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# Low fraction of fetal haemoglobin is associated with retinopathy of prematurity in the very preterm infant

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## ABSTRACT

**Background** Blood loss and adult blood transfusions are common during the neonatal period in preterm infants. The objective of the study was to clarify if degree of loss of fetal haemoglobin (HbF) was associated with later retinopathy of prematurity (ROP).

**Methods** Retrospective observational cohort study. In total, 452 infants born <30 gestational weeks at a tertiary level neonatal intensive care unit in Sweden in 2009–2015 were included, 385 of whom had final ROP outcome. Mean fractions of HbF (%) during the first postnatal week were calculated from 11 861 arterial blood gas analyses. The relationship between fractions of HbF (%) and ROP was evaluated.

**Results** The mean (SD) gestational age (GA) at birth was 26.4 (1.8) weeks. In total, 104 (27 %) infants developed ROP. Higher fraction of HbF (%) was associated with a lower prevalence of ROP, OR by a 10% increase 0.83 (95% CI: 0.71 to 0.97;  $p=0.019$ ), following adjustment for GA at birth, small for GA and sex. Infants with HbF (%) in the lowest quartile had OR of 22.0 (95% CI: 8.1 to 59.2;  $p<0.001$ ) for ROP development compared with those in the highest quartile. The predictive ability (area under the curve) of HbF (%) in the full model during the first week was 0.849 for ROP.

**Conclusions** Early low fraction of HbF is independently associated with abnormal retinal neurovascular development in the very preterm infant. The potential benefit of minimising blood loss on development of ROP will be investigated in a multicenter randomised trial (NCT04239690).

at 6 months after term age.<sup>3</sup> In transfused newborns, HbA substitutes HbF during periods, while preterm erythropoiesis produces RBC with HbF at a postmenstrual age (PMA)-dependent rate.<sup>4</sup> Both volume and number of RBCs transfusions are associated with increased ROP risk in infants with very and extremely ( $\leq 1000$  g) low birth weight.<sup>5–7</sup>

Recent preliminary evidence suggests that a low fraction of HbF is an independent predictor for ROP in preterm neonates.<sup>8</sup> Given the association between low HbF and ROP, it has been hypothesised that HbF per se might protect from oxidative stress.<sup>8,9</sup> The decreased oxygen affinity of HbA results in increased availability of oxygen in the tissues of transfused compared with non-transfused extremely preterm infants and is therefore likely to contribute to hyperoxia<sup>2</sup> and fluctuating oxygenation which are known risk factors for ROP.<sup>10</sup> Other effects of HbF are largely unknown.

The relationship between administered blood transfusions and decreased fractions of HbF was shown recently in a relatively small cohort of very preterm infants. That study also indicated an association between lower fraction of HbF and the development of ROP. An underlying mechanism was proposed involving a shift of the oxygen-dissociation curve due to replacement of HbF by HbA, resulting in a higher fraction of oxygen dissolved in plasma and increased exposure of tissue to oxygen.<sup>8</sup>

We hypothesised that early postnatal lower HbF is a risk factor for ROP. This was evaluated in 452 consecutively admitted inborn infants.

## INTRODUCTION

Despite significant advances in neonatology during the last decades, the morbidity in extremely preterm and low birth weight preterm infants remains high. In Europe, 12% of infants born at 24–28 weeks of gestation developed severe retinopathy of prematurity (ROP) requiring treatment.<sup>1</sup> Repeated adult red blood cell (RBC) transfusions in preterm infants are acknowledged risk factors for prematurity-associated diseases and constitute an independent predictor of adverse outcome, in particular for ROP and bronchopulmonary dysplasia (BPD). The current source of RBCs for transfusions to preterm infants is blood derived from adult donors. Fetal RBCs contain mainly fetal haemoglobin (HbF) which binds oxygen with greater affinity than adult haemoglobin (HbA).<sup>2</sup> The fraction of HbF is approximately 70%–80% during the third pregnancy trimester with a gradual decrease to 3%–4%

## MATERIAL AND METHODS

### Patients and clinical characteristics

This single-centre retrospective study included 452 live-born infants born before 30 weeks of gestational age (GA) at Skåne University Hospital in Lund, admitted to the neonatal intensive care unit (NICU), tertiary level, between 2009 and 2015. Infants were included, and data collected according to the ethical approval. GA was determined at 17–18 postmenstrual weeks by routine ultrasound fetometry. Birth weight (BW) small for GA (SGA) was defined as a BW >2SD below the GA-related mean of the population according to the Swedish reference curve for normal fetal growth.<sup>11</sup> During the study period, infants received supplemental oxygen aiming to achieve an SpO<sub>2</sub> of 91%–95%, when needed. Blood transfusions were administered to maintain Hb concentrations above 140 g/L during the first postnatal week. ROP was classified



