

Intravenous iron treatment in the puerperium

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THE FOUR ORIGINAL PAPERS ARE

1. Holm C, Thomsen LL, Nørgaard A, Langhoff-Roos J: Intravenous iron isomaltoside 1000 administered by high single-dose infusions or standard medical care for the treatment of fatigue in women after postpartum haemorrhage: study protocol for a randomised controlled trial. *Trials* 2015;16:5.
2. Holm C, Thomsen LL, Nørgaard A, Langhoff-Roos J: Single-dose intravenous iron infusion or oral iron for treatment of fatigue after postpartum haemorrhage: a randomized controlled trial. *Vox Sang.* 2017 Apr;112(3):219-228.
3. Holm C, Thomsen LL, Nørgaard A, Langhoff-Roos J: Single-dose intravenous iron infusion versus red blood cell transfusion for the treatment of severe postpartum anaemia: a randomised controlled pilot study. *Vox Sang.* 2017 Feb;112(2):122-131.
4. Holm C, Thomsen LL, Nørgaard A, Markova V, Michaelson KF, Langhoff-Roos J: Iron concentration in breast milk normalised within one week of a single high-dose infusion of iron isomaltoside in randomised controlled trial. *Acta Paediatr.* 2017 Feb;106(2):256-260.

PREFACE

This PhD thesis was conducted during my employment as industrial PhD student at Pharmacosmos A/S and the Department of Obstetrics, Rigshospitalet, University of Copenhagen. An industrial PhD is a research project and PhD education, conducted jointly by a company (Pharmacosmos A/S) and a university (University of Copenhagen). The project was assessed and approved for funding by the Industrial Researcher Committee, Innovation Fund Denmark (Ministry of Higher Education and Science) (<http://innovationsfonden.dk/en/application/erhvervsphd>).

BACKGROUND

According to The World health organization (WHO) anaemia affects approximately 2 billion people worldwide or 30% of the world's population. Iron deficiency is by far the most common cause, and is the most significant nutrient deficiency in industrialised countries.¹ Due to high iron requirements in pregnancy, pregnant women are vulnerable to iron deficiency and the global prevalence of anaemia in pregnancy is 42%.²

Postpartum haemorrhage (PPH) remains a common cause of maternal morbidity and mortality worldwide, and there is an increasing trend in the incidence of PPH over time in high-resource countries.³ Pre-partum iron deficiency combined with PPH is a major cause of postpartum anaemia.⁴

This thesis focuses on clinical consequences of iron deficiency and anaemia in the puerperium, specifically the efficacy and safety of treatment with intravenous (i.v.) iron.

In this background section the normal course of maternal haematological physiology and basic knowledge about the erythropoiesis and iron homeostasis are described. This must be clarified to enable diagnosis and rational treatment of postpartum iron deficiency and anaemia. Current treatment practice is described, and i.v. iron is suggested as an alternative. In the studies conducted, primary focus is on the effect of i.v. iron treatment on patient reported outcomes, and the background for this approach is described. Lastly, as a background for evaluating the safety aspects of treating lactating women with i.v. iron, the relation between maternal iron status and iron concentration in breast milk is described.

Maternal haematological physiology

During pregnancy the maternal haematological system changes to meet maternal and fetal needs.

According to a review by Jansen (2005), the circulating blood volume increases during pregnancy resulting in a total blood volume of 6 to 7 L. Several blood components contribute differently to this increase: plasma increases by 40%, whereas erythrocyte volume increases 15-20%. Consequently, the haemoglobin (Hb) level decreases by approximately 10%. This natural process of haemodilution improves the placental circulation.⁵

Immediately after delivery the blood volume will decrease corresponding to the amount of blood loss at delivery. Mean Hb levels decrease further in the first days after delivery, due to a redistribi-

bution of extracellular fluid. Plasma volume then decreases as a result of increased diuresis, and from the third day postpartum, Hb levels usually start to increase and in general return to pre-pregnancy values after approximately 6 weeks postpartum.^{4,6,7}

Erythropoiesis

The process of differentiation and maturation from stem cells to erythrocytes (red blood cells) takes place in the bone marrow, and erythropoietin (EPO) (a glycoprotein hormone) and iron are both necessary for erythropoiesis (Fig. 1). According to Besarab (2009), erythropoietin is important during the first approximately 10- to 13-day period by protecting haematological precursor cells from apoptosis. During the subsequent short stage (3-4 days) of erythropoiesis when erythroblasts develop into reticulocytes, iron is incorporated into Hb synthesis and the reticulocytes emerge into the circulating blood.⁸ The reticulocytes contain netlike structures, consisting of the remains of cell organelles and ribosomal DNA, which persist 1-2 days. Any rise in the circulating blood reticulocyte count indicates a rise in erythropoiesis. The functional availability of iron for the haemoglobin synthesis and erythropoiesis can be assessed by the reticulocyte mean haemoglobin content (CHr), and values <26-28 pg may indicate iron-deficient erythropoiesis.⁹

iron binds to the transferrin receptor on the surface of the hepatocyte and is taken up by the cell through endocytosis.¹¹ The iron can be released from the hepatocyte and return into the circulation by the ferroportin transporter. Aged or damaged red blood cells are phagocytosed by macrophages in the spleen and the iron from haemoglobin is transported back into circulation by the ferroportin transporter.¹⁰ There is no known regulated mechanism for excretion of excess iron. About 1-2 mg of iron is lost daily due to bleeding, sweating, skin desquamation and urinary excretion.¹⁵ The iron concentration in plasma and the systemic iron bioavailability are primarily regulated by hepcidin, a peptide hormone produced in the liver. Nemeth (2004) describes how hepcidin inhibits the function of ferroportin, i.e. inhibits the release of iron into circulation resulting in decreased plasma iron concentration. Hepcidin also inhibits the absorption of iron from the lumen of the small intestine. Hepcidin levels decrease in cases of iron deficiency, but increase in response to inflammation. Thus, hepcidin acts as both an acute phase protein and a regulator of iron metabolism.¹⁶

Pavord (2012) describes the spectrum of iron deficiency ranging from iron depletion to iron deficiency anaemia. In iron depletion, the amount of stored iron is reduced, but in mild cases the

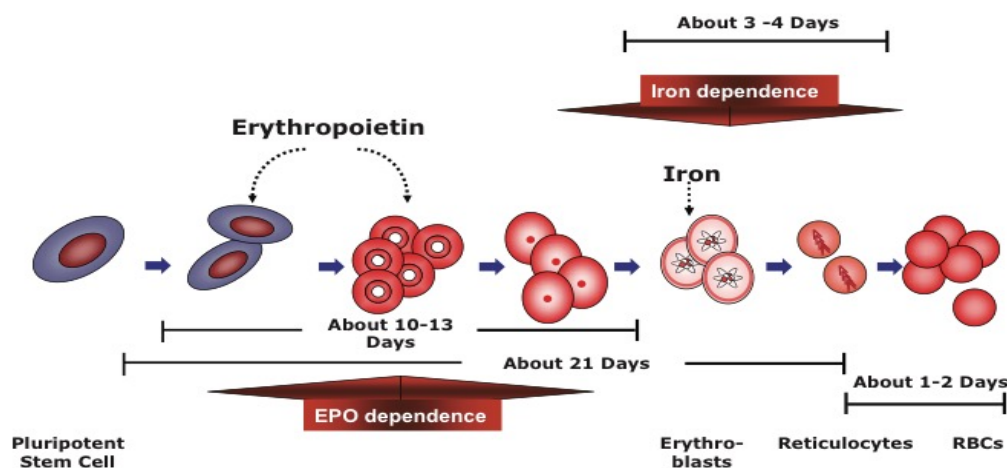


Figure 1 Erythropoietin (EPO) and iron in the erythropoiesis (Modified from Besarab *et al*⁸).

Iron

Iron is essential for many vital biological functions, including oxygen transport in the blood and muscles, mitochondrial energy metabolism, and for other biological processes such as enzyme reactions, neurotransmitter production and immune system functions.¹⁰ According to a review by Munoz (2009), the iron absorption occurs mainly in the duodenum, and the daily absorption is estimated to be 1-2 mg, which under normal conditions balances the loss (Fig. 2). Ferrous iron is absorbed by enterocytes through a divalent metal transporter (DMT1) on the apical surface of these cells.

The iron leaves the enterocyte through the transporter ferroportin on the basolateral surface of the cell. The iron is transported in the circulating blood bound to the carrier protein transferrin. Approximately 75% of the absorbed iron is used in the erythropoiesis.¹³ Iron is stored in the liver as ferritin and in the reticuloendothelial system in the spleen. Normally the liver contains approximately 500-1000 mg of iron. The transferrin-bound

amount of iron transported to the bone marrow and functionally available iron may not be affected. Those with iron depletion have no iron stores to mobilise if the body requires additional iron, e.g. during pregnancy or after blood loss. In iron-deficient erythropoiesis, stored iron is depleted and transport iron is reduced further. The amount of iron absorbed is not sufficient to replace the amount lost, and the shortage of iron limits red blood cell (RBC) production, which may result in the formation of small erythrocytes with reduced Hb content and reduced oxygen-transporting capacity. In iron deficiency anaemia, the most severe form of iron deficiency, there is both decreased iron stores, transport and functional iron, as well as low Hb levels.¹⁷

A gold standard for diagnosing depleted iron stores is the iron content of the bone marrow.¹⁸ An aspiration of bone marrow is, however, too invasive and painful for routine diagnostic purposes in iron deficiency conditions. Hence, in clinical practice and research, a variety of other parameters are used to assess the iron

status with differing degrees of precision and reliability. These parameters include serum or plasma ferritin, iron, transferrin, transferrin saturation (TSAT), total iron-binding capacity (TIBC), soluble transferrin receptor (sTfR), percentage of hypochromic reticulocytes (hypo%) and Chr.¹⁰ In the trials conducted in this project we measured ferritin, serum iron, transferrin, TSAT, and Chr (Tab. 1).

