Understanding the role of intravenous iron to treat anaemia following critical care

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For Charlotte, Amélie, Élodie & Cecily

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Statement of originality

All of the work reported in this thesis is my own unless otherwise stated.

I designed and led the systematic reviews and meta-analyses in Chapters 2 and 3. Dr Carolyn Doree designed the search strategies. Dr Sheila Fisher and Mrs Susan Brunskill provided overall technical and advisory support. Data were extracted in duplicate for all meta-analyses and I am grateful to Dr Killian Donovan, Dr Claire Seeley, Mr Edward Dickson, Mr Antony Palmer, Dr Henna Wong and Dr Sheila Fisher for their help extracting data. I performed the statistical analyses.

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I designed, setup, secured funding and carried out the INTACT trial (Chapters 5 and 6) with the assistance of the Oxford Clinical Trials and Research Unit and members of the Trial Management Group. I co-designed the statistical analysis plan with Mrs Susan Dutton and Miss Ioana Marian. Miss Mae Chester-Jones performed the statistical analysis.

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Abstract

The overarching aims of the work to be presented in this thesis are to investigate the role of intravenous iron for treating anaemia in survivors of critical illness and to improve our understanding of iron status and erythropoiesis in critical illness. I plan to achieve these aims by undertaking systematic reviews and meta-analyses, experimental work and a feasibility randomised controlled trial (RCT).

Intravenous iron is a biologically plausible treatment option for the anaemia of inflammation that accompanies critical illness. However, the evidence of efficacy for intravenous iron in this cohort is limited and there may be some associated harm, in particular the risk of causing or exacerbating infection. Guidelines do not recommend the routine use of iron therapy in critically ill patients, but clinician surveys have highlighted variation in practice. Expert panels have called for further research into the optimal timing, dose and route of administration of iron therapy in critically ill patients.

The objectives of this research are to determine:

1. The current evidence of the safety and efficacy of iron therapy in critically ill patients
2. The evaluate safety data for intravenous iron on the risk of infection across all patient groups
3. If newer markers of iron status, such as hepcidin, can better identify iron deficiency in the context of inflammation (or systemic iron requirement) than current routine tests
4. The variability in subpopulations of erythroid progenitors, as a marker of erythropoiesis, obtained from peripheral blood in a heterogenous cohort of critically ill patients at two distinct time points (acute illness and recovery)
5. If it is feasible to conduct a clinical trial of intravenous iron to treat anaemia in patients recovering from critical illness

I systematically evaluated the safety and efficacy of iron therapy, by any route, on treating anaemia in critically ill patients. I identified six RCTs comparing iron therapy with either no iron or placebo. Overall, there was no evidence of an effect of iron therapy on transfusion requirements (Risk Ratio (RR) 0.91; 95% Confidence Interval (CI): 0.80 to 1.04). There was no difference in infection (RR 0.95; 95%

CI: 0.79 to 1.19). However, iron therapy did result in a modest increase in haemoglobin (Hb), particularly at longer follow-up time points (mean different 0.31 g/dl, 95% CI: 0.04 to 0.59). The clinical implications of this increase require further investigation. Trial sequential analysis suggested the current evidence is inadequate to detect or reject a clinically important benefit or harm from iron therapy. No trials evaluated participant health-related quality of life (HRQoL) or functional outcomes.

Many clinical practice guidelines, across a range of clinical conditions, advocate the use of intravenous iron to treat anaemia. Intravenous iron administration can lead to increased levels of free circulating iron which can promote pathogen growth and result in clinically important infection. As part of a comprehensive systematic review aiming to characterise the risk of infection across all clinical settings, I found an increased risk of infection with intravenous iron (RR 1.17; 95% CI: 1.04 to 1.31; 64 RCTs; 19,480 participants). I judged the certainty of evidence to be moderate (i.e. the true effect is probably close to the estimate effect). However, there was significant variation in the definitions and reporting of infection across included studies. Only fifteen RCTs attempted to define infection (guideline/consensus based (n = 8), investigator-defined (n = 7)). The increased risk of infection associated with intravenous iron must be considered alongside the potential benefits such as treatment of anaemia. There is a need for consistent and standardised reporting of this potential harm in ongoing and future RCTs.

In order to characterise iron status in patients recovering from critical illness, I analysed blood samples taken from a RCT evaluating the effect of a rehabilitation programme delivered to patients discharged from critical care. These samples were taken as close as possible to hospital discharge and were available for 117 participants. I measured hepcidin and soluble transferrin receptor using enzyme-linked immunoassay, along with other routine markers of iron status such as ferritin, serum iron and transferrin saturation. Using linear regression and area under the curve (AUC) receiver operator characteristic (ROC) analysis, I found that a hepcidin concentration of <19 ng/ml achieved the maximum AUC^{ROC} (0.72) and Youden index (0.47) with a sensitivity of 73% and specificity of 74%, for identifying true iron deficiency in the context of inflammation. Approximately one-third of patients (45/110) would be classified as being iron deficient based on this definition compared with only 10% using routine tests of iron status.

In a separate exploratory study, I aimed to determine the variability in subpopulations of erythroid progenitors, as a marker of erythropoiesis, obtained from peripheral blood in a heterogenous cohort of critically ill patients at two distinct time points (acute illness and recovery). This would enable a more precise investigation of the impaired erythropoiesis that accompanies the anaemia of critical illness. The study has been temporarily paused due to the restrictions placed by the COVID-19 pandemic. However, the important first phase of the study is complete. I have collected peripheral blood samples from 30 patients within 72 hours of an intensive care unit admission (ICU), with a follow-up sample available for 17 patients. CD34+ cells from all samples have been extracted and are currently stored in liquid nitrogen. The second phase will involve performing multi-colour flow cytometry to help establish the colony forming potential of the Megakaryocyte Erythroid Progenitor, with a particular focus on the erythroid output, as a marker of erythropoiesis. This work will form part of my post-doctoral training.

Building on the findings of my systematic reviews and the laboratory work, I hypothesised that the recovery phase of critical illness, when the iron and erythropoietic profiles are more favