

Changes in circulating red cell volume during the first 6 weeks of life in very-low-birth-weight infants

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BACKGROUND: Little is known about the change in circulating red cell volume (RCV) of very-low-birth-weight (VLBW) infants during the first weeks of life.

METHODS: RCV was measured during the first 5 d in 35 VLBW infants using chromium-51 labeling of the infants' red blood cells (RBCs). RCV was measured again at 6 wk of age in 12 infants, and the volumes of RBCs lost by phlebotomy and those gained by transfusion were recorded between the RCV measurements. In six infants, the volume of waste blood on materials contaminated with blood during phlebotomy, which would usually be discarded, was measured by radioactive counting.

RESULTS: The mean RCV in the first several days of life was 39.6 ml (35.7 ml/kg; range: 20.1–58.7 ml/kg). Of the 12 infants whose RCV was measured twice, all but one had a decrease in absolute RCV. The mean RCV initially and at 6 wk were 37.3 and 26.6 ml, respectively. The mean volume of RBCs lost through phlebotomy was 29.2 ml, and the mean volume of RBCs given by transfusion was 34.5 ml.

CONCLUSION: During the first 6 wk of life, when the anemia of prematurity is evolving, the RCV falls despite complete replacement of RBCs lost by diagnostic phlebotomy with transfused RBCs.

The circulating red cell volume (RCV) was first measured in newborn infants by Mollison *et al.* (1) in 1950 using phosphorus-32 (³²P). Others have subsequently reported their experiences measuring RCV directly using chromium-51 (⁵¹Cr) (2–6), chromium-50 (⁵⁰Cr) (7), technitium-99 (⁹⁹Tc) (8), and biotin (9–11) as red blood cell (RBC) labels. In addition, several other methods have been used to calculate RCV based on changes in fetal hemoglobin concentration (12–14) or mismatched RBC antigens (14,15) following a transfusion of packed RBCs, or by calculation from measured plasma volume and hematocrit (16).

Faxelius *et al.* (7) measured RCV in newborn infants, including some very-low-birth-weight (VLBW) infants, using ⁵⁰Cr labeling, which requires *ex vivo* neutron activation analysis. Their studies did not include repeated measurements of RCV in the same patients. Bratteby (5) measured RCV repeatedly

on several occasions in a group of 15 infants; 8 of these infants were preterm, but none weighed less than 1.5 kg at birth. Serial determination of RCV during the first weeks of life in VLBW infants might shed light on the anemia of prematurity.

Little is known about the role of changing RCV in the evolution of anemia of prematurity. The study reported here was undertaken to measure the circulating RCV in a group of VLBW infants during the first few days of life and, in some of these infants, again at 6 wk of age.

RESULTS

RCV measurements were performed during the first 5 d of life in 35 infants who weighed less than 1.5 kg at birth. Their mean birth weight was 1.10 kg (range: 0.60–1.45 kg), and their mean gestational age was 29 wk (range: 25–34 wk). The median age at the time of the initial RCV measurement was 36 h; 30 of the 35 infants were less than 72 h old. The mean RCV at initial measurement was 39.6 ml (SD: 15.4 ml; range: 14.3–75.2 ml), and when expressed per kilogram body weight, it was 35.7 ml/kg (SD: 9.4 ml/kg; range: 20.1–58.7 ml/kg) (Table 1).

In 12 infants, the RCV was measured again at 6 wk of age (Table 2). The mean RCV for these infants fell from 37.3 ml (SD: 11.4 ml) in the first 5 d of life to 26.6 ml (SD: 7.1 ml) at 6 wk of age ($P < 0.01$). When expressed as ml/kg, the RCV fell from 37.3 ml/kg (SD: 9.5 ml/kg) to 21.6 ml/kg (SD: 6.0 ml/kg). The RCV (in ml) fell in 11 of 12 patients and increased in 1 patient. The mean volume of RBCs removed for laboratory analysis was 29.2 ml (SD: 13.2 ml), and the volume of RBCs given by transfusion was 34.5 ml (SD: 25.9 ml). The mean net RBC production (P-D) was –15.9 ml (SD: 18.7 ml), which means that RBC production was not sufficient to keep up with endogenous destruction. All infants except one had negative values for P-D. However, that infant had a negative value when the RBC gains and losses from an exchange transfusion were included in the calculation.

For the six infants in whom the quantity of waste blood loss was assessed by counting of radioactivity, the mean measured phlebotomy loss for laboratory analyses was 0.81 ml of RBCs per kg per day, and the normally occult, waste loss averaged 0.08 ml/kg/d, or an additional 10% above the measured phlebotomy loss.