According to Munoz (2011), measurement of ferritin provides the most useful indirect estimate of body iron stores, and ferritin,

and not due to delivery induced inflammatory response.^{4,20}

According to Huch (2005), serum iron is subject to considerable diurnal fluctuations, and therefore of secondary diagnostic value. The normal range for women is 37-145 µg/dL. Transferrin, the iron transport protein, has a normal range between 200 to 400 mg/dL. The ratio between the serum iron concentration and the transferrin concentration, called TSAT is used to determine how much iron is carried by the transferrin. The reference range is 15-

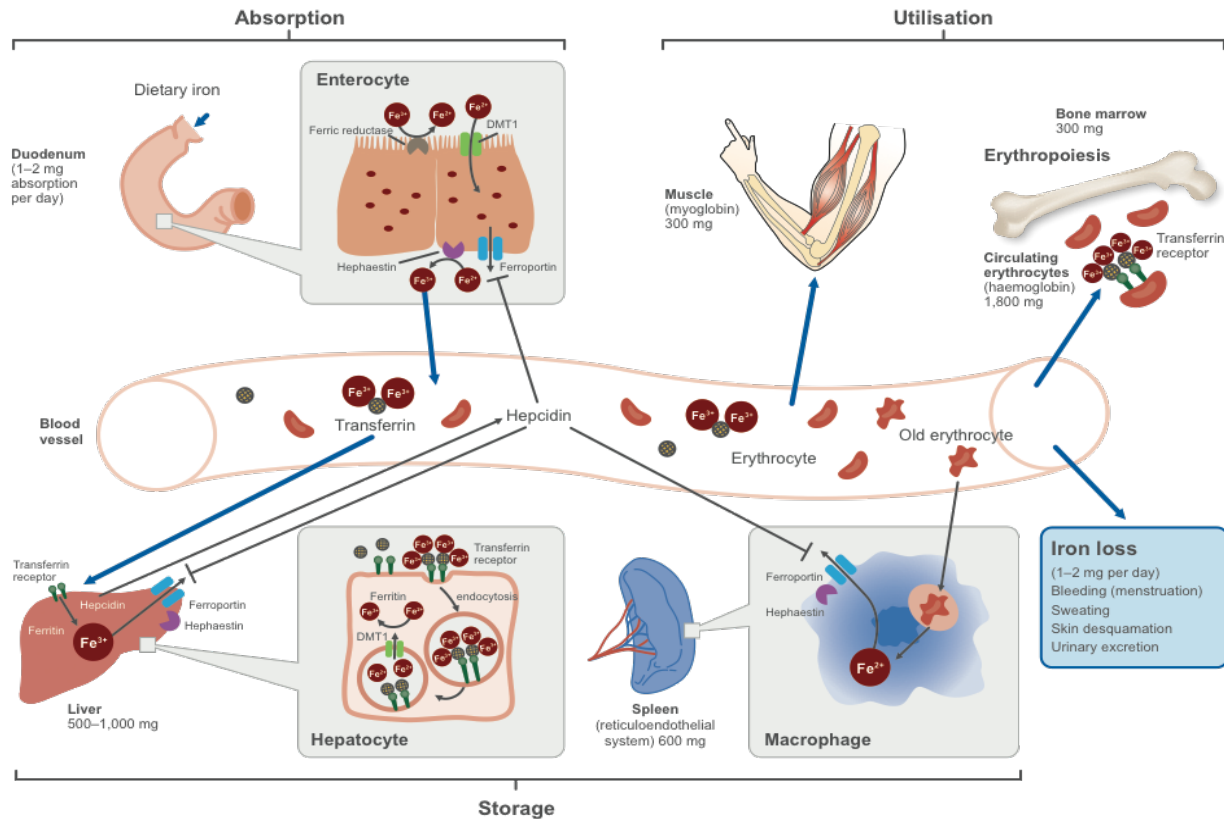


Figure 2 Overview of iron homeostasis and distribution in adults. The total amount of iron in adults is typically 3-5 g. The daily absorption is 1-2 mg, which balances the loss. Ferrous iron (Fe^{2+}) is absorbed in by duodenal enterocytes through a divalent metal transporter (DMT1) and leaves the cell through ferroportin. Hephaestin converts the ferrous iron to ferric iron (Fe^{3+}), which is transported in blood bound to transferrin. Iron is primarily used in the erythropoiesis, and additionally used in muscle (myoglobin) and other tissues (e.g. in enzymes). Iron is stored in the liver as ferritin and in the reticuloendothelial system of the spleen. Iron uptake in the hepatocyte and macrophages is described in details in the text. Hepcidin is the primary regulator of iron metabolism through inhibition of the function of ferroportin, i.e. inhibits the release of iron into circulation. (Modified from Andrews¹¹⁻¹³ and Hasudungan¹⁴)

levels <15-30 µg/L indicate iron deficiency. In the absence of inflammation, the ferritin concentration correlates with total body iron stores and by comparison to the gold standard for assessing bone marrow iron content it has the highest diagnostic precision.¹⁹ In a random sample of 38-year-old women a specificity of 99% for assessing the body's iron stores was found, however, the sensitivity was only 59% as ferritin also serves as an acute phase protein and can increase during inflammation.¹⁸ Thus, inflammation may mask iron deficiency. According to a study by Milman (1991), ferritin increases within the first week after delivery. However, no significant difference was found between the ferritin levels at 1 week compared with 8 weeks postpartum. Milman (2011) suggests that these findings indicate that increased ferritin 1 week after delivery is due to the disappearance of haemodilution

45% in healthy women.²¹ Milman (2011) describes a decrease in serum iron from delivery to 1 week postpartum, followed by an increase from 1 week to 8 weeks postpartum. Milman suggests that these findings indicate that the decrease in serum iron within the first week postpartum is induced by the inflammatory response at delivery, and due to serum iron's role in calculating TSAT, concludes that TSAT is an inappropriate marker in the assessment of iron status 1 week postpartum.⁴

Postpartum haemorrhage

One of the most frequent complications of delivery is postpartum haemorrhage. Traditionally, PPH is defined as a blood loss of 500 ml or more within 24 hours after birth, while severe PPH is defined as a blood loss of 1000 ml or more within the same timeframe.²²

Laboratory test	Normal value
Ferritin	30-300 µg/dL**
Serum iron	37-145 µg/dL* (female)
Transferrin	200-400 mg/dL*
TSAT	15-45%* (female)
CHr	28-35 pg*

*Huch²¹, **Munoz¹⁰, TSAT: Transferrin saturation, CHr: reticulocyte mean haemoglobin content

Table 1 Normal values of the laboratory tests for iron status measured in the trials

A blood loss of 500 mL equals a 250 mg iron loss, approximately.²³ PPH is an important cause of maternal morbidity and mortality. There is no universally accepted definition of PPH between countries, and therefore its reported incidence varies depending on how it is defined.³ Williams' Obstetrics, a classical textbook, mentions a prevalence of 15% of women with blood loss >500 mL at the time of delivery.²⁴

According to the above described physiology, acute blood loss such as PPH without a precondition of iron deficiency or anaemia will lead to an acutely reduced blood volume, which is quickly restored by fluid, hereafter the Hb level falls. Under normal conditions, the reduced oxygen transport will trigger the formation of erythropoietin and quickly accelerate maturation of new erythrocytes. The iron stores in the body might be adequate initially, but can be expected to be reduced or even depleted when erythropoiesis increases. If the PPH is severe, the normal iron stores are exhausted in this process. Hence, an important clinical effect of PPH is the development of iron deficiency and anaemia.

Postpartum iron deficiency and anaemia

Pregnancy creates a large demand for iron, which is needed to support fetal and placental development and to expand the maternal blood volume. The changes in the haematological system during pregnancy are important for maternal and fetal homeostasis, but they also increase the woman's need for iron and place her at risk for developing iron deficiency and anaemia.⁴

Between 1993 and 2005 WHO estimated that, the prevalence of anaemia in pregnancy in Europe and North America were 19% and 6%, respectively, and in Africa and Asia the prevalence was estimated 56% and 42%, respectively.²⁵ In addition to the consequences of maternal anaemia for the fetus, anaemia in pregnancy also increases the risk of subsequent postpartum anaemia and need of peripartum blood transfusions.²⁶

The WHO defines postpartum anaemia as Hb <10.0 g/dL, however, the level of Hb to define postpartum anaemia has been discussed excessively, and higher levels have been suggested.⁴ A Danish study of healthy women with normal deliveries showed a 14% prevalence of anaemia (Hb <11.0 g/dL) one week postpartum in iron-supplemented women and of 24% in women with no iron intake.²⁰ A study from Berlin in 1993–2008 examined women 24–48 hours after delivery; postpartum anaemia defined by Hb <10.0 g/dL was found in 22% and defined by Hb <11.0 g/dL in 50% of

the women.²⁷ During the study, there were no official guidelines in Germany on iron supplementation in pregnancy.

Among Danish women of reproductive age, approximately 40% have low body iron reserves (i.e. ferritin <30 µg/L).²⁸ Combined with the previously described increased iron demands during pregnancy, this makes pre-partum iron deficiency combined with PPH the major cause of postpartum anaemia.⁴

Postpartum iron deficiency anaemia is associated with several clinical consequences, most prominently maternal fatigue.²⁹ Other important consequences include impaired quality of life, reduced physical and mental performance, headaches, orthostatic dizziness, exhaustion, reduced immune function and reduced duration of breastfeeding.^{6,30}

The clinical consequences of postpartum iron deficiency without anaemia are not known in detail and there is a lack of systematic studies on the subject. Iron deficiency without anaemia in women of reproductive age is associated with impaired physical work capacity, fatigue, and deficits in cognitive function and mood.^{6,31} Hence, postpartum iron deficiency without anaemia might contribute significantly to the fatigue experience by women in the puerperium, and sufficient iron treatment might be beneficial.

Treatment options

RBC transfusion

Approximately 2% of mothers in Denmark receive RBC transfusion as a result of severe PPH.³² RBC transfusion may be a lifesaving treatment for ongoing severe bleeding, but it is also widely used for treating severe postpartum anaemia. Specific recommendations for a transfusion trigger for puerperal women in the time shortly after delivery rely on the general recommendations, i.e. clinical signs of severe anaemia and a Hb below 7.0 g/dL (4.3 mmol/L).³³ However, this does not take into account the previously described physiological fluid balance and haemodynamic changes that occur shortly after birth. In practice, the transfusion trigger depends on the clinician, and a number of studies and audits have shown that the transfusion level varies widely between medical teams and institutions, and that a significant proportion of transfusions are given inappropriately.³⁴ A low postpartum Hb level is the most important reason to prescribe RBC transfusions.⁵ The aim of RBC transfusion, however, is not to increase postpartum Hb levels, but to reduce subacute morbidity, particularly severe anaemia symptoms which accompany the course of acutely developed anaemia, such as angina pectoris, severe dyspnoea, and syncope. There is also a need to improve the health-related quality of life, especially longer term fatigue that may influence the mothers ability to care for and bond to a newborn.

