Neonatal outcomes following intrauterine transfusion for hemolytic disease of the fetus and newborn: a twenty-year service review

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Objective: The primary objective was to explore perinatal and neonatal outcomes amongst infants who received intrauterine transfusion (IUT) for the management of hemolytic disease of the fetus and newborn (HDFN). The secondary objective was to evaluate the role of key investigations in the fetus at risk of HDFN and assess the relationship with neonatal outcomes. We hypothesized that middle cerebral artery peak systolic velocity (MCA-PSV) and corresponding multiples of the median (MoM) would be predictive of neonatal course.

Methods: This was a retrospective observational study conducted at a tertiary center in the United Kingdom between January 2000 and August 2020. Trust approval was obtained to conduct this service review. Pregnancies requiring IUT for HDFN were identified using the fetal medicine department database. Inclusion criteria were infants who received IUT for HDFN. 67 pregnancies were eligible for inclusion in the study with 156 IUT events. Data were extracted using healthcare records. Statistical analysis was performed using SPSS version 28.0, data were assessed for normality and Spearman’s correlation analysis was performed with p values < .05 considered significant.

Results: 67 pregnancies were included in the study which led to the live birth of 68 infants (one twin pregnancy). There were no fetal deaths following IUT. There was one neonatal death due to extreme prematurity following spontaneous vaginal delivery at 23 + 4 weeks gestation, occurring three days following IUT. 97% of infants required admission to the neonatal intensive care unit and 88% required phototherapy. 25% of infants required readmission for red blood cell transfusion due to anemia. There was a significant correlation between maternal anti-D antibody levels and length of neonatal admission: r = 0.477, p = .014. MCA-PSV and MoM measured prior to the last IUT had a significant positive correlation with the duration of phototherapy: r = 0.527 (p < .001) and r = 0.313 (p < .05) respectively. Linear regression analysis demonstrated a significant positive relationship between MCA-PSV and corresponding MoM recorded prior to the last IUT with r² = 0.177 (p = .030) and r² = 0.101 (p = .029).

Conclusion: HDFN is an important cause of fetal anemia associated with significant neonatal morbidity. MCA-PSV and MoM may be predictive of neonatal phototherapy requirements. The predictive value of MCA-PSV appears to be dependent on the timing of measurement during the antenatal period and more research is needed. Multicentre collaboration is required to generate a reliable large-scale database to further delineate the value of MCA-PSV and MoM and predict neonatal outcomes in cases of HDFN requiring IUT. This data would assist clinicians in antenatal planning and enable more informed counseling of parents in the antenatal period.

Introduction

Hemolytic disease of the fetus and newborn (HDFN) is a form of anemia caused by the destruction of fetal red blood cells by the maternal immune system. HDFN has significant fetal and neonatal morbidity and mortality with complications including hydrops fetalis, postnatal hyperbilirubinemia and perinatal death [1]. Following the introduction of anti-D prophylaxis, it is estimated that 520 pregnancies per year are affected by HDFN in the United Kingdom [2]. Antenatal management of HDFN requires prompt identification and monitoring of fetal anemia and intrauterine transfusions (IUT) are the mainstays of treatment in severe cases [3]. The aim of IUT is to prevent, or reverse fetal hydrops, a consequence of severe fetal anemia, and minimize the need for preterm delivery [4]. The middle cerebral artery peak systolic velocity (MCA-PSV) and corresponding
multiple of the median (MoM) are used as noninvasive indicators of fetal anemia severity \[3,5,6\]. A MoM $\geq 1.5$ is the recommended threshold for giving IUT \[3\]. Risks of IUT include fetal bradycardia, fetal hemorrhage, pre-term rupture of membranes, induced labor and fetal loss \[7\]. Management in the neonatal period involves monitoring for hyperbilirubinemia and anemia with treatments including phototherapy, exchange or top-up transfusions and intravenous immunoglobulins \[4\].

