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Blood transfusion and chronic lung disease in preterm infants

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Abstract Frequent blood transfusions may produce changes in iron status which can give rise to oxygen-derived free-radical (ODFR) generation and oxidative injury. Preterm infants developing chronic lung disease (CLD) receive significantly more transfusions. A total of 73 very preterm infants had weekly estimations of serum iron, transferrin, transferrin saturation, ferritin, caeruloplasmin, bleomycin detectable ('free') iron (BDI), and thiobarbituric acid reacting substances (TBARS) made over the first 28 days. Thirty infants remained oxygen dependent at 36 weeks postmenstrual age and were termed as having CLD. They were significantly lighter and less mature at birth and received more than twice as many transfusions during the 1st month. They had significantly lower transferrin levels initially but similar total iron and transferrin saturations as non-CLD infants. Ferritin and caeruloplasmin levels rose to significantly higher levels over the 1st month in CLD infants, and ferritin levels were significantly related to the number of transfusions given. Infants with higher ferritin levels were more likely to show BDI, although this was not associated with increased lipid peroxidation as evidenced by higher TBARS.

Conclusion It is unlikely that oxidative injury from ODFRs induced by blood transfusion contributes to the risk of developing CLD in preterm infants.

Key words Blood transfusion · Chronic lung disease · Oxygen derived free radicals · Newborn

Abbreviations *BDI* bleomycin detectable iron · *CLD* chronic lung disease · *ODFR* oxygen derived free radicals · *TBARS* thiobarbituric acid reacting substances

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Introduction

Chronic lung disease (CLD) in preterm infants is widely believed to be related at least in part to oxidant injury of the immature lung [12]. Not only is the lung in preterm infants lacking in antioxidant protective mechanisms, but tissue injury from mechanical ventilation, ischaemia/reperfusion injury, exposure to high environmental oxygen and neutrophil and macrophage activity, all increase the generation of oxygen derived free radicals (ODFRs).

A further source of free radical activity may be through Fenton reactions catalysed by free iron and this mechanism has been proposed to be important in the genesis of complications of neonatal care [2, 6]. A possible source of free iron is frequent blood transfusion, widely used in the management of sick preterm infants. While much of the time these transfusions are simply replacing blood losses from sampling, they are also used to correct the anaemia of prematurity in oxygen dependent infants caused by the period of depressed erythropoiesis seen post-natally. In adults and mature infants, free iron is not usually detectable in the blood due to the very high affinity of transferrin for iron, but preterm infants have considerably lower levels of transferrin. To investigate the possibility that blood transfusion therapy in preterm infants might contribute to the generation of free iron, increased free radical generation and risk of chronic lung disease through oxidative injury, we investigated the iron status of a cohort of preterm infants over the 1st month after birth in relation to evidence for lipid peroxidation and chronic lung disease.

Subjects and methods

Infants admitted to the neonatal intensive care unit were included in the study if they were of less than 34 weeks gestation, and remained ventilated in more than 40% at 12 h of age. A high proportion of the subjects were extremely preterm, and as a result many were still mildly oxygen dependent at 28 days. Because of this a stricter criterion for chronic lung disease was taken as oxygen dependency at 36 weeks post-menstrual age.

The criteria for blood transfusion used on the unit during the study were that infants were transfused if their haemoglobin level fell below 14 g/dl and were receiving ventilatory support, or more than 30% inspired oxygen during the first 10 days; at 12 g/dl if they required added oxygen or had recurrent apnoea; and at 10 g/dl or below in any infant.