There are increasing concerns about adverse effects of transfusions, and the costs of current transfusion practice.^{35,36} The classical risks of transfusion, i.e. administration errors and blood-borne infections, are now rare, although not zero in high-resource countries. However, the potential dangers of RBC transfusions are still numerous and include increased health-care associated infections, volume overload and a number of immunologic adverse effects, including an increased risk of formation of red cell allo-antibodies which may complicate future transfusions and pregnancies.^{17,37,38}

Oral iron

The current standard treatment of mild to moderate iron deficiency anaemia is oral iron supplementation. Gastrointestinal absorption of oral iron supplementation depends on several factors including the administered dose, the haematological iron status, and whether or not the iron supplementation is consumed with food.³⁹ A significant disadvantage of oral iron preparations are the dose-limiting gastrointestinal side-effects such as constipation, gastric irritation and nausea occurring in up to 30% of patients leading to a lack of compliance and reduced long-term efficacy.⁴⁰

Oral iron therapy is the treatment of choice for the majority of patients with iron deficiency anaemia. However, 3 to 6 months of treatment are required for the repletion of iron stores and the normalisation of ferritin levels, and oral iron is often not sufficient in replenishing severe iron deficiencies.⁴¹

Intravenous iron

The currently available intravenous iron preparations are generally considered equally efficacious but vary in terms of molecular size, kinetics, bioavailability and toxicology.^{42,43} Isomaltoside 1000, the carbohydrate component in iron isomaltoside (Monofer, Pharmacosmos A/S, Holbaek, Denmark), has a mean molecular weight of 1,000 Da and consists predominantly of 3 to 5 glucose units. The carbohydrate isomaltoside is linear and unbranched with a low immunogenic potential. Iron isomaltoside has strongly bound iron within the iron isomaltoside matrix formulation, which enables a controlled slow release of bioavailable iron to the iron-binding proteins with minimal risk of free iron toxicity allowing high and rapid dosing.⁴⁴ Following intravenous administration, iron isomaltoside is rapidly taken up by the cells in the reticuloendothelial system, particularly in the liver and spleen.

To date, iron isomaltoside has been approved in more than 30 countries worldwide. The indications are iron deficiency and iron deficiency anaemia when oral iron preparations are ineffective or cannot be used, or where there is a clinical need to deliver iron rapidly. Several studies of iron isomaltoside treatment of iron deficiency anaemia have been published supporting a robust efficacy and safety profile.⁴⁵⁻⁵⁰

Iron isomaltoside was chosen for the clinical trials conducted as part of this project. The drug profile seemed optimal for treating postpartum iron deficiency and anaemia, as it is approved for high single dosing, which is preferable in the treatment of puerperal women. These women need fast correction of their iron deficit without several visits to the obstetric unit for additional infusions.

Previous studies on treatment of postpartum anaemia

A recent Cochrane review on treatment of women with postpartum iron deficiency anaemia concluded that there is limited evidence for treatment with recombinant EPO, and that the previous studies have focused on haematological and iron biomarkers, rather than clinical outcomes. They recommend further research with focus on evaluating the treatment effect by clinical outcomes.⁴⁰

Mild to moderate postpartum anaemia

Ten randomised controlled studies compared i.v. iron to oral iron in women with postpartum iron-deficiency anaemia.⁵¹⁻⁶⁰ They found that i.v. iron was superior as judged by Hb and iron parameters, but none of the trials primarily measured the effect of i.v.

iron administration on patient reported outcomes. Fatigue and psychological well-being were reported as secondary outcomes in two studies. Westad (2008) reported a statistically significant improvement in the mean values from baseline to week 4, 8 and 12 for physical, mental and total fatigue in the group receiving i.v. iron measured by the Fatigue Scale.⁵² Van Wyck (2007) used the Fatigue Linear Analogue Scale Assessment for a mean total fatigue score measured at week 2, 4 and 6, and showed no difference in fatigue.⁵⁴ Both studies used the SF-36 questionnaire to measure psychological well-being, and found overall no difference between the treatment groups.

Severe postpartum anaemia

The WOMB study (Well-being of Obstetric patients on Minimal Blood transfusion) compared RBC transfusion to expectant management of severe postpartum anaemia in a randomised controlled trial with 500 women and showed that RBC transfusion led to a small improvement in physical fatigue measured 3 days after intervention compared to the non-transfused. Hence, the study did not confirm the hypothesis of expectant management being non-inferior to RBC transfusion with relevance to the primary endpoint of physical fatigue.⁶¹

In a retrospective study in women with severe postpartum anaemia, administration of i.v. iron reduced the number of women receiving RBC transfusions with 65%.⁶² However, there are no published randomised controlled trials comparing i.v. iron with RBC transfusion in the treatment of severe postpartum anaemia.

Patient reported outcomes

Randomised clinical trials have traditionally primarily considered objective clinical outcome measures. More recently, investigators and patients have argued that subjective indicators (patient reported outcomes) should be considered.⁶³

It is generally accepted that fatigue is associated with anaemia.⁶⁴ Fatigue is often described as a complex concept in which psychological, biochemical and physiological mechanisms play a role.⁶⁵ A large number of instruments have been developed for fatigue assessment. Most of these are condition-specific measures, i.e. developed for use among specific patient populations (e.g. chronic illness often cancer). One-dimensional measures of fatigue such as the Visual Analogue Scale (VAS) are often used in research. However, according to Smets (1995) a one-dimensional measure limits the full description of the fatigue experienced by the responder. For example, a person that feels physically exhausted and mentally alert might have the same score as a different person that feels mentally tired but physically fit.⁶⁶

The instrument used for measuring the primary endpoints in the trials in this thesis is the Multidimensional Fatigue Inventory (MFI). The MFI was developed with the intention to construct a short, easy to administer questionnaire to assess fatigue and provide information on the different dimensions of the experience, and its intensity.⁶⁶ The MFI evaluates five dimensions of fatigue: general fatigue, physical fatigue, reduced activity, reduced motivation, and mental fatigue, and consists of 20 statements for which the participant indicates, on a five-point scale, the extent to which the particular statement applies with regard to aspects of fatigue experienced during the previous days. Higher scores indicate a higher degree of fatigue.

We selected this generic fatigue questionnaire, the MFI, due to its previous use for evaluation in a postpartum population, where the findings demonstrated a significant correlation between the Hb and fatigue scores.⁶⁷

However, no validated questionnaires have been specifically developed for measuring postpartum fatigue. Therefore, we took the initial steps to develop an instrument to measure fatigue in women in the puerperium. Based on interviews with women with postpartum anaemia we constructed the Postpartum Questionnaire (PPQ) and pre-tested this self-reported tool for feasibility. The PPQ consists of 6 items. First item is a VAS, where the respondent specifies her level of fatigue by indicating a position on a 10 cm continuous line between two end-points. In the second item the respondent is asked to indicate what she experiences as most troublesome, pain or fatigue. In the third item the respondent is asked to indicate whether the fatigue she experiences is mostly physical or mental. For the latter 2 items the respondent is asked to underline 1 of 5 possible responses. The last 3 items explore how different tasks specific for the puerperium are influenced by fatigue (breastfeeding, contact to the newborn and to what extent the respondent needs help with nappy changing). The respondent is asked to indicate, on a four-point scale, the extent of the influence of the fatigue. Higher scores from the last 3 items indicate a higher degree of fatigue.

Postpartum anaemia has been associated with postpartum depression.^{68,69} The Edinburgh Postnatal Depression Scale (EPDS), a self-reported questionnaire, was developed as a tool to screen for postpartum depression.⁷⁰ The validated questionnaire has been used in several clinical trials to assess symptoms of depression in the puerperium.⁷¹ The EPDS consists of 10 questions to detect symptoms of depression in puerperal women during the previous 7 days. The maximum score is 30 and a score of 10 or higher indicates possible depression.

Iron concentration in breast milk

The primary objectives of the trials conducted as part of this thesis are the patient reported outcomes. However, in lactating women given iron treatment we also focused on the safety and potential benefits of treatment for the breastfed infant by laboratory indicators of the iron level in breast milk.

Studies have shown that low maternal iron status and mild to moderate anaemia have no effect on milk iron concentration, except in cases of severe maternal anaemia.⁷²⁻⁷⁷ This general independence of milk iron from maternal Hb and iron status supports the likelihood of an active, rather than passive transfer of iron into breast milk.⁷⁸

In previous studies on iron concentration in breast milk from mothers given iron supplementation diverging results were found. In a comparative study by Zavaleta (1995), anaemic lactating women were treated with oral iron supplementation, and treatment was found not to affect the iron levels in the breast milk.⁷⁵

In a study by Breymann (2007), lactating women with mild anaemia and functional iron deficiency (defined by the study authors as Hb 10-12 g/dL and TSAT <15%) were treated with either 100 mg i.v. iron sucrose or no iron treatment over 4 days. Milk iron concentration was measured before the treatment and every day afterwards. No significant difference between the groups was found at any time point.⁷⁹ In contrast, in a subsequent study by Breymann (2008), lactating women were randomised to 1000 mg i.v. iron carboxymaltose or oral ferrous sulphate 100 mg twice daily, the mean iron concen-

tration in breast milk were measured 48 hours after iron infusion and was found to be significantly higher in the i.v. iron group.⁸⁰

According to Quinn (2014), excess iron in infant nutrition poses two different hypothetical risks. First potential risk is iron overload, where excess iron absorption by the immature intestine of the infant might lead to iron accumulation in tissues and subsequently organ damage.⁷⁸ Second potential risk is the unabsorbed excess iron in the intestine. The majority of the iron will be lost in the stool, however, it might provide as a nutrient for pathogenic iron-requiring bacteria in the intestine, thus change the bacterial composition of the gut flora and hypothetical cause neonatal infection.⁸¹⁻⁸⁴

Information is needed regarding the effect of a high single-dose infusion of iron isomaltoside on milk iron concentration in lactating women, as well as the association between milk iron concentration and maternal iron status and Hb.

