The effect of delayed cord clamping on haematological status in low birth weight infants

A randomised controlled trial in Stanger, South Africa

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May 28th – December 6th 2012

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“Another thing very injurious to the child, is the tying and cutting 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 much weaker than it ought to be, a portion of the blood being left in the placenta, which ought to have been in the child.”

Erasmus Darwin, Zoonomia, 1801
ENGLISH ABSTRACT

OBJECTIVES To evaluate whether delayed cord clamping (DCC) is effective in improving the haematological status of low birth weight infants in a middle-income country, and whether this is associated with an increased risk of adverse effects in mothers or infants.

METHODS Women who were expected to deliver a low birth weight infant (as measured by a symphysal-fundal height ≤34 cm) in Stanger Hospital, South-Africa, were randomly assigned to receive early (within 1 minute) or delayed (between 2 and 3 minutes) umbilical cord clamping. Infants were reassessed at 24 hours (n=104) and 2-3 months after birth (n=68).

RESULTS Delayed compared to early cord clamping (ECC) resulted in a higher mean haemoglobin (18.0 g/dL v 16.9 g/dL, p=0.014) and haematocrit (0.460 L/L v 0.490 L/L, p=0.013) 24 hours after birth. Mean bilirubin levels after 24 hours were comparable between groups (DCC 94 μmol/L v ECC 83 μmol/L, p=0.570). Eight (14%) ECC infants and two (4%) DCC infants required phototherapy. Polycythaemia or postpartum haemorrhage did not occur. At the follow-up 2 to 3 months after birth, there were no differences in mean haemoglobin levels (DCC 9.9 g/dL v ECC 9.8 g/dL, p=0.626), iron status, or prevalence of anaemia (DCC 34% v ECC 42%, p=0.452) between groups.

CONCLUSION Despite evidence of successful placental transfusion on the first day after birth, a beneficial effect of DCC on the haematological status of low birth weight infants could not be detected 2 to 3 months later. These results, however, may be biased by a high attrition rate. DCC was not associated with increased adverse events.

DUTCH ABSTRACT

DOELEN Evalueren of de hematologische status van Zuid-Afrikaanse zuigelingen met een laag geboorte gewicht verbeterd kan worden door middel van verlate afklemming van de navelstreng, en of dit gepaard gaat met nadelige effecten voor de moeder of de zuigeling.

METHODEN Vrouwen die naar verwachting zouden bevallen van een kind met een laag geboorte gewicht (op basis van een symfyse-fundus hoogte ≤34 cm) in een overheidsziekenhuis te Stanger, Zuid-Afrika, werden gerandomiseerd in groepen om de navelstreng na de geboorte vroeg (binnen 1 minuut) of laat (tussen 2 en 3 minuten) af te klappen. Follow-up vond plaats na 24 uur (n=104) en na 2-3 maanden (n=68).

RESULTATEN Late vergeleken met vroege afnaveling resulteerde in een hoger gemiddeld hemoglobine (18.0 g/dL v 16.9 g/dL, p=0.014) en hematocriet (0.460 L/L v 0.490 L/L, p=0.013) 24 uur na de geboorte. Het gemiddelde bilirubine gehalte 24 uur na de geboorte was vergelijkbaar tussen beide groepen (late afnaveling 94 μmol/L v vroege afnaveling 83 μmol/L, p=0.570). Fototherapie was nodig voor 8 (14%) zuigelingen in de vroege en 2 (4%) zuigelingen in de late groep. Polycythemie of hemorragie postpartum waren niet opgetreden. Bij de follow-up 2 tot 3 maanden na de geboorte waren er geen verschillen tussen de late en vroege groep in gemiddeld hemoglobine (9.9 g/dL v 9.8 g/dL, p=0.626), de ijzerstatus, of de prevalentie van anemie (34% v 42%, p=0.452).

CONCLUSIE Ondanks dat latere afklemming van de navelstreng leidde tot meer placenta transfusie, had dit na 2 tot 3 maanden geen zichtbaar effect op de hematologische status van kinderen met een laag geboorte gewicht. Bias van de resultaten is mogelijk door een hoge loss to follow-up. Late afnaveling had geen nadelige effecten voor moeder of kind.
# TABLE OF CONTENTS

Citation .................................................................2  
Abstract ..............................................................3  
Table of contents .....................................................4  

## Introduction
- Iron deficiency (anaemia) ..................................5  
- Physiology .......................................................5  
- Current practice ..............................................6  
- RCTs comparing DCC vs ECC ..............................7  
- Problem definition ...........................................8  
- Research question ......................................... 8  

## Material and methods
- Study population ...........................................9  
- Patient enrollment and eligibility ....................9  
- Study procedures ..........................................9  
- Outcomes .......................................................10  
- Sample size calculation ..................................11  
- Statistical analyses .....................................11  
- Ethical approval ...........................................11  

## Results
- Recruitment ...............................................13  
- Participant flow ..........................................13  
- Peripartum results .......................................13  
- Results 1 day postpartum ...............................14  
- Results 2-3 months postpartum .......................15  
- Post-hoc analyses ........................................15  

## Discussion
- Key findings ...............................................18  
- Comparison with other studies .....................18  
- Strengths and limitations ............................19  
- Implications ...............................................20  

## Conclusion ...................................................20  

## References ....................................................21
Introduction

Iron deficiency (anaemia)
Iron is required for normal brain development. Iron deficiency leads to impaired psychomotor and/or mental development. Symptoms only manifest in an advanced stadium, when detrimental effects have already started. Since iron is a component of haemoglobin (Hb), iron deficiency anaemia can develop after iron stores are depleted. The World Health Organization (WHO) estimated that anaemia affects one quarter of the world's population and is concentrated within pre-school age children and adult women. Iron deficiency anaemia is the most common cause and is a serious problem in developing countries. The current study is performed in South-Africa. Here the prevalence of infant anaemia is estimated between 21% and 50% depending on the haemoglobin (Hb) cut-off point, and even higher in children infected with HIV. An estimated 7.3% of perinatal deaths was attributed to iron deficiency anaemia in 2000.

