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The role of delayed umbilical cord clamping to control infant anaemia in resource-poor settings

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Publication date

2007

Document Version

Final published version

[Link to publication](#)

Citation for published version (APA):

van Rheenen, P. F. (2007). *The role of delayed umbilical cord clamping to control infant anaemia in resource-poor settings*. [Thesis, fully internal, Universiteit van Amsterdam].

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STATEMENTS CONCERNING THE THESIS:

The role of delayed umbilical cord clamping to control infant anaemia in resource-poor settings

1. A majority of normal birth weight infants living in a malaria-endemic region of Zambia already have low iron stores at birth (this thesis)
2. Delayed cord clamping should be considered in every infant born in a resource-poor setting, regardless of gestational age (this thesis)
3. When a normal birth weight is anticipated, the cord should be clamped three minutes after placing the baby on the mother's abdomen or between her legs (this thesis)
4. Calling 'delayed cord clamping' an intervention indicates that modern medicine has forgotten about the physiological delivery
5. Delaying clamping of the umbilical cord reduces the decline in haemoglobin in term infants living in a malarious area at four months of age (this thesis)
6. There is no sense in promoting the use of ultrasonography for assessing fetal growth retardation in developing countries, knowing that the majority of infants are not even weighed at birth
7. The ABCD approach in HIV/AIDS prevention in Africa (abstinence, be faithful, condoms, drugs) has the same hierarchical order as the ABCD in basic life support
8. Although one would expect the contrary, delaying clamping of the umbilical cord does not interfere with a quick delivery of the placenta (this thesis)
9. In term infants from a malaria-endemic area the beneficial haematological effect of delayed cord clamping disappears between the ages of 4 to 6 months (this thesis)
10. The task of the modern educator is not to cut down jungles, but to irrigate deserts (CS Lewis – The Abolition of Man (1943))
11. The lap of a graduating father is more often occupied by his notebook than by his children
12. It takes a village to raise a child (African proverb)

Delay clamping of the umbilical cord for ≥ 3 minutes for optimal blood transfusion from the placenta, regardless of fetal weight

Combine delayed cord clamping with the administration of oxytocin immediately after delivery of the infant to reduce maternal blood loss

When the state of the infant does not allow a clamping delay of 3 minutes, aim for a delay of ≥ 60 seconds with the infant placed between the mother's legs

Proceed with controlled cord traction after clamping the umbilical cord

Tropical science, as it relates to developing countries, must address as a priority those problems whose solutions would contribute to the urgent reductions needed in neonatal and infant mortality.

Patrick van Rheezen made a landmark contribution to this effort through his research on the timing of umbilical cord clamping in low resource settings. His work has systematically established the essential requirement for delayed cord clamping and demonstrated the substantial nutritional benefits this provides through reduction in anaemia to the young infant. He developed the first published Practice Guidelines for developing countries related to the timing of umbilical cord clamping.

The *British Medical Journal* correspondence on this recently published work confirmed this as a milestone in research on obstetric and paediatric practice. This research is of wide practical relevance for improving child health and nutrition in developing countries.

ISBN 978 90 5170 956 8
NUR 870

ROZENBERG
Publishers

Patrick van Rheezen

The role of delayed umbilical cord clamping to control infant anaemia in resource-poor settings

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Patrick van Rheezen

STELLINGEN BEHORENDE BIJ HET PROEFSCHRIFT:

The role of delayed umbilical cord clamping to control infant anaemia in resource-poor settings

1. Een groot deel van de kinderen met een normaal geboortegewicht in een malaria-endemisch gebied in Zambia hebben bij de geboorte al een uitgeputte ijzervoorraad (dit proefschrift)
2. Bij ieder kind dat in een ontwikkelingsland geboren wordt dient even gewacht te worden met het afnavelen, ongeacht de postconceptionele leeftijd (dit proefschrift)
3. Wanneer een kind met een normaal geboortegewicht wordt verwacht, dient het afnavelen te gebeuren drie minuten na plaatsing van het kind op de buik van de moeder (dit proefschrift)
4. Uit het feit dat 'even wachten met afnavelen' een *interventie* wordt genoemd blijkt dat de moderne geneeskunde niet meer weet wat een fysiologische bevalling is.
5. Bij a terme geboren kinderen in een malaria-endemisch gebied zorgt 'even wachten met afnavelen' voor een minder sterke daling van het hemoglobine gehalte in de eerste vier levensmaanden (dit proefschrift)
6. Promoten van het gebruik van echo-apparatuur in ontwikkelingslanden voor het vaststellen van foetale groeivertraging is onzinnig wanneer men beseft dat de meerderheid van de baby's in deze landen niet eens gewogen kunnen worden bij geboorte
7. De ABCD benadering in het voorkomen van een HIV besmetting in Afrika
A abstinence (=onthouding)
B be faithful (=wees trouw aan je partner)
C condoms (=gebruik een condoom bij seksueel contact)
D drugs (=gebruik anti-retrovirale middelen rondom de bevalling)
heeft een vergelijkbare volgorde van belangrijkheid als de ABCD-systematiek bij reanimatie
8. Ofschoon het tegendeel vaak wordt gedacht interfereert 'even wachten met afnavelen' niet met het snel geboren laten worden van de placenta (dit proefschrift)
9. Het gunstige hematologische effect van 'even wachten met afnavelen' verdwijnt tussen de vierde en zesde levensmaand bij a terme geboren kinderen in een malaria-endemisch gebied (dit proefschrift)
10. De taak van de moderne leraar is niet het kappen van oerwoud, maar het irrigeren van woestijnen (CS Lewis – De Afschaffing van de Mens (1943))
11. De promoverende vader heeft zijn kinderen minder vaak op schoot dan zijn laptop.
12. Om een kind groot te brengen heb je een dorp nodig (Afrikaans gezegde)

The role of delayed umbilical cord clamping to control
infant anaemia in resource-poor settings.

De publicatie van dit proefschrift werd mede
mogelijk gemaakt door financiële steun van:
Nutricia Nederland B.V.
Schering-Plough B.V.
Ferring Pharmaceuticals

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Coverdesign & DTP: Ingrid Bouws, Amsterdam

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Rozenberg Publishers
Bloemgracht 82
1015 TM Amsterdam
The Netherlands

Tel: +31 (0) 20 625 54 29
Fax: +31 (0) 20 620 33 95

E-mail: info@rozenbergps.com
www.rozenbergps.com

ISBN 978 90 5170 956 8
NUR 870

The role of delayed umbilical cord clamping to control infant anaemia in resource-poor settings

ACADEMISCH PROEFSCHRIFT

Ter verkrijging van de graad van doctor
aan de Universiteit van Amsterdam
op gezag van de Rector Magnificus
Prof. dr. J.W. Zwemmer
ten overstaan van een door het college voor promoties
ingestelde commissie,
in het openbaar te verdedigen in de Aula der Universiteit
op woensdag 30 mei 2007, te 14.00 uur

door

Patrick Ferry van Rheenen
geboren te Groningen

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1 INTRODUCTION AND RESEARCH QUESTIONS

INFANT ANAEMIA

The prevalence of anaemia among infants in malaria-endemic countries is high. In sub-Saharan Africa it affects as many as 75% of children in the second half of infancy.¹ The mean haemoglobin (Hb) level in infancy is age dependent. In term infants from an iron-supplemented USA reference population not exposed to malaria, Hb levels decline from birth, reaching a nadir at 2–3 months, and subsequently rise to reach a plateau around 6 months of age.² Hb levels in preterm infants follow a similar pattern, but the nadir is earlier (around 6 weeks), reaches lower levels and takes longer subsequently to plateau (Figure 1). In sub-Saharan Africa Hb levels continue to decline after the physiological nadir, to reach its lowest point only 6 to 12 months after birth.^{1:3:4}

Figure 1. Mean (± 2 SD) haemoglobin levels in term and preterm infants (adapted from Dallman 1988)

CAUSES OF INFANT ANAEMIA IN DEVELOPING COUNTRIES

Anaemia is defined as a Hb concentration greater than two standard deviations below the age-specific mean of the USA reference population. It is often multifactorial in origin in low resource countries and malaria plays a key etiologic role in sub-Saharan Africa. In holoendemic malarious areas, infants are exposed to malaria from birth although clinical malaria in infants mostly presents from about 4 months of age, when immunity acquired from the mother is diminishing.⁵ In these infants the highest burden of anaemia occurs towards the end of the first year of life.⁶

However, the basis for infant anaemia is frequently established during the antenatal period. Maternal factors such as placental malaria, human immunodeficiency virus (HIV) infection, undernutrition and anaemia are associated with poor infant haematological status.^{1;3;7-11}

ANTENATAL FACTORS

Placental malaria

Over a 50 million pregnancies are exposed annually to *Plasmodium falciparum* malaria parasites.¹⁰ Accumulation of malaria parasites in the placenta can cause serious health problems for both the mother and her baby, especially in first pregnancies.¹² Maternal anaemia and low birth weight (LBW) are frequently mentioned consequences of placental malaria.¹³⁻¹⁷ Less known is that maternal placental infection has been positively associated with infant anaemia and increased risk for malaria morbidity..^{9;14;18} The immunological mechanism behind these associations are unclear.

HIV infection

Maternal HIV infection aggravates the risk for placental malaria and dual infection further increases the risk for both LBW and infant anaemia. ^{1;19}

Poor maternal nutrition and micronutrient deficiencies

Maternal undernutrition during pregnancy has adverse consequences for the fetus which may persist throughout the life cycle with their influence continuing through to the next generation.²⁰ Stunted or underweight women are at increased risk of delivering LBW infants, who are themselves at risk of poor growth and development and anaemia in childhood and adolescence, as depicted in figure 2.

Figure 2. Nutrition throughout the life cycle (adapted from the 4th report of ACC/SCN 2000)

In developing countries anaemia in pregnancy is primarily caused by dietary iron deficiency.²¹ Adequate iron supply is critical for the rapidly developing fetal and neonatal organ systems. In iron deficient states iron is prioritized to haemoglobin synthesis to the detriment of non-haem tissues such as the skeletal muscle, heart and brain.²² The combined factors of placental malaria, HIV infection and maternal iron deficiency anaemia all contribute to the 30% fetal anaemia prevalence (cord Hb < 125 g/L) in babies born in sub-Saharan Africa^{3;7;9;11;23}.

POSTNATAL FACTORS

Malaria and iron deficiency are thought to be the two main factors which play an important role in the development of anaemia after birth. As a result, diagnosis, treatment, and preventive measures are focused on these two causes, but other factors also contribute to the complex aetiology of anaemia in developing countries.

Malaria

Over 50% of infant mortality from malaria relate to severe anemia.²⁴ Malaria can cause anaemia through haemolysis, increased splenic clearance of infected red blood cells and cytokine-induced dyserythropoiesis.²⁵

Poor child nutrition and iron deficiency.

In the normal term infants iron stores are adequate to maintain iron sufficiency for approximately four months of postnatal growth.²⁶ Dietary iron supplementation should compensate for the iron needed for growth and to replace normal losses. Iron losses from the body are small and relatively constant, except during episodes of diarrhoea, when losses may be increased. In the normal infant, iron losses average approximately 20 µg per kilogram per day. An infant who weighs 3 kg at birth and 10 kg at one year of age will require approximately 270 to 280 mg of additional iron during the first year of life to maintain normal iron stores. Infants with low total body iron as a consequence of LBW or severe maternal iron deficiency anaemia are particularly prone to iron deficiency, which develops in infancy by two to three months of age. Early introduction of complementary foods,

with bioavailability as low as 5%, accelerates the depletion of iron stores. Breast milk has a uniquely high bioavailability of iron (20-50%), which compensates for its low iron concentration.²⁷ In infants not receiving iron-fortified complementary foods, exclusive breastfeeding for at least the first six months of life improves iron intake.

HIV infection

HIV-infection increases anaemia risk²⁸ and vulnerability to the adverse consequences of malaria in infancy.¹ The exact mechanism is unknown, but cytokine-mediated inflammation with iron sequestration in macrophages and decreased iron absorption in the small intestine might play a role (anaemia of chronic disease).

Hookworm infection

Helminthic infection is unusual before the age of 6 months,²⁹ although infestation with *Ancylostoma duodenale* sometimes cause significant intestinal blood loss in young infants.³⁰

Inherited red cell disorders

Inherited red cell disorders commonly occur in malarious areas. The high frequencies of sickle cell trait (heterozygous HbAS) and glucose-6-phosphate-dehydrogenase (G6PD) deficiency in sub-Saharan Africa occur due to selection pressure as they confer partial protection against severe *Plasmodium falciparum* malaria.

Sickle cell trait is a benign carrier condition with no haematologic manifestations and it has no effect on prevalence of symptomless parasitaemia. It is reported as almost 90% protective against severe or complicated *P. falciparum* malaria.³¹ A reduction in all-cause mortality is observed during the period when infants are most at risk of severe malarial anaemia (2-16 months).³² In vitro investigations for both homozygous and heterozygous HbS erythrocytes have shown reduced invasion and growth of *P. falciparum* under conditions of low oxygen. Furthermore, HbAS red cells are phagocytosed more avidly when infected with ring-stage parasites, which could also contribute to malaria protection in children with sickle cell trait.^{33;34}

G6PD deficiency is an X-linked enzyme deficiency which affects hemizygous males and homozygous females by causing major haemolysis after exposure to oxidants or infection. Female heterozygotes and male hemizygotes have a 50% risk reduction for severe anaemia.³⁵ Under conditions of oxidative stress, in vitro growth of *P. falciparum* seems to be inhibited. Similar to the situation with sickle cell trait, G6PD deficient red cells are more susceptible to phagocytosis when infected by ring-stage parasites.³⁴

Another red cell disorder, α -thalassaemia, is also known to confer protection from severe falciparum malaria. Silent carriers ($-\alpha/\alpha\alpha$) are clinically normal. People with α -thalassaemia trait ($-\alpha/-\alpha$ or $--/\alpha\alpha$) have mild microcytic anaemia.³⁶

CONSEQUENCES OF ANAEMIA

Iron deficiency anaemia has been related to impaired mental and motor development among infants and young children and there are indications that with iron therapy anaemic children fail to catch up to non-anaemic children.^{37;38} Severe anaemia (Hb below 50 g/L) is associated with increased mortality risk. A survival analysis of Malawian infants indicated that a decrease in Hb by 10 g/L after 6 months of age increased the risk of dying before 12 months of age by 72%.^{23;39} In view of this morbidity and mortality risk, primary prevention of iron deficiency and malaria in infants could have a substantial effect on improving infant survival.

ANAEMIA CONTROL STRATEGIES

To control antenatal factors associated with infant anaemia every effort must be made to prevent maternal *Plasmodium falciparum* infection and to treat anaemia effectively during the antenatal period. The World Health Organization recommends a package of interventions during pregnancy⁴⁰ that comprise:

- (1) intermittent preventive antimalarial treatment (IPT) to address the heavy burden of asymptomatic infections among pregnant women from malaria-endemic areas;
- (2) use of insecticide treated nets (ITNs); and
- (3) access to effective case management for malaria illness and anaemia.

Presently, sulfadoxine-pyrimethamine is the only antimalarial medicine for which data on efficacy and safety for IPT is available from controlled clinical trials.⁴¹ At least 2 doses of sulfadoxine-pyrimethamine should be given during regularly scheduled antenatal visits after the first trimester.⁴²

Iron supplementation in pregnancy prevents low maternal Hb values at birth and six weeks post-partum, but there is little information on the effect on the infant.⁴³

There is good evidence that the use of ITNs for infants is effective in reducing malaria deaths and malarial anaemia, but the efficacy is compromised if re-treatment with insecticide is delayed beyond six months. Widespread access to ITNs will therefore require major financial, technical and operational inputs.⁴⁴⁻⁴⁶

A randomized controlled trial from an area of intense transmission of malaria in Tanzania showed that a single dose of sulfadoxine-pyrimethamine given to asymptomatic infants attending for routine vaccination at 2,3 and 9 months of age reduced episodes of clinical malaria and anaemia.^{47;48} Although this strategy is attractive because delivery may be achieved through the expanded programme on immunization, which increases sustainability, many important questions still need to be addressed before this intervention can be included in national malaria control policies.

The role of iron in the prevention and treatment of anaemia in malaria-endemic regions remains a highly contentious issue. Iron is a key functional component of a wide range of biologic systems, and is therefore an essential element for nearly all living organisms. Excessive iron can, however, cause tissue damage, since it has the ability to catalyze the generation of reactive free radicals. In settings with high malaria transmission supplementation of iron might worsen the outcome

of infectious illnesses.^{49;50} However, a short period of iron supplementation in the first few months of life will replenish iron stores at a time when there is still little pressure from infectious diseases such as malaria, and is therefore likely to be beneficial. In low-income areas where iron deficiency is common and malaria absent, routine iron plus folic acid supplementation does no harm, and is likely to have long-term benefits.⁵⁰⁻⁵²

THE NEED FOR CHEAP AND EFFECTIVE PREVENTIVE MEASURES

The insidious nature of infant anaemia results in mild-to-moderate degrees of anaemia frequently remaining undetected and untreated. When the condition of the infant has deteriorated so much that blood transfusion has become inevitable, the patient is exposed to the risk of infection with human immunodeficiency virus (HIV) and other blood-borne pathogens. Prevention of infant anaemia is clearly of critical importance, yet current coverage with antimalarial interventions and micronutrient supplementation is poor in many African countries due to financial, logistic and technical constraints.⁵³ In these settings cheap and effective interventions are needed to reduce the risk of infant anaemia and improve child survival.

DELAYED UMBILICAL CORD CLAMPING

One intervention that has not been adequately studied is delayed umbilical cord clamping, which may help to prevent or slow the onset of infant anaemia by augmenting the infant's red cell mass at birth. Delayed cord clamping (DCC) is a simple and cost-free delivery procedure that makes use of readily available blood from the fetoplacental compartment. It is estimated that the total fetoplacental blood volume is roughly 120 ml/kg of fetal weight. After immediate cord clamping the distribution of blood reflected in the fetus:placenta ratio is approximately 2:1. Allowing placental transfusion to occur for three minutes results in a larger fetal blood volume (ratio 5:1).^{54;55} After three minutes the transfer of blood will stop with approximately 20 ml/kg of blood remaining in the placenta (figure 3). Compared with immediate clamping, a clamping delay of 3 minutes provides an additional 20-35 ml/kg of body weight. For a 3 kg infant with a packed cell volume of approximately 0.50 at birth, this amounts to an additional 45 mg of iron added to iron stores. This is a sufficient amount of iron to meet the requirements of a 6 month-old infant for more than 3 months.²⁶

Figure 3. Distribution of blood in fetoplacental compartment depending of timing of cord clamping (adapted from Linderkamp 1982 and Yao 1969)

In addition to the timing of cord clamping, three other birth-related factors might influence the amount of placental transfusion at birth:

- (1) the position of the baby in relation to the placenta;
- (2) the method of delivery (vaginal vs caesarean section); and
- (3) uterine contractions during the third stage

The rate of placental transfusion is markedly influenced by the position of the delivered infant. An infant held 50-60 cm above the placenta will not receive any blood from the placenta. At 10 cm above or 10 cm below the level of the placenta, infants receive the maximum possible amount within 3 minutes of birth. Keeping the infant 40 cm below the placenta hastens placental transfusion to almost completion within 1 minute.^{54;56}

Infants born by caesarean section do not participate in placental transfusion when they are laid on the mother's abdomen for about 40 s after birth. Failure of the incised and possibly atonic uterus to squeeze the placenta might explain why infants delivered by CS are at greater risk than vaginally born infants to lose blood when lifted above the placenta.⁵⁴ Intravenous administration of oxytocin to the mother after delivery of the first shoulder and lowering the infant below the level of the placenta guarantee placental transfusion.

After vaginal delivery, uterine contractions usually start again between 1 and 3 minutes postpartum. When oxytocics drugs are administered immediately after delivery of the first shoulder, uterine contractions will start earlier and hasten the transfer of blood into the baby.^{57;58}

Table 1. The paediatricians' debate about effects of delayed cord clamping

Issue	Pro	Con
Increased blood volume	Prevents hypovolaemia and reduces IRDS risk ^{59;59-61}	Volume overload in preterms ⁶²⁻⁶⁴
Increased red cell mass	Prevents anaemia ⁶⁵⁻⁶⁸ Better tissue oxygenation	Polycythaemia and hyperviscosity syndrome ^{62;69} Hyperbilirubinaemia ^{62;68}
Thermal control	Increases skin temperature ⁷⁰	Hampers prevention of hypothermia
Resuscitation	Initiation of resuscitation with intact umbilical cord reduces hypovolaemia and improves tissue oxygenation ^{71;72}	Obstructs immediate resuscitation

After decades of debate there is still little agreement among paediatricians about the optimal time to clamp the umbilical cord after birth. Similarly, consensus regarding potential harmful effects of DCC is lacking (table 1). Intuition, unsystematic clinical experience, and pathophysiologic rationale are insufficient grounds for clinical decision-making. Many paediatric procedures have had a long history of being introduced into clinical practice without undergoing careful evaluation necessitating clinical trials. Evidence based medicine stresses the examination of evidence from clinical research. Before DCC can be adopted as a useful strategy to prevent or slow down the onset of infant anaemia in malaria-endemic areas, this intervention needs to be justified in terms of quantitative outcome measures. Maternal parameters should not be overlooked in future research on cord clamping, as maternal safety, in particular postpartum haemorrhage, is said to be associated with the timing of cord clamping.⁷³

RESEARCH QUESTIONS AND OUTLINE OF THE THESIS

This thesis aims to evaluate DCC as a possibly useful strategy to prevent or slow down the onset of infant anaemia in malaria-endemic areas. The focus in this thesis is on the sub-group of infants who are born appropriate-for-gestational-age (AGA).

Objectives

- (1) To follow a cohort of Zambian mother-infant pairs from birth to six months postpartum to determine predictors of iron deficiency anaemia in term (AGA) infants
- (2) To summarize previous research findings on DCC in a systematic review, with special attention for short and long term effects, in
 - a. term AGA infants, and
 - b. small-for-gestational-age (SGA) infants
- (3) To assess short term haematological effects of DCC in term (AGA) infants
- (4) To ascertain whether DCC at birth is effective in reducing anaemia in term (AGA) babies in a malaria-endemic area
- (5) To assess the accuracy of the Haemoglobin Colour Scale as a simple and cheap method to estimate Hb values in neonatal and infant blood

- (6) To propose a practice guideline on cord clamping for resource poor countries taking account of the safety of both mothers and infants.

Outline of the thesis

In *chapter 2* the iron status of Zambian term infants in the first six months of life is described and its relation to the obstetrical procedure, used infant food intake, malaria parasite prevalence and growth.

Chapter 3 contains two systematic reviews summarising previous DCC research findings. As the potential adverse effects of DCC are considered to affect SGA infants more than AGA babies, we decided to evaluate the intervention separately for both groups. Part A focussed on DCC in term AGA infants and formed the basis for the design of new research initiatives that are described in chapters 4, 5 and 7. Part B is a systematic review on DCC in LBW infants. In developing countries the group of LBW babies is predominantly formed by SGA infants, which might have different pathophysiological responses to the extra amount of red blood cells received during placental transfusion. The available evidence is critically appraised and forms the prologue for a future randomised controlled trial in SGA babies in developing countries.

Chapter 4 reports the results of a randomised controlled trial that was performed in a non-malarious setting (Libya) and focussed on the short term effects of DCC. No significant difference was found between the DCC group and the immediately clamped group with respect to total serum bilirubin levels at 24 h, the number of infants requiring phototherapy, and the prevalence of symptomatic polycythaemia-hyperviscosity syndrome.

The long term haematological effects of DCC are described in *Chapter 5*, which reports the findings of a randomised controlled trial from rural Zambia, where malaria is endemic. During this trial the safety of the mother was also closely monitored.

Due to the insidious nature of infant anaemia, the disorder frequently remains undetected and untreated by health care workers. Furthermore, blood transfusions for severe anaemia may be prescribed on the basis of inaccurate haemoglobin measurement, thus exposing the patient unnecessarily to the risk of infection with HIV or other blood-borne pathogens. The haemoglobin colour scale (HCS) is a simple, low-cost and sustainable method that might give a more accurate estimation of the Hb-level than using clinical signs. In *Chapter 6* the diagnostic accuracy of the HCS is evaluated for use in early infancy.

In *Chapter 7* new knowledge on DCC is translated into an evidence-based practice guideline for resource-poor countries. A practical and simple flow chart for quick reference is included.

Finally, in *chapter 8*, the limitations of the studies in this thesis are discussed and future perspectives are outlined.

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2 A COHORT STUDY OF HAEMOGLOBIN AND ZINC-PROTOPORPHYRIN LEVELS IN TERM ZAMBIAN INFANTS: EFFECTS OF IRON STORES AT BIRTH, COMPLEMENTARY FOOD, AND PLACENTAL MALARIA

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ABSTRACT

Objective: To examine zinc-protoporphyrin (ZPP) and haemoglobin levels, and to determine predictors of iron deficiency anaemia (IDA) in Zambian infants.

Design: A cohort of infants was followed bi-monthly during the first 6 months of life, and iron status, food intake, malaria parasitaemia and growth were monitored. At 4 months the infants were divided into two groups, and the data were analysed according to whether or not they were exclusively breastfed.

Setting: Mpongwe District, Copperbelt Province, Zambia.

Subjects: 91 women and their normal birth weight (NBW) infants.

Results: Almost two thirds of infants were born with low iron stores as defined by ZPP levels, and this proportion increased with age. Over 50% had developed IDA by 6 months. Exclusive breastfeeding at 4 months could be a protective factor for IDA (odds ratio (OR): 0.2; 95% confidence interval (CI): 0.0-1.1). Exclusively breastfed infants had higher haemoglobin values at 4 and 6 months (mean difference 0.6; 95% CI: 0.1-1.2 g/dL and 0.9; 95% CI: 0.2-1.7 g/dL, respectively), compared to infants with early complementary feeding. In univariate analysis past or chronic placental malaria appeared to be a predictor of IDA at 4 and 6 months, but the significance was lost in multivariate analysis.

Conclusions: Zambian NBW infants are born with low iron stores and have a high risk to develop IDA in the first 6 months of life. Continuation of exclusive breastfeeding after 4 months is associated with a reduction of anaemia. The effect of placental malaria infection on increased risk of infant IDA could not be proven.

INTRODUCTION

Early infancy is a period of rapid growth with high iron requirements. In the absence of iron supplementation over 50% of infants in resource-poor countries develop iron deficiency anaemia by the age of six months (Crawley, 2004; Schellenberg *et al*, 2003; van Eijk *et al*, 2002). Iron deficiency anaemia is associated with increased mortality (Brabin *et al*, 2001; Brabin *et al*, 2003), and has been related to impaired mental and motor development (Grantham-McGregor & Ani, 2001). Iron endowment at birth is necessary to protect the child from iron deficiency during the first months of life, when breast milk is the only available source of iron (Michaelsen *et al*, 1995). Thereafter, dietary iron becomes critical. From a macronutrient point of view exclusive breastfeeding is recommended for the first 6 months of life for infants in resource-poor settings (Kramer & Kakuma, 2002). However, this recommendation might not be beneficial to infants born with low iron stores (Dewey *et al*, 1999), as breast milk contains little iron. In sub-Saharan Africa complementary foods and family foods are often introduced at much earlier ages than recommended. The implications of this for infant iron status and anaemia are unclear.

Zinc-protoporphyrin (ZPP) levels can be used as a proxy for total body iron contents. Raised ZPP levels indicate incomplete iron incorporation into protoporphyrin, as zinc substitutes for iron when stores are low. ZPP is regarded as a sensitive indicator of iron deficiency (Kling, 2006; Juul *et al*, 2003; Lott *et al*, 2006; Miller *et al*, 2003; Rettmer *et al*, 1999).

We followed a cohort of Zambian mother-infant pairs from birth to six months postpartum and examined their ZPP and haemoglobin levels in relation to their feeding pattern. The aim of this analysis was to determine factors associated with iron deficiency anaemia in these normal birthweight infants.

METHODS

Study area, enrolment and study population

The study location was Mpongwe District, a rural region of the Copperbelt Province in Zambia, approximately 1000m above sea level. Malaria transmission in this area is holoendemic. Peak malaria transmission occurs from November to April. A total of 91 pregnant women in the latent phase of labour attending the labour ward at Mpongwe Mission Hospital were recruited from May to July 2004 as part of a randomised controlled trial investigating the effects of delayed cord clamping on haematological status in full term infants. This trial, registered under the controlled-trials.com identifier ISRCTN48735857, has been described in detail elsewhere (van Rheen *et al*, 2007). The women had uncomplicated singleton pregnancies and their babies were included in the study when the birth weight was over 2500 g. The cohort of mother-infant pairs was followed two monthly during the first 6 months post-partum. Except for the cord clamping, no other interventions were introduced. At 4 months the infants were divided into two groups, according to whether or not they were exclusively breastfed.

