The effect of delayed cord clamping on haematological status in low birth weight infants: an interim analysis of an ongoing randomised controlled trial in South Africa

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Abstract

Background: Delayed cord clamping (DCC) is a cost-effective and natural solution to prevent anaemia in infancy. The intervention has not been studied in low birth weight infants in resource-poor settings.

Objective: To evaluate the haematological effects of delayed and early cord clamping (ECC) in low birth weight infants

Methods: We report the interim results of a randomised controlled trial. Infants born from mothers with an intrapartum symphysal fundal height (ISFH) ≤ 34 cm were eligible for inclusion. So far we have included 57 infants. Infant blood samples were taken from the umbilical cord immediately after birth, after 24 hours and two months by venepuncture. Primary outcomes of the trial are infant haemoglobin levels and iron status at two months of age. As the follow-up data at two months were still incomplete during this interim-analysis, we focussed on the side effects within the first 24 hours after delivery including hyperbilirubinemia and polycythaemia.

Results: After 4 months of recruitment the inclusion rate was satisfying. We included 11% more than expected. 35% of the included infants actually had a low birth weight (< 2500 g), approximately ⅓ of the total group already returned for the two months follow-up. 7 infants were lost to follow-up so far. In the group that was subjected to ECC haematocrit on second day post-partum was 0.47 ± 0.06 compared to 0.48 ± 0.08 in the DCC group. The proportion of children that required phototherapy on the first day after birth was 12%.

Conclusion: Polycythaemia did not occur. There was no difference in occurrence in hyperbilirubinemia between the two groups. We decided that there were no reasons to end this study prematurely. Recruitment of study participants will continue till we have included 102 infants.
Samenvatting

Achtergrond: Wachten met afnavelen navelstreng (DCC) kan een kosten-effectieve en een natuurlijke oplossing zijn om anemie op kinderleeftijd te voorkomen. DCC is bij baby’s met een laag geboortegewicht in landen met minder capaciteit nog niet bestudeerd.

Objectief: de hematologische effecten DCC vergelijken met het direct afnavelen (ECC) bij baby’s met een laag geboortegewicht.

Methoden: Dit is een interim analyse van een gerandomiseerd controloerd onderzoek. Ouders met een intrapartum symfyse fundus hoogte (ISFH) van ≤ 34 cm, kwamen in aanmerking voor inclusie. Tot dus ver hebben we 57 baby’s geïncludeerd. Bloed werd afgenomen van de navelstreng, na 24 uur en na twee maanden bij de baby’s. Primaire uitkomst was de hematologische status bij de baby’s op de leeftijd van 2 maanden. Omdat nog niet alle gegevens van de follow up data compleet zijn tijdens deze interim analyse concentreren we op de bijwerkingen binnen de eerste 24 uur na de geboorte. We nemen polycythaemie en hyperbilirubinemie daarin mee.

Resultaten: Na 4 maanden was het tempo van rekruteren bevredigend. We hebben 11% meer dan verwacht geïncludeerd. 35% van de baby’s had een geboortegewicht van < 2500 gram. Ongeveer ⅓ van de baby’s zijn teruggekomen voor follow up. Tot nu toe zijn 7 baby’s niet voor follow up terug gekomen. De ECC groep was het hematocriet op de tweede dag post partum 0.47 ± 0.06 in vergelijking tot 0.48 ± 0.08 in de DCC groep. Het deel van de baby’s dat fototherapie nodig had op de eerste dag na de geboorte was 12%.

Conclusie: Polycythaemie kwam niet voor. Er was geen verschil in hyperbilirubinemie tussen de groepen. Er zijn geen redenen om dit onderzoek voortijdig te beëindigen. We zullen het onderzoek voortzetten totdat we 102 kinderen hebben geïncludeerd.
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Introduction

Anaemia: a major problem

Anaemia is a major public health problem. Both strong and weak economies are faced with this invisible health problem. The World Health Organization (WHO) estimates that one in four people are anaemic, corresponding with 1.62 billion people in the world. Approximately 24% of the South-African pre-school children are anaemic, and 40% of them have iron deficiency anaemia. In KwaZulu Natal, the setting of this study, over 50% have iron deficiency anaemia. Preschool children, women of child bearing age and pregnant women are most affected by iron deficiency anaemia, as they have high daily iron requirements.

Iron and the fetus

During pregnancy, maternal iron is actively transported across the placenta to the fetus. Transportation of iron takes place mostly in the third trimester of pregnancy. 70% of total body iron is found in haemoglobin, 25% is bound to ferritin and 5% in myoglobin and enzymes. Because of the low oxygen level in utero fetal haemoglobin levels are higher than at any later stage in life. The total iron capacity of a healthy infant is estimated to be 75 mg/kg at birth. After birth fetal hemoglobin (with a high affinity to oxygen) is no longer needed. Erythropoietin synthesis and red cell production decrease, the intravascular volume expands and haemolysis occurs. This causes the haemoglobin levels to drop from 17 g/dL at birth to 12 g/dL at the end of the first week of life.

Healthy full-term infants, born from healthy mothers, are not likely to develop anaemia in the first six months of life. This is due to sufficient iron stores at birth and high bioviability of iron in breast milk. After 6 months iron stores may become depleted unless the infant is fed with iron-fortified weaning food.

Prematurely born infants have a 25 to 85% increased risk of developing anaemia, caused by a rapid catch up growth in the first few weeks of life that exhausts their already limited iron stores. Maternal conditions including placenta insufficiency, iron deficiency, HIV infection, and malnutrition also contribute to the high risk of iron deficiency anaemia in infancy.

Prematurely born infants have their haemoglobin nadir at 6 to 8 weeks. The more premature, the earlier and deeper their nadir.

Consequences of iron deficiency

Iron deficiency in early childhood is associated with poor psychomotor development and cognitive function impairments. Children with iron deficiency anaemia in childhood score significantly lower on IQ tests than iron-sufficient children. These differences can still be found after replenish their iron stores. Iron-deficient children also seem to have more behavioural problems.

