloblastic anemia, transfusion of packed erythrocytes or washed erythrocytes invariably resulted in a decline in plasma iron concentration to a range of 20–90 μg/dl (3.6–16 μmol/l) after 36 to 48 hours. The same phenomenon was observed in two of six cases of ineffective erythropoiesis without megaloblastosis and in none of five cases of aplastic anemia. The observed changes did not result from a specific hematinic response or from iron uptake by a non-erythroid compartment. In megaloblastic anemia, alteration in marrow function in response to transfusion was reflected by plasma iron kinetics and serum lactate dehydrogenase values, which indicated marked reductions in both marrow hyperplasia and ineffective erythropoiesis. Transfusion in megaloblastic anemia was also responsible for a 50% reduction in platelet count after 2 to 6 days. The significance of these changes is discussed. (Key words: Megaloblastic anemia; Transfusion; Plasma iron; Serum lactate dehydrogenase; Platelets.)

WITH THE INTRODUCTION of therapy with folic acid and vitamin B_{12}, indications for transfusion in megaloblastic anemia have been considerably reduced. However, transfusion may be needed when anemia is unusually severe, when the patient is debilitated, or in case of cardiovascular failure. The effects of transfusion therapy on bone marrow cytology were documented several years ago, but no clear modifications of blood constituents in that condition have been reported.[3,11] This report shows that transfusion markedly influences plasma iron and serum lactate dehydrogenase (LDH) concentrations, as well as platelet count.

Received June 9, 1976; received revised manuscript October 5, 1976; accepted for publication October 5, 1976.
Dr. Fillet is a recipient of a Chargé de Recherches Fellowship from the Fonds Belge de la Recherche Scientifique.
Address reprint requests to Dr. Fillet.

From the Section of Hematology, Department of Clinics and Medical Pathology, University of Liège, 4020 Liège, Belgium

Materials and Methods

The hematologic values, age, sex and diagnosis of the 27 patients are listed in Table 1. In the first 16 patients, morphologic examination of bone marrow showed typical megaloblastic hyperplasia. Ten had pernicious anemia and six were folate-deficient due to anticonvulsant therapy or malnutrition. Unless otherwise stated, hematinic agents were not given, and anticonvulsant drug therapy and nutritional habits were not modified prior to or during the study. The distinction between pernicious anemia and folate deficiency was made on the basis of a previous diagnosis and by the Schilling test and a therapeutic trial performed after transfusion. The other 11 patients had various other types of anemia and served as controls. Six were characterized by marked erythroid hyperplasia and ineffective erythropoiesis, and five had extreme erythroid hypoplasia.

The first 11 patients with megaloblastic anemia and the 11 patients who had other anemias received transfusions of packed or washed erythrocytes. Changes in plasma iron concentration were then determined. Erythrocytes from 2 to 6 units of blood were given within a 12–36-hour period to increase hemoglobin levels from 4–8 to 8–14 g/dl (.62–1.24 to 1.24–2.17 mmol/l). Twenty milligrams of furosemide were given intravenously in five cases. No transfusion reaction was observed. Three additional patients with pernicious anemia (Patients 12–14) each received a single dose of 0.5–0.75 μg vitamin B_{12} and 500–700 μg pteroylglutamic acid intramuscularly instead of

