Blood Transfusion Increases Functional Capillary Density in the Skin of Anemic Preterm Infants

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ABSTRACT

Direct visualization of the microcirculation at the level of the skin capillaries may provide information on the quality of tissue perfusion. Orthogonal polarization spectral imaging (OPS) enables noninvasively direct observation of those blood vessels. OPS was applied to the upper arm of 13 preterm anemic infants [median (95% confidence interval) gestational age: 26 wk (25–26 wk); birth weight: 730 g (652–789 g)] before and 2 and 24 h after transfusion (Tx). OPS images of skin perfusion were continuously recorded on video. Off-line quantitative data of microvascular perfusion were obtained by measuring functional capillary density, vessel diameter, red blood cell velocity, and flow. We found a significant increase in functional capillary density 2 h after transfusion with an additional significant rise after 24 h [before: 142 (134–155); 2 h after Tx: 185 (166–196); 24 h after Tx: 206 (185–219) cm/cm^2; $p < 0.001$], thus indicating improved microvascular perfusion. Vessel diameter, red blood cell velocity, and flow did not change significantly. There were no significant changes in clinical variables, such as blood pressure, heart rate, or body temperature. Whereas conventional monitoring methods did not show any changes after transfusion, quantitative analyses of OPS images indicated improved perfusion; hence, it seems a useful monitor for assessing the response to therapies aimed to improve tissue perfusion. (*Pediatr Res* 56: 751–755, 2004)

Abbreviations
FCD, functional capillary density
MAP, mean arterial blood pressure
OPS, orthogonal polarization spectral imaging
RBC vel, red blood cell velocity
Tx, transfusion

Transfusion practices for neonates are controversial, variable, and based on limited scientific information. This inconsistent approach stems from incomplete knowledge of the effects of anemia in infants and a lack of controlled studies. This results in no clearly established indications for transfusions (Txs) in neonates. The decision to transfuse is frequently based on clinical signs, such as episodes of apnea and bradycardia or failure to gain weight. Data on the effects of blood Tx on these clinical parameters, however, are conflicting (1,2). Peripheral fractional oxygen extraction may be a better indicator for the need of Tx. This concept, however, did not seem applicable in the clinical setting, because in a pilot study of anemic infants, indication for Tx was not influenced by the fractional oxygen extraction value (3).

The effect of hemodilution on capillary perfusion has been investigated extensively in the hamster skinfold model using intravital microscopy (4–7). Orthogonal polarization spectral imaging (OPS) has been developed as a noninvasive technique to investigate the human microvasculature and can be used even in small infants (8). The technology has been described in detail elsewhere (9). In brief, through the use of special optics, a virtual light source is created within the tissue and the light is absorbed by Hb, yielding an image of the illuminated Hb-carrying structures in negative contrast. We have previously shown that microvessel diameter, red blood cell velocity (RBC vel), and functional vessel density can be measured noninvasively by OPS in the skin of term and preterm infants (8). The aim of the study was to determine whether RBC Txs in anemic preterm infants results in an improved microvascular perfusion as indicated by a higher functional capillary density (FCD).

METHODS

Patients. The Ethics committee of the Medical Faculty of the Ludwig-Maximilians University Munich approved the study, and parental consent was sought before measurements.