The iron status of an infant in the first few months of life mainly depends on the iron status at birth. Factors that are of influence are the maternal iron status before and during pregnancy, infant gestational age, birth weight and the timing of umbilical cord clamping. After birth, gender, rate of weight gain, nutrition and iron supplementation are the most important factors.

Low birth weight infants have an increased risk of developing iron deficiency and anaemia due to the smaller iron storages at birth and increased postnatal growth.

Efforts have been made to prevent iron deficiency. Pregnant women with anaemia benefit from iron supplementation. Developing countries face problems of high costs, an underdeveloped health care system and non-compliance to prenatal health care and/or prescribed medication. Iron supplementation in infants faces these same problems and comes with side-effects, as constipation and a higher risk of infection. Delayed cord clamping (DCC) has shown its benefit in multiple studies (as discussed further on). This procedure seems of special interest in resource-poor settings since it is cost-free and easy to perform.

Iron studies - Ferritin is the cellular storage protein for iron. Low serum ferritin levels are the earliest indication of low body iron stores. Ferritin is also an acute phase reactant, helping the cellular defense against oxidative stress and inflammation. Serum ferritin levels are decreased in iron deficiency.

Transferrin is a protein that binds one or two ferric molecules and is the major transporter for iron transport through the plasma. Its synthesis is increased in states of iron deficiency by mechanisms that are unknown. Circulating transferrin is normally about one-third saturated with iron, this is called the transferrin saturation. In iron deficiency the transferrin saturation is reduced.

Hb represents one of the last indicators of iron deficiency. A microcytic, hypochromatic anaemia with a low mean corpuscular volume (MCV) is observed.

Physiology
The placenta and umbilical cord – The fetus absorbs iron from the mother across the placenta. Transferrin-bound iron is taken up by the transferrin receptor on the placental membrane, which is then taken into the cell and released into the cytoplasm. The released iron is taken into the fetal circulation. Although the placenta can absorb iron from the maternal circulation for the fetus at the expense of maternal iron stores by increasing transport proteins (transferrin), fetal iron storage will suffer from maternal iron deficiency.
The total estimated feto-placental blood volume in term infants is about 120 ml/kg fetal weight. After birth of the infant, the umbilical cord is clamped and cut to separate the baby from the mother. After immediate cord-clamping, the distribution of blood reflected in the fetus:placenta ratio is approximately 2:1. But if the cord is not clamped, there is still circulation for a period of time between the infant and placenta through the umbilical vein and arteries.

The rate of placental transfusion is rapid at first and then slows down, with approximately 25% of the transfer occurring in the first 15-30 seconds, 50-78% of the transfer by 60 seconds and the remaining transfer by 3 minutes. Studies performed to estimate the actual blood transfer show an increase of blood volume of 20-35 ml/kg body weight with DCC. The amount of iron provided by DCC would represent a 30–33% increase in total body iron (TBI). Iron is found in the body in Hb (75%), in stores as ferritin (20%) and in myoglobin and enzymes.

Fetal and infantile Hb - Intrauterine the fetus is exposed to a relatively hypoxic environment. The fetus has several mechanisms to overcome this lower oxygen availability. The Hb is predominantly fetal haemoglobin (Hbf), which has a greater affinity for oxygen than adult haemoglobin (HbA). The fetal production of erythrocytes is high, with a maximum Hb and haematocrite (Ht) in term newborn infants.

Due to a newborn’s high Hb and a relatively higher oxygen availability extraterine, the erythropoietin production and erythropoiesis decline after birth. The Hb concentration decreases from approximately 170 g/l at birth to a low of 112 g/l at about 2 months of age. This ‘physiological anaemia of infancy’ is a result of a combined effect of the shorter lifespan of fetal erythrocytes (70-90 instead of 120 days), decreased erythrocyte production and a dilution effect from increased blood volume related to growth. From 2 to 3 months onward Hb usually starts increasing again.

Iron released during the breakdown of erythrocytes is stored or re-used in the formation of new erythrocytes. Because of a higher breakdown than formation of erythrocytes in the first few weeks postpartum, a temporary increase in storage iron is expected. As mentioned before, the infant’s iron status in the first few months of life mainly depends on the amount of iron at birth. This is because breast milk and non-iron-fortified formula contain little iron. Bleeding or gastro-intestinal infections can lead to loss of iron from the body. Rapid postnatal growth leads to exhaustion of the body’s iron at an earlier age.

Physiology of delayed cord clamping – During the time that the umbilical cord is unclamped, blood transfusion from the placenta to the infant still occurs. In the short term this would lead to an increased volume of blood. In the first few hours after birth, a shift of fluid from the plasma to extravascular sites occurs, leading to a relatively higher amount of erythrocytes. Due to reasons explained before, the Hb and Ht levels decrease in the first few weeks after birth. It is expected that infants who start life with a higher Hb and Ht, will have more erythrocytes to be broken down, and the total release of iron (for stores) will be increased until erythrocyte formation starts again.

Current practice
The third stage of labour is the period from birth of the baby until delivery of the placenta. The most important complication of the third stage of labour is postpartum haemorrhage (PPH, blood loss >500 ml). To reduce the risk of PPH, a more active management of the third stage of labour has become standard practice in many countries over the last decade, including: administration of a prophylactic oxytocic after delivery of the baby to enhance
uterine contraction, early cord clamping (ECC) and cutting, and controlled traction of the umbilical cord.

Previous to the current study, an observational study was performed at Stanger Provincial Hospital, which showed that 70% of the cords were clamped within 30 seconds and around 90% of the cords were clamped before 60 seconds.

