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Darbepoetin alfa to reduce transfusion episodes in infants with haemolytic disease of the fetus and newborn who are treated with intrauterine transfusions in the Netherlands: an open-label, single-centre, phase 2, randomised, controlled trial

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For the Dutch translation of the abstract see **Online** for appendix

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Summary

Background Up to 88% of infants with haemolytic disease of the fetus and newborn who are treated with intrauterine transfusions require erythrocyte transfusions after birth. We aimed to investigate the effect of darbepoetin alfa on the prevention of postnatal anaemia in infants with haemolytic disease of the fetus and newborn.

Methods We conducted an open-label, single-centre, phase 2 randomised controlled trial to evaluate the effect of darbepoetin alfa on the number of erythrocyte transfusions in infants with haemolytic disease of the fetus and newborn. All infants who were treated with intrauterine transfusion and born at 35 weeks of gestation or later at the Leiden University Medical Center, Leiden, Netherlands, were eligible for inclusion. Included infants were randomised by computer at birth to treatment with 10 µg/kg darbepoetin alfa subcutaneously once a week for 8 weeks or standard care (1:1 allocation, in varying blocks of four and six, with no stratification). Treating physicians and parents were not masked to treatment allocation, but the research team, data manager, and statistician were masked to treatment allocation during the process of data collection. The primary outcome was the number of erythrocyte transfusion episodes per infant from birth up to 3 months of life in the modified intention-to-treat population. This trial is registered with ClinicalTrials.gov (NCT03104426) and has been completed.

Findings Between Oct 31, 2017, and April 31, 2022, we recruited 76 infants, of whom 44 (58%) were randomly assigned to a treatment group (20 [45%] were allocated to receive darbepoetin alfa and 24 [55%] were allocated to receive standard care). Follow-up lasted 3 months and one infant dropped out of the trial before commencement of treatment. A significant reduction in erythrocyte transfusion episodes was identified with darbepoetin alfa treatment compared with standard care (median 1·0 [IQR 1·0–2·0] transfusion episodes *vs* 2·0 [1·3–3·0] transfusion episodes; p=0·0082). No adverse events were reported and no infants died during the study.

Interpretation Darbepoetin alfa reduced the transfusion episodes after intrauterine transfusion treatment for haemolytic disease of the fetus and newborn. Treatment with darbepoetin alfa or other types of erythropoietin should be considered as part of the postnatal treatment of severe haemolytic disease of the fetus and newborn.

Funding Sanquin Blood Supply.

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Introduction

Haemolytic disease of the fetus and newborn is a condition in which maternal erythrocyte alloantibodies lead to the destruction of foetal erythrocytes and can also impair erythropoiesis. The main antenatal treatment of foetal anaemia due to haemolytic disease of the fetus and newborn is intrauterine erythrocyte transfusion or serial transfusions, which can aggravate hyporegenerative anaemia in these infants.[1,2] The main treatment of haemolytic disease of the fetus and newborn in the first week of life is intensive phototherapy and, if necessary, exchange transfusion to treat hyperbilirubinaemia and prevent kernicterus and erythrocyte transfusion because of low haemoglobin concentration.[3]

Up to 88% of infants with haemolytic disease of the fetus and newborn who are treated with intrauterine transfusion require at least one erythrocyte transfusion during the first 3 months of life, with a median of 2 (IQR 2–3) erythrocyte transfusion episodes.[4–6] The postnatal course of haemolytic disease of the fetus and newborn therefore requires a long follow-up time with weekly blood draws, a minimum of two hospital admissions per infant, and potential complications related to transfusion. The need for erythrocyte transfusion in infants with haemolytic disease of the fetus and newborn during the first 3 months of life is thought to be due to ongoing haemolysis by persisting, and only gradually declining, maternal antibodies

Research in context

Evidence before this study

We searched PubMed from database inception to Sept 9, 2023, for published randomised controlled trials assessing the effect of erythropoiesis stimulating agents (ESAs) among infants with haemolytic disease of the fetus and newborn. The search terms used were ("Haemolytic disease of the foetus and newborn" or "hemolytic disease of the fetus and newborn" or "haemolytic disease" or "hemolytic disease") AND ("erythropoietin" or "darbepoetin"or "EPO") AND ("clinical trial" or "randomised controlled trial" or "randomized controlled trial"). We identified one pilot study, which suggested that infants with haemolytic disease of the fetus and newborn might benefit from treatment with ESAs to reduce the risk of anaemia and subsequent erythrocyte transfusion episodes. Several other small non-randomised studies and case reports support this theory. However, other studies have reported that ESAs might be less effective than expected. Due to the absence of convincing evidence, and as there are no published clinical randomised trials on the effect of ESAs specifically in the population of infants affected by haemolytic disease of the fetus and newborn, ESAs are currently not recommended in the treatment of haemolytic disease of the fetus and newborn.

