The Effect of Intrauterine Intravascular Blood Transfusion on Iron Metabolism in Fetuses With Rh Alloimmunization

HASSAN A. NASRAT, MD, UMBERTO NICOLINI, MD, PETER NICOLAIDIS, MD, ELIZABETH A. LETSKY, MD, GILLIAN GAU, MD, AND CHARLES H. RODECK, MD

Fetal plasma ferritin concentrations were measured in 43 normal fetuses at 18–36 weeks and in 78 blood samples collected before transfusion from 23 fetuses with Rh alloimmunization. Among controls, there was a significant correlation between fetal serum ferritin and gestational age (r = 0.39, P = .009), consistent with an increase in fetal storage of iron during normal pregnancy. In Rh-alloimmunized fetuses, the ferritin concentration was above the reference range in 63% of the samples. Before the first transfusion, the fetal ferritin concentration was increased compared with controls (mean multiples of the mean = 2.6, range 1–26) and showed a negative correlation with fetal hematocrit (r = −0.43, P < .05), suggesting that the worse the fetal anemia, the higher the iron store. Serial transfusions were associated with further increase in serum ferritin, which correlated primarily with the total volume of blood transfused. Three fetuses had plasma serum ferritin concentrations above 1 mg/L, a level compatible with a diagnosis of iron overload in children. These observations suggest that there is a potential risk of iron overload in Rh-alloimmunized fetuses undergoing intrauterine blood transfusion. *(Obstet Gynecol 77:558, 1991)*

In normal pregnancy, the mother's plasma is the only source of fetal iron. Although the exact mechanism of maternal-fetal iron transport is not fully understood, in normal pregnancy iron is transported across the placenta against a concentration gradient, unidirectionally from the mother to the fetus.[1,2] It has been suggested that the human placenta has a dual function of transporting iron when it is deficient and storing it when it is in excess.[2] The latter function might be significant, as the developing fetal liver cells, the main site of iron storage,[3] are particularly vulnerable to iron toxicity in the early stages of differentiation.[4] However, when maternal iron levels are markedly increased, as by iron dextran transfusion, the fetal iron is also significantly raised.[5,6] Excessive deposition of iron in parenchymal cells may result in cellular damage and functional insufficiency of the organs involved.[7,8]

Because iron overload can be recognized at an early precirrhotic stage,[9] the definition of hemochromatosis relies on the presence of an increase in total body iron rather than on evidence of parenchymal tissue damage and fibrosis.[8] Chronic anemia with ineffective erythropoiesis and multiple blood transfusions are among the important causes of secondary hemochromatosis.[10] Anemic fetuses with Rh alloimmunization undergoing repeated intravascular transfusion may be vulnerable to similar risks of iron overload.

Iron is stored mainly as ferritin, a high-molecular-weight, water-soluble protein.[11] Although ferritin is an intracellular compound, a small circulating fraction reflects the level of body store of iron.[12–14] Because there is no active mechanism for iron excretion, parenteral iron administration, as in transfused red cells, may result in iron overload.

The aim of this study was to investigate the effects of serial blood transfusions on iron stores of Rh-alloimmunized fetuses, as measured by the fetal serum ferritin level.

Materials and Methods

Serum ferritin was measured in 43 control fetuses undergoing blood sampling at 18–36 weeks' gestation for rapid karyotyping or prenatal diagnosis of congenital disease and who were later found to be unaffected. In another 23 fetuses with Rh alloimmunization, 78 samples were collected before transfusion: 20, 17, 19, 13, eight, and one sample before the first, second,

From the Institute of Obstetrics and Gynecology, Royal Postgraduate Medical School, Queen Charlotte's and Chelsea Hospital, London, United Kingdom.