In summary, iron deficiency and anaemia occurs frequently in women after delivery. The condition has several clinical consequences and a high single-dose infusion of iron isomaltoside seems relevant as an alternative to current treatment practice with oral iron and RBC transfusion. In order to evaluate the efficacy of the treatment the primary focus should be on the patient reported outcomes, and as part of the assessment of safety, also focus on the iron concentration in breast milk.

AIMS

The overall aim of the studies conducted as part of this PhD project is to explore the clinical aspects of i.v. iron in the treatment of women with postpartum iron deficiency and anaemia.

Study I - Intravenous iron versus oral iron

The aim of the study was to compare i.v. iron with the current treatment practice of giving oral iron supplementation to women after postpartum haemorrhage. We hypothesised that i.v. administration of iron isomaltoside is clinically superior to currently used oral iron supplementation as measured by patient-reported physical fatigue as the primary outcome.

Study II - Intravenous iron versus red blood cell transfusion

The aim of the study was to evaluate the feasibility and exploratory outcomes of a high single-dose infusion of iron isomaltoside compared with RBC transfusion for the treatment of severe postpartum anaemia in haemodynamically stable women.

Study III - Intravenous iron and iron concentration in breast milk

The aim of the study was to measure the concentration of iron in breast milk after treatment with a high single-dose iron isomaltoside compared with current treatment practice with oral iron supplementation from lactating women after postpartum haemorrhage.

METHODS

Study I & III

Design

Study I & III were conducted in a single centre, parallel, open-label randomised controlled trial carried out at the Department of Obstetrics, Rigshospitalet, University of Copenhagen, Denmark.

The participants were randomly assigned to either i.v. iron or current treatment practice with oral iron using an interactive web response system.

Participants

Eligible participants were healthy women with PPH ≥ 700 and $\leq 1,000$ mL; or PPH $>1,000$ mL and Hb >6.5 g/dL measured at least 12 hours after delivery.

Intervention

The participants were enrolled within 48 hours after delivery and if allocated to i.v. iron they received a single dose infusion of 1200 mg iron isomaltoside administered during approximately 15 minutes. The participants allocated to current treatment practice with oral iron received a recommendation to continue oral iron supplementation as they had done during pregnancy or to take 100 mg oral iron one or two times daily for a variable time period. The individual intake of elemental oral iron, including the type of preparation, dose and treatment duration, was monitored throughout the study period. Participants completed the MFI and EPDS, and blood samples were drawn at inclusion and during five visits in the following 12 weeks (study I). Maternal milk samples were collected from participants with sufficient breast milk production three days and one week after inclusion (study III).

Outcomes

The primary outcome in study I was aggregated change in physical fatigue from baseline to 12 weeks measured by a subscale of the MFI. Secondary outcomes included changes in haematological parameters (Hb, reticulocyte count, CHr) and iron biochemical parameters (ferritin, iron, transferrin, and TSAT) and assessment of other dimensions of fatigue and symptoms of postpartum depression. We monitored vital signs before, during, and after infusion, and recorded any adverse events and laboratory safety parameters in both groups.

The primary outcome in study III was the iron concentration in breast milk 3 days and 1 week after intervention.

Statistical analysis

The sample size for study I was determined with reference to the primary outcome. The physical fatigue subscale of MFI allows a maximum change of 16 points, and we set the minimal clinically relevant difference to 1.8 with a SD of 4.2, based on a previous study.⁶⁷ Based on these presumptions, we needed 87 women for each treatment group to demonstrate superiority with a power of 80%. Due to the risk of missing data and drop-outs, 100 women per treatment arm were included.

All statistical tests were two-sided and performed on a 5% significance level. The primary endpoint for study I, aggregated change in physical fatigue score, was calculated as the area under the curve (AUC) of the change from baseline to 12 weeks, using the trapezoidal method. The primary endpoint was analysed using an analysis of variance model (ANOVA), with treatment and bleeding volume (700 mL-1000 mL, >1000 mL) as factors, and baseline MFI physical fatigue score as the covariate. Continuous secondary endpoints were analysed by a mixed model for repeated measurements, and the estimation method was a restricted maximum likelihood-based approach. 'Proportion' endpoints were analysed using logistic regression.

The mean iron concentration in breast milk (Study III) was compared between treatment groups by a two-sided t-test.

Study II

Design

In a second single centre parallel, open-label randomised controlled pilot trial carried out at the Department of Obstetrics, Rigshospitalet, University of Copenhagen, Denmark, the participants were randomly assigned to either i.v. iron or RBC transfusion using an interactive web response system.

Participants

Eligible participants were healthy women with PPH $>1,000$ mL and Hb between 5.6 and 8.1 g/dL measured at least 12 hours after delivery.

Intervention

The participants were included within 48 hours after delivery and if allocated to i.v. iron received a single-dose infusion of 1500 mg iron isomaltoside in approximately 15 minutes. The participants allocated to RBC transfusion received RBC transfusion dosed according to the following trigger Hb levels: Participants with Hb 5.6-6.3 g/dL received 2 units of RBC and participants with Hb 6.4-8.1 g/dL received 1 unit of RBC. Oral iron intake was prohibited in the follow up period.

Participants completed the MFI and EPDS, and blood samples were drawn at inclusion in the hospital and daily visits during the first week after inclusion at the hospital or at home, and at 3, 8, and 12 weeks at home.

Outcomes

The primary outcome in study II was the same as in study I the aggregated change in physical fatigue from baseline to 12 weeks measured by a subscale of the MFI. Secondary outcomes included other dimensions of fatigue, symptoms of postpartum depression, and changes in haematological and iron biochemical parameters (Hb, reticulocyte count, CHr, ferritin, iron, transferrin and TSAT). We monitored vital signs before, during, and after infusion, and recorded any adverse events and laboratory safety parameters in both groups.

Statistical analysis

The nature of the trial was considered explorative and regarded as a pilot for future powering of confirmatory studies. Hence, no power calculation was performed. We estimated that it would be possible to enrol 20 women within the planned recruitment period.

The primary endpoint for study II aggregated change in physical fatigue score was calculated as the AUC of the change from baseline to 12 weeks, using the trapezoidal method and analysed as described above. No formal statistics were planned for the secondary endpoints as there were only planned to be a maximum of 10 women in each treatment group, hence descriptive statistics were applied. Exceptions were change in Hb and change in ferritin that were analysed by a mixed model for repeated measurements, and the estimation method was a restricted maximum likelihood-based approach.

For further details about exclusion criteria, additional outcomes and the statistical methods, the respective articles are referred to.

Ethics

In order to ensure that health research projects such as the randomised controlled trials in this thesis would be carried out in a responsible manner, and that the rights, safety and wellbeing of trial participants were protected, an approval from the National Committee on Biomedical Research Ethics before trial start was required and obtained (approval number: H-4-2013-019 and H-4-2013-023).⁸⁵

Clinical trials with medicinal products in Denmark must only be conducted when the Danish Medicines Agency has given its authorisation (approval number: EudraCT 2012-005782-12 and EudraCT 2012-005783-10), and the trials must be conducted in accordance with the Declaration of Helsinki and Good Clinical Practice Guidelines.^{86,87}

In addition, the trials were registered at the EU Clinical Trials Register before trial start, www.clinicaltrialsregister.eu (EudraCT Number: 2012-005782-12 and 2012-005783-10) and the trial protocol (Article 1) were submitted to the journal *Trials* before completion of patient recruitment.

CONSORT stands for Consolidated Standards of Reporting Trials and the CONSORT statement is an evidence-based, minimum set of recommendation for reporting randomised trials.⁸⁸ These recommendations were followed in the reporting of studies in this thesis.

RESULTS

Study I - intravenous iron versus oral iron

Two hundred women met the eligibility criteria and were randomised (i.v.-iron group, n=100 and oral iron group, n=100).

In all, 196 received the study drug and had at least one post-baseline physical fatigue score, and were included in the full analysis set. Overall, there was no difference in baseline characteristics between the two treatment groups.

The primary endpoint of the difference between the i.v.-iron group and oral iron group with regards to the change in aggregated physical fatigue from baseline to 12 weeks postpartum was -0.97 (CI 95% -1.65; -0.28) ($p=0.006$) in favour of i.v. iron. The difference was greater in the subgroup of women with PPH >1,000 mL -1.13 (CI 95% -2.10; -0.15) ($p=0.02$)

The mean fatigue and depression scores measured by the MFI and EPDS decreased continuously in both treatment groups during the 12 weeks, with overall statistically significant lower scores in the i.v.-iron group particularly in the first weeks (Fig. 3).

Post hoc analysis of the aggregated change in physical fatigue from baseline to 1 week, 3 weeks, and 8 weeks did not meet the minimal clinical relevant difference between the two treatment groups.

The mean Hb increased continuously from baseline to 12 weeks in both treatment groups; however, the increase was significantly higher in the i.v.-iron group (Fig. 4). The reticulocyte count and CHr increased significantly more in the i.v.-iron group compared with the oral iron group especially within the first week.

Ferritin increased promptly and significantly in the i.v.-iron group, but remained unchanged at all time points in the oral iron group (Fig. 5). At 12 weeks, the mean ferritin remained at a level indicating replenished iron stores in the i.v.-iron group, whereas iron stores remained low in the oral iron group (176 ng/mL vs 37

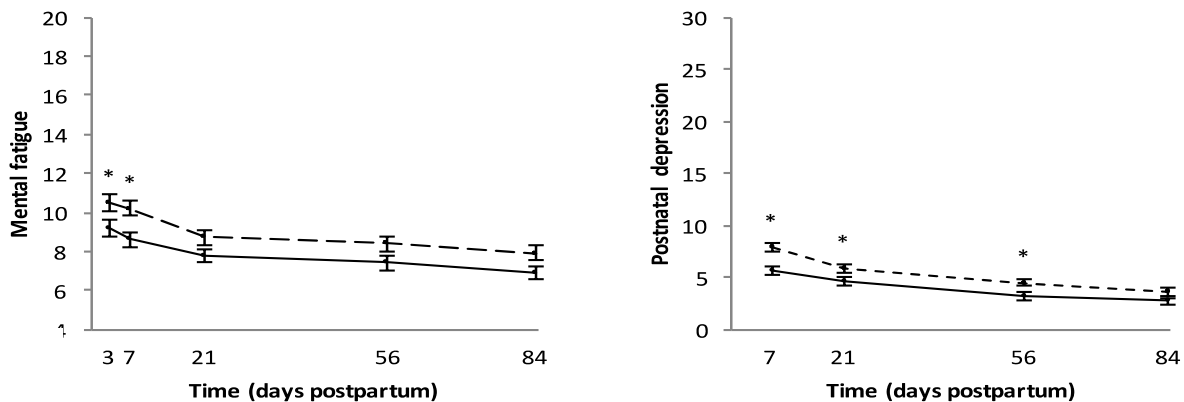


Figure 3 **Physical fatigue and depression.** Results are shown as mean scores of the physical fatigue subscale of the MFI and EPDS in the i.v.-iron and oral iron groups from baseline to 12 weeks postpartum. Whiskers indicate standard error. Between-group comparisons: * $p<0.05$. Solid line = i.v.-iron group; dashed line = oral iron group.