Data collection

Labour ward:

A structured survey questionnaire was used to gather past obstetrical and medical details and a venous blood sample was taken from the mother in the first stage of labour for determination of haematological status and to obtain a malaria smear. Following vaginal delivery of the baby and clamping of the umbilical cord a sample of cord blood was collected for laboratory analysis, while the placenta was still inside the uterus. Birth weight was measured using a Salter balance (nearest 10 grams). One cubic centimetre tissue samples were obtained from a central position of the maternal surface of the placenta, half the distance from the centre to the edge of the organ and placed in 10% neutral buffered formalin for fixation. From the blood present in the placenta biopsy site a malaria smear was made. Assessment of gestational age was done using the Ballard-ext method (Verhoeff *et al*, 1997) shortly before discharge from hospital.

Infant follow-up:

Mothers living within a radius of 4 kilometres from the hospital were asked to return to the hospital-based Mother and Child Health Clinic every two months. Non-attendees and mother-infant pairs living further away were visited in their villages by the investigators to reduce loss to follow-up. At two months baseline data concerning socio-economic and demographic background were collected. Literacy status was assessed by asking the mother to read a simple sentence in the local language. Maternal weight was estimated in bare feet (nearest kg) using a standing weigh scale and height (nearest cm) using a height board. Mid-upper-arm circumference (MUAC) was measured on the right arm, hanging loosely, with a TALC insertion tape (TALC, St. Albans, UK) and recorded to the nearest 0.1 cm. At each follow-up visit information was collected on infant feeding practices and morbidity in the previous two months. Infants' finger prick blood was collected at two, four and six months to assess Hb, ZPP and malaria smear. Infants with Hb concentrations below 7 g/dL at follow-up were given therapeutic iron supplements for two months. Infants with malaria were treated with a therapeutic dose of sulfadoxine-pyrimethamine following local guidelines. All other illnesses were treated or referred as required.

Laboratory investigations

Blood samples were collected in EDTA microtainers (Becton Dickinson, Franklin Lakes, USA) and immediately stored at +4 degrees Celsius. Within 6 hours the Hb level was measured using a HemoCue (HemoCue AB, Ängelholm, Sweden) and packed cell volume (PCV) by Micro-haematocrit. Zinc-protoporphyrin (ZPP) level, in $\mu\text{mol/mol}$ haem, was assessed using a ZP Haematofluorometer (Aviv Biomedical, Lakewood, NJ, USA) within 48 hours of collection. Blood smears were examined for malaria parasites. A thick smear was considered negative if 100 microscopic fields revealed no parasites. For positive smears, malaria parasites were counted against 300 leucocytes. The placenta tissue samples were kept up to 9 months until processed and embedded in paraffin

wax by standard techniques. Paraffin sections 4 µm thick were stained with haematoxylin and eosin.

Definitions

Exclusive breastfeeding was defined as breast milk with no other liquids or solids. Fetal anaemia was defined as a cord Hb less than 12·5 g/dL, which is two standard deviations below the mean cord Hb for non-malarious western populations (Brabin, 1992). Infant anaemia was defined as a Hb concentration more than two standard deviations below the mean of similarly aged infants from an iron-supplemented USA reference population not exposed to malaria (Dallman, 1988). Reference values for the ages of 2, 4 and 6 months were respectively 9·4 g/dL, 10·3 g/dL, and 11·0 g/dL. Anaemia in pregnant women was defined as Hb less than 11 g/dL (WHO, 2001). Iron deficiency was defined as ZPP above 80 µmol/mol haem for adults and infants (Domellof *et al*, 2002; Hinchliffe, 1999; Juul *et al*, 2003; Kling, 2006; Lott *et al*, 2006; Rettmer *et al*, 1999; Soldin *et al*, 2003). No published ZPP cut-off values are established to diagnose iron deficiency in cord blood. A recently published cross-sectional study among babies born to non-anaemic mothers found that infants born after 35 weeks completed weeks of gestation had mean ZPP levels in cord blood of 73 µmol/mol haem (Lott *et al*, 2006). As this result corresponded to normal values in older infants, we decided also to use 80 µmol/mol haem as cut-off for fetal iron deficiency. The combination of abnormal values for both ZPP and Mean Cell Haemoglobin Concentration (MCHC) is a more sensitive indicator of iron deficiency (IN-ACG, 1985). We used MCHC cut-offs of 32 g/dL for pregnant women (Letsky, 1991) and 28 g/dL for newborns (Saarinen & Siimes, 1978; Serjeant *et al*, 1980). MCHC estimates were not available at 4 and 6 months follow-up. Iron deficiency anaemia was defined as Hb < -2 SD in combination with ZPP > 80 µmol/mol haem.

Malaria was defined as the presence of asexual stage parasites in thick smears at follow-up, independent of the presence of clinical signs and symptoms. A history of a positive blood smear result at a local health facility together with clinical signs or symptoms of malaria in the last two weeks before infant review was considered as evidence for a recent malaria infection. The histopathological slides of placenta tissue were classified according to the criteria of Ismail *et al* (2000). Presence of parasites without pigment deposition is considered an *acute infection*, presence of parasites and a significant amount of pigment deposition a *chronic infection*, and presence of pigment deposition without parasites a *past infection*. Adequate intermittent presumptive treatment was defined as at least two doses of sulfadoxine-pyrimethamine during the second and third trimesters of pregnancy. Adolescence was defined as less than 20 years of age.

Statistical methods

Data were collected on standardised forms, and analysed with SPSS for Windows, version 12.0.1 (2003). Student *t* tests and Chi-square tests were used to compare baseline characteristics between groups. For non-parametric data the Mann-Whitney *U*-test was used. All tests were two-tailed. A *P*-value < 0·05 was considered significant.

The haematological follow-up data were analysed as continuous variables with analysis of covariance (ANCOVA) and as categorical variables with logistic regression. Baseline imbalances which could influence infant haematological outcome (maternal Hb, parity, cord Hb and cord clamping time) were controlled for in these analyses. The longitudinal development of weight-for-age z-scores was analysed with use of a multivariate ANOVA for repeated measurements. The multivariate ANOVA tested an age effect, a feeding practice effect, as well as the interaction between age and feeding practice.

Stepwise logistic regression with backward elimination was used to analyse factors related to iron deficiency anaemia in early infancy (up to six months of age). Only cases with a complete haematological follow-up (birth and 4 and 6 months) were included. Explanatory variables with a p-value < 0.10 in univariate analysis were kept in the final multivariate model. This level was chosen because of the limited number of infants in the analysis. Effect size was expressed as odds ratio (OR) and 95% confidence interval (CI).

Ethical approval

The women were contacted in the latent stage of labour to obtain informed consent. The consent information sheet was written in Lamba, the local language. In cases of illiteracy, the midwife on duty read the form. Mothers gave written consent or a thumb impression. The research protocol was approved by the Research Ethics Committee of the Liverpool School of Tropical Medicine and by the Board of Management of Mpongwe Mission Hospital. The Mpongwe District Health Management Team, the Chairpersons of the health neighbourhoods (lay people representing the community for health issues) and the local chiefs were informed and all provided signed consent.

RESULTS

Figure 1. Study profile

Figure 1 shows the study profile. Between May and July 2004, 91 mother-infant couples entered the study and were actively followed on a two monthly basis until the infants reached the age of six months. Infant haematological data were complete in 60 cases. The follow-up period ended in January 2005. During the follow-up period four children died: one in the neonatal period, the others after four months of age. In two of these infants mild anaemia had been diagnosed during the follow-up visit prior to death ($Hb > 7.0$ g/dL).

Table 1 shows haematological characteristics, weight and feeding pattern of the cohort of infants. Almost two third of the infants had low iron stores at birth, indicated by a ZPP value above the cut-off of $80 \mu\text{mol/mol}$ haem, and 13% of infants had fetal anaemia. Hb levels declined throughout the observation period and did not show a rise after the physiological nadir at 2-3 months. At 4 months 73% of infants had iron deficiency, and 24% were anaemic. By 6 months half of the infants were anaemic. Three infants had Hb levels below 7 g/dL at 6 months and received iron supplementation.

Table 1. Infant haematological characteristics, growth, feeding pattern, and disease incidence during the observation period

	Birth	2 months	4 months	6 months
Haemoglobin (g/dL)	14.6 (1.6)	12.0 (1.5)	11.4 (1.4)	10.4 (1.8)
Anaemia (Hb < -2 SD)*	13%	2%	24%	52%
Zinc-protoporphyrin (mcmol/mol/haem)	96 (26)	-	127 (81)	163 (115)
Iron deficiency (ZPP > 80 μ mol/mol haem)	65%	-	73%	75%
Iron deficiency anaemia (Hb < -2 SD and ZPP > 80 μ mol/mol haem)	9%	-	24%	47%
Weight (kg)	3.130 (0.326)	5.5 (0.6)	6.9 (0.7)	7.6 (0.8)
Weight gain during previous interval (g/day)	-	38 (9)	23 (10)	12 (7)
Exclusively breast-fed (proportion)	100%	98%	34%	3%
Partially breast-fed and:				
Maize-based porridge	-	2%	66%	83%
Maize-based porridge and fruits	-	-	-	14%
Proportion reported to have had diarrhoea in the previous two weeks	-	5%	15%	27%
Proportion with positive malaria smear	0%	0%	0%	13%

Data are mean (SD) unless indicated otherwise

* Cut-offs: birth: < 12.5 g/dL; 2 months: < 9.4 g/dL; 4 months: < 10.3 g/dL; 6 months: < 11.0 g/dL

In the majority of infants complementary foods were introduced early: by four months only a third of the children were exclusively breastfed. The complementary foods used in the study population were maize-based porridges. At introduction the porridge was thin with low energy density. The consistency became thicker (and more energy-dense) with increasing age of the infant. The early introduction of complementary foods was associated with a gradual increase in the number of diarrhoea episodes, as reported by the mother. At six months more than a quarter of the infants suffered from diarrhoea in the two weeks prior to the follow-up visit. Malaria infections were not diagnosed before the age of four months. At six months, malaria was diagnosed in 13% of cases.

At 4 months the cohort was divided into two groups, based on complementary feeding practice. Table 2 shows that the mothers who were exclusively breast-feeding at 4 months and who introduced complementary foods early were comparable in terms of age, nutritional state, antenatal iron supplementation, the use

of intermittent preventive antimalarial treatment, socio-economic status and literacy level. Mothers who introduced early complementary foods were more likely to be primigravidae ($p=0.031$). Maternal haematological baseline characteristics and placental histopathology did not differ between groups and infants were comparable at birth in terms of anthropometric and haematological parameters and cord clamping times.

Table 2. Baseline characteristics of subjects in groups based on feeding practice at 4 months

	Exclusively breast-fed at 4 months (n=27)	Compl. foods introduced before 4 months (n=64)	P-value
Age at introduction of complementary foods (wks)	21 (4)	13 (4)	
Maternal characteristics			
Age (years) - median (range)	22.0 (15.9-39.9)	21.7 (15.5-46.0)	
Adolescents	48%	39%	
BMI (kg/m ²)	22.2 (2.2)	22.8 (2.9)	
MUAC (cm)	24.9 (2.2)	25.1 (1.9)	
Primigravidae	19%	42%	0.031
Proportion iron deficiency anaemia	26%	17%	
Infant characteristics			
Proportion delayed cord clamping	48%	50%	
Cord clamping time (secs)	134 (137)	174 (188)	
Gestational age (weeks)	39.9 (1.7)	40.0 (1.5)	
Birthweight (g)	3114 (335)	3138 (324)	
Females	56%	45%	
Cord haemoglobin (g/dL)	14.2 (1.8)	14.7 (1.5)	
Proportion fetal anaemia	22%	8%	
Zinc-protoporphyrin (μmol/mol haem)	98 (21)	95 (27)	

Data are mean (SD) unless indicated otherwise

Table 3. Comparison of haematological results between exclusively and partially breast-fed infants

	Exclusively breast-fed at 4 months	Compl. foods Introduced before 4 months	Mean difference (95% CI)	Odds ratio (95% CI)	P-value
Age 4 months					
Haemoglobin (g/dL)	11.8 (1.0)	11.2 (1.5)	0.6 (0.1; 1.2)		0.033
Anaemia (Hb < 10.3 g/dL)	8% (2/24)	33% (16/49)		0.19 (0.04; 0.90)	0.024
Zinc-protoporphyrin (mcmol/mol haem)	107 (46)	136 (91)	-29 (-61; 4)		0.081
Iron deficiency (ZPP ≥ 80 mcmol/mol haem)	67% (16/24)	73% (37/51)		0.76 (0.27; 2.16)	0.602
Iron deficiency anaemia (Hb < 10.3 g/dL and ZPP ≥ 80 mcmol/mol haem)	8% (2/24)	33% (16/49)		0.19 (0.04; 0.90)	0.024
Positive malaria smear	0%	0%		-	-
Age 6 months					
Haemoglobin (g/dL)	10.9 (1.1)	10.0 (2.0)	0.9 (0.2; 1.7)		0.015
Anaemia (Hb < 11.0 g/dL)	52% (14/27)	59% (26/44)		0.75 (0.28; 1.96)	0.550
Zinc-protoporphyrin (mcmol/mol haem)	137 (80)	180 (131)	-43 (-93; 6)		0.087
Iron deficiency (ZPP ≥ 80 mcmol/mol haem)	78% (21/27)	78% (35/45)		1.00 (0.32; 3.15)	1.000
Iron deficiency anaemia (Hb < 11.0 g/dL and ZPP ≥ 80 mcmol/mol haem)	44% (12/27)	48% (21/44)		0.88 (0.33; 2.29)	0.788
Positive malaria smear	7% (2/27)	16% (7/45)		0.43 (0.08; 2.26)	0.311

Data are mean (SD) unless indicated otherwise. Effect size is expressed as mean difference for continuous variables and as odds ratio for categorical variables, and are controlled for maternal Hb, parity, cord Hb and cord clamping time.

Haematological follow-up data show that for exclusively breast-fed infants Hb was significantly higher at 4 and 6 months (mean difference 0.6; 95% CI: 0.1, 1.2 g/dL and 0.9; 95% CI: 0.2, 1.7 g/dL, respectively, table 3). The proportion of

cases with Hb-values below the cut-off for anaemia was significantly lower in the exclusively breast-fed group at 4 months (OR: 0.19; 95% CI: 0.04, 0.90), but not at 6 months (OR: 0.75; 95% CI: 0.28, 1.96). Although absolute ZPP-values in exclusively breast-fed infants were lower at 4 and 6 months (mean difference -29; 95% CI: -61, 4 $\mu\text{mol/mol}$ haem and -43; 95% CI: -93, 6 $\mu\text{mol/mol}$ haem, respectively), the proportion of infants with ZPP-values above the cut-off level for iron deficiency did not differ significantly. The proportion of infants with iron deficient anaemia was significantly lower in the exclusively breast-fed group at 4 months (OR: 0.19; 95% CI: 0.04, 0.90), but not at 6 months (OR: 0.88; 95% CI: 0.33, 2.29). At 6 months the proportion of infants with a positive malaria smear was not different, but diarrhoea incidence was significantly lower in the exclusively breast-fed group (OR: 0.23; 95% CI: 0.04, 0.84).

There was a trend with higher weight-for-age z-scores in exclusively breast-fed infants, but this difference was not significant in multivariate ANOVA for repeated measurements (figure 2).

Figure 2. Mean weight for age Z scores in relation to complementary feeding pattern. Only infants with a complete follow-up were included (n = 60)

Table 4 shows the results of the univariate and multivariate logistic regression analysis. In univariate analysis past or chronic placental malaria was significantly associated with iron deficiency anaemia at 4 months (OR: 4.0; 95% CI: 1.1, 14.4) and at 6 months (OR: 3.8; 95% CI: 1.2, 12.5). A positive malaria smear at 6 months (OR: 8.5; 95% CI: 1.0, 75.2), maternal iron deficiency anaemia (OR: 3.5; 95% CI: 0.9, 14.1), and exclusive breast-feeding at 4 months (OR: 0.2; 95% CI: 0.0, 1.1),

showed borderline significant associations with iron deficiency anaemia at 4 or 6 months. Iron deficiency anaemia at 4 months was a highly significant predictor of iron deficiency anaemia at 6 months (OR: 25.1; 95% CI: 3.0, 210). In multivariate analysis the influence of iron deficiency anaemia at 4 months remained significantly associated with iron deficiency anaemia at 6 months. The significance of placental malaria was lost in multivariate analysis, and exclusive breast feeding at 4 months remained marginally associated with protection from iron deficiency anaemia.

Table 4. Estimates from logistic regression analysis of factors influencing infant haematological status.

Dependent variable	Analysis	Explanatory variables	Regression coefficient	Odds ratio (95% CI)	P-value
Age 4 months					
Iron deficiency anaemia	Univariate	Positive placenta histology for malaria	1.386	4.0 (1.1; 14.4)	0.033
		Maternal IDA	1.258	3.5 (0.9; 14.1)	0.076
		Exclusive breast-feeding at 4 mo	-1.517	0.2 (0.0; 1.1)	0.065
	Multivariate	Positive placenta histology for malaria	1.235	3.4 (0.8; 14.4)	0.091
		Maternal IDA	1.768	5.9 (0.9; 37.3)	0.061
		Exclusive breast-feeding at 4 mo	-1.948	0.1 (0.0; 1.1)	0.057
Age 6 months					
Iron deficiency anaemia	Univariate	Positive placenta histology for malaria	1.344	3.8 (1.2; 12.5)	0.026
		IDA at 4 months	3.224	25.1 (3.0; 210)	0.003
		Positive malaria smear at 6 months	2.135	8.5 (1.0; 75.2)	0.056
	Multivariate	Positive placenta histology for malaria	1.187	3.3 (0.8; 13.4)	0.099
		IDA at 4 months	3.100	22.2 (2.5; 194)	0.005

Only cases with a complete haematological follow-up (birth and 4 and 6 months) were included. Explanatory variables with a p-value < 0.10 in univariate analysis were kept in the final multivariate model.

Iron deficiency anaemia is defined as haemoglobin lower than -2 SD and zinc-protoporphyrin > 80 µmol/mol haem.

Iron deficiency is defined as zinc-protoporphyrin > 80 µmol/mol haem.

DISCUSSION

This cohort study showed that in absence of iron supplementation over 50% of Zambian normal birth weight infants developed iron deficiency anaemia by the age of 6 months. Almost two thirds of the infants were born with low iron stores, and this proportion increased with infant age. Hb levels in the study population continued to decline after the physiological nadir at two months. This pattern is observed in other studies from sub-Saharan Africa with many infants reaching their lowest Hb values 6 to 12 months after birth (le Cessie *et al*, 2002; van Eijk *et al*, 2002; Kitua *et al*, 1997).

Introduction of complementary foods before the age of 4 months was associated with a higher incidence of iron deficiency anaemia in these normal birth weight Zambian infants who were not supplemented with iron. The decision to start introducing complementary foods early was not related to maternal health or nutritional status or to infant health, as study characteristics were similar between exclusively breast fed babies and those with early complementary feeding. The group who stopped exclusive breastfeeding early contained a higher proportion of primigravid women. This may be a chance finding or could indicate that their perception of insufficient milk supply was stronger. The average weight of infants was lower in the early complementary feeding group. Early complementary feeding has been associated with growth faltering in breastfed Malawian infants and with increased risk of respiratory infection (Kalanda *et al*, 2006). Growth faltering could also lead to interruption of breastfeeding with earlier introduction of complementary foods. In that case the reason to supplement with porridge affects the outcome, rather than the supplementation itself: *confounding by the indication* (Miettinen, 1983). The solution to this problem would be a randomised controlled design. However, it is very difficult to randomise a behaviour such as breastfeeding, which is inherently a part of a woman's motherhood. This issue has been discussed in a Kenyan study in relation to mother-to-child transmission of HIV in breastfed infants (Nduati *et al*, 2000).

Our findings support the conclusion of other investigators that complementary feeding before 6 months of age does not prevent infant anaemia in developing countries (Dewey *et al*, 2004). Poorer haematological outcomes with early complementary feeding could relate to low bioavailability of food iron. In this study population the complementary foods used were maize-based porridges. Although the iron contents of these porridges is higher than estimates for human milk (Dorea, 2000; Picciano, 2001) bioavailability is much lower (<5%) than human milk (20-50%) (Griffin & Abrams, 2001). Poor haematological outcomes with early introduction of complementary foods could also relate to an increased incidence of diarrhoea (Arifeen *et al*, 2001). Almost two thirds of iron losses in infancy occur when cells are extruded from the intestinal mucosa with the remainder from cells shed from the skin and urinary tract. For this reason during episodes of diarrhoea iron losses may be increased (Osaki, 1993). This is consistent with the significantly higher population of infants with diarrhoea at 6 months in the group who received early complementary foods. The haematological benefits of continuation of exclusive

breast-feeding after 4 months disappeared at 6 months, when the need for iron for growth and to replace losses can no longer be met by human milk alone

Infant anaemia is usually multi-factorial in origin, but malaria probably plays a key etiologic role in endemic countries. The presence of histological evidence of past or chronic malaria infection in the placenta biopsy was a predictor for iron deficiency anaemia in infants aged 4 or 6 months in the univariate analysis. In the multivariate analysis this effect disappeared. The existence of a direct and causal relationship can not be proven in this observational study, but others have also reported the effect of placental malaria infection on increased risk of infant anaemia (Cornet *et al*, 1998; Redd *et al*, 1994). It is uncertain whether this association was due to comparable malaria exposure in mothers during pregnancy and their infants after birth, or whether it related to recrudescence of infant malaria or immune sensitisation occurring as a consequence of congenital infection. A further explanation is that transplacental transport of iron is disturbed by pathological changes of the syncytiotrophoblast with placental malaria. Although there is general agreement that iron is bound to transferrin at the maternal side of the placenta, very little is known about how iron is subsequently transported across the syncytiotrophoblast (Brabin *et al*, 2004a; Fuchs & Ellinger, 2004; Srai *et al*, 2002). Seasonal factors could also alter anaemia risk, but in view of the short study duration it was not possible to assess these. As mothers in this study delivered in hospital they do not represent the majority of women who deliver at home where both maternal and infant anaemia prevalence may be much higher.

A total of 13% of Zambian normal birth weight infants had fetal anaemia. These infants had significantly higher mean ZPP values than those with no fetal anaemia, indicating poorer iron status. Fetal anaemia is rare in affluent countries and is mostly caused by red cell alloimmunization, parvovirus infection or chronic fetomaternal haemorrhage. In poor-resource countries fetal anaemia is more common and associated with severe maternal iron deficiency anaemia. In malarious areas the degree of fetal anaemia is out of proportion to the level of maternal anaemia and is likely to relate to the density of placental malaria as well as the risk of developing infant anaemia (Brabin, 1992; Brabin *et al*, 2004b). Glucose-6-phosphate dehydrogenase deficiency (G6PDD) might contribute to fetal anaemia. It is reported as occurring in 23.5% (95% CI: 16.7, 30.1%) of Malawian infants (Brabin *et al*, 2004).

We used ZPP levels at birth as a proxy for total body iron contents, and found that normal birth weight Zambian infants already had low iron stores at birth. Although ZPP has the advantage of low cost and simplicity, its specificity may be low as it can be increased by malaria and other infections, chronic inflammation and haemoglobinopathies (Asobayire *et al*, 2001; Stoltzfus *et al*, 2000), which may have led to overestimation of ZPP values at 6 months, when a small number of infants had positive malaria smears (13%).

In conclusion, Zambian normal birth weight infants are born with low iron stores and have a high risk to develop iron deficiency anaemia in their first six months of life. Continuation of exclusive breast-feeding after 4 months could reduce this risk compared to earlier introduction of complementary foods. The importance of these findings for evaluation and introduction of nutritional interventions to reduce in-

fant anaemia risk needs to be assessed in particular in the context of traditional feeding practices and exclusive breast feeding, as the proportion of exclusively breast-fed infants remains persistently low in many African countries (Jones *et al*, 2003).

ACKNOWLEDGEMENTS

We thank the management board of Mpongwe Mission Hospital for their hospitality; and maternity and laboratory staff for their assistance.

This study received financial support from the Amsterdam-Liverpool Programme for research in Tropical Child Health and from the Bemmels Support Group.

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3a LATE UMBILICAL CORD CLAMPING AS AN INTERVENTION FOR REDUCING IRON DEFICIENCY ANAEMIA IN TERM INFANTS IN DEVELOPING AND INDUSTRIALISED COUNTRIES: A SYSTEMATIC REVIEW

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SUMMARY

This review evaluates the potential of delayed cord clamping for improving iron status and reducing anaemia in term infants, and for increasing the risk of polycythaemia and hyperbilirubinaemia. We applied a strict search protocol to identify controlled trials of early versus late cord clamping. Four trials from developing and four trials from industrialised countries were finally assessed. Two of the four studies from developing countries found a significant difference in infant haemoglobin levels at 2-3 months of age in favour of delayed cord clamping. This difference was more marked when mothers were anaemic. Three of four studies from industrialised countries showed a significant difference in haematocrit levels in favour of delayed clamping. Although meta-analysis showed an increased risk for hyperbilirubinaemia of 12%, no studies reported the need to apply phototherapy or perform exchange transfusion. We conclude that delayed cord clamping in term infants, especially those with anaemic mothers, increases infant haemoglobin concentration by 2-3 months of age and reduces the risk of anaemia, without an associated increased risk of perinatal complications. In developing countries where fetal anaemia is common, the advantages of delayed cord clamping might be especially beneficial.

INTRODUCTION

It is estimated that 3.6 billion people are iron-deficient and that two billion of these are anaemic.¹ The most vulnerable groups are women of reproductive age and children under five years in developing countries.² Iron deficiency anemia has been related to impaired mental and motor development in infants and young children and there are indications that with iron therapy anaemic children fail to catch up with non-anaemic children.^{3,4} Generally, it is considered that the most important factors that cause iron deficiency are nutritional in origin. The two main interventions aimed at reducing iron deficiency, supplementation and iron fortification, have had limited success in reducing global iron deficiency, particularly in developing countries. Iron supplementation in infants in developing countries has been shown to be beneficial in reducing anaemia, even in malarious areas, but is logistically difficult.⁵

In view of this there is interest in evaluating the potential for improving the iron status of infants by enhancing their red cell mass with late umbilical cord clamping. It is estimated that in full-term infants, the total feto-placental blood volume is roughly 120 ml/kg of fetal weight. After immediate cord clamping the distribution of blood reflected in the fetus:placenta ratio is approximately 2:1. Allowing placental transfusion to occur for three minutes results in a larger fetal blood volume (ratio 4:1).^{6,7} To evaluate the haematological benefits of late cord clamping, infants need to be followed to at least two months of age, when a nadir occurs for mean haemoglobin (Hb) concentration in healthy term infants.⁸

The main objective of this analysis was to complete a systematic review of randomised controlled trials of early versus late cord clamping in term infants, in order to examine the effect of time of umbilical cord clamping on infant haematological status after two months of age. A second objective was to assess whether the time of cord clamping was associated with altered risk of polycythaemia and hyperbilirubinaemia in the first week of life.

In malarious areas of developing countries and in populations where maternal iron deficiency anaemia (IDA) is common, fetal anaemia (venous cord Hb < 125 g/L) is also common, occurring in up to 30% of babies. The advantages of placental transfusion are therefore potentially particularly beneficial as it is known that fetal anaemia is a risk factor for infant anaemia and, when associated with low birth weight, is related to post-neonatal infant mortality.⁹⁻¹³

METHODS

Searching

Trials were identified by searching MEDLINE (1966 to January 2003) and EMBASE (1988 to January 2003) using the search strategy developed by the Cochrane Collaboration adapted for use in PubMed.¹⁴ The optimal search strategy was combined with the MeSH terms “umbilical cord” AND “anaemia” OR “polycythaemia” OR “hyperbilirubinaemia”. In MEDLINE, articles related to selected studies were also examined. The Cochrane Database of Systematic Reviews, Database of Abstracts of Reviews of Effectiveness, The Cochrane Central Register of Controlled Trials, The Cochrane Database of Methodology Reviews, The Cochrane

Methodology Register, Health Technology Assessment Database and NHS Economic Evaluation Database (Cochrane Library, Issue 1, 2003) were searched using the key words “delayed cord clamping”. Reference lists of published trials were examined for other potentially relevant trials.

Selection

Controlled Trials that compared delayed cord clamping (DCC) with early cord clamping (ECC) in term infants were eligible for inclusion. ECC is usually done immediately after the baby is delivered and DCC after placental descent into the vagina, or when the cord stops pulsating. The minimum age at follow-up for participants was set at two months to ensure that the mean age when infant Hb is at its lowest was achieved. Trials looking at potential adverse effects of DCC (hyperbilirubinaemia or polycythaemia) were not excluded when the age of the infant was below two months, as these side effects largely occur in the first neonatal week.