The primary objective of this study was to evaluate the morbidity and mortality amongst babies who required IUTs due to HDFN. The secondary objective was to evaluate the role of key investigations in the fetus at risk of HDFN and assess the relationship of these investigations with neonatal outcomes. This information could be used to facilitate evidence-based counseling of parents of babies who require IUT for HDFN and allow the identification of high-risk pregnancies to ensure appropriate monitoring and clinical planning in the antenatal and neonatal period.

Methods and materials
This was a retrospective observational study of babies who had received IUT for HDFN at the Royal Victoria Infirmary, Newcastle, United Kingdom between January 2000 and August 2020. Trust approval was granted to conduct this service review. The fetal medicine department database was used to identify women who had received IUTs for HDFN between 2000 and 2020. Inclusion criteria were babies who had confirmed HDFN and had received IUTs. Exclusion criteria were: fetal anemia caused by other pathologies and inaccessible medical records. Data were collected using maternal and neonatal electronic and archived healthcare records. Obstetric data of interest included: maternal antibodies (type and titre), number of IUTs, MCA-PSV measurements, IUT complications, and mode of delivery. Neonatal data collected included: cord hemoglobin, weight at delivery, length of neonatal admission, top-up and exchange transfusions, duration of phototherapy, neonatal complications and readmissions. Data were statistically analyzed using IBM SPSS statistics version: 28.0.0.0 (190). Data were assessed for normality and Spearman’s correlation analysis was performed. Linear regression analysis was performed on factors that demonstrated a significant correlation with phototherapy duration or length of neonatal admission. A $p$ value of $<.05$ was considered statistically significant.

Results
Between January 2000 and August 2020 there were 94 babies affected by HDFN that required IUTs. 26 babies were excluded due to inaccessible medical records, including six babies delivered at other hospitals. 68 babies were eligible for inclusion, there were 67 mothers (one twin pregnancy) and 156 IUTs were performed. Anti-D was the most commonly detected maternal antibody present in 84% of mothers (56 out of 67). Anti-D antibodies were detected in isolation in 32% of mothers (21 out of 67) and in combination with other antibodies in 52% of mothers. Anti-D and Anti-C were the second most common combination, detected in 28% of mothers (19 out of 67). Maternal antibodies detected in maternal blood samples are outlined in Supplementary Table 1.

Antenatal management
The indication for the first IUT was MoM $\geq 1.5$ \[3\]. Subsequent transfusions and timing of delivery were decided using clinical judgment with factors including gestational age, timings of the previous transfusion and estimated rate of fetal hemoglobin decline considered \[3\]. The average gestational age at the time of IUT was 28 weeks, ranging between 18 and 34 weeks. The median number of IUTs per pregnancy was two (range 1–5). The mean hematocrit measured prior to the first IUT was 19% (range 2.6%–32%). The majority of IUTs were complication free (81.4%). Complications following IUT are outlined in Figure 1. All pregnancies in this group resulted in a live birth. The most common mode of delivery was elective lower segment cesarean section (49%, 33 out of 68). The mean gestational age at the time of delivery was 35 weeks to the nearest week, the median of 35 $\pm$ 2 weeks, with a range between 23 $\pm$ 4 and 38 $\pm$ 1 weeks. 64 of 68 babies (94%) were born prematurely: 2 infants were extremely premature at 23 and 25 weeks gestation, but most premature infants were born between 32 and 36 weeks of gestational age. The mean cord hemoglobin at the time of delivery was 118 g/L (range 39–199 g/L). The mean birth weight was 2553 g (range 500–4100 g). There was one case of fetal hydrops and two cases of moderate ascites identified on antenatal ultrasound scan: outcomes were comparable with the rest of the cohort.

