Hepatic iron storage in very low birthweight infants after multiple blood transfusions

P C Ng, C W K Lam, C H Lee, K F To, T F Fok, I H S Chan, E Wong

Abstract

Objective—To investigate the effect of multiple blood transfusions on hepatic iron storage in very low birthweight (VLBW) infants.

Methods—Seventeen VLBW infants who died within the first six months of life and underwent postmortem examination were studied. Serum ferritin, iron, and total liver iron binding capacity were measured within the week before the infants’ death. Liver iron concentration was quantitatively determined by atomic absorption spectrophotometry and semiquantitatively assessed by histochemical liver iron grading. The clinical characteristics and the iron results were compared between infants receiving < 100 ml of blood (group A) and those receiving ≥ 100 ml (group B). Spearman’s correlation coefficient was used to evaluate the relation between the volume of blood transfused and serum/liver iron concentrations. Statistically significant variables associated with liver iron concentration were further subjected to multivariate stepwise regression analysis.

Results—Infants in group B had significantly higher serum iron (p < 0.01), serum ferritin (p < 0.01), and liver iron concentration (p < 0.01) than those in group A. The total and net volume of blood transfused were significantly associated with liver iron concentration (p < 0.001, r = 0.86; p < 0.001, r = 0.71 respectively), semiquantitative histochemical liver iron grading (p < 0.001, r = 0.80; p < 0.005, r = 0.71 respectively), and serum ferritin (p < 0.001, r = 0.84; p < 0.01, r = 0.69 respectively). In addition, both liver iron concentration and liver iron grading were found to be significantly associated with serum ferritin (p < 0.001, r = 0.76; p < 0.005, r = 0.68 respectively). Multivariate stepwise regression analysis indicated that the (log) liver iron concentration was significantly associated with the (log) volume of blood transfusion (p < 0.001; regression coefficient 0.39, SE 0.09), after adjustment for gestational age (R^2 = 0.84).

Conclusions—This study showed a significant positive relation between the volume of blood transfused and the liver iron concentration in preterm VLBW infants. Although the transfusional blood volume correlated closely with the amount of iron deposited in hepatic tissues, clinical manifestations of iron overload were not observed. Carers should be aware of this potential harmful effect before prescribing blood or routine iron supplement to vulnerable preterm infants.

Keywords: blood transfusion; ferritin; liver; iron; preterm; very low birthweight

Anaemia is a common problem in newborn infants. The haemoglobin concentration and red blood cell count fall precipitously after birth, and this phenomenon is known to be exaggerated in preterm neonates. 1 The cause of this anaemia is not fully understood, but undoubtedly in very low birthweight (VLBW) preterm infants, it is probably multifactorial. 2 Haemolysis, shortened red blood cell lifespan, low circulating erythropoietin, blood sampling, and blood loss attributable to medical and surgical conditions all contributed to the anaemia in premature infants. 3 Although most infants manage to tolerate low levels of haemoglobin without major difficulty, sick VLBW infants requiring mechanical ventilation may have considerable problems with low oxygen carrying capacity, and a significant proportion of them require repeated blood transfusions. In addition, there has been a consensus of opinion that all preterm infants, but, in particular those weighing < 1500 g at birth, will inevitably develop iron deficiency anaemia in the first few months of life if iron supplementation is not given at an early stage. 3 This recommendation has resulted in the routine use of oral iron for prevention of late anaemia in many neonatal centres. However, as more preterm infants with extreme gestations survive with better neonatal care and new technology, we have observed that most VLBW infants would receive repeated blood transfusions during their stay in intensive care. Serum ferritin concentrations measured in some of these infants are abnormally high. However, as ferritin is also an acute phase reactant and its level may be influenced by extrinsic factors such as haemolysis, infection, inflammation, and liver disease, it would be difficult to determine whether high levels of serum ferritin represent excess iron burden or merely reflect the concurrent illness. This study was therefore undertaken to investigate the effect of repeated blood transfusions on the body iron status in VLBW infants. Liver biopsy with quantitative iron measurement and semiquantitative histochemical iron grading was performed at the postmortem examination to assess hepatic iron storage. The results could...
Results are number of patients or median (interquartile range). The duration of hospital stay is the same as the age of death.

*p<0.05 and **p<0.001.

RDS, respiratory distress syndrome.

POSTMORTEM EXAMINATION AND HISTOPATHOLOGY

About 1 cm³ of hepatic parenchymal tissue was obtained at autopsy from each of the 17 infants. Standard histological liver sections were stained with haematoxylin and eosin. Iron was also histochemically disclosed using the Perl's Prussian Blue staining technique, and graded according to the reference criteria. The histopathologist who stained the liver sections was unaware of the amount of blood transfused to these infants.

