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Fetal Hemoglobin from Newborn Screening Signals Postnatal Anemia After Intrauterine Transfusion

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Abstract

Fetal anemia is a rare but potentially life-threatening condition requiring intrauterine transfusion (IUT). Neonates treated with IUT remain at risk of late postnatal hyporegenerative anemia. We aimed to evaluate whether fetal hemoglobin (HbF) measured through routine newborn screening (NBS) could provide information about the risk of postnatal anemia and red blood cell (RBC) transfusion in neonates treated with IUT. Fifty fetuses who underwent at least one IUT between 2016 and 2025 at two tertiary referral centers were included. HbF measured through NBS was compared between IUT-treated neonates who did and did not require postnatal RBC transfusion and healthy newborn controls. Of the 43 live-born neonates, 35% required postnatal RBC transfusion; 27% of these developed anemia during follow-up after not being anemic at birth. HbF levels were lower in neonates requiring postnatal RBC transfusion than in those who did not and were also lower than in healthy controls ($p < 0.001$). The interval between the last IUT and birth was positively associated with HbF ($\beta = 3.7$, $p = 0.001$). Lower HbF levels at NBS were associated with a higher risk of postnatal anemia and RBC transfusion. HbF may help identify neonates who require closer hematologic monitoring after IUT.

Keywords: fetal hemoglobin; anemia; intrauterine transfusion; newborn screening

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1. Introduction

Intrauterine transfusion (IUT) is a widely used therapeutic procedure for the treatment of moderate to severe fetal anemia, particularly when fetal hemoglobin (HbF) levels are critically low or signs of fetal compromise, such as hydrops, are present.

Red-cell alloimmunization has historically been the leading indication for IUT, with RhD alloimmunization representing a major cause of hemolytic disease of the fetus and newborn (HDFN) [1]. Although anti-D immunoglobulin prophylaxis has substantially

reduced the incidence of RhD-related disease [2,3], IUT remains necessary in pregnancies complicated by other red-cell antibodies, including anti-Kell, which may cause both hemolysis and suppression of fetal erythropoiesis [4,5]. Other indications include fetal infections, particularly parvovirus B19, which can cause severe anemia through suppression of erythroid progenitor cells; congenital red-cell disorders, such as alpha-thalassemia and pyruvate kinase deficiency; and significant fetomaternal hemorrhage. IUT may also be required in selected cases of anemia associated with twin-to-twin transfusion complications or other less common fetal conditions [4]. These differences in etiology may be relevant to postnatal hematologic recovery and the risk of late anemia after IUT.

Some neonates who undergo IUT are not anemic at birth and may therefore appear clinically stable during the immediate postnatal period, despite remaining at risk of developing late-onset hyporegenerative anemia several weeks later. This delayed presentation complicates early risk stratification and may result in missed opportunities for closer monitoring or timely intervention. While immediate neonatal complications are uncommon [6–8], late postnatal hyporegenerative anemia remains a clinically relevant and incompletely understood outcome [9].

Reticulocyte count is the reference marker for assessing erythropoietic activity [10]; however, it is not systematically available at birth in many centers, particularly in neonates who are not anemic at birth. HbF measured routinely through newborn screening (NBS) is influenced by endogenous erythropoiesis, the proportion of transfused adult red blood cells (RBCs), and the underlying cause of fetal anemia. Previous work from our group suggested that persistently low or absent HbF at birth was associated with severe late hyporegenerative anemia and the need for postnatal treatment, including erythropoietin therapy [11]. The aim of the present study was to evaluate the association between HbF levels measured through NBS and postnatal anemia outcomes in a cohort of neonates treated with IUT, testing the hypothesis that lower HbF at birth is associated with an increased need for postnatal transfusion and erythropoietin therapy.

2. Materials and Methods

2.1. Study Design and Population

We conducted a retrospective observational cohort study including neonates who underwent at least one IUT for fetal anemia between January 2016 and December 2025 and received postnatal follow-up at two tertiary fetal and neonatal medicine centers: Hospital Clínic de Barcelona and Hospital Sant Joan de Déu (BCNatal), Barcelona, Spain. Neonates referred from other centers in Spain were also included when postnatal follow-up data were available.