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Table 1. Circulating RCV in the first 5 d of life in VLBW infants

Patient number	Birth weight (g)	Gestational age (wk)	Age at study (d)	Weight at study (kg)	RCV (ml)	RCV (ml/kg)	Hct (%)
1	1,120	29	5	0.99	24.0	24.2	52
2	720	25	0	0.72	28.0	38.9	57
3	1,140	29	1	1.14	38.6	33.8	66
4	1,315	27	2	1.31	61.4	46.8	59
5	1,100	27	3	1.09	44.6	40.8	38
6	1,210	27	1	1.18	50.0	42.4	48
7	980	Unknown	0	1.00	36.0	36.0	70
8	780	29	2	0.78	45.8	58.7	66
9	960	28	1	0.96	26.1	27.2	43
10	920	26	0	0.92	36.2	39.3	50
11	950	32	5	0.92	28.0	30.5	59
12	985	28	3	0.98	28.4	29.0	61
13	1,360	31	2	1.30	36.2	27.9	44
14	1,320	33	2	1.32	41.1	31.1	38
15	1,180	33	2	1.18	36.2	30.7	38
16	680	27	4	0.68	14.3	22.5	46
17	1,170	27	2	1.20	48.8	40.7	65
18	1,070	28	3	1.07	40.8	38.1	61
19	960	27	2	0.94	31.0	33.0	48
20	1,400	32	1	1.40	72.2	51.5	69
21	1,140	27	1	1.14	38.0	33.3	49
22	600	Unknown	5	0.60	15.7	26.2	37
23	1,220	Unknown	5	1.22	43.6	35.7	40
24	1,330	Unknown	2	1.35	65.2	48.3	46
25	1,200	29	2	1.20	31.0	25.9	55
26	1,330	Unknown	1	1.34	27.0	20.1	50
27	1,450	Unknown	3	1.44	75.2	52.2	74
28	1,320	32	1	1.32	48.0	36.4	63
29	1,380	32	2	1.38	47.6	34.5	54
30	1,240	32	2	1.30	30.1	23.1	38
31	1,370	34	3	1.41	74.8	53.7	72
32	850	25	2	0.82	24.7	30.2	48
33	960	26	1	0.94	35.4	37.6	46
34	1,170	29	3	1.13	37.1	32.8	NA
35	685	27	1	0.68	24.7	36.4	NA
Mean	1,102	28.9	–	1.10	39.6	35.7	–
SD	230	2.6	–	0.23	15.4	9.4	–

Hct, hematocrit; NA, not available; RCV, red cell volume; VLBW, very low birth weight.

DISCUSSION

The mean RCV in our patients, 35.7 ml/kg soon after birth and 21.6 ml/kg at 6 wk, is similar to the results previously reported for newborn infants (1,4,5,7,8,10,11).

Bratteby (5) demonstrated a fall in RCV expressed in ml/kg during the first several months in a group of patients that included eight preterm infants, all with higher birth weights than our study infants. Our results demonstrate the same

phenomenon in VLBW infants. To our knowledge, there have been no other reports of repeated RCV measurements in the same VLBW infants during the first weeks of life. The fall in RCV/kg parallels the fall in hemoglobin concentration seen in preterm infants during the first several months (17).

The mean volume of phlebotomy losses for the 12 infants with two measurements of RCV, 29.2 ml of RBCs over ~40 d, is equivalent to ~0.7 ml/kg/d. This figure is similar to other

Table 2. RBC balance in VLBW infants

Patient number	RCV ₁ (ml)	Weight at RCV ₁ (kg)	Hct at RCV ₁ (%)	RCV ₂ (ml)	Weight at RCV ₂ (kg)	Hct at RCV ₂ (%)	Phlebotomy RBC losses (ml)	Transfused RBCs (ml)	P-D (ml)
1	24.0	0.99	52	33.4	1.24	36	26.8	60.8	−24.6
2	28.0	0.72	57	27.0	0.83	46	47.8	86.4	−39.6
3	38.6	1.14	66	26.1	1.53	36	24.3	31.3	−19.5
4	61.4	1.31	59	26.1	1.40	43	46.4	64.1	−53.0
5	44.6	1.09	38	35.0	1.13	44	32.9	54.7	−31.4
6	50.0	1.18	48	40.6	1.66	38	23.4	20.0	−6.0
7	36.0	1.00	70	19.4	1.13	30	21.6	12.0	−7.0
8	45.8	0.78	66	15.4	1.10	34	32.8	17.4	−15.0
9	26.1	0.96	43	24.3	1.04	44	49.1	33.5	+13.8 ^a
10	36.2	0.92	50	28.3	1.46	32	24.5	16.7	−0.1
11	28.0	0.92	59	18.8	1.22	30	11.7	7.0	−4.5
12	28.4	0.98	61	25.3	1.32	37	9.1	9.9	−3.9
Mean	37.3	1.00	56	26.6	1.26	38	29.2	34.5	−15.9
SD	11.4	0.16	10	7.1	0.23	6	13.2	25.9	18.7

All volumes are expressed as ml of RBCs.

Hct, hematocrit; P-D, RBC production minus destruction *in vivo* (net RBC production).

^aThis patient also had five exchange transfusions, with a total of 320.4 ml of RBCs transfused and 287.7 ml of RBCs removed; including these volumes, the net RBC production for this patient (P-D) was −18.9 ml.

reports (18–21), including some from recent years (22). At the time of our study, microanalytical techniques were already in use in the clinical laboratories at McMaster University Medical Centre (Hamilton, Ontario, Canada).

