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Pediatric RESEARCH

CLINICAL RESEARCH ARTICLE

Altered erythropoiesis in newborns with congenital heart disease

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BACKGROUND: Fetal hypoxia has been implicated in fetal growth restriction in congenital heart disease (CHD) and leads to stress erythropoiesis in utero. The objective is to assess erythropoiesis and its association with growth in newborns with CHD.
METHODS: Fetuses with prenatally diagnosed CHD from 2013 to 2018 were retrospectively reviewed. Pregnancies with multiple gestation, genetic abnormalities, major extra-cardiac anomalies, and placental abruption were excluded. Complete blood count tests at birth were compared to published normative values. Spearman correlation assessed associations of red blood cell (RBC) indices with birth anthropometrics and prenatal Doppler measures.
RESULTS: A total of 160 newborns were included. Median gestational age was 38.3 (37.3, 39.0) weeks. Infants ≥37 weeks gestation had lower hemoglobin (Hgb), hematocrit, and elevated nucleated RBC (nRBC), mean corpuscular volume, and mean corpuscular hemoglobin compared to reference. No differences in RBC indices were observed in infants <34 and 34–37 weeks gestation. There was no difference in Hgb and nRBC between CHD subgroups. Neither Hgb nor nRBC were associated with birth anthropometrics or Doppler patterns.
CONCLUSIONS: Term infants with CHD demonstrated multiple alterations in erythrocyte indices suggesting ineffective stress erythropoiesis in late gestation resulting in lower Hgb at birth. Altered erythropoiesis was not correlated to growth or Doppler patterns.

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IMPACT:

- Newborns with congenital heart disease (CHD) born at term gestation demonstrated altered erythropoiesis.
- Term newborns with CHD have decreased hemoglobin levels despite having red blood cell indices consistent with stress erythropoiesis, suggesting an incomplete compensatory response to in utero physiologic disturbances associated with CHD.
- The etiology is unknown; however, it may be influenced by multiple risk factors during pregnancy in the maternal–fetal dyad.
- Alterations in red blood cell indices were not associated with outcomes of fetal growth.

INTRODUCTION

Gestational age (GA) and birth weight (BW) are important predictors of survival outcomes in newborns with congenital heart disease (CHD).[1–3] Fetal growth restriction (FGR) and small for gestational age (SGA) status are common in this population.[4,5] Fetal hypoxia has been implicated as a possible causal mechanism; however, it is unclear whether hypoxia occurs secondary to placental abnormalities or hemodynamic circulation changes secondary to underlying CHD. Chronic hypoxia from placental insufficiency is known to be associated with intrauterine growth restriction and placental abnormalities have been demonstrated in pregnancies complicated by fetal CHD.[6–8] Additionally, fetuses with different types of CHD exhibit varying growth trajectories throughout gestation.[9–11]

The evaluation of fetal hypoxia remains difficult. Direct measurements of oxygen levels can be made with invasive methods such as umbilical vein sampling; however, the procedure carries risks.[12] Indirect measurements that evaluate for secondary signs of hypoxia have been established with Doppler ultrasound and fetal magnetic resonance imaging (MRI).[13,14] Fetal asphyxia can lead to increased impedance of blood flow in the umbilical artery (UA), which manifests as absent or reversed end diastolic flow.[15] With decreased fetal oxygen saturation, there are higher UA resistance indices (RIs) and lower middle cerebral pulsatility indices (PIs) and RIs as a result of redistribution of blood flow.[16,17] Increased umbilical PIs have also been noted in pregnancies at high altitude.[18] Recently, MRI phase-contrast imaging and T2 mapping have been used to determine oxygen saturation in fetuses.[19] A higher T2 signal, which has been demonstrated in the umbilical vein, represents a higher oxygen saturation of blood compared to a lower T2 signal seen in the descending aorta that returns blood to the placenta.