In preterm infants iron deficiency can lead to abnormal neurological reflexes at 37 weeks post menstrual age compared to infants born at 37 weeks postmenstrual age. In full term infants with iron deficiency, cognitive problems seem to be predominant, while in preterm infants motor neurological problems seem to be predominant.

Solutions to iron deficiency anaemia in infancy.

Interventions that prevent iron deficiency anaemia include oral iron supplementation in liquid form (as long as the child is breastfed) and iron-fortified infant formula. It is
recommended to supplement low birth weight infants from the age of two months with a daily dosage of 2 mg/kg iron. Adverse effects like oxidative stress and an increased risk of infection are not likely to occur in iron-deplete infants. When the child is given solid food, advice can be given about preparation of food and which food contains most iron. Children can also be given foods fortified with iron. Fortification of food is an effective approach to treat iron deficiency anaemia. This is however an intervention which takes a lot of cooperation from governments, consumers and the food industry. It is also a solution for older infants, premature infants who often need iron supplementation earlier, can’t use this.

In premature infants red blood cell transfusions are often used to improve haemoglobin status. Transfusions are given when the haemoglobin level is below the ill-defined minimum. Transfusion is not without risk, infection is still a concern. An alternative used for red cell transfusions is the simple limitation of blood sampling. In the 80’s the use of recombinant human erythropoietin (EPO) was studied. According to a Cochrane review from 2010 EPO did not significantly decrease the use of one or more red blood cell transfusions or the number of transfusions per infant. They did however, find a significant increase risk of retinopathy of prematurity.

Delayed cord clamping
A solution for anaemia that in fact is very old but wasn’t practised anymore is the delaying of cord clamping (DCC). A few centuries ago, delayed cord clamping was standard practise. Erasmus Darwin (the grandfather of Charles Darwin) even said in his work Zoonomia: ‘Another thing very injurious to the child, is the tying and cutting of the navel-string too soon; which should always be left till the child has not only repeatedly breathed, but till all pulsation in the cord ceases. As otherwise the child is weaker than it ought to be; a part of the blood being left in the placenta, which ought to have been in the child’. Several decades ago giving birth moved from the homes to the hospital. At this time the active management of cord clamping started. With active management the cord was clamped earlier. It was thought to prevent post-partum haemorrhage. Post-partum haemorrhage is the most fatal complication of pregnancy and childbirth in the world. Standard practise of delaying of the cord clamping was discarded without being thoroughly studied. Now, with new studies about this subject, physicians are reconsidering delaying cord clamping again. The best timing of cord clamping has long been debated, and until recently, research has been ambiguous. Early cord clamping is defined as clamping the cord between 10 seconds and 60 seconds after birth. Delayed cord clamping is clamping of the cord between 2 minutes and cessation of cord pulsations. Late cord clamping is mainly seen in traditional home deliveries, where the cord is cut after placental descent into the vagina. Blood flow from the placenta to the infant continues through the umbilical vein up to 3 minutes after delivery. About 25% of the transfer will occur in the first 15-30 seconds and the other 50-78% will occur after 1 minute. The flow will cease after approximately 3 minutes. With delaying cord clamping with 2 to 3 minutes in a full term infant, the infant receives 23-35 mL blood/kg bodyweight from the placenta. This means that the infant will have an additional 30% more blood volume and 60% more red blood cells. An infant with a weight of 3 kg would receive 46-60 mg iron in the form of haemoglobin from the placenta. Mentioned disadvantages are the increase of hyperbilirubinaemia. Also the higher incidence of post-partum haemorrhage has been mentioned. However, the level of evidence for these disadvantages is low.
Other studies on delayed cord clamping

A Cochrane review from 2008 concluded that delayed cord clamping should be taken into consideration. It does warn that phototherapy must be available since the risk of jaundice with delayed cord clamping is increased. The relative risk of jaundice requiring phototherapy was 0.59 with a 95% confidence interval of 0.38 to 0.92 according to the five studies evaluated in the Cochrane review. The review recommended to further investigate the long term effects of delayed cord clamping on iron status on children and to include PPH in study results. A recent study in Sweden showed that infants in the delayed cord clamping group had significantly higher ferritin levels at the age of 4 months than the infants in the early cord clamping group. The study had 400 infants participating. This study was conducted with normal birth weight infants in a country with good health care access. A study in Mexico showed that infants who had delayed cord clamping at birth had significantly higher ferritin, mean corpuscular volume, transferrin receptor to ferritin ratio, estimated total body iron and storage iron at the age of 6 months.

Few studies focussed on the group of low birth weight infants. Low birth weight infants possibly have more advantages of delayed cord clamping than infants born full-term as described before. Linderkamp et al found that preterm infants had less respiratory distress with delaying of cord clamping. Disadvantages have also been described. Earlier we already mentioned the risk of jaundice. For the vulnerable group of preterm infants other disadvantages mentioned are delay in resuscitation, polycythaemia and possible...
intraventricular haemorrhage\textsuperscript{36}. However, there is no conclusive evidence that these disadvantages occur more in delayed cord clamping groups than in early cord clamping groups. Mercer et al found that delayed cord clamping reduced the risk of male onset sepsis\textsuperscript{37}. The study of A. Kugelman et al included 65 low birth weight infants born before 35 weeks\textsuperscript{38} and did not show any adverse effects. The infants had a higher haematocrit a higher blood pressure, less mechanical ventilation and less need for surfactant. This study had no long term follow up.

The study of Uittee et al. was one of the few who included low birth weight infants and did a follow up at the age of 10 weeks. This study found the haemoglobin levels of the late cord clamping group to be consistently higher compared to the control group at both 1 hour of birth and 10 weeks after birth. \textsuperscript{39} A recent Cochrane study concluded that delayed cord clamping seemed to be associated with less need for transfusion, better circulatory stability, less intraventricular haemorrhage and lower risk for necrotising enterocolitis in preterm infants. But more studies are necessary for reliable conclusions.