Neonatal management
66 out of 68 babies (97%) were admitted to the neonatal intensive care unit (NICU). Interventions during
the neonatal period are outlined in Supplementary Table 2. The average duration of admission was 10 days, ranging between 1 and 63 days. There was one neonatal death in NICU due to prematurity, this baby was born at 23 \(\pm\) 4 weeks following a spontaneous vaginal delivery. This delivery occurred three days following the second IUT, given at 23 \(\pm\) 1 weeks with no immediate IUT complications reported. Phototherapy was the most common intervention required, given to 60 out of 68 babies (88%), with an average duration of four days, ranging between 1 and 11 days. Exchange transfusions were given to 44 babies (65%) with 14 babies requiring top-up transfusions of red blood cells. Three babies required platelet transfusions. 29 babies required respiratory support, five of these required intubation and ventilation. One baby had an extra-axial hemorrhage detected on a cranial ultrasound scan. Necrotizing enterocolitis (NEC) occurred in two babies. One of the babies who developed NEC was born at 35 \(\pm\) 2 weeks with no respiratory support. The other was born at 32 \(\pm\) 3 weeks and had complex cardiac anomalies and required respiratory support. Hepatosplenomegaly noted at the time of delivery was recorded for two babies. One of these babies had a 12 days admission, required five exchange transfusions and was readmitted for a repeat red blood cell transfusion aged seven days.

Following discharge 19 babies were readmitted (28%). 17 were readmitted for additional blood transfusion: 14 of these were detected at a routine six-week blood test, and three presented with jaundice prior to the six-week check. The average hemoglobin at the time of readmission was 68.4 g/L, a range between 44 and 90 g/L. Four of the babies readmitted required multiple blood transfusions and one baby required five top-up transfusions and received input from hematology due to prolonged reticulocytopenia. Two babies were readmitted to the hospital with a respiratory syncytial virus. One baby with complex cardiac anomalies required multiple admissions under the cardiology team. In the group of babies readmitted requiring blood transfusion the combination of maternal Anti-D and Anti-C antibodies was the most common maternal antibody pattern, present in 41% of cases, followed by the combination of anti-D, anti-C and anti-E in 18% of readmissions.

**Antenatal predictors of neonatal outcomes**

The correlation between MCA-PSV measurements, MoMs, maternal antibody levels, fetal hematocrit, cord hemoglobin, gestational age at delivery and the neonatal outcomes including length of admission and duration of phototherapy are outlined in Table 1. Linear regression analysis of factors with significant correlation is displayed in Table 1. A significant positive correlation between maternal anti-D antibody levels pre-delivery and length of neonatal admission was identified, regression analysis however was not significant. There was a significant positive correlation between MCA-PSV and MoM measured before the last IUT and duration of phototherapy, this relationship was also identified with linear regression analysis. There was no significant correlation between the last MoM or MCA-PSV measured prior to delivery and the duration of phototherapy. A positive correlation was identified between MCA-PSV prior to first IUT and duration of phototherapy. A positive correlation was identified between MCA-PSV prior to first IUT and duration of phototherapy. A positive correlation was identified between MCA-PSV prior to first IUT and duration of phototherapy. A positive correlation was identified between MCA-PSV prior to first IUT and duration of phototherapy.

![Intrauterine Transfusion Complication](image)

Figure 1. Intrauterine transfusion complications. \(n = 156\) transfusion events.
Table 1. Correlation and regression analysis of antenatal predictors of neonatal length of admission and phototherapy duration.

| Predictive factor | Phototherapy | Length of stay |
|-------------------|--------------|----------------|
|                   | Correlation  | P value | Correlation  | P value |
| Maternal Anti-D levels | 0.092 | .677 | 0.477 | .014 |
| MCA-PSV before first IUT | 0.509** | <.001 | 0.172 | .228 |
| MoM before first IUT | 0.155 | .297 | 0.021 | .885 |
| MCA-PSV before last IUT | 0.527** | <.001 | 0.138 | .338 |
| MoM before last IUT | 0.313* | .032 | 0.007 | .961 |
| MCA-PSV last recorded | 0.092 | .543 | 0.195 | .179 |
| MoM last recorded | 0.030 | .842 | 0.143 | .327 |
| Fetal Hct(%) before first IUT | 0.135 | .446 | -0.148 | .374 |
| Fetal Hct(%) post last IUT | -0.179 | .277 | -0.113 | .476 |
| Gestational age at delivery | -0.114 | .427 | -0.216 | .110 |
| Cord Hb at delivery | 0.103 | .478 | -0.117 | .476 |