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Hemoglobin (Hgb) and hematocrit (Hct) are measurable variables that can reflect oxygenation. Hypoxia leads to accelerated stress erythropoiesis in utero and typically results in elevated levels of erythropoietin, Hct, and nucleated red blood cell (nRBC).[20–23] Similarly in older children with cyanotic CHD, chronic hypoxia leads to elevated erythropoietin, Hct, and Hgb levels in order to maintain higher oxygen delivery.[24] Evidence of such an erythropoiesis response in newborns would provide support to imaging findings suggestive of fetal hypoxia in the setting of CHD. The primary aim of this study was to assess for altered erythropoiesis in newborn infants with CHD and determine the relationship between erythrocyte indices and fetal growth. The secondary aim was to evaluate for changes in placental function on ultrasound Doppler and for a correlation with changes in erythropoiesis.

METHODS

This was a retrospective study of routinely collected data from infants with prenatally diagnosed CHD whose mothers' received obstetric care at the University of Cincinnati Medical Center and received fetal cardiology prenatal care at Cincinnati Children's Hospital Medical Center between January 2013 and November 2018. Additional inclusion criterion was the presence of a complete blood count (CBC) laboratory test obtained at birth. Exclusion criteria included multiple gestation pregnancies, prenatally known genetic abnormality or syndrome that may be a confounder for growth, major extra-cardiac anomalies, outcome of intrauterine death, and pregnancies complicated by placental abruption. Approval was obtained from the Institutional Review Board at Cincinnati Children's Hospital Medical Center.

The electronic medical record was used to collect demographic characteristics of mother and infant pairs, obstetrical and delivery history, and identify additional risk factors for FGR such as diabetes, preeclampsia, hypertension, and smoking status. Birth anthropometrics including BW, length, and head circumference (HC) were converted to GA adjusted percentiles and *z*-scores for analysis (BWz and HCz). FGR was defined as a prenatally estimated fetal weight <10th percentile for GA and SGA was defined as BW <10th percentile for GA.[25] Patients were subdivided into GA groups of <34 weeks, 34–37 weeks, and ≥37 weeks gestation for group analysis.

Cardiac anatomy was obtained from postnatal echocardiography reports. Infants were grouped by cardiac anatomy for subgroup analysis: single ventricle (SV), conotruncal, left ventricular outflow tract obstruction (LVOTO), and all other CHD (other). These subgroups were created based on anatomic considerations and physiologic properties of the patient's CHD. In fetuses with normal cardiac anatomy, oxygenated blood from the placenta enters the heart through the umbilical vein, ductus venosus, and inferior vena cava. A portion of this blood crosses the patent foramen ovale into the left heart and is sent to the aorta, providing oxygen-rich blood to the brain and upper body. The rest of the blood from the inferior vena cava enters the right heart and leaves the right ventricle through the ductus arteriosus, bypassing the lungs and supplying oxygen to the lower body. Anatomic abnormalities affect this normal circulation and result in physiologic changes and re-distribution of blood flow. In our study, SV CHD included functional univentricular hearts that would be expected to proceed with single ventricle palliation (i.e., hypoplastic left heart syndrome, tricuspid atresia, and double inlet left ventricle). Conotruncal CHD included defects of the conus and truncus arteriosus, such as transposition of the great arteries, double outlet right ventricle, and tetralogy of Fallot. These patients will often achieve biventricular repair. LVOTO CHD encompasses left-sided abnormalities, including aortic valve stenosis and aortic arch obstruction. The remaining types of CHD were grouped into other CHD, which included ventricular septal defects and total anomalous pulmonary venous return.

Our institution's standard of care is to obtain CBC testing at the time of admission after birth. Thus the first CBC obtained after birth was evaluated for the following indices: red blood cell (RBC) count, white blood cell (WBC) count, Hgb, Hct, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), red cell distribution width (RDW), and nRBC. The nRBC levels were reported as values relative to 100 WBC and converted to absolute nRBC measurements (nRBC/μl) for analysis. This conversion was performed to account for variability in WBC count after birth and to allow for direct comparison between patients. Elevated nRBC was defined as values >638 nRBC/μl (75th percentile nRBC level in healthy newborns ≥37 weeks of age).[26] CBC testing was performed with Sysmex XN-3100 with an internal DI-60 Cell Imaging Analyzer. Cord blood gas data were collected when available.