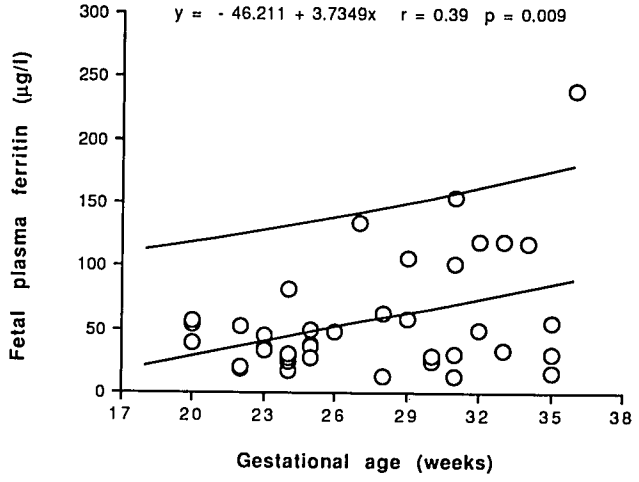

Figure 1. Plasma ferritin concentration during gestation in normal fetuses (mean and 95th percentile).

third, fourth, fifth, and sixth transfusions, respectively. For 16 fetuses, complete data for serial transfusions from the first to the third transfusion were available. Fetal blood sampling and transfusions were performed under ultrasonic guidance using a 20-gauge needle. The site of sampling/transfusion was the umbilical vein, either at the placental cord insertion or the intrahepatic tract.[15,16]

In both groups, we collected 2–3 mL maternal venous blood just before fetal blood sampling for measurement of serum ferritin. Immediately before and after completion of each transfusion, the fetal hematocrit was checked (Coulter Channelyzer; Luton, Bedford, UK). Rh-negative donor blood, crossmatched with maternal blood and packed to a hematocrit of 60–80%, was transfused intravascularly to achieve a final fetal hematocrit in the range of 40–50%.[17] Transfusions were performed at intervals of 2–4 weeks according to our management protocol for Rh alloimmunization.[18]

The plasma concentration of ferritin was measured by radioimmunoassay.[19] Intra- and inter-batch variations were 4.9 and 10.4% at 32 µg/L ferritin, 3.4 and 6.5% at 111 µg/L ferritin, and 2.6 and 4.1% at 1126 µg/L ferritin, respectively.

From the controls, we constructed a reference range (mean and 95% data intervals) of plasma ferritin concentration throughout gestation. The least-squares method was used to assess the correlation between fetal and maternal plasma ferritin concentrations, fetal ferritin and gestational age, and fetal hematocrit and fetal ferritin concentration before the first transfusion. Multiple regression analysis was used to assess the relation between fetal plasma ferritin concentration and gestational age, number of transfusions, and cumulative volume of transfused blood. The value of plasma ferritin concentration in the Rh group at a given gestational age was also expressed as multiples of the mean, constructed from the control group. In fetuses for whom the complete data of serial transfusions were available (N = 16), one-way analysis of variance was performed to test longitudinal changes in the absolute value of serum ferritin.

Results

Figure 1 displays the mean and 95th percentile of fetal plasma ferritin concentration in the controls. Fetal plasma ferritin concentration correlated significantly with gestational age (r = 0.39, P = .009), whereas there was no significant correlation between fetal and maternal plasma ferritin concentrations (r = −0.14). Fetal hematocrit showed a nonsignificant correlation with plasma ferritin concentration (r = 0.33, P = .053).

In Rh fetuses, the fetal ferritin concentration was above the reference range in 63% of the samples (Figure 2). Before the first transfusion, the fetal plasma ferritin concentration was increased over that of controls (mean multiples of the mean = 2.6, range 1–26) and showed a significant negative correlation with fetal hematocrit (r = 0.43, P < .05) (Figure 3).

Following transfusions, there was a significant positive correlation between plasma ferritin concentration and both the cumulative volume of transfused blood (r = 0.52, P < .0001) (Figure 4) and the number of transfusions (r = 0.51, P < .001) independent of gestational age. However, multiple regression analysis showed that the correlation of plasma ferritin with the number of transfusions depended on the cumulative volume of blood transfused.