Decision for Tx was independent of the study and up to the discretion of the attending neonatologist. Infants usually received a Tx when the Hb was <12 g/dL in ventilated infants with oxygen requirement, Hb <10 g/dL nonventilated infants but with oxygen requirement >30%, Hb 8–9 g/dL in infants with oxygen requirement <30% but not feeding or not gaining weight well, and any infants with Hb levels <7 g/dL. Because Hb levels are measured in each blood gas, Hb values were more consistently available than spun hematocrit values. Infants received a Tx of 10–15 mL/kg body weight of packed cytomegalovirus-negative irradiated RBC over 4–5 h. Microcirculatory parameters were measured within 4 h before and 2 and 24 h after the Tx. Systolic, diastolic, and mean arterial blood pressure (MAP) were recorded simultaneously with the OPS measurements either continuously *via* an arterial line (Becton Dickinson Critical Care systems with an Ohmeda Statham Transducer, Singapore) if in place or every 5 min oscillometrically (Dinamap Critikon Vital Data Monitor, Norderstedt, Germany). Heart rate and body core temperature with a skin probe over the liver were also recorded, as was incubator temperature. Measurements were obtained in 15 infants, but two infants had to be excluded as a result of incomplete measurements. The probe was placed next to the infants' skin close to the axilla with the least possible contact to avoid pressure against the skin. Lubricating the skin with normal saline improved imaging. Measurements were started when the infants settled down and went to sleep. Sedation was not necessary. Measurements were done over 5–10 min to obtain as many possible sequences without movements artifacts. Data were calculated for all sequences without movement artifacts, and values were averaged. At each time point (before and 2 and 24 h after Tx), FCD was measured three to five times with a variance of <10%. It is not possible to measure exactly the same vessels repeatedly, but measurements before and after the Tx were always obtained on the upper arm close to the axilla.

Imaging. In OPS imaging, a virtual light source is created at a depth of ~1 mm within the tissue through the use of special optics. The light shining back through the tissue is absorbed by Hb, yielding an image of the illuminated vessel in negative contrast with a resolution of 1 pixel $\cong$ 1 μm. Because no fluorescent dyes are needed, it can be used in humans. The method has been validated for quantitative measurements of microcirculatory parameters in an animal model against intravital microscopy (10).

Calculation of vessel diameter, RBC vel, and vessel density. Images were created using the CYTOSCAN A/R, stored on videotape and analyzed off-line. Vessel diameter, vessel density, and RBC vel were measured with the CapiScope program in as many vessels as possible per observation area (Figs. 1 and 2). To measure the diameter, a vessel is selected from the image on the video screen, and a line is placed in the vessel.
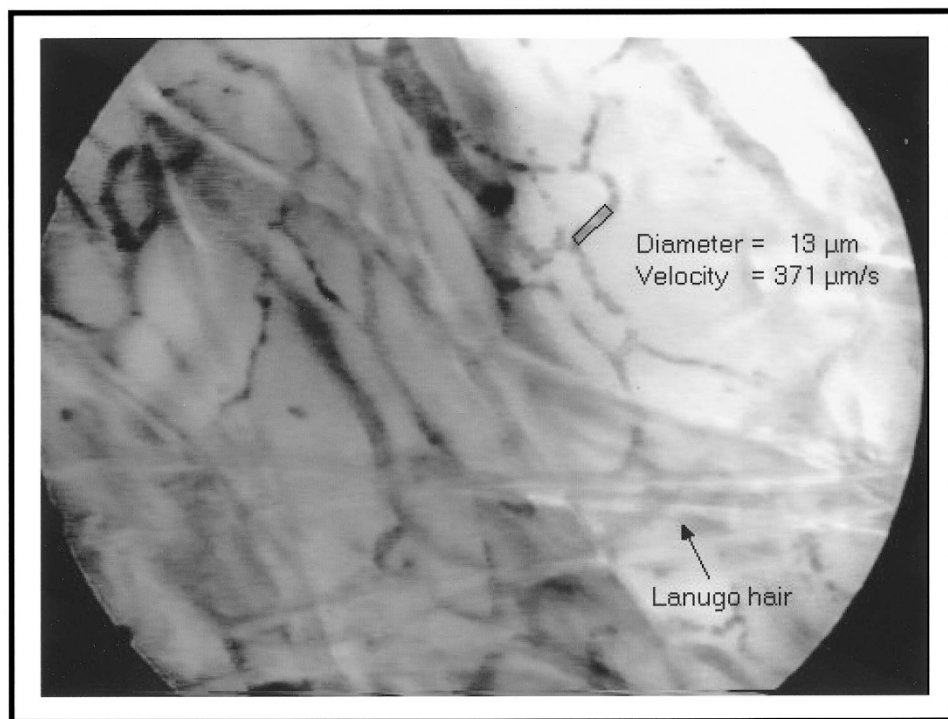