All patients had at least one prenatal fetal echocardiogram performed at Cincinnati Children's Hospital Medical Center. Biometrics such as HC, estimated fetal weight, and abdominal circumference were obtained from fetal echocardiograms. Our standard fetal echocardiography protocol includes Doppler evaluation of the UA and middle cerebral artery (MCA). Peak systolic, end diastolic, and mean velocities of UA and MCA Doppler were measured and PIs and RIs were calculated for the UA and MCA.[27] Available maternal obstetrical ultrasound reports were reviewed for growth biometrics and UA and MCA Doppler data. These Doppler patterns were secondary outcomes of interest as previous data have demonstrated changes in UA and MCA Doppler in pregnancies with placental insufficiency and hypoxia (non-CHD pregnancies), although a relationship to altered erythropoiesis has not been demonstrated.[13]

Statistical analysis

Statistical analyses were conducted using the SAS version 9.4 (SAS Institute Inc., Cary, NC) software. Descriptive statistics were reported as frequencies and percentages for categorical variables and as median (25th, 75th percentile) for continuous variables. RBC indices are reported as mean ± standard deviation with the exception of nRBC, which was reported as median (25th, 75th percentile). This was to allow for comparison with equivalent published reference values from large population studies.[28–30] One-sample *t* tests were performed to compare RBC indices in each GA group to published reference values. Differences in indices according to anatomy subgroups were evaluated using analysis of variance. Spearman correlation was used to examine associations of RBC indices with GA-adjusted BWz and HCz or PIs and RIs of both UA and MCA. Chi-square test was used to assess the association between categorical variables.

RESULTS

A total of 160 mother–infant pairs were included. Patient characteristics can be found in Table 1. Maternal obstetrical history of the cohort included diabetes in 30 (19%), hypertension in 45 (28%), and preeclampsia in 18 (11%), and there was no difference in the incidence of co-morbidities between the anatomic subgroups. Mothers with infants born at <34 weeks and 34–37 weeks were more likely to have diabetes, hypertension, and preeclampsia ($p < 0.05$). The median GA at birth was 38.3 (37.3, 39.0) weeks, and the majority of the infants were born at ≥37 weeks gestation (83%). Within the cardiac anatomy subgroups, 85 (53%) infants had SV CHD, 30 (19%) infants had conotruncal defects, 19 (12%) infants had LVOTO CHD, and 26 (16 %) infants were categorized as other CHD. In our cohort, 82 newborns (51%) were delivered by cesarean section and half of these patients had SV CHD (43/82, 52%). A two-vessel cord was noted in 20 (13%) infants and delayed cord clamping was

Table 1. Patient characteristics.

Variable	Total, $n = 160$
Maternal age (years)	27.3 ± 5.5
Race	
White	129 (81%)
African American	22 (14%)
Other	9 (6%)
Ethnicity	
Hispanic	7 (4%)
Non-Hispanic	153 (96%)
Maternal factors	
Hypertension	45 (28%)
Diabetes	30 (19%)
Preeclampsia	18 (11%)
Smoking	18 (11%)
Two-vessel cord	20 (13%)
Delayed cord clamping	48 (30%)
Mode of delivery	
Spontaneous vaginal	74 (46%)
Operative vaginal	4 (3%)
Cesarean section	82 (51%)
Gestational age	38.3 (37.2, 39.0)
<34 weeks	6 (4%)
34–37 weeks	21 (13%)
≥37 weeks	133 (83%)
Infant sex, male	102 (64%)
Apgars (1 and 5 min)	8 (8, 8) and 8 (8, 9)
Birth weight (g)	2960 (2590, 3340)
Birth weight *z*-score (BWz)	−0.438 (−1.06, 0.24)
Birth weight percentile (%)	33.1 (14.6–59.4)
Small for gestational age	28 (18%)
Birth head circumference (cm)	32.8 (31.5, 34.5)
Birth head circumference percentile (%)	24.0 (9.55, 58.20)
Birth head circumference *z*-score (HCz)	−0.706 (−1.31, 0.21)
Birth length (cm)	48.5 (46.0, 51)
Birth length percentile	33.3 (16.2, 61.4)
Birth length *z*-score	−0.43 (−0.99, 0.29)
Cardiac subtype	
Single ventricle	85 (53%)
Conotruncal	30 (19%)
LVOTO	19 (12%)
Other	26 (16%)
Cord blood gas	
pH	7.26 (7.22–7.30)
pCO_2	57 (49, 63)
paO_2	18 (14, 24)
Base	−2.4 (−4.1, −0.5)
Prenatal Doppler patterns	
UA PI ($n = 150$)	1.1 ± 0.24
UA RI	0.66 ± 0.08
MCA PI ($n = 111$)	1.67 ± 0.44
MCA RI	0.77 ± 0.08