\textit{Rational of this study}

The studies done on delayed cord clamping for low birth weight infants are performed in developed countries where all the resources for treatment are widely available. There are few studies which study the potential benefits of delayed cord clamping in developing countries on low birth weight infants. Because of the limited resources, delayed cord clamping could be of great-relevance. Follow up studies are also rare. Delayed cord clamping could especially be an important intervention in developing countries. Developing countries often cope with low birth weight infants and poor resources for their treatment. Delayed cord clamping could be a safe and cost-effective intervention to treat anaemia and to prevent possible transfusions. A follow up is needed to study the effect of delayed cord clamping on iron status two months after birth. Favourably the follow up should be a few years after birth, neurological outcome, behaviour problems and intelligence could be evaluated.

To our knowledge this is the first study done on low birth weight infants, in a rural setting and with a follow up of two months after delivery. This is an interim analysis of an ongoing study. This report will focus on the early adverse effects and safety of this study. The study will focus on the effect of delayed cord clamping on haematological status of low birth weight infants two months after delivery in a rural setting in KwaZulu Natal, South Africa.

\textbf{Material and methods}

\textit{Participants}

The setting of this study is the maternity ward of Stanger hospital in KwaZulu Natal, a regional hospital in the Ilembe district in the rural area of KwaZulu Natal, South Africa. Stanger hospital has 500 beds and serves an estimated population of 600.000 people. According to the monthly statistics the maternity ward has about 550 deliveries every month. All women between 18 and 40 years who were admitted to maternity ward in Stanger Hospital in an early stage of labour and who were suspected of carrying a low birth weight infant were eligible for inclusion. As birth weight can only be measured after delivery we used the intrapartum symphysial fundal height (ISFH) as a proxy. Women with an ISFH ≤ 34 cm or a gestational age of ≤ 36 weeks were eligible for inclusion. Exclusion criteria included 1. Twin pregnancy, 2. History of post-partum haemorrhage (PPH), 3. (gestational) diabetes, 4. Pre-eclampsia, 5. abruptio placentae, 6. Caesarean section, 7. Necessity of early clamping due to
tight nuchal cord, 8. Neonatal resuscitation directly postpartum, 9. Major congenital abnormalities, 10. Infant ≥ 3000g. The criteria 1-4 were assessed before delivery and before randomisation, the criteria 5-10 could only be assessed after delivery and were therefore assessed after randomisation.

On admission women eligible for inclusion were asked to participate in the study. The aim of the study was explained and the woman received additional information on paper in either Zulu or English. When the mother agreed to participate in the study she was asked to sign the informed consent form, complete a questionnaire to gather socio-economic and obstetrical and medical details. The nurses in the antenatal ward were notified when mothers agreed to participate.

**Intervention**

When the cervix is 4 cm dilated the woman is transferred to the labour ward. At this point the researcher is notified by the midwives. When it is clear that the woman is progressing in labour and that she will not have a caesarean section, the researcher opens an opaque envelope to allocate the patient to either the delayed cord clamping group or the control group of early cord clamping. In the delayed clamping group the cord will be clamped after 2-3 minutes. In the early clamping group the cord will be clamped within one minute. One of the researchers monitors the delivery. The midwife conducting the delivery received information about the group assignment. The researcher present measured the clamping time with a stopwatch. After vaginal delivery the infant is placed on the mother’s abdomen, where it is dried off and breathing of the infant is monitored. In the case of a tight nuchal cord or need for resuscitation, no intervention was administered. The mother then receives an intramuscular injection with oxytocin.

After clamping the cord, the researcher takes a venous blood sample from the umbilical cord. The infant is then placed on the mother’s chest for bonding. The attending midwife estimated the maternal blood loss visually.

The infant is checked by either the researcher or a midwife. Weight, length and head circumference are measured and vitamin K and eye drops are given to the infant. The scale used to measure weight had an accuracy of 100 grams.

Healthy infants with a weight above 1800 grams were transferred to the postnatal ward. Infants below 1800 grams or with other health problems were transferred to the nursery. During the first 24 hours after delivery, the infant was observed for signs of hyperbilirubinaemia or hyperviscosity by the nurses of the postnatal ward and the investigator present at the delivery. After 24 hours hyperbilirubinaemia and hyperviscosity were evaluated by venapuncture or finger prick. Hyperbilirubinaemia was visually assessed and with a transcutaneous bilirubin measuring device, the Dräger JM103. The investigators used a Ballard score to assess the gestational age of the infant. A venous blood sample from the baby was collected to measure Hb (hemoglobin) level, Ht (hematocrit) level, MCV (mean cell volume) and TSB (total serum bilirubin). The blood samples were collected in EDTA micro containers and directly transported to the laboratory. If the child was HIV exposed, a PCR test for HIV was done to assess for intrauterine transmission. In order to prevent post-natal HIV transmission Nevirapine was given to the child. The child is given Nevirapine for the period in which the mother is breastfeeding. If the mother is not breastfeeding nevarapine was given for at least 6 weeks. MCV, Ht, Hb, PCR and bilirubin were tested in the laboratory of Stanger hospital. C-reactive protein (CRP), Ferritin, transferrin and serum iron were analysed at Inkosi Albert Luthuli Central Hospital in Durban.
Infants were discharged when their weight was over 1800 grams and when there were no signs of hyperbilirubinemia and hyperviscosity. Before discharge an outpatient folder for the infant was made and a date was set for follow up.

Follow up
After two months the children were seen back by an investigator in Stanger hospital at the outpatient department of paediatrics. The children’s weight, length and head circumference were measured and a general physical examination was done. A questionnaire focussing on maternal and infant health was completed, including information on emergency clinic visits and feeding of the child.