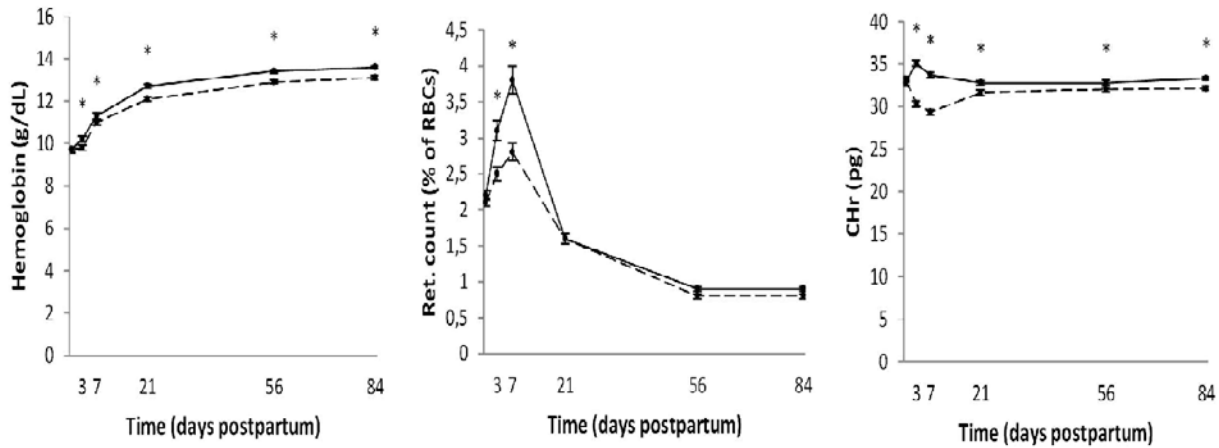


Figure 4 Mean haematological parameters in the i.v.-iron and oral iron groups from baseline to 12 weeks postpartum Whiskers indicate standard error. Between-group comparisons: * $p < 0.05$ in analysis of change from baseline. Solid line = i.v.-iron group; dashed line = oral iron group.

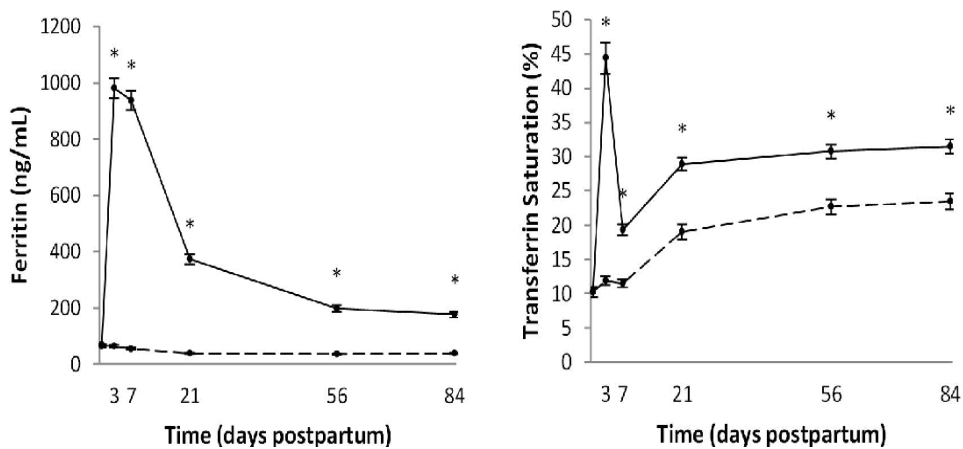


Figure 5 Mean ferritin and transferrin saturation in the i.v.-iron and oral iron groups from baseline to 12 weeks postpartum. Whiskers indicate standard error. Between-group comparisons: * $p < 0.05$ in analysis of change from baseline. Solid line = i.v.-iron group; dashed line = oral iron group.

ng/mL). Significant difference in favour of iron isomaltside was noted in the mean change from baseline to all study time points inTSAT.

In terms of safety assessment, the body system with the highest incidence of reported adverse events was gastrointestinal disorders (33.7% in the i.v.-iron group and 30% in the oral iron group). Overall, adverse drug reactions were experienced by 13.3% and 22.0%, respectively. The body systems with the highest incidence of adverse drug reactions were the gastrointestinal (1.0% and 22.0%, respectively), and administration-site conditions (9.2% and 0.0%, respectively). Two women in the i.v.-iron group presented with back and chest pain during infusion that abated spontaneously over a few minutes without change in vital signs.

Study II - intravenous iron versus red blood cell transfusion

Sixty-two women met the eligibility criteria and 13 were randomised to the i.v.-iron group ($n=7$) or the transfusion group ($n=6$) (Fig. 6). The baseline characteristics were similar in the two treatment groups except age, where women in the transfusion group were on average 4 years older than women in the i.v.-iron group, approximately. All the participants received the planned treatment according to the protocol and no participants were lost to follow-up. In the transfusion group, 5 women received 1 unit and 1 woman received 2 units according to protocol. 'Rescue' RBC transfusion were given to women in both treatment groups.

There was no statistically significant difference in aggregated changes with regard to physical fatigue from baseline to 12 weeks (-0.63 (95% CI: -3.28;2.02, $p=0.61$).

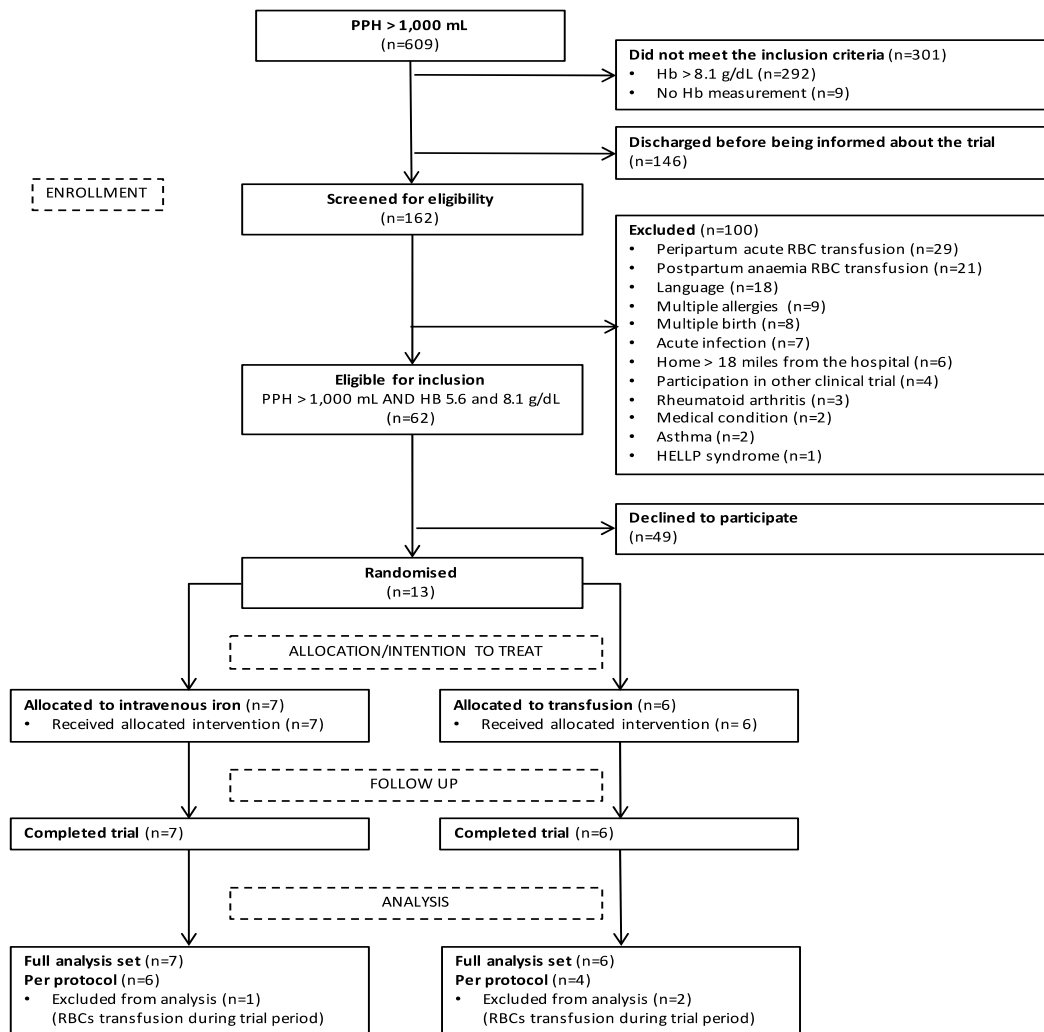
Sensitivity analysis with exclusion of the participants who received 'rescue' RBC transfusion after the randomised treatment at baseline (per protocol population) resulted in an unchanged non-statistically significant difference between the two treatment groups (0.30 (95% CI: -3.26;3.86, $p=0.85$).

No change over time or between the treatment groups could be demonstrated in the fatigue outcomes within the first week (Fig. 7). Overall, the mean scores of all five dimensions of the MFI and EPDS decreased over time from one week and 12 weeks in both treatment groups. In the transfusion group the mean Hb increased significantly from baseline to day 1 and continued increasing slowly over the following 12 weeks to 12.0 g/dL (Fig. 8). The reticulocyte count remained unchanged within the first week, and decreased from 1 week to 12 weeks. The mean CHR decreased within the first week followed by an increase throughout the remaining study period.