Figure 1. Measurement of vessel diameter and RBC vel. Image of the skin of a preterm infant (gestational age: 25 wk; birth weight: 685 g) at day 16 and current weight of 850 g. Vessel diameter, vessel density, and RBC vel were measured with the CapiScope program in as many vessels as possible per observation area. The RBC vel is measured by drawing a line along the vessel and projecting the image of the vessel onto a 1-pixel thick line. The gray profile along the line is taken for each field every 1/50th of a second. The comparison is performed by calculating the correlation coefficient for every possible shift of the previous gray level profile relative to the new profile. The shift, which produces the highest correlation, indicates the distance that the pattern traveled between the two gray level profile measurements. Because the time lapse between the two gray level profiles is known, the velocity can be calculated. For measuring the diameter, a vessel is selected from the image on the video screen and a line is placed in the vessel. The computer calculates the diameter from 1-pixel perpendicular lines placed over the whole length of the drawn line, based on the gray level change. The program then places a box over the measured vessel as depicted. The arrow points at lanugo hair, which is frequently seen in images of very premature infants.

Figure 2. Measurement of FCD. Vessel density is defined as the length of RBC-perfused vessels per observation unit area and is given as cm/cm^2. Using the CapiScope Software program, the perfused vessels within the defined area are traced and the length of the marked vessels is calculated per area of observation.

The computer calculates the diameter from 1-pixel perpendicular lines placed over the whole length of the drawn line, based on the gray level change. RBC vel is measured using a spatial correlation technique, which requires a 10-s video sequence in which the tissue reveals little movement (for details, see Fig. 1). Vessel density is defined as the length of RBC-perfused vessels per observation unit area and is given as cm/cm^2 (Fig. 2). "Vessel density" refers to perfused vessel density for this article. In neonates, because of the small size of the vessel, arterioles, capillaries, and postcapillary venules cannot be distinguished clearly.

Quantitative measurement of vessel density, vessel diameter, and RBC vel using OPS imaging all have been validated against intravital microscopy in the hamster dorsal skinfold model and the rat liver and human sublingual tissue (9–11). Validation of the CapiScope Software program can be found at www.bridleways.uklinux.net/Videoscopy.com/index.php.

Statistics. Data are presented as median and 95% confidence interval (CI). Data were analyzed using ANOVA with a *post hoc* test for linear trend, followed by paired *t* test for parametric data. For correlation, the Spearman rank two-way test was applied. All calculations were done with GraphPad Prism 3.0 for Windows 95 (GraphPad Software, Inc., San Diego, CA).

RESULTS

The 13 infants [median (95% CI) gestational age: 26 wk (25–26 wk); birth weight: 730 g (652–789 g)] received a Tx of 10 mL (9.3–14.2 mL) packed RBCs/kg body weight at age 30 d (19–39 d) and current body weight of 1075 g (897–1338 g). As expected, Hb values rose after Tx, but none of the clinical variables, such as blood pressure, heart rate, or body temperature, changed significantly (Table 1). FCD increased significantly 2 h after the Tx (Fig. 3) with an additional significant rise after 24 h, whereas vessel diameter, RBC vel, and flow did not change (Table 2). We found no correlation between Hb levels or heart rate and microcirculatory parameters. As a measure of potential oxygen transport capacity, we calculated the product of RBC flow and FCD but found no linear correlation with heart rate or Hb levels.

Table 1. *Clinical data (median and 95% CI) before and 2 and 24 h after Tx*

	Hb (g/dL)	Heart rate (beats/min)	MAP (mmHg)	Infant temperature (°C)	Incubator temperature (°C)
Before Tx	8.3*†	165	42	37.0	32.4
	7.9–10.4	157–174	38–51	36.9–37.3	31.2–34.2
2 h after Tx	10.7	164	42	37.2	31.7
	9.9–12.9	159–179	39–51	37.0–37.5	30.8–33.6
24 h after Tx	11.8	164	39	37.0	32.1
	10.8–12.9	155–175	35–45	36.5–37.2	30.6–33.6

* $p = 0.04$ before *vs* 2 h after Tx.