Outcome measures were the change in haemoglobin or haematocrit and the change in indicators of iron status (serum ferritin, free erythrocyte protoporphyrin, total iron binding capacity, serum transferrin receptors, serum iron, transferrin, or transferrin saturation). Secondary outcome measures were a rise in total bilirubin and haematocrit in the first week. Trials were separated into those from developing and those from industrialised countries.

Validity assessment

The Delphi list was used as a means of quality assessment.¹⁵ Details of the studies included are given in Table 1. Studies were assessed for control or exclusion of relevant confounding factors that could influence infant haematological status: exact time of cord clamping, gravity enhancement, use of oxytocics prior to placental delivery, maternal anaemia, maternal or infant use of iron supplements, and infant feeding pattern. Confounding factors for hyperbilirubinaemia and polycythaemia were also assessed. These included post-maturity, intrauterine growth retardation, haemolytic disease and septicaemia. Four studies were from non-malarious, urban areas of developing countries¹⁶⁻¹⁹ and four from urban areas of non-malarious, industrialised countries, that were published in five articles.²⁰⁻²⁴

Table 1. Details of studies included

Reference	Country	Rural vs. urban	Malaria endemicity	Duration of follow-up	Number of study subjects	Study groups	Inclusion criteria	Exclusion criteria	Maternal mean Hb ± SD at enrolment (g/L)
Developing countries									
Geethanath et al, 1997 ¹⁶	India New Delhi	Urban	Very low	3 months	107 neonates	ECC (n=48) DCC (n=59)	Vaginally born term singleton neonates (GA not specified)	Eclampsia, cardiac failure, antepartum haemorrhage, Hb < 100 g/L	ECC: 125 ± 17 DCC: 127 ± 18 NS
Grajeda et al, 1997 ¹⁷	Guatemala Amatitlán	Semi-urban	None	2 months	88 term babies	ECC (n=29) DCC-1 (n=30) DCC-2 (n=29) <i>DCC-1: baby at placenta level</i> <i>DCC-2: baby below placenta</i>	Vaginally born term singleton neonates, birth weight ≥ 2000 g, GA ≥ 37 wk	Gestational diabetes, antepartum haemorrhage, major congenital anomalies, perinatal complications	ECC: 116 ± 21 DCC-1: 122 ± 18 DCC-2: 125 ± 14 NS
Gupta et al, 2002 ¹⁸	India New Delhi	Urban	Very low	3 months	102 babies from anaemic mothers	ECC (n=53) DCC (n=49)	Vaginally born term singleton neonates from anaemic mothers (Hb < 100 g/L)	Eclampsia, cardiac failure, antepartum haemorrhage, need for resuscitation, major congenital malformations	ECC: 89 ± 8 DCC: 92 ± 6 NS
Lanzkowsky, 1960 ¹⁹	South Africa Capetown	Urban	None	3 months	133 term Caucasian babies	ECC (n=63) DCC (n=70)	Vaginally born term singleton neonates	Rhesus negatives, antepartum haemorrhage, instrumental delivery	ECC: 115 ± 13 DCC: 115 ± 14 NS
Industrialised countries									
Linderkamp et al, 1996 ²⁰	Germany Heidelberg	Urban	None	5 days	30 term neonates	ECC (n=15) DCC (n=15)	Vaginally born term singleton neonates	-	-
Nelle et al, 1993 ²¹	Germany Heidelberg	Urban	None	5 days	30 term neonates	ECC (n=15) DCC (n=15)	Vaginally born term singleton neonates	-	-
Nelle et al, 1995/ 1996 ^{22,23}	Germany Heidelberg	Urban	None	5 days	30 term neonates	ECC (n=15) DCC (n=15)	Vaginally born term singleton neonates	-	-
Saigal et al, 1972 ²⁴	Canada Montreal	Urban	None	3 days	45 term neonates	ECC (n=15) DCC-1 (n=15) DCC-2 (n=15) <i>DCC-1: 1 min</i> <i>DCC-2: 5 min</i>	Vaginally born term singleton neonates	Gestational diabetes, congenital malformations, IUGR, neonatal infection, haemolytic disease	-

GA: gestational age; ECC: early cord clamping; DCC: delayed cord clamping; NS: not significant; IUGR: intrauterine growth retardation; Hb: haemoglobin; SD: standard deviation

The four trials in developing countries all assessed infant haematological status at or after two months of age. They were adequately randomised, and the most important baseline factors between study groups were similar. There were differences in definitions of time of delayed cord clamping and in the use of gravity enhancement. None reported whether oxytocics were used prior to cord clamping, which would stimulate uterine contraction and could increase placental transfusion. Maternal use of iron supplements during pregnancy was appropriately reported, but postnatal use of iron supplements was not. None of the infants received iron supplementation during follow-up. There were no significant differences in feeding patterns between the groups. The majority of infants were still exclusively breastfed at follow-up in three of the four studies.¹⁶⁻¹⁸ In the study from South Africa, only 40% of cases were still breastfed at follow-up. However, none of the artificially fed babies had received iron-fortified milk.¹⁹ No authors commented on malaria exposure, which would have been unlikely in the locations of these studies. Malaria would only have occurred in migrant women travelling from rural, malarious areas in other parts of the country.

The study of Geethanath et al.¹⁶ in India reported no loss to follow-up at three months. Grajeda et al.¹⁷ in Guatemala included 88 women and infants; 15 infants were lost to follow-up and a follow-up blood sample was not obtained from another four. The drop-out rates in the three study groups ranged from 13 to 27% and were not significantly different. There were no significant differences in socio-economic or demographic characteristics between the 19 drop-outs, except for a significantly lower birth weight (2.9 kg vs. 3.1 kg; $p < 0.05$). Baseline data did not include information on lost participants. Outcome was a continuous variable and corrections for missing data could not be made.

In a second study from India reported by Gupta et al., 102 mother-infant pairs were enrolled.¹⁸ At the three month follow-up, only 58 infants were available. The drop out rate was equal in both study groups. Mothers from the lost-to-follow-up group had significantly fewer antenatal visits and lower mean ferritin than mothers who attended for review.

Of the original 133 infants in the South African study by Lanzkowsky,¹⁹ 112 were seen at three months. Loss to follow-up did not differ between groups.

All but one of the studies from industrialised countries were controlled clinical trials. All assessed for polycythaemia and hyperbilirubinaemia up to five days of age. There was no loss to follow-up in these studies. Three trials were conducted by the same research group from Germany.²⁰⁻²³ The single trial from Canada examined both pre-term and term infants.²⁴ Only data for term infants has been analysed in this review.

Because of the type of intervention, blinding was not possible in any of the eight studies, and none stated whether the mother was informed of the baby's assigned group for cord clamping.

Data extraction

PvR applied inclusion criteria to potentially relevant trials and he and BB assessed trial quality. Data extracted included: country where the study was con-

Table 2a. Confounding factors: studies from developing countries

Reference	Timing of DCC	Gravity enhancement	Oxytocics prior to cord clamping	Maternal anaemia	Fetal growth retardation	Maternal use of iron supplements antenatally	Feeding practices at follow-up	Maternal use of iron supplements postnatally	Infant use of iron supplements
Geethanath et al, 1997 ¹⁶	After placental descent into vagina	Baby within 10 cm below introitus	Unknown	Exclusion factor	Unknown	No difference between ECC and DCC group	Majority exclusive breastfeeding. No difference between ECC and DCC group	Unknown	None
Grajeda et al, 1997 ¹⁷	When cord stopped pulsating	-Baby at level of placenta (DCC-1) -baby below level of placenta (DCC-2)	Unknown	Equal in all three groups	Unknown	Higher in DCC -1	Majority exclusive breastfeeding. No difference between ECC and DCC group	Unknown	None
Gupta et al, 2002 ¹⁸	After placental descent into vagina	Baby within 10 cm below introitus	Unknown	Inclusion factor	Unknown	No difference between ECC and DCC group	Majority exclusive breastfeeding. No difference between ECC and DCC group	No difference between ECC and DCC group	None
Lanzkowsky, 1960 ¹⁹	After signs of placental separation and after cord stripping 4-5x*	Baby between mother's legs	Unknown	Equal in both groups	Unknown	None	Approximately 40% exclusive breastfeeding. No difference between ECC and DCC group. In the remainder no iron-fortified infant formula	Unknown	Exclusion factor

* Stripping 4-5 times from vulva to infant's umbilicus

Table 2b. Confounding factors: studies from industrialised countries

Reference	Timing of DCC	Gravity enhancement	Oxytocics prior to cord clamping	Maternal anaemia	Fetal growth retardation	Maternal use of iron supplements antenatally	Postdate pregnancy	Haemolytic disease	Septicaemia
Linderkamp et al, 1992 ²⁰	3 min after delivery	Baby at level of introitus	Unknown	Unknown	None	Unknown	Exclusion factor	Unknown	Exclusion factor
Nelle et al, 1993 ²¹	3 min after delivery	Baby on mother's abdomen	Unknown	Unknown	None	Unknown	Exclusion factor	Unknown	Exclusion factor
Nelle et al, 1995/1996 ^{22,23}	3 min after delivery	Baby on mother's abdomen	Unknown	Unknown	None	Unknown	Exclusion factor	Unknown	Exclusion factor
Saigal et al, 1972 ²⁴	1 and 5 min after delivery	Baby 30 cm below introitus	No	Unknown	Exclusion factor	Unknown	None	Exclusion factor	Exclusion factor

ECC: early cord clamping; DCC: delayed cord clamping

ducted, malaria endemicity, duration of follow-up, number of study subjects, study groups, inclusion and exclusion criteria, and maternal mean haemoglobin at enrolment (Table 1). Data on possible confounding factors for infant haematological status were also extracted: exact timing of cord clamping, gravity enhancement, oxytocics prior to placental delivery, maternal anaemia, maternal or infant use of iron supplements, and infant feeding pattern (Tables 2a and 2b). Confounding factors for hyperbilirubinaemia and polycythaemia were also extracted: post-mature pregnancy, intrauterine growth retardation, haemolytic disease of the newborn and septicaemia.

RESULTS

Trial flow

Overall, 26 potentially relevant studies were identified, 17 of which were excluded because they adopted different outcome measures, were in pre-term babies, or included groups that were dissimilar at baseline.²⁵⁻⁴¹ Eight trials (one of them was described in two articles) were included in the systematic review (Figure 1). References for excluded studies are quoted as superscripts, and the reasons for exclusion are given.

Figure 1. Trial flow chart

Study characteristics

The study results are summarized in Tables 3a and 3b.

Table 3a. Study results: trials from developing countries

Reference	Maternal Hb Mean $\pm$ SD (g/L)	Cord Hb Mean $\pm$ SD (g/L)	Infant Hb Mean $\pm$ SD (g/L)	Proportion of group with anaemia at follow-up	Infant ferritin Mean $\pm$ SD (μ g/L)
Geethanath et al, 1997 ¹⁶	ECC: 125 $\pm$ 17 DCC: 127 $\pm$ 18 NS	ECC: 161 $\pm$ 22 DCC: 155 $\pm$ 23 NS	ECC: 89 $\pm$ 16 DCC: 83 $\pm$ 21 (at 3 months) NS	Not reported	ECC: 55.7 $\pm$ 3.7 DCC: 73.6 $\pm$ 3.1 (at 3 months) NS
Grajeda et al, 1997 ¹⁷	ECC: 116 $\pm$ 21 DCC-1: 122 $\pm$ 18 DCC-2: 125 $\pm$ 14 NS	Not reported	ECC: 100 $\pm$ 9 DCC-1: 108 $\pm$ 11 DCC-2: 106 $\pm$ 9 (at 2 months) ECC vs. DCC-1: P = 0.03	ECC: 15/17=88% DCC-1: 10/ 24=42% DCC-2: 11/ 20=55% Anaemia at 2 months defined as Ht<33% <p=0.01	ECC: 119.3 $\pm$ 91.6 DCC-1: 131.2 $\pm$ 55.4 DCC-2: 130.8 $\pm$ 69.8 (at 2 months, excluding those positive for CRP test) NS
Gupta et al, 2002 ¹⁸	ECC: 89 $\pm$ 8 DCC: 92 $\pm$ 6 NS	ECC: 139 $\pm$ 15 DCC: 141 $\pm$ 14 NS	ECC: 88 $\pm$ 8 DCC: 99 $\pm$ 9 (at 3 months) P < 0.001	ECC: 25/29=86% DCC: 13/ 29=45% Anaemia at 3 months defined as Hb<100 g/L	ECC: 73.0 (range 15-180) DCC: 118.4 (range 30-500) (at 3 months) P = 0.02
Lanzkowsky, 1960 ¹⁹	ECC: 115 $\pm$ 13 DCC: 115 $\pm$ 14 NS	Not reported	ECC: 111 $\pm$ 10 DCC: 111 $\pm$ 9 NS	Not reported	-

Table 3b. Study results: trials from industrialised countries

Reference	Cord Ht Mean $\pm$ SD	Ht at 2-4 hours after delivery Mean $\pm$ SD	Ht at 24 hours after delivery Mean $\pm$ SD	Ht at 120 hours after delivery Mean $\pm$ SD	Proportion of group with bilirubin > 15 mg/dL
Linderkamp et al, 1992 ²⁰	ECC: 48 $\pm$ 4 DCC: 50 $\pm$ 4 NS	ECC: 47 $\pm$ 5 DCC: 63 $\pm$ 5 P < 0.005	ECC: 43 $\pm$ 6 DCC: 59 $\pm$ 5 P < 0.005	ECC: 44 $\pm$ 5 DCC: 59 $\pm$ 6 P < 0.005	ECC: 0/15 (0%) DCC: 3/15 (20%)
Nelle et al, 1993 ²¹	ECC: 49 $\pm$ 5 DCC: 48 $\pm$ 5	ECC: 48 $\pm$ 6 DCC: 58 $\pm$ 6 P < 0.05	ECC: 44 $\pm$ 5 DCC: 56 $\pm$ 7 P < 0.05	ECC: 44 $\pm$ 5 DCC: 54 $\pm$ 5 P < 0.05	ECC: 0/15 (0%) DCC: 4/15 (27%)
Nelle et al, 1995/ 1996 ^{22,23}	ECC: 49 $\pm$ 4 DCC: 51 $\pm$ 5 NS	ECC: 53 $\pm$ 7 DCC: 61 $\pm$ 6 P < 0.05	Not reported	ECC: 50 $\pm$ 7 DCC: 57 $\pm$ 2 P < 0.05	ECC: 0/15 (0%) DCC: 2/15 (13%)
Saigal et al, 1972 ²⁴	ECC: 50 $\pm$ 5 DCC-1: 48 $\pm$ 7 DCC-2: 43 $\pm$ 4 NS	ECC: 50 $\pm$ 4 DCC-1: 63 $\pm$ 5 DCC-2: 65 $\pm$ 5	Not reported	Not reported	ECC: 0/15 (0%) DCC-1: 0/15 (0%) DCC-2: 0/15 (0%)

ECC: early cord clamping; DCC: delayed cord clamping; NS: not significant; Hb: haemoglobin; Ht: haematocrit; $\pm$ SD: standard deviation; CRP: c-reactive protein

Mean infant haemoglobin

Two of the four studies from developing countries found a significant difference in infant haemoglobin levels in favour of DCC.^{17;18} The study by Gupta et al. also found a significant difference in infant ferritin levels at three months. The remaining two studies showed no difference in indicators for infant iron status.^{16;19} A meta-analysis for anaemia reduction showed an absolute risk reduction of 15% (Table 4).

Table 4. Meta-analysis of effect of delayed cord clamping on postnatal anaemia in developing countries

Study	Proportion of infants with anaemia after ECC	Proportion of infants with anaemia after DCC	Relative risk reduction (95% CI)	Absolute risk reduction (95% CI)	Number needed to treat (95% CI)
Grajeda et al, 1997 ¹⁷	15/17	21/44	0.31 (-0.15, 0.59)	0.15 (-0.06, 0.35)	6.9 (3, 16)
Gupta et al 2002 ¹⁸	25/29	13/29	0.33 (-0.14, 0.61)	0.15 (-0.04, 0.35)	6.5 (3, 25)
Total (95% CI)	40/46	34/73	0.32 (0.02, 0.52)	0.15 (0.01, 0.29)	6.8 (4, 100)

Polycythaemia in the first week

Three of four studies from industrialised countries showed a significant difference in haematocrit levels in favour of DCC.²⁰⁻²³ This difference was already seen by 2-4 hours after delivery, and remained significant during the following five days. None of these trials reported any clinical manifestations of polycythaemia. The Canadian trial provided no statistical results, so it is unclear whether the difference in haematocrit was statistically significant.²⁴

Hyperbilirubinaemia in the first week

All the German trials reported that some newborns with cords clamped late had bilirubin levels > 15 mg/dL. It was not stated how many days after delivery these neonates were assessed. No studies reported the need to perform phototherapy or exchange transfusions. Meta-analysis showed an increased risk for hyperbilirubinaemia of 12% (Table 5).

Table 5. Meta-analysis of effect of delayed cord clamping on hyperbilirubinaemia

Study	Proportion of neonates with bilirubin > 15 mg/dL after ECC	Proportion of neonates with bilirubin > 15 mg/dL after DCC	Relative risk increase (95% CI)	Absolute risk increase (95% CI)	Number needed to harm (95% CI)
Linderkamp et al, 1992 ²⁰	0/15	3/15	6.00 (-0.61, 123.83)	0.19 (-0.03, 0.41)	5 (2, 31)
Nelle et al, 1993 ²¹	0/15	4/15	8.00 (-0.47, 152.80)	0.25 (0.01, 0.49)	4 (2, 71)
Nelle et al, 1995/ 1996 ^{22,23}	0/15	2/15	4.00 (-0.74, 95.13)	0.13 (-0.07, 0.32)	8 (3, 14)
Saigal et al, 1972 ²⁴	0/15	0/30	Not applicable		
Total (95% CI)	0/60	9/75	14.25 (-0.09, 255.83)	0.12 (0.04, 0.20)	9 (5, 26)

DISCUSSION

A significant improvement in mean haemoglobin was reported after two months of infant follow-up in two of the four selected studies.^{17,18} In one of the studies which observed significant improvement,¹⁸ the prevalence of maternal anaemia was high because only anaemic mothers were recruited. Although this study found a significant increase in haemoglobin with DCC, the absolute magnitude of this change was small (11 g/L). A larger period of follow-up to six months of age, or later, might have resulted in a larger difference in haemoglobin between study groups. As there is a greater risk of iron deficiency anaemia occurring after 3-4 months of age owing to the large increase in red cell mass which occurs in mid-infancy, it might be expected that the potential benefits of late cord clamping would be more apparent in older infants. DCC was associated with significantly improved ferritin values at 3 months in one Indian study.¹⁸ In developing countries weaning foods are often introduced to breastfed babies as early as 2-3 months of age. If the iron content of these foods is low or poorly bio-available, then the risk of anaemia in later infancy would be even greater.

There is good evidence that iron status in small-for-gestational-age infants is compromised,⁴² and fetal growth retardation is an important confounding factor that would influence the effect of DCC. In the four studies from developing countries, no information on growth retardation or low birth weight was given. There are indications that African neonates have lower haematological values than their European and North American counterparts,^{43,44} even after excluding children with haemoglobinopathies. This can be a confounding factor in a multi-ethnic study population but did not play a role in the studies selected.

Polycythaemia, hyperviscosity, and hyperbilirubinaemia are frequently mentioned potential harmful consequences of placental transfusion.⁴⁵ There is little evidence that these effects are harmful in term neonates. Their occurrence at the levels reported might be within physiological limits. Although a significant dif-

ference in haematocrit was observed in three of four studies from industrialised countries, with higher levels in the DCC groups, no clinical manifestations of polycythaemia were noticed. The three German trials showed that delaying the timing of cord clamping increased the risk of higher bilirubin levels in term infants, although phototherapy or exchange transfusion were not required.

There was considerable heterogeneity between studies in the actual timing of cord clamping. There was some heterogeneity also in positioning of the baby after delivery. The rate of placental transfusion is influenced by the position of the delivered infant in relation to the level of the placenta.⁴¹ These factors might have influenced the outcomes measured.

CONCLUSION

We conclude that delayed cord clamping, especially in anaemic mothers, increases haemoglobin concentration by 2-3 months of age and reduces the anaemia risk of term infants. Longer follow-up studies are required to establish whether this delivery procedure is effective in reducing anaemia in later infancy. There is an urgent need to assess the potential value of DCC in malarious areas where fetal anaemia is common. This delivery procedure might offer an additional, sustainable, low-cost and safe strategy within integrated programmes aimed at reducing iron deficiency anaemia in infants in developing countries.⁴⁶⁻⁴⁸

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3b DELAYED UMBILICAL CORD CLAMPING FOR REDUCING ANAEMIA IN LBW INFANTS – IMPLICATIONS FOR DEVELOPING COUNTRIES

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ABSTRACT

Background: Cheap and effective interventions are needed to reduce the risk of infant anaemia in developing countries. Delayed cord clamping (DCC) has been shown to be a simple, safe and cost-free delivery procedure that augments red cell mass in appropriate-for-gestational-age term and preterm infants. It is unknown, however, whether DCC is similarly safe and effective in small-for-gestational-age (SGA) infants. We analysed the available evidence to generate a balanced inference on the use of DCC in developing countries.

Objectives: To examine the short- and long-term effects in SGA infants of DCC compared to immediately clamping; and to assess the relationship between time of clamping time and the potential postnatal haematological complications of DCC in SGA infants.

Search strategy: PubMed (1966 to January 2006), EMBASE (1988 to January 2006), and The Cochrane Library (Issue 1, 2006) were searched. Reference lists of published trials were examined and major perinatal and tropical medicine journals were handsearched.

Selection criteria: Randomised and quasi-randomised trials comparing delayed with immediate cord clamping in infants born between 30 and 42 completed weeks of gestation and which included a proportion of SGA-infants.

Data collection and analysis: Three reviewers assessed eligibility and trial quality

Main results: To date, no trials have specifically reported the effects of DCC in SGA infants. Three trials were included, of 190 term and 40 preterm infants, a proportion of whom were SGA. DCC was associated with higher haemoglobin levels in term infants at follow-up (two trials, 127 infants, weighted mean difference (WMD) 9.17 g/L, 95% confidence interval (CI) 5.94 to 12.40). In preterm infants, the proportion who required a blood transfusion in the first 6 weeks after birth was lower after DCC (one trial, 38 infants, RR 0.56, 95% CI 0.34 to 0.94). It was not possible to infer from the available data whether SGA infants were at greater risk of adverse effects in the early neonatal period.

Authors' conclusions: DCC in a group that contains both AGA and SGA infants was associated with higher haemoglobin levels at two to three months of age in term infants and a reduction in the number of blood transfusions needed in the first 4-6 weeks of life in preterm infants. No reliable conclusions could be drawn about the potential adverse effects of DCC. The paucity of information on DCC in SGA infants justifies further research, especially in developing countries where the baseline risk for polycythaemia-hyperviscosity syndrome is likely to be lower than in industrialised countries.

BACKGROUND

The prevalence of anaemia in infants in developing countries is high, especially where malaria is endemic. In the second half of the first year of life it affects up to 75% of all children.¹⁻³ The basis for anaemia is frequently laid during the antenatal period and low birthweight (LBW <2,500 g) is a significant predictor of anaemia in later infancy.^{4,5} Severe anaemia is associated with an increased risk of death, while iron deficiency anaemia has been related to impaired mental and motor development in infants and young children.^{6,7} There are indications that anaemic children given iron therapy fail to catch up with non-anaemic children.⁸

Prevention of infant anaemia is of critical importance, yet current coverage with antimalarial interventions and micronutrient supplementation is poor in many African countries because of financial, logistic and technical constraints.⁹ In these settings, cheap and effective interventions are needed to reduce the risk of infant anaemia and improve child survival.

Delaying clamping of the umbilical cord at birth is a simple and cost-free delivery procedure that augments red cell mass in infants. It is estimated that the total fetoplacental blood volume is roughly 120 ml/kg of fetal weight. After immediate cord clamping, the distribution of blood reflected in the fetus:placenta ratio is approximately 2:1. Delaying the clamping for three minutes results in a larger fetal blood volume (ratio 4:1).^{10,11} For a 3 kg infant with a haemoglobin concentration of 170 g/L at birth, this amounts roughly to an additional 75 mg of iron, sufficient to meet the requirement of 1 mg/kg/day for approximately three months.¹²

Historically, there has been much debate on the potential harmful effects of delayed cord clamping (DCC). Frequently mentioned adverse effects were polycythaemia and hyperviscosity syndrome, hyperbilirubinaemia and hypothermia. Today, the safety of DCC in appropriate-for-gestational-age (AGA) infants is indisputable. Recently, two systematic reviews considered the short- and long-term effects of placental transfusion.^{13,14} In the first, which focussed on term AGA infants, it was concluded that DCC reduces the risk of anaemia in infants without an increased clinical risk of perinatal complications.¹³ The second concentrated on preterm AGA infants in industrialised countries and concluded that delaying clamping by 30-120 seconds was associated with less need for blood transfusion and a reduction in the risk of intraventricular haemorrhage.¹⁴

DCC could be of particular relevance in developing countries in view of the high prevalence of LBW infants born where resources are limited and in situations with an increased risk of transmitting infection through blood transfusion. Many LBW infants born in developing countries are small-for-gestational-age (SGA).^{15,16}

Whether DCC is a safe and effective intervention in SGA infants has not been evaluated. We present a critical appraisal of available evidence on DCC in SGA term and preterm infants as a basis for evaluating this intervention for use in developing countries.

OBJECTIVES

The objectives were (1) to examine the short- and long-term effects of DCC in SGA infants, and (2) to assess the relationship between the time of cord clamping and the potential postnatal complications (polycythaemia-hyperviscosity syndrome and hyperbilirubinaemia) and the need to treat these conditions.

CRITERIA FOR CONSIDERING STUDIES FOR THE REVIEW

Types of studies

Randomised and quasi-randomised trials.

Types of participants

Infants born between 30 and 42 completed weeks' gestation (delivered vaginally or by caesarean section), and with a birthweight > 1000 g. The group should contain a proportion of SGA infants, defined as infants with a birthweight below the 10th percentile of a birthweight-for-gestational-age, sex-specific, singleton curve.^{17,18} Infants born before 30 completed weeks' gestation or with birthweights < 1000 g were not included as mortality is high in very low birthweight infants who need artificial ventilation in resource-poor countries.^{19,21} To evaluate the haematological effects of delayed cord clamping, infant follow-up was required to at least 6 weeks after birth, when a nadir occurs for mean haemoglobin Hb concentration in LBW infants.²²

Types of interventions

Delayed (≥ 30 sec) versus immediate umbilical cord clamping with or without oxytocics, gravity enhancement or milking the cord.

Types of outcome measures

Long term beneficial haematological effects:

Prevention of infant anaemia

- Haemoglobin level at two months postpartum or later
- Occurrence of anaemia in infancy
- Number and volume of blood transfusions in neonatal period

Short term adverse haematological effects:

- (1) Polycythaemia or hyperviscosity syndrome
 - Haematocrit in first 24 hours
 - Clinical signs of hyperviscosity present
 - Treatment for polycythaemia with partial exchange transfusion
- (2) Hyperbilirubinaemia
 - Peak serum bilirubin
 - Treatment for hyperbilirubinaemia with phototherapy or exchange transfusion
- (3) Hypothermia
 - Body temperature in the first hour after birth

SEARCH STRATEGY FOR IDENTIFICATION OF STUDIES

Trials were identified by searching PubMed (1966 to January 2006) and EMBASE (1988 to January 2006) using the search strategy developed by the Cochrane Collaboration adapted for use in PubMed.²³ The optimal search strategy was combined with the MeSH terms “umbilical cord” AND “anaemia” OR “polycythaemia” OR “hyperbilirubinemia”. In PubMed, articles related to selected studies were also examined. The Cochrane Database of Systematic Reviews, Database of Abstracts of Reviews of Effectiveness, The Cochrane Central Register of Controlled Trials, The Cochrane Database of Methodology Reviews, The Cochrane Methodology Register, Health Technology Assessment Database and NHS Economic Evaluation Database (Cochrane Library, Issue 1, 2006) were searched using the key words “delayed cord clamping”. Reference lists of published trials were examined for additional relevant trials. Major journals of perinatal and tropical medicine journals were hand-searched.