Table 1. Initial Laboratory Findings

	Age (Yr.), Sex	Hemoglobin g/dl	Erythrocyte Count 1,000/μl	Reticulocytes %	Platelets /μl	Plasma μg/dl	Plasma (μmol/l)	Lactate Dehydrogenase mIU/ml*	Diagnosis
Megaloblastic anemia									
Patient 1	45, F	5.3	1,670	.8	96,000	277	(49.6)	10,900	Pernicious anemia
Patient 2	61, M	4.9	1,260	1.9	72,500	340	(60.9)	6,800	Pernicious anemia
Patient 3	79, F	3.9	1,410	—	51,000	300	(53.7)	12,400	Folate deficiency (nutritional)
Patient 4	65, M	5.6	1,550	.9	140,000	317	(56.7)	—	Pernicious anemia
Patient 5	42, F	6.1	1,640	1.5	170,000	197	(35.3)	1,550	Folate deficiency (nutritional)
Patient 6	45, M	7.6	1,950	.1	16,000	241	(43.1)	1,085	Folate deficiency (anticonvulsant drugs)
Patient 7	45, F	3.7	1,330	—	228,000	267	(47.8)	—	Pernicious anemia
Patient 8	65, F	6.1	1,510	1.9	169,000	355	(63.5)	8,000	Pernicious anemia
Patient 9	69, F	7.6	1,850	1.4	176,500	225	(40.3)	1,770	Pernicious anemia
Patient 10	24, F	4.0	1,300	.6	92,600	320	(57.3)	—	Folate deficiency (oral contraceptives)
Patient 11	57, F	6.9	1,740	1.1	180,000	207	(37.0)	1,990	Pernicious anemia
Patient 12	56, F	8.9	2,580	.6	148,000	120	(21.5)	4,770	Pernicious anemia
Patient 13	52, F	6.4	1,590	5.9	161,000	88	(15.8)	8,100	Pernicious anemia
Patient 14	73, F	8.7	1,991	1.4	151,000	190	(34.0)	1,830	Pernicious anemia
Patient 15	54, M	4.5	1,500	.1	33,000	250	(44.7)	4,900	Folate deficiency (anticonvulsant drugs)
Patient 16	24, M	2.4	—	1.6	39,000	220	(39.4)	3,600	Folate deficiency (anticonvulsant drugs)
Other ineffective erythropoiesis†									
Patient 17	42, M	6.5	2,110	.4	77,000	325	(58.2)	286	Sideroblastic anemia
Patient 18	65, M	7.2	1,990	—	375,000	263	(47.0)	—	Refractory anemia
Patient 19	43, F	7.7	2,310	1.4	21,500	175	(31.3)	655	Refractory anemia
Patient 20	8, M	8.7	3,190	—	396,000	175	(31.5)	—	Thalassemia major
Patient 21	69, M	7.5	2,240	.8	160,000	183	(32.7)	172	Refractory anemia
Patient 22	62, M	6.1	1,880	2.0	48,500	145	(25.9)	300	Refractory anemia
Aplastic anemia									
Patient 23	17, F	7.7	2,310	.3	91,200	245	(43.8)	350	Aplastic anemia (preleukemic)
Patient 24	12, M	6.9	2,440	.1	181,000	225	(40.3)	345	Immunologic erythroblastopenia
Patient 25	16, M	3.5	860	.1	3,500	330	(59.0)	335	Aplastic anemia (idiopathic)
Patient 26	69, M	6.5	1,860	.4	9,000	210	(37.6)	660	Aplastic anemia (idiopathic)
Patient 27	47, F	4.0	1,040	—	10,500	182	(32.6)	187	Aplastic anemia (gold salts)

* Normal range 30–450 mIU/ml.

† Ineffective erythropoiesis in those patients was evidenced by iron kinetic studies demonstrating increased plasma iron turnover, delayed radioiron release from the marrow, and a marked decreases in erythrocytic incorporation of radioiron.

transfusion. In the cases of two other patients with megaloblastosis (Patients 15 and 16), suboptimal specific therapy was combined with transfusion.

Plasma iron, hemoglobin and erythrocyte count were measured sequentially in every case. For ten patients who had megaloblastic anemia, platelet and leukocyte counts were also recorded. Eight patients had repeated LDH determinations.* In six cases, reticulocyte counts, determined on 3,000 erythrocytes, were measured repeatedly. Plasma iron turnover was measured sequentially in two patients after intravenous injection of ^{59}Fe-transferrin. Standard hematologic measurements were made with Coulter Counters®. Plasma iron was measured by a Technicon AutoAnalyzer®.[12] Plasma iron turnover was determined according to the method of Finch and associates.[6]

Whole blood for transfusion purposes was collected by the hospital blood bank. A 63-ml volume of citrate-phosphate-dextrose solution was used as an anticoagulant for each 450 ml of blood obtained. Packed erythrocytes were prepared by centrifuging fresh blood and withdrawing most of the plasma. Washed erythrocytes were prepared by adding cold saline solution to the cell concentrate in an amount at least equal to that of the plasma withdrawn, and the blood

* DHL Merckotest, E. Merck Diagnostica, Darmstadt, West Germany.