Added value of this study

This is the first randomised controlled trial evaluating an ESA (ie, darbepoetin alfa) in a population of infants with severe haemolytic disease of the fetus and newborn. Overall, there was a significant effect of darbepoetin alfa administration on transfusion episodes in severe haemolytic disease of the fetus and newborn.

Implications of all the available evidence

The postnatal burden of haemolytic disease of the fetus and newborn is still high due to a high transfusion dependency seen in infants treated with intrauterine transfusions. Treatment with darbepoetin alfa or other types of ESAs as part of the postnatal treatment of severe haemolytic disease of the fetus and newborn should be considered.

and transfusion-related suppressed erythropoiesis (ie, hyporegenerative anaemia).[7–9] Other contributing factors have been reported, such as severity of haemolytic disease of the fetus and newborn, most likely because of high maternal antibody concentrations, and the declining use of exchange transfusion over time due to more restrictive guidelines on exchange transfusion and more aggressive phototherapy (hence less removal of maternal alloantibodies from the neonatal circulation than if exchange transfusion was more readily used).[3,10]

Hyporegenerative anaemia occurs particularly in infants who are treated with multiple intrauterine transfusions.[4,6,7] The foetal reticulocyte count shows an exponential decline over the course of consecutive intrauterine transfusions, with near disappearance of foetal reticulocytes after two intrauterine transfusions.[9] Although suppression of foetal haematopoiesis decreases haemolysis by the offending maternal antibody, haematopoiesis needs to recommence to prevent anaemia after birth as maternal antibodies decrease. Transfusion-induced erythropoietin deficiency is considered to be a possible contributing factor to long-lasting low erythrocyte counts in infants with haemolytic disease of the fetus and newborn.[11–16] Transfusion-induced erythropoietin deficiency might be reversed by treatment with exogenous erythropoietin, which is increasingly used in full-term and preterm (ie, born at <37 weeks of gestation) infants to prevent or reduce neonatal anaemia without adverse effects in the short term or the long term.[11–18] Several small studies and case reports have suggested that infants with haemolytic disease of the fetus and newborn might benefit from treatment with erythropoiesis stimulating agents (ESAs) to reduce the risk of anaemia and subsequent erythrocyte transfusion episodes.[11–16] However, other studies have reported that ESAs might be less effective than expected.[19] Due to the absence of convincing evidence, routine use of ESAs in infants with haemolytic disease of the fetus and newborn is currently not recommended.[3]

We hypothesised that administration of a long-acting ESA, darbepoetin alfa, in near term and term (ie, born at ≥35 weeks of gestation) infants with haemolytic disease of the fetus and newborn would decrease the number of erythrocyte transfusions needed. We aimed to evaluate the effect of exogenous erythropoietin on the prevention of postnatal anaemia in infants with haemolytic disease of the fetus and newborn.

Methods

Study design and participants

We conducted an open-label, single-centre, phase 2, randomised controlled trial to investigate whether darbepoetin alfa is effective in reducing the incidence of late anaemia in infants with haemolytic disease of the fetus and newborn who are treated with intrauterine transfusion, and therefore in decreasing the number of erythrocyte transfusion episodes per infant, compared with infants who received standard of care without darbepoetin alfa.

All near-term and term infants (ie, born at ≥35 weeks of gestation) with haemolytic disease of the fetus and newborn (due to D, C, c, E, or K antigen or other type of erythrocyte alloimmunisation) who were treated with intrauterine transfusion and admitted to the Leiden University Medical Center (LUMC), Leiden, Netherlands, on or after Oct 31, 2017 were eligible for the study.

The LUMC is the national referral centre for pregnancies complicated by maternal erythrocyte alloimmunisation.

The prenatal national screening programme in the Netherlands recommends referral to the LUMC in case of increased antibody titres tested in maternal serum of at least 1/2 in K immunisation and at least 1/16 in D or other types of alloimmunisation or in case of an increased antibody-dependent cell-mediated cytotoxicity test result of more than or equal to 50% in case of D immunisation or more than or equal to 30% in case of other type of alloimmunisation. These pregnancies are monitored by serial Doppler measurements to assess the velocity of the blood in the middle cerebral artery. If middle cerebral artery Doppler exceeds 1·55 multiples of the median, or if signs of hydrops are present, then treatment with intrauterine transfusion is indicated. Intrauterine transfusion is usually continued until 34–35 weeks of gestation. The intrauterine transfusion technique has been previously described.[1,20] Subsequently, birth is planned in the LUMC, and the infant is admitted to the neonatal intensive care unit. Approximately 15–20 eligible infants are born annually after intrauterine transfusion treatment at the LUMC. All pregnancies complicated by maternal erythrocyte alloimmunisation that required intrauterine transfusions were monitored by the research team to ensure complete screening for patient recruitment. Parents or caregivers received initial information regarding the study before birth.