Since the effects of early cord clamping were noted, the authors of the latest Cochrane review on the topic of active versus expectant management of the third stage of labour state that future studies should consider leaving the cord unclamped until it has stopped pulsating. The WHO now recommends that the umbilical cord should not be clamped earlier than 1 minute after birth in newly-born term or preterm babies who do not require positive-pressure ventilation.

**Randomized controlled trials comparing DCC versus ECC**

The definitions of ECC and DCC differ in the literature. ECC varies between within 10 seconds to within 60 seconds and DCC varies between after 60 seconds to the cessation of cord pulsations (up to 15 minutes).

**In term infants in high income countries**

Multiple randomized trials have compared the effects of ECC versus DCC. A recent study with term infants in an industrialized country found that infants subjected to DCC had a higher mean ferritin concentration and a lower prevalence of iron deficiency at the age of 4 months, although they found no difference in Hb count. The prevalence of neonatal anaemia at 2 days of age was lower in the DCC group. There were no significant differences between groups in potential side effects, namely postnatal respiratory symptoms, polycythaemia, or hyperbilirubinaemia requiring phototherapy. A study performed in Mexico showed that infants who received DCC had higher levels of ferritin and iron at the age of 6 months. Longer term effects, with a special interest in neurological development, are currently being studied.

**In term infants in middle and low income countries**

A meta-analysis of randomized controlled trials performed in middle and low income countries showed that Hb concentration in term infants who underwent DCC is significantly higher 2 to 4 months after birth, and the proportion of infants with anaemia significantly lower. A beneficial effect of DCC was especially seen in infants born to anaemic mothers.

**In preterm infants in high income countries**

A meta-analysis of randomized trials in preterm infants showed that DCC caused an increase in blood volume during the first 24 hours of life, fewer transfusions for anemia or low blood pressure and a lower incidence of intraventricular haemorrhage. The peak bilirubin concentration was higher for infants who received DCC, without an increase in the need for phototherapy. The authors concluded that a DCC time of at least 30 seconds is safe.

In hospital deliveries of preterm infants the cord is usually clamped directly, mainly to quickly hand the infant over to the neonatal team for resuscitation. The increase in cardiac output to the lung from 8% during fetal life to the 45% immediately after birth necessitates transfer of an adequate blood volume. When the cord is clamped before an adequate placental transfusion to the infant has occurred, blood volume needed to fill the lung vasculature may be taken from other capillary beds resulting in relative hypoperfusion.
In the premature infant, total body iron is lower than in the term newborn, although the proportion of iron to body weight is similar. Premature infants have a faster rate of postnatal growth than infants born at term, so they become iron-depleted more rapidly than fullterm infants. In developing countries, with limited resources and a high risk of transmitting infection through blood transfusion, a reduced need for blood transfusion would be of particular interest.

**Problem definition**
Up to now there have been no trials that specifically reported the effects of DCC in low birth weight infants in middle or low income countries. Neither have previous cord clamping studies reported on the prevalence or effects of HIV. Results between high and low income countries may differ, as the risk of anaemia is much higher in low income countries due to a high prevalence of maternal iron deficiency and HIV. As mentioned above, anaemia is a critical factor contributing to neonatal and infant mortality in developing countries. Low birth weight infants have an increased risk of developing anaemia and DCC might improve haematological outcome in these children. The effects of DCC are of special importance in these resource-poor settings as it is a simple and cost-free procedure.

Since the current study is performed in a resource-poor setting, where gestational age is mostly estimated by palpation of the abdomen only and thus making the estimation less accurate, we chose to focus on ‘low birth weight’ infants rather than ‘premature’ infants.

**Research question**
The main aim of this study is to assess if DCC is beneficial for the haematological status of low birth weight infants in a middle-income country. The primary outcomes are the Hb level of low birth weight infants at the age of 2 months and the number of anaemic infants. Secondary outcomes are infant iron status at 2 months, the occurrence of possible adverse effects in the first 24 hours after birth and the HIV-transmission rate.
Material & methods

Study population
The study was conducted in Stanger Provincial Hospital, a 500-bed public hospital in Stanger, Kwazulu-Natal, South-Africa. Medical care was provided free of charge to the patients. Five-to sixhundred infants were born per month. About 90-95% of the women delivering were of African origin and 5-10% of Indian origin. The percentage of HIV-positive women delivering in Stanger hospital was 39%. Kwazulu-Natal is the province with the highest rate of HIV-infection in South-Africa. Malaria is not endemic in the study area.

Patient enrollment and eligibility
Patients were included between February and October 2012. Pregnant women delivering in Stanger Hospital were eligible for inclusion if they were expected to vaginally deliver a low birth weight infant, defined as a birth weight below 2500 grams. Women with a symphysal-fundal-height (SFH) measurement ≤ 34 cm, gestational age < 37 weeks (by dates or palpation) or an estimated fetal weight of less than 2,5 kg by ultrasound were informed about the study and were asked to participate. Since the actual birth weight can only be assessed after delivery, infants with a birth weight up to 3000 grams were included and followed for 2 months.

Prenatal exclusion criteria were: maternal age <18 years, twin pregnancy, history of postpartum haemorrhage, (pre-)eclampsia, pregnancy-induced hypertension, gestational diabetes, abruptio placentae, antepartum haemorrhage and caesarian section.

Prenatal exclusion criteria were: tight nuchal cord necessitating early umbilical cord cutting, need for neonatal cardiopulmonary resuscitation, or major congenital abnormalities.