Data reported as median (25th, 75th percentile), mean ± standard deviation, and frequencies *N*(%).
UA umbilical artery, *MCA* middle cerebral artery, *PI* pulsatility index, *RI* resistance index.

reported in 48 infants (30%), with no difference between the GA and anatomy subgroups.

Birth anthropometrics for the entire cohort are presented in Table 1 and for each cardiac anatomy subgroup in Table 2. Twenty-eight (18%) newborns met criteria for SGA status. When comparing all the four cardiac anatomy subgroups, the absolute BW was significantly different ($p < 0.05$). However, this difference was no longer present when adjusted for GA. There were no other differences in age-adjusted birth anthropometrics between infants with SV CHD, conotruncal defects, LVOTO CHD, or other CHD.

Laboratory studies

Results from cord blood gases are found in Table 1. CBC laboratory tests were obtained at a median of 4.00 (2.33, 5.13) hours of life. Of the entire cohort, mean Hgb was 16.5 ± 2.06 g/dl, mean Hct was 49.0 ± 6.36%, and median absolute nRBC was 400/μl (46.5–1050.5/μl). Mean values of RBC indices and reference values for GA groups can be found in Table 3. Only MCV differed between the cardiac anatomic subgroups. There were no differences in RBC, WBC, MCH, and RDW between the cardiac anatomy groups (data not shown).

With further analysis in each GA subgroups, there were observed differences in multiple RBC indices between infants with CHD compared to reference values in the GA group ≥37 weeks (Table 3). The Hgb and Hct levels were both significantly lower compared to the reference (16.3 vs 18.0 g/dl and 48.6 vs 52.0% respectively, $p < 0.001$). Median nRBC level was elevated compared to the reference (384 vs 0/μl, $p < 0.001$). Additionally, there were higher MCV ($p < 0.001$) and MCH ($p = 0.03$) levels in infants ≥37 weeks with CHD. When evaluating these RBC indices for infants in the <34- and 34–37-week GA groups, we did not find any differences between our cohort and GA-matched normative values; however, our ability to detect small or moderate differences in these GA subgroups was limited.

There were no significant associations between Hgb, Hct, and nRBC with growth parameters of BWz or HCz based on Spearman correlation. However, there was a modest relationship between other RBC indices and BWz. Univariate correlations for RDW demonstrated a weak positive correlation with BWz ($r = 0.16$, $p < 0.05$) and weak negative correlation with increasing GA ($r = -0.31$, $p < 0.01$). MCH demonstrated a weak negative correlation with BWz ($r = -0.16$, $p < 0.05$). WBC demonstrated a positive correlation with BWz ($r = 0.22$, $p < 0.01$) and positive correlation with increasing GA ($r = 0.38$, $p < 0.01$).