Inclusion criteria were gestational age at birth of at least 35 weeks, treatment with at least one intrauterine transfusion for erythrocyte alloimmunisation, and birth in the LUMC. Exclusion criteria were early onset proven neonatal sepsis or a language barrier (ie, people who did not speak Dutch or English, due to the availability of written study information in only these languages).

We obtained written consent from parents within the first week after birth. The study protocol was approved by the Dutch Central Committee on Research Involving Human Subjects, protocol number NL60858.058.17. The protocol is available at ClinicalTrials.gov.

For the **protocol** see https://clinicaltrials.gov/ct2/show/NCT03104426

Randomisation and masking

Included infants were randomly assigned at birth by computer to treatment with darbepoetin alfa or standard care (ie, phototherapy, follow-up of blood values, and erythrocyte transfusion if haemoglobin concentration fell beneath predefined thresholds), with 1:1 allocation, in varying blocks of four and six. No stratification was applied. Patients were enrolled by a member of the research team, who was masked to treatment allocation.

Treating physicians and parents were not masked to treatment allocation. The research team, data manager, and statistician were masked to treatment allocation during the process of data collection. Data were collected into an online secured database, Castor EDC. The database was unmasked to all parties involved after completion of data collection and double checking of data. We did not use a placebo. As predefined guidelines exist to decide on the use of erythrocyte transfusion, the potential bias of the non-blinded design on the primary outcome was considered small and not enough to outweigh the burden of a subcutaneously administered placebo.

For more on **Castor EDC** see https://www.castoredc.com

Procedures

In the treatment group, darbepoetin alfa (Amgen, Breda, Netherlands) was administered subcutaneously at a dosage of 10 µg/kg once a week, starting at approximately day 7 after birth, for 8 weeks, or at least 4 weeks in case of a haemoglobin concentration above 13 g/dL. The half-life of darbepoetin alfa is approximately 72 h. For the control group, phototherapy and, if necessary, erythrocyte transfusions were given as usual. No additional measures were done in this group as part of the study protocol. Darbepoetin alfa was administered by a member of the research team, either at the LUMC, at a local hospital in case of clinical transfer after discharge from the LUMC, or at home when discharged completely. After discharge home, follow-up measures were done by local hospitals for infants in the treatment group, but also as part of standard care. Weekly measurements of complete blood counts (including haemoglobin concentration, haematocrit, and reticulocyte count) were performed in both groups and are usually done by capillary blood draws. The neonatal erythropoietin concentration was measured at birth from cord blood in both groups, as is standard practice at our centre.

Monthly assessment of potential ferritin overload was recommended to the local hospitals. As infants in the treatment group received weekly home visits by a member of the research team for administration of darbepoetin alfa, blood pressure was also measured during these home visits for safety reasons at onset of treatment, after 4 weeks, and after 8 weeks. Hypertension was defined as repeated systolic measures more than the 95th percentile for gestational age. The study team was accessible to parents and local physicians at all times to discuss any concerns regarding treatment or potential side effects, and contact for these reasons was encouraged. Parents and local physicians were obliged to inform the research team of unexpected hospital readmissions.

The number of transfusion episodes during the first 3 months after randomisation and haemoglobin concentrations before each transfusion were recorded retrospectively after 3 months by use of data from medical records. Infants received transfusions if their haemoglobin concentration fell below the cutoff values according to the Dutch national transfusion guideline.[21]

Anaemia was defined as requiring erythrocyte transfusion according to the Dutch national transfusion guideline. The guideline recommends transfusion of 15 mL/kg erythrocytes when haemoglobin concentrations are lower than 10·5 g/dL (6·5 mmol/L, at day 0–6 of life), below 8·9 g/dL (5·5 mmol/L, at day 7–13 of life), and below 7·2 g/dL (4·5 mmol/L, from day 14 of life onwards) and was communicated to all involved local hospitals after discharge from the LUMC. Decisions on whether to

transfuse were made by local physicians, not by the study team. Concomitant therapy with folate or iron therapy are not standard practice in the Netherlands for these infants. Neonatal clinical records from the LUMC, and from additional admissions and outpatient clinic visits in other hospitals, were collected for each included infant with written consent of parents.

Outcomes

The primary outcome was the number of erythrocyte transfusion episodes per infant from birth up to 3 months of age in the modified intention-to-treat (ITT) population. Secondary outcomes were proportion of infants receiving an erythrocyte transfusion up to 3 months of age; number of postnatal transfusions per infant from 7 days to 3 months of life; time from birth to first erythrocyte transfusion; number of days of hospitalisation and readmissions associated with erythrocyte transfusions; course of haemoglobin and reticulocytes up to 3 months of age; haemoglobin concentration at first postnatal transfusion (g/dL); proportion of infants in the treatment group with a systolic blood pressure at least 2 SD above the age-adjusted mean systolic blood pressure during treatment; and proportion of infants with ferritin concentration higher than 200 µg/L during treatment.

We performed a post-hoc analysis to examine the interaction between treatment allocation and endogenous erythropoietin concentration in patients with erythropoietin measurements at baseline.