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according to the International Classification of Retinopathy of Prematurity<sup>12</sup> and treatment followed recommended guidelines.<sup>13</sup> Severe ROP was defined as stage 3 and/or type 1 ROP. Infants included in this cohort have previously been investigated when examining the role of HbF and the association to BPD.<sup>14</sup> Efforts to minimise bias included continuous inclusion of all consecutively born infants during the study period, and a high frequency of infants with complete data. Ninety-eight of the included infants were part of a previous bi-centre study in Sweden investigating sampling-related blood volumes and blood transfusions.<sup>15</sup>

### Blood gas analyses

In total, 11 861 HbF measurements were retrieved from clinical arterial blood gases obtained and analysed during the first 7 postnatal days (PNDs). Data on arterial HbF were retrieved from the local hard drive of the blood gas analyzer (Radiometer 800, Copenhagen, Denmark) in the NICU. Arterial HbF analyses from each individual were sorted according to timepoint and date of analysis. Fraction of HbF (%) of total Hb for each individual was calculated and averaged for each PND (1–7). Accumulated mean values for each day were then used when calculating a weekly mean of HbF.

### Statistics

Binary regression models assessed in the statistical analyses were verified in terms of multicollinearity; variance inflation factor score >1, <10, for linearity between the predictor and log-odds, independence of observations and outliers; Cook's distance <1 and leverage below a threshold level of <3 times (k+1)/N. If any outlier was present, it was excluded and models rerun. Rerun analyses yielding non-similar results as compared with the original model were displayed if present. The mean of each blood gas variable during postnatal week 1 was used in logistic regression analysis. GA at birth, SGA and sex were assessed as independent confounding variables included in the full multiple variable analysis model. Infants with incomplete ROP data were excluded from the analysis. Inclusion of confounding variables in the model was based on clinical relationship to ROP development. Predicted probabilities were obtained by using logistic regression. Area under the curve (AUC) values using ROC analysis were used to evaluate independent predictive abilities. In all analyses, p values <0.05 were considered significant. For comparison between groups, independent samples t-test was used. When investigating thresholds of HbF (%) and the development of ROP, quartiles were used, thus generating similar group sizes.

### RESULTS

Overall, 452 live-born infants were included in the study with a mean (SD) GA of 26.4 (1.8) weeks and a mean (SD) birth weight of 876 (272) grams. The overall mortality rate prior to home discharge was 61/452 (13.5%). Six (1.3%) infants were lost to follow-up ROP screening at PMA 36 weeks. In total, 385 infants participated in the analyses and 104 (27%) developed any and 37 (9.6%) severe ROP. Clinical characteristics are shown in table 1. The number of blood samples available for analysis on PNDs 1–7 were 1386, 2389, 2112, 1773, 1523, 1366 and 1312, respectively. The median (IQR) number of blood samples per subject and PND on PNDs 1–7 were 3 (2–4), 5 (3–7), 4 (3–7), 3 (2–6), 3 (1–5), 3 (1–5) and 3 (1–5). Mean values of HbF (%) in relation to postnatal age (days) in infants without ROP and infants developing any ROP are displayed in figure 1.

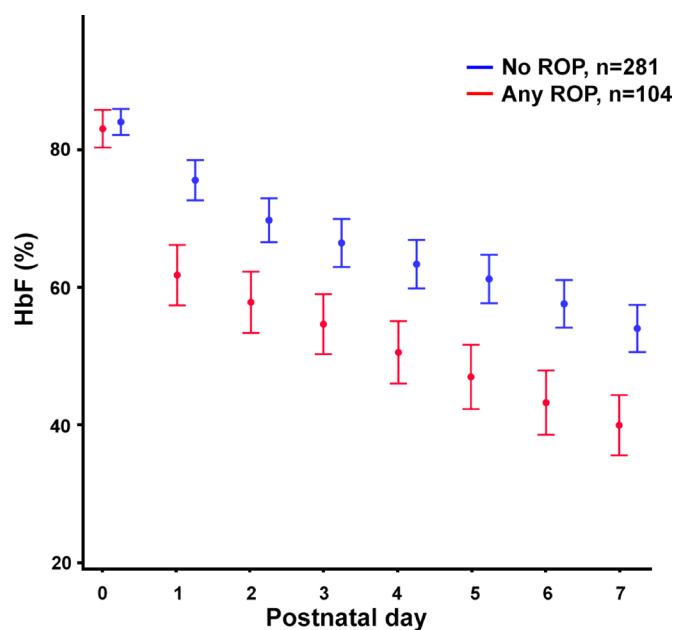
**Table 1** Clinical characteristics and blood gases per subject PND 1–7