The indication for IUT was determined on an individual basis according to the etiology and severity of fetal anemia, as assessed by middle cerebral artery peak systolic velocity (MCA-PSV), relevant fetal ultrasound findings, and clinical evaluation.

Collected antenatal data included gestational age at diagnosis of fetal anemia; anemia etiology; gestational age at each IUT; HbF and hematocrit levels before and after transfusion; MCA-PSV; and transfused blood volume. Neonatal data included gestational age at birth, birth weight, hematologic parameters at birth, and postnatal clinical outcomes.

2.2. HbF Analysis and Availability of HbF Results

HbF levels were obtained from routine NBS for hemoglobinopathies. In Spain, NBS is performed using dried blood spot (DBS) samples collected 24–72 h after birth. NBS is coordinated through regional early diagnosis centers, where hemoglobin profiles are analyzed as part of the NBS program for sickle cell disease (SCD) [12].

During the study period, HbF levels were measured as part of the SCD screening by the regional NBS laboratories. Families were routinely informed only when screening results were suggestive of a hemoglobinopathy, and affected newborns were subsequently referred to specialized reference hospitals for hematologic evaluation and follow-up. HbF values were not systematically incorporated into hospital electronic medical records and were not used for routine neonatal clinical decision-making during the study period. For the purposes of this study, HbF values were retrospectively retrieved from NBS records and linked to perinatal and postnatal clinical data. Healthy term and near-term newborns included in the Catalan NBS program during the study period were used as a control group for HbF values.

Hemoglobin profiles were analyzed using either capillary electrophoresis (Capillarys™ 2 and Capillarys™3 DBS, Sebia, Lisses, France) or high-performance liquid chromatography (VARIANT™ NBS System, Bio-Rad Laboratories, Hercules, CA, USA), depending on the newborn's place of birth. For this study, an "adult hemoglobin pattern" was operationally defined as a predominance of adult hemoglobin (HbA) with absent or markedly reduced HbF on NBS, in the absence of structural hemoglobin variants.

2.3. Postnatal Follow-Up and Clinical Management

Postnatal clinical and hematological outcomes were retrospectively reviewed during a predefined follow-up period of six months after birth. Follow-up consisted of regular outpatient visits, including growth assessment, complete blood counts, and clinical evaluation for signs of anemia.

Anemia during follow-up was defined as a hemoglobin concentration and/or hematocrit below the age-specific lower reference limit (mean −2 standard deviations), according to established pediatric hematological reference values [13,14]. This definition accounted for the physiological changes in hemoglobin and hematocrit during early infancy. Reticulocyte counts were not routinely measured at birth and were obtained during follow-up when clinically indicated to assess erythropoietic activity.

Exchange transfusion was performed according to local protocols in cases of severe hyperbilirubinemia or ongoing hemolysis not adequately controlled with intensive phototherapy. RBC transfusions were administered for isolated anemia in the absence of significant hemolysis. In symptomatic neonates, RBC transfusion was performed according to institutional protocols, which evolved over the study period in line with increasingly restrictive neonatal transfusion guidelines. In clinically stable, asymptomatic neonates, transfusion was consistently indicated when hemoglobin levels were <7 g/dL and/or hematocrit was <21%.

Recombinant human erythropoietin (rHuEPO) therapy was initiated from the third week of life in neonates with hemoglobin levels < 7.5 g/dL and reticulocyte counts < 2%, consistent with hyporegenerative anemia. rHuEPO was administered subcutaneously at a dose of 250 IU/kg three times weekly for 4–6 weeks.

2.4. Outcomes

The primary outcome was the need for postnatal RBC transfusion during follow-up. Secondary outcomes included erythropoietin treatment, timing of anemia onset, and mortality within the first 6 months of life. HbF levels at NBS were analyzed in relation to postnatal outcomes.

2.5. Ethical Considerations

The study was approved by the Institutional Review Boards of Hospital Clínic de Barcelona and Hospital Sant Joan de Déu (approval codes HC-TIU-01 and PIC-178-23). Given the retrospective nature of the study and the use of routinely collected clinical data, the requirement for informed consent was waived. The study was conducted in accordance with the Declaration of Helsinki.