Statistical analysis

A statistical analysis plan was formulated and formalised before inclusion of the first infant and submitted to our local medical ethics committee. On the basis of the literature, we expected a 50% reduction in the median number of total erythrocyte transfusion episodes per infant with darbepoetin alfa treatment, from a median of two transfusions per infant in the control group to one transfusion per infant in the treatment group. Group sample sizes of 21 infants would yield 81% power to detect a difference of 1·1 between the null hypothesis that both groups would receive a mean of 1·9 transfusions and the alternative hypothesis that the mean of the treatment group would be 0·8, with a significance level (alpha) of 0·05 using a two-sided Mann-Whitney test. The drop-out percentage was estimated at 5%, so we added (42 / 0·95=44) one infant to the sample size of each group, making a total of 22 per group. The sample size was calculated using the Mann-Whitney test procedure from PASS, version 11e (NCSS, Kaysville, UT, USA).[22]

We planned to conduct a modified ITT and per-protocol analysis to study the differences in all outcome measures between the intervention and control group, if there was at least 10% difference between the modified ITT and per-protocol analysis. The modified ITT analysis set consisted of all infants who were randomly assigned to a group and who did not withdraw before day 7, classified according to allocated treatment regardless of whether they received the assigned treatment or had any protocol deviations. Infants with major protocol deviations were excluded from the per-protocol analysis set. For infants assigned to the treatment group, receiving fewer than four darbepoetin alfa injections was considered to be a major protocol violation.

The total number of postnatal transfusions per infant from birth to the end of the follow-up period, the number of postnatal transfusions per infant from 7 days (ie, the start of intervention in the treatment group) until 3 months of life, haemoglobin concentration at first postnatal transfusion (g/dL), and the number of days of additional admissions for erythrocyte transfusions were compared between the groups using a Mann-Whitney U test. The time from birth to first erythrocyte transfusion was compared between the groups using a log-rank test. The proportion of infants requiring a postnatal transfusion up to 3 months of life was compared between the groups using a Fisher's exact test.

A multiple linear regression analysis was performed to correct the association between treatment allocation and the primary outcome (ie, number of postnatal transfusions) for type of alloimmunisation (categorical) and number of intrauterine transfusions (continuous).

A post-hoc analysis was performed with univariable analysis of treatment allocation and multivariable analysis of treatment allocation and log-transformed endogenous erythropoietin concentration, with both restricted to the subset of 33 patients with erythropoietin measurements at baseline.

A linear mixed model was fitted to assess the course of haemoglobin concentration in the treatment and control group over time. The haemoglobin concentration and reticulocyte count course were graphically depicted. To account for the correlation between repeated measurements in the same participant, a random intercept and random time effect were included. The fixed effects were treatment, time, and the interaction between treatment and time. To adequately capture non-linear progression over time, natural cubic splines (with one interior and two boundary knots) both in the fixed and random time effects were used. To examine whether the two groups differed in their haemoglobin concentration course over time, we studied an interaction term of treatment and time.

Throughout the analyses, a p value below 0·05 was regarded as statistically significant. Statistical analysis was performed using SPSS (version 23.0) and R (version 4.2.1). A data monitoring committee evaluated the trial proceedings. This study was registered prospectively on ClinicalTrials.gov (NCT03104426).

Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

Results

Infants were recruited between Oct 31, 2017, and April 31, 2022. A total of 76 pregnant women were treated with one or more intrauterine transfusions for haemolytic disease of the fetus and newborn at the LUMC and assessed for eligibility (figure 1). Of the 76 babies born to these women, 32 (42%) infants were excluded from participation. Parents either did not consent to participation (n=18), or the inclusion criteria were not met (n=14). Parents who declined to provide consent for their infant to participate were not obliged to explain their decision but were invited to share their reasons. Among the answers were hesitation due to the experimental nature of the study (n=4), expected burden of home visits and prolonged medical interference after tiresome pregnancy and hospital admission (n=3), fear and reluctance regarding the method of administration (ie, subcutaneous injections) of darbepoetin alfa (n=2), and fear for potential side effects (n=2). No reasons were provided for the other seven infants.