Blood samples were taken from the infant by the investigator. Either venous or arterial blood was taken. The blood was checked for Hb, Ht, MCV, ferritin, transferrin, transferrin saturation, serum iron and CRP. If the child had an Hb below 9.5 g/dL, iron supplements were given. Other health issues like contact with tuberculosis were also monitored and, when necessary, prophylaxis was given. After this visit the patient was discharged from the research.

Objectives
In this study we want to evaluate the haematological effects of delayed cord clamping compared to early cord clamping on low birth weight infants in a setting with poor resources. Our main focus is the haemoglobin of the infant at two months. We will test the hypothesis that infants in the delayed cord clamping group to have higher haemoglobin on two months of age compared to the early cord clamping group. The follow up data was incomplete during this interim analysis. We focussed on the side effects within the first 24 hours after delivery including hyperbilirubinemia and polycythaemia.

Definitions
Low birth weight was defined as weight below 2500 grams. We accept an error of 500 grams, there for all children with a birth weight below 3000 grams were included. Maternal anaemia was defined as an Hb concentration of < 11.0 g/dL. Post-partum haemorrhage was defined as blood loss > 500 mL. Fetal anaemia was defined as Hb concentration of < 12.5 g/dL, this is two standard deviations below the mean. Infants at 2 months were considered anaemic when their Hb-levels were below 9.5 g/dL. Iron deficiency anaemia was defined as MCV <77 fl, ferritin < 12µ/g/L (<30 µg/dL in the presence of infection with a CRP > 10 mg/L) and transferrin saturation <16%.

Phototherapy was initiated according to the South African neonatal Academic Hospital guidelines from 2006(Appendix 1).

Outcomes
The primary outcome of this study is the haemoglobin level at two months after birth. During the time of this interim analysis we did not have a sufficient number of patients for the two month follow up. Therefor our focus is on the potential side effects of the intervention within the first 24 hours. Potential side effects are hyperbilirubinemia and polycythaemia measured by haematocrit and total serum bilirubin at 24 hours post-partum of the infant. Secondary outcome measures are hematocrit, MCV, ferritin (linked to serum CRP), Transferrin, transferrin saturation and serum iron at the two month follow up. Also
secondary outcome measures were the haemoglobin, haematocrit, ferritin and transferrin of the umbilical cord and the haemoglobin and mean cell volume 24 hours post-partum of the infant. Apart from that we monitored the infant morbidity, which includes anaemia, HIV transmissions, hyperviscosity syndrome and the need for phototherapy. Other outcomes were the social-economic and the medical obstetrical details of the mothers and the general health of the infants at 2 months old. To enhance the reliability of the measurements, all measurements were done by one of the researchers.

Sample size
In order to detect a clinically significant difference in mean haemoglobin between the delayed cord clamping group and the immediate cord clamping group at two months of 1 g/dL with an SD 1.4 a power analysis was performed with $\alpha = 0.05$ (two sided) and $1-\beta=80\%$. With a maximum drop-out of 40% we will have to include 51 infants in each group, 102 in total. One interim analysis was performed in order to determine the study early if any harmful or convincing beneficial effects would emerge.

Randomization and blinding
The randomisation was performed in a 1:1 ratio for the DCC and the control group using website (www.randomization.com). Block randomisation was used to create an equal allocation for this interim analysis. Closed opaque envelopes were made by only one researcher, who was not involved in the inclusion of infants, to ensure concealment of the allocation. Envelopes were only opened when it was clear that the participant was in labour. The researcher who enrolled the participants was also the person who would open the envelope and assigned participants to their group. A different researcher however generated the allocation sequence and made the envelopes. Extra envelopes were made in case of higher drop-out expectancy. The two month follow up was done by the researcher also present at the delivery. As part of the exclusion criteria could only be applied after randomization, which is after delivery, data were analyzed according to the per-protocol principle. The researchers in this study were not blinded. The laboratory staff that performed the analyses of blood samples was blinded to the infant’s allocation group.

Statistical methods
Patient’s characteristics and baseline data were described using mean and standard deviation in the case of normal distribution; if not normally distributed median and range were used. In order to detect significant differences between the intervention and the control group we used a student – $t$ test for independent samples for normally distributed samples and the Mann-Whitney rank-sum test for parametric samples. For the comparison of the means of the two groups the student- $t$ test for independent samples was used. When the data was not normally distributed the Mann-Whitney rank –sum test for independent samples was used. We used Fisher’s exact test to compare categorical variables between groups. We considered a $p$ value of $< 0.05$ to be significant.

We used SPSS for Windows, version 19 for the analysis.
The Human Sciences Research Council, Pretoria South Africa gave ethical approval for this study.
Results
Figure 2 shows the inclusion rate in the first 4 months of this study. Figure 3 shows the patient flow. Between February 2012 and June 2012 a total of 183 women were assessed for eligibility. Prior to delivery 105 singleton pregnancies were potentially eligible for inclusion.
After delivery 48 (46%) had to be excluded, amongst others for having an actual birthweight over 3000 g (n=30) and for having been born by Caesarean Section (n=9). So far, 8 of 28 infants (29%) in the DCC group returned for follow-up two months after birth, and 10 of 29 infants (34%) in the control group. One infant in the early cord clamping group died during the follow up period, and one mother–infant pair moved out of the study area and was lost to follow-up. Five participants did not show up for their follow up appointment, these participants were all allocated to the delayed cord clamping group.