The volumes of RBCs lost by phlebotomy and gained by transfusion were roughly equal in magnitude to the initial RCV. This means that the removal of RBCs by phlebotomy and their replacement by transfusion produces a volume of RBC turnover equivalent to what would occur in a single-volume exchange transfusion but over a period of 6 wk.

Our method of measuring RCV used a radioactive RBC label, ⁵¹Cr. At the time these studies were conducted, research using radioisotopes in infants was an accepted practice. Saenger and Kereiakes (23) suggested 5,000 millirads (50 mSv) as an upper limit for exposure of newborn infants to γ irradiation for research purposes. The dose required for our RCV method, 0.16 mSv, was far below this limit. Nevertheless, the use of radioactive substances for research in infants would be considered ethically unacceptable in the 21st century. Fortunately, biotin has been developed as a safe, nonradioactive RBC label for studies of RCV and RBC survival in infants (10,11,14,24,25), allowing continued research in this important area.

To our knowledge, even 36 y after the studies were done, this is still the first report documenting the change in RCV that occurs in individual VLBW infants during the first weeks of life, when anemia of prematurity is evolving. As such, our study shows by direct measurement of RCV that the postnatal fall in hemoglobin and hematocrit in VLBW infants results not only from a decrease in RCV expressed in ml/kg but also, in most patients, from a decrease in the absolute RCV expressed in ml.

METHODS

The subjects were born weighing less than 1.5 kg and were patients in the McMaster University Neonatal Unit between October 1976 and June 1977.

All infants had at least one measurement of their circulating RCV during the first 5 d of life, and a convenience sample of these infants had their RCV measured again at 6 wk of age, a time that corresponds to the usual nadir in hemoglobin concentration resulting from anemia of prematurity (17). For these infants, the volume of blood removed for laboratory monitoring, including blood incidentally wasted during phlebotomy, and the volume of blood given as transfusions were determined and recorded.

The method of determining RCV was a scaled-down adaptation of a technique that was well established in adults (26). One milliliter of the infant's blood was collected by venipuncture or from an indwelling umbilical catheter and then injected into a vial containing 0.25 ml of acid-citrate-dextrose anticoagulant. The sample was centrifuged, and the plasma was discarded. The red cells were incubated with 2 microcuries of ⁵¹Cr sodium chromate at room temperature for 20 min, then washed twice with 2 ml of normal saline, and resuspended in 1 ml of saline. The labeling efficiency was determined to be greater than 95%.

The labeled cells were reinjected through the umbilical catheter, if present, or a 25-gauge butterfly needle. Fifteen and 30 min after the reinjection of the labeled RBCs, 0.1-ml blood samples were obtained by heel prick using heparinized microhematocrit tubes. The hematocrit was determined, and the sample was transferred quantitatively into a counting tube. The weight of the sample of blood was determined, and 1 ml of water was added to the counting tube to lyse the cells and reduce the counting errors arising from inhomogeneous samples. Each sample was counted for 30 min; the counting error was less than 1%. The mean difference in RCV calculated from the 15- and 30-min samples was 3.5%. Two infants had their RCV measured twice within 4 h with separate injections of ⁵¹Cr. The mean difference in RCV calculated from the two injections was 5%.

Injection of 2 microcuries of ⁵¹Cr into a 1-kg infant provided an effective dose of ~0.16 mSv. The International Commission on Radiological Protection recommends that the total effective dose from a procedure be compared with the radiation exposure from natural background sources such as the sun, outer space, and radioactive materials found in the earth's air and soil, which is 3 mSv per year (27).

For the infants whose RCV was measured twice, records were kept of the volume of blood removed for laboratory analyses and the volume of blood transfused between the two RCV measurements. These volumes of blood were converted to volumes of RBCs using the infant's hematocrit for the blood removed and the hematocrit of the transfused blood product. In addition, in six infants, all materials containing any of the patient's blood, which would normally be discarded—such as needles, syringes, gauze pads, cotton balls, tissues, and bandages—were collected in a bag attached to the bedside, and the volume of blood in these materials was determined by radioactive counting.

The net RBC production (production minus destruction *in vivo*, designated as P-D) was calculated as $RCV_2 - RCV_1 + S - T$, where RCV_2 is the RCV at 6 wk, RCV_1 is the initial RCV, S is the volume of RBCs sampled by phlebotomy for laboratory analysis between RCV_1 and RCV_2 , and T is the volume of RBCs transfused between the RCV measurements. Descriptive statistics were calculated for the RCV measurements and RBC volumes removed and transfused. RCV was expressed both as ml and as ml/kg. The decrease in RCV during the first 6 wk was tested for significance using the Wilcoxon signed-rank test.

The study was approved by the McMaster University institutional review board. Informed consent for study participation was obtained in writing from one or both parents of each patient.

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Red cell volume measurements from some of the patients included in this series were also included in a previous non-peer-reviewed publication (Blanchette VS, Zipursky A. Clin Perinatol. 1984;11:489), which did not address the central theme of this report, the change in red cell volume over time and the red cell balance during evolution of anemia of prematurity.

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