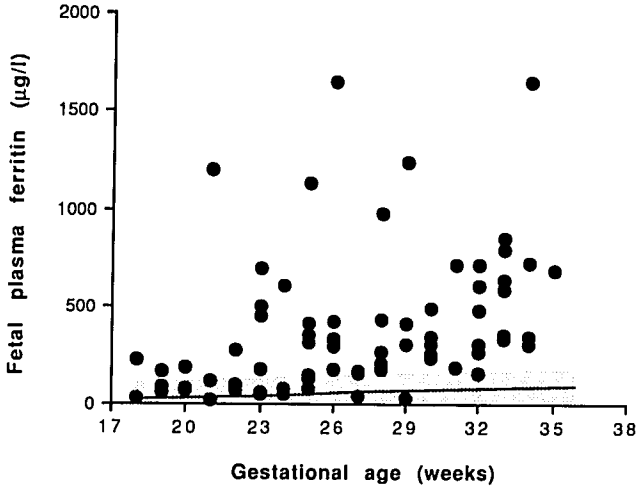

Figure 2. Plasma ferritin concentration in Rh fetuses (78 samples) in relation to the reference range (*shaded area*).

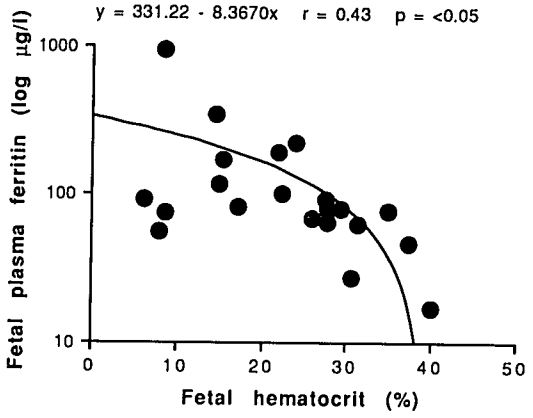

Figure 3. Correlation between hematocrit and plasma ferritin concentration (log scale) before the first transfusion in Rh fetuses.

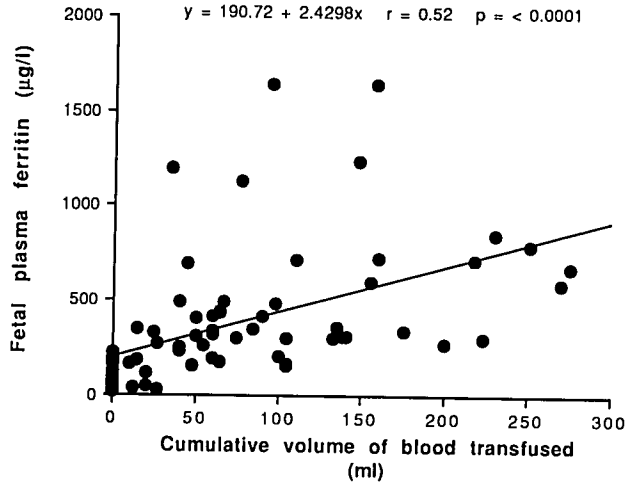

Figure 4. Correlation between the cumulative volume of transfused blood and plasma ferritin concentration in Rh fetuses.

In the 16 fetuses studied longitudinally, we found a significant increase ($F = 5.5$, $P < .001$) in the absolute value of fetal ferritin with serial transfusions.

One fetal death occurred a few hours after the third transfusion at 33 weeks because of cord tamponade. Postmortem histology showed extensive iron deposition throughout the liver. This was present in the majority of parenchymal cells (Figures 5 and 6). There was no placental iron deposition in the sections examined. The fetal serum ferritin concentration measured 268 µg/L (3.5 multiples of the mean) before the last transfusion following a cumulative total volume of 188 mL of donor blood.