We further investigated a subset of patients in the ≥37-week GA group who were found to have elevated nRBC levels. Using the threshold of 638 nRBC/μl, there were 45 (34%) infants with elevated nRBC values ranging from 651 to 54,810 nRBC/μl. Half of these infants (51%) had a history of antenatal maternal comorbidities, including diabetes, hypertension, smoking status, and/or preeclampsia (Fig. 1). When individual comorbidities were assessed, the incidence of preeclampsia remained significantly higher in infants with >638 nRBC/μl compared those with <638 nRBC/μl. There were no significant differences in Hgb levels when stratified by maternal comorbidities (data not shown). Lastly, we compared Hgb, nRBC level, and presence of elevated nRBC between modes of delivery in our cohort and found no significant difference between infants born by spontaneous vaginal delivery, operative vaginal delivery, and cesarean section, suggesting no significant effect of mode of delivery on erythropoiesis (data not shown). There was no difference in nRBC levels between infants with Apgar scores 0–3, 4–6, and 7–10 at 5 min of life ($p = 0.11$).

Fetal echocardiography and ultrasound

For the entire cohort, mean UA PI was 1.10 ± 0.24 and RI was 0.66 ± 0.08, and the mean MCA PI was 1.67 ± 0.44 and RI was 0.77 ± 0.08. There was no difference in UA or MCA PI and RI between the GA groups. There was a significant difference in MCA PI between the cardiac anatomy subgroups; infants with LVOTO lesions had lower MCA PI, consistent with a decrease in cerebral resistance that has been demonstrated previously in this type of cardiac lesion. When comparing RBC indices with Doppler patterns, no significant relationship was identified between any RBC indices and MCA and UA PI and RI.

Table 2. Birth anthropometrics between cardiac anatomy subgroups.

	SV, $n = 85$	Conotruncal, $n = 30$	LVOTO, $n = 19$	Other, $n = 26$	p value*
Birth weight (g)	2990 (2580, 3300)	2970 (2720, 3440)	3120 (2730, 3360)	2720 (2310, 3060)	<0.05
Birth weight *z*-score	−0.43 (−1.09, 0.12)	−0.61 (−0.95, 0.26)	−0.24 (−0.86, 0.36)	−0.65 (−1.31, −0.12)	0.48
Head circumference (cm)	32.5 (31, 34)	33 (32, 34.6)	34 (32, 35)	32 (31.5, 33)	0.09
Head circumference *z*-score	−0.80 (−1.39, 0.09)	−0.52 (−1.08, 0.23)	−0.09 (−1.1, 0.48)	−0.77 (−1.08, 0.03)	0.23
Length (cm)	48.5 (46.0, 51.0)	49 (47, 50)	49.5 (47.5, 51.5)	48 (45, 49)	0.21
Length *z*-score	−0.463 (−0.986, 0.289)	−0.473 (−1.18, 0.371)	−0.184 (−0.846, 0.4)	−0.233 (−0.821, 0.236)	0.96

Differences in birth anthropometrics between anatomy subgroups were assessed with ANOVA testing. Results reported as median (25th, 75th percentile). The *z*-scores are GA-adjusted *z*-scores.
SV single ventricle, *LVOTO* left ventricular outflow tract obstruction.
**p* value represents difference between the four-group comparison.

DISCUSSION

This analysis of newborns with CHD identified both increases and decreases in multiple RBC indices on the first clinical laboratory examination. These findings were observed in newborns with CHD born at GA ≥37 weeks. Although these changes are demonstrated, the values fall within the wide, expected range of reference values for newborns. In infants with CHD, the distribution curves of these RBC indices may be shifted, particularly left shifted for Hgb and Hct levels and right shifted for nRBC levels, when compared to newborns with no CHD. We were unable to demonstrate any differences in RBC indices in our small cohort of infants born <37 weeks compared to reference values, preliminarily suggesting that there is no evidence of altered erythropoiesis in preterm infants with CHD. These findings support the hypothesis that stress erythropoiesis develops during late gestation in the setting of fetal CHD. Stress erythropoiesis is a regenerative reaction to acute or chronic, real or "perceived" anemia, developing as an adaptive response to hypoxia.[23] In the case of persistent and chronic stressors, stress erythropoiesis may fail to provide an adequate compensatory response with a net result of persisting chronic anemia and/or hypoxia.[23]