REVIEW METHODS

Validity assessment and data extraction

Authors PvR and SG applied inclusion criteria to potentially relevant trials and, together with BB, independently assessed trial quality using the Delphi list.²⁴ Any discrepancies were resolved by discussion. Data presented in tables and graphs were extracted. Data extracted included study location, study groups, inclusion and exclusion criteria, and outcome measures. If the outcomes of interest were not reported, efforts were made to contact the authors of the trial to obtain the data, if available. Additional data were provided for eight trials.²⁵⁻³² Data were entered into the Review Manager software.³³

Statistical methods

Adverse outcomes were described as relative risks (RR), the ratio of adverse events between the treated and control groups. Treatment effect was expressed as RR, relative risk reduction (1-RR), risk difference (RD) and number needed to treat (1/RD). For continuous data, we used the difference between the means of each group and the standard deviations of the original data. Weighted mean difference (WMD) and its confidence interval were obtained by using the inverse-variance method of meta-analysis. This method assumes that all of the trials have measured the outcome on the same scale.

DESCRIPTION OF STUDIES

Three trials qualified for inclusion into this review (Grajeda *et al.*²⁵, Gupta & Ramji³⁴, and Rabe *et al.*³¹) (Table 1). We excluded 18 studies (reasons given in Table 2).^{26-30;32;35-46} The included trials were assessed for control or exclusion of relevant confounding factors that could influence infant haematological status: gestational age, route of delivery, exact time of cord clamping, gravity enhancement, use of oxytocics prior to cord clamping, maternal anaemia, infant use of iron supplements, and cumulative blood loss by sampling (Table 3).

Table 1. Characteristics of included studies

Study	Grajeda et al. ²⁵
Study location	Guatemala, Amatitlán (semi-urban, no malaria)
Methods	Randomized controlled trial, quasi-randomisation: alternate days
Participants	88 term infants; including SGA infants Inclusion: birth weight ≥ 2000 g Exclusions: gestational diabetes, antepartum haemorrhage, major congenital abnormalities
Interventions	Control: immediate cord clamping Intervention 1: clamping after cord stops pulsating; infant at level of placenta Intervention 2: clamping after cord stops pulsating; infant below level of placenta
Outcomes	Primary outcome: serum ferritin, serum iron, TIBC, transferring saturation and haematocrit 2 months after birth Secondary outcome: haematocrit 24 hrs after birth
Notes	Considering inclusion criteria SGA infants likely to be present. Authors were contacted, but no further details were given
Allocation concealment	C
Study	Gupta & Ramji ²⁴
Study location	India, New Delhi (urban, malaria endemicity very low)
Methods	Randomized controlled trial (maternal haemoglobin below 100 g/L)
Participants	102 term infants; including SGA infants Exclusions: eclampsia, heart failure, antepartum haemorrhage, Rhesus-incompatibility, major congenital malformations, need for resuscitation
Interventions	Control: immediate cord clamping Intervention: cord clamping after placental descent into Vagina; position of baby within 10 cm below introitus; drying and wrapping in dry, warm linen – thereafter under preheated radiant warmer
Outcomes	Primary outcome: serum ferritin 3 months after birth Secondary outcome: haemoglobin in cord blood and at 3 months
Notes	mean (SD) birth weight in both groups 2.7 (0.4) kg SGA infants likely to be present. Authors were contacted, but no further details were given
Allocation concealment	A

Study		Rabe et al.³¹
Study location		Germany, Münster (urban, no malaria)
Methods		Randomized controlled trial, opaque sealed envelopes
Participants		40 preterms < 33 weeks; including SGA infants Exclusions: multiple pregnancy, Rhesus incompatibility, fetal hydrops, congenital abnormalities, Apgar < 3 at 0 minutes
Interventions		Control: immediate cord clamping (20 s) Intervention: cord clamping 45 s after birth; infant below level of placenta; oxytocin at delivery of first shoulder
Outcomes		Primary outcome: number of blood transfusions during first 6 weeks of life Secondary outcomes (amongst others): temperature on admission in NICU, volume resuscitation during first 24 h, phototherapy
Notes		Author confirmed the proportion of SGA infants to be 36%
Allocation concealment		A
<hr/>		
A	indicates adequate concealment of the allocation (e.g. by telephone randomisation, or use of consecutively numbered, sealed, opaque envelopes).	
B	indicates uncertainty about whether the allocation was adequately concealed (e.g. where the method of concealment is not known).	
C	indicates that the allocation was definitely not adequately concealed (e.g. open random number lists or quasi-randomisation such as alternate days, odd/even date of birth, or hospital number).	
D	indicates the score was not assigned.	

Table 2. Characteristics of excluded studies

Name	Reason for exclusion
Aladangady <i>et al.</i> ³⁶	No infant baseline characteristics available
Baenziger <i>et al.</i> ³⁷	No infant baseline characteristics available
Geethanath <i>et al.</i> ³⁸	No infant baseline characteristics available
Hofmeyr <i>et al.</i> ²⁶	Main interest was peri- and intraventricular haemorrhage. Author was not able to retrieve haematological data
Hofmeyr <i>et al.</i> ²⁷	Main interest was peri- and intraventricular haemorrhage. Author was not able to retrieve haematological data
Hudson ³⁹	Sequential allocation procedure. Gestational age and birth weight not given
Ibrahim <i>et al.</i> ⁴⁰	Extremely low birth weight infants (< 1000 g and below 30 weeks of gestation)
Kinmond <i>et al.</i> ²⁸	No SGA infants
Lanzkowsky ⁴¹	No SGA infants
McDonnell & Henderson-Smart ⁴²	No SGA infants
Mercer <i>et al.</i> ²⁹	No SGA infants
Nelle <i>et al.</i> ³⁰	No SGA infants
Oh <i>et al.</i> ⁴³	Extremely low birth weight infants (< 1000 g and below 30 weeks of gestation)
Oxford Midwives ³⁵	No SGA infants
Pao-Chen & Tsu-Shan ⁴⁴	No SGA infants
Saigal <i>et al.</i> ³²	No SGA infants
Strauss <i>et al.</i> ⁴⁵	No data available for the group of interest of 31 to 36 weeks. No real placental transfusion, but harvesting of red blood cells and retransfusion
Taylor <i>et al.</i> ⁴⁶	Inadequate randomization

Table 3. Confounding factors

Study	GA (wks)	Method to assess GA	Proportion SGA	Delivery	Timing of DCC	Position of baby in relation to placenta	Oxytocics prior to cord clamping	Maternal anaemia	Infant use of iron supplements	Cumulative bloodloss by sampling
Grajeda <i>et al.</i> ²⁵	≥ 37	Clinical examination (Dubowitz)	Unknown	Vaginal	When cord stopped pulsating	Intervention 1: Baby at level of placenta Intervention 2: Baby below level of placenta	Unknown	Equal in all three groups	None	
Gupta & Ramji ³⁴	≥ 37	Unknown	Unknown	Vaginal	After placental descent into vagina	Baby within 10 cm below introitus	Unknown	Inclusion factor	None	
Rabe <i>et al.</i> ³¹	< 33	Last menstrual period and early sonography	Control: 40% Intervention: 33%	Majority by caesarean section (Control: 19/20 Intervention: 15/19)	45 s after delivery	Baby 20 cm below level of placenta	9 IU I.V. after delivery of first shoulder	Unknown	2 mg/kg from 4 weeks onwards	Control: 30.4 ml/kg Intervention: 27.1 ml/kg (NS)

GA=gestational age; SGA=small-for-gestational-age; DCC= delayed cord clamping; FiO2= fraction of inspired oxygen; Hb= haemoglobin; NS=not significant

The three included trials were from non-malarious countries. All assessed infant haematological status, and the follow-up period was sufficiently long. Since Grajeda *et al.*²⁵ and Gupta & Ramji³⁴ enrolled only term infants (including SGA), and Rabe *et al.*³¹ only preterms (including SGA), comparability was limited. Definitions of DCC varied from 45 seconds (Rabe *et al.*³¹), the moment when cord pulsations cease (Grajeda *et al.*²⁵; mean 1.5 – 2 minutes), to the moment when the placenta appeared in the vagina (Gupta & Ramji³⁴; no exact indication of time). The term infants were all delivered vaginally, while the majority of preterms were delivered by caesarean section. In all trials, the infants were held below the level of the placenta to enhance placental transfusion. Rabe *et al.*³¹ reported the use of oxytocin after delivery of the baby's first shoulder. We consider it unlikely that this enhanced placental transfusion, as uterine contractions begin only approximately 45 sec after intravenous administration of oxytocin⁴⁷ and the cord was clamped after 45 sec exactly. Grajeda *et al.*²⁵ and Gupta & Ramji³⁴ did not report the use of oxytocin. Maternal use of iron supplements during pregnancy was not reported appropriately. In the study by Rabe *et al.*³¹ infants received iron supplementation from 4 weeks of age and the follow-up period was 6 weeks. This might have influenced infant haematological outcome at the end-point.

In the trial by Grajeda *et al.*,²⁵ follow-up haematological data were available for 69 of the 88 recruited infants. The drop out rate did not differ between the allocation groups. Gupta & Ramji³⁴ reported a total drop-out rate of 43%, equally distributed in both groups. In both trials the drop-outs were excluded from the analysis. Rabe *et al.*³¹ reported one death in the control group. Another child who was allocated to the intervention group was clamped earlier because of a deteriorating condition and was excluded from the analysis.³⁴

QUALITY OF THE METHODOLOGY

The Delphi list was used to assess quality (table 4). Randomisation was appropriate and well described. Rabe *et al.*³¹ made efforts to blind the outcome assessor. Grajeda *et al.*²⁵ and Gupta & Ramji³⁴ make no statement on blinding. In none of the three studies was it stated whether the mother was informed of the baby's assigned group for cord clamping.

Table 4. Delphi assessment for included trials

Question	Grajeda <i>et al.</i> ²⁵	Gupta & Ramji ³⁴	Rabe <i>et al.</i> ³¹
Method of randomisation performed?	Yes (quasi: alternate days)	Yes	Yes
Treatment allocation concealed?	No	No	No
Groups similar at baseline regarding the most important prognostic indicators?	Yes	Yes	Yes
Eligibility criteria specified?	Yes	Yes	Yes
Outcome assessor blinded?	No	No	Yes
Care provider blinded?	No	No	Yes
Patient blinded?*	Yes	Yes	Yes
Point estimates and measures of variability presented for the primary outcome measures?	Yes	Yes	Yes
Analysis includes an intention-to-treat analysis?	No	No	No

*All of the subjects can be considered to be blind to the intervention as newborns are unaware of the clamping status

RESULTS

The three included studies involved 190 term infants and 40 preterms. Only some infants were characterised as SGA. Thus, stratification by size-for-gestational-age was not possible. As clinical diversity between the studies was large, the results of Rabe *et al.*³¹ were not combined with those of Grajeda *et al.*²⁵ or Gupta & Ramji.³⁴ Rabe *et al.*³¹ studied preterm infants of < 33 weeks of gestation, whereas Grajeda *et al.*²⁵ and Gupta & Ramji³⁴ focussed on term infants. Furthermore, the preterms were mainly delivered by caesarean section, which limited the duration of DCC to 45 sec.

Long term haematological outcome

After DCC, Hb levels in term infants at follow-up were higher (two trials, 127 infants, weighted mean difference (WMD) 9.17 g/L, 95% confidence interval (CI) 5.94 to 12.40). The proportion of term infants with anaemia at follow-up was lower in the DCC groups (two trials, 127 infants, relative risk (RR) 0.54, 95% CI 0.41 to 0.71). However, Grajeda *et al.*²⁵ used a Ht cut-off value of 0.33, the equivalent of a Hb level of 110 g/L, which probably caused an over-estimation of anaemia incidence since the cut-off level in an iron-supplemented USA reference population at two months was 94 g/L.⁴⁸ After DCC, fewer preterms infants required a blood transfusion in the first 6 weeks postpartum (one trial, 38 infants, RR 0.56, 95% CI 0.34 to 0.94). Rabe *et al.*³¹ used well-defined, strict guidelines for ordering blood transfusions. The two allocation groups were comparable in terms of the need for artificial ventilation and grade of infant respiratory distress syndrome.

Short term adverse effects:

Only Grajeda *et al.*²⁵ reported the short-term effects of DCC in term infants. Polycythaemia (venous Ht > 0.65) was found to be higher 24 hours after DCC, but the study was too small to provide reliable conclusions (30 infants, RR 3.42, 95% CI 0.18 to 65.58). None of the infants with polycythaemia had symptoms of hyperviscosity. In the same study hyperbilirubinaemia was assessed visually and no difference was found between the groups. Rabe *et al.*³¹ reported higher Ht levels one hour after DCC but, again, the study was too small to be conclusive (39 infants, WMD 3.00, 95% CI -0.66 to 6.66). None of the preterms showed clinical signs of hyperviscosity, such as sluggish peripheral perfusion, jitteriness and lethargy. There were insufficient data for a reliable conclusion about the risk of hyperbilirubinaemia after DCC in preterms (one trial, 39 infants, RR 1.05, 95% CI 0.64 to 1.73). There was no difference in temperature on admission to the neonatal intensive care unit (one trial, 39 infants, WMD 0.20, 95% CI -0.03 to 0.43).

It was not possible to infer from the available data whether SGA infants were at greater risk of short-term adverse effects. None of the studies provided data on cord Hb levels or Ht values.

DISCUSSION

The main objective of this analysis was to examine the short- and long-term haematological effects of DCC in SGA infants. To date, no trials have specifically reported the effects of DCC in SGA infants. Only a minority of cases in the selected studies were SGA. The available data were insufficient to perform a sub-group analysis. The selected studies had a sufficiently long follow-up period of at least 6 weeks, but the drop-out rate among term infants was high. As iatrogenic blood loss and frequent blood transfusions are important confounders in preterm infants, we chose not to use the proportion of anaemic infants, Ht or Hb levels as the only indicators. In LBW infants the total number of required blood transfusions can also be used as a reflection of their haematological status. The two trials that focussed on term infants (including some SGA) showed significantly higher Hb levels two to three months after DCC, and a significantly lower anaemia incidence. In the preterm infants (either AGA or SGA), DCC was associated with fewer blood transfusions in the first six weeks after birth. Only one trial in term infants examined possible short-term adverse effects of DCC, but the sample size was too small to make reliable conclusions. The selected study of DCC in preterms was also too limited in size to draw reliable conclusions on the potential harmful effects in the first few days after birth.

It is reasonable to postulate that possible postnatal haematological complications of DCC (polycythaemia-hyperviscosity syndrome and hyperbilirubinaemia) in SGA infants depend on the volume of placental blood received. We tried to determine the optimal time of clamping for maximum haematological benefit and minimum adverse effects, but we could not provide an analysis because of insufficient data.

The safety of DCC in AGA term infants has been demonstrated,¹³ but there is paucity of information on DCC in SGA infants. In SGA infants in industrialised

countries, there is often an increased incidence of polycythaemia because of chronic hypoxaemia *in utero* leading to increased erythropoiesis. In the presence of sufficient iron this leads to increased red blood cell volume. Although most polycythaemic infants remain asymptomatic, SGA infants are considered to be at greater risk of symptoms and clinical consequences of altered viscosity.⁴⁹ Because of the fear of worsening polycythaemia, many health workers are reluctant to use DCC in SGA infants. However, the baseline risk for polycythaemia-hyperviscosity syndrome in SGA infants from developing countries might be considerably lower than in SGA infants in the industrialised world. The prevalence of infants born with low cord Hb is high in areas where malaria and iron deficiency anaemia in pregnancy are common.^{1:50;51} Up to 30% of infants can present with fetal anaemia, which is defined as cord Hb below 125 g/L.⁵⁰ The association of SGA with fetal anaemia is related to increased post-neonatal infant mortality^{1:6;50;52;53}. A survival analysis of Malawian infants indicated that a decrease in Hb by 10 g/L at 6 months of age increased the risk of dying before 12 months of age by 72%.⁵⁴ This group of SGA infants in particular should be the focus of future research on DCC.

AUTHORS' CONCLUSIONS

Implications for practice

To date, no trials have specifically reported the effects of DCC in SGA infants. Delayed clamping of the umbilical cord in a group of term infants that includes both AGA and SGA infants is associated with a significant rise in haemoglobin levels two to three months after birth, and a significant reduction in the incidence of anaemia. It was not possible to infer from the available information if these results apply to the SGA group alone. DCC in a group of preterm infants (both AGA and SGA) can reduce the number of blood transfusions needed in the first 4-6 weeks of life. No reliable conclusions can be drawn about the potential adverse effects of DCC in SGA infants in the early neonatal period.

Recommendations for future research

The paucity of information on DCC in SGA infants justifies further research in this topic. In resource-poor countries, where the baseline risk for haematological adverse effects is likely to be lower than in industrialised countries, DCC is of particular relevance. SGA infants should be the focus of future trials, irrespective of their gestational age, and they should be closely monitored in hospital for at least 3 days for polycythaemia-hyperviscosity syndrome and hyperbilirubinaemia. Clamping times varying from 1 to 2 minutes in SGA infants need to be assessed to determine the optimal duration, and a number of interim analyses should be done to enable the study to be stopped early if a clear harmful effect should emerge. During monthly follow-up, attention should be paid to intercurrent diseases, feeding practices and haematological status. Reduction of loss to follow-up by tracing defaulters will improve the reliability of the results. There is an urgent need to assess the value of DCC in developing countries, as this delivery procedure might offer a simple, cost-free strategy to reduce infant anaemia and improve child survival in developing countries.

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4 THE EARLY EFFECTS OF DELAYED CORD CLAMPING IN TERM INFANTS BORN TO LIBYAN MOTHERS

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SUMMARY

This study was conducted to evaluate the haematological effects of the timing of umbilical cord clamping in term infants 24 h after birth in Libya. Mother-infant pairs were randomly assigned to early cord clamping (within 10 s after delivery) or delayed clamping (after the cord stopped pulsating). Maternal haematological status was assessed on admission in the delivery room. Infant haematological status was evaluated in cord blood and 24 h after birth. Bilirubin concentration was assessed at 24 h. 104 mother-infant pairs were randomized to delayed (n=58) or early cord clamping (n=46). At baseline the groups had similar demographic and biomedical characteristics, except for a difference in maternal haemoglobin, which was significantly higher in the early clamping group (11.7 g/dL (SD 1.3) versus 10.9 g/dL, (SD 1.6); $P=0.0035$). Twenty-four hours after delivery the mean infant haemoglobin level was significantly higher in the delayed clamping group (18.5 g/dL versus 17.1 g/dL; $P=0.0005$). No significant differences were found in clinical jaundice or plethora. Surprisingly, blood analysis showed that two babies in the early clamping group had total serum bilirubin levels ($>15\text{mg/dL}$) that necessitated phototherapy. There were no babies in the late clamping group who required phototherapy. Three infants in the delayed clamping group had polycythaemia without symptoms, for which no partial exchange transfusion was necessary. Delaying cord clamping until the pulsations stop increases the red cell mass in term infants. It is a safe, simple and low cost delivery procedure that should be incorporated in integrated programmes aimed at reducing iron deficiency anaemia in infants in developing countries.

INTRODUCTION

Iron deficiency anaemia (IDA) is the most common nutritional disorder worldwide. Its prevalence is highest among children aged under five years in developing countries, where approximately 50% are affected.¹ Severe anaemia in infancy is a life threatening condition and a major contributor to infant mortality in developing countries.²⁻⁴ IDA has been associated with impaired cognitive development in children under five. These children fail to catch up with iron therapy.^{5,6}

Strategies to reduce IDA in infants include iron supplementation and iron fortification. Although these measures have been shown to be clinically effective,⁷⁻⁹ they are either cost-ineffective or difficult to implement, especially in developing countries.

A first step towards reducing anaemia in infancy can be taken during birth. Delayed cord clamping or placental transfusion could be a cost-effective intervention to improve the iron status of infants by enhancing their red cell mass.

The main objective of this study was to examine the effect of the time of umbilical cord clamping in term infants on haematological status 24 hours after birth. A second objective was to assess possible adverse effects, especially hyperviscosity and hyperbilirubinaemia. There have been controlled trials evaluating the short-term haematological effects of delayed cord clamping in term infants,¹⁰⁻¹⁴ but as far as we know this is the first randomised controlled trial.

SUBJECTS AND METHODS

Term infants delivered at Tripoli Medical Centre (TMC) in Libya between April and June 2003 were enrolled. Their mothers were contacted while in their first stage of labour to obtain informed consent. After giving consent, and prior to vaginal delivery, the infants were randomly assigned by means of sealed opaque envelopes to either delayed cord clamping (DCC) or immediate cord clamping (ICC). In the DCC group the umbilical cord was clamped after it stopped pulsating. The exact time was recorded by use of a stopwatch, with complete expulsion of the infant as the starting point. In the ICC group clamping was done within 10 seconds after delivery, which was the standard delivery practice at that time in TMC. Following vaginal birth the infant was placed on the mother's abdomen and dried and wrapped in a warm towel. Oxytocin was given to the mother intramuscularly after cord clamping. Common practice is to give oxytocics with the delivery of the anterior shoulder. This delivery practice was adapted for the trial period to minimise confounders, as the administration of oxytocin in the third stage might speed up placental transfusion.¹⁵ The nurse midwives attending the deliveries were closely monitored by one of the authors (MOE).

All subjects meeting the following selection criteria were included: expected birth weight ≥ 2500 g, gestational age between 37 and 42 weeks (estimated by early ultrasound) and singleton birth. Mother-infant pairs were excluded when the baby had low birth weight (< 2500 g) or when the gestational age (as assessed by Ballard-external method) was less than 37 weeks. Other exclusion criteria were maternal gestational diabetes or (pre)eclampsia, instrumental

delivery, serious haemorrhage during pregnancy or delivery, major congenital abnormalities (neural tube defects, respiratory distress syndrome) and the need for early cord clamping (medical history of post-partum haemorrhage; tight nuchal cord; resuscitation).

One venous blood sample was taken from the mother upon arrival in the labour ward for estimation of maternal haemoglobin (Hb) and haematocrit (Ht). Women with Hb < 10 g/dL were considered to be anaemic. Before delivery a small structured survey questionnaire was used to gather socio-economic and demographic details from the mothers, as well as information on reproductive health. A sample of cord blood was collected from the placental side after clamping and ligating the fetal side for Hb and Ht estimation. Babies with cord Hb < 12.5 g/dL were considered to have fetal anaemia. Before discharge home (16-24 hours after birth) the babies were assessed for clinical signs of polycythaemia, hyperviscosity or hyperbilirubinaemia, and, if necessary, an estimation of the gestational age was done by using the Ballard-external method. Finally, an infant venous blood sample was taken for Ht and bilirubin analysis. Polycythaemia was defined as venous Ht > 65%. Phototherapy was considered to be necessary when bilirubin levels exceeded 15 mg/dL on day 1.

Based on the results of a recent systematic review,¹⁶ we expected a difference in mean haemoglobin between the ICC and DCC group at 24 hours of 1.5 g/dL with a standard deviation of 2.0 g/dL. On this assumption with a power of 90 % and a confidence level of 95%, a sample size of 40 babies was required in each group.

As the aim of this study was to evaluate the effects of late cord clamping under ideal circumstances, data were analysed according to the per-protocol principle. Epi Info 2002 (Centers for Disease Control and Prevention, CDC, USA) was used for data analysis. The t-test for independent samples was used to compare group means for normally distributed data. When variances were not homogeneous the Mann-Whitney rank-sum test was used. A P-value < 0.05 was considered significant.

Ethical approval for the study was given by the Ethics Committee of the Liverpool School of Tropical Medicine and by local authorities of the departments of Obstetrics & Gynaecology and Paediatrics in TMC.

RESULTS

There were 112 mother-infant pairs eligible for inclusion (figure 1); 62 were randomised to DCC and 50 to ICC. Eight mother-infant pairs, equally distributed over both randomisation groups, were excluded from the final analysis. The infants needed resuscitation for intrapartum asphyxia. As a consequence, 58 mother-infant pairs remained in the DCC group (mean clamping time: 215 seconds (SD 51), and 46 in the immediate clamping group (mean 13 seconds (SD 6)). The mothers in the ICC and DCC groups were comparable in terms of age, parity, gestational age, ultrasound confirmation, level of education and antenatal iron supplementation. The mean maternal (Hb) level on admission to the labour ward was higher in the ICC group (11.7 g/dL (SD 1.3)) than in the

DCC group (10.9 g/dL (SD 1.6), and this difference was significant ($p = 0.0035$). The proportion of women with anaemia at delivery was also higher in the DCC group: 29% versus 9% ($p=0.0096$). Other maternal baseline characteristics were not significantly different (Table1).

Figure. 1 Recruitment into the study. DCC=delayed cord clamping; ICC=immediate cord clamping

Table 1. Maternal baseline characteristics

Variable	Early clamping (n=46)	Delayed clamping (n=58)	P-value
Maternal age in yrs	28.9 (4.8)	28.4 (4.7)	0.64
Parity (mean $\pm$ SD)	2.2 (2.4)	1.7 (1.7)	0.55
Primigravida (%)	26	22	
Multigravida (%)	74	78	
Iron supplementation in pregnancy (%)	78	81	0.73
Number of antenatal visits to clinic	6.8 (3.2)	6.2 (3.2)	0.36
Maternal schooling			
None (%)	6.5	3.4	
Primary (%)	8.7	12.1	0.32
Secondary (%)	71.7	60.3	
Tertiary (%)	13.0	24.1	
Ultrasound in first trimester (%)	91	93	0.74
Oxytocics prior to clamping (%)	39	45	0.56
Hb on admission to labour ward (g/dL)	11.7 (1.3)	10.9 (1.6)	0.0035
Proportion maternal anaemia (%) (Hb < 10 g/dL)	9	29	0.0096

Data are mean (SD) unless stated otherwise

Infant baseline characteristics did not differ significantly (Table 2). Low birth weight was not observed in any infant. Cord Hb and Ht levels were comparable in both groups. The prevalence of fetal anaemia was higher in the DCC group, although this difference was not significant. Polycythaemia was not observed at birth. There was no correlation between maternal and cord haemoglobin in either the ICC or DCC group.

Table 2 Infant baseline characteristics

Variable	Early clamping (n=46)	Delayed clamping (n=58)	P-value
Clamping time (sec)	12.8 (5.5)	214.6 (50.6)	0.0000
Gestational age (weeks)	40.0 (1.4)	39.8 (1.4)	0.36
Birthweight (grams)	3428 (424)	3390 (421)	0.65
Female sex (%)	46	55	0.33
Cord haemoglobin (g/dL)	15.4 (1.4)	14.9 (1.7)	0.12
Proportion Fetal Anaemia (%) (Hb < 12.5 g/dL)	4.3	6.9	0.58
Cord haematocrit	45.0 (4.6)	44.1 (5.8)	0.37

Data are mean (SD) unless stated otherwise

A single child was lost to follow-up in each group, as their parents had taken them home before they could be reviewed. Evaluation after 24 hours revealed no significant differences in clinical jaundice or the proportion of infants with clinical plethora. There were no significant differences in serum total bilirubin levels at 24 hours, although surprisingly 4.6% of the infants in the ICC group had bilirubin levels (>15 mg/dL) that necessitated phototherapy.

The Ht level was significantly higher in the DCC group ($p = 0.0037$), but only 5% of infants in this group had polycythaemia. Hb levels after 24 hours showed higher values in the DCC group (18.5 g/dL (SD 2.1) versus 17.1 g/dL (SD 1.9)) and these differences were significant ($p = 0.0005$). The infants' haematological outcomes are summarised in Table 3.

Table 3. Infant haematological outcome after 24 hours

Variable	Early clamping (n=45)	Delayed clamping (n=57)	P value
Clinical jaundice			
None (%)	68.9	73.7	-
Mild-moderate (%)	28.9	26.3	
Severe (%)	2.2	0	
Serum bilirubin (mg/dL)	6.1 (3.0)	5.8 (1.3)	0.38
Proportion (%) above phototherapy threshold (bilirubin > 15 mg/dL)	4.6	0	0.11
Clinical plethora (%)	24.4	40.4	0.09
Haemoglobin (g/dL)	17.1 (1.9)	18.5 (2.1)	0.0005
Difference in cord haemoglobin and at 24 hrs (g/dL)	1.7 (1.7)	3.5 (1.9)	0.0000
Haematocrit (%)	49.3 (5.7)	52.9 (6.3)	0.0037
Difference in cord haematocrit and at 24 hrs (%)	4.4 (5.6)	8.7 (5.7)	0.0002
Proportion polycythaemia (%) (Hct > 65%)	0	5.3	0.12

Data are mean (SD) unless stated otherwise

DISCUSSION

The aim of this study was to evaluate whether DCC could enhance red cell mass in term infants in Libya. We found a difference in the mean Hb and Hct levels of infants at 24 hours after delivery in favour of the DCC group. This difference is statistically significant and is possibly of clinical importance. This result is in accordance with earlier published studies on the short-term effects of placental transfusion.¹⁶ It should be noted that the incidence of moderate maternal anaemia (Hb < 10 g/dL) was significantly higher in the group randomised for DCC, while both groups were comparable for all other variables. No women had severe anaemia (Hb < 7 g/dL). The fact that a considerable number of babies (6 out of 104) were found to have fetal anaemia, can possibly be explained by the existence of α -thalassaemia in the study population, which is known to reduce cord Hb levels.