Table 1 Maternal baseline characteristics

<table>
<thead>
<tr>
<th>Variable</th>
<th>Delayed cord clamping (n=28)</th>
<th>Controls (n=29)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Obstetrical-medical details</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years) median (range)</td>
<td>24 (18-34)</td>
<td>22 (18-39)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>65.6 ± 8.9</td>
<td>66.15 ± 8.1</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>155 ± 4.8</td>
<td>156.3 ± 5.4</td>
</tr>
<tr>
<td>BMI</td>
<td>27.3 ± 3.6</td>
<td>26.6 ± 3.5</td>
</tr>
<tr>
<td>MUAC (cm) median (range)</td>
<td>25 (23-52)</td>
<td>25 (22-34)</td>
</tr>
<tr>
<td>SFH (cm) median (range)</td>
<td>32 (27-34)</td>
<td>32 (27-35)</td>
</tr>
<tr>
<td>Primigravidae</td>
<td>36 %</td>
<td>66 %</td>
</tr>
<tr>
<td>Iron supplementation in pregnancy</td>
<td>68 %</td>
<td>72 %</td>
</tr>
<tr>
<td>Folic acid in pregnancy</td>
<td>64 %</td>
<td>76 %</td>
</tr>
<tr>
<td>HIV infection</td>
<td>39 %</td>
<td>38 %</td>
</tr>
<tr>
<td><strong>Socio-economic details</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grade of education median (range)</td>
<td>11 (0-postgrad)</td>
<td>12 (0-postgrad)</td>
</tr>
<tr>
<td>Literacy</td>
<td>100 %</td>
<td>100 %</td>
</tr>
<tr>
<td>Employment:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unemployed</td>
<td>54 %</td>
<td>66 %</td>
</tr>
<tr>
<td>Employed</td>
<td>32 %</td>
<td>17 %</td>
</tr>
<tr>
<td>Student</td>
<td>14 %</td>
<td>17 %</td>
</tr>
<tr>
<td>Married</td>
<td>11 %</td>
<td>10 %</td>
</tr>
<tr>
<td>Unmarried</td>
<td>89 %</td>
<td>90 %</td>
</tr>
<tr>
<td>Informal housing</td>
<td>61 %</td>
<td>55 %</td>
</tr>
<tr>
<td>Formal housing</td>
<td>39 %</td>
<td>45 %</td>
</tr>
<tr>
<td>Water from the tap</td>
<td>61 %</td>
<td>55 %</td>
</tr>
<tr>
<td><strong>Hematological details</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hb (g/dL)</td>
<td>10.9 ± 1.6</td>
<td>10.8 ± 1.5</td>
</tr>
<tr>
<td>Proportion of maternal anaemia</td>
<td>43 %</td>
<td>45 %</td>
</tr>
</tbody>
</table>

Data are mean ± SD unless indicated otherwise. BMI= body mass index; MUAC = mid upper arm circumference; SFH=symphysis-fundal height; HIV=human immunodeficiency virus; Hb = haemoglobin

Table 1 shows the characteristics of the mothers in the study. There was no significant difference between the groups for age and anthropometry. The control group contained more primigravidae, but this difference was not statistically significant. Table 2 shows that the infant baseline characteristics between the groups were not different, with the exception of the median clamping time.
The median clamping time in the delayed cord clamping group was 135 seconds. Most infants (97%) had their cords clamped between 2 and 3 minutes. In the control group the umbilical cord was mostly clamped (in 97%) within one minute (median 23 seconds). The control group has 51, 7% male infants where the delayed cord clamping group has 36, 3%.

The umbilical cord ferritin in the control group (mean 179, 3 ± 95) and the umbilical cord transferrin (mean 2, 05 ± 0, 46) were not significantly different compared to the delayed cord clamping group.

There was no maternal post-partum hemorrhage noted, the estimated amount of blood loss between the two groups was not significantly different. The number of admissions to the nursery was not statistically different per group. Infants were admitted for having difficulty of breathing or low birth weight.

### Table 2 Baseline characteristics infant

<table>
<thead>
<tr>
<th>Variable</th>
<th>Delayed cord clamping</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clamping time (s) median (range)</td>
<td>135 (115-210)</td>
<td>23 (4-75)</td>
</tr>
<tr>
<td>Gestational age (weeks) median (range)</td>
<td>36.5 (29-40)</td>
<td>36 (27-40)</td>
</tr>
<tr>
<td>Males</td>
<td>39.3 %</td>
<td>51.7%</td>
</tr>
<tr>
<td>Birth weight (grams) median (range)</td>
<td>2645 (1400 – 2980)</td>
<td>2600 (1200-2980)</td>
</tr>
<tr>
<td>Length (cm) median (range)</td>
<td>48 (33-55)</td>
<td>49 (40-56)</td>
</tr>
<tr>
<td>Head circumference (cm) median (range)</td>
<td>33 (30-39)</td>
<td>33 (29-36)</td>
</tr>
<tr>
<td>Apgar after 1 minute median (range)</td>
<td>9 (7-9)</td>
<td>9 (2-9)</td>
</tr>
<tr>
<td>Apgar after 5 minutes median (range)</td>
<td>10 (9-10)</td>
<td>10 (5-10)</td>
</tr>
<tr>
<td>Umbilical cord Hb (g/dL)¹</td>
<td>17.78 ± 2.90</td>
<td>17.02 ± 2.38</td>
</tr>
<tr>
<td>Umbilical cord Ht (%)²</td>
<td>0.48 ± 0.08</td>
<td>0.43 ± 0.06</td>
</tr>
<tr>
<td>Umbilical cord Ferritin (µg/L)³</td>
<td>160.67 ± 84.78</td>
<td>179.3 ± 95</td>
</tr>
<tr>
<td>Umbilical cord transferrin (mg/dL)⁴</td>
<td>1.86 ± 0.38</td>
<td>2.05 ± 0.46</td>
</tr>
<tr>
<td>Admitted to nursery</td>
<td>3 (11%)</td>
<td>6 (21%)</td>
</tr>
</tbody>
</table>

Data are mean ± SD unless stated otherwise. Hb= haemoglobin; Ht= hematocrit; CI= confidence interval; MCV= mean cell volume; PCR= poly chain reaction * Using Fisher’s exact test.