CLINICAL BIOCHEMICAL MEASUREMENTS

Blood for assessment of liver function and body iron status was obtained within the week before the infant's death. Serum ferritin was measured by microparticle immunoassay (IMx analyser; Abbott Laboratories, Abbott Park, Illinois, USA), and serum iron and total iron binding capacity (TIBC) were measured by dye binding colorimetry using the Vitros 250 analyser (Ortho-Clinical Diagnostics Inc, Rochester, New York, USA). The liver specimens were thoroughly rinsed with saline before being digested with concentrated (69%) nitric acid. Liver iron concentration was quantitatively determined using graphite furnace atomic absorption spectrophotometry (SIMAA 6000 analyser; Perkin-Elmer Corp, Norwalk, Connecticut, USA). The interassay coefficients of variation of the measurements were: 3.2% at 420 pmol/l for serum ferritin, 2.5% at 13.5 µmol/l for serum iron, 4.6% at 47.2 µmol/l for TIBC, and 6.8% at 3.46 µmol/g dry weight for liver iron. The biochemist who performed these analyses was blinded to the clinical details of the patients.

STATISTICAL ANALYSIS

The results for clinical variables and body iron status were expressed as median and interquartile range. Mann-Whitney U test and Kruskal-Wallis Exact test were used to compare the clinical and iron results between infants receiving <100 ml of blood transfusion (group A) and those receiving ≥100 ml (group B). Spearman's correlation coefficient was used to evaluate the relation between the volume of blood transfused and serum/liver iron concentrations or other clinical variables. Statistically significant variables associated with liver iron concentration were further subjected to multivariate regression analysis using the forward stepwise selection strategy. In this process, the most significant risk factor—that is, the factor
Table 2 Results of blood transfusion and body iron status of the infants studied (n=17)

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*Double volume exchange transfusion.
TIBC, total iron binding capacity; RDS, respiratory distress syndrome; BPD, bronchopulmonary dysplasia; NEC, necrotising enterocolitis.

that will give the largest likelihood ratio statistic—is added to the model at each step, and this selection process repeats itself until no further factor can make a significant (p < 0.05) contribution to the model. However, when two or more potential closely related risk factors are present, the one that is clinically or biologically more significant is selected for entry. Data not normally distributed were logarithmically transformed before the analysis. The statistical tests were performed by SPSS for Windows (Release 9.0, SPSS Inc, Chicago, Illinois, USA). The level of significance was set at 5% in all comparisons.

Results

Table 1 compares the clinical characteristics between group A and group B infants. Infants in group B had significantly higher serum iron (p < 0.01), serum ferritin (p < 0.01), liver iron grading (p < 0.05), and liver iron concentration (p < 0.01) than those in group A. They also had significantly longer duration of mechanical ventilation (p < 0.001), oxygen supplementation (p < 0.001), and hospital stay (p < 0.001) than infants in group A. Table 2 summarises the results of blood transfusions and body iron status of each infant. This table allows cross reference of the clinical and biochemical variables between and within individual patients.

When the results of the infants studied were pooled and analysed, the total and net volume of blood transfused to these infants were significantly associated with liver iron concentration (p < 0.001, r = 0.86; p < 0.001, r = 0.71 respectively), liver iron grading (p < 0.001, r = 0.80; p < 0.005, r = 0.71 respectively), and serum ferritin (p < 0.001, r = 0.84; p < 0.01, r = 0.69 respectively). In addition, liver iron concentration was also significantly associated with the liver iron grading (p < 0.001, r = 0.93), serum ferritin (p < 0.001, r = 0.76), and % saturation (p < 0.05, r = 0.54). With regard to other clinical variables, the total and net volume of blood transfused to the infants, liver iron concentration, liver iron grading, and serum ferritin were significantly correlated with gestational age (p < 0.05, r > 0.55), birth weight (p < 0.05, r > 0.50; with the exception of the net amount of blood transfused), duration of mechanical ventilation (p < 0.05, r > 0.60), oxygen supplementation (p < 0.05, r > 0.58), and hospital stay (p < 0.01, r > 0.61). Multivariate stepwise regression analysis showed that the (log) liver iron concentration was significantly associated with the (log) total volume of blood transfusion (p < 0.001; regression coefficient 0.39, SE 0.09), after adjustment for gestational age (R² = 0.84).

Discussion

Iron overload develops when there is an intrinsic defect in the regulation of iron absorption such as in hereditary haemochromatosis but, more commonly, it is due to medical interventions, such as chronic red blood cell transfusion in malignancies, chronic infections, and refractory anaemia.7 Repeated blood transfusions result in iron overload because the human body does not have the physiological means of excreting iron.7 8 Excess iron therefore accumulates in reticuloendothelial cells and parenchymal cells of the liver, heart, and endocrine tissues.8 It causes tissue damage by generating free radicals that destroy intracellular organelles, DNA, and cellular membranes.9 10 Although most clinical manifestations of iron overload in patients with β thalassaemia do not appear until after many years of red blood cell transfusions, serial liver biopsies show that iron could be deposited in parenchymal tissues within one year of repeated blood transfusions.11 Furthermore, a newborn with Rhesus

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haemolytic disease who received multiple intrauterine transfusions was subsequently found to develop liver disease consistent with iron overload. Sick preterm infants who require multiple blood transfusions and have immature endogenous antioxidant systems for organ protection are at increased risk of multiple blood transfusions and have immature endogenous antioxidant systems for organ protection are at increased risk of developing iron overload and free radical oxidative tissue damage. To our knowledge, the impact of repeated blood transfusions on the body iron status has not been accurately quantified, by liver iron measurement—the reference standard for body iron evaluation, in this category of patients.