Maternal iron deficiency has also been related to fetal anaemia in a study from Malawi.¹⁷ Four randomised controlled trials have been published that examined longer-term effects of DCC in developing countries have been published. Two found a significant difference in Hb levels at 2-3 months in favour of the DCC group.^{18,19} The other two studies showed no difference in indicators for infant iron status.^{20,21} Three controlled trials from Germany (published in four papers) showed a significant increase in Ht levels in favour of DCC.¹⁰⁻¹³ This difference was seen by 2-4 hours after delivery, and remained significant during the following five days.

Hyperbilirubinaemia, polycythaemia and hyperviscosity syndrome are frequently mentioned adverse effects of placental transfusion. In our study in term infants no significant difference was found between the DCC and ICC groups in total serum bilirubin levels at 24 hours, the number of infants requiring phototherapy, or the prevalence of plethoric skin and polycythaemia. None of the children showed signs of hyperviscosity syndrome (cyanosis, feeding difficulties, tachypnea or neurological depression). Blood glucose levels were not checked routinely, but none of the children showed signs of hypoglycaemia. Placental transfusion in term infants was not associated with perinatal complications in this study.

No previously published trials reported any clinical manifestations of polycythaemia.^{10-13,18-21} The German trials reported that some newborns with DCC had bilirubin levels > 15 mg/dL. It was not stated how many days after delivery these neonates were assessed. Neither did they report whether phototherapy or exchange transfusions were needed. A trial from Canada examined both pre-term and term infants.¹⁴ When analysing the data for term infants separately no cases of hyperbilirubinaemia were found.

The major objective of this trial was to evaluate whether DCC could improve the haematological status of infants. The difference we found in the mean Hb and Ht levels of infants at 24 hours after delivery in favour of the DCC group is possibly of clinical importance. It is estimated that in full-term infants placental transfusion can increase red cell mass by 25-33%.^{22,23} Iron stores in the term newborn are normally adequate to maintain iron sufficiency for approximately four months of postnatal growth.²⁴ Improved iron status from these additional red cells might increase the stores sufficiently to cover the first 5-6 months.

A recent systematic review showed that DCC in premature babies should be done with more caution. Delaying cord clamping in premature babies for more than 1 minute increases the risk of complications such as polycythaemia and hyperbilirubinaemia. It seems advisable to delay clamping of the umbilical cord in this group of neonates for not more than 45-60 seconds.²⁵

The policy of delayed cord clamping is in contrast with active management of the third stage of labour to reduce post-partum haemorrhage. Active management involves ICC, prophylactic use of oxytocic drugs before delivery of the placenta and controlled cord traction.²⁶ In our opinion delaying cord clamping can be done safely in selected groups. Women who have a history of post-partum haemorrhage should be exempted from this delivery procedure. Although the administration of oxytocin was postponed until after cord clamping in this study, this is not necessary in practice. Early intramuscular injection of oxytocin can even speed up placental transfusion.¹⁵

Delaying cord clamping until the pulsations stop is a physiological way of treating the cord and is not associated with adverse effects, at least in term vaginal deliveries. Many children living in less developed countries belong to anaemia risk groups (low birthweight; severe maternal anaemia, chronic infection, iron deficient diets after 5-6 months) and should therefore be given the opportunity to boost their iron stores at birth. DCC, which is a safe, simple and low-cost delivery procedure, should be incorporated in the routine labour management. It could serve as an additional cost-effective intervention within integrated programmes aimed at reducing IDA in infants in developing countries.

ACKNOWLEDGEMENTS

We thank all doctors and nurse midwives of Tripoli Medical Centre who have contributed to this study with their warm dedication and cooperation.

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5 DELAYED CORD CLAMPING AND HAEMOGLOBIN LEVELS IN INFANCY: A RANDOMISED CONTROLLED TRIAL IN TERM BABIES

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ABSTRACT

Objectives: This study was carried out to assess whether delaying umbilical cord clamping is effective in improving the haematological status of term infants living in a malaria-endemic area, and whether this is associated with complications in infants and mothers.

Methods: We randomly assigned women delivering term babies in Mpongwe Mission Hospital, Zambia, to delayed cord clamping (DCC, n=46) or immediate cord clamping (controls, n=45) and followed their infants on a bi-monthly basis until the age of six months. We compared the haemoglobin (Hb) change from cord values and the proportion of anaemic infants. Secondary outcomes related to infant and maternal safety.

Results: Throughout the observation period infant haemoglobin levels in both groups declined, but more rapidly in controls than in the DCC group (difference in Hb change from baseline at four months 1.1 g/dL, 95% confidence interval (CI) 0.2; 2.1). By 6 months, this difference had disappeared (0.0 g/dL, 95% CI -0.9; 0.8). The odds ratio for iron deficiency anaemia in the DCC group at four months was 0.3 (95% CI 0.1; 1.0), but no differences were found between the groups at six months. No adverse events were seen in infants and mothers.

Conclusion: Our findings indicate that DCC could help improve the haematological status of term infants living in a malaria-endemic region at 4 months of age. However, the beneficial haematological effect disappeared by 6 months. This simple, cost-free and safe delivery procedure might offer a strategy to reduce early infant anaemia risk, when other interventions are not yet feasible.

INTRODUCTION

One of the most critical factors contributing to neonatal and infant mortality in developing countries is anaemia (Brabin *et al.* 2001; Brabin *et al.* 2003b), which has been repeatedly shown to be an intractable problem even with anti-malarial and iron interventions. One of the most important trials which studied primary anaemia prevention in Tanzania observed a 20% incidence of severe anaemia even in infants who had received prophylactic antimalarials and iron supplements (Menendez *et al.* 1997). Although iron supplementation in infants from malarious regions has been shown to be beneficial it remains a highly contentious issue, as it might worsen the outcome of infectious illnesses (Openheimer 2001; English & Snow 2006; Sazawal *et al.* 2006).

In view of this there is interest in evaluating the potential for improving the iron status of infants by enhancing their red cell mass with delayed umbilical cord clamping. In traditional African home deliveries the umbilical cord is cut after placental descent into the vagina (Lefebvre & Voorhoeve 1997), but in hospital deliveries in resource-poor settings immediate cord clamping is the routine standard of care. It is estimated that the total fetoplacental blood volume is roughly 120 ml/kg of fetal weight. After immediate cord clamping the distribution of blood reflected in the fetus:placenta ratio is approximately 2:1. Allowing placental transfusion to occur for at least three minutes results in a larger fetal blood volume with 15 ml/kg of blood remaining in the placenta (Linderkamp 1982; Yao *et al.* 1969). Compared with immediate clamping, a clamping delay of 3 minutes provides an additional blood volume of 20-35 ml/kg of body weight (Linderkamp *et al.* 1992; Yao *et al.* 1969). For a 3 kg infant with a packed cell volume (PCV) of approximately 0.50 at birth, this amounts to an additional 45 mg of iron added to iron stores. Theoretically this amount of iron should be sufficient to meet the requirements of an infant for more than 3 months (Osiki 1993).

Previous trials indicated that delayed cord clamping (DCC) prevents a steep decline in haemoglobin (Hb) concentration at 2-3 months of age, especially in infants born to anaemic mothers (van Rheeën & Brabin 2004). A recent trial from Mexico showed that at 6 months of age, infants who had DCC had a better iron status compared to early clamped infants (Chaparro *et al.* 2006).

It has never been evaluated whether this delivery procedure is effective in reducing anaemia in infants from malaria-endemic regions. We performed a randomised controlled trial in a highly malarious rural area of Zambia to assess whether DCC is effective in reducing anaemia in term infants up to the age of 6 months, and is associated with complications in infants and mothers.

METHODS

Study area, enrolment and study population

The study location was Mpongwe District, a rural region of the Copperbelt Province in Zambia, approximately 1000m above sea level. Malaria transmission in this area is holoendemic. Peak malaria transmission occurs from November to April. Like in other parts of Zambia, over 50% of women deliver at

home (Stekelenburg *et al.* 2004). Especially nulliparous and grand multiparous women, and women with a prior caesarean section, are advised to deliver in hospital. Those who are able to pay the user fees and live in the vicinity are more likely to deliver in hospital. The caesarean section rate in Mpongwe Mission Hospital is 5%, with the majority of sectioned women being transferred from peripheral health units for obstructed labour. Approximately ten percent of the babies are born with birth weight below 2500 g.

Full term pregnant women delivering in Mpongwe Mission Hospital were candidates for inclusion in the study. Prespecified exclusion criteria were: (1) twin pregnancy, (2) history of post partum haemorrhage (PPH), (3) gestational diabetes, (4) pre-eclampsia, (5) placental separation before delivery, (6) caesarean section, (7) tight nuchal cord necessitating early cutting, (8) need for neonatal resuscitation, or (9) major congenital abnormalities (e.g. neural tube defects). Criteria 1-4 were applied before randomisation. Criteria 5-9 were assessed after randomisation. Infants who weighed less than 2500 g, or with gestational age below 37 weeks, were excluded.

Study design

We investigated in a partially blinded randomised controlled trial, firstly, whether DCC affected the haematological status of term infants in a malaria-endemic region and, secondly, whether DCC is associated with complications in infants and mothers. Primary endpoints included Hb change from cord values and the proportion of anaemic infants at four months after birth, and the duration infants remained free of anaemia during follow-up. Secondary outcomes included possible side effects of DCC in infants (PCV changes 1 day postpartum; clinical signs of hyperviscosity syndrome or hyperbilirubinaemia) and mothers (Hb change one day after delivery in relation to antenatal values and blood loss in the third stage of labour). Infants were followed two monthly until six months of age.

Initially this trial had a follow-up of 4 months and “Hb change from baseline at 4 months” was one of the pre-specified summary measures to analyse serial Hb-values.

This summary measure was of clear clinical and biological relevance, as no previous cord clamping trial had done a follow-up beyond three months after birth. During the trial we wished to extend the follow-up period from 4 to 6 months, to evaluate how long the potential beneficial effects of DCC would be measurable. The Research Ethics Committee of the Liverpool School of Tropical Medicine and all other parties involved gave their approval for the extension. As the shape of the “time-response” curve was uncertain, we could only choose a new summary measure after the data had been examined, and we decided to stick to the initial summary measures.

Pregnant women were randomised to either DCC or ICC. ICC was the routine standard of care in Mpongwe Mission Hospital at the time of the trial, and was usually completed within 20 s of delivery. In the DCC group the umbilical cord

was clamped after the cord stopped pulsating. The cord was palpated every other 20 seconds for cessation of pulsations and clamped when pulsations were no longer observed. The exact time was recorded by stopwatch.

For allocation concealment, the randomisation instructions were given to study midwives in sequentially numbered, opaque, sealed envelopes with unpredictable allocation code, which were only opened when a mother had consented to enrol. Used envelopes with the assignment instruction enclosed were sent to the study coordinator and were regularly audited. Randomisation was done on admission to the labour ward, when the women were in the first stage of labour and the attending midwife was convinced that uterine contractions would persevere. Although study staff did not inform the mothers of their assignment, the nature of the intervention made it impossible to blind them. If an already randomised mother later became ineligible, the assigned allocation code was not re-used. One of the investigators (De Moor) monitored the delivery procedure, and was therefore not blinded to treatment assignment.

A structured survey questionnaire was used to gather obstetrical and medical details and a venous blood sample was taken from the mother for laboratory investigations in the first stage of labour. Following vaginal birth all infants were placed between the legs of the mother (approximately 10 cm below the vaginal introitus), dried, and wrapped in a warm towel. The infants remained in this position until the cord was clamped. Intramuscular oxytocin was administered to the mothers after clamping of the cord.

A sample of cord blood was collected for laboratory analysis. Maternal blood loss was estimated visually by the attending midwife. A more objective measure of blood loss was Hb concentration 24 h post partum compared to Hb in the first stage of labour.

Birthweight was measured using a Salter balance (nearest 10 grams). In the first 24 hours after delivery the child was observed for clinical signs of hyperviscosity syndrome and hyperbilirubinaemia by one of the investigators, who was not blinded to the assigned intervention. The icterometer, a Perspex ruler with yellow stripes, was used to estimate the degree of hyperbilirubinaemia. In the darkly pigmented newborns in our study population the icterometer was used to blanch the gum. This method has been shown to be as useful as the original (Hamel 1982; Morley 1973). Assessment of jaundice was performed in daylight at a window. The presence of hyperviscosity syndrome was assessed whenever the babies vital signs were measured by examining for plethora, apathy, tachypnea, poor sucking and hypoglycemia. Before discharge (16-24 hours after delivery), assessment of gestational age was completed by the Ballard-ext method (Verhoeff *et al.* 1997), a capillary heel-prick blood sample was collected from the baby after pre-warming and a venous sample was taken from the mother.

For mother-infant pairs living within a radius of 4 kilometres from Mpongwe Mission Hospital follow-up took place at the hospital-based Mother and Child Health Clinic, where vaccinations and growth monitoring were routinely undertaken. Mothers who indicated a preference to attend a more local health facility for vaccination and growth monitoring and non-attendees were traced in their vil-

lages by the investigators to minimise loss to follow-up. At two months baseline data concerning socio-economic and demographic background were collected to assess possibly confounding factors. Literacy status was assessed by asking the mother to read a simple sentence in the local language. Weight of the mothers was estimated in bare feet (nearest kg) using a standing weigh scale and height (nearest cm) using a height board. Mid-upper-arm circumference (MUAC) was measured on the right arm, hanging loosely, with a TALC insertion tape (TALC, St. Albans, UK) and recorded to the nearest 0.1 cm. At each follow-up visit information was collected on infant feeding practices and morbidity in the previous two months. Infants' finger prick blood was collected at two, four and six months. Infants with Hb concentrations below 7 g/dL at follow-up were given therapeutic iron supplements for two months. Infants with malaria were treated with a dose of sulfadoxine-pyrimethamine as per local guidelines. All other illnesses were treated or referred if appropriate.

Laboratory investigations

Blood samples were collected in EDTA microtainers (Becton Dickinson, Franklin Lakes, USA) and immediately stored at +4 degrees Celsius. Within 6 hours the Hb level was measured using a HemoCue (HemoCue AB, Ångelholm, Sweden). Zinc-protoporphyrin (ZPP) level, in $\mu\text{mol ZPP/mol heme}$, was assessed using a ZP Haematofluorometer (Aviv Biomedical, Lakewood, NJ, USA) within 48 hours of collection, PCV by Micro-Haematocrit and blood glucose level in the first 24 hours by glucometer (Ascensia Elite XL, Bayer B.V., Mijdrecht, The Netherlands). Blood smears were examined for malaria parasites. A thick smear was considered negative if 100 microscopic fields revealed no parasites. For positive smears, malaria parasites were counted against 300 leucocytes. The placenta tissue samples were kept up to 9 months until processed and embedded in paraffin wax by standard techniques. Paraffin sections 4 μm thick were stained with hematoxylin and eosin.

Definitions

Anaemia was defined as a Hb concentration more than two standard deviations (SD) below the mean of similarly aged infants from an iron-supplemented USA reference population not exposed to malaria (Dallman 1988). Reference values for the ages of 2, 4 and 6 months were 9.4 g/dL, 10.3 g/dL, and 11.0 g/dL, respectively. A cut-off of 10.5 g/dL for 6 months old infants has been shown to be more appropriate, and we therefore considered infants with Hb measurements below this value as anaemic (Domellof *et al.* 2002). Fetal anaemia was defined as a cord Hb less than 12.5 g/dL, which is 2 SD below the mean cord Hb for non-malarious western populations (Brabin 1992). Anaemia in pregnant women was defined as Hb less than 11 g/dL (WHO 2001). Iron deficiency was ZPP above 80 $\mu\text{mol/mol haem}$ for adults and infants (Domellof *et al.* 2002; Hinchliffe 1999; Juul *et al.* 2003; Kling 2006; Lott *et al.* 2006; Rettmer *et al.* 1999; Soldin *et al.* 2003). A recently published cross sectional study among babies born to non-anaemic mothers found that infants born after 35 weeks completed weeks of gestation had

mean ZPP levels in cord blood of 73 $\mu\text{mol/mol}$ haem (Lott *et al.* 2006). As this result corresponds to normal values in older infants, we decided to also use 80 $\mu\text{mol/mol}$ haem as cut-off for fetal iron deficiency. The combination of abnormal values for both ZPP and Mean Cell Haemoglobin Concentration (MCHC) is a more sensitive indicator of iron deficiency (INACG 1985). We used MCHC cut-offs of 32 g/dL for pregnant women (Letsky 1991) and 28 g/dL for newborns (Saarinen & Siimes 1978; Serjeant *et al.* 1980). MCHC estimates were not available at 4 and 6 months follow-up. Iron deficiency anaemia in mothers and newborn infants was defined as the combination of ZPP above the cut off level and MCHC below the cut off level, together with Hb more than 2 SD below the reference mean. For infants aged 4 and 6 months the combination of ZPP above the cut off level and Hb more than 2 SD below the reference mean was used.

Malaria was defined as the presence of asexual stage parasites in thick smears at follow-up, independent of the presence of clinical signs and symptoms. A history of a positive blood smear result at a local health facility together with clinical signs or symptoms of malaria in the last two weeks before review, was also considered as evidence for a recent malaria infection.

The histopathological slides were classified as *acute infection* when parasites without pigment deposition were seen, as *chronic infection* when parasites and a significant amount of pigment deposition were seen, and as *past infection* when pigment deposition without parasites were seen (Ismail *et al.* 2000).

Neonatal tachypnea was defined as a respiratory rate over 60/min, hypoglycaemia as glucose below 45 mg/dL (or 2.5 mmol/L) on the first day after birth, polycythaemia as a PCV above 0.70 in capillary blood. The threshold for phototherapy was set at a bilirubin level above 6 mg/dL (grade 2 of the icterometer) at 12 hours after birth (Subcommittee on Hyperbilirubinemia 2004).

Underweight was more than two standard deviations below the mean of the 2000 CDC reference curves for weight-for-age (Kuczmarski *et al.* 2000). Intermitent presumptive treatment with antimalarials was defined as at least two doses of sulphadoxine-pyrimethamine during the second and third trimesters of pregnancy. Adolescence was less than 20 years of age.

Statistical methods

The sample size was determined on the basis of a mean Hb change from cord values of 4 (SD 1) g/dL and a difference in mean Hb change between the DCC and control group at three months of 1 g/dL (van Rheenen & Brabin 2004). The 1 g/dL was chosen on the basis of clinical relevance: a survival analysis of Malawian infants indicated that a decrease in Hb by 1 g/dL after 6 months of age increased the risk of dying before 12 months of age by 72% (Brabin *et al.* 2003a). With a study power of 95% and in order to detect a significant difference with $P=0.05$ (two-sided), 28 babies were required for each study group. Allowing for a maximum drop-out of 40%, we planned to enrol 47 babies per group, or 94 in total. A total of 120 envelopes were prepared for the trial with a 1:1 randomisation ratio. As not all envelopes were expected to be used, we could not ensure that comparison groups were exactly the same size.

Data were collected on standardised forms, and analysed with SPSS for Windows (version 12.0.1 (2003), SPSS Inc., Chicago, IL, USA) and Epi Info (version 3.2 (2004), CDC, Atlanta, GA, USA). Student *t* tests and Chi-square tests were used to compare baseline characteristics between treatment groups, and between infants who completed the study and those who were lost to follow-up. For non-parametric data the Mann-Whitney *U*-test was used. Time to event data were analysed by Kaplan-Meier and logrank test. All tests were two-tailed. As part of the exclusion criteria could only be applied after randomisation, which is after delivery, data were analysed according to the per-protocol principle. Infants with tight nuchal cord necessitating early cutting, perceived need for neonatal resuscitation, and major congenital abnormalities were excluded regardless of group assignment, thus reducing the likelihood of introducing bias. Furthermore, an intention-to-treat analysis would not have been appropriate for examining adverse effects (Altman *et al.* 2001).

The haematological follow-up data were analysed as continuous variables with analysis of covariance (ANCOVA) and as categorical variables with logistic regression. Baseline imbalances which could influence infant haematological outcome (maternal Hb, parity, age and cord Hb) were controlled for in these analyses. The level of significance used was a *P*-value < 0.05. This study is registered with the controlled-trials.com identifier ISRCTN48735857.

Ethical approval

The women were contacted while in their first stage of labour to obtain informed consent. The consent information sheet was written in Lamba, the local language. In case of illiteracy, the midwife on duty read the form. Mothers gave written consent or a thumb impression. The research protocol was approved by the Research Ethics Committee of the Liverpool School of Tropical Medicine and by the Board of Management of Mpongwe Mission Hospital. The Mpongwe District Health Management Team, the chairpersons of the health neighbourhoods (lay people representing the community for health issues) and the local chiefs were informed and all provided signed consent.

RESULTS

Figure 1 shows the trial profile. Between May and July 2004, 105 pregnant women were assessed for eligibility before entering the labour ward and randomised to either DCC (*n*=55) or control group (*n*=50). Nine cases in the DCC group and four cases in the control group were excluded from the analyses. No women delivered by caesarean section after randomisation. A total of 91 mother-infant couples entered the trial who were actively followed on a two monthly basis until the infants reached the age of six months. The follow-up period ended in January 2005.

35 of 46 mother-infant pairs in the DCC, and 37 of 45 pairs in the control group completed six months of follow-up (total drop out rate respectively 24% and 18%, not significant).

During the follow-up period four children died: one in the neonatal period, the others after four months of age. In two of these infants mild anaemia had been diagnosed during the follow-up visit prior to death (Hb > 7.0 g/dL). Baseline charac-

teristics for women and infants who completed the study did not differ significantly from those lost to follow-up.

Table 1 shows that the mothers in the DCC and control groups were comparable in terms of age, parity, nutritional state, antenatal iron supplementation, the use of intermittent preventive antimalarial treatment, socio-economic status and literacy level. None of them had a prior caesarean delivery. The median number of visits to the antenatal clinic was higher in the DCC group than in the control group (resp. 4 and 3; $P=0.045$). This factor, however, was not associated with outcome. Maternal haematological baseline characteristics and histopathological classification of placenta tissue did not significantly differ.

Figure 1. Trial profile

Table 1. Maternal baseline characteristics

Variable	Delayed Cord Clamping (n=46)	Controls (n=45)
Obstetrical-medical details		
Age (years) - <i>median (range)</i>	20.5 (15.5-41.9)	22.9 (15.6-46.0)
Adolescents	46%	38%
Weight (kg)	56.3 (8.1)	58.2 (8.6)
Height (cm)	159.3 (5.9)	158.7 (5.5)
BMI (kg/m ²)	22.1 (2.4)	23.1 (2.9)
MUAC (cm)	24.7 (2.0)	25.4 (2.0)
Chronic energy deficiency	2%	0%
Primigravidae	43%	27%
Last interpartum interval (months) - <i>median (range)</i>	40 (11-90)	36 (10-120)
Number of antenatal visits - <i>median (range)</i>	4 (1-11)	3 (1-6)
Intermittent presumptive treatment for malaria	100%	100%
Iron supplementation in pregnancy	89%	93%
Socio-economic details		
Literacy	54%	67%
Bednet use	33%	38%
Haematological details		
Hb (g/dL)	11.8 (1.4)	12.1 (1.3)
Proportion maternal anaemia	24%	16%
ZPP (mcmol/mol haem)	68 (25)	66 (25)
Proportion iron deficient erythropoiesis (ZPP > 80 µmol/mol haem)	26%	27%
Histopathological details of placenta		
Past malaria	24%	32%
Chronic malaria	10%	5%

Data are mean (SD) unless indicated otherwise.

BMI=body mass index; MUAC=mid upper arm circumference; Hb=haemoglobin; PCV=packed cell volume; MCHC=mean cell haemoglobin concentration; ZPP=zinc-protoporphyrin

In the DCC group the mean (SD) clamping time was 305 s (136) and in the control group 15 s (8). Although ICC was usually done within 20 s after delivery, in four cases the delay was longer than intended (range 22-45 seconds). Both groups of infants were comparable at birth in terms of anthropometric parameters. At baseline, mean cord Hb was slightly, but not significantly lower in babies in the DCC group.

Table 2 shows that eighteen percent of newborns in the DCC group had fetal anaemia compared to 7% in the control group ($p>0.05$), and over 70% of the infants had iron deficient erythropoiesis at birth, indicated by a ZPP value above the cut-off of 80 $\mu\text{mol/mol}$ haem. In 4 out of 10 cases of fetal anaemia there was associated maternal anaemia. There was a marginally significant relationship between maternal and cord Hb, $r = .175$, p (one-tailed) = 0.053.

Table 2. Infant baseline characteristics

Variable	Delayed Cord Clamping (n=46)	Controls (n=45)
Clamping time (s)	305 (136)	15(8)
Gestational age (weeks)	40.0 (1.5)	40.0 (1.7)
Birthweight (g)	3142 (326)	3119 (328)
Weight-for-Age z score	-0.62 (0.64)	-0.59 (0.62)
Females	46%	51%
Apgar-score		
After 1 min – <i>median (range)</i>	9 (8-10)	9 (6-10)
After 5 min – <i>median (range)</i>	10 (9-10)	10 (9-10)
Cord Hb (g/dL)	14.3 (1.7)	14.9 (1.5)
Proportion fetal anaemia	18%	7%
Cord PCV	48 (6)	50 (5)
Cord MCHC (g/dL)	30.0 (2.0)	29.8 (1.6)
Cord ZPP ($\mu\text{mol/mol}$ haem)	102 (41)	97 (28)
Proportion with iron deficient erythropoiesis (ZPP > 80 $\mu\text{mol/mol}$ haem)	75%	71%

Data are mean (SD) unless indicated otherwise

Hb=haemoglobin; PCV=packed cell volume; MCHC=mean cell haemoglobin concentration; ZPP=zinc-protoporphyrin

Up to a mean (SD) postnatal age of 16 (8) hours both mother and child were assessed for possible side-effects of the intervention. Maternal blood loss in the third stage of labour was comparable in both groups and PPH did not occur. Manual removal of the placenta was not required for any of the women involved. The difference between ante- and postnatal maternal Hb levels was small and comparable in both groups (data not shown).

Table 3 shows that the mean increase in PCV on the first day post partum was significantly higher in newborns from the DCC group (0.13 vs. 0.07; $p < 0.001$). The proportion of neonates with polycythaemia (PCV > 0.70) was 5% in each group. None of the infants showed clinical signs of hyperviscosity syndrome or hyperbilirubinaemia before discharge.

Table 3. Infant haematological outcome on first day post-partum

Variable	Delayed Cord Clamping (n=46)	Controls (n=45)	Effect size (95% CI)	p-value
PCV	0.61 (0.05)	0.57 (0.07)	0.04 (0.01-0.06)	0.008
PCV increase compared to cordblood	0.13 (0.06)	0.07 (0.08)	0.06 (0.03-0.09)	< 0.001
Proportion polycythaemia	5%	5%		1.000
Proportion above phototherapy threshold	0%	0%		1.000

Data are mean (SD) unless indicated otherwise

PCV=packed cell volume

Table 4 shows that infant Hb-levels in both the DCC and control groups continued to decline throughout the observation period. By four months the decrease in Hb compared to cord blood values was significantly smaller in the DCC group (mean difference 1.1 g/dL, 95% confidence interval (CI) 0.2-2.1 g/dL). By four months more infants in the control group had Hb levels below -2 SD cut-off level and were classified as having iron deficiency anaemia than those with delayed clamping, but the differences were marginally significant (odds ratio 0.3, 95% CI 0.1; 1.0). At four months no infants had Hb levels below 7.0 g/dL.

At 6 months, the difference in Hb change from cord values had disappeared and half of the infants in both groups were anaemic. Three infants were given therapeutic iron supplements as their Hb levels dropped below 7.0 g/dL. Results did not differ when analyses were done either with or without control for maternal baseline differences (anaemia, age and parity). The results also did not change when additional analyses were done by actual clamping time rather than by randomisation group. Malaria infections were not diagnosed before the age of four months. At six months, malaria was diagnosed evenly in both groups. Controlling for infant malaria infections did not change the results at six months. Mean ZPP levels and proportion of infants with iron deficient erythropoiesis were similar in the allocation groups, at four and six months.