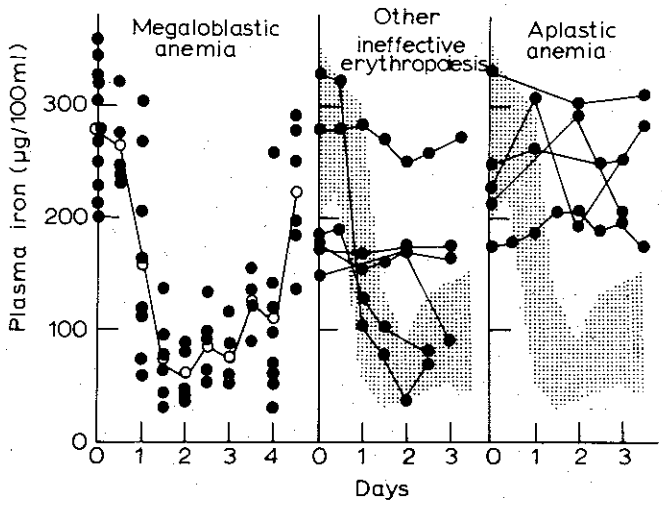

FIG. 1. Plasma iron changes following transfusion in 11 cases of megaloblastic anemia compared with 11 anemias of other etiology (non-megaloblastic ineffective erythropoiesis and aplastic anemias). Transfusion was started at day 0. Mean values of plasma iron in megaloblastic anemia are represented by the open circles. The shaded areas cover the range of values recorded in megaloblastic anemia.

was then centrifuged for 10 minutes, after which the saline solution was withdrawn. The blood used was always less than ten days old, and in most instances was drawn two to five days before transfusion.

Results

In megaloblastic anemia, transfusion invariably resulted in reduction of plasma iron (Fig. 1). Initial plasma iron values ranged from 197 to 355 μg/dl (35.3 to 63.5 μmol/l) in 11 patients, with a composite mean of 278 μg/dl (49.8 μmol/l). The decrease of plasma iron was obvious 24 hours after starting transfusion. After 36 and 48 hours, the mean values were 72 and 58 μg/dl (12.9 and 10.4 μmol/l), respectively. Following this, a gradual return towards pretransfusion levels was observed. A decline in plasma iron similar to that seen in megaloblastic anemia was observed twice in six cases of ineffective erythropoiesis of other etiology. No systematic variation occurred in aplastic anemia (Fig. 1).

To determine plasma iron exchange in patients with megaloblastic anemia who had received transfusions, transferrin-bound ^{59}Fe was injected intravenously into two patients before and after transfusion of washed erythrocytes. The results are shown in Table 2. In Patient 1, plasma iron had decreased from 325 to 42 μg/dl (58.2 to 7.6 μmol/l) after 36 hours and had reverted to 150 μg/dl (26.8 μmol/l) after 84 hours. The initial plasma iron turnover of 183 mg Fe/day (3.3 mmol/day) had decreased to 70 and 66 mg Fe/day (1.25 and 1.18 mmol/day) after 36 and 84 hours, respectively. Similar results were obtained for Patient 6.

Systematic reductions of LDH and platelets were also observed in megaloblastic anemia. From a mean initial value of 5,600 ± 3,500 mIU/ml (SD), LDH decreased to 2,450 ± 1,260 mIU/ml after four to six days ($t = 2.43$, $P < 0.05$). Platelets decreased from an initial count of 124,000 ± 75,000 to 59,000 ± 43,000 after three to five days ($t = 2.34$, $P < 0.05$). The time course of these changes, expressed as percentages of the initial values, is shown in Figure 2. No systematic variation was observed for granulocytes or reticulocytes.

Plasma iron, reticulocyte and platelet responses were followed in three patients with megaloblastic anemia who each received a single intramuscular injection of 0.5–0.75 μg vitamin B_{12} and 500–700 μg pteroylglutamic acid. Their responses were compared with those following transfusion. Following suboptimal doses of hematinics, the lowest values of plasma iron were observed only after three days. There was a reticulocyte crisis after hematinics unlike the absence of response after transfusion. Hematinics induced an increase in platelets, whereas transfusions were followed by a decrease in platelet count. To determine whether transfusion could inhibit platelet and reticulocyte responses following suboptimal doses of therapy, hematinics and transfusion were both administered to two patients. Pteroylglutamic acid, 200 μg/day, was started one day before a transfusion that brought the hemoglobin to 9.5 g/dl (1.47 mmol/l), a level similar to that obtained in the patients described