The ferritin level decreased throughout the study period and at 12 weeks the mean ferritin was 12.2 ng/mL. No overall change over time was seen for the mean TSAT throughout the study period with all low values between 8.8 and 15.5%. In the i.v.-group the mean Hb increased significantly from one week to 3 weeks and continued an increase to 12 weeks when the mean Hb was 13.2 g/dL. The mean reticulocyte count and CHR increased within the first week followed by an overall decrease throughout the remaining study period. Ferritin and TSAT increased promptly after infusion followed by a decrease throughout the remaining study period. At 12 weeks the mean ferritin was 141.1 ng/mL and the mean TSAT was 25.1%.

There were no serious adverse reactions. The iron isomaltoside infusion was interrupted in 1 participant with back and chest pain. The symptoms occurred within the first 2 minutes of infusion and abated spontaneously over a few minutes without change in vital signs, and the infusion was restarted without recurrence of the symptoms.

Figure 6 Flow diagram from Study II



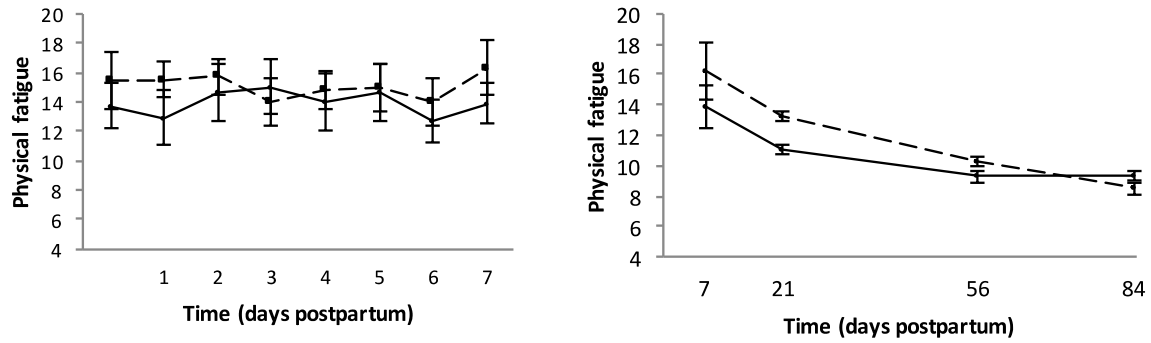


Figure 7 **Physical fatigue**. Results are shown as mean scores of the physical fatigue subscale of the MFI in the i.v.-iron and transfusion groups from baseline to 12 weeks postpartum. Whiskers indicate standard error. Solid line = i.v.-iron group; dashed line = transfusion group.

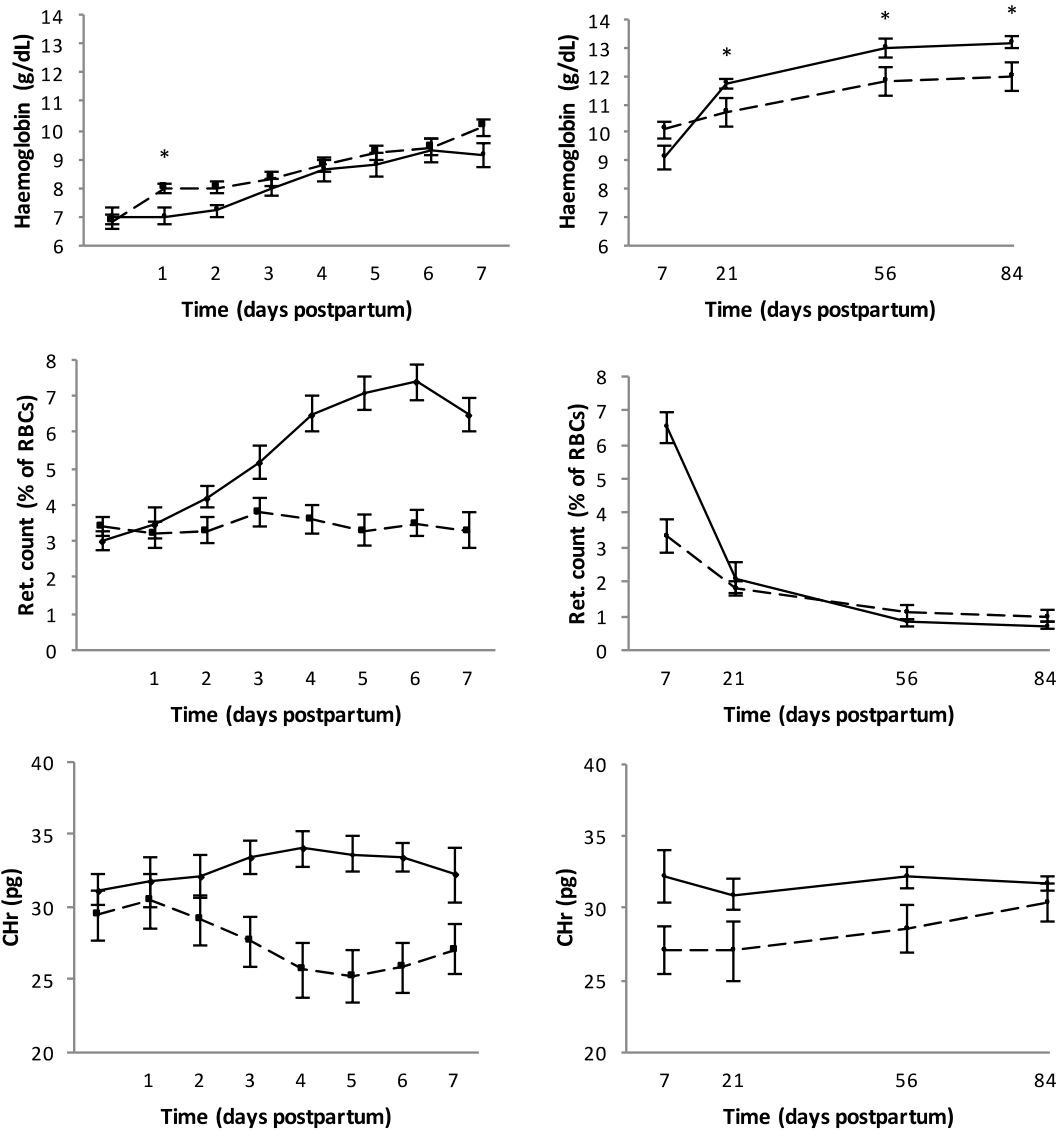


Figure 8 **Mean haematological parameters in the i.v.-iron and transfusion groups from baseline to 12 weeks postpartum**. Whiskers indicate standard error. Between-group comparisons: * $p < 0.05$ in analysis of change from baseline. Solid line = i.v.-iron group; dashed line = transfusion group.

Study III - Intravenous iron and iron concentration in breast milk

Sixty-five women had sufficient milk production 3 days after inclusion; 30 women from the i.v.-iron group and 35 women from the oral iron group.

The mean iron concentration in maternal milk 3 days after intervention was significantly different between groups; 0.72 mg/L in the IV-iron group and 0.40 mg/L in the oral iron group ($p < 0.001$). No statistically significant difference between the groups was seen in mean iron concentration 1 week after intervention: 0.47 mg/L in the i.v.-iron group and 0.44 mg/L in the oral iron group ($p = 0.64$).

The i.v. iron treatment resulted in a normalisation of TSAT in the maternal blood, and repleted the maternal iron stores with a mean ferritin level that was 16 times larger than in the oral iron group (Tab. 2). The mean serum iron level peaked on the third day after treatment with i.v. iron with an increase that was 3.8 times greater than the serum iron level in the oral iron group. However, the mean iron concentration in breast milk only increased with a factor of 1.8 in the i.v. iron group compared to the oral iron group.

ratio. I.v. iron has in previous studies shown fast improvement of Hb and iron biochemical markers in the treatment of postpartum anaemia.

The primary focus in this thesis were the patient reported outcomes when comparing treatment efficacy of i.v. iron with oral iron supplementation in women after postpartum haemorrhage, and test the feasibility and exploratory outcomes of i.v. iron compared with RBC transfusion for the treatment of severe postpartum anaemia.

In order to evaluate safety of i.v. iron with respect to maternal milk content, we further compared the iron concentration in breast milk after treatment between a single high-dose i.v. iron and oral iron supplementation.

Intravenous iron versus oral iron

We compared a single high-dose infusion of iron isomaltoside with current treatment practice with oral iron supplementation primarily measured by the aggregated change in physical fatigue within 12 weeks postpartum, and found a between-group difference that was statistically significant, but less than our consensus-

	IV iron	Oral iron
Full analysis set (n, %)	30 (100.0)	35 (100.0)
Haemoglobin (g/dL)		
Baseline	9.36 (1.27)	9.49 (1.34)
Day 3	10.01 (1.32)	9.71 (1.28)
Week 1	11.03 (1.21)	10.90 (1.24)
Ferritin (ng/mL)		
Baseline	72.24 (52.03)	66.23 (37.20)
Day 3	1017.52 (340.62)	62.59 (36.62)
Week 1	1013.13 (352.33)	50.5 (23.14)
Iron (µg/dL)		
Baseline	41.30 (25.99)	44.04 (26.93)
Day 3	190.90 (93.48)	50.12 (15.90)
Week 1	74.87 (29.58)	50.42 (25.17)
Transferrin (mg/dL)		
Baseline	325.56 (58.45)	306.39 (59.46)
Day 3	311.98 (52.29)	324.51 (54.06)
Week 1	298.06 (44.08)	330.83 (45.64)
Transferrin saturation (%)		
Baseline	9.25 (5.47)	10.31 (5.89)
Day 3	45.45 (22.25)	11.29 (3.82)
Week 1	18.5 (7.03)	11.21 (5.75)

Values are expressed as mean (SD) unless indicated otherwise
 IV: intravenous, n: Number of participants, SD: standard deviation

Table 2 Summary of haematological parameters in the two treatment groups at baseline, and three days and one week after intervention.