2.6. Statistical Analysis

Continuous variables are presented as means ± standard deviations or medians and interquartile ranges, as appropriate. Categorical variables are presented as counts and percentages. Associations between HbF levels at NBS and postnatal outcomes were assessed using appropriate parametric or non-parametric tests. Between-group differences in HbF levels were assessed using Welch's one-way ANOVA, followed by pairwise Welch's t-tests with Bonferroni correction for multiple comparisons. Linear regression analyses were performed to assess the association between HbF levels and antenatal variables. Because of the limited number of postnatal transfusions, we used a Firth penalized logistic regression model, adjusting for the interval between the last IUT and delivery. Receiver operating characteristic (ROC) curve analysis was performed to evaluate the ability of HbF levels to identify neonates requiring postnatal RBC transfusion. Statistical analyses were performed using SPSS Statistics version 22.0 (IBM Corp., Armonk, NY, USA) and GraphPad Prism version 5 (GraphPad Software, San Diego, CA, USA). All tests were two-sided, and a p-value < 0.05 was considered statistically significant.

3. Results

3.1. Patients

During the study period, a total of 50 mother–fetus dyads were treated at the two participating centers, of whom 19 (38%) were referred from other regions of Spain. The median gestational age (GA) at diagnosis of fetal anemia was 23 weeks (IQR, 20–26). Maternal ABO blood groups were distributed as follows: group O, 20 cases (40%); group A, 20 (40%); group B, 7 (14%); and group AB, 3 (6%). Regarding maternal Rh status, 31 mothers (62%) were Rh-positive and 19 (38%) were Rh-negative. Among the newborns, ABO blood groups were distributed as follows: group O, 21 cases (42%); group A, 17 (34%); group B, 4 (8%); and group AB, 2 (4%), with six missing values. Rh status was positive in 43 newborns (86%) and negative in 1 (2%).

A total of 99 IUT procedures were performed, with a median of 1 IUT per patient (IQR, 1–2.5). The median GA at the first transfusion was 25 weeks (IQR, 22–28), and the median transfused volume was 57 mL (IQR, 39.5–86.5). Before IUT, the median hemoglobin level was 7.4 g/dL (IQR, 4.1–8.5), hematocrit was 22% (IQR, 13.1–26), and MCA-PSV was 65 cm/s (IQR, 57–86), corresponding to 1.81 multiples of the median (MoM) (IQR, 1.41–2.21). Following IUT, the median MCA-PSV decreased to 41 cm/s (IQR, 33–63). The median GA at birth was 37.3 weeks (IQR, 35.0–38.5), and the median birth weight was 2840 g (IQR, 2530–3361).

A total of 32,689 healthy term and near-term newborns were included as controls for HbF comparisons.

3.2. Etiologies of Fetal Anemia

As shown in Figure 1, red-cell alloimmunization was the leading cause of fetal anemia requiring IUT, accounting for 52% of cases (n = 26). Anti-D was the most frequent antibody (69.2%, n = 18), followed by anti-K (19.2%, n = 5). Other alloantibodies included anti-C, anti-Duffy, and anti-M, with one case each (3.8%). Infectious causes accounted for

34% of cases (n = 17), predominantly due to parvovirus B19 infection (n = 16, 94%), while one case was attributed to toxoplasmosis (n = 1, 6%). Three cases (6%) involved genetic disorders: alpha-thalassemia, pyruvate kinase deficiency, and GATA1-related cytopenia. Alpha-thalassemia was diagnosed prenatally by targeted genetic testing of chorionic villus samples, because both parents were carriers and had a previously affected child. Pyruvate kinase deficiency and GATA1-related cytopenia were diagnosed prenatally by molecular testing using an anemia gene panel on amniotic fluid.

One case occurred in the context of twin anemia-polycythemia sequence (TAPS) in a monochorionic twin pregnancy previously treated with fetoscopic laser ablation of placental vascular anastomoses for twin-to-twin transfusion syndrome (TTTS). Another case was due to fetomaternal hemorrhage. No underlying cause of anemia was identified in two patients.