Table 4. Infant haematological status at four and six months

	Unadjusted analysis				Analyses adjusted for maternal haemoglobin, age, parity, and cord haemoglobin [†]			
	Delayed Cord Clamping	Controls	Difference or OR (95% CI)	p-value	Delayed Cord Clamping	Controls	Difference or OR (95% CI)	p-value
Four months								
Hb (g/dL)	11.5 (1.5)	11.2 (1.3)	0.3 (-0.3; 0.9)	0.344	11.5 (1.5)	11.2 (1.3)	0.3 (-0.2; 1.2)	0.330
Hb change from baseline (g/dL)	-2.5 (2.1)	-3.7 (2.1)	1.2 (0.2; 2.3)	0.016	-2.5 (2.1)	-3.4 (2.1)	1.1 (0.2; 2.1)	0.024
Proportion anaemia (cut-off 10.3 g/dL)	21%	41%	0.4 (0.1; 1.1)	0.067			0.4 (0.1; 1.0)	0.059
ZPP (μmol/mol haem)	117 (81)	138 (80)	-22 (-60; 16)	0.253	115 (81)	140 (80)	-24 (-63; 14)	0.213
Iron deficient erythropoiesis (cut-off > 80 μmol/mol haem)	68%	73%	0.8 (0.3; 2.2)	0.665			0.8 (0.3; 2.1)	0.592
Proportion iron deficiency anaemia (ZPP>80 μmol/mol haem and Hb<10.3)	16%	35%	0.3 (0.1; 1.0)	0.054			0.3 (0.1; 1.0)	0.054
Proportion with positive malaria smear	0%	0%		..				..
	Unadjusted analysis				Analyses adjusted for maternal haemoglobin, age, parity, cord haemoglobin and infant malaria infection [†]			
	Delayed Cord Clamping	Controls	Difference or OR (95% CI)	p-value	Delayed Cord Clamping	Controls	Difference or OR (95% CI)	p-value
Six months								
Hb (g/dL)	10.2 (2.1)	10.6 (1.5)	-0.3 (-1.2; 0.5)	0.431	10.6 (2.1)	10.6 (1.5)	0.0 (-0.7; 0.7)	0.992
Hb change from baseline (g/dL)	-3.6 (2.7)	-4.3 (1.9)	0.6 (-0.5; 1.7)	0.262	-4.0 (2.7)	-4.0 (1.9)	0.0 (-0.9; 0.8)	0.992
Proportion anaemia (cut-off 10.5 g/dL)	50%	46%	1.4 (0.3; 2.0)	0.733			1.3 (0.4; 4.0)	0.612
ZPP (μmol/mol haem)	168 (109)	157 (121)	11 (-43; 65)	0.692	148 (109)	155 (121)	-7 (-56; 43)	0.789
Iron deficient erythropoiesis (cut-off > 80 μmol/mol haem)	77%	76%	1.1 (0.4; 3.2)	0.884			1.5 (0.4; 5.2)	0.538
Proportion iron deficiency anaemia (ZPP>80 μmol/mol haem and Hb<10.5)	50%	46%	1.2 (0.5; 3.0)	0.733			1.5 (0.5; 4.4)	0.495
Proportion with positive malaria smear	14%	11%	1.4 (0.3; 5.6)	0.656				..

Data are mean (SD) unless indicated otherwise. Effect size is expressed as mean difference for continuous variables and as odds ratio for categorical variables.

[†]Data are adjusted means and SD of unadjusted means

Hb=haemoglobin; ZPP=zinc-protoporphyrin ; OR=odds ratio; 95% CI=95% confidence interval

Table 5. Follow-up dietary data

	Two months		Four months		Six months	
	Delayed cord clamping n=43	Controls n=41	Delayed cord clamping n=39	Controls n=39	Delayed cord clamping n=35	Controls n=37
Proportion exclusively breastfed	95%	100%	23%	32%	0%	3%
Proportion on iron supplements	0%	0%	3%	0%	6%	3%
Proportion reported to have had diarrhoea in the previous two weeks	5%	5%	16%	14%	26%	27%

Table 5 shows that infant feeding patterns were similar in both groups, with early introduction of complementary foods. By four months less than a third of the children were exclusively breastfed. The early introduction of complementary foods was associated with a gradual increase in the number of diarrhoea episodes, as reported by the mother. At six months more than a quarter of the infants suffered from diarrhoea in the two weeks prior to the follow-up visit. Figure 2 shows that in the first four months weight gain was above the reference for both DCC and control infants (Kuczmarski *et al.* 2000). Hereafter a reduction in mean weight increments was observed. Weight for age Z scores showed a steep fall between four and six months.

In the DCC group seven babies had fetal anaemia, of which five remained free of anaemia throughout the follow-up period. In the control group three babies were anaemic at birth, and one of them was free of anaemia at six months follow-up. All fetal anaemia cases were included in the Kaplan-Meier survival curves (Figure 3). The logrank test showed that there was no significant difference in the proportion of anaemia cases between the groups.

Figure 2. Changes in weight-for-age Z scores in ICC and DCC groups
Error bars: standard error

Figure 3. Kaplan-Meier plot of anaemia-free infants in ICC and DCC group

DISCUSSION

Delaying clamping of the umbilical cord till the cord pulsations cease improved the haematological status of term infants living in a highly malarious area at four months of age, as measured by Hb change from cord blood values and anaemia incidence. This intervention was not associated with increased risk of maternal blood loss or with hyperviscosity syndrome in the baby. However, the beneficial effect of extra red cell mass and disappeared between the age of 4 to 6 months, also when controlled for malaria infections.

Two previous randomised controlled trials on DCC have reported beneficial effects on infant Hb, but the infants were not followed beyond three months of age (Grajeda *et al.* 1997; Gupta & Ramji 2002). A recently published trial from Mexico had a follow-up of six months, but did not find a difference in Hb, although the iron status at six months was significantly higher in the DCC group. This lack of difference in Hb is most likely due to the fact that iron deficiency was relatively uncommon in the Mexican study population. Hb is normally not affected until iron stores are depleted (Chaparro *et al.* 2006). All three trials were done in non-malarious areas. This study is the first to evaluate the haematological effects of DCC in infants living in a malaria-endemic area. We were able to continue following the infants after complementary feeding had commenced, and malaria incidence had increased.

Infant Hb levels in the study population continued to decline after the physiological nadir around two months. This pattern is observed in other trials from sub-Saharan Africa and many infants reach their lowest point only 6 to 12 months after birth (le Cessie *et al.* 2002; van Eijk *et al.* 2002; Kitua *et al.* 1997). Anaemia is usually multi-factorial in origin, but it is clear that malaria plays a key etiologic role in endemic countries. In holoendemic malarious areas, infants are infected with malaria from birth onwards but clinical malaria usually only develops from about 4 months of age, when immunity acquired from the mother is wearing off. In these regions the highest burden of anaemia occurs towards the end of the first year of life (Schellenberg *et al.* 2003).

The disappearance of the haematological benefits of DCC after 4 months can possibly be explained by two pathophysiologic mechanisms. Either the infant outgrows its iron stores, or the infant suffers increased loss of iron via the gastrointestinal tract during episodes of diarrhoea or during the feeding of whole cow's milk (Oski 1993).

Neonatal polycythaemia, hyperviscosity, and hyperbilirubinaemia are mentioned as potential consequences of placental transfusion (Behrman *et al.* 2000), but the safety of DCC in appropriate-for-gestational-age term infants has been demonstrated in several trials (Linderkamp *et al.* 1992; Nelle *et al.* 1993; Nelle *et al.* 1995; Nelle *et al.* 1996; van Rheenen & Brabin 2004; Grajeda *et al.* 1997; Emhamed *et al.* 2004). However, there is paucity of information on DCC in small-for-gestational-age (SGA) infants. SGA infants from industrialised countries often manifest an increased incidence of polycythaemia due to chronic hypoxaemia in utero leading to increased erythropoiesis. These infants are considered to be at greater risk of symptoms and clinical consequences of altered viscosity (An-

derson & Hay 1999). However, the baseline risk for polycythaemia-hyperviscosity syndrome in SGA infants from developing countries might be considerably lower. The prevalence of infants born with low cord haemoglobin is high in areas where malaria and iron deficiency anaemia in pregnancy are common (Brabin 1992; Brabin *et al.* 2004; le Cessie *et al.* 2002).

Mean bilirubin concentrations were higher in the DCC group, but they did not reach the levels requiring phototherapy or exchange transfusion. We did not observe clinical hyperbilirubinaemia in the first 24 hours after birth, although visual estimation of bilirubin levels using the icterometer could lead to misclassification. Neonatal admissions for a longer period in order to observe later onset of neonatal jaundice was not possible, as early discharge within 24 hours after an uncomplicated delivery was expected by mothers and was hospital policy.

We used ZPP levels at birth as a proxy for total body iron contents, and found that normal birth weight infants from Zambia already had low iron stores at birth. ZPP is regarded as a sensitive indicator of iron deficient erythropoiesis (Kling 2006; Juul *et al.* 2003; Lott *et al.* 2006; Miller *et al.* 2003; Rettmer *et al.* 1999). Raised ZPP levels indicate incomplete iron incorporation into protoporphyrin, as zinc substitutes for iron when stores are low. Although ZPP has the advantage of low cost and simplicity, its specificity may be low as it can be increased by malaria and other infections (Asobayire *et al.* 2001; Stoltzfus *et al.* 2000), which could have caused an overestimation of ZPP values at 6 months, when a small number of infants had positive smears.

Two recently published randomised controlled trials (Ceriani Cernadas *et al.* 2006; Chaparro *et al.* 2006) evaluated the effect of cord clamping on maternal blood loss. Major limitations of these trials were diversity in measuring blood loss (visual estimation versus measuring jar), diversity in mode of delivery (100% vaginal versus >25% caesarean section), and in definition of delayed cord clamping. No differences were found between DCC and immediate clamping. In our study there was no significant difference between groups in the midwives' assessment of maternal bleeding, although the sample size was too small to adequately detect modest differences. A further limitation is that we were not able to quantitatively measure maternal blood loss. However, we tried to quantify the blood loss indirectly by comparing maternal haemoglobin levels in the first stage of labour with levels on average 16 hours postpartum and found no differences.

The strengths of this study are its randomised design, the low drop-out rate for a rural area, and the control for confounding factors which could influence infant haematological status, including obstetrical procedures, maternal or infant use of iron supplements, intercurrent (malaria) infections, and complementary infant feeding practice. A limitation is that mothers who were already assigned to a treatment group could later become ineligible. This could not be avoided in view of timing and nature of the intervention. In view of this the data are analysed as per-protocol and not on an intention-to-treat basis. A second limitation was that the investigators, assessors and mothers were not blinded to the assigned intervention. Theoretically this could have biased the clinical estimation of maternal blood loss, or the clinical evaluation for the hyperviscosity syndrome and hyperbilirubinaemia.

In this trial a sample of cord blood was collected for Hb analysis immediately after clamping and cutting the umbilical cord. This baseline measurement was used to compare the Hb changes between the DCC and control group. There was a slight baseline imbalance which influenced our chosen summary measure. We corrected for this using the analysis of covariance.

The trial aimed to determine the effect only of DCC on infant anaemia and did not assess other known causes of infant anaemia, including human immunodeficiency virus (HIV) infection. We do not have HIV prevalence estimates for Mpongwe District. HIV prevalence among antenatal clinic attendees 1998-1999 in Kapiri Mposhi District, a neighbouring district, was 8.3%, and in Ibenga 9.3% (Fylkesnes *et al.* 2001). Mpongwe District has a similar low rural population density that is dispersed and village-based.

DCC has previously been shown to be more beneficial in babies of anaemic mothers (Gupta & Ramji 2002; van Rheenen & Brabin 2004). We were not able to confirm this, as maternal anaemia prevalence was lower than expected in this study population. All women had received two intermittent preventive doses of sulfadoxine-pyrimethamine during their pregnancy to control malaria in pregnancy which should reduce maternal anaemia prevalence (Garner & Gulmezoglu 2003). The majority of women in Mpongwe District deliver at home and the prevalence of maternal anaemia could be much higher in these community deliveries. A larger community based trial with this type of intervention would be difficult to conduct. The relevance of the present study findings for community deliveries needs to be assessed in the context of traditional delivery practices. This is an important area for further research.

Infant anaemia at six months was highly prevalent in both the DCC and control group, indicating that DCC alone was not adequate to prevent this. Combining DCC with intermittent antimalarial treatment in infants and use of impregnated bed nets requires further assessment. Low birth weight and pre-term babies have a higher incidence of infant anaemia, because of lower body iron stores and higher iron requirements for catch-up growth. Evaluation of delayed cord clamping is required in this higher risk group, especially in developing countries, from where there have been no studies of DCC in low birth weight babies.

We have shown that delaying clamping of the umbilical cord till cord pulsations stop significantly increased Hb levels at the age of four months in term babies. The decision to clamp after cessation of pulsations was based on the fact that in many delivery rooms in resource-poor settings a clock is absent or not functioning (own observations). The rate of placental transfusion is markedly influenced by the position of the delivered infant. An infant held 50-60 cm above the placenta will not receive net blood from the placenta (Yao & Lind 1969). From 10 cm above to 10 cm below the level of the placenta, infants receive the maximum possible amount within 3 minutes of birth. Keeping the infant 40 cm below the placenta hastens placental transfusion to almost completion within 1 minute (Linderkamp 1982; Yao & Lind 1969). Cord clamping should be delayed for at least three minutes for the optimal volume of placental transfusion, provided that the position of the term infant before clamping is on the mothers' abdomen or lower (van Rheenen & Brabin 2006).

Effective interventions are urgently needed to improve child survival in malaria-endemic areas. DCC is a simple, cost-free and safe delivery procedure that might offer a sustainable strategy to reduce early infant anaemia risk when other interventions are not yet feasible. It should be included in integrated programmes aimed at reducing anaemia in young children in developing countries.

ACKNOWLEDGEMENTS

We thank the management board of Mpongwe Mission Hospital for their hospitality; maternity and laboratory staff for their assistance; Mr J. Western (Technical Department, Mpongwe Mission Hospital) for maintenance of our equipment; Mr J. de Witte for the logistical support; and Prof. Dr. H.J. Verkade (University Medical Center Groningen) and Dr F. ter Kuile (Liverpool School of Tropical Medicine) for their critical review of the manuscript. This study received financial support from the Amsterdam-Liverpool Programme for research in Tropical Child Health and from the Bemmelen Support Group.

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6 DIAGNOSTIC ACCURACY OF THE HAEMOGLOBIN COLOUR SCALE IN NEONATES AND YOUNG INFANTS IN RESOURCE POOR COUNTRIES

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Tropical Doctor. In press.

SUMMARY

Due to the insidious nature of infant anaemia, this disorder frequently remains undetected and untreated by health care workers in resource-poor settings. We assessed the accuracy of a low-cost and simple diagnostic tool, the Haemoglobin Colour Scale (HCS), in estimating haemoglobin (Hb) values in infants between 0 and 4 months of age. In a rural hospital in Zambia blood samples were analysed for Hb concentration by HCS and HemoCue method. Bland-Altman plots were used to express agreement between the two methods. The mean difference between HCS and HemoCue at birth (n=94), two months (n=87) and four months (n=69) was -0.39, 0.20 and -0.11 g/dL, respectively. Limits of agreement were -2.39 to 1.51, -1.80 to 2.20, and -1.98 to 1.75 g/dL, respectively. Disagreement with HemoCue measurements of more than 2 g/dl was noted in only 4% of all blood samples. We conclude that the HCS provides Hb estimations in infants aged zero to four months that are sufficiently accurate to improve timely recognition of anaemia in settings where there is no laboratory.

INTRODUCTION

Anaemia is a common disorder in pregnant woman, infants and preschool children.^{1,2} In resource-poor settings, where calorimetric methods are not readily available, anaemia is usually diagnosed on the basis of clinical signs. The sensitivity of this method varies widely (27-97%) and is lowest in untrained staff.³⁻⁶ The lower the actual haemoglobin (Hb) level, the more accurate the clinical diagnosis. However, due to the insidious nature of infant anaemia, the disorder frequently remains undetected and untreated by health care workers. Furthermore, blood transfusions for severe anaemia may be prescribed on the basis of an inaccurate haemoglobin assessment, thus exposing the patient unnecessarily to the risk of infection with HIV or other blood-borne pathogens. The Haemoglobin Colour Scale (HCS) is a simple, low-cost and sustainable method that might give a more accurate estimation of the Hb level than by using clinical signs. The HCS consists of six standard colours varying from pale to dark red, corresponding to haemoglobin levels of 4, 6, 8, 10, 12, and 14 g/dl. To estimate the Hb value, a drop of blood absorbed on a paper test strip is matched against the scale of standards. Readers can also report intermediate values (e.g. 11 g/dl, 13 g/dl) if they judge that the colour falls between two standards.

The accuracy of the HCS method has been tested by several research groups⁶⁻¹², especially in blood from pregnant women and pre-school children, but never in neonatal and infant blood samples. As the cut-off values for neonatal anemia are much higher than in older patients, the accuracy of the HCS in neonates and young infants may not be as good as those reported by others. Neonatal anaemia is defined as Hb < 12.5 g/dl,¹³ and this value is at the top end of the scale, which might influence the reliability of reading. The question to be addressed in this study was whether the HCS provides Hb estimations in neonatal and infant blood that are sufficiently accurate to be of material benefit in resource-poor settings.

MATERIAL AND METHODS

Subject selection

Blood specimens were collected from a group of term, singleton newborns born in Mpongwe Mission Hospital, a district hospital in the rural part of the Copperbelt Province in Zambia. Blood sampling was done between April and November 2004. The mothers were contacted while in their first stage of labour to obtain informed consent, and their infants were then prospectively enrolled in the study. The normal birth weight babies that participated in the trial were born after an uncomplicated singleton pregnancy and had a gestational age ≥ 37 weeks as assessed by Ballard-ext method.¹⁴ The trial protocol was approved by the Research Ethics Committee of the Liverpool School of Tropical Medicine and by the Board of Management of Mpongwe Mission Hospital. The neonates and young children who were identified as anaemic (12.5, 9.4 and 10.3 g/dL at respectively 0, 2 and 4 months)^{13,15} were treated conform the protocol in the local hospital. Clinical decision for medical treatment was left to the medical staff.

Blood sampling

For the purpose of the evaluation of the HCS, blood samples were collected from the umbilical cord at birth, and by heel prick method at 2 and 4 months post partum. Because of the pressure of activities during delivery it was impracticable to analyse the blood at the bedside. A total of 250 µl of whole blood was therefore collected in an ethylenediaminetetraacetate microtainer tube (Becton Dickinson, Franklin Lakes, NJ, USA) and immediately stored at +4 degrees Celsius. At a later moment the blood was put on a paper test strip and in a HemoCue cuvette. At the age of 2 and 4 months blood sampling was done at the mother and child clinic in Mpongwe. Mothers who indicated a preference to attend a local health facility for vaccination and growth monitoring and non-attendees were traced in their villages. The capillary blood was also collected in microtainers and analysed later on (maximum delay 24 hours). Before the skin was punctured, it was cleaned with an alcohol swab and a film of silicon paste (Biolyon Hemade, Lyon, France) was applied to allow easy scooping of blood drops. The first drop of blood was discarded. Degradation of blood in the microtainers with consequent errors in the estimation of Hb values was ruled out in preliminary experiments.

Lab analysis

Hb estimation by HCS reading and HemoCue method were done by one of the authors (LdM). To avoid diagnostic review bias, HCS was read before HemoCue analysis. The time interval from the HCS reading to the HemoCue analysis was approximately 30 minutes. To estimate Hb with the HCS (Copack GmbH, Oststeinbek, Germany), a drop of blood was taken from the microtainer with an adjustable pipette, and put on filtration paper. The paper was directly taken outside in daylight to compare the colour with the standard colours printed on the HCS chart, as indicated by Ingram & Lewis.¹⁰

HCS estimations were compared with the HemoCue method (Hb 210 analyzer, HemoCue AB, Ängelholm, Sweden). The accuracy of the instrument was checked on a weekly basis using standard controls.

Data analysis

HCS observations were expressed without decimals, HemoCue estimates were expressed with one decimal. Since a large proportion of the cord Hb estimations was expected to exceed 15, which is the highest possible value on the HCS, we decided to treat all HemoCue observations above 15.0 as 15's, as has been done by other research groups as well.¹⁶ Data were analysed using SPSS for Windows, version 12.0.1 (2003). Agreement between HCS and HemoCue method were measured using Bland and Altman's analysis, following the recommendations of White & Van den Broek¹⁶. For each pair of observations, this method uses the difference between the two measurements (HCS – HemoCue) together with the mean ((HCS + HemoCue)/2). The 95 percent prediction interval is indicated by the limits of agreement.

RESULTS

A total of 250 blood samples were analysed by HCS and HemoCue method. Not all infants were seen at follow-up and as a consequence 38 % of samples was taken from cord blood (n=94), 35 % of 2 month old infants (n=87) and 28 % of 4 month old infants (n=69). Mean Hb (SD) at birth, as measured by HemoCue method, was 14.7 g/dL (1.6), at 2 months 12.0 g/dL (1.4) and at 4 months 11.4 g/dL (1.3). Figure 1 shows that HCS category 15 is relatively prevalent, and this was largely the case in cord blood. The differences in Hb estimations between HCS and HemoCue for all age groups are summarized in table 1. Figure 2 shows Bland-Altman plots by age group.

Figure 1. Histogram showing overall results of Hb estimation by HCS (n=250 samples)

Table 1. Mean differences and limits of agreement by age

Age	Difference (HCS-HemoCue)		Limits of agreement
	Mean (g/dL)	SD	Estimate
Cord	-0.39	0.97	-2.39 : 1.51
2 Months	0.20	1.02	-1.80 : 2.20
4 Months	-0.11	0.95	-1.98 : 1.75

Figure 2. Bland-Altman plots showing difference against mean for Hb estimation with 95% limits of agreement.

The limits of agreement indicate simply that discrepancies of up to 2 g/dL can occur in either direction. Accuracy can also be expressed as the proportion of HCS observations lying within 1 g/dL from the HemoCue measurements (that is the nearest category in the HCS). Agreement within 1 g/dL was 77%, 66% and 74% at birth, two months and four months of age, respectively (table 2). Disagreement with HemoCue measurements of more than 2 g/dl was noted in only 4% of all blood samples.

Table 2. Agreement between Haemoglobin Colour Scale (HCS) and HemoCue method expressed as the nearest category of HCS.

	Within 1 g/dl		Within 2 g/dl	
	1 HCS category away from the real Hb value		2 HCS categories away from the real Hb value	
Age	N	Proportion	N	Proportion
Cord (n=94)	72	77 %	91	97 %
2 Months (n=87)	58	66 %	83	95 %
4 Months (n=69)	51	74 %	67	97 %
Overall (n=250)	181	72 %	241	96 %

DISCUSSION

We evaluated whether the HCS provides Hb estimations in neonatal and infant blood that are sufficiently accurate to be of material benefit in resource-poor settings. Our findings indicate that there is an acceptable agreement of HCS and HemoCue in infant blood collected between birth and the age of four months. Disagreement with HemoCue measurements of more than 2 g/dl was noted in only 4% of all blood samples. Although this discrepancy of measurements might not be acceptable for affluent countries, HCS is far superior to clinical assessment alone and therefore currently the best available method to diagnose anaemia where there is no laboratory.

A limitation of our study was that all HemoCue observations above 15.0 were treated as 15's, and this especially applied to cord Hb. Approximately 40% of cord blood samples exceeded the Hb value of 15.0 g/dL, which is the highest possible value that can be measured by HCS. Omitting this would have resulted in an underestimation of Hb by HCS in the higher ranges, but this is less relevant for anaemia screening programmes. However, HCS should not be used when high Hb levels need to be specified, for example in polycythaemia.

Another limitation was that blood sampling and HCS reading were done by the same researcher. Intra-assessor and inter-assessor agreement have not been evaluated in this study. Earlier studies already found that inter-assessor discrepancies mostly result from incorrect use of the HCS, namely inadequate or excessive blood; reading the results too soon or too late (beyond the limit of two minutes); and poor lighting.^{6,10} The accuracy improves dramatically when HCS readings are trained under supervision with 95% of estimations within 1 g/dL.¹⁰

We did not aim to calculate sensitivity and specificity of the HCS, as the probability of anaemia being present in our study subjects was expected to be low. In term infants from sub-Saharan Africa Hb levels continue to decline after the physiological nadir and start falling below the cut-off for anaemia around 4 months,¹⁷⁻¹⁹ when malaria "immunity" acquired from the mother is wearing off and iron stores from birth are exhausted.¹ Furthermore, care needs to be taken in interpreting sensitivity and specificity, as HCS measurements that are close

to the anaemia cut-off value are at greater risk of being misclassified than those that are more distant.¹⁶

In sub-Saharan Africa, the highest burden of anaemia occurs towards the end of the first year of life²⁰ when up to 75% of all infants are anaemic.¹⁸ Although the disorder is common, it frequently remains undetected and untreated by health-care workers. Use of the HCS as a screening tool, e.g. during regular visits to the mother and child health clinic, could improve timely recognition of anaemia and initiation of treatment.

We conclude that the HCS is sufficiently accurate in estimating Hb levels in infants between zero and four 4 months of age to be of benefit for anaemia screening programmes in resource-poor countries.

ACKNOWLEDGEMENTS

We thank the management board and the staff of the maternity ward and laboratory of Mpongwe Mission Hospital for their hospitality and for facilitating this study. The support of Mrs Hannah de Grooth (General Medical Officer) and Susan Hutton (Financial Advisor) is much appreciated. We thank the mothers and their infants in Mpongwe District for their cooperation and interest in the study.

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7 A PRACTICAL APPROACH TO TIMING CORD CLAMPING IN RESOURCE POOR SETTINGS

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SUMMARY POINTS

- There is little agreement about the optimal time to clamp the umbilical cord after birth
- Delaying clamping of the umbilical cord is a cheap and effective strategy to reduce infant anaemia and improve child survival in resource poor settings
- This practice guideline for singleton vaginal deliveries takes into account the safety of both mothers and infants
- Delayed cord clamping should be considered in every infant born in a resource poor setting, regardless of gestational age
- It should be combined with the administration of oxytocin immediately after delivery to reduce maternal blood loss in the third stage of labour
- Cord clamping should be delayed for at least three minutes for optimal placental transfusion, regardless of fetal weight
- When the state of the infant does not allow a clamping delay of three minutes, aim for a delay of at least 60 seconds with the infant placed between the mother's legs

INTRODUCTION

There is little agreement among doctors and midwives about the optimal time to clamp the umbilical cord after birth. The most important points of difference relate to maternal and infant safety. Many health care workers worldwide tend to clamp the cord and pass the baby off as quickly as possible. Infants in resource poor settings are the main victims of immediate clamping, as this prevents a cost-free means of boosting their small iron stores.

Infant anaemia is common in poor communities, especially where malaria is endemic. In sub-Saharan Africa more than 75% of infants are anaemic before six months of age.^{w1-w3} Infant anaemia is associated with increased mortality,^{w4,w5} and with impaired mental and motor development.^{w6} Its prevention is of critical importance, and delaying clamping of the umbilical cord could be an effective strategy to reduce anaemia and improve child survival.

We propose a practice guideline on cord clamping for resource poor countries for singleton vaginal deliveries, based on published systematic reviews, randomised controlled trials, and biological evidence. Taking account of the safety of mothers and infants, we provide evidence about inclusion and exclusion criteria for delayed cord clamping, optimal timing of clamping, infant position during placental-fetal transfusion, and potential side effects. We present the evidence as a series of structured clinical questions, which identify the population concerned (mothers and infants from resource poor countries), the options being compared (mostly delayed versus immediate cord clamping), and the outcome measures used to measure effectiveness and safety of delayed cord clamping. We also present a practical and simple flow chart for quick reference.

METHODS

Search strategy

We identified systematic reviews from the *Cochrane Library* (Issue 2, 2006). We identified randomised controlled trials from the *Cochrane Library* (Issue 2, 2006), PubMed (1966 to June 2006) and EMBASE (1988 to June 2006) using the terms “umbilical cord” and “clamp*”. We examined related articles and reference lists of published trials, and hand searched major journals on perinatal and tropical medicine. Any new evidence was used to update existing meta-analyses. When a systematic review or meta-analysis did not exist, we completed our own following published methods.^{w7}

Criteria for considering trials for meta-analysis

We included randomised and quasi-randomised trials comparing immediate (within 20 s of birth) with delayed clamping in infants born vaginally at 30-42 weeks' gestation and with birth weight > 1000 g. We excluded infants born before 30 completed weeks' gestation or with birth weights < 1000 g because their mortality is high in resource poor countries.^{w8-w10}

Quality assessment

To minimise bias and to aid interpretation of the guidelines, we used the systematic approach to grading the strength of recommendations developed by the GRADE working group¹:

- Levels of evidence

High—Further research is very unlikely to change our confidence in the estimate of effect

Moderate—Further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate;

Low—Further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate;

Very low—Any estimate of effect is very uncertain.