Study procedure
We performed a randomized controlled trial comparing early versus delayed clamping of the umbilical cord. Randomization was performed in a 1:1 ratio using an online computer program (http://www.randomization.com). Sequentially numbered, closed, opaque envelopes ensured concealment of allocation. The envelopes were opened when the women in labour were almost about to deliver. The midwife conducting the delivery was informed about the allocation. Blinding of the clinicians and mothers was not possible due to the nature of the intervention. After birth, the baby was dried and placed on the mother’s abdomen. The time between birth of the baby (full body) and the first clamp was recorded with a stopwatch. Early cord clamping was defined as within 60 seconds, delayed cord clamping as between 120 and 180 seconds. If a mother, already assigned to a treatment group, later became ineligible (e.g., infant birthweight more than 3 kg), the assigned treatment card was not re-used. If an infant assigned to the delayed cord clamping group needed to be resuscitated, the cord was clamped and cut early and the patient was excluded from the study.

As standard procedure, an oxytocin injection was given to the mother shortly after birth of the baby to stimulate uterine contraction, thereby reducing the risk of postpartum haemorrhage. After the cord was clamped and cut, 3x0.5 ml blood was drawn from the maternal side of the umbilical cord. Haemoglobin (Hb), haematocrit (Ht), mean corpuscular volume (MCV), ferritin and transferrin levels were assessed. The former three were analysed in the hospital’s own laboratory. Ferritin and transferrin were analysed at the Inkosi Albert Luthuli Central Hospital.

Data regarding maternal age, parity, body-mass index (BMI), Hb, HIV-status and if positive, the compliance to the Prevention of Mother-To-Child Transmission (PMTCT) protocol, use of medication (including iron supplements) and socio-economic status was collected on standardized forms.
Included in the PMTCT protocol are Voluntary Counselling and Testing (VCT) during antenatal care, the use of Nevirapine (antiviral medication) by the mother during delivery, the administration of Nevirapine in the infant, education about feeding practices, and HIV-testing in the infant and treatment accordingly.\textsuperscript{32}

The infants were examined 20-26 hours postpartum for clinical signs of hyperviscosity (apathy, tachypnea, hypoglycaemia, poor sucking, plethora) or hyperbilirubinaemia. The Ballard-score was used to assess gestational age. Bloodsamples (2x0.5ml) were taken to measure the Hb, Ht, MCV and total serum bilirubin (TSB). Infants with hyperbilirubinaemia (according to South-African bilirubin curves) were admitted in nursery and treated with phototherapy according to local guidelines. An HIV-PCR was performed in infants born to HIV-positive mothers. Healthy mother-infant pairs were discharged around 24 hours after birth.

Follow-up of the infant took place two months after birth. The infant’s weight, length and head circumference were measured, the medical and nutritional history were recorded, the road-to-health card was checked to see if immunisations were up to date, and a physical examination was performed. Bloodsamples (4x0.5ml) were taken to evaluate the haematological status of the infant (Hb, Ht, MCV, iron, ferritin, transferrin, transferrin saturation and C-reactive protein (CRP)). Infants with a Hb-level <9.5 g/dL (more than two standard deviations below the mean of similarly aged infants from an iron-supplemented USA reference population, see table 1) were defined as having anaemia. Care/medication was provided as required. The result of the HIV-PCR test, which was done in the local clinic in six-week-old infants born to HIV-positive mothers, was recorded. Mothers who did not return after two months were contacted per phone and were given a new follow-up appointment. In case the mother did not have sufficient money to pay for transport to the hospital, arrangements were made (where possible) to see the patients in their local clinic instead. Patients with a follow-up between two and three months postpartum were included.

Table 1. Laboratory tests in iron deficiency anaemia.\textsuperscript{33-35}

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Normal</th>
<th>Iron deficiency anaemia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- maternal</td>
<td>12.0-16.0</td>
<td>&lt; 11.0 g/dL</td>
</tr>
<tr>
<td>- fetal (cord blood)</td>
<td>14.0-20.0 g/dL</td>
<td>&lt; 12.5 g/dL</td>
</tr>
<tr>
<td>- infants at 2 months</td>
<td>10.0-14.5 g/dL</td>
<td>&lt; 9.5 g/dL</td>
</tr>
<tr>
<td>MCV</td>
<td>80-100 fl</td>
<td>&lt; 77 fl</td>
</tr>
<tr>
<td>Ferritin</td>
<td>87-430 µg/L</td>
<td>&lt; 12 µg/L *</td>
</tr>
<tr>
<td>Transferrin saturation</td>
<td>20-50 %</td>
<td>&lt; 16%</td>
</tr>
</tbody>
</table>

* < 30 µg/L in the presence of infection: CRP > 10 mg/L

Outcomes
Primary endpoints are the Hb levels in the infant at the age of two months and the amount of infants with anaemia. Secondary endpoints are the iron status of the infant after two months (iron, ferritin, transferrin and transferrin saturation) and possible adverse effects such as hyperviscosity syndrome, hyperbilirubinaemia (requiring phototherapy), mortality, postpartum haemorrhage and the transmission of HIV.
**Sample size calculation**

To detect a clinical significant difference in mean Hb between the delayed and early cord clamping group at two months of 1 g/dl with SD 1.5\textsuperscript{25,36} a power analysis was performed with \( \alpha = 0.05 \) (two-sided) and \( 1-\beta = 80\% \). Expecting a maximum drop-out of 40% we had to include 51 infants in each group.\textsuperscript{a}

\textsuperscript{a} Formula used: \( (2 \times SD^2 \times (Z_{alpha} + Z_{beta})^2)/\delta^2 \) \( \rightarrow (2 \times 1.4^2 \times (0.84 + 1.96)^2)/1.02 = 31. 31 + 40\% = 51 \) per group

**Statistical analysis**

SPSS (version 19.0) for Windows was used. Data about patient characteristics of mothers and infants are presented using standard statistical descriptives: mean [SD] or median [range] if not normal distributed. For group comparisons of continuous variables, the Student’s t-test was used for variables with a normal distribution, and the Mann-Whitney U test for variables without a normal distribution. Categorical variables were compared between groups using the \( X^2 \)-test. A P-value <0.05 was considered significant.