Clinical data concerning gestation, birth weight, number of blood transfusions during the first 28 days, antenatal steroid prophylaxis, surfactant therapy and clinically evident arterial duct were noted from the intensive care records. In order to assess iron, a 1 ml aliquot of blood was drawn on days 1, 7, 14, 21, and 28, centrifuged and serum kept at 4° C for transferrin, and iron measurements and –20° C for ferritin and free iron measured as bleomycin-detectable iron (BDI). BDI was measured using the method described by Gutteridge and Hou [7]. The method is based on the fact that the antibiotic bleomycin requires the presence of iron salts in order to degrade DNA. If other agents are present to excess, the extent of DNA degradation is proportional to the amount of iron in the system that is available to be bound by bleomycin. The products of DNA degradation include base propenals that react on heating with thiobarbituric acid at low pH to form a pink thiobarbituric acid-malondialdehyde adduct. Bleomycin has a fairly low affinity for iron and cannot remove iron at physiological pH from pure iron proteins such as transferrin and ferritin. BDI is thought to represent iron bound to low molecular mass chelating agents such as citrate, or loosely bound to proteins such as albumin. In iron overload in cases of haemachromatosis, BDI correlates with plasma ferritin but not total plasma iron. BDI probably represents that iron which is available to stimulate free radical reactions. Because of the high sensitivity of the assay, care must be taken to avoid contamination from iron-containing reagents and containers. Iron-free plastic materials were used throughout. In our laboratory we achieved an intra-assay coefficient of variation of 10% and an inter-assay variation of 12%. The limit of detection was 0.1 μmol/l.

Thiobarbituric acid reactive substances (TBARS) were also quantified concurrently as an index of lipid peroxidation, on 60 samples during the 1st month using the thiobarbituric test by fluorometry as described by Yagi [13]. The samples chosen were those large enough to permit TBARS estimation after the other assays had been completed. The method used fluorometric detection of the adduct which tends to give a higher value for TBARS than methods using HPLC. Ferritin was measured by enzyme linked immunoassay (Cambridge Life Sciences MELISA Ferritin kit). Total serum iron was determined by a modified ferrozine method, and transferrin and caeruloplasmin were analysed by rate nephelometry (Beckman Array 360 system). Transferrin saturation was calculated.

Descriptive data are presented as median with range or mean ± SD for continuous data and percentages with 95% confidence intervals for categorical data. Differences between those patients who were oxygen dependent at week 36 (CLD) compared with those who were not, were tested using the Wilcoxon Rank Sum test for continuous data and the Chi squared test for categorical data. Variables associated with CLD on univariate analysis were entered into logistic regression analyses as independent variables with CLD as the dependent variable. Correlation between variables was calculated using Kendall's Rank Correlation.

Results

Ninety-eight infants were initially included in the study. Of these, 25 either died or were discharged back to a referring hospital before 28 days. The remaining 73 infants form the patient group described in this report. Of these, 30 (41%) were still oxygen dependent at 36 weeks postmenstrual age and form the CLD group, and the remainder the non-CLD group. Clinical data for the two groups are shown in Table 1.

Gestational age and birth weight were both significantly lower in the CLD group ($P < 0.0001$). The number of transfusions given during the 1st month was significantly greater in the CLD group ($P < 0.0001$). Statistically significant differences were not observed between the groups in the frequency of antenatal steroid or surfactant administration. A clinically evident arterial duct was diagnosed more frequently in the CLD group ($P = 0.03$)

Table 2 shows median and range for total iron, transferrin, transferrin saturation, ferritin, BDI, and caeruloplasmin in the two groups over the first 28 days after birth. Ferritin levels were significantly higher in the CLD infants on day 14, day 21 and day 28 ($P = 0.02$, 0.0004 and < 0.0002 respectively) and transferrin levels on day 1, day 7 and day 14 were significantly lower ($P = 0.03$, < 0.0001 and 0.01 respectively). There were no significant differences in either total serum iron or transferrin saturation. BDI was significantly higher in CLD infants only on day 21 ($P = 0.01$). However, BDI was undetectable in 95 of 170 (65.9%) of all estimations in non-CLD infants, but in only 42 of 125 (33.6%) estimations in those babies who developed CLD (chi-square 14.3, $P = 0.00015$).

Ferritin levels in all infants related to the number of blood transfusions given. In those infants receiving two transfusions or fewer, no significant change in plasma ferritin occurred over the 1st month. Infants receiving 3–6 or 7–16 transfusions over the same period had significantly higher ferritin levels from 14 days onwards. Ferritin levels were also correlated with BDI (Kendall's tau = 0.2, $P = 0.03$). Caeruloplasmin levels rose equally in both groups from birth to day 14, but were significantly higher in the CLD group on day 28.