### Table 3 Infant hematological status on the second day post-partum

<table>
<thead>
<tr>
<th>Variable</th>
<th>Delayed cord clamping</th>
<th>Control</th>
<th>P value</th>
<th>Difference 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb (g/dL.)</td>
<td>17.78 ± 2.90</td>
<td>17.02 ± 2.38</td>
<td>0.30</td>
<td>- 2.21 – 0.69</td>
</tr>
<tr>
<td>Hb compared to cord blood</td>
<td>2.85 ± 2.68</td>
<td>1.89 ± 1.86</td>
<td>0.15</td>
<td>- 2.28 – -0.36</td>
</tr>
<tr>
<td>Ht (%)</td>
<td>0.48 ± 0.08</td>
<td>0.47 ± 0.06</td>
<td>0.50</td>
<td>- 0.05 – 0.03</td>
</tr>
<tr>
<td>Ht compared to cord blood</td>
<td>0.06 ± 0.06</td>
<td>0.04 ±0.06</td>
<td>0.16</td>
<td>-0.06 – 0.01</td>
</tr>
<tr>
<td>Serum bilirubin</td>
<td>107 ± 46.65</td>
<td>99.75 ± 58.56</td>
<td>0.61</td>
<td>-35.9 – 21.5</td>
</tr>
<tr>
<td>MCV (fl/L)</td>
<td>99.6 (88-114)</td>
<td>98.1 (86-125)</td>
<td>0.36</td>
<td>-4.89 – 2.68</td>
</tr>
<tr>
<td>Proportion phototherapy*</td>
<td>7.14%</td>
<td>17.24 %</td>
<td>0.4</td>
<td>-</td>
</tr>
<tr>
<td>Proportion polycythaemia</td>
<td>0 %</td>
<td>0%</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Positive PCR for HIV</td>
<td>0 %</td>
<td>0%</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Data are mean ± SD unless stated otherwise. CI= confidence interval; Hb= haemoglobin; Ht= hematocrit; MCV= mean cell volume; PCR= poly chain reaction * Using Fisher’s exact test.
Table 3 shows the hematological status of the infants on the second day after delivery. The mean values of Hb, Hb change from baseline and Ht were not statistically different between the groups, although there may be a trend to higher values in the delayed clamping group. The number of children receiving phototherapy in the control group was higher than in the delayed cord clamping group, the differences were not significantly different. No HIV exposed infants had a positive PCR on the second day post-partum. All HIV-exposed children were treated with nevirapine monotherapy to prevent mother-to-child transmission.

**Discussion**

**Key results**

We report the early effects of delayed clamping in 57 low birth weight infants in KwaZulu-Natal, South Africa. This study should be considered as an interim analysis of an on-going project. For this analysis we focussed on the potential adverse effect of delayed cord clamping. We hope to have included a total of 102 mother-infant pairs at the end of this study. We hypothesized that delaying cord clamping for at least two minutes would improve the infant haematological status at the age of two months in infants with a low birth weight. Although patient recruitment progressed well till the point of the interim analysis, we were not able to test our primary hypothesis. We therefore focussed on the early side effects of the intervention. Up to now delayed cord clamping has not been associated with an increased risk on post-partum haemorrhage, HIV transmission or mortality. Although there is a trend to higher Ht and bilirubin levels in de intervention group, hyperviscosity and need for phototherapy were not observed.

**Minor mothers**

In April 2012 we were confronted with an unexpected drawback. Until that moment we had included all mothers with an ISFH ≤ 34 cm. A significant proportion of this group (4 out of 22 mother-infant pairs) turned out not to have reached their 18th year of life. The institutional review board of the University of Pretoria strongly advised against inclusion of these minors into the study. We had to exclude all minor mother-infant pairs that were already participating subsequently.

The prevalence of teenage pregnancies in KwaZulu-Natal is higher than the nation-wide figure. In 2011 almost 33000 registered hospital deliveries in South-Africa were by girls aged between 15 and 19 years. Local hospital data show 10% of new-borns in Stanger hospital were delivered by mothers younger than 18 yrs.

**Seasonal influences**

The decline in recruitment in April may also be explained by lack of human resources and the lack of mothers who fitted all the inclusion criteria. If we look at the overall birth rate per month over the years 2007 to 2011 you also see that there is a slight decline in birth rate in the months April and May.
Maternal baseline characteristics
As we compare the two groups, we don't see differences between the groups of mothers. The groups also look healthy. Their BMI is in between the normal ranges (20-25). The average height in both groups is around 155 cm. The average height of women in South Africa is about 159 cm. This difference in average of height could be explained with stunted growth of the mothers. A stunted growth could be caused by malnutrition at an early age. Currently the percentage of malnutrition in children in KwaZulu-Natal now is estimated 19,8% in preschool children. We also see that the percentage of primigravida is high in the control group. Primigravida have been associated with a higher risk of lower birth weight than multiparas. Another explanation for this is that primigravida would fulfil the inclusion criteria sooner than multigravids.

Infant baseline characteristics
The median weight of the infants is 2645 gram in the delayed cord clamping group and 2600 gram in the delayed cord clamping group. This is above our initial definition of low birth weight of 2500 gram or less. Because birth weight could only be determined after randomisation, we used a margin of error of 20%. This means that we include children with a birth weight of less than 3000 grams. Infants who were born with a weight under 2500 grams usually were born after pregnancies where the mother had pre-eclampsia. Once a woman was diagnosed with pre-eclampsia, she couldn't be included in the study anymore. The difference in birth weight between the two groups could also be explained by transfusion of the placenta. Infants in the DCC group received more blood from the placenta than the infants in the delayed cord clamping group. Another possible reason was that infants born prematurely were also often born at home. This means that we had to rely on a fairly healthy group of infants.
Maternal baseline characteristics
The unemployment rate in South Africa and especially KwaZulu-Natal is 22.3%\textsuperscript{45} We found higher unemployment rates in the study population. The people who work have more money to spend. They will sooner go to a private hospital. Just under half of all mothers have anaemia. Anaemia is especially a major problem in KwaZulu-Natal with a rate of 57% of pregnant women being anaemic\textsuperscript{3}. This anaemia occurs despite iron supplementation. The mean of haemoglobin level of the women in the study is 10.9 g/dL in the DCC group and 10.8 g/dL in the ECC group. These Hb values are below the threshold of anaemia. This means that the iron supplements aren't sufficient enough. The study of M. Hogue et al also suggests that anaemia in pregnancy can be caused by other causes like HIV-infection and schistosomiasis.\textsuperscript{46} The rate of HIV infection in our study was 39% in the DCC group and 38% in the ECC group. HIV infection could be a good explanation for the high incidence of maternal anaemia in our study groups.