**Ethical approval**

The study is approved by the ethical council at the University of KwaZulu-Natal (UKZN), Durban, KwaZulu-Natal, South Africa and the Liverpool School of Tropical Medicine (LSTM), Liverpool, United Kingdom.
Mothers assessed for eligibility (n=282)

Excluded (n=102)
- Caesarean section (n=17)
- Age below 18 years (n=16)
- Pre-eclampsia (n=15)
- Twin pregnancy (n=4)
- Intra-uterine death (n=3)
- Antepartum haemorrhage (n=1)
- History of postpartum haemorrhage (n=1)
- Pregnancy induced diabetes mellitus (n=1)
- Successfully tocolysed (n=1)
- No consent (n=43)

Assigned to early cord clamping (n= 92)
Excluded (n=25)
- Birthweight >3000 g (n=20)
- Need for acute caesarian section (n=4)
- Unexpected stillborn (n=1)

Discharge before follow-up, blood sample was withdrawn (n=11)
Follow-up 1 day postpartum (n=56)
Lost to follow-up (n=12)
- Died (n=1)
- Moved (n=4)
- Unknown (n=7)
Follow-up at 2 months (n=36)
Still expected for follow-up (n=8)

Assigned to delayed cord clamping (n=88)
Excluded (n=33)
- Birthweight >3000 g (n= 23)
- Need for acute caesarean section (n=2)
- Major congenital abnormalities (n=1)
- Need for resuscitation and early cord clamping (n=7)

Discharge before follow-up, blood sample was withdrawn (n=7)
Follow-up 1 day postpartum (n=48)
Lost to follow-up (n=13)
- Died (n=1)
- Moved (n=2)
- Unknown (n=10)
Follow-up at 2 months (n=32)
Still expected for follow-up (n=3)
Results

Recruitment
Participants were enrolled between February and October 2012. Infants were seen for follow-up after 2-3 months. The follow-up period will end in December 2012. We hope to include the follow-up data of 11 more trial participants, but were not able to present these data in the current report.

Participant flow
Figure 1 shows the participant flow chart. During the study period, 283 women admitted in the antenatal and labour ward of Stanger Hospital were expected to deliver a low-birth weight infant, based on the (intrapartum) symphysal-fundal height. A total of 181 women met the inclusion criteria and agreed to participate in the study. Ninety-two mother-infant pairs were assigned to ECC and 88 to DCC. Immediately after delivery 26 infants in the ECC and 33 infants in the DCC group were excluded, based on prespecified postrandomization criteria. In 7 mother-infants pairs assigned to DCC, the infants’ condition was unsatisfying and the umbilical cord was clamped early to allow a quick transfer to the resuscitation table. In most cases drying and stimulation was all that was needed. As there was only one midwife per patient and no space on the delivery bed the first steps of the neonatal resuscitation were preferably done away from the mother.

Seventeen infants who successfully received the assigned intervention were discharged before the follow-up assessment at 20-24 hours postpartum could take place. Reasons for this were preference of the mother (after an uncomplicated delivery) and lack of transport home at the earlier agreed-upon time of discharge. Fifty-six infants in the ECC and 48 in the DCC group were assessed 20- to 24 hours after delivery. In both groups one infant died after showing symptoms of neonatal sepsis and despite administration of intravenous antibiotics. Thirty-six infants in the ECC and 32 in the DCC group were assessed after 2-3 months. The drop-out rate was 36 and 33% respectively for the ECC and DCC group. Reasons to drop-out were that participants moved, had no money for transport, had no time to come to the hospital, or had forgotten their appointment and could not be reached by phone.

Peripartum results
Table 1 shows maternal and neonatal baseline characteristics. Maternal baseline characteristics did not differ between the early and delayed cord clamping groups. Of the neonatal baseline data, only the umbilical cord transferrin levels were different, with significantly lower levels in the DCC group (p=0.032). Median clamping time was 28 s (range 4 to 75) in the ECC group and 138 s (range 115 to 210) in the DCC group. In the ECC group one cord was clamped later than intended (75 s), and in the DCC group one cord was clamped too early for no reason (115 s). Both infants were primarily included in the group to which they were randomly assigned (intention-to-treat analysis). Assessment of maternal blood loss in the third stage of labour was by visual estimation by the midwife. Postpartum haemorrhage was not observed. Manual removal of the placenta was required for one woman in the DCC group. Two of 56 newborns (4%) in the ECC group and 4 of 48 (9%) in the DCC group had fetal anaemia. All infants with a birth weight below 1800 grams were admitted in the Paediatric Department, as well as five newborns in the ECC and 1 in the DCC group with a birth weight above 1800 grams because of respiratory distress. Healthy newborn babies were admitted to the postnatal ward together with their mothers.
Table 1. Maternal and newborn characteristics.