Median levels of TBARS were higher in those samples with any BDI, than in those containing no free iron (3.55 μmol/l vs 2.72 μmol/l) although there was wide variability, and these differences were not statistically significant. After standardising for gestational age, birth weight and days of ventilation in a logistic regression analysis, the number of transfusions given in the first 28 days remained significantly associated with CLD (Table 3).

Table 1 Clinical data and statistical comparison. Descriptive data are mean ± SD, categorical data are percentages (*n*)

	CLD *n* = 30	No CLD *n* = 43	*P*
Gestational age (weeks)	25.9 ± 1.9	28.3 ± 1.9	< 0.0001
Birth weight (g)	862 ± 239	1176 ± 294	< 0.0001
Number of transfusions	8.5 ± 3.7	3.3 ± 2.3	< 0.0001
Prenatal steroids	93% (28)	79% (34)	0.17
Surfactant	93% (28)	74% (32)	0.07
Patent Ductus Arteriosus	50% (15)	23% (10)	0.03
Ventilator			
Days (median)	25	4	< 0.0001
(range)	1–71	1–38	

Table 2 Summary of biomedical data in CLD and noCLD subjects over first 28 days

		Total serum iron (μmol/l)	Transferrin (g/l)	Transferrin saturation (%)	Ferritin (μg/l)	Bleomycin detectable iron (μmol/l)	Caeruloplasmin (g/l)
Day 1	Chronic lung disease	4.5 (3.6–10.3)	1.06*[1] (0.97–1.21)	15 (1–83)	155 (53.4–468)	0.18 (0–2.53)	0.06 (0.04–0.13)
	No chronic lung disease	4.9 (4.3–8.5)	1.25 (1.23–1.62)	11 (4–93)	168 (17–1 000)	0 (0–2.20)	0.05 (0.01–0.31)
Day 7	Chronic lung disease	14.4 (12.6–18.8)	1.32*[2] (1.19–1.39)	41 (17–108)	468.8 (176.6–1 000)	0.22 (0–2.46)	0.08 (0.05–0.22)
	No chronic lung disease	18.6 (15.5–22.8)	1.65 (1.54–1.79)	42 (7–87)	454.1 (83.5–1 000)	0 (0–2.20)	0.09 (0.02–0.19)
Day 14	Chronic lung disease	16.8 (16.0–23.3)	1.64*[2] (1.46–1.71)	41 (12–85)	416.3*[3] (161.5–2 000)	0.30 (0–2.70)	0.12 (0.03–0.40)
	No chronic lung disease	18.5 (15.3–21.8)	1.88 (1.67–1.94)	38 (4–88)	314.6 (88.7–1 000)	0 (0–1.48)	0.11 (0.04–0.30)
Day 21	Chronic lung disease	18.3 (15.4–25.8)	1.62 (1.43–1.74)	43 (9–93)	532.0*[4] (220.8–2 000)	0.38*[5] (0–2.46)	0.15 (0.03–0.36)
	No chronic lung disease	17.0 (14.8–18.2)	1.65 (1.58–1.95)	40 (16–84)	266.4 (30.9–1 000)	0.24 (0–1.70)	0.11 (0.05–0.35)
Day 28	Chronic lung disease	15.6 (12.9–20.5)	1.46 (1.30–1.67)	48 (22–116)	512.2*[4] (241.2–1 000)	0.30 (0–2.17)	0.16*[6] (0.02–0.35)
	No chronic lung disease	17.0 (15.2–20.0)	1.54 (1.35–1.73)	43 (11–87)	228.3 (40.2–1 132)	0 (0–2.77)	0.09 (0.06–0.32)

Values shown as median (range)
*[1] $P = 0.009$
*[2] $P < 0.0001$
*[3] $P = 0.05$
*[4] $P = 0.0001$
*[5] $P = 0.01$
*[6] $P = 0.02$

Table 3 Summary table of logistic regression with CLD as the dependent variable and gestation, birth weight, transfusions and days of ventilation as independent variables

	Correlation coefficient	Standard error	Coefficient/SE	*P*
Gestation	–0.645	0.576	–1.119	0.25
Birth weight	0.001	0.004	0.295	0.77
Transfusions	0.665	0.266	2.500	0.01
Ventilator Days	0.102	0.053	1.914	0.06

Discussion

CLD is likely to be the end result of the interaction of a variety of insults to the immature lung. Some of these may relate to the form of management received by the infant for the respiratory distress syndrome. Differences in incidence of CLD have been observed in clinical trials and epidemiological studies comparing alternative ventilation strategies [8], nutritional support [3] and surfactant therapy [5]. The number of blood transfusions given to an infant has been linked to later morbidities such as retinopathy of prematurity, but the causal nature of this association has not been established [4]. It may simply be that sicker infants receive more transfusions and are at greater risk of later morbidities.