- Grades of recommendations

The strength of a recommendation indicates the extent to which one can be confident that adherence to the recommendation will do more good than harm

Strong—Do it

Weak—Probably do it

CLINICAL QUESTIONS

► *Is delayed cord clamping associated with improved haematological status in infancy?*

Four randomised controlled trials, all from developing countries, evaluated haemoglobin concentrations in term infants 2-4 months after birth.²⁻⁵ Meta-analysis showed that Hb levels were significantly higher after delayed cord clamping (317 infants, weighted mean difference 4.9 g/L (95% confidence interval 2.6 to 7.2 g/L)). The proportion of infants with anaemia was lower with delayed cord clamping (three trials, 127 infants, relative risk 0.53 (95% confidence interval 0.40 to 0.69)).^{2,3,5} A large randomised controlled trial from Mexico showed a beneficial effect of delayed cord clamping on infant iron status could be measured six months after birth, although haemoglobin levels were no longer different.⁶

The haematological effects of delayed cord clamping in preterm infants were studied in four randomised controlled trials from industrialised countries.⁷⁻¹⁰ The observation period lasted four to six weeks and comprised the time the infants were admitted to the neonatal intensive care unit. Many of these infants born before 30 weeks of gestation would not have survived in resource poor countries. After delayed cord clamping, fewer of these infants required blood transfusion in the first six weeks after birth (183 infants, relative risk 0.64 (95% confidence interval 0.46 to 0.88)).

► *Is delayed cord clamping associated with side effects that require treatment?*

Four controlled trials and one randomised controlled trial, all from industrialised countries,¹¹⁻¹⁶ and two randomised controlled trials from resource poor countries^{5,17} evaluated the incidence of hyperbilirubinaemia and hyperviscosity in term neonates. Packed cell volume was significantly higher after delayed cord clamping, but infants showed no evidence of hyperviscosity syndrome and partial

exchange transfusion was never needed. Although peak bilirubin concentrations tended to be higher after delayed cord clamping, the phototherapy threshold was never exceeded and none required exchange transfusion. Meta-analysis showed that delayed cord clamping in healthy term infants caused no side effects requiring treatment (seven trials, 583 infants, relative risk 0.20 (95% confidence interval 0.01 to 3.97)).

Three randomised trials and one quasi randomised controlled trial in preterm neonates, all from industrialised countries, measured peak bilirubin, and found significantly higher concentrations after delayed cord clamping (259 infants, weighted mean difference 25 $\mu\text{mol/L}$ (95% confidence interval 14 to 36 $\mu\text{mol/L}$)).^{8-10,15} Two of these four trials reported the incidence of hyperbilirubinaemia necessitating treatment and found no difference between delayed cord clamping immediate clamping (138 infants, relative risk 1.09 (95% confidence interval 0.66 to 1.81)).^{10,15} It is unclear whether these results can be extrapolated to resource poor countries, where low birth weight babies are predominantly growth retarded. About a third of the preterm infants in the study by Rabe et al¹⁰ were growth retarded and equally distributed between delayed cord clamping and immediate clamping. Necrotising enterocolitis was examined in two trials, and the incidence did not differ between delayed and immediate clamping (111 infants, RR 0.76 (confidence interval 0.37 to 1.58)).^{9,10}

Body temperature on admission to the neonatal intensive care unit was studied in only one trial, which found no difference between delayed and immediate clamping (39 infants, weighted mean difference 0.20 degrees Celsius (95% confidence interval -0.03 to 0.43)).¹⁰ This should be studied further and advice is required about keeping the baby wrapped and warm.

Recommendation

Delayed cord clamping should be considered in every infant born in a resource poor setting, regardless of gestational age

Grade of recommendation: Strong

► *Is delayed cord clamping associated with increased maternal blood loss?*

Two randomised controlled trials^{5,16} (one from a resource-poor country⁵) evaluated the effect of cord clamping on maternal blood loss. Major limitations of these trials were the differences in the method of measuring blood loss (visual estimation versus measuring jar), the mode of delivery (100% vaginal versus >25% caesarean section), and the definition of delayed cord clamping. The risk of postpartum haemorrhage, defined as blood loss > 500 ml, was not different after delayed cord clamping or immediate clamping (363 participants, RR 0.89 (95% confidence interval 0.58 to 1.36)). A Mexican trial did not quantitatively measure maternal blood loss but classified the bleeding as normal, high or severe, and found no differences between delayed or immediate clamping.⁶

► *How does delayed cord clamping affect obstetric management of the third stage of labour?*

The third stage of labour is defined as the period from expulsion of the fetus to the expulsion of the placenta. In active management the aim is to keep this period as short as possible to reduce maternal blood loss, but the time frame is not exactly specified. In expectant management the aim is to deliver the placenta within one hour without medical interference. A Cochrane review compared active management with expectant management and included five randomised controlled trials from industrialised countries.¹⁸ Active management involves clamping the cord as soon as possible, as well as routinely using prophylactic uterotonic drugs and controlled cord traction. Expectant management is a “hands off” policy, in which signs of placental separation are awaited and spontaneous delivery of the placenta is allowed. Active management was associated with a reduction in clinically estimated maternal blood loss (2 trials, 2941 participants, weighted mean difference -79 ml (95% confidence interval -94 to -64)),^{w11:w12} and a reduced risk of postpartum haemorrhage (four trials, 6284 participants, relative risk 0.38 (95% confidence interval 0.32 to 0.46)).^{w11-w14}

The administration of uterotonic drugs immediately after delivery of the baby, which forms the mainstay of active management, would hasten the transfer of blood into the baby, and increase the infant's red cell mass.^{w15:w16} Immediately after placental transfusion is completed, after about three minutes, the cord can be clamped and cut, and delivery of the placenta by controlled cord traction can commence.

The authors of the Cochrane review concluded that routine “active management is superior to expectant management” in terms of maternal complications, but considered that research was needed in the developing world, with its higher incidence of maternal and infant mortality. Whether all components of full active management are useful should also be investigated.^{w17} The International Confederation of Midwives and the International Federation of Gynaecologists and Obstetricians have not waited for these studies before acting and have removed immediate cord clamping from their recommendations.^{w18} Oxytocin is the uterotonic drug of choice, but there are problems with its universal availability and storage conditions.

Recommendation

Delayed cord clamping should be combined with the administration of oxytocin immediately after delivery of the infant to reduce maternal blood loss in the third stage of labour

Grade of recommendation: Strong

► *Is delayed cord clamping in growth retarded infants associated with more adverse effects?*

A systematic review has established the safety of delayed cord clamping in normal birthweight babies,¹⁹ but there is little information about growth retarded babies.²⁰ Those born in industrialised countries often have an increased inci-

dence of polycythaemia due to chronic hypoxaemia in utero and increased fetal erythropoiesis. In the presence of sufficient iron this leads to increased packed cell volume, although half of growth retarded newborn babies have ferritin concentrations below the fifth centile.^{w19} Most polycythaemic infants remain asymptomatic, although growth retarded babies may be at greater risk of symptoms and the clinical consequences of altered blood viscosity.^{w20}

The baseline risk for polycythaemia and hyperviscosity in growth retarded babies in resource poor countries should be low because many infants have low cord haemoglobin concentration in areas where malaria and maternal iron deficiency anaemia are common.^{w21-w23} In these areas up to 30% of babies have fetal anaemia, defined as cord haemoglobin concentration below 125 g/L.^{w21}

► *What is the optimal delay for cord clamping in infants in relation to their position during placental transfusion?*

Vaginally born, normal birthweight infants

The total fetoplacental blood volume is about 120 ml/kg of fetal weight.^{w24-w27} At birth, the distribution of blood between fetus and placenta is roughly in a ratio of 2:1, and this distribution remains unchanged if the cord is clamped immediately. Figure 1 shows that allowing placental transfusion to occur for at least three minutes results in a larger infant blood volume (ratio 5:1).^{w24;w25} The rate of placental transfusion is markedly influenced by the position of the delivered infant. An infant held 50-60 cm above the placenta will not receive any blood from the placenta. From 10 cm above to 10 cm below the level of the placenta, infants receive the maximum possible amount after at least three minutes of birth. Keeping the infant 40 cm below the placenta hastens placental transfusion to near completion within one minute.^{w24;w28}

Figure 1. Distribution of blood between infant and placenta depending on time of cord clamping after birth (adapted from Linderkamp^{w24} and Yao et al^{w25}). The term infants are at the level of the introitus, about 10 cm below the placenta

The randomised and quasi-randomised controlled trials that studied delayed cord clamping in vaginally born, healthy, term infants differed in clamping time and infant position before clamping (see table 1). All the trials showed that placental transfusion occurred after delayed cord clamping by showing higher packed cell volume or haemoglobin concentration in the first 24 hours after birth compared with immediate clamping.^{2-6;11-16;21;22}

Table 1. Position of infant in relation to timing of cord clamping in trials that studied vaginally born, healthy, term infants.

Position of infant in relation to placenta	Time to cord clamping (minutes)			
	1-2	3	5	>5
>10 cm above	Ceriani Cernades et al ¹⁶	Nelle et al ¹²⁻¹⁴ , Ceriani Cernades et al ¹⁶	-	-
0-10 cm below	Chaparro et al ⁶	Linderkamp et al ¹¹	Grajeda et al ² , van Rheenen et al ⁵ , Pao-Chen et al ²²	Gupta et al ³ , Lanzkowsky ⁴ , Geethanath et al ²¹
30 cm below	Saigal et al ¹⁵	-	Saigal et al ¹⁵	-

Recommendation
 Cord clamping should be delayed for at least three minutes for the optimal volume of placental transfusion, regardless of fetal weight
 Grade of recommendation: Weak

Vaginally born, low birthweight infants

Most of the low birthweight infants in the cord clamping trials were born by caesarean section. Six trials had a sufficient number of vaginally born preterm infants.^{7-9;15;23-25} There was considerable heterogeneity in clamping time and infant position before clamping. In five trials the cord was clamped after a delay of 30-60 seconds,^{7-9;23;25} and all but one compensated for the relatively short delay by lowering the infant as much as cord length permitted to ensure placental transfusion. The trial in which infants were not lowered failed to show placental transfusion.⁸ In the trial with a clamping delay of 1-2 minutes the infants were also not lowered, but the longer delay was sufficient for placental transfusion.²⁴

► *What is the optimal time of clamping when neonatal resuscitation is required?*
 The vast majority of newborn infants do not require resuscitation—immediate drying and keeping them warm is all that is required. Less than 10% of newborns require help to start breathing at birth (stimulation, positioning, clearing the airway), and about 1% require extensive resuscitation. When respiratory efforts are absent or inadequate despite initial stabilisation, positive pressure ventilation with a self inflating bag is the priority.^{w29} The earliest time to assess whether ventilation is successful is approximately 60 seconds after delivery.

All these steps can be done while the umbilical cord is intact. When resuscitation is required the preferred position for the infant should be between the mother's legs, as bag-mask ventilation is not feasible if the infant is placed on the mother's abdomen. Immediate cord clamping to enable resuscitation away from the mother could deprive the infant of much needed extra blood volume, and the resulting hypovolaemia might adversely affect tissue perfusion. Furthermore, as long as the uterus is not contracting and the placenta has not been detached, the infant may still receive oxygen via the intact placental-fetal circulation.

Recommendation

When the state of the infant does not allow a clamping delay of three minutes, aim for a delay of at least 60 seconds with the infant placed between the mother's legs

Grade of recommendation: Weak

CONCLUSIONS

The evidence we reviewed can be summarised as follows:

1. Delayed cord clamping in term infants is safe and, compared with immediate clamping, is associated with higher haemoglobin concentrations and lower incidence of anaemia in the first four months of life, and higher iron stores up to at least six months

Level of evidence: Moderate

2. Although delayed cord clamping in preterm infants is associated with higher peak bilirubin concentrations, the need to give treatment for hyperbilirubinaemia was not different after immediate clamping. Delayed cord clamping is safe in preterm infants and is associated with fewer blood transfusions in the first six weeks of life compared with immediate clamping.

Level of evidence: Moderate

3. Delayed cord clamping in combination with administration of oxytocin immediately after delivery of the baby is safe for mothers.

Level of evidence: Moderate

4. There is little information on delayed cord clamping in growth retarded babies, but the presumed baseline risk for polycythaemia in these infants is probably lower in resource poor countries than in industrialised countries.

Level of evidence: Low

5. In normal birthweight babies, for the maximum possible volume of placental transfusion cord clamping should be delayed for at least three minutes, provided that the position of the baby before clamping is on the mothers' abdomen or lower.

Level of evidence: Moderate

6. In low birthweight babies delaying clamping for 30-60 s without lowering the baby is probably not effective.

Level of evidence: Moderate

7. When immediate neonatal resuscitation is required, place the child between the legs of the mother, start positive pressure ventilation with the umbilical cord intact and delay clamping for at least 60 seconds

Level of evidence: Very low

We also provide a flow chart of our guidelines on cord clamping in resource poor settings for quick reference (figure 2).

Figure 2. Guidelines for cord clamping in resource poor settings

ACKNOWLEDGEMENTS

We thank the following for their critical review of the manuscript (in alphabetical order):

R Broadhead, College of Medicine, University of Malawi, Blantyre, Malawi; J Bunn, Department of Paediatrics, College of Medicine, Blantyre, Malawi; C Chintu, Department of Paediatrics and Child Health, University Teaching Hospital, Lusaka, Zambia; R Cooke, Neonatal Unit, Liverpool Women's Hospital, Liverpool, UK; P Kazembe, Department of Paediatrics, Kamuzo Central Hospital, Lilongwe, Malawi; E Molyneux, Paediatric Department, Queen Elizabeth Central Hospital, College of Medicine, Blantyre, Malawi; JJM van Roosmalen, Department of Obstetrics, Leiden University Medical Centre, Leiden, the Netherlands; DAA Verkuyl, Department of Gynaecology and Obstetrics, Bethesda Hospital, Hoogeveen, the Netherlands.

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8 GENERAL DISCUSSION

Iron deficiency anaemia is one of the most intractable public health problems in poor communities, especially where malaria is common. In sub-Saharan Africa over 50% of infants become anaemic by the age of six months¹⁻³. Iron deficiency anaemia is associated with increased mortality,^{4,5} and has been related to impaired mental and motor development.⁶ Its prevention is of critical importance, and delaying clamping of the umbilical cord could be an effective strategy to reduce these risks and improve child survival.

The studies in this thesis aimed to (1) define the magnitude and main risk factors of iron deficiency anaemia in early infancy, (2) to assess the early and late effects of delayed cord clamping in term, appropriate-for-gestational-age infants, and (3) to design a simple practice guideline on cord clamping for resource poor countries.

CONCLUSIONS

We showed that almost two thirds of Zambian normal birth weight infants were born with low iron stores. In absence of iron supplementation over 50% of them developed iron deficiency anaemia by the age of 6 months. Introduction of complementary foods before the age of 4 months was a risk factor for iron deficiency anaemia in early infancy. Continuation of exclusive breast-feeding after 4 months reduces this risk, but was not sufficient to prevent iron deficiency anaemia. Therefore, adequate iron endowment at birth is essential to reduce anaemia risk during the first months of life in these Zambian babies.

Delaying clamping of the umbilical cord has the potential of improving the iron status of infants by enhancing their red cell mass. However, in hospital deliveries in resource-poor settings immediate cord clamping is the routine standard of care, which prevents a cost-free means of boosting small iron stores. Reasons for immediate clamping relate to safety of mothers (reduction of postpartum haemorrhage) and infants (reduction of polycythaemia and hyperviscosity syndrome; no obstacles for immediate resuscitation), but are based on insufficient evidence. Conversely, the evidence presented in this thesis, based on systematic reviews and randomised controlled trials, shows that delayed cord clamping is

safe for term, normal birth weight infants. Compared with immediate clamping, it is associated with higher haemoglobin concentrations and lower incidence of anaemia in the first 4 months of life, and improved infant iron status up to at least 6 months. Delayed cord clamping in combination with the administration of oxytocin immediately after delivery of the baby is also safe for mothers. No reliable conclusions could be drawn about the potential adverse effects of delayed cord clamping in small-for-gestational-age infants, but the presumed baseline risk for polycythaemia in these infants is probably lower in resource poor countries than in industrialised countries.

The new knowledge on delayed cord clamping presented in this thesis was translated into an evidence-based practice guideline, which recommends that delayed cord clamping should be considered in every infant born in a resource poor setting, regardless of gestational age. It should be combined with the administration of oxytocin immediately after delivery to reduce maternal blood loss in the third stage of labour. Cord clamping should be delayed for at least three minutes, regardless of fetal weight, for optimal redistribution of fetal blood.

COMMENTS

Placental malaria and iron deficiency anaemia

Although our cohort study does not allow assessment of causality, placental malaria infection was identified as an important factor associated with iron deficiency anaemia in early infancy. Other investigators have also reported this association.^{7,8} It is not known whether this association is due to similar malaria exposure in mothers during pregnancy and their infants after birth, or whether it relates to recrudescence of infant malaria or immune sensitisation occurring as a consequence of congenital infection. Another possible explanation is that transplacental transport of iron is disturbed by pathological membrane changes of the syncytiotrophoblast due to placental malaria infection. Excess of perivillous fibrinoid deposits and thickening of the syncytiotrophoblastic basement membrane are the lesions most frequently associated with malarial infection,⁹⁻¹⁴ and thickening of the basement membrane may alter materno-fetal exchange of nutrients.¹⁵

Although there is agreement that iron is bound to transferrin at the maternal side of the placenta, very little is known about how iron is subsequently transported across the syncytiotrophoblast and basement membrane.¹⁵⁻¹⁷

Figure 1. Transmission electron micrograph demonstrating the fetomaternal barrier in the third trimester of pregnancy. The thin syncytiotrophoblast (STB) is in close contact with the fetal vessel, separated only by the basement membrane. Note that the maternal surface of the STB is enlarged by numerous microvilli. (Fuchs and Ellinger, 2004)¹⁶

Limited duration of beneficial haematological effect of delayed cord clamping in malarious areas

We found that delaying clamping of the umbilical cord improved the haematological status of term infants living in a highly malarious area at four months of age, as measured by Hb change from cord blood values or from reduction in anaemia incidence. The beneficial effect of increased red cell mass was no longer apparent between the ages of 4 to 6 months after controlling for the effect of infant malaria infections. A recently published large randomised controlled trial from Mexico, with a similar study design and follow-up time of six months, reported that iron status at six months remained significantly better in infants in the delayed clamped group.¹⁸ In the Mexican trial serum ferritin was used as an indicator of iron status, whereas in the Zambian study zinc-protoporphyrin was used. This may explain these different findings. Figure 2 outlines schematically the relationship between haematological parameters of iron status with increasing depletion of body iron and shows the inverse relationship between serum ferritin and zinc-protoporphyrin.

Maternal haematological baseline characteristics in the Mexican and Zambian trials were comparable, but baseline infant haematological characteristics differed. High ferritin concentration in the cord blood of Mexican infants indicated iron sufficiency, whereas raised levels of zinc-protoporphyrin in the Zambian infants suggested iron deficient erythropoiesis. The poor haematological baseline status of the Zambian infants could relate to placental malaria infection affecting transplacental

iron transport. As a consequence the Zambian infants outgrew their iron stores more quickly. Although the beneficial haematological effect of delaying cord clamping is of shorter duration in malaria-endemic areas, it offers a sustainable strategy to reduce early infant anaemia prevalence, when other interventions are not yet feasible.

Figure 2.
Blood levels of iron indicators with increasing depletion of body iron

Delayed cord clamping in small-for-gestational-age infants

There is good evidence that in small-for-gestational-age (SGA) infants iron status is compromised.¹⁹ Their risk of acquiring anaemia is increased compared to infants born appropriate-for-gestational-age (AGA). This is due to their higher iron requirement during the catch-up growth. In view of the high prevalence of SGA infants born in developing countries^{20;21} delayed cord clamping could be of particular relevance to this group. The safety of delayed cord clamping in AGA (both term²² and preterm²³) infants is reasonably well established, but whether the procedure is safe and effective in SGA infants is unclear. The literature search identified three trials that evaluated delayed cord clamping in term or preterm infants, a proportion of whom were SGA.²⁴ The available data were insufficient to draw reliable conclusions on potential harmful effects in SGA infants. The paucity of information on delayed clamping in SGA infants justifies further research, especially in developing coun-

tries where the baseline risk for polycythaemia-hyperviscosity syndrome is likely to be lower than in industrialised countries.²⁴ A randomised controlled trial of early versus delayed clamping is required in this particular risk group.

Delayed cord clamping and mother-to-child-transmission of HIV

It is not known whether delayed clamping of the umbilical cord increases the risk for mother-to-child HIV transmission. There is no biological evidence that the intervention itself increases transfer of virus particles to the child. At the time of placental separation the integrity of the syncytiotrophoblast and the fetal endothelium might become interrupted with transfer of virus. This is unlikely to occur before the recommended time for delaying clamping of 3 minutes.²⁵ Without prophylactic oxytocic drugs, controlled cord traction or cord drainage the median duration until placental separation is 15 minutes.²⁶ However, in order to minimize perinatal transmission during delayed cord clamping, it is essential to avoid leaving the baby in close contact with the mothers blood.

Umbilical cord blood banking

The present thesis examined the effect of placental transfusion (with the infants own blood) to improve early infant's haematological status. Currently umbilical cord blood is utilised for additional purposes:

(1) Umbilical cord blood transplantation

The haematopoietic stem cells in umbilical cord blood are used with increasing frequency as an alternative to bone marrow or peripheral stem cells for transplantation in the treatment of malignant and non-malignant conditions.²⁷ Umbilical cord blood transplantation has several advantages, including prompt availability, decreased risk of transmissible viral infections and graft-versus-host disease, and ease of collection.²⁸ The number of commercial cord blood banks is rapidly growing and offer long-term storage for possible autologous transfusion in the future, a practice commonly referred to as biological insurance.²⁹ Potential limitations of umbilical cord blood transplantation include insufficient stem cell dose to reliably treat larger children and adult recipients, slower rate of engraftment, and the potential for transfer of genetically abnormal haematopoietic stem cells.

(2) Umbilical cord blood transfusion

In umbilical cord blood transplantation only 0.01% of the nucleated cells of cord whole blood is used, but the other 99.99% could still be used as a viable source of blood for transfusion. At the time of World War II, stored placental blood was explored as a source of blood for transfusion, and found to be similar in effect to fresh adult blood.^{30;31} Recently, cord blood has been used successfully in India and Ghana as an alternative emergency source of blood for severely anaemic patients.³²⁻³⁵ Despite a higher oxygen affinity of fetal haemoglobin, oxygen delivery to the tissues is increased in this condition due to the local hypoxia and the accompanying acidosis, both causing a right shift in the haemoglobin dissociation curve.³⁶

The maximum volume of umbilical cord blood for the above mentioned procedures is obtained after immediate cord clamping to force a larger placental residual volume. Although there has been little agreement among doctors and midwives about the optimal time to clamp the umbilical cord, the decision has always been based on a choice of practice to benefit the infant or the mother. Immediate clamping to harvest more neonatal blood for public banking is not for the benefit of the infant or the mother, but to collect larger volumes of neonatal blood for other children or adults. Quality standards for harvesting neonatal blood reject samples smaller than 70 or 100 mL,^{37,38} while the natural residual placental volume is only 15 mL/kg.³⁹ It is my opinion that the practice of deliberately taking the newborn infant's own blood, knowing that delaying clamping can boost their small iron stores, should be criticized. In addition, the long-term effect(s) for the infant or older child of deliberately removing 25% of the circulating stem cells remains to be studied. Parents who are requested to donate the blood remaining in the placenta should be made aware of the difference between *natural* residual placental blood volume and *forced* residual placental blood volume. A reduction in neonatal blood donation is foreseeable if parents understand that the natural residual placental blood is insufficient for banking and a decrease in infant iron reserves may result from forced volume.⁴⁰

RECOMMENDATIONS FOR FUTURE RESEARCH

The studies in this thesis have lead to a recently published Practice Guideline,²⁵ which can be summarised as follows:

- Delayed cord clamping should be considered in every infant born in a resource poor setting, regardless of gestational age
- It should be combined with the administration of oxytocin immediately after delivery to reduce maternal blood loss in the third stage of labour
- Cord clamping should be delayed for at least three minutes for optimal placental transfusion, regardless of fetal weight
- When the state of the infant does not allow a clamping delay of three minutes, aim for a delay of at least 60 seconds with the infant placed between the mother's legs

These recommendations for singleton vaginal deliveries were based on published systematic reviews and randomised controlled trials, but the level of evidence for the last two statements is moderate. Further research is likely to have an important impact on our confidence in the estimate of effect and may even change the magnitude of the estimate.

Putting evidence into practice

This thesis has collated the research evidence from systematic reviews and primary research and the next step is to ensure that our findings are widely used in clinical practice in low resource settings. Therefore we need to “globalise the evidence, and localise the decision”.⁴¹ At an international level the evidence based Practice Guidelines have been brought to the attention of the World Health Organisation Child and Adolescent Health Group and several professional organisa-

tions. There is a need for an extensive audit in urban and rural health facilities in developing countries to assess current practice and the requirements for implementation of these Practice Guidelines. As part of the audit a small scale research project is to commence in the Caribbean region in the first half of 2007. This will pilot an assessment of issues related to introduction of these new Practice Guidelines.

Placental malaria and iron deficiency anaemia in the infant

There is much we do not know about the pathogenesis of placental malaria and its influence on the transplacental transfer of iron to the infant. No published studies of maternal iron status and placental malaria have been reported, nor is there information on maternal iron supplementation and placental malaria, except in a single study from the Gambia in multigravidae.⁴² Nutrient-placental interactions are an important theme for further research in the context of placental malaria and fetal nutrition.

Limited duration of beneficial haematological effect of delayed clamping in malarious areas

Delaying cord clamping alone was not adequate to prevent iron deficiency or anaemia in the 6 month old infant. Combining delayed clamping with strategies to introduce intermittent antimalarial treatment in infants and the use of impregnated bed nets require further assessment as anaemia control strategy in infants.

Delayed cord clamping in small-for-gestational-age infants in developing countries

The paucity of information on delayed cord clamping in SGA infants, irrespective of their gestational age, justifies further research on this topic. The infants should be closely monitored in hospital for at least 3 days for hyperbilirubinaemia and polycythaemia and hyperviscosity syndrome. Clamping times should vary from 1 to 3 minutes to determine the optimal duration, and a number of interim analyses should be done to enable the study to be stopped early for adverse effects. Frequent infant follow-up is required with attention to intercurrent diseases, feeding practices and haematological status. A randomised controlled trial is in preparation to be undertaken in India, where the prevalence of SGA infants is approximately 20%.²⁰

Delayed cord clamping and mother-to-child-transmission of HIV

Although there is no biological evidence that delaying umbilical cord clamping increases the risk of transfer of HIV virus particles to the child, there are concerns about this possibility. To determine the risk of mother-to-child-transmission of HIV with delayed cord clamping, polymerase chain reaction (PCR) techniques can be used. PCR is able to amplify viral genetic material and the presence of HIV infection in young infants can be established.

Umbilical cord blood banking

Stem cell transplantation is no option for resource-poor countries, but cord blood banking for emergency transfusion is.^{33;35} Cord clamping practice will influence the role of cord blood collection for transfusion purposes. At present a study is being completed in Kenya examining the safety of cord blood collection for both the recipient and the infant donor (Hassall, personal communication).

Delayed cord clamping in caesarean section

The studies in this thesis focussed on delayed cord clamping after vaginal deliveries. Very little research has been done on cord clamping practices in the subgroup of infants delivered by caesarean section. One of the common reasons for emergency caesarean section is fetal distress,⁴³⁻⁴⁵ which is often due to cord compression or nuchal cord. As blood continues to be pumped out by the fetal heart, while at the same time the low pressure return of oxygenated blood from the placenta is obstructed, the net result can be a congested placenta and a depleted fetal blood volume.⁴⁶ If the cord is clamped immediately after caesarean delivery, the excess of oxygenated blood can not return to the infant and the resulting hypovolaemia might adversely affect tissue perfusion.²⁵

In these circumstances it is particularly important that resuscitation is started while the umbilical cord is intact. As long as the uterus is not contracting and the placenta has not been detached, the infant may still receive oxygen via the placental-fetal circulation.

There is no agreement about the optimal position of the infant after caesarean delivery.^{47;48} In most randomised controlled trials which have studied delayed cord clamping in caesarean section deliveries, the infant was held as low as the cord length permitted to enhance placenta-fetal transfusion by gravity.⁴⁹⁻⁵² Certain modifications in the operating theatre may be necessary to facilitate positioning of the infant below the level of the placenta. When the baby is born, a reception table covered with a sterile drape may be rolled near the operating table on which the baby is placed without clamping the cord to observe immediate neonatal adaptation and initiate care by the neonatologist if needed. For control of uterine bleeding during placental transfusion IV oxytocics can be administered to the mother and haemostatic clamps should be placed on the edges of the uterine incision. The maximum possible delay in cord clamping during caesarean section is approximately 90 seconds (Nelle, personal communication), but gravity and oxytocin-stimulated uterine contractions will enhance transfer of blood to the infant.

CONCLUDING REMARKS

The cutting of the umbilical cord is essential and its timing is of critical importance for early child health. The timing of umbilical cord clamping relates to the babies biological and immunological status, and its health during infancy and probably later childhood.