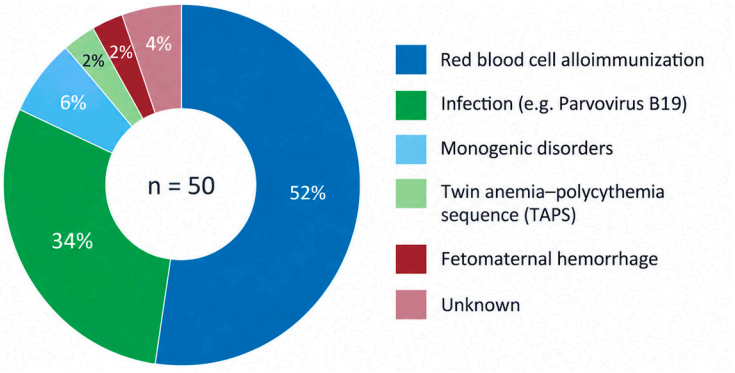


Figure 1. Etiologies of fetal anemia.

3.3. Description of Postnatal Anemia and Outcomes

Death occurred in 11 patients (22%), including 7 antenatal deaths (1 Kell alloimmunization, 1 Parvovirus B19 infection, 1 TAPS following laser ablation, 1 fetal hydrops of unknown etiology associated with maternal mirror syndrome, 1 fetal hydrops due to Parvovirus B19 infection, 1 intraparenchymal cerebral hemorrhage likely secondary to fetomaternal hemorrhage, and 1 elective termination of pregnancy secondary to fetal intracranial hemorrhage) and 4 postnatal deaths (1 from Parvovirus B19 infection following birth at 21 weeks of gestational age [GA], who received palliative care in the delivery room; 1 patient with anemia of unknown etiology who developed early sepsis and died at 6 days of life; 1 patient with RhD alloimmunization who died at 5 months of life due to mitochondrial disease and multiorgan failure; and 1 patient with Parvovirus B19 infection who died within the first days of life due to severe hydrops fetalis).

Among the 43 live-born neonates, the median hematocrit at birth was 46.9% (IQR, 32.1–59.3). Anemia at birth, defined as a hematocrit <45% or hemoglobin level <15 g/dL, was identified in 11 newborns (26%), with no significant differences after stratification by etiology. Clinically significant hemolysis, as indicated by the need for phototherapy (PT), was observed in 9 patients (21%). Among these, 8 cases were due to RhD alloimmunization and 1 to pyruvate kinase deficiency (PKD). Four patients (9%) required exchange transfusion, 3 with RhD alloimmunization and 1 with PKD. Postnatal RBC transfusions were required in 15 patients (35%), including 11 with alloimmunization (8 RhD, 2 Kell, and 1 Duffy), one with hemoglobin H disease, one with PKD, one with toxoplasmosis infection, and one with an unknown cause. Among these 15 patients, 4 (27%) were not anemic at birth but developed anemia during the follow-up period. rHuEPO therapy was initiated in 10 of these 15 patients (67%), all of whom had alloimmunization (8 RhD and 2 Kell).

3.4. Association of Intrauterine Transfusion with Fetal Hemoglobin at NBS

All surviving neonates completed the predefined six-month follow-up, with no losses. Observation was shorter only for neonates who died before six months of age. Among the 43 live-born neonates, NBS HbF results were available for 34 (79.1%) but unavailable for the remaining nine (20.9%). Missing results may have been attributable to early postnatal death, which could have precluded NBS, or to difficulties retrieving results from other regions despite contacting the corresponding regional NBS services. Patient inclusion and HbF result availability are illustrated in Figure 2.

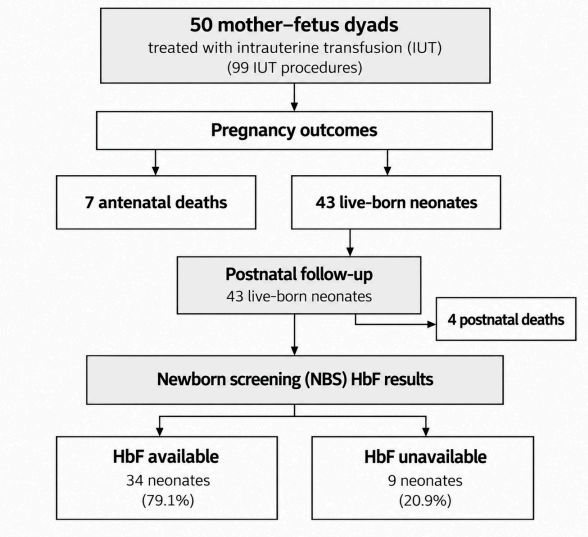


Figure 2. Flow diagram of the study cohort, pregnancy outcomes, postnatal follow-up, and availability of HbF results from routine NBS.