<table>
<thead>
<tr>
<th>Maternal characteristics</th>
<th>ECC (n=56)†</th>
<th>DCC (n=48)†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>24 (18-38)</td>
<td>23 (18-33)</td>
</tr>
<tr>
<td>Parity (incl. study child)</td>
<td>2 (1-6)</td>
<td>2 (1-5)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27 (18-42)</td>
<td>27 (21-37)</td>
</tr>
<tr>
<td>MUAC (cm)</td>
<td>26 (21-36)</td>
<td>25 (22-32)</td>
</tr>
<tr>
<td>Hb (g/dL)</td>
<td>10.8 (1.5)</td>
<td>11.2 (1.5)</td>
</tr>
<tr>
<td>Maternal anaemia (Hb &lt; 11 g/dL)*</td>
<td>42%</td>
<td>36%</td>
</tr>
<tr>
<td>Antenatal iron supplementation</td>
<td>82%</td>
<td>73%</td>
</tr>
<tr>
<td>HIV-positive</td>
<td>47%</td>
<td>43%</td>
</tr>
<tr>
<td>Grade of education</td>
<td>12 (2-13)</td>
<td>11 (5-13)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Infant characteristics</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Time to clamping (sec)</td>
<td>28 (4-75)</td>
<td>138 (115-210)</td>
</tr>
<tr>
<td>Gestational age (weeks)</td>
<td>37 (27-40)</td>
<td>36 (29-40)</td>
</tr>
<tr>
<td>Female</td>
<td>52%</td>
<td>65%</td>
</tr>
<tr>
<td>Birthweight (g)</td>
<td>2605 (1200-2980)</td>
<td>2600 (1400-2980)</td>
</tr>
<tr>
<td>Birth length (cm)</td>
<td>48 (3)</td>
<td>48 (3)</td>
</tr>
<tr>
<td>Head circumference (cm)</td>
<td>33 (2)</td>
<td>33 (2)</td>
</tr>
<tr>
<td>No of infants with Apgar score &lt;7 at 1 min</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Ballard-score</td>
<td>35 (14-44)</td>
<td>35 (17-42)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Cord blood</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Umbilical cord Hb (g/dL, n=97)*</td>
<td>15.0 (1.9)</td>
<td>14.9 (1.7)</td>
</tr>
<tr>
<td>No of infants with fetal anaemia (Hb &lt; 12.5 g/dL)</td>
<td>2 (4%)</td>
<td>4 (9%)</td>
</tr>
<tr>
<td>Umbilical cord Ht (L/L, n=96)*</td>
<td>0.420 (0.054)</td>
<td>0.422 (0.054)</td>
</tr>
<tr>
<td>Umbilical cord ferritin (µg/L, n=78)</td>
<td>173 (17-821)</td>
<td>163 (36-513)</td>
</tr>
<tr>
<td>Umbilical cord transferrin (µg/dL, n=97)*</td>
<td>2.05 (0.44)</td>
<td>1.86 (0.39)</td>
</tr>
</tbody>
</table>

Data are median (range) unless stated otherwise

# mean (SD)
† in case of missing data, the numbers of successful analyses are presented separately
* according to the definition of the World Health Organization
ᶺ based on palpation (symphysal-fundal height), antenatal data and/or ultrasound

BMI = body mass index, MUAC= mid-upper arm circumference, Hb = haemoglobin, Ht = haematocrit

Results 1 day postpartum
After a median postnatal age of 24 hours (range 14 to 54) both mother and child were reassessed. Infants in the DCC group had a higher Hb, respectively 16.9 and 18.0 g/dL (p=0.014) and a higher Ht, respectively 0.46 and 0.49 (p=0.013). Polycythaemia did not occur. Bilirubin levels were comparable between groups. Eight of 56 (14%) newborns in the ECC group compared to 2 of 48 (4%) newborns in the DCC group had hyperbilirubinaemia requiring phototherapy. Eight of 10 newborns requiring phototherapy had a birth weight less than 2000 g. One infant had a positive HIV-PCR 1 day postpartum.
Table 2. ECC vs DCC results 1 day postpartum, all participants.

<table>
<thead>
<tr>
<th>1 day postpartum</th>
<th>ECC (n=56) †</th>
<th>DCC (n=48) †</th>
<th>P-value of difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (hours)</td>
<td>24 (20-48)</td>
<td>24 (14-54)</td>
<td>0.014</td>
</tr>
<tr>
<td>Hb (g/dL, n=98)#</td>
<td>16.9 (2.4)</td>
<td>18.0 (1.9)</td>
<td>0.014</td>
</tr>
<tr>
<td>Hb increase compared with cord blood (g/dL, n=92)</td>
<td>2.1 (-2.3 – 5.4)</td>
<td>3.6 (-6.1 – 7.7)</td>
<td>0.003</td>
</tr>
<tr>
<td>Ht (L/L, n=96)#</td>
<td>0.460 (0.063)</td>
<td>0.490 (0.051)</td>
<td>0.013</td>
</tr>
<tr>
<td>Ht increase compared with cord blood (L/L, n=90)</td>
<td>0.039 (0.049)</td>
<td>0.065 (0.063)</td>
<td>0.007</td>
</tr>
<tr>
<td>Infants with polycythaemia (Ht &gt; 0.65 L/L)</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Mean corpuscular volume (fl, n=97)</td>
<td>98 (86-125)</td>
<td>98 (87-114)</td>
<td>0.809</td>
</tr>
<tr>
<td>Total bilirubin (µmol/L)</td>
<td>83 (14-285)</td>
<td>94 (11-222)</td>
<td>0.570</td>
</tr>
<tr>
<td>No of infants with bilirubin &gt; phototherapy threshold †</td>
<td>8 (14%)</td>
<td>2 (4%)</td>
<td></td>
</tr>
</tbody>
</table>

Data are median (range) unless stated otherwise
# mean (SD)
† in case of missing data, the numbers of successful analyses are presented separately
* according to South-African bilirubin curve
Hb=haemoglobin, Ht=haematocrit

Results 2-3 months postpartum

At a median age of 61 days (range 45 to 91), 36 children in the ECC and 32 in the DCC group returned for assessment. The target sample size of 31 infants per group was achieved. Mean Hb levels two months after delivery were comparable in both groups (table 4). Also the markers for iron deficiency (mean corpuscular volume, ferritin, serum iron, transferrin, and transferrin saturation) were comparable. Fifteen of 36 (42%) infants in the ECC group and 11 of 32 (34%) infants in the DCC group had anaemia, a non-significant difference. None of the infants had iron deficiency (defined by ferritin levels). Two infants of the ECC group who had no ferritin levels measured (missing data) did have transferrin saturation levels suggestive of iron deficiency. The Hb levels in both groups had decreased compared to Hb levels at birth and at day 1. The results did not change when additional analyses were done by actual cord clamping time rather than by intervention group.