Discussion

Ferritin, the main iron storage compound in the body, is concentrated in the reticuloendothelial cells of the liver, spleen, and bone marrow.[20,21] Measurement of serum ferritin reliably reflects the total body iron stores in both adults and newborns.[12,13]

At birth, the infant's plasma ferritin concentration is normally much higher than in the mother or in normal adults[22]: Mean concentrations of 183 µg/L[23] and 128 µg/L[24] have been reported, values that are compatible

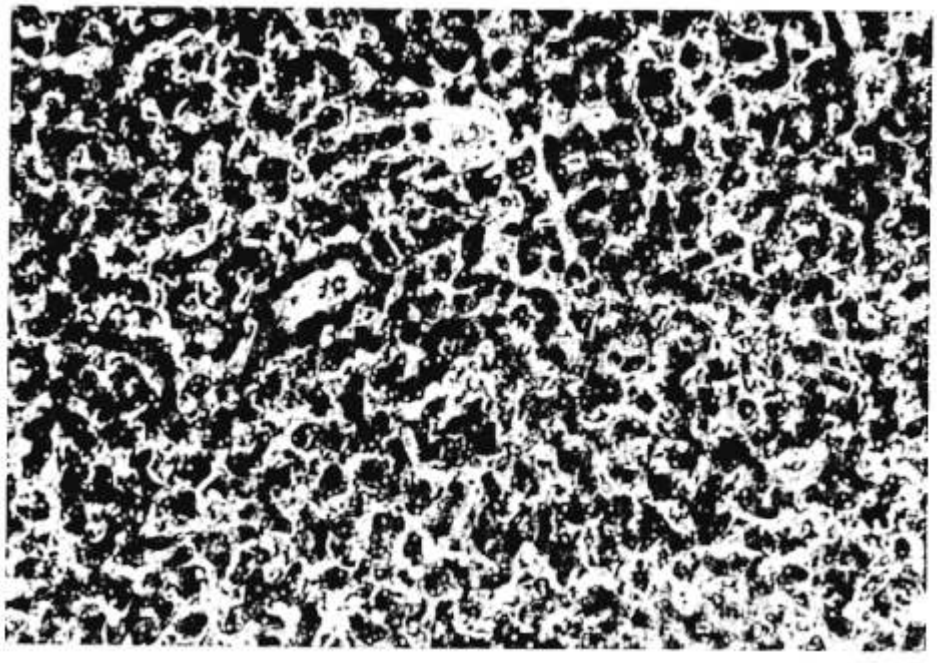

Figure 5. Liver section from a non-Rh fetus that died at 33 weeks from placental abruption. Small aggregates of iron can be seen in occasional Kupffer cells (*arrow*).

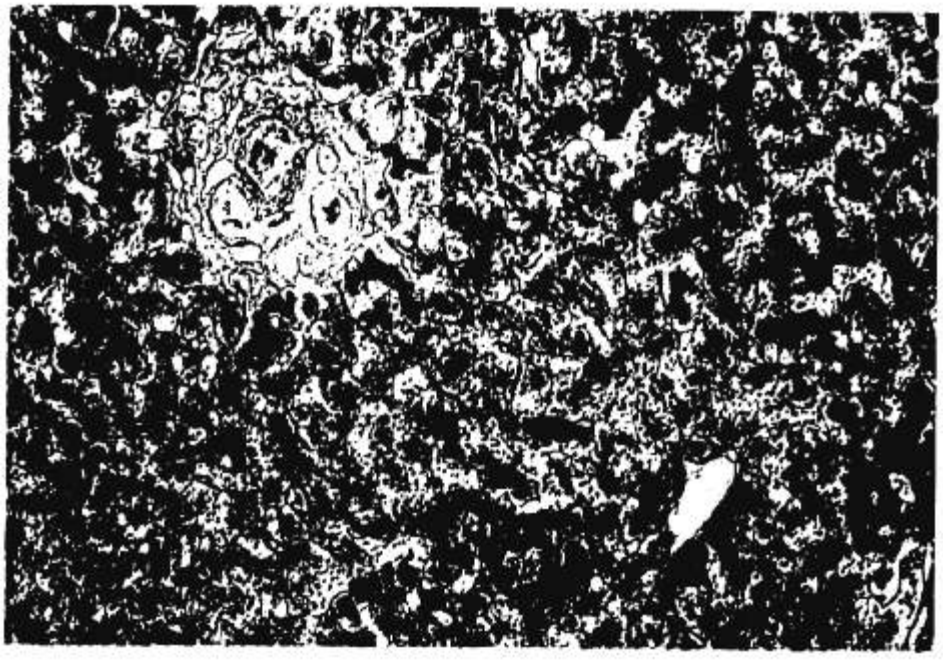

Figure 6. Liver section from the Rh fetus that died at 33 weeks after the third transfusion. Iron can be seen in rough aggregates throughout the liver parenchymal cells. (Perl reaction followed by Gordon and Sweet's silver impregnation for reticulin fibers. Original magnification × 30).