Our cohort demonstrated elevated nRBC levels at birth, which is consistent with physiologic stress erythropoiesis that results in a rapid production of erythrocytes. The normal trend of nRBC level is to decrease as GA increases.[31] In the third trimester, a fetus normally exhibits rapid growth and concurrently a high RBC production, 3–5 times the normal adult steady state.[32] Thus there is a natural progressive increase in erythrocytes and Hgb concentration throughout gestation, with normal high values at birth.[33,34] In infants with CHD, the low Hgb levels at birth could represent the failure of stress erythropoiesis to maintain normal erythrocyte production in late gestation in the setting of in utero chronic stressors. This would result in an accelerated cell cycle and/or decreased number of cell divisions from proerythroblast to enucleating orthochromatic erythroblast to allow for an increase in cytoplasm prior to erythroblast division, resulting in an increase in MCV of the produced RBCs, which was also seen in our cohort.

It is known that nRBCs are produced in response to acute stressors and that they have also been detected in pregnancies complicated by diabetes.[21,31,35] Although not exactly known, studies have suggested that nRBCs may be seen in the circulation as soon as 30 minutes to a few hours after significant hypoxic or catastrophic events.[21] One third of our patients ≥37 weeks gestation had elevated nRBC levels and half of these were product of a pregnancy complicated by preeclampsia, hypertension, diabetes, or smoking. However, there were many infants with elevated nRBC levels who did not have a history of any of these maternal comorbidities (Fig. 1). The median time of CBC testing from birth in our cohort was 4 hours of life and is likely a reasonable surrogate for assessment of in utero environment and delivery. Chronic maternal comorbidities, fetal hypoxia during labor, or significant delivery complications may explain the elevated nRBC levels seen in this study; however, it would not explain the decrease in Hgb in term infants. Changes in erythropoiesis that would affect Hgb level would occur over the course of days and more likely weeks. There is also no evidence in our population of red cell loss at delivery or hemodilution, as all laboratory reports are obtained by standard clinical protocol.

Although maternal comorbidity may play a role in erythropoiesis, it does not explain all the observed changes. Additionally, the lack of differences in RBC indices between the CHD anatomy subgroups may implicate associated placental pathology as a cause of fetal stress erythropoiesis rather than the underlying fetal heart defect.[8,36] Cord blood evaluation of nRBCs in a prospective study would be useful to answer this question.

The biological mechanisms explaining these abnormalities in RBC indices remain unclear. Mothers with fetal CHD have complicated pregnancies and studies have demonstrated that the maternal–fetal environment is complex and that multiple factors may play a role in outcomes in this population.[37] Placental abnormalities leading to dysfunction should be considered, as evidence suggests that the placenta may have a regulatory effect on erythropoiesis.[38] The role of inflammation must also be considered as other studies have demonstrated that in utero inflammation as a result of infection may alter iron homeostasis at the maternal–fetal interface.[39] Maternal stress during pregnancy, comorbidities such as hypertension, diabetes, and preeclampsia, or chronic hypoxia may contribute to a chronic stress state in utero. Forms of fetal stress such as the distinction between an elective or emergency cesarean section were not able to be made in this study but may play a role as well. The underlying etiology is likely multifactorial, indicating the importance of the maternal–fetal environment in fetal CHD.[37] These findings are hypothesis generating and would be better addressed by future studies to determine whether these changes are affected more by fetal or maternal factors.

Infants with CHD are at increased risk for FGR and low BW has a significant impact on surgical results and outcomes.[1–5] Chronic hypoxia has been associated with FGR in altitude studies and in pregnancies with placental insufficiency.[6,7,40] Our findings did not support a relationship between disturbances in erythropoiesis and late gestation fetal growth and GA-adjusted BWz in infants with CHD. There was also no association between RBC indices and UA and MCA Doppler measures. Specific Doppler pattern changes of the UA and MCA in response to placental insufficiency and hypoxia have been demonstrated.[13] In utero hypoxia has been associated with stress erythropoiesis in non-CHD fetuses and thus our aim was to evaluate whether CHD patients had evidence of

Table 3. Red blood cell indices across gestational age groups compared to reference values.