An HIV-PCR-test was performed at the age of 6 weeks in infants born to HIV-positive mothers. The test result in the single patient with a positive test at day 1 was indeterminate at 6 weeks. The other test results were negative (n=23) or unknown (n=7, tests failed or could not be traced).

Post-hoc analyses

Baseline characteristics of participants who completed the follow-up (n=68) were different from the baseline characteristics of all participants (n=104). In a subgroup including only participants who completed the follow-up, maternal Hb was significantly higher in the DCC group compared to the ECC group (mean 11.5 g/dL (SD 1.4 g/dL) vs mean 10.9 (SD1.4), p=0.045). Umbilical transferrin levels were still significantly higher in the DCC group (p=0.012).
## Table 3. Results 2 months postpartum.

<table>
<thead>
<tr>
<th>2 months postpartum</th>
<th>ECC (n=36) †</th>
<th>DCC (n=32) †</th>
<th>P-value of difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (days)</td>
<td>61 (45-85)</td>
<td>61 (46-91)</td>
<td>0.734</td>
</tr>
<tr>
<td>Infant weight (g, n=63)</td>
<td>4700 (1900-5800)</td>
<td>4700 (3000-6100)</td>
<td>0.091</td>
</tr>
<tr>
<td>Weight gain since birth (g)#</td>
<td>1953 (594)</td>
<td>2199 (574)</td>
<td>0.582</td>
</tr>
<tr>
<td>Length (cm, n=63)</td>
<td>55 (42-58)</td>
<td>55 (47-60)</td>
<td>0.094</td>
</tr>
<tr>
<td>Head circumference (cm, n=63)</td>
<td>38 (32-40)</td>
<td>39 (31-41)</td>
<td>0.924</td>
</tr>
<tr>
<td>Exclusively breastfed</td>
<td>69%</td>
<td>59%</td>
<td>0.641</td>
</tr>
<tr>
<td>Infection in past 2 weeks*</td>
<td>54%</td>
<td>53%</td>
<td>0.626</td>
</tr>
<tr>
<td>C-reactive protein (mg/L)</td>
<td>2 (0-10)</td>
<td>2 (0-12)</td>
<td>0.452</td>
</tr>
<tr>
<td>Hb (g/dL)#</td>
<td>9.8 (1.0)</td>
<td>9.9 (1.1)</td>
<td>0.663</td>
</tr>
<tr>
<td>Proportion of anaemic infants (Hb &lt; 9.5 g/dL)</td>
<td>42%</td>
<td>34%</td>
<td>0.204</td>
</tr>
</tbody>
</table>

Hb = haemoglobin, Ht = haematocrit

Data are median (range) unless stated otherwise

# mean (SD)

† in case of missing data, the numbers of successful analyses are presented separately

* diarrhea, cough or fever in the last 2 weeks, according to mother’s history

Subgroup analysis of infants with a complete follow-up showed that there were no differences (anymore) between cord clamping groups in Hb and Ht at day 1 (table 4 and figure 2).

## Table 4. ECC vs DCC results 1 day postpartum, only including participants who completed the study.

<table>
<thead>
<tr>
<th>1 day postpartum</th>
<th>ECC</th>
<th>DCC</th>
<th>P-value of difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb (g/dL, n=64)</td>
<td>17.2 (2.3)</td>
<td>18.0 (2.0)</td>
<td>0.134</td>
</tr>
<tr>
<td>Ht (L/L, n=62)</td>
<td>0.471 (0.060)</td>
<td>0.489 (0.052)</td>
<td>0.212</td>
</tr>
</tbody>
</table>

Data are mean (SD)

Hb = haemoglobin, Ht = haematocrit
Figure 2. Differences in infant Hb 1 day postpartum between groups (ECC, DCC and complete/incomplete follow-up).

P-value of difference: 1=0.134, 2=0.012, 3=0.893
DISCUSSION

Key findings
Our results show that a 2-minute delay in clamping of the umbilical cord resulted in a higher Hb and Ht 24 hours postpartum compared to cord clamping within 1 minute, suggesting that more placental transfusion occurred in the DCC group. This effect, however, did not improve the infants’ haematological status at the age of 2-3 months.

There were no differences between groups in bilirubin levels, the need for phototherapy, or the incidence of vertical HIV-transmission. PPH or polycythaemia did not occur.

Comparisons with other studies
This study was the first randomized controlled study assessing the effects of DCC versus ECC on the haematological status beyond the neonatal period in low birth weight infants in a middle-income country. Previous studies with preterm or low birth weight infants comparing ECC versus DCC mainly focused on the short term haematological effects of DCC. The results showed higher Hb and Ht values in the first 24 hours after birth, thus suggesting more placental transfusion with DCC. The only study with preterm infants which assessed Hb and iron status beyond the neonatal period was performed in a high income country and showed a higher Hb in the DCC group (n=18) at the age of 10 weeks. Many more studies in term infants have shown that DCC results in a higher Hb and better iron status during the first half year of infancy. But these results cannot simply be extrapolated to preterm or low birth weight infants because they have smaller iron storages at birth and increased postnatal growth leading to an increased risk of developing iron deficiency and anaemia.

We found the amount of infants with anaemia to be comparable between the ECC and DCC group. Only 2 infants had laboratory findings suggestive of iron deficiency, making other causes of anaemia at this age more likely. Although not further studied, other possible causes are: physiological anaemia of infancy, anaemia of prematurity, intercurrent infections, or the use of nevirapine. The Hb cut-off point used in this study was 2 standard deviations below the mean of similarly aged infants from an iron-supplemented USA reference population. By using this Hb cut-off we might have overestimated the prevalence of anaemia, as some studies have shown that Hb levels in the black population are normally 0.5 g/dL lower than in the white population, and because our study included infants with a low birth weight who tend to have a nadir that reaches lower levels and occurs earlier than in normal weight infants.