| Predictive factor | Phototherapy | Length of stay |
|-------------------|--------------|----------------|
|                   | R² | P value | R² | P value |
| Maternal Anti-D levels | 0.110 | .099 |
| MCA-PSV before first IUT | 0.266** | <.001 |
| MoM before first IUT | 0.058 | .104 |
| MCA-PSV before last IUT | 0.177* | .003 |
| MoM before last IUT | 0.101* | .029 |

* Significant at p < .05. **Significant at p < .001.

Discussion

HDFN has significant morbidity amongst those babies who require IUT as a result of the disease, however, there was a low mortality rate in this group.

Antenatal management

IUT is the mainstay of clinical treatment of severe HDFN; whilst current techniques have improved the safety of IUTs the risks remain serious and adequate counseling is important to ensure informed decision-making [8,9]. The IUT complication rate in this study was 28%. There was one neonatal loss following spontaneous vaginal delivery three days following IUT in this group (1.5%), in line with the national quoted risk of 2% [2,9]. There is a lack of up-to-date available data regarding current IUT complication rates. The infographic created in Figure 1 may have a useful role in clinical practice as a visual aid to facilitate evidence-based counseling of parents prior to IUT. It is important that specialized centers report IUT outcomes and complication rates to enable the identification and implementation of gold standard practices.

Neonatal outcomes

The use of IUT has improved neonatal outcomes for babies with HDFN however high levels of morbidity remain [9]. It has been reported that IUT has the effect of suppressing fetal erythropoiesis, which can increase the requirement for subsequent transfusions in the intrauterine and postnatal periods [7]. There are increased rates of preterm delivery amongst babies with HDFN, either planned or as an emergency. This prematurity increases the neonatal burden and is a confounding factor when interpreting the impact of HDFN on neonatal outcomes [10]. In our cohort, 98% of babies required admission to NICU with 88% requiring phototherapy; comparable admission rates were reported by Birchenhall et al. with a 97% admission to NICU and 96% phototherapy rate. There was a high rate of readmission for repeat blood transfusions (25%) in this group, this is comparable with the readmission rate of 20% reported by Birchenhall et al. [11].

Maternal antibodies

Anti-D, -C and -Kell are the most common maternal antibodies in cases of HDFN[12]. This was echoed in our sample population with Anti-D, Anti-C and Anti-Kell detected, in isolation or combination with one another, in 72% of cases. Anti-D was the most common antibody in our sample causing 32% of cases of HDFN and identified in combination with other maternal antibodies in 83.5% of cases. The combined presence of Anti-D and Anti-C was the most common amongst the group of babies requiring a readmission for additional blood transfusions. The significance of multiple maternal antibodies on the severity of HDFN is not fully understood [13,14]. There is evidence that specific combinations of maternal antibodies may result in more severe disease including the combination of anti-D and anti-C [10,13,14]. This may explain the increased rates of readmission amongst these infants. This is clinically relevant as increased outpatient monitoring could be considered in babies with known high-risk maternal antibody combinations to ensure early detection of anemia.

Antenatal predictors of neonatal course

There is limited available data regarding antenatal factors predictive of neonatal outcomes in HDFN. The identification of such factors could assist with informed counseling of parents and ensure adequate planning and coordination between obstetric and neonatal teams prior to delivery. We assessed maternal antibody levels, MCA-PSV, MoM, fetal hematocrit, cord hemoglobin at time of delivery and gestational age at delivery with a length of neonatal admission and duration of phototherapy treatment (Table 1). We hypothesized that these factors could indicate the
severity of HDFN on the fetus and thus be predictive of neonatal course.