	≥37 weeks, $n = 133$			34–37 weeks, $n = 21$			<34 weeks, $n = 6$		
	CHD	Ref	*p* value	CHD	Ref	*p* value	CHD	Ref	*p* value
Hgb (g/dl)	16.3 ± 2.0	18.0 ± 2.0	<0.001	17.1 ± 2.4	17.0 ± 2.0	0.80	16.7 ± 3.0	16.0 ± 2.0	0.55
Hct (%)	48.6 ± 6.0	52.0 ± 6.4	<0.001	51.2 ± 7.0	50.0 ± 6.4	0.47	50.5 ± 9.9	48.0 ± 6.4	0.52
nRBC/µl	384 (0, 1044)	0 (0, 638)	<0.001	513 (156, 702)	696 (0, 1672)	0.17	663 (464, 1155)	1901 (492, 5970)	0.13
RDW (%)	16.6 ± 2.0	17.3 ± 2.3	<0.001	17.7 ± 1.9	17.5 ± 2.3	0.68	17.8 ± 1.5	17.8 ± 2.3	0.94
MCV (fl)	109.1 ± 5.8	106.5 ± 5.0	<0.001	109.8 ± 5.7	108.0 ± 5.0	0.18	112.4 ± 4.1	112.0 ± 5.0	0.79
MCH (pg)	36.7 ± 2.1	36.3 ± 2.0	0.03	36.8 ± 2.3	37.0 ± 2.0	0.77	37.4 ± 1.9	38.0 ± 2.0	0.41

Results of one-sample *t* test between congenital heart disease (CHD) and Reference (Ref)[21,23–25] across each gestational age group. Nucleated red blood cell (nRBC) is reported as median (25th, 75th percentile) and all other values are reported as mean ± standard deviation.
Hgb hemoglobin, *Hct* hematocrit, *RDW* red cell distribution width, *MCV* mean corpuscular volume, *MCH* mean corpuscular hemoglobin.

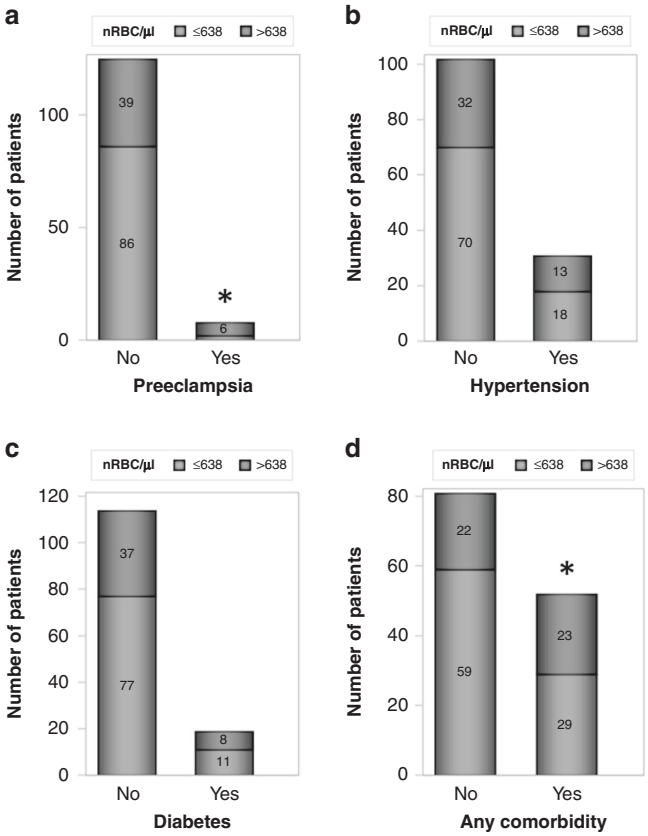

Fig. 1 Maternal comorbidities in infants with CHD ≥37 weeks gestation ($n = 133$) with elevated nucleated red blood cells (>638 nRBC/µl, $n = 45$, red) and nRBC <638 nRBC/µl ($n = 88$, blue).[26] a Preeclampsia; **b** Hypertension; **c** Diabetes; **d** Any maternal comorbidity. Asterisk (*) denotes $p < 0.05$ significance.