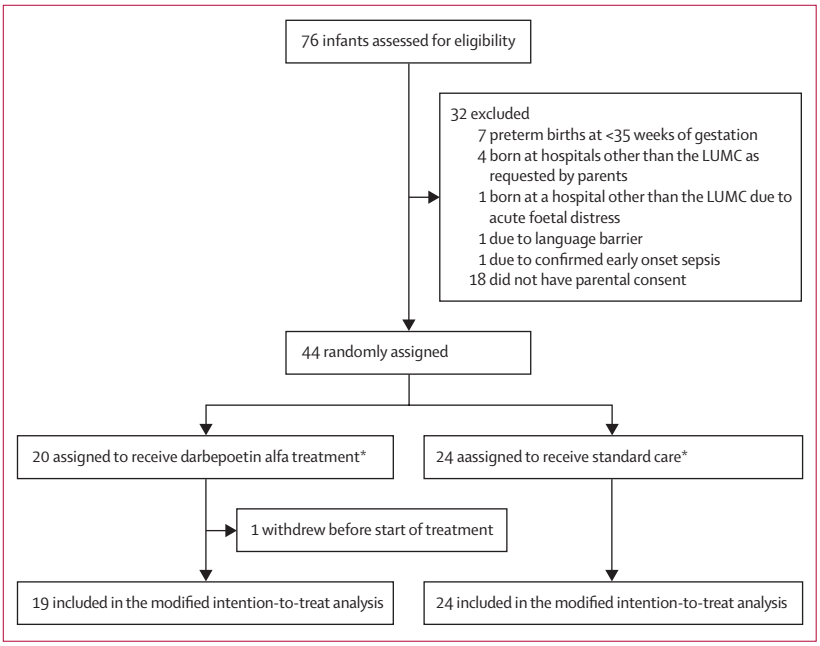

Figure 1: **Trial profile**
*There were two randomisation errors (failure to remove a fictional test case and one participant being allocated to a group twice) leading to the disparity in the number of infants randomly assigned to the two groups.

	Treatment group (n=19)	Control group (n=24)
Sex		
Female	11 (58%)	16 (67%)
Male	8 (42%)	8 (33%)
Caesarean delivery	5 (26%)	9 (38%)
Gestational age at birth, weeks	37 (36–37)	37 (36–37)
Birthweight, g	2845 (2685–3091)	2773 (2561–3276)
Apgar score at 5 min	10 (9–10)	10 (9–10)
Number of intrauterine transfusions per infant	3·0 (1·0–4·0)	2·9 (1·3–4·0)
Gestational age at first intrauterine transfusion, weeks	28 (24–33)	30 (26–32)
Type of alloimmunisation		
D-mediated alloimmunisation	12 (63%)	21 (88%)
c-mediated alloimmunisation	1 (5%)	1 (4%)
K-mediated alloimmunisation	6 (32%)	2 (8%)
Haemoglobin concentration at birth, g/dL	12·6 (11·4–14·3)	12·7 (11·2–14·3)
Reticulocyte count at birth, %*	1·9% (0·2–4·7)	1·3% (0·2–5·0)
Endogenous erythropoietin at birth, units per L†	110 (44–362)	65 (22–280)
Ferritin at birth, µg/L†	828 (665–828)	811 (634–1078)
Phototherapy, days	3 (2–4)	4 (3–6)
Treatment with exchange transfusion	1 (5%)	1 (4%)
Maximum bilirubin, mg/dL	8·8 (7·5–14·3)	14·9 (10·8–17·5)

Data are n (%) or median (IQR). *One missing value in each group. †Five missing values in each group.

Table 1: **Baseline demographic and clinical characteristics by treatment group**

A total of 44 infants were randomly assigned to a group: 20 were allocated to treatment with darbepoetin alfa and 24 infants were allocated to the control group. Directly after the randomisation process, on day 2 after birth, one couple withdrew consent to start treatment and the infant was excluded from the modified ITT population, resulting in 19 infants in the treatment group and 24 infants in the control group.

The disparity in group sizes is due to block randomisation and the addition of a fictional test case, which was used to validate the randomisation process of the database. The number of this test case was erroneously not removed. Additionally, one child was randomly assigned to a group twice in the database. We chose not to replace these randomisations with new participants, but instead to leave the allocated treatment unused, to protect randomisation concealment. No clinical data or any other data were included for these two randomisation lots and they were not included in any analysis.

Treatment with darbepoetin alfa was discontinued in nine (47%) of 19 treated infants when haemoglobin concentrations increased to 13 g/dL or more after at least 4 weeks of treatment, as was predetermined in the protocol to prevent polycythaemia or hyperviscosity. These infants received four (n=4), five (n=1), six (n=3), or seven injections (n=1). None of these infants received an erythrocyte transfusion after discontinuation of treatment.

Baseline characteristics of the cohort are shown in table 1. In both groups, D-mediated alloimmunisation was the predominant type of alloimmunisation, although more infants in the treatment group had K-mediated alloimmunisation than in the control group.

Outcome measures regarding erythrocyte transfusion are shown in table 2. A significant reduction in erythrocyte transfusion episodes was identified with darbepoetin alfa treatment compared with standard care. Mann-Whiney test confirmed a shift in the distributions between the two treatment groups. This effect was maintained when excluding the erythrocyte transfusion episodes that were administered in the first week after birth, before darbepoetin alfa treatment was started. The number of

transfusions in both groups are depicted in figure 2. Two infants seemingly did not respond to darbepoetin alfa treatment (one infant had three transfusion episodes and the other infant had four transfusion episodes).