Table 2. Iron Kinetics Following Transfusion

	Time in Relation to Starting Transfusion Hours	Hemoglobin g/dl	Plasma Iron		Plasma ^{59}Fe $t_{\frac{1}{2}}$ Min	Plasma Iron Turnover	
			μg/dl	(μmol/l)		mg/day	(mmol/day)
Patient 1	−10	5.3	325	(58.2)	60.1	183	(3.27)
	+36	12.5	42.5	(7.6)	19.6	70	(1.25)
	+84	10.6	150	(26.8)	76.6	66	(1.18)
Patient 6	−10	7.6	241	(43.1)	90.7	120	(2.15)
	+36	12.6	72.5	(13.0)	28.8	44	(0.79)

above. Reticulocyte and platelet crises were obtained after four to six days.

Discussion

Cytologic studies in pernicious anemia have shown that transfusion decreases bone marrow cellularity and the number of megaloblasts.[3,11] In this report, the reduction of erythroid hyperplasia following transfusion was evidenced by a reduced plasma iron turnover. Although iron supply to the bone marrow was probably suboptimal 36 hours after transfusion, the plasma iron was not sufficiently low to account for a reduction of half of the plasma iron turnover. In fact, this value remained decreased after 84 hours, when plasma iron had returned to a high value.

To the best of our knowledge, the hematologic changes reported here have not been previously described to occur in patients with megaloblastic anemia who have received transfusions. The finding that transfusion markedly depressed plasma iron despite a reduced uptake by tissues indicates a shortage of iron supply to the plasma. Previous iron kinetics studies have established that the plasma compartment in pernicious anemia is mostly fed by iron wasted by ineffective erythropoiesis.[5] The recircuiting of this iron from nonviable erythroid precursors occurs via the marrow reticuloendothelial cells.[2,7] Specific therapy that converts ineffective erythropoiesis into effective production of erythrocytes is known to decrease plasma iron within 24–30 hours.[5] The possibility that the transfused normal blood could have provided sufficient hematinic factor was therefore considered. However, the erythrocytes have a B_{12} content of only 200 pg/ml[10] and the folate bound to erythrocytes (200 ng/ml) does not seem available for erythropoiesis before cell lysis.[1,8] Furthermore, in three patients in this study who received suboptimal doses of vitamins, the decline in plasma iron was less than after transfusion, although the amounts of vitamin B_{12} and pteroylglutamic acid injected were 5–7 times and 2–3 times greater, respectively, than the amounts present in 2 units of packed erythrocytes. In addition, suboptimal doses of vitamins also increased the platelet and reticulocyte counts, a phenomenon not recorded after transfusion, although transfusion itself could not prevent completely the platelet and reticulocyte responses after vitamin therapy. Finally, it was observed that transfusion induced typical reductions of plasma iron in two patients who had ineffective erythropoiesis refractory to vitamin B_{12} or pteroylglutamic acid. From these facts, it may reasonably be concluded that the reduction of plasma iron in patients with megaloblastic anemia who have received transfusions is independent of a hematinic effect.

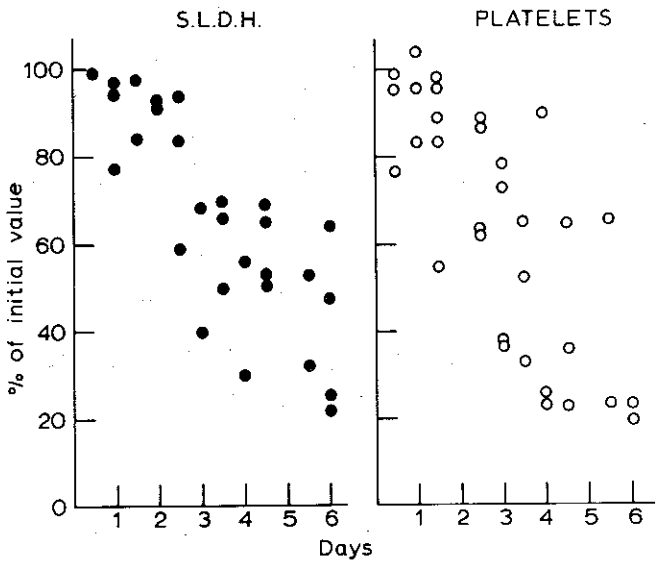

FIG. 2. Serum lactate dehydrogenase (LDH) and platelet changes after transfusion in megaloblastic anemia, expressed as percentages of their initial values. Transfusion was started at day 0.