DISCUSSION

Iron deficiency and anaemia are known to have several clinical important consequences. Oral iron frequently causes gastrointestinal side-effects with subsequent poor compliance and accumulating evidence has questioned the RBC transfusion's risk-benefit

based pre-defined minimal clinically relevant difference for claiming superiority.

Across visits, particularly in the first weeks postpartum, we found differences in the fatigue and depression scores, as well as hae-

matological and iron-related biochemical parameters, all statistically significant in favour of i.v. iron.

As mentioned in the background section, previous studies have shown that measures of both Hb and iron parameters are inaccurate to determine iron and haematological status within the first week postpartum. When treating women after delivery we do, however, not want to wait 1 week to treat these women as the potential consequences of iron deficiency and anaemia might occur already in the immediate time after delivery. We therefore chose to include women based on their amount of blood loss at delivery instead of biochemical parameters.

We chose an unconventional cut off for postpartum haemorrhage (≥ 700 mL). The reason for this was a change in the method of estimating the blood loss after vaginal delivery at the Department of Obstetrics, Rigshospitalet prior to enrolment of women in the trial. Previously the amount of blood loss had been estimated visually, but practice changed to quantification of the blood loss by weighing the delivery items. After the change in practice the rates of PPH defined as blood loss ≥ 500 mL increased to 23%. To avoid overtreatment of women with a non-pathological amount of blood loss, we chose to include women with blood loss ≥ 700 mL which occurred in 13% of all deliveries (data from our local obstetric database).

To our knowledge this is the first randomised controlled trial that primarily focused on clinical outcomes in comparing treatment options for postpartum iron deficiency and anaemia, and a major strength of this study is the thorough follow-up with minimal missing data.

The choice of an individualised oral iron treatment regimen as comparator to i.v. iron in this study may be seen as a limitation due to the low mean iron intake in the study period.

As gastrointestinal side effects to oral iron supplementation are well-known and results in poor compliance, especially in a population of women in the puerperium where constipation and haemorrhoids are often present,⁸⁹ we chose an individualised oral iron treatment regimen as a comparator to simulate normal clinical practice as opposed to a fixed dosing regimen that would result in poorer compliance outside a clinical study setting.

An inherent weakness was the open-label design. I.v. iron is a black fluid, and oral iron is known to colour the stools. Both facts are challenges when conducting a double-blind trial and no absolute blinding guarantee could be achieved. The individualised oral iron treatment regimen design implies that we were not able to blind the randomisation, and we assessed that a double-blinded design would be unfeasible. The clinical outcomes from the self-reported questionnaires may be influenced by the participants' knowledge of which treatment they received, and a placebo effect in the i.v.-iron group cannot be discarded. The study might have been strengthened by including physical tests such as exercise capacity or muscle strength to measure physical capacity after childbirth and accompanying the subjective outcomes.

Intravenous iron versus red blood cell transfusion

We conducted a pilot study to test feasibility and exploratory outcomes of a high single-dose infusion of iron isomaltoside

compared with RBC transfusion for the treatment of severe postpartum anaemia.

No change over time could be demonstrated in the fatigue outcomes within the first week. Overall the fatigue and depression scores decreased over time from 1 to 12 weeks, and no between-group differences could be demonstrated. RBC transfusion showed an immediate effect on Hb, however iron biochemical outcomes remained low. I.v. iron improved iron biochemical outcomes significantly and provided a long-term normalisation of Hb.

This pilot trial showed that it was possible, but difficult and time-consuming, to include eligible women with severe postpartum anaemia and randomise them to one of two treatment arms and give the planned treatment according to the protocol. A large part of the women did not meet the eligibility criteria of this pilot study (Fig. 6). To enhance the inclusion in a future larger study we would consider including women who received acute peripartum RBC transfusion before inclusion in the trial. This pilot study showed that a significant proportion of the women with severe anaemia after PPH had already received acute RBC transfusion during the acute treatment of the bleeding or shortly after due to low Hb. If these women are severely anaemic after the acute transfusion, they could be considered eligible in future studies. One must of course keep in mind that by including these women in a trial the safety assessment will be biased and the 'clean' comparison of RBC transfusion and i.v. iron will not be possible.

We also found that a large part of the eligible women declined to participate. Due to the explorative pilot nature of this study we chose to measure both clinical and biochemical outcomes daily in the first week. However, the intense follow-up visits were noted as a reason for the women to decline participation, hence fewer follow-up visits should be considered in the design of a larger trial.

To minimise the loss to follow-up we performed the follow up visits in the participants' home after discharge from the hospital, resulting in no loss to follow-up.

'Rescue' RBC transfusions were given to women in both treatment groups with symptoms of severe anaemia. This might indicate that the planned treatment in both treatment groups was insufficient, or more likely confirm previous findings that a significant proportion of transfusions are given inappropriately in the postpartum period.³⁴ Adding a clear definition of absolute indication for RBC transfusion (e.g. chest pain, orthostatic hypotension or tachycardia unresponsive to fluid resuscitation⁹⁰) might improve 1) the determination of eligibility to the trial and 2) the decision whether or not a 'rescue' RBC transfusion is indicated after the randomised treatment at baseline.

The strengths of the study include that this to our knowledge is the first randomised controlled trial comparing i.v. iron with RBC transfusion in the treatment of severe postpartum anaemia.

The participants had no RBC transfusion prior to inclusion and no oral iron intake in the follow-up period. This 'clean' design is unique in randomised controlled trials with RBC transfusion.

The daily clinical scores with simultaneous blood sampling during the first week after intervention is a very good exploration in time

variation in both types of measurements during the first week and gives important information on when and what to measure early on.

Even though this was a pilot study, the very low number of participants was a major weakness. Hence the results should be considered with caution. A limitation was also the 'rescue' RBC transfusion given in both treatment groups, causing interference in the 'clean' group comparison.

Patient reported outcomes

In research and subsequent treatment guidelines there is at present increased emphasis on patient reported outcomes. The recent published Cochrane review of treatment of women with postpartum iron deficiency anaemia suggested a need for trials with clinical outcomes.⁴⁰

The primary clinical aims of the studies in this thesis were to assess and compare patient reported outcomes in women with postpartum iron deficiency and anaemia, and as Jansen (2007) found a low Hb to be associated with the physical fatigue subscale and not the mental fatigue subscale, we chose the physical fatigue subscale of the MFI for our primary outcome.⁹¹

We found that physical fatigue improved over time from baseline to week 12, however, the difference between the two treatment groups in study I in terms of aggregated change in physical fatigue from baseline to week 12 was not as large as the pre-specified minimal clinically relevant difference to proclaim clinical superiority. The question is to what extent the treatment of iron deficiency and anaemia influence fatigue in women postpartum, where many other factors influence their fatigue level. The birth of a child has a major impact on the new mother. Her self-reported fatigue is affected by a variety of medical, psychological, social, and obstetric factors, such as her age, general physical health, health of the baby, length of labour, parity, mode of delivery, maternal expectations, and quality of sleep.⁶⁷

No validated questionnaires have been specifically developed for measuring postpartum fatigue, and we took the initial steps to develop an instrument to measure fatigue in women in the puerperium. Based on interviews with women with postpartum anaemia we constructed the Postpartum Questionnaire (PPQ) and pre-tested the self-reported questionnaire for feasibility (i.e. missing answers, reported difficulties, and completion time). The PPQ was further tested in the trials in this thesis and some construction issues were detected with the response options for some of the items. In item 2 the respondent was asked to discriminate between pain and fatigue, and in item 3 to discriminate between mental and physical dimensions of fatigue. If a respondent felt no pain or no fatigue, she did not have a response option that could accurately capture her response, i.e. the response options were not collectively exhaustive. This must be corrected by applying additional response option before further testing of the tool.

We found statistically significant differences in the mean fatigue and depression scores measured by the MFI and EPDS between i.v. iron and oral iron supplementation, but the question is if these differences also represent a clinical significance.

In order to interpret the clinical outcomes, a pre-definition of the

smallest difference in the patient reported outcome would be required so that we as clinicians (alternatively the patients) would identify this as important (the minimal clinically relevant difference). The minimum clinically relevant difference for the MFI has not been established for women in the puerperium. Based on clinical judgment we chose a difference greater than 10% in the patient's perception of physical fatigue for claiming clinically relevant superiority. In order to design future clinical relevant trials using the MFI to measure the primary outcome there is a need to establish the minimal clinically relevant difference for the MFI in women in the puerperium.

The primary endpoint of the trials was predefined as the difference in the aggregated change in physical fatigue from baseline to 12 weeks between the two treatment groups. The aggregated change in physical fatigue was calculated as the AUC from baseline to 12 weeks. The AUC measure is often used in clinical pharmacology, but has also been used for clinical endpoints, for example, in pain assessment.^{92,93} We decided to use the AUC as our primary endpoint as it included all physical fatigue scores measured in the study period. It can be interpreted as an integral measure of total physical fatigue score rather than using a physical fatigue score only at a specific time point, which it can be difficult to predefine.

In retrospect, one might question whether aggregated change in physical fatigue from baseline to 12 weeks was the proper primary endpoint for a randomised trial comparing iron treatment options immediately after delivery. Previous studies on biochemical parameters suggested that i.v. iron had a faster response than oral iron,⁵¹⁻⁶⁰ and from a clinical point of view the primary aim would be to improve the symptoms within the first weeks after delivery.

However, post hoc analysis of the aggregated change in physical fatigue from baseline to 1 week, 3 weeks and 8 weeks did not meet our pre-defined minimal clinical relevant difference between the two treatment groups.

Biochemical outcomes

The Hb increased from baseline to all following time points significantly more after a single high-dose iron infusion compared to treatment with oral iron after PPH. Transfusion of 1-2 units of RBCs increased the Hb significantly more 1 day after treatment compared to a single high-dose iron infusion, whereas after 3, 8 and 12 weeks the mean Hb was significantly higher in the women treated with i.v. iron.