similar Doppler patterns consistent with hypoxia, to support an association of hypoxic stress in the fetus as an etiology of altered erythropoiesis. UA and MCA Doppler have been validated to detect vascular resistance changes in response to hypoxia and the lack of abnormal Doppler in our population indicates that the typical uteroplacental hemodynamic response to placental insufficiency was not present in our cohort.[41–43]

Although low Hgb was not found to be related to SGA status in our cohort, low Hgb levels may have implications on oxygen delivery and organ development in late gestation. This may also be important in the context of patients with complex cyanotic CHD who rely on relatively high Hgb and Hct for optimal oxygen delivery postnatally. This was not within the scope of our study; however, the evaluation of low Hgb levels and its relationship to outcomes in this population could be pursued in the future.

Limitations include the single-center nature of this study, which resulted in reduced sample sizes with subgroup analysis between the GA and cardiac anatomy groups and is expected to have contributed to variability and limited ability to detect small or moderate differences from reference values in the preterm GA groups. A local control population was lacking, as CBC testing is not routine ordered on healthy newborns in our center. With prospective studies, a control population can be included and our findings can be validated. The source of blood for the CBC (venous, arterial, or capillary) was not documented in the clinical records and thus not known. However, standard practice at our institution is to establish umbilical venous access immediately after birth for newborns with complex CHD and transfer the infant to our tertiary care center where laboratory testing is performed. It is typical that baseline laboratory testing is obtained from venous lines or peripheral venous access and is likely consistent across our cohort. Therefore, it is unlikely that source variability has confounded our results.[44,45] Lastly, steady state and stress erythropoiesis has been differentiated by specific signaling pathways.[23] Cytokine level evaluation and molecular and cellular studies would need to be conducted prospectively to determine whether our findings are secondary to stress erythropoiesis or another process. The evaluation of cord blood in future studies would be ideal as this would provide a more accurate representation of the in utero environment.

CONCLUSION

Term infants with CHD demonstrated altered erythropoiesis and have decreased Hgb levels despite having RBC indices consistent with stress erythropoiesis. These findings suggest an incomplete compensatory response to in utero physiologic disturbances associated with CHD. This may be secondary to ineffective stress erythropoiesis response due to maternal stressors, placental insufficiency, or chronic hypoxia that results in lower Hgb levels at birth. Additionally, RBC indices of erythropoiesis did not differ between CHD anatomy suggesting an etiology beyond fetal hemodynamics. There was no association between RBC indices and growth or prenatal UA or MCA Doppler patterns. Further studies should be performed to confirm these findings, determine its effect on outcomes, and to better elucidate the role of maternal or fetal factors impacting fetal erythropoiesis in CHD.

AUTHOR CONTRIBUTIONS

Each author has met authorship requirements. S.Y.T. conceptualized and designed the study, acquired data, performed data interpretation, and drafted and critically revised the manuscript. Z.G. and N.J.O. assisted with study design, carried out data analyses and interpretation, and critically revised the manuscript. T.A.K. and S.T. assisted with study design and critically revised the manuscript. R.K. collected data and reviewed the manuscript. J.F.C. conceptualized and designed the study, coordinated and supervised data collection and data analysis, performed data interpretation, and critically revised the manuscript. All authors approved the final version for publication.

ADDITIONAL INFORMATION

Competing interests: The authors declare no competing interests.

Patient consent: Patient consent was not required for this study. Approval was obtained from the Institutional Review Board at Cincinnati Children's Hospital Medical Center with waiver of consent due to the retrospective nature of the study.

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