Results infants second day post partum
The haemoglobin on the second day post-partum is higher than the haemoglobin in the umbilical cord blood. This increase is probably due to the fact that the system of the infant is adapting to the outside world. Being outside the uterus the circulation of the infant changes, going from the fetal circulation to an adult circulation. Hemoconcentration occurs due to fluid shift from circulation to tissues and the limited fluid intake in the first 24-48 hours because of lack of breast milk production by the mother. As we look at the possible side effects we see that the delayed cord clamping group in our study has a higher median of bilirubin level than the control group. However, the delayed cord group had a lower percentage of infants who needed phototherapy than the control group. A reason for this could be that there are more infants in the control group who have a lower birth weight than in the delayed cord clamping group without the difference being significant. Lower birth weight and preterm labour is a risk factor for unconjugated hyperbilirubinaemia. Almeide et al even found that 50 to 80% of the preterm neonates have to be treated-for their high bilirubin\textsuperscript{47}. Tests show a relationship between birth weight and total bilirubin. There is also a significant correlation between birth weight and total bilirubin. Pearson’s correlation has a value of –0.51 with a p-value of 0.001. There are more infants with birth weight lower than 2000 grams in the control group than in the delayed cord clamping group. All the children (6 in total) below 2000 grams in the control group had to have phototherapy. In the delayed cord clamping group there are 4 children with a birth weight below 2000 grams, only two had to have phototherapy. We can’t explain the reason for this difference.

In our study we couldn’t find a significant difference in Hb and Ht between the DCC group and the ECC group at the second day post-partum. There are however, a few studies which do see a difference between the DCC and ECC group in Hb and Ht in the first few days in low birth weight infants. A study from Kugelman et al showed that the haematocrit is significantly higher in infants who had delayed cord clamping although they only included 65 infants.\textsuperscript{40} Their delaying of the cord clamping however had a median of 32.8 seconds and they also included Caesarean sections. A more recent study with larger groups also found a significant difference in hematocrit between DCC and ECC groups. In this study the caesarean sections were also included and the cord clamping time in the DCC group had a mean of 45 seconds\textsuperscript{48}. 

\textsuperscript{17}
Rabe et al did a study in preterm infants with a gestational age < 33 weeks. 34 out of 40 infants were delivered by caesarean section. The delayed cord clamping group had a delay of 45 seconds before clamping the cord. In the control group the cord was clamped before 20 seconds. Rabe et al found that the delayed cord clamping group had significant fewer blood transfusions than the control group. There was no difference between adverse effects between the two groups.

In a study of Oh et al infants born after a pregnancy of 24 weeks till <28 weeks were included. They included 33 infants in their study. In the control group the cord was clamped <10 seconds and in the delayed cord clamping group the cord was clamped between 30 and 45 seconds. They included normal deliveries as well as caesarean deliveries. However, their results do not reflect the number of caesarean deliveries and normal deliveries. The primary outcome was haematocrit at 2, 4 and 6 weeks of life. At the age of 4 hours, they found a significant difference in haematocrit favouring the delayed cord clamping group. In this study there were also no differences in adverse effects between the two groups. What these studies all have in common is that they all also included caesarean sections, that their groups are generally small and that their clamping time does not reach 2 minutes. Our study has tried to improve these studies by recruiting more patients, having only vaginal deliveries and to delay the cord clamping with 2 -3 minutes.

The study of Ultree et al resembles our study. Ultree et al did a study on low birth weight infants. This study only included children being vaginally delivered and the delaying time was at least 180 seconds versus < 30 seconds for early clamping. The difference in Hb and Ht in the first hour between the two groups is significant. The two groups are smaller than the groups used in this study with 19 infants in the early clamping group and 19 in the late clamping group. Big differences with our study are that these studies did not include maternal characteristics and these studies are all performed in developed countries.

One of the main arguments not to delay cord clamping is because of potential side effects like hyperbilirubinaemia, polycythaemia in infants and post-partum haemorrhage in mothers. For this study, potential HIV transmission is also a possible effect. A recent Cochrane review showed that infants who were in a DCC group had a higher peak bilirubin concentration. However, there was no difference in occurrence of phototherapy between the two groups. Unfortunately polycythaemia was not reported in this review. None of the articles mentioned in this discussion reported polycythaemia.

**HIV Transmission**

No current study on cord clamping mentions HIV transmission. In our study HIV transmission didn’t occur. This was due to PMTCT. This regime was initiated at 14 weeks of pregnancy with HIV infected mothers. At 14 weeks they are given Zidovudine twice a day. At the onset of labour they are given one dose of Nevirapine. During delivery and a week postpartum they are given a combination of Zidovudine and Lamivudine. Nevarapine prophylaxis is given to the child for a period of 6 weeks after birth if the infant is not breastfed. If the infant is breastfeeding, prophylaxis should be given till one week after the end of the period of breastfeeding.

**Strengths**

This study is the first study comparing delayed and early cord clamping in infants with low birth weight in a poor resource setting. This study is also the first that follows up these infants after 2 months. With this study two of the most fragile groups are combined: infants
with a low birth weight and infants who are born in an area with poor resources. Strength of this study was that the same researcher was present at every delivery, so the same stopwatch was used and the same method of timing. The researcher was also available in the evenings, to maximize the inclusion rate.