Although cord cutting and clamping is essential its provision has been marred by confusion. Clarity on its implementation is of primary importance for obstet-

ric care and the promotion of appropriate practice guidelines will reinforce good perinatal care. As a cost-free intervention its optimal application should have good “cost-effectiveness”. There is an urgency to promote operational research on this issue and to complement this with relevant biological research. Charles Darwin’s grandfather over 200 years ago promoted physiological clamping and perhaps his early insight has inherent meaning for the “origins of good infant health”.⁵³

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EFFECT OF TIMING OF CORD CLAMPING ON NEONATAL
VENOUS HEMATOCRIT VALUES AND CLINICAL OUTCOME AT TERM:
A RANDOMIZED, CONTROLLED TRIAL

To the Editor.—

We read the article by Ceriani Cernadas et al¹ with great interest. The authors studied the effects of delayed cord clamping (DCC) on neonatal hematocrit values and maternal blood loss in the third stage of labor. Both vaginal deliveries and cesarean sections were included in the study. It was concluded that DCC is effective in increasing hematocrit values in newborns and that the increase was positively related to the delaying time. Furthermore, the intervention is said to be safe for term infants and their mothers irrespective of the duration of the delay in clamping.

These statements may not be true for the subgroup of infants delivered by cesarean section. After delivery by cesarean section, newborns were placed on their mother's lap while waiting for their cords to be clamped. It is unlikely that a substantial augmentation of red cell volume took place in this position unless oxytocic drugs were administered intravenously with or immediately after delivery of the infant. Linderkamp² postulated in 1982 that infants born by cesarean section do not participate in placental transfusion when they are held in a position that is level with the placenta. Failure of the incised and possibly atonic uterus to squeeze the placenta were mentioned as possible explanations. In most randomized, controlled trials that have studied DCC in cesarean-section deliveries, the infant was held as low as the cord length permitted to enhance placenta-fetal transfusion by gravity.³⁻⁶ We suggest a subgroup analysis by mode of delivery to analyze for possible differences in treatment effect of DCC.

Second, we noticed that the prevalence of postpartum hemorrhage (PPH, maternal blood loss > 500 mL) was high in all study groups (27% in the early-cord-clamping group, 22% in the 1-minute cord-clamping group, and 25% in the 3-minute cord-clamping group). Could it be that blood loss during cesarean section was misclassified as PPH? It would be relevant to complete another subgroup analysis for PPH by mode of delivery.

After decades of debate, there is still little agreement among doctors about the optimal time to clamp the umbilical cord after birth, and practice varies widely, especially in developing countries. The time has come to no longer waste precious umbilical cord blood by routine immediate clamping. Obstetricians and

pediatricians require cord-clamping practice guidelines that are based on high-quality systematic reviews and randomized, controlled trials. The study of Ceriani Cernades et al provides a valuable contribution toward developing these guidelines, especially if the influence of cesarean-section practices can be clarified in relation to delivery outcomes.

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Pediatrics 2006; 118; 1317-1318

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In Reply.—

We really appreciate the comments from van Rheenen and Brabin, which undoubtedly have enriched the discussion about this important subject (the timing of umbilical cord clamping).

Our primary hypothesis was to demonstrate that delayed cord clamping (DCC) produced an increase of venous hematocrit within physiologic ranges, with no harmful effects for the neonate. Therefore, the sample size was estimated for this hypothesis, which was later demonstrated.¹ van Rheenen and Brabin led us to another aspect, stating that placental transfusion with DCC is unlikely to occur in cesarean delivery, when the infant is placed at the same level of the maternal uterus. We do not agree with this opinion. In designing the protocol, we already considered this issue, and the scarce available evidence was inconclusive on the level at which to place the neonate in relation to the mother. We prefer to maintain a similar position in both: above the mother's abdomen after vaginal birth and above the legs after cesarean delivery. In both situations, the difference with the placental level was no higher than 10 cm.

It must be noted that, regarding the references indicated by van Rheenen and Brabin, the studies were conducted in very low birth weight preterm infants²⁻⁵ (except for the Linderkamp study⁶); therefore, these are populations extremely different than ours, which only included term newborns. In fact, there is no solid evidence in this respect. In general, the published studies suffer from methodologic pitfalls and also include a reduced number of patients. Sisson et al,⁷ in one of their first published studies regarding this issue, included only 23 neonates divided into 3 groups. In 2 of the groups, the umbilical cord was clamped at 3 minutes: 1 group of neonates was placed 15 cm above the placenta and the other group was placed 15 cm below it. In the group placed above the placenta, transfusion was less compared with those placed below it. The 10-cm difference of level above the placenta may decrease placental transfusion but does not interrupt it, as was described by Yao and Lind⁸ many years ago. In our study, newborns were placed at the same level of the placenta.

Likewise, we considered as very relevant the suggestion made by van Rheenen and Brabin to perform a subgroup analysis according to mode of delivery. In our study, we stratified the randomization by mode of delivery; thus, the analyses are not prone to selection bias, but in view of the small sample size, we could have random error. In Table 1, it is shown that there were no differences in the venous hematocrit at 6 hours among the neonates born by vaginal and cesarean deliveries at

Table 1 Neonatal Hematocrit at 6 Hours According to Mode of Delivery

	Early Clamping (n = 90)	Clamping at 1 min (n = 90)	Clamping at 3 min (n = 92)
Vaginal (n = 193)			
Mean (SD), %	54.7 (7.1)	57.4 (6.0)	60.0 (6.2)
Minimum–Maximum, %	39.7–68.0	43.5–71.0	45.0–75.0
Cesarean section (n = 77)			
Mean (SD), %	50.4 (6.0)	56.3 (5.5)	58.0 (5.7)
Minimum–Maximum, %	41.0–60.0	45.5–66.3	49.0–68.0

Table 2 Differences in Neonatal Hematocrit at 6 Hours According to Mode of Delivery

Comparison	Mean Difference	95% CI
Vaginal (n = 193)		
Early clamping vs clamping at 1 min	-2.729	-5.483 to 0.024
Clamping at 1 min vs 3 min	-2.574	-5.317 to 0.170
Cesarean section (n = 77)		
Early clamping vs clamping at 1 min	-5.879	-9.830 to -1.928
Clamping at 1 min vs 3 min	-1.669	-5.539 to 2.200

Table 3 Neonatal Polycythemia and Anemia at 6 Hours Of Life According to Mode of Delivery

	Early Clamping	Clamping at 1 min	Clamping at 3 min
Vaginal (N = 193), n	65	62	66
Polycythemia (hematocrit > 65%), n (%)	4 (6.2)	4 (6.5)	10 (15.2)
Anemia (hematocrit < 45%), n (%)	3 (4.6)	1 (1.6)	0 (0.0)
Cesarean section (N = 77), n	24	27	26
Polycythemia (hematocrit > 65%), n (%)	0 (0.0)	1 (3.7)	3 (11.5)
Anemia (hematocrit < 45%), n (%)	5 (20.8) ^a	0 (0.0)	0 (0.0) ^a

^a Clamping at 3 minutes versus early clamping: P = .02.

1 and 3 minutes in relation to the main results of the trial. Thus, the hypothesis addressed by van Rheenen and Brabin is not verified in light of our data. On the contrary, we should note that the hematocrit increase between the early-cord-clamping group and the 2 DCC groups was significantly higher in the neonates born by cesarean section than in those born by vaginal delivery, as shown by the mean difference. In Tables 2 and 3, we show interesting results; all anemic neonates (hematocrit < 45%), at both 6 and 24 to 48 hours, are from the immediate-cord-clamping group born by cesarean section. Thus, the anemic neonates were exclusively confined to this group. We do not have an explanation for this phenomenon; there are probably antenatal mechanisms that stimulate

the passage of blood from the placenta to the fetus in vaginal labor but not in cesarean section. It is also necessary to point out that, because the number of neonates in these groups is small, the results should be viewed cautiously because random error can arise.

Our results clearly show that placental transfusion in the DCC newborns takes place in similar ways at vaginal and cesarean deliveries. Moreover, it is evident that, at least in our population, DCC in cesarean-delivered newborns could prevent early neonatal anemia.

In summary, we have clarified the doubt presented by van Rheenen and Brabin. The DCC indication in cesarean section must be the usual practice because, in light of our data, it becomes singularly valuable to increase the blood volume of the newborns and, thus, their iron stores. We hope these findings help to decrease the still-remaining controversies regarding the benefits of DCC and that the practice will be extended to all term deliveries.

With regard to maternal blood loss, we must remark that the measurement of the postpartum hemorrhage was performed only in women with vaginal delivery. Therefore, it is impossible to carry out a subgroup analysis.

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Pediatrics 2006; 118; 1318-1319

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SUMMARY

Early infancy is a period of rapid growth with high iron requirements. In the absence of iron supplementation and proper malaria case management more than 50% of infants in resource-poor countries develop anaemia by the age of six months. Iron deficiency anaemia in infancy is associated with increased mortality and has been related to impaired mental and motor development. Due to the insidious nature of infant anaemia, the disorder frequently remains undetected and untreated by health care workers. Prevention of infant anaemia is of critical importance, yet current coverage with antimalarial interventions and micronutrient supplementation is poor in many countries due to financial, logistic and technical constraints. In these settings cheap and effective interventions are needed to reduce the risk of infant anaemia.

The studies in this thesis aim to evaluate delayed cord clamping (DCC) as a possibly useful strategy to prevent or slow down the onset of infant anaemia in resource-poor settings. DCC is a simple and cost-free delivery procedure that augments the infant's red cell mass at birth. During the delay a redistribution of blood takes place in the fetoplacental compartment, with an additional 20-35 ml/kg of body weight running into the child. The focus in this thesis is on the subgroup of infants who are born appropriate-for-gestational-age (AGA).

In *chapter 2* a cohort of Zambian mother-infant pairs was followed from birth to six months postpartum to determine predictors of iron deficiency anaemia in normal birthweight infants. Almost two thirds of infants were born with low iron stores, and this proportion increased with age. By the age of 6 months, over 50% had developed iron deficiency anaemia. Continuation of exclusive breastfeeding after 4 months could reduce iron deficiency anaemia compared to early introduction of complementary foods, but was not sufficient to prevent anaemia. The presence of histological evidence of past or chronic malaria infection in the placenta biopsy was a predictor for iron deficiency anaemia in infants aged 4 or 6 months in the univariate analysis. In the multivariate analysis this effect disappeared.

In *chapter 3* two systematic reviews are presented that summarise earlier trials on DCC. As the potential adverse effects of DCC are considered to affect small-for-gestational-age (SGA) infants more than AGA babies, we decided to evaluate

the intervention separately for both groups. In the first review all (randomised) controlled trials on DCC in term AGA infants that were published before the year 2003 were evaluated. Polycythaemia, hyperviscosity syndrome and hyperbilirubinaemia are frequently mentioned early complications of DCC, but this systematic review showed that the haematocrit and bilirubin levels were within physiological limits in the DCC group and phototherapy or exchange transfusion were not required. Two of the four trials with a follow up of 2-3 months found a significant difference in infant haemoglobin levels at the end of the study period in favour of delayed cord clamping. This difference was more marked when mothers were anaemic. We concluded that DCC is a safe procedure in term AGA infants and that this obstetric procedure has the potential to reduce the risk of early infant anaemia. These conclusions formed the basis for the design of the research initiatives that are further described in chapters 4, 5 and 7.

The second systematic review focussed on DCC in infants who were SGA, defined as a birthweight below the 10th percentile of a birthweight-for-gestational-age curve. Infants born before 30 completed weeks' gestation or with birthweights < 1000 g were not included as mortality is high in very low birthweight infants who need artificial ventilation in resource-poor countries. To date, no trials have specifically reported the effects of DCC in SGA infants. Three trials were included, of 190 term and 40 preterm infants, a proportion of whom were SGA. The two trials that focussed on term infants showed significantly higher Hb levels 2-3 months after DCC and a significantly lower anaemia incidence. In the preterm group DCC was associated with fewer blood transfusions in the 1st 6 weeks after birth. It was not possible to infer from the available data whether SGA infants were at greater risk of adverse effects in the early neonatal period. The paucity of information on DCC in SGA infants justifies further research, especially in resource-poor countries, where the baseline risk for polycythaemia-hyperviscosity syndrome is likely to be lower than in industrialised countries. We describe the design for a future randomised controlled trial in SGA infants in a resource-poor setting.

Chapter 4 reports the results of a randomised controlled trial that was performed in a non-malarious setting (Libya) and focussed on the short term effects of DCC. Twenty-four hours after delivery the mean infant haemoglobin level was significantly higher in the DCC group. No significant difference was found between the DCC group and the immediately clamped group with respect to total serum bilirubin levels at 24 h, the number of infants requiring phototherapy, and the prevalence of symptomatic polycythaemia-hyperviscosity syndrome.

In *Chapter 5* we assessed whether DCC is effective in improving the long term haematological status of normal birth weight infants in a malaria-endemic area. It was found that infant haemoglobin levels declined throughout the observation period, but more rapidly in controls than in the DCC group. However, the beneficial haematological effect disappeared by six months. DCC offers a strategy to increase iron endowment at birth and can reduce early infant anaemia risk, when antimalarial interventions and micronutrient supplementation are not yet feasible. We advise to include DCC in integrated programmes aimed at reducing anaemia in young children in resource-poor settings.

Due to the insidious nature of infant anaemia, the disorder frequently remains undetected and untreated by health care workers. The haemoglobin colour scale (HCS) is a simple, low-cost and sustainable method that might give a more accurate estimation of the haemoglobin level than using clinical signs. In *Chapter 6* the diagnostic accuracy of the HCS is evaluated for use in early infancy. Our findings indicate that there is an acceptable agreement of HCS and the technically advanced HemoCue method in infant blood collected between birth and the age of four months. Disagreement with HemoCue measurements of more than 2 g/dl was noted in only 4% of all blood samples. Although this discrepancy of measurements might not be acceptable for affluent countries, HCS is far superior to clinical assessment alone and therefore currently the best available method to diagnose anaemia where there is no laboratory. Use of the HCS as a screening tool, e.g. during regular visits to the mother and child health clinic, could improve timely recognition of anaemia and initiation of treatment.

In *chapter 7* a cord clamping practice guideline is presented for resource poor countries for singleton vaginal deliveries, that is based on the latest published systematic reviews, randomised controlled trials, and biological evidence. The recommendations can be summarised as follows:

- Delayed cord clamping should be considered in every infant born in a resource poor setting, regardless of gestational age
- It should be combined with the administration of oxytocin immediately after delivery to reduce maternal blood loss in the third stage of labour
- Cord clamping should be delayed for at least three minutes for optimal placental transfusion, regardless of fetal weight
- When the state of the infant does not allow a clamping delay of three minutes, aim for a delay of at least 60 seconds with the infant placed between the mother's legs

This simple flow chart should hang on the wall in every labour ward in a resource poor setting to remind healthworkers how to give the newborn infant the best possible start of life.

Een van de belangrijke factoren die bijdraagt aan sterfte bij pasgeborenen en zuigelingen in ontwikkelingslanden is bloedarmoede, een aandoening die daarom wel “de bleke dood” wordt genoemd. Zelfs wanneer getracht wordt malaria infecties te controleren en de kinderen extra ijzer toegediend krijgen, blijkt bloedarmoede moeilijk te behandelen. Het toedienen van ijzer aan kinderen in malariagebieden is overigens een zeer controversieel onderwerp, aangezien het de kans op het krijgen van een malaria-infectie zou kunnen vergroten.

Het geleidelijke ontstaan van bloedarmoede is er de oorzaak van dat het lang duurt voordat de aandoening herkend wordt. Wanneer de conditie van het jonge kind zodanig is verslechterd dat een bloedtransfusie onvermijdelijk is, bestaat de kans dat het wordt blootgesteld aan via het bloed overdraagbare ziekten, zoals HIV (het virus dat AIDS veroorzaakt). Het is duidelijk dat het voorkomen van bloedarmoede bij zuigelingen in ontwikkelingslanden van groot belang is. De voorkeur gaat uit naar een goedkope en effectieve oplossing.

Een van die gepaste oplossingen is het wachten met afklemmen van de navelstreng na de geboorte van het kind, zodat er meer bloedcellen vanuit de moederkoek naar het kind kunnen stromen. De pasgeborene krijgt als het ware een transfusie met eigen bloed, dat tijdens de zwangerschap voor de aanvoer van zuurstof en voedingsstoffen heeft gezorgd. In de meeste ziekenhuizen in ontwikkelingslanden wordt het kind direct na de geboorte afgenveld en wordt kostbaar bloed met de moederkoek weggegooid. Verondersteld wordt dat door drie minuten te wachten met afnavelen het kind voldoende ijzer krijgt voor de eerste maanden na de geboorte. Langer wachten met afnavelen heeft geen zin, omdat de bloedstroom in de navelstreng daarna tot stilstand komt. Het is niet bekend hoe lang het effect van “even wachten met afnavelen” voortduurt.

In dit proefschrift wordt beschreven of “even wachten met afnavelen” het ontstaan van bloedarmoede bij kinderen in ontwikkelingslanden kan voorkomen of vertragen. Het zwaartepunt van dit proefschrift ligt bij kinderen met een normaal geboortegewicht.

In *hoofdstuk 2* wordt de uitkomst van een cohort onderzoek beschreven dat plaatsvond in Zambia. Een groep pasgeborenen met een normaal geboortegewicht werd gedurende 6 maanden gevolgd, waarbij geobserveerd werd of zij bloedarmoede

ontwikkelden. Bij de geboorte bleek 2/3 van de pasgeborenen al een te kort aan lichaamsijzer te hebben, zonder dat er op dat moment sprake was van bloedarmoede. Het hemoglobine, een eiwit in de rode bloedcel dat betrokken is bij het transport van zuurstof, is een maat voor bloedarmoede. Na 6 maanden bleek meer dan de helft van de kinderen een te laag hemoglobinegehalte te hebben. Voor de leeftijd van 4 maanden beginnen met bijvoeden leek het risico op bloedarmoede te vergroten. Echter, het geven van uitsluitend borstvoeding tot 6 maanden kon bloedarmoede niet voorkomen. Wanneer er in de moederkoek aanwijzingen waren voor een in de zwangerschap doorgemaakte malaria infectie, hadden de kinderen een vier maal zo grote kans op het ontwikkelen van bloedarmoede.

Hoofdstuk 3 bestaat uit twee delen. In het eerste deel wordt een systematisch overzicht gegeven van alle wetenschappelijke studies naar het effect van “even wachten met afnavelen” tot aan het jaar 2003. Er wordt geconcludeerd dat een transfusie van bloed uit de moederkoek direct na de geboorte veilig is voor kinderen met een normaal geboortegewicht. Onderzoek in India en Guatemala toonde aan dat het hemoglobine gehalte van zuigelingen in de eerste 2 tot 3 maanden na “even wachten met afnavelen” minder afnam dan na direct afnavelen. Of deze bevindingen ook gelden voor kinderen in malaria-gebieden en of het effect langer blijft bestaan dan drie maanden is niet bekend.

Aan de hand van de in dit proefschrift beschreven onderzoeken wordt getracht deze vragen te beantwoorden.

Het tweede deel is een systematisch overzicht van alle onderzoeken die tot 2006 gepubliceerd zijn over de effecten van “even wachten met afnavelen” bij kinderen met een vertraagde groei in de baarmoeder. Er wordt beweerd dat deze groep kinderen een grote kans heeft op het krijgen van bijwerkingen door extra bloed uit de moederkoek. Het blijkt echter dat er tot nu toe nauwelijks *gerandomiseerd onderzoek met een controlegroep* gedaan is bij deze categorie pasgeborenen. Het zou heel goed kunnen dat groeivertraagde kinderen in malaria gebieden juist een lager risico hebben. Dit hoofdstuk wordt besloten met een voorstel hoe toekomstig onderzoek naar de effecten van afnavelen zou moeten worden opgebouwd voor deze categorie kinderen in ontwikkelingslanden.

In *hoofdstuk 4* wordt verslag gedaan van een *gerandomiseerd onderzoek met een controlegroep*, dat in 2003 in Libië werd verricht. Deze vorm van onderzoek is ideaal om de effecten van “even wachten met afnavelen” te evalueren. Hiervoor werd een groep pasgeborenen waarbij de navelstreng laat werd afgeklemd vergeleken met een controlegroep die direct na geboorte was afgenaamd. De toewijzing van de kinderen aan de verschillende groepen werd *door het lot* bepaald om de kans op beïnvloeding van de resultaten te minimaliseren. Dit is de betekenis van het woord *gerandomiseerd*. Het onderzoek in Libië richtte zich op de mogelijke nadelen van de extra toevoer van bloed in de eerste 24 uur na geboorte. Zo werd er gekeken naar de mate van stroperigheid van het bloed en naar geel worden van de kinderen. Er werden geen verschillen gevonden tussen de beide groepen pasgeborenen.

De lange termijn effecten van “even wachten met afnavelen” worden geevalueerd in *hoofdstuk 5*, waarin een *gerandomiseerd onderzoek met een controlegroep* wordt beschreven dat in 2004 in Zambia werd uitgevoerd. De moeders en kinderen die meededen aan het onderzoek wonen in een malaria gebied. Opvallend was dat het hemoglobine gehalte in beide groepen kinderen daalde, maar minder na laat afnavelen. De kans op bloedarmoede op de leeftijd van vier maanden was twee keer zo klein als na direct afnavelen. Echter, zes maanden na geboorte leek het gunstige effect verdwenen en werden er geen verschillen meer gevonden tussen de groepen. Zowel de kinderen als de moeders hadden geen bijwerkingen van het laat afnavelen.

De hemoglobine waarde speelt een centrale rol in de keuze van behandeling bij bloedarmoede. In landen met beperkte middelen is er vaak geen mogelijkheid om een betrouwbare hemoglobine bepaling te verrichten. De hemoglobine kleurenkaart is een eenvoudige goedkope methode voor het vaststellen van het hemoglobine gehalte, waarbij de kleur van een druppel bloed op een filtreerpapierje wordt vergeleken met een standaard die varieert van licht tot donkerrood. In *hoofdstuk 6* wordt beschreven dat deze methode voldoende nauwkeurig is om bloedarmoede bij pasgeborenen en zuigelingen vast te stellen wanneer er geen laboratorium voor handen is. De kleurenkaart kan bijdragen aan het vroegtijdig herkennen en behandelen van bloedarmoede op de zuigelingenleeftijd.

In *hoofdstuk 7* worden alle wetenschappelijke artikelen die tot juli 2006 zijn verschenen over het effect van “even wachten met afnavelen” (inclusief de onderzoeken behorend bij dit proefschrift) samengevat in een richtlijn voor de praktijk:

- Bij ieder kind dat in een ontwikkelingsland geboren wordt dient even gewacht te worden met het afnavelen
- Dit zou gecombineerd moeten worden met het toedienen van een medicijn aan de moeder, dat het samentrekken van de baarmoeder stimuleert en de kans op bloedverlies in het nageboortetijdperk vermindert
- Het “even wachten” moet ten minste 3 minuten duren zodat het kind optimaal gebruik kan maken van het in de moederkoek aanwezige bloed
- Wanneer de conditie van het kind een wachttijd van 3 minuten niet toestaat, dan dient gestreefd te worden naar een wachttijd van tenminste 1 minuut, waarbij het kind tussen de benen van de moeder blijft liggen

Het eenvoudige stroomdiagram dat in dit hoofdstuk wordt besproken zou in iedere verloskamer in de tropen aan de muur moeten hangen.

ACKNOWLEDGEMENTS

I would like to thank:

my promotores,

Prof Dr Bernard Brabin, for his inspiring positivism and creativity, his encouragements and constructive supervision, and for being an example of modesty and politeness, Prof Dr Henkjan Verkade, for his stimulating keen analytic powers, his support, guidance and advice,

in Liverpool (United Kingdom),

Greg Harper, for teaching the laboratory analyses and providing the essential laboratory supplies, Feiko ter Kuile, for his very useful critical comments on chapter 5 and his hospitality, Jean Taylor, for typing Bernard's amendments and facilitate *long distance supervision*,

in Amsterdam (the Netherlands),

Bert Bos and Hennie Knoester, for giving me the freedom to combine a tight clinical duty-roster in the Academic Medical Centre with supervision trips to Libya and Zambia, Sebastian Gruschke, for his assistance in analysing earlier published trials on delayed cord clamping, and for setting up a randomised controlled trial in India, to further study the intervention in small-for-gestational age infants, my fellow paediatric registrars in the Academic Medical Centre, for taking over my clinical duties while being away, Hans Wendte, for drawing the attention of the medical students to the cord clamping trial in Zambia,

in Tripoli (Libya),

Musbah Emhamed, for performing the cord clamping trial in Tripoli Medical Centre and for his generous Libyan hospitality, the doctors and nurse midwives of Tripoli Medical Centre who have contributed to the cord clamping trial with their warm dedication and cooperation, the mothers and infants, for participating,

in Mpongwe (Zambia),

Lette de Moor, Sanne Eschbach, and Hannah de Grooth, for their essential role in the Cord Clamping Trial in Mpongwe, their ambition to make the project work, and their endless efforts to trace participating mothers and infants in remote villages, the Board of Management of Mpongwe Mission Hospital, the Mpongwe District Health Management Team, the chairpersons of the health neighbourhoods and the local chiefs for allowing me to perform the Cord Clamping Trial on their grounds, the staff of maternity ward, mother and child health clinic, and laboratory department of Mpongwe Mission Hospital for their assistance and dedication, John Western, for maintenance of our equipment, Joost de Witte, for the logistical support, the mothers and infants, for participating,

in Barcelona (Spain),

Jaume Ordi, for teaching placenta biopsy reading, and for his hospitality,

in Blaricum (the Netherlands),

Erik Prins and his team at the Pathological Laboratory, for processing placenta tissue and making high quality paraffin sections,

in Groningen (the Netherlands),

Frank Bodewes, Edmond Rings, René Scheenstra, Ekkehard Sturm (paediatric gastroenterologists), who are the best colleagues I can think of with their dedication, unity, sincerity and great sense of humour, the Department of Pathology of the University Medical Centre Groningen, for allowing me to use their light microscope with polarized light,

Robin Broadhead, James Bunn, Chifumbe Chintu, Richard Cooke, Peter Kazembe, Elisabeth Molyneux, Jos van Roosmalen, and Douwe Verkuy, for their critical review of chapter 7,

the Bemmelen Support Group, for their financial support,

The members of the “promotiecommissie” Prof Dr Martien Borgdorff, Prof Dr Hugo Heymans, Prof Dr Piet Kager, Prof Dr Joke Kok, Dr Albert Mantingh, and Prof Dr Pieter Sauer, for their critical review of the manuscript,

and, last but not least,

Katja, for love and understanding, and for continuously showing me the real essence of life, and Adam, Sofia, Hanna, and Jonas (who are gifted with the same sense of perspective as their mother), and Lucia (who is too young to be a critic) for being my children.

CURRICULUM VITAE

The author of this thesis was born on January 16th, 1968 in Groningen, the Netherlands. He finished secondary school in 1986 (Atheneum Nienoordcollege Leek) after which he studied Medicine at the University of Groningen. Early 1994 he worked as a Junior House Officer in Sengerema Hospital in Tanzania. Later that year he was appointed Medical Doctor (Cum Laude) and started his training in Tropical Medicine in the Twenteborg Hospital in Almelo. He worked at the Department of General Surgery (head Dr. R.P. Bleichrodt) and Obstetrics and Gynaecology (head Dr. D. Smit). In 1996 he moved to Zambia to work in St. Theresa's Hospital Ibenga as a tropical doctor. He is still proud of the Network Project, an HIV awareness programme aiming at youths, that combined drama and sports. The project still runs to date and the concept has been copied by many other HIV/AIDS programmes in developing countries. Next to the busy clinical duties, he started doing research, realizing that this was an important way to contribute to a better health care in less privileged countries. During his training as a specialist in paediatrics in St. Lucas Andreas Hospital (head Dr. B. Wolf) and the Emma Children's Hospital – Academic Medical Center in Amsterdam (head Prof. Dr. H.S.A. Heymans) from 2000 to 2004, he kept his fascination in International Child Health. From 2003 onwards he has been working on the studies as described in this thesis. After his registration as Paediatrician he worked a short while in Gooi-Noord Hospital in Blaricum. In August 2005 he started his training in Paediatric Gastroenterology at the University Medical Center in Groningen (head Dr. E.H.H.M. Rings). At the same time he received a scholarship to follow the Postgraduate Epidemiology Programme at the Free University in Amsterdam. He is an active member of the Working Group 'Tropical Paediatrics and International Child Health' of the Dutch Society of Pediatricians (NVK) and board member of the NVK Working Group 'Paediatric Gastroenterology and Nutrition'.

He is happily married to Katja Meijknecht and they have 5 children: Adam (1993), Sofia (1996), Hanna (1998), Jonas (2002), and Lucia (2007).