	Infants with ROP outcome, n=385
GA, weeks; mean (SD)	26.6 (1.7)
Birth weight, g; mean (SD)	900 (261)
BW SGA; n (%)	90 (23.4)
Male; n (%)	217 (56.4)
Twins; n (%)	87 (22.6)
Triplets; n (%)	18 (4.7)
Morbidities	
IVH grades 3–4; n (%)	27 (7.0)*
Any IVH; n (%)	96 (25.1)*
NEC; n (%)	14 (3.7)*
BPD; n (%)	212 (55.9)†

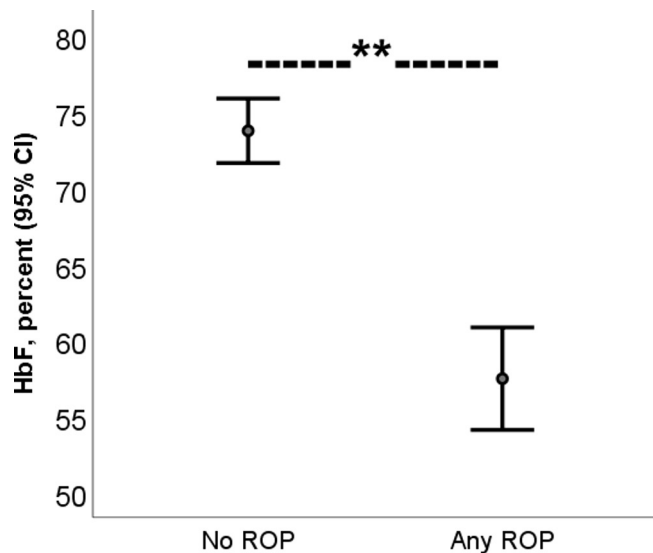
\*383 available for analysis.  
†379 available for analysis.  
BPD, bronchopulmonary dysplasia; GA, gestational age; IVH, intraventricular haemorrhage; NEC, necrotising enterocolitis; PMA, postmenstrual age; PND, postnatal day; BW SGA, birth weight small for gestational age.

Infants with any ROP displayed significantly lower mean fractions of HbF (%), during the first postnatal week, figure 2. Higher fraction of HbF (%) during the first postnatal week was associated with a lower prevalence of any ROP, OR by a 10% increase 0.83 (95% CI: 0.71 to 0.97; p=0.019), following the adjustment for GA at birth, SGA and sex. The fraction of HbF (%) was also inversely associated with severe ROP, both unadjusted (p<0.001), and in the full statistical model, OR by a 10% increase 0.79 (95% CI: 0.63 to 0.99, p=0.041).

When dividing the complete cohort in quartiles based on HbF%, the infants with an HbF (%) in the lowest quartile had an OR of 22.0 (95% CI: 8.1 to 59.2; p<0.001) for developing any ROP, compared with infants an HbF in the highest quartile.



**Figure 1** Mean fraction (%) of HbF during postnatal days 1–7 in relation to development of any ROP. Infants with no ROP (n=281) in blue; Infants with any ROP (n=104) in red. The bars represent 95% CI. HbF, fetal haemoglobin; ROP, retinopathy of prematurity.



**Figure 2** Significantly higher fraction of HbF during postnatal week 1 in infants without ROP compared with infants developing any ROP, in a cohort consisting of 452 very preterm infants. \*\* Indicating  $p < 0.001$ . HbF, fetal haemoglobin; ROP, retinopathy of prematurity.

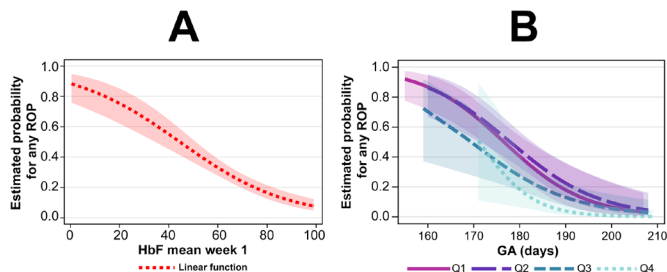
Estimated probability plots for ROP and mean HbF (%) and for each quartile of HbF (%) during postnatal week 1 considering GA at birth are illustrated in [figure 3A,B](#).

The predictive ability (AUC) of HbF (%) in the full model during the first week was 0.849 for any ROP, [figure 4](#).