Limitations
A limitation of the study was that the researcher didn’t speak the local language; Zulu. Not every participant spoke English. This meant that the researcher had to ask an interpreter to help with translating. Because of this interpreter, we couldn’t quite control what was being said to these women. Sometimes it turned out that the women didn’t understand what was going on. To prevent this from happening, we also offered the women a pamphlet with information about the study in Zulu and we also always made sure that the women could call us with questions when they wanted. The women could also withdraw their child from the study at any given moment.

A month into the study we heard that under aged women couldn’t participate in the study. This was a loss because most under aged women were perfect candidates for the study; the reason for this is mentioned earlier in this discussion.

We also had some miscommunication with our laboratory. Sometimes blood went missing so they couldn’t perform test on them anymore. Other times the form that ordered the test was read wrongly which gave us the wrong results. Because of these incidents we now have quite a few missing results. To try and minimize the missing results the researcher would immediately take the blood to the laboratory. If there were extra tests to be ordered the researcher made sure that the lab-assistant knew about these tests.

About 40% of the pregnant women in KwaZulu-Natal are HIV infected. Because of this high percentage we also see a lot of women with severe conditions like extensive genital warts and vaginal abscesses. Sometimes these conditions are the reason why a woman can’t deliver vaginally. This was also one of the reasons why inclusion didn’t always go as planned.

The caesarean section rate of Stanger hospital is high: 30% of all deliveries are from a caesarean section. A lot of those caesarean sections were emergency sections for poor progression of delivery. Often women agreed to participate, but still had to be excluded because of an emergency caesarean section.

Post-partum haemorrhage was not reported in any of the previous mentioned articles. We did report post-partum haemorrhage, but we used a midwife to visually asses the amount of blood loss. This is not an accurate measurement. A method to improve this limitation could be to weigh the amount of blood loss caught. Most of the blood is caught on towels and in trays underneath the bed. The midwife or researcher could weigh the amount of blood after delivery and make a better judgement of blood loss.

Because the researchers lived next to the hospital, they could also include in the evening and nights. But they needed the help of the midwives on the labour ward for this. The researchers made up a calling regime so that they could be called day and night. The midwives however didn’t use this regime and the researchers were rarely called. When asked for the reasons to not be called the midwives usually said it was too busy or that the woman already came in fully dilated and that there was no time to call. To promote calling we asked the midwives every night and every morning to call and to improve recruitment we were there during the day and sometimes during the evening as well. Later in the study we also did night shifts.
Post-partum haemorrhage was not reported in any of the previous mentioned articles. We did report post-partum haemorrhage, but we used a midwife to visually assess the amount of blood loss. This is not an accurate measurement. A method to improve this limitation could be to weigh the amount of blood loss caught. Most of the blood is caught on towels and in trays underneath the bed. The midwife or researcher could weigh the amount of blood after delivery and make a better judgement of blood loss.

After two months the mothers had to come back with their children to the outpatient department of paediatrics. A lot of mothers didn’t come at the date they were asked to come back. If the mothers don’t come at the set date, they are called the day after to ask why they didn’t come. Most women forgot, some didn’t have the money and some women couldn’t come. A new appointment was made on the date that the mothers could come. If it was impossible to come to the hospital arrangements were made to be seen at a local clinic nearby. We stopped calling the mothers after 3 months after delivery of the infant. Because of the delay in women coming back, this interim analysis has a small sample size of the follow up. We didn’t include the haematological results of the infants at the two month follow up in this study because the numbers were still too small to conclude anything from them. The rate of the infants seen at follow up is going as expected.

This interim analysis is an analysis of an on-going study. The sample size isn’t big enough to give solid conclusions about the results. Even though the results aren’t statistically significant yet, we do see difference between the groups when it comes to haematological status favouring the delayed cord clamping group. If our hypothesis proves to be correct, it could have a big impact on the obstetrical method of delivery in South Africa. It could also have a high impact on the outcome of premature infants in rural areas, increasing their chances of survival and better their overall outcome. Delayed cord clamping is a cost effective method which seems to be improving haematological status of low birth weight infants. However, even if these results would plead for standard delaying cord clamping instead of the standard of early cord clamping more research is needed to back up ours and to look for more benefits and disadvantages.

**Conclusion**

This interim analysis doesn’t show any significant difference in occurrence of hyperbilirubinaemia between the two groups. Polycythaemia did not occur. All of the PCR’s for HIV of the infants were negative. Post-partum haemorrhage did not occur. Negative effects of the intervention have not been observed. We decided that there are no reasons to end this study prematurely. The recruitment of study participants will continue when we have included 102 infants.
References


Aher SM, Ohlsson A. Early versus late erythropoietin for preventing red blood cell transfusion in preterm and/or low birth weight infants. Cochrane Database Syst. Rev. 2006;19:CD004865.


Chaparro CM. Timing of umbilical cord clamping± effect on iron endowment of the newborn and later iron status. Nutr Rev. 2011;69:30-6


Appendix

PHOTOTHERAPY

In presence of risk factors use one line lower (the gestation below) until <1000g.
If gestational age is accurate, rather use gestational age (weeks) instead of body weight.

Infants > 12 hours old with TSB level below threshold, repeat TSB level as follows:
1-20 μmol/l, below line repeat TSB in 6hrs or start phototherapy and repeat TSB in 12-24hrs.
21-30 μmol/l, below line: repeat TSB in 12-24hrs.
>30 μmol/l, below line: repeat TSB until it is falling and/or until jaundice is clinically resolving.

Infants under phototherapy:
Check TSB 12-24 hrs but if TSB >30 μmol/l, above the line, check TSB 4-6hrs.
STOP phototherapy:
If TSB > 30 μmol/l, below the line. Recheck TSB in 12-24hrs.

Start intensive phototherapy when TSB is 2 the line according to gestation or weight.

Appendix 1