The high LDH levels in megaloblastic anemia result from intramedullary destruction of megaloblasts, with little contribution from the circulating erythrocytes. Following adequate therapy, LDH gradually returns to normal, reaching 50% of the initial value after five to six days.[4] The sequence of changes of LDH activity described here is similar, and indicates that transfusion markedly reduced ineffective erythropoiesis. This finding is in agreement with the regression of megaloblastic abnormalities described by Davidson[3] and Mason.[11] The decline in LDH implies that transfusion depresses the flow of iron from nonviable erythroid precursors to the plasma, and by this mechanism has a tendency to reduce the plasma iron concentration. However, transfusion also inhibits the uptake of iron by bone marrow, an effect that certainly antagonizes the reduction in plasma iron. Therefore, there are reasons to believe that the abatement of intramedullary hemolysis is not the only factor responsible for the decline in plasma iron. Transfusions were given over 12–36-hour periods, whereas plasma iron decreased over 24–36 hour periods. Thereafter, the continuing decline in LDH is in contrast to the return of plasma iron towards pretransfusion values. In view of the rapid decrease in plasma iron concentration in the presence of a reduced plasma iron turnover, we suggest that the release of iron by the reticuloendothelial system is also impaired by transfusion. This hypothesis is not ruled out by the lack of variation in plasma iron seen in aplastic anemia, since under this circumstance the iron input from the reticuloendothelial cell to plasma is basically reduced.[6]

The progressive decline in platelets after transfusion

has some practical importance. From the data presented, it is clear that transfusion in megaloblastic anemia induces or enhances thrombocytopenia. Although no hemorrhagic incident was observed in the present study, it is emphasized that platelets may, in some patients, reach potentially dangerous levels. The mechanism producing this thrombocytopenia can only be a matter of speculation. However, the time course of the platelet count changes after transfusion of erythrocytes might suggest inhibition of platelet production rather than hyperdestruction or increased sequestration, which would be expected to occur earlier. This view would be consistent with a recent report indicating that controls of erythropoiesis and of thrombopoiesis are not entirely independent.[9]

Acknowledgements. Technical assistance was provided by Mr. L. Baldelli and Mrs. J. Van Roy.

References

1. Chanarin I: The Megaloblastic Anemias. First edition. Oxford and Edinburgh, Blackwell, 1969, p 318
2. Cook, JD, Marsaglia G, Eschbach JW, et al: Ferrokinetics: A biologic model for plasma iron exchange in man. J Clin Invest 49:197–205, 1970
3. Davidson CS, Murphy JC, Watson RJ, et al: Comparison of the effects of massive blood transfusions and of liver extract in pernicious anemia. J Clin Invest 25:858–869, 1946
4. Emerson PM, Wilkinson JH: Lactate dehydrogenase in the diagnosis and assessment of response to treatment of megaloblastic anemia. Br J Haematol 12:678–688, 1966
5. Finch CA, Coleman DH, Motulsky AG, et al: Erythrokinetics in pernicious anemia. Blood 11:807–820, 1956
6. Finch CA, Deubelbeiss K, Cook JD, et al: Ferrokinetics in man. Medicine 49:17–53, 1970
7. Goodman JR, Wallerstein RO, Hall SG: The ultrastructure of bone marrow histiocytes in megaloblastic anemia and the anemia of infection. Br J Haematol 14:471–475, 1968
8. Herbert V: Experimental nutritional folate deficiency in man. Trans Am Assoc Physicians 75:307–312, 1962
9. Jackson CW, Simong JV, Edwards CC: The relationship of anemia and thrombocytosis. J Lab Clin Med 84:357–368, 1974
10. Kelly A, Herbert V: Coated charcoal assay of erythrocyte vitamin B_{12} levels. Blood 29:139–143, 1967
11. Mason JD, Leavell BS: The effect of transfusion of erythrocytes on untreated pernicious anemia. Blood 11:632–640, 1956
12. Young DS, Hicks JM: Method for the automatic determination of serum iron. J Clin Pathol 18:98–102, 1965