We found a significant positive association between the interval from the last IUT to birth and HbF levels at NBS (β = 3.7; 95%CI, 1.9–5.5; p = 0.001; R^2 = 0.401; Figure 3), indicating that HbF levels at NBS increased by 3.7 percentage points for each additional week elapsed since the last transfusion.

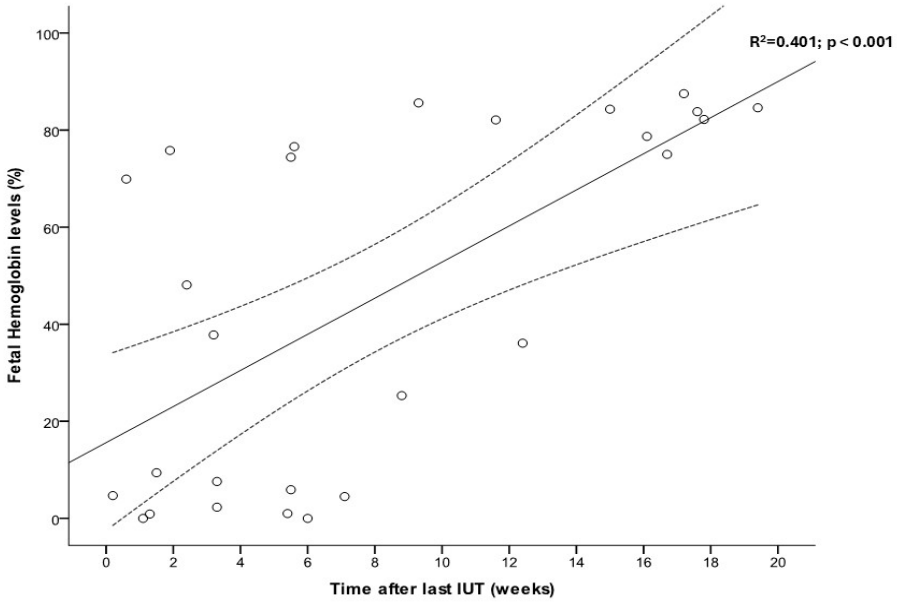


Figure 3. Linear regression analysis of the interval in weeks between the last IUT and birth and HbF measured during routine NBS.

Patients with alloimmunization had significantly lower mean HbF levels at NBS than those with infectious fetal anemia (19% vs. 81%, respectively; $p < 0.001$). The interval between the last IUT and birth was shorter in the alloimmunization group than in the infection group (20 vs. 108 days, respectively; $p = 0.003$). After adjustment for the interval between the last IUT and birth, alloimmunization remained independently associated with lower mean HbF levels at NBS, with a mean difference of 62 percentage points (95%CI, 45–79; $p < 0.001$).

3.5. Association of Fetal Hemoglobin at NBS with Postnatal Outcomes

Compared with healthy controls, our cohort had significantly lower HbF levels measured through routine NBS ($p < 0.001$; Figure 4). Stratification of the cohort according to postnatal transfusion requirement revealed that patients who underwent postnatal transfusion had significantly lower mean HbF levels at NBS than those who did not require transfusion (21 ± 27% vs. 64 ± 31%, respectively; $p = 0.005$; Figure 4), corresponding to a mean difference of 44 percentage points (95%CI, 23–65). After adjustment for the transfusion-to-delivery interval, each 10-percentage-point increase in HbF levels was associated with lower odds of postnatal transfusion (adjusted OR = 0.79, 95%CI 0.58–1.04; $p = 0.092$), although the association did not reach statistical significance.