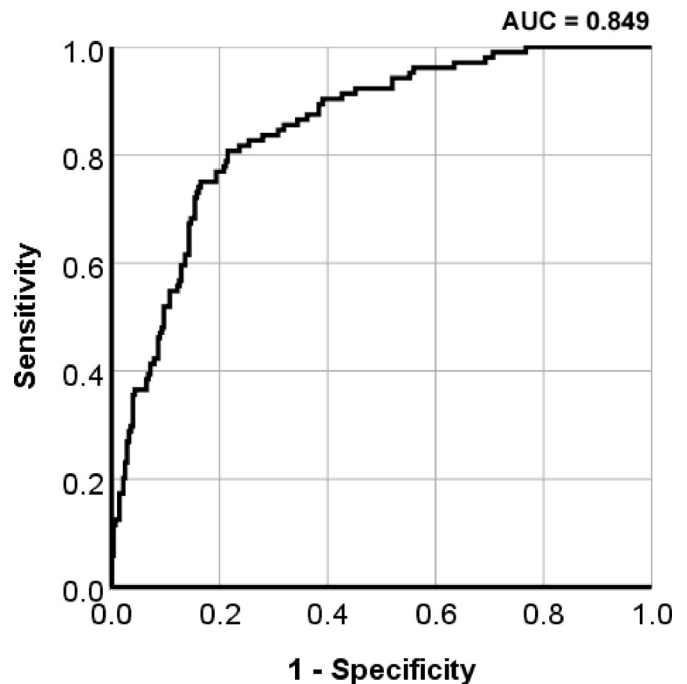
### DISCUSSION

The difference in fraction of HbF between infants who did or did not develop any ROP was pronounced the very first PND and persisted throughout the first postnatal week.

The probability of any ROP was inversely related to mean HbF during the first postnatal week, with a probability of near 60% for development of any ROP at a mean HbF of 40%, compared with only a 10% probability at a mean HbF of 90%. A similar pattern was also seen for severe ROP (data not shown). The potential causal mechanisms in ROP development involving low HbF thus appear to be operating during early postnatal development.



**Figure 3** (A,B) Estimated probability for any ROP in relation to HbF (A) and GA at birth (B). Illustrated by linear function by mean HbF (%) in postnatal week 1 in the complete cohort for any ROP (A) and by GA at birth illustrated per quartile of HbF (%) in postnatal week 1 for any ROP (B). Q1 represents infants with a mean HbF% in the lowest quartile in B, and Q4 represent the infants with a mean HbF% in the highest quartile. Q4; HbF% > 77.6%, Q3; 74%–77.6%, Q2; 58.8%–73.9% and Q1 < 58.8. The shadowed area illustrates a 95% CI. ROP, retinopathy of prematurity; HbF, fetal haemoglobin; GA, gestational age; Q, quartile.



**Figure 4** ROC curve for any ROP (A) and adjusted fraction of HbF (%) during postnatal week 1. HbF (%) adjusted for GA at birth, SGA and sex in the ROC curve. The AUC for HbF was 0.849. ROP, retinopathy of prematurity; HbF, fetal haemoglobin; GA, gestational age; SGA, small for gestational age; AUC, area under the curve.

Importantly, the relationship between low HbF and later ROP reported here was independent of GA at birth and other potential confounders studied (SGA and sex). The probability for development of ROP also differed for the respective HbF quartiles across the range of GA at birth. The retina is part of the central nervous system and higher fractions of HbF in preterm infants have been shown to have beneficial effects in the brain such as increased oxygen transport, due to the specific oxygen-carrying properties of HbF.<sup>16</sup>

It has been suggested that replacing HbF by HbA, thus lowering HbF levels may lead to high oxygen exposure to the developing retina thereby increasing the risk of acute ROP.<sup>8,17</sup>

Studies in animals and humans receiving repeated intrauterine transfusions found that HbA retains its low oxygen binding properties also in the fetal circulation.<sup>18</sup> Notably, as compared with HbA, HbF has a greater capacity to generate nitric oxide (which plays an important role in regulating tone and blood flow),<sup>19</sup> and is less prone to the DNA cleavage activity,<sup>20</sup> HbF seems to protect from reactive oxygen species.<sup>21</sup> On the other hand, birth is accompanied by oxidative stress and cord blood contains more non protein bound iron and its erythrocytes have higher membrane esterified F2-isoprostane levels and increased superoxide anion production than adult erythrocytes<sup>22</sup> and, in addition, low levels of antioxidants.<sup>23</sup> Based on all the above-mentioned considerations, we hypothesised that fetal RBCs containing HbF might be more physiologically relevant than RBCs from adults containing HbA in relation to ROP development.