with our finding of a mean fetal plasma ferritin concentration of 88 μg/L at 36 weeks.

Human and animal experiments have yielded conflicting results regarding the influence of maternal iron balance on fetal iron stores.[2,13,22–24] We found no correlation between maternal and fetal serum ferritin concentrations during pregnancy, due most likely to the active placental mechanism of iron transfer which allows the fetus to maintain a normal hemoglobin concentration even if the mother is iron-deficient.[25,26]

Fletcher and Suter,[1] by labeling maternal plasma with radioactive iron, calculated that the average daily transfer of iron across the placenta increases from approximately 0.4 mg/day at 16 weeks to 4.7 mg/day at 30 weeks. This increase in placental iron transfer may explain our observation of a more than fourfold increase in the mean fetal ferritin concentration between 18–36 weeks' gestation, despite the parallel increase in fetal size.

Infants affected with severe alloimmunization have increased parenchymal iron stores,[27] probably because of an increased rate of fetal hemolysis. This is confirmed by our finding of raised serum ferritin in Rh fetuses before the first transfusion and by the negative correlation between fetal plasma ferritin and fetal hematocrit, suggesting that the worse the degree of fetal anemia, the higher the iron stores. Serial intravascular transfusions further increased the fetal ferritin concentration, thus causing tissue iron overload. This rise in fetal ferritin was dependent on the total volume of transfused blood.

For detection of iron overload, regardless of its location, measurement of serum ferritin is the most valuable test.[9] In adults, plasma ferritin levels above 300 μg/L in men and 200 μg/L in women indicate increased stores and require further investigation.[21] In a study of children between 6 months and 15 years of age with chronic hemolytic anemia, the median value of serum ferritin was 850 μg/L before transfusion,[21] indicating iron overload.

At levels greater than 1000 μg/L, transferrin becomes almost fully saturated.[28] In the present study, three fetuses exhibited serum ferritin values above 1000 μg/L following transfusion of 30–170 mL of donor blood. Although these neonates did not show any untoward effects in the immediate postpartum period, the long-term consequences of such high fetal ferritin concentrations remain unknown.

In fetuses with hemolytic diseases, iron loading is temporary and indeed may be beneficial because iron stores normally become depleted during the first year of life. Nevertheless, there may be a concern about the ability of fetal liver cells to cope with excessive iron load at a premature stage. Excessive cellular ferritin itself may cause functional and cellular damage.[11] Moreover, non-protein-bound iron "free radical" is a potentially toxic compound known to increase in the serum of patients with iron overload when transferrin is nearly saturated.[29] A recent study[30] suggested that iron toxicity occurs in newborns with severe Rh disease because of increased ferritin concentration and limited transferrin binding, causing increased production of free radicals. Severe bradycardia and unexpected neonatal death were attributed to iron-induced cardiac damage.

We have previously reported abnormalities of glucose metabolism[31] and recently demonstrated disturbances of liver enzymes in Rh-alloimmunized fetuses.[32] Both of these abnormal findings might be related to iron overload.

Although repeated fetal transfusions have been a standard treatment for many years, long-term side effects have not come to light.[33] However, many of the clinical sequelae of iron overload can take up to 20 years before they become evident,[7,20] and treatment has been advocated even in asymptomatic cases.[20]

Our results suggest that follow-up studies of iron metabolism in Rh-alloimmunized newborns are urgently needed. In the meantime, in Rh newborns, who are often preterm, iron supplementation should be withheld until serum ferritin returns to the normal range.

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