Furthermore, an HbF level > 50% obtained through NBS was associated with a lower likelihood of postnatal transfusion (AUC 0.87, 95%CI, 0.75–0.99; $p < 0.001$). At this threshold, sensitivity was 86.7% (95%CI, 59.5–98.3%), specificity was 72.2% (95%CI, 46.5–90.3%), PPV was 72.2% (95%CI, 46.5–90.3%), and NPV was 86.7% (95%CI, 59.5–98.3%).

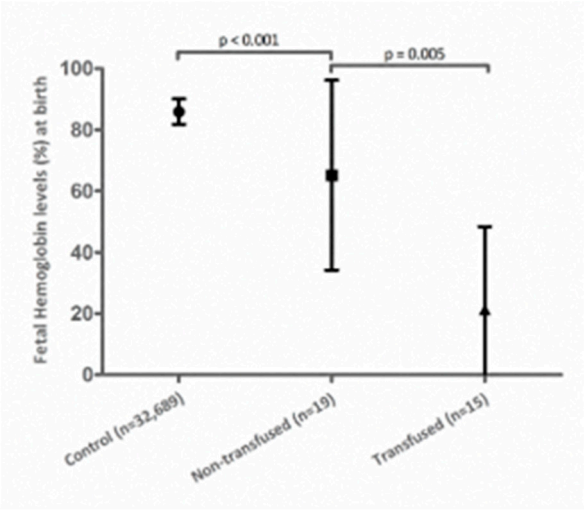


Figure 4. HbF levels at birth in healthy controls, non-transfused neonates, and neonates who received postnatal RBC transfusions. Data are presented as mean ± standard deviation. Between-group differences were assessed using Welch's one-way ANOVA, followed by pairwise Welch's t-tests with Bonferroni correction for multiple comparisons. $p < 0.001$ for the comparison between controls and non-transfused neonates; $p = 0.005$ for the comparison between non-transfused and transfused neonates.

Among patients treated with rHuEPO, the median HbF at NBS was 4.5% (IQR, 0–8.5). Notably, all but one patient required postnatal transfusion before the initiation of rHuEPO treatment; the exception involved an earlier initiation of treatment at 21 days of life.

4. Discussion

This study suggests that HbF measured through routine NBS may provide additional information on the risk of postnatal anemia and transfusion requirements in neonates treated with IUT. Our findings highlight the potential clinical value of leveraging routinely collected NBS data to support risk stratification in this high-risk population, particularly among neonates who are not anemic at birth but subsequently develop late hyporegenerative anemia.

Since the introduction of IUT, survival and perinatal outcomes in severe fetal anemia and fetal hydrops have improved substantially [15,16]. However, postnatal outcomes remain heterogeneous [17], and predicting which neonates will require closer monitoring or additional treatment continues to be challenging [18–20]. Given the rarity of this condition and the limited neonatal experience in many centers, considerable variability in postnatal management still exists. Late-onset anemia after IUT is a well-recognized complication [21–23], and postnatal RBC transfusions are more frequently required in infants treated with IUT than in those who are not [18,19,24,25]. Importantly, this increased transfusion requirement may occur even in the absence of anemia at birth, suggesting that these infants could particularly benefit from early identification and treatment with erythropoietin [26,27]. In our cohort, 27% of neonates who required postnatal RBC transfusion were not anemic at birth but subsequently developed anemia during follow-up and received simple top-up transfusions. These transfusions were not exchange transfusions and occurred beyond the immediate postnatal period, reflecting delayed-onset hyporegenerative anemia rather than acute hemolysis. This observation highlights the limitations of relying solely on hemoglobin levels at birth to guide postnatal monitoring and underscores the potential value of additional parameters for early risk stratification.

HbF is the predominant hemoglobin at birth, but its levels progressively decline during the first months of life as fetal hemoglobin synthesis transitions to adult hemoglobin production [28]. HbF is routinely measured in NBS programs that include SCD screening. In neonates undergoing intrauterine transfusion (IUT), HbF levels are further influenced by the replacement of fetal RBCs with transfused adult RBCs, a phenomenon previously described [29]. Beyond this transfusion-related effect, the underlying cause of fetal anemia may also impair erythropoiesis, either transiently, as in parvovirus B19 infection, or more persistently, as in anti-Kell alloimmunization. Together, these mechanisms may contribute to the variability in postnatal hematologic outcomes among neonates treated with IUT.