The infants in this study who developed CLD received on average more than twice as many transfusions in the 1st month as those infants who did not develop CLD. A more exact estimate of the effect of blood transfusions may have been achieved if the total volume of blood transfused had been recorded. However, the haematocrit of each transfusion varied considerably, so that such an estimate may not have been any more precise. The association between number of transfusions and CLD persisted after standardisation for gestation, birth weight and days of ventilation, in a logistic regression analysis, although these variables may be insufficient to account for all differences in disease severity on admission between the two groups of infants (Table 3).

Total serum iron and transferrin saturation rose rapidly in all infants in the first 7 days, but remained steady for the rest of the 1st month. This rise probably results from iron gained from initial blood transfusions minus that lost through sampling for clinical purposes. There was no difference between infants with or without CLD. Most transfusions were given early in the month, while growth and erythropoesis later may have utilised excess iron. Transferrin levels were however, lower on days 1, 7 and 14 in those developing CLD, indicating the relative immaturity of these infants.

The presence of BDI seems to be a paradox in the coexistence of transferrin iron-binding capacity, given its high affinity for free iron (dissociation constant $10^{22} \times M^{-1}$). Such an observation has been made in iron overloaded patients after venesection treatment [1] and in premature and full-term infants [6]. An explanation is that iron may exist as chelates that are poor donors of iron to transferrin. The absence of (theoretical) 100% transferrin saturation does not necessarily mean that there are sites available to bind iron. Other metals may block up to 30% of binding sites. In addition it has been shown that high

ascorbate levels in premature infants may inhibit caeruloplasmin ferroxidase activity, significantly reducing the oxidation of Fe^{2+} to Fe^{3+} required for iron binding to transferrin, such that even if there were iron binding sites available, the iron would be unable to bind in its Fe^{2+} state [10].

More frequent blood transfusions in CLD infants, whilst not increasing total iron, were associated with raised ferritin levels, possibly indicating an acute phase response in sicker infants. Total serum iron includes iron in transferrin but not in ferritin. The significant late rise in caeruloplasmin would also suggest an acute phase response, as it was also only seen in the sicker CLD group, and caeruloplasmin is known to act as an acute phase reactant. It is of interest that it is also an inhibitor of iron-dependent lipid peroxidation because of its ferroxidase activity.

Ferritin level increased over the 1st month in infants receiving more than two transfusions. In those receiving seven or more, the increase was nearly fourfold. Ferritin can release iron in a form capable of stimulating free radical reactions such as hydroxyl radical production and lipid peroxidation, when subjected to acidosis, which may have been more frequent in the CLD infants [9]. BDI was more frequently observed in infants with CLD and was significantly correlated with ferritin levels suggesting that this process may be occurring in frequently transfused infants. However, the presence of BDI was not significantly associated with evidence for lipid peroxidation such as elevated TBARS at any time during the 1st month, neither were higher measurements of TBARS associated with an increased risk of CLD. It is possible that plasma TBARS is too crude a measure of ODFR activity, especially when measured using the fluorometric method. Urinary TBARS has been shown to differ in preterm infants with differing degrees of lung disease and oxygen exposure, although the more specific HPLC method was used [11]. Alternatively, lipid peroxidation could have been inhibited by the higher levels of caeruloplasmin present in the CLD infants. The significance of BDI in preterm infants remains unclear, although it may simply be associated with high haemoglobin turnover, as it is in severe Rhesus isoimmunisation [2].

In conclusion, whilst infants who subsequently develop CLD are likely to receive more blood transfusions than those who do not, and consequently develop higher ferritin levels, we have not been able to demonstrate that this increases the risk of ODFR production and lipid peroxidation, and as such contributes to the risk of developing CLD.

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