Previous studies have used varied definitions for DCC and ECC (sometimes even called immediate cord clamping, ICC). In a meta-analysis with preterm infants, a cord clamping time of at least 30 seconds was (already) considered delayed cord clamping and early cord clamping was described as in the first few seconds after birth. As in our study the median cord clamping time in the ECC group was 28 seconds, and only 2 infants had their cords clamped in less than 10 seconds, our “early” cord clamping was relatively “late” compared to other studies.

Infants in our study were placed on the mother’s abdomen, whereas most studies comparing ECC to DCC in high income countries placed the infant on a lower extension of the delivery bed while cord clamping was delayed. This difference, however, should not be of influence on the total amount of placental transfusion but could enhance the rate at which transfusion occurs.

Our study was not primarily powered for assessing possible adverse effects, but our findings support other studies in terms of safety during the neonatal period.

The possible effect of DCC on vertical HIV-transmission has not been studied before. The Prevention of Mother-To-Child-Prevention guideline of the WHO is silent on the matter of
cord clamping time. In The British HIV Association guideline of 2008 early cord clamping was recommended when caesarean section was performed. This recommendation was based on experts’ opinions without further reasoning. Their 2012 guideline does not mention cord clamping time. In a study in Zambia, midwives were interviewed and observed regarding the practice of cord clamping. In the majority of observed deliveries the cord was clamped within a minute, reasoned by many midwives as being a measure to prevent maternal to child transmission of HIV. However, one might argue that it is unlikely for DCC to affect HIV transmission, since it is only more blood from the infant itself that is being transfused and that the cord clamping technique seems to be more important than the cord clamping time. Special attention should be made not to perforate the umbilical cord, thus preventing maternal blood and secretions to enter the infant’s circulation. In our study, only 1 infant in the ECC group had an indeterminate test at the age of 6 weeks. Most HIV-positive mothers who delivered in Stanger hospital were treated according to the PMTCT protocol. When this protocol is fully applied, the vertical HIV-transmission rate can be reduced to 1.5% in South-Africa. Larger studies are needed to assess the safety of DCC regarding HIV transmission.

Strengths and limitations
This is the first randomized controlled study assessing the effects of DCC versus ECC on the haematological status beyond the neonatal period in low birth weight infants in a middle-income country. We were also the first cord clamping study with a known (high) HIV prevalence and the first to assess the possible effect of DCC on vertical HIV-transmission. Another strength of this study was its realistic nature. Other than the intervention of DCC, there was no change of practice during the delivery period, and other than the researcher recruiting participants and informing the midwife about the allocation, there was no special trained or extra staff. Observations prior to the study showed that 90% of the cords were clamped within 1 minute. Cord clamping within a few seconds was in our setting often not possible and was not particularly intended, thus making the ECC group (cord clamping within 1 minute) a realistic control group.

The randomized design resulted in inclusion of participants with comparable maternal and neonatal baseline characteristics between study groups, except for significantly higher umbilical cord transferrin levels in the DCC group. Unfortunately we faced a relatively high loss to follow-up rate, with only 68 of the 104 (65%) infants being assessed at the age of 2-3 months. Subgroup analysis of infants who completed the follow-up showed comparable Hb and Ht levels 24 hours after birth between the ECC and DCC group (as opposed to significantly higher levels that were found in the DCC group compared to the ECC group when all infants were included). Maternal Hb levels were in this subgroup significantly higher in the DCC group compared to the ECC group. These 2 differences may lead to bias when interpreting the results after 2-3 months.

The target sample size was reached, which was calculated as to detect a significant difference of 1 g/dL in mean Hb at 2 months. The 1 g/dL was chosen on the basis of clinical significance (decreased mortality) and the result of a previous study. That study, however, only included mothers with anaemia during pregnancy, a factor which probably enhanced the beneficial effect of DCC. For this reason, our study sample might have been too small to detect such a difference.

We intended to include infants with a birth weight below 2.5 kg, but with the inclusion criteria we used (SFH ≤ 34 cm or estimated fetal weight by ultrasound < 2.5 kg) more infants weighed between 2.5 and 3 kg (67%) than below 2.5 kg. By lowering the SFH cut-off and by using ultrasound more often, a higher percentage of actual low birth weight infants could have been included.
Blood samples sometimes got lost, were not analyzed as requested, or were not sufficient to perform all analyses, thereby leading to missing data. Transferrin receptor levels were not measured. Combined with ferritin we could have calculated the total amount of body iron per body weight, making a better evaluation of iron deficiency (anaemia) than with ferritin alone.\textsuperscript{47}

**Implications**
Based on our results, we cannot conclude that DCC has a beneficial effect on the haematological status of low birth weight infants at the age of 2-3 months. More research is needed in this area. Future studies should try to minimize the loss to follow-up. This could possibly be reached by having a setting where participants live close to the hospital or in a safe area so that they can be visited. Ideally, infants should be assessed more often and for a longer period of time, so that Hb, Ht and iron levels and their development in time could be more closely monitored. Missing data should be minimized by double-checking if the right tests are requested, or by storing extra blood for in case analyses should be re-done. Stricter inclusion criteria should be used to include more infants with a low birth weight. Large sample sizes are needed to assess the safety regarding vertical HIV-transmission.

**CONCLUSION**
Despite evidence of successful placental transfusion on the first day after birth, a beneficial effect of DCC on the haematological status of low birth weight infants could not be detected 2 to 3 months later. Our results may be biased by a high loss to follow-up. DCC in low birth weight infants did not cause increased adverse effects.
REFERENCES

29. Maternity Register, Stanger Hospital.
32. Summary MTCT protocol, Western Cape Province.
47. Cook JD, Flowers CH, Skikne BS. The quantitative assessment of body iron. Blood. 2005 May 1;101(9):3359-64.