When corrected for type of alloimmunisation (D *vs* other types of alloimmunisation) and total number of intrauterine transfusions, darbepoetin alfa treatment was associated with fewer postnatal transfusions per infant compared with standard care (unstandardised β=0·74; p=0·033). The number of infants with missing values for endogenous erythropoietin (n=10; five infants in each group) would have reduced the significance of the multivariable analysis to an extent that we decided not to include this variable in the multivariable analysis. A post-hoc analysis was performed with univariable analysis of treatment allocation (control or erythropoietin) and multivariable analysis of treatment allocation and log-transformed endogenous erythropoietin concentration, both restricted to the subset of 33 patients with erythropoietin measurements at baseline. In these analyses, the effect of treatment allocation was materially unaltered with and without correction for baseline erythropoietin values (and was significant in both analyses), with p=0·68 for baseline endogenous erythropoietin values.

Boxplots of haemoglobin concentrations and reticulocyte count course after birth of both groups are presented in figure 3. An initial decline in haemoglobin concentration after birth was observed in both groups, reaching a nadir around the age of 5–6 weeks, after which concentration gradually increased. Although numerical values of haemoglobin concentration are higher in the treatment group, the difference with the control group was not significant (p=0·12 for the interaction between treatment and time). Reticulocyte count after birth also showed an initial decrease followed by gradual increase, which was numerically more pronounced in the treatment group, but not significantly different.

No side-effects of darbepoetin alfa treatment were reported and no hypertension was observed in treated infants. Hospital readmission for reasons other than erythrocyte transfusion occurred in five (12%) of 43 infants in the first 3 months after birth. These reasons were infection with respiratory syncytial virus (n=2; one infant in the treatment group and one in the control group), feeding difficulties (n=2; one infant in the treatment group and one in the control group), and diagnostics for cholestasis (n=1; control group). None of the included infants died during the study period.

In the study population, 79 erythrocyte transfusion episodes occurred (24 in the treatment group and 54 in the control group), of which 28 (35%) were administered at haemoglobin concentrations that were higher than the advised cutoff concentrations. In the treatment group, six (25%) of 24 transfusion episodes occurred at higher haemoglobin concentrations than the recommended cutoff, with a mean deviation of 0·4 g/dL (SD 0·3) above the advised cutoff level. In the control group, 22 (41%) of 54 transfusion episodes occurred at higher haemoglobin concentrations than the recommended cutoff, with a mean deviation of 0·5 g/dL (SD 0·5). These deviations were, in most cases, motivated by clinical signs of fatigue, feeding problems, or both. A transfusion was withheld for one infant in the control group with a haemoglobin concentration below the advised threshold. The infant's haemoglobin concentration was 0·2 g/dL below the threshold, but because the infant showed a good clinical condition and a high reticulocyte count, haemoglobin concentration was measured again after 2 days and showed a spontaneous increase above the transfusion threshold.

Ferritin assessments were recommended to all local hospitals but were rarely done and so are not reported.

	Darbepoetin alfa treatment group (n=19)	Control group (n=24)	p value
Total number of erythrocyte transfusion episodes per infant	1·0 (1·0–2·0)	2·0 (1·3–3·0)	0·0082
Number of erythrocyte transfusion episodes per infant at least 7 days after birth	1·0 (0·0–1·0)	2·0 (1·0–3·0)	0·0033
Treatment with postnatal erythrocyte transfusion*	15 (79%)	22 (92%)	0·38
Time between birth and first transfusion, days†	29 (6–61)	22 (8–29)	0·14
Haemoglobin concentration at first transfusion episode, g/dL‡	7·9 (6·5–10·2)	8·1 (6·8–9·2)	0·75
Total length of hospitalisation, days	8 (6–16)	12 (7–16)	0·29
Initial hospitalisation	6 (4–7)	6 (5–7)	0·58
Additional hospitalisation	4 (0–9)	5 (0–10)	0·40

Data are median (IQR) or n (%), unless otherwise stated. *Fisher's exact test. †Median calculated by Kaplan-Meier estimator and corresponding p value calculated by log-rank test. ‡One missing value in each group.