In the early haematopoietic response, the reticulocyte count is expected to increase after acute blood loss to compensate for the low Hb. We found an increase within the first week after iron treatment, and the increase was significantly higher after i.v. iron compared to oral iron. We found differences in the reticulocytes count based on daily measurements, suggesting that transfusion of 1-2 units effectively inhibits the haematopoietic response to acute anaemia whereas i.v. iron effectively supports the haematopoietic response. Additionally, the i.v. iron significantly increased the functional bioavailability of iron for the erythropoiesis as assessed from the CHr, compared to oral iron and RBC transfusion which both resulted in decreased functional iron bioavailability. We found that current treatment practice with oral iron and RBC transfusion failed to replete iron stores,

whereas i.v. iron ensured prompt replenishment of iron stores that persisted throughout the 12 weeks. This long term benefit may reduce the risk of re-occurrence of iron-deficiency anaemia once menses resume and during subsequent pregnancies. From a clinical perspective, these could be significant findings.

Safety

Comparison of the frequency in adverse events and adverse drug reactions between trials is difficult. In study I, we found a similarly high reporting of gastrointestinal symptoms in both treatment groups (reporting of adverse events of any cause). However, in reporting of drug-related reactions (adverse drug reactions) the rate differed significantly between the treatment groups. As gastrointestinal symptoms are well-known side effects to oral iron supplementation, these symptoms were categorised as related or possible related in this group, whereas gastrointestinal symptoms in the i.v.-iron group were not considered drug related. So even though the frequency of gastrointestinal symptoms was the same in the two treatment group, when reporting adverse drug reactions only the symptoms in the oral iron group were reported. The GCP definition of adverse events is: "Any untoward medical occurrence in the patient or clinical trial subject administered a medicinal product and which does not necessarily have a causal relationship with this treatment", and it is recommended to ask the participants with an open question whether they experienced adverse events since the last follow-up.⁸⁷ The investigator interprets the information in a biomedical framework and might filter some of it, especially if he/she believes the event is not drug related.⁹⁴ This discrepancy in reporting adverse events and classifications of relation to the study drug makes comparison of the frequency in adverse events and adverse drug reactions between trials difficult.

The focus on safety related to i.v. iron infusion has always been a debated issue and the European Medicines Agency recently reviewed the safety of i.v. iron. It concluded that the benefits of i.v. iron are greater than the risks and was unable to establish a difference in the safety profiles of the products.⁹⁵ The single high-dose infusion in the two randomised controlled trials was well tolerated and not associated with any serious adverse reactions.

In both trials we observed the reaction described by Dr. Fishbane.^{96,97} Acute back or chest pain during infusion that abate spontaneously without change in vital signs. When the reaction occurs, it initially simulates the beginning of a hypersensitivity reaction with chest compression and flushing followed by back pain. However, the symptoms abate spontaneously over a few minutes, and do not reoccur when infusion is restarted. The pathogenesis remains unknown, but according to Rampton (2014), it is believed to be a complement activation-related pseudo-allergy (CARPA) triggered by iron nano-particles. The reaction only appears when the i.v. iron is first infused, and does not reoccur in subsequent exposures, as opposed to an immunological IgE-mediated reaction that would reoccur and worsen in subsequent exposures and require treatment to resolve.⁹⁸

Infusion site discolourations occurred in 4 of the 105 women treated with iron isomaltoside in the two trials. The discolourations appeared in various circumferences surrounding the infusion site without accompanying symptoms, and the discolourations abated slowly with complete remission after approximately one year. The participants felt bothered by the discomfort of the

cosmetic appearance on the often very visible body part of the lower arm or hand.

Intravenous iron and iron concentration in breast milk

A single dose infusion of iron isomaltoside lead to a significant increase in the iron concentration in breast milk within the first week after treatment compared to the iron milk concentration in women treated with oral iron supplementation. The increase was transient and one week after onset of treatment the between-group and within-group difference had disappeared.



Figure 9 **Infusion site discolouration** (photographed one day after infusion).

The maternal blood samples showed significant differences between the treatment groups in the iron metabolism in blood compared to the moderate variations in the iron concentration in breast milk. These results support the assumption that the mammary glands actively regulate the iron uptake even after treatment with high single-dose i.v. iron.^{80,75,99}

The mean levels of iron concentration in breast milk found in study III were all within what is considered the normal range of iron concentration in breast milk.¹⁰⁰⁻¹⁰³ The question is if a transient elevation in iron concentration in colostrum after treatment with i.v. iron either raises a safety concern or has benefits for the infant. The risks associated with excess iron such as neonatal infections or iron overload are considered irrelevant taking into account that the mean iron concentration at the peak still remained within the normal range of iron in colostrum, and the fact that the iron level in infant formula is 10-fold higher.^{104,105}

In terms of benefit, we do not expect the short transient elevation of the iron concentration in colostrum to be of any benefit for the infant's iron status.

This is to our knowledge the first randomised controlled trial in which the iron concentration in breast milk from women receiving high single-dose i.v. iron or oral iron supplementation has been compared using concurrent collection of breast milk and maternal blood samples. This thereby adds to the knowledge on maternal regulation of milk iron concentration and provides important information about the safety of i.v. iron treatment of lactating women.

A limitation to study III was that we had no baseline measurement of iron concentration in breast milk. The women were included within 48 hours after delivery, i.e. before onset of the postpartum lactogenesis, and therefore we were unable to collect the breast milk samples of 3 mL required for analysis. Overall, the maternal characteristics and blood measurements at baseline did not differ between the two groups, and we would expect the same for the iron concentration in breast milk. A second limitation was the timing of the breast milk collection. We might have missed a higher peak of the iron concentration in the breast milk

before the collection of milk samples 3 days after i.v. iron treatment.

CONCLUSIONS

This project aimed to advance the knowledge of clinical aspects of i.v. iron treatment in the puerperium and evaluate the safety by the iron concentration in breast milk.

We found statistically significant improvement in patient reported outcomes when comparing i.v. iron to oral iron treatment after PPH. The clinical significance of these findings needs to be discussed.

We confirmed previous findings of a fast haematopoietic response and a prompt replenishment of iron stores after treatment with i.v. iron. The difference between i.v. iron and oral iron persisted for 12 weeks.

The pilot study of i.v. iron vs. RBC transfusion showed that enrolment and allocation was possible. The preliminary results of patient reported outcomes and biochemical data suggest that i.v. iron may be an attractive alternative to RBC transfusion. With some adjustments to the eligibility criteria we consider a larger trial feasible.

No serious adverse reactions were found after treatment with a single high-dose infusion of iron isomaltoside, and the increase in iron concentration in breast milk after treatment was within the normal range.

PERSPECTIVES

The studies conducted in this thesis have added new knowledge regarding i.v. iron treatment in the puerperium, and uncovered areas for further research.

In order to assess clinical consequences and efficacy of treatment of postpartum iron deficiency and anaemia, there is a need for better tools to measure patient reported outcomes.

We evaluated the efficacy and safety of i.v. iron as an alternative to current treatment practice. However, rising healthcare costs and limited budgets demands economic evaluation of the costs and health effects, and a proper health technology assessment is warranted.

The treatment dependent difference in the iron stores at 12 weeks may result in long term clinical consequences. We need to know if this influences iron status and patient reported outcomes in subsequent pregnancies.

The results from the pilot study provided important information on how to design a proper randomised controlled trial to determine whether a high single-dose infusion of i.v. iron is an attractive alternative to RBC transfusion in the treatment of severe postpartum anaemia.

Even though iron concentration in breast milk after i.v. iron infusion was within the normal range, the transient peak calls for further investigation of biochemical outcomes including the free iron component in breast milk and health of the infants.

ABBREVIATIONS

ANOVA	Analysis of variance
AUC	Area under the curve
CARPA	Complement activation-related pseudo-allergy
CI	Confidence interval
CHR	Reticulocyte mean haemoglobin content
CONSORT	Consolidated Standards of Reporting Trials
DMT	Divalent metal transporter
EPDS	Edinburgh Postnatal Depression Scale
EPO	Erythropoietin
GCP	Good clinical practice
Hb	Haemoglobin
HELLP	Haemolysis, elevated liver enzymes, low platelet count
Hypo%	Percentage of hypochromic reticulocytes
i.v.	Intravenous
MFI	Multidimensional Fatigue Inventory
PPH	Postpartum haemorrhage
PPQ	Postpartum Questionnaire
RBC	Red blood cell
SD	Standard deviation
sTfR	Soluble transferrin receptor
TIBC	Total iron binding capacity
TSAT	Transferrin saturation
VAS	Visual Analogue Scale
WHO	World Health Organization

SUMMARY

Iron deficiency and anaemia in the puerperium are associated with several important clinical consequences, most prominently physical fatigue. Current treatment practice with oral iron supplementation is associated with gastrointestinal side-effects and subsequent poor compliance. Red blood cell transfusion is also widely used to treat severe postpartum anaemia, though accumulating evidence questions its risk-benefit ratio. Intravenous iron has in previous studies been associated with fast improvement of haemoglobin and iron biochemical markers in the treatment of postpartum anaemia, but there is a lack of studies on patient reported outcomes.

The thesis is based on three studies of intravenous iron (Monofer®, iron isomaltoside) as an alternative to current treatment practice in postpartum iron deficiency and anaemia.

The first study is a randomised controlled trial comparing a high single-dose iron infusion with oral iron in women after postpartum haemorrhage without severe anaemia. The primary outcome was the aggregated change in physical fatigue within 12 weeks postpartum. We found a difference that was statistically significant, but less than the consensus-based and pre-defined minimal clinically relevant level. Across visits, particularly in the first weeks postpartum, we found statistically significant differences in fatigue and depression scores, all in favour of intravenous iron. We confirmed previous findings of a fast haematopoietic response and prompt replenishment of iron stores that persisted throughout the 12 weeks of follow-up.

The second study, a randomised controlled pilot study, tested feasibility and exploratory outcomes of a high single-dose iron infusion compared with red blood cell transfusion for the treatment of severe postpartum anaemia. We found that randomisa-

tion could be feasible with some adjustments for a future study design. The difference in biochemical markers was larger than the patient-reported outcomes in the first week. A larger trial is needed to determine whether a high single-dose iron infusion is non-inferior to red blood cell transfusion in severe postpartum anaemia.

The third study compared iron concentration in breast milk in a randomised sample of women receiving high single-dose iron infusion or oral iron. A high single-dose iron infusion lead to a transient increase in the iron concentration in breast milk, which remained within the normal range.

In conclusion, iron isomaltoside seems to be associated with improved patient-reported outcomes compared to oral iron treatment, and in severe postpartum anaemia intravenous iron seems promising as an alternative to red blood cell transfusion.

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