The association between low HbF proportion and later ROP and changes in HbF might also reflect the loss of other endogenous blood components essential for normal organ development. A main contributor to anaemia in very preterm infants is excessive blood sampling. A significant reduction in iatrogenic blood

loss in very preterm infants might maintain circulating growth factors, thereby improving the outcome of these infants.<sup>24</sup> The concentration of circulating haematopoietic stem cells is inversely related to GA at birth.<sup>25</sup> Historically, blood transfusions have been used as therapy for apnea and bradycardia, poor feeding, poor weight gain, and pallor. Although many studies have examined these outcomes, few are methodologically sound.<sup>26</sup> A recent report from a Swedish national population-based cohort showed that extremely preterm infants receive a mean of seven adult blood transfusions per infant during the neonatal period with a considerable between-centre variation (three to nine transfusions per infant).<sup>27</sup> Guidelines at Swedish units use reference values of haemoglobin (g/l) at different postnatal ages as markers of need of transfusion.

Delayed cord clamping at birth is by far the most favourable intervention for preventing anaemia. As shown in a Cochrane meta-analysis, the resulting autotransfusion of fetoplacental blood, reduces the number of blood transfusions and decreases rate of intraventricular haemorrhage and necrotising enterocolitis in very preterm infants.<sup>26</sup> The impressive effects of delayed cord clamping strongly supports the hypothesis that fetal blood components are essential for prevention of morbidity in the extremely preterm infant. The excessive decrease in HbF observed during the first postnatal week is to a large extent reflecting current blood sampling strategies and existing laboratory infrastructures for blood sample analysis. It was recently shown that sample-related blood loss during neonatal clinical care is linked to the number of administered blood transfusions.<sup>15</sup> The study showed a median sample-related withdrawal of whole blood of 58% of total blood volume (42 mL/kg) during the first two postnatal weeks and a median transfused volume of more than 100% of total blood volume (85 mL/kg) with adult donor blood during the corresponding period.

This finding emphasises the importance of developing and implementing blood-saving sampling methodology in the clinical care of very preterm infants. Recently a multicenter study was initiated with the aim to study the impact of microsampling to preserve HbF and thereby reduce neonatal morbidities including ROP, [www.clinicaltrials.gov/NCT04239690](http://www.clinicaltrials.gov/NCT04239690).

As previously mentioned, decreased fraction of HbF is highly correlated with the volume of administered blood from adult donors. Several studies have shown associations between administered blood transfusions and increased rates of neonatal morbidity. However, prospective studies comparing liberal or restrictive transfusion strategies based on differing Hb thresholds have not, as yet, been able to show a clear impact on either short-term or long-term morbidity.<sup>28 29</sup>

Regarding whether the pathophysiological mechanisms associated with our present finding of low HbF being associated with higher risk of abnormal retinal neurovascular development is low HbF itself, or a proxy of reduction in other endogenous fetal blood components, or adult transfusions, or a combination cannot at this stage be elucidated. In a recent publication aiming to titrate out the risk of early anaemia versus transfusions for ROP development, it was suggested that early anaemia, rather than blood transfusions, was a risk factor for ROP warranting treatment.<sup>30</sup> Further, although the degree of immaturity, SGA and sex were adjusted for, sicker infants are more prone to a more frequent blood sampling rate.

This study has a number of limitations, one of which is its retrospective design. This study included all consecutively admitted infants born before 30 gestational weeks from 2009 to 2015. The data from this single-centre study reflect the medical routines and regimes at one NICU (Swedish level III). This study

population, consisting of very preterm infants, is heterogeneous due to the high frequencies of morbidities, clinical interventions, and the integration of new clinical routines. Priorly, an in-depth analysis of the local sampling regimes have been illustrated, based on a subcohort of this study population.<sup>15</sup>

The main strength of this study is the high intensity of data points and high number of retrieved blood gas analyses along with the size of the study. By using the same blood gas analyser during the study period, data on HbF was downloadable directly from the analyser's hard disk, the risk of errors was minimised.

## CONCLUSION

Low fraction of HbF during the first postnatal week was independently associated with the development of any and severe ROP in very preterm infants. Conversely, maintaining a higher percentage of HbF may be protective for developing ROP. The potential beneficial role of minimising the loss of endogenous blood components in a clinical setting will be further investigated in an ongoing multicenter randomised trial.

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**Data availability statement** Data are available upon reasonable request. Deidentified data will be available on reasonable request.

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