In our cohort, HbF levels measured through NBS increased with longer intervals between the last IUT and delivery. This finding is consistent with a decreasing proportion of transfused adult RBCs over time, potentially accompanied by recovery of endogenous erythropoiesis. Notably, neonates who required postnatal RBC transfusion had significantly lower HbF levels than both non-transfused neonates and healthy controls. These results suggest that low or absent HbF at birth may help identify infants at increased risk of clinically significant postnatal anemia. The observation that all neonates treated with erythropoietin had HbF levels below 10% at NBS further supports this possibility. Nevertheless, HbF levels are influenced by several factors, including the timing of the last IUT and the underlying etiology of fetal anemia. Our data do not allow us to distinguish the relative contributions of transfusion-related effects and impaired erythropoietic recovery. Similarly, we considered an HbF threshold of 50% because it provides a clinically interpretable binary classification that may facilitate the identification of neonates at increased risk of requiring postnatal transfusion, although this threshold should be considered exploratory and requires validation in larger cohorts.

The reticulocyte count remains the reference marker for assessing erythropoietic activity; however, it is not systematically available at birth, particularly in neonates who are clinically stable and not anemic. Moreover, its interpretation is further limited by the need

for serial measurements, as reticulocyte counts vary during the first weeks of life. HbF, in contrast, is systematically measured at a population level through NBS, although it was not incorporated into routine neonatal clinical decision-making during the study period. Our findings suggest that retrospective access to HbF values in patients who received IUTs may provide clinically relevant information. In the future, the integration of HbF results into hospital records could support postnatal risk stratification when reticulocyte data are unavailable. HbF should therefore be considered an adjunctive marker, to be interpreted alongside reticulocyte count and markers of hemolysis when available.

This study has several limitations. Its retrospective design and small sample size, reflecting the rarity of fetal anemia requiring IUT, limited analyses by disease etiology. Reticulocyte counts were not routinely available at birth, precluding comprehensive assessment of erythropoietic activity. Differences in postnatal follow-up and management among referring centers, including the use of exchange transfusions, intravenous immunoglobulin (IVIG), erythropoiesis-stimulating agents, and RBC transfusions, may also have affected outcome assessment. Such variability has been reported internationally in neonates with HDFN [30].

Furthermore, HbF measurements were obtained using two analytical platforms (Sebia capillary electrophoresis and Bio-Rad HPLC). Although previous studies have reported generally comparable results [31,32], inter-method variability cannot be excluded. HbF results were available for only 34 neonates, and potential selection bias due to missing data cannot be ruled out. Nevertheless, these limitations reflect real-world clinical practice and support further investigation of routine NBS HbF as a potential tool for postnatal risk stratification and hematologic monitoring.

5. Conclusions

In conclusion, HbF measured through routine NBS may provide information about the risk of postnatal anemia and subsequent transfusion requirements in neonates treated with IUT. Because HbF is already assessed routinely through NBS, these results could help identify neonates at increased risk of late-onset hyporegenerative anemia and prompt closer hematologic follow-up. Prospective studies in larger, multicenter cohorts are needed to confirm these findings and evaluate the clinical utility of NBS HbF in guiding postnatal care.

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Informed Consent Statement: Informed consent was waived by the ethics committees due to the retrospective nature of the study and the use of routinely collected clinical data.

Data Availability Statement: The data that support the findings of this study are not publicly available due to restrictions related to patient confidentiality and data protection regulations. De-

identified individual participant data are available from the corresponding author upon reasonable request and subject to approval by the relevant institutional review boards and data protection authorities.

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Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

CI	Confidence interval
GA	Gestational age
HbA	Adult hemoglobin
HbF	Fetal hemoglobin
IQR	Interquartile range
IUT	Intrauterine transfusion
MCA-PSV	Middle cerebral artery peak systolic velocity
MoM	Multiples of the median
NBS	Newborn screening
PKD	Pyruvate kinase deficiency
PT	Phototherapy
RBC	Red blood cell
rHuEPO	Recombinant human erythropoietin
TAPS	Twin anemia–polycythemia sequence
TTTS	Twin-to-twin transfusion syndrome

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