Maternal antibodies
A significant correlation was identified between maternal anti-D antibody levels detected prior to delivery and length of neonatal admission however this was not replicated with linear regression, suggesting this correlation is not cause and effect. There was no significant correlation between anti-D levels and duration of phototherapy. The role of maternal anti-D antibody levels as markers of the severity of HDFN, and thus as predictors of neonatal outcomes, is complex [15–17]. Maternal antibody titers are used clinically to provide threshold values for further investigation and high maternal antibody titers may be associated with increased severity of HDFN [13,15–17]. Subclasses of IgG, particularly IgG1, have been implicated in predicting the severity of HDFN however these subclass data were not available in our study group [16,18]. Confounding factors including gestational age, type of antibody and individual variations in immune response mean that maternal antibody titers cannot be used in isolation to predict the severity of HDFN [15,16].

MCA-PSV and MoM
We hypothesized that increased MCA-PSV and MoM could be predictive of length of neonatal admission, duration of phototherapy and readmission. There was evidence in this study of a positive correlation between MCA-PSV and MoM with a longer duration of phototherapy. The significance of this correlation varied depending on the timing of MCA-PSV measurement. The MCA-PSV prior to the first transfusion was associated with a significant correlation with neonatal phototherapy requirements however the corresponding MoM was not, this was also seen on regression analysis. The MoM accounts for gestational age and therefore provides a useful value that can allow comparison between individuals of different gestational ages and between institutions. The disparity between the significance between MoM value and the MCA-PSV may be due to the small sample size. Large-scale multicentre studies would assist in determining whether MCA-PSV measurements prior to the first IUT are predictive of neonatal phototherapy requirements. Based on these findings we cannot confirm the predictive value of MCA-PSV and MoM measured prior to the first IUT on neonatal course. The MCA-PSV and corresponding MoM recorded prior to the last transfusion gave the most significant correlation with neonatal phototherapy requirements, linear regression analysis was also significant, and this warrants more research. The predictive value of MoM is limited by the complex confounding factors that impact neonatal outcomes including gestational age at the time of delivery, maternal age, maternal BMI and mode of delivery. MoM also improves in the immediate aftermath of an IUT, making the MoM less accurate at predicting the severity of fetal anemia. There is evidence MoM may be useful after one transfusion [5,19]. There is no proven predictive value following two IUTs [19–21]. It is possible that this improvement following IUT masks ongoing severe disease which has a negative impact on fetal condition by the time of delivery. It is important to note that HDFN occurs as a result of maternal antibody destruction of red blood cells and MCA-PSV may not reflect antibody activity at the time of delivery and in the neonatal period.

Limitations and recommendations
This study gives a useful insight into the antenatal and neonatal outcomes following IUT, however, there is a need for caution when interpreting these findings. This was a retrospective study using medical records which led to some gaps in the data available, including in the follow-up information due to patient movement between differing trusts. The sample size is small which is in keeping with the rarity of antenatal IUTs. Long-term neonatal outcomes were beyond the scope of this study however remain an important consideration. The relationship between MCA-PSV, MoM and neonatal outcomes requires more research. Large multicentre prospective observational studies will provide a more robust evidence base. It is important that centers providing IUTs make complication rates and outcomes available to allow the identification of gold standard practice.

Conclusion
HDFN requiring IUT is associated with neonatal morbidity, though mortality is low following advances in fetal medicine. MCA-PSV may be a predictive factor of neonatal phototherapy requirements, however, the value is affected by the timing of measurement and more research is indicated. Identifying antenatal predictive factors of neonatal outcomes is complicated by maternal and neonatal confounding factors such as gestational age at delivery, birth weight and mode of delivery. More research is required to enable the identification of useful predictors of neonatal outcomes. These predictors could be used by clinicians to guide
monitoring and management in the antenatal and neonatal periods, as well as enable evidence-based counseling of parents.

**Authors contributions**

UB: data collection, statistical analysis, writing. ML: data collection, editing, writing. AB: data collection, statistical analysis, editing and writing. NA: conception of the project, data collection, editing and writing.

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