Table 2: **Outcomes by treatment group**

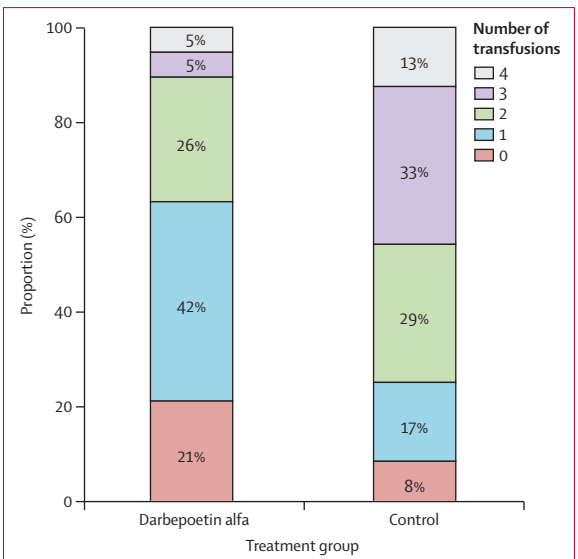

Figure 2: **Stacked bar chart of total transfusions after birth**

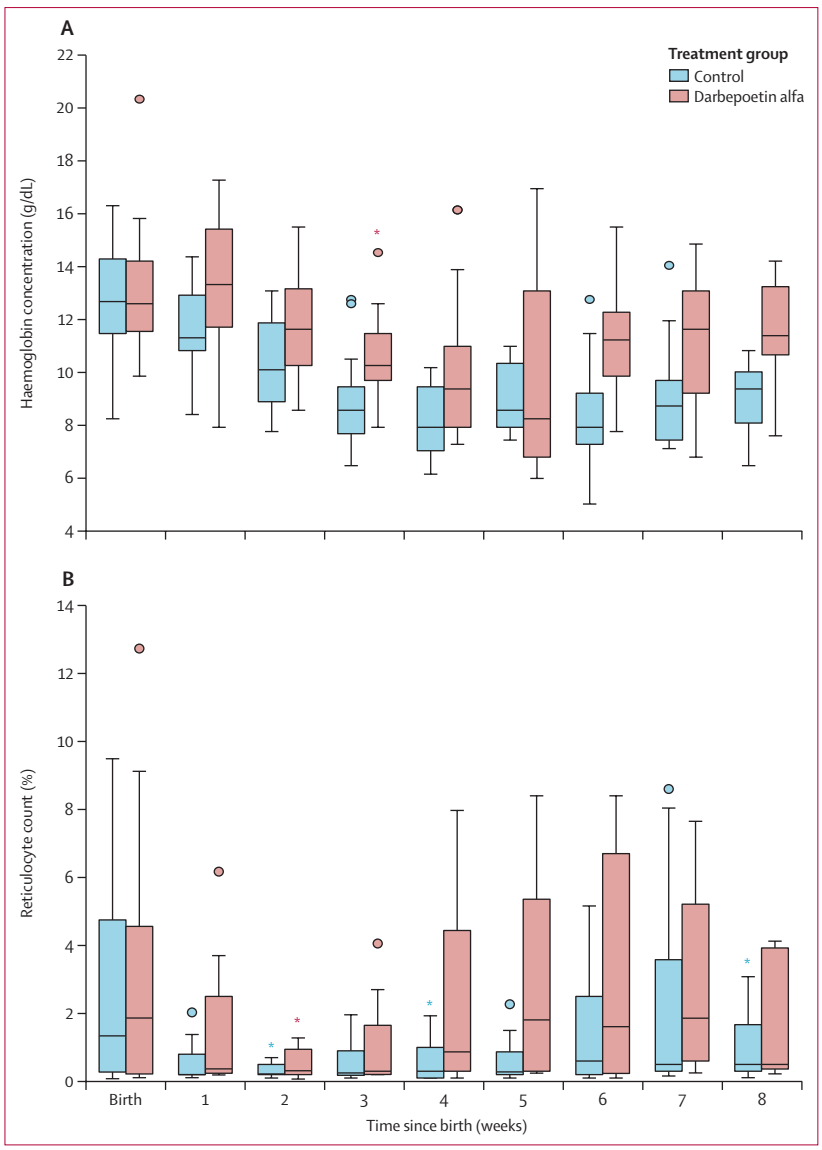

Figure 3: **Boxplots of haemoglobin and reticulocyte course after birth**
(A) Haemoglobin course after birth. (B) Reticulocyte course after birth. Minimum and maximum values are represented by the bottom and top of the vertical line. The boxes represent the IQR and the horizontal lines represent the median values. Circles represent mild outliers, with values more than 1·5 times the IQR below Q1 or above Q3, asterisks are extreme outliers, with values more than 3 times the IQR below Q1 or above Q3.

Discussion

Darbepoetin alfa treatment during the first 8 weeks after birth in infants with haemolytic disease of the fetus and newborn who were prenatally treated with intrauterine transfusion resulted in significant reduction in erythrocyte transfusion episodes in this randomised controlled trial. Correspondingly, darbepoetin alfa prevented one hospital admission per infant. All secondary outcomes were not significantly different between the treatment groups. The study was not powered for these outcomes, which might explain why secondary outcome comparisons did not show significance. Importantly, the number of transfusions and transfusion dependency in the control group were in line with previous findings of our group.[6,9] Although K-mediated alloimmunisation is associated with fewer postnatal transfusions[23] and the treatment group consisted of more K-mediated immunisations, the effect persisted when corrected for type of alloimmunisation and number of intrauterine transfusions.