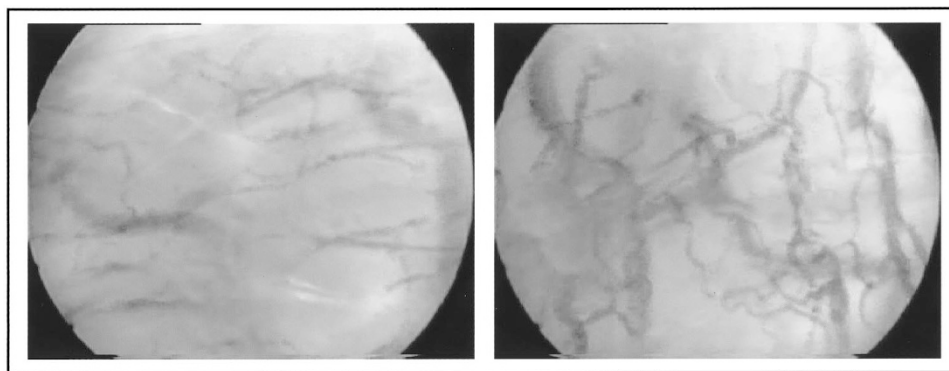† $p = 0.002$ before *vs* 24 h after Tx.

Figure 3. Image before and after Tx. The image on the left was taken 3 h before and the one on the right 2 h after Tx of packed RBCs to a 16-d-old premature infant with a gestational age of 25 wk, birth weight of 686 g, and current body weight of 850 g.

Table 2. *Vessel diameter, density, flow, and RBC vel (median and 95% CI) before and 2 and 24 h after Tx*

	Diameter (μm)	RBC vel (μm/s)	Flow (μm^3/s)	FCD (cm/cm^2)
Before Tx	10.8	349	29 392	142
	9.7–11.2	336–402	26 625–37 616	134–155
2 h after Tx	10.1	343	27 797	185*
	9.4–10.7	316–378	24 228–31 366	166–190
24 h after Tx	10.3	371	30 425	206*†
	9.2–11.3	323–392	24 506–36 343	185–219

* $p < 0.0001$ *vs* before Tx.

† $p < 0.0001$ 2 *vs* 24 h after Tx.

DISCUSSION

After birth, Hb concentration decreases markedly in premature infants, partially as a result of a physiologic reduction in erythrocyte number but mostly as a result of iatrogenic blood loss. However, there is no universally accepted definition of anemia requiring Tx in infants, because there is only a poor relationship between Hb level and the occurrence of pathologic symptoms, *e.g.* tachycardia, poor weight gain, breathing abnormalities (2). However, an effective microvascular perfusion is essential for an adequate nutritional supply, which finally guarantees appropriate organ function. In the present study, we showed for the first time that functional vessel density increases after raising Hb values by 2.5 g/dL, equivalent to a 30% rise. FCD is well validated and currently seems to be one of the best quantitative indicators of microvascular perfusion (12). The observed rise in FCD 2 h after the transfusion could be an effect of volume only rather than RBC Tx but not the further increase after 24 h. In this context, perfusion means erythrocytes participating in nutritive exchange. This is a function of the number of erythrocytes present, the velocity of the erythrocytes, and the vessel density. FCD is currently the best parameter to assess the quality of perfusion, because we cannot evaluate the quality of nutritive exchange. Erythrocyte velocity measures only the mean speed of erythrocytes in a vessel.

In adults with a lower heart rate, the pulse variation is clearly visible in a good-quality OPS sequence. In fact, one can measure peak and lowest erythrocyte velocity and variability during the cardiac cycle. Theoretically, effects of cardiac output could probably be detected in a similar manner as used in pulse wave analysis based on photoplethysmography (13). In neonates, the vessels visualized with OPS in the skin are too small and the heart rate is too high to see these phenomena.