A significant association between the total volume of blood transfused and the liver iron concentration and/or liver iron grading suggests that iron deposition in parenchymal tissues increases with the amount of blood transfused. This finding is further supported by the observation that the accretion of fetal serum ferritin in Rhesus alloimmunised fetuses was primarily correlated with the total volume of intrauterine blood transfused. In older children and adults, liver iron concentration is considered normal under 20 µmol/g dry weight (< 1.12 mg/g dry weight), and values in excess of 40 µmol/g dry weight (2.25 mg/g dry weight) and 265 µmol/g dry weight (15 mg/g dry weight) represent iron overload and significant increase in risk of associated complications respectively. As premature infants have significant extramedullary haematopoiesis which may alter the liver iron concentration, a reference standard for hepatic iron has not been established in this category of infants. Georgieff and colleagues measured neonatal liver iron concentration in a small number of preterm and term infants. The results (mean (SE)) were 5.30 (0.66) and 3.86 (0.67) mg/g dry weight respectively. This study, however, did not give details of blood transfusion, gestational age, or birth weight, and hence a direct comparison with our results would not be meaningful. Faa and coworkers have also studied liver iron concentrations in 22 stillborns, newborns, and infants who did not receive blood transfusion. A substantial interindividual variation in iron content was observed, and the hepatic iron concentrations ranged from 3.3 to 66.4 µmol/g dry weight. Similarly, our results showed a wide range of liver iron concentrations from 6.3 µmol/g dry weight (0.4 mg/g dry weight) to 168.3 µmol/g dry weight (9.2 mg/g dry weight). In addition, preterm infants who received < 100 ml of blood transfusion (group A) had a mean liver iron concentration (15.1 µmol/g dry weight) similar to that reported by Faa (21.6 µmol/g dry weight). However, we again urge caution when interpreting these results, as the cohort of infants described by Faa et al encompassed a heterogeneous group of babies with different gestational and postnatal ages, and thus their results may not be strictly comparable with those of preterm VLBW infants. Alternatively, if the aforementioned criteria for patients with β thalassaemia are extrapolated so as to evaluate our newborns, all five infants who received > 180 ml of packed cells had liver iron concentrations > 40 µmol/g dry weight (> 2.25 mg/g dry weight). Judging by this standard, our patients would be considered to have a mild degree of iron overload. Semiquantitative evaluation of hepatic iron storage using the Perl’s Prussian Blue staining technique also indicated that four of the above five infants had histochemical liver iron grading ≥ 2. Moreover, the presence of stainable iron primarily in hepatic Kupffer cells further suggested that excess iron was probably imported from an external source. None of these cases showed any histological changes suggestive of hepatic architectural damage or fibrosis. The liver iron concentration of the studied infants did not reach the threshold for chelation treatment nor did any of the infants develop clinical manifestations indicative of iron overload. We suspect that the relatively high demand of iron for growth and haemoglobin synthesis in the postnatal period, the delay in introduction of enteral feeding, the relatively short period of exposure to transfusion therapy, the significant amount of blood removed for laboratory investigations, and blood loss due to various medical or surgical procedures and complications all contribute to minimising the risk of iron accumulation in body tissue organs. However, the small sample size of this study precludes us from predicting exceptional cases, such as those receiving an excessive amount of blood or repeated intrauterine transfusions.

Serum ferritin, a commonly used indirect index of body iron store, was also found to correlate significantly with liver iron concentration. Despite the association, there was a wide variation in serum ferritin levels, and individual results did not necessarily reflect the quantity of iron deposited in the liver (table 2). A low or normal serum ferritin would suggest no excessive accumulation of body iron, whereas an elevated level may indicate acute inflammation, particularly in severely ill patients, or an increase in body iron storage.

In conclusion, this study shows a significant positive relation between the volume of blood transfused and the liver iron concentration in preterm VLBW infants. Although the transfusional blood volume correlated closely with the amount of iron accumulated in the hepatic tissues, clinical manifestations of iron overload were not observed. Measures to minimise blood loss and promote erythropoiesis, such as reducing the number of unnecessary laboratory investigations, judicious use of blood transfusion, and utilisation of recombinant human erythropoietin would almost certainly decrease the frequency and volume of transfusion, and the amount of iron transfused to these infants. As VLBW infants who received ≤ 180 ml of packed cells did not exhibit excessive hepatic iron storage (table 2), and those who received > 180 ml had hepatic iron concentrations > 40 µmol/g dry weight and/or histochemical liver iron grading ≥ 2, routine iron supplementation in the latter group of infants would probably be unnecessary. However, further studies are required to determine the body iron status in the postnatal period, the need for routine iron supplementation, and the long term risk of...
transfusion related iron overload in this category of patients.

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