Earlier studies suggested that, despite a reduction of erythrocyte transfusion episodes with darbepoetin alfa treatment in preterm infants, the eventual clinical importance might be modest.[17,18] However, in this population of infants affected by severe haemolytic disease of the fetus and newborn, the postnatal burden of transfusion is huge. It encompasses demanding monitoring after birth, with frequent hospital readmissions for one or more transfusion episodes, in these otherwise healthy infants. Neonatal transfusion dependency has been reported to affect long-term neurodevelopment, with worse neurodevelopmental outcomes in preterm infants with a higher number of transfusions.[24] A cost-effectiveness analysis is needed to weigh the positive effects properly against the effort and cost of treatment.

Two infants appeared not to respond to darbepoetin alfa treatment. They had three and four transfusion episodes, despite darbepoetin alfa treatment. Clinical characteristics of these infants did not help to find an underlying explanatory common factor, such as erythropoietin concentration at birth. Neither infant had known predictors of high transfusion dependency: both infants had D-mediated alloimmunisation and neither infant had treatment with exchange transfusion.[6] High antibody concentrations and specific type of IgG antibody in the infants could possibly be an explanatory factor in the apparent unresponsiveness to darbepoetin alfa treatment. Known factors for darbepoetin alfa unresponsiveness in other patient groups, such as in people with chronic kidney disease, do not occur in this population.[25] The small group size does not allow for further speculations as to whether these infants were really unresponsive to darbepoetin alfa treatment or whether a higher dose or earlier start of the treatment would have led to different results.

The chosen dosage of 10 U/kg in this study is in agreement with previous studies.[16,26] A lower dose is used in patients with chronic kidney disease who are anaemic, supporting the effectiveness of this dose to enhance erythropoiesis. When used for its neuroprotective potential, higher doses are commonly used to reach adequate concentration of darbepoetin alfa to pass the blood–brain barrier and are reported to be safe.[27] Although a minority might show insufficient response to darbepoetin alfa, treatment at this dose appears to be effective and safe, without short-term harmful side-effects. The results of the long-term follow-up of neurological development of the study population will be reported elsewhere.

We expected to observe an association with endogenous erythropoietin concentrations measured immediately

after birth and response to darbepoetin alfa treatment, but identified a very broad range of endogenous erythropoietin concentrations, to the extent where it is unclear how useful and accurate these cord blood measures of erythropoietin are. Additionally, the number of infants with missing values for endogenous erythropoietin would hugely reduced any significance of the multivariable analysis; therefore, we decided not to include this variable in the multivariable analysis.

In line with the primary endpoint, the course of haemoglobin concentrations and reticulocyte counts after birth appeared to be favourable in the treatment group; however, differences were not significant. Among healthy term infants, haemoglobin concentrations show a physiological decline from 14·6–22·5 g/dL at birth to 10·0–12·0 g/dL by 8–10 weeks of age.[28] Infants affected by haemolytic disease of the fetus and newborn have lower haemoglobin concentrations at birth compared with healthy infants and show a deeper overall decline before reaching haemoglobin concentrations similar to healthy infants. Infants who received darbepoetin alfa appeared to have a more rapid recovery of haemoglobin concentrations compared with the control group, with an accompanying increase in reticulocyte count, but these changes were not significant.

This is the first randomised controlled trial evaluating darbepoetin alfa treatment in infants with haemolytic disease of the fetus and newborn, and its greatest strength is the homogenous treatment of the study population in one centre of expertise. A major limitation was the observation of more protocol violations than anticipated regarding erythrocyte transfusions administered above predefined haemoglobin concentrations, which might reflect bias due to unmasking (ie, infants in the treatment group were transfused more conservatively than infants in the control group due to awareness of treatment allocation). Nonetheless, these violations occurred in both groups, and we therefore deemed it unlikely that these violations grossly altered the comparison between total number of transfusion episodes per infant. Additionally, the sample size of the study was modest, and patients were included from a single centre, possibly affecting the generalisability of the results.

Overall, there was a significant effect of darbepoetin alfa administration on the number of transfusion episodes after intrauterine transfusion treatment for haemolytic disease of the fetus and newborn. Results on long-term follow-up are expected to further clarify whether treatment with darbepoetin alfa or other types of erythropoietin as part of the postnatal treatment of severe haemolytic disease of the fetus and newborn should be considered, but no short-term concerns regarding safety or efficacy were observed.

Contributors

All authors had full access to all the data in the study and had final responsibility for the decision to submit for publication. IMCR and NvG accessed the data and were responsible for data curation, verification, formal analysis, and visualisation. DdW contributed to investigation and critical review of the original draft. IMCR was also responsible for investigation, methodology, project administration, and writing and editing of the original draft. MdH, SEJ, EJTV, DO, and EL were responsible for conceptualisation of the work. MdH, EL, and JGvdB oversaw the supervision of the project and were also involved in critical appraisal of the methodology involved and reviewing. MdH and EL were further responsible for funding acquisition and aided in project administration. All contributors actively reviewed and edited the various drafts of the manuscript.

Declaration of interests

We declare no competing interests.

Data sharing

De-identified participant data can be made available on request to the corresponding author.

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