Relevance of measurement of skin perfusion. Cardiac output normalized to mass is much higher in the newborn than in the adult. Perfusion pressure is maintained by redistributing marginal cardiac output and oxygen supply to brain, heart, and adrenal gland. Under nonstress conditions, the skin has a high blood flow in relation to its oxygen requirement, whereas stress results in a profound vasoconstriction mainly of skin arteriovenous shunts. Whereas this holds true for all humans, this compensatory mechanism is particularly profound in neonates. Keeping this in mind, it is less surprising that most studies in premature infants have not found any significant changes in heart rate in anemic infants (2,14). We would argue that in neonates, disturbance of skin perfusion can be an early sign of global tissue perfusion impairment as a result, for example, of insufficient blood volume. In this context, OPS imaging seems more suitable, because the shorter wave used in OPS (green light) allows estimations of true capillary perfusion, whereas longer wave length instrumentation such as laser Doppler instruments and infrared photoplethysmographs mainly represent vessels from a greater depth and measure a parameter more representative of total blood flow (15).

Bauer *et al.* (16) investigated the effects of RBC Tx on blood volume, blood pressure, and resting peripheral blood flow in the leg with strain-gauge plethysmography and blood viscosity in the first week of life. Their infants with pre-Tx hematocrits of 38% were significantly less anemic than in our study. They found an increase in resting peripheral blood flow by 25%, whereas systolic blood pressure increased by only 12%. They also found that peripheral resistance decreased by 9% and

blood viscosity increased by 21% with a decrease in vascular hindrance by 24%, indicating vasodilation of limb arteries. The increase in resting peripheral blood flow and the decrease in hindrance were particularly pronounced in infants with high pre-Tx blood volume. One could speculate that the increase of blood viscosity led to recruitment of capillaries with an increase in FCD, which also might explain the increase in resting peripheral blood flow. These findings are in good agreement with our own report, because both indicate a better tissue perfusion after RBC Tx.

Studies in hemorrhagic shock show that survival is primarily determined by the maintenance of FCD and secondarily by tissue oxygenation (7). FCD is maintained as hematocrit is reduced beyond the Tx trigger by increasing plasma viscosity, which transmits systemic pressure to the capillaries and induces vasodilation through the increased shear stress-dependent release of vasodilators. Consequently, the Tx trigger is also a "viscosity trigger" indicating when blood and plasma viscosity are too low. In this condition, increasing plasma viscosity is beneficial and extends the Tx trigger, reducing the use of blood Txs (7). Blood viscosity at a given hematocrit is lower in preterm infants than in term neonates and adults as a result of low plasma viscosity and low RBC aggregation, because total plasma protein levels correlate with gestational age. Consequently, the exchange of neonatal plasma with adult plasma increases blood viscosity values in the neonate to adult values. Conversely, the exchange of neonatal RBCs for adult RBCs does not affect blood viscosity. Neonatal RBCs therefore do not possess specific properties that influence blood viscosity (17). Opposite to exchanging neonatal RBCs with adult RBCs, transfusing anemic preterm infants does increase blood viscosity by 33% and also blood volume (18).

Limitations. One problem of OPS imaging for microvascular monitoring in humans is the variability of the vessels measured. Identical vessels cannot be examined over time in contrast to intravital microscopy in animal experiments (*e.g.* hamster skinfold chamber) or capillaroscopy of the nail fold. Hence, many more sites of interest must be evaluated to obtain statistically significant data. Movement artifacts may impair the measure velocity with the CapImage software. An additional limiting factor is the application pressure that is uncontrolled and may lead to pressure artifacts. This may result in an underestimation of the microvascular RBC vel values. Great care was undertaken to reduce these pressure artifacts by placing the probe next to the infant with basically minimal touch.

Harris *et al.* (19) demonstrated in the dorsal skinfold chamber of the awake Syrian golden hamster that OPS imaging technology can be used to make accurate and reproducible quantitative measurements of diameter and FCD over a wide variety of hematocrits. They found a very good agreement between measurements obtained by OPS and by standard intravital fluorescence microscopy during isovolemic hemodi-

lution down to hematocrits of 25% of the initial values. In this model of isovolemic hemodilution, FCD decreased significantly at very low hematocrit values, and values of both methods were in good agreement.

CONCLUSION

We demonstrated that blood Tx in anemic neonates results in a better perfusion of skin microvessels as indicated by FCD data from OPS imaging. We suggest that OPS imaging can be used to measure microvascular perfusion in neonates and allows the assessment of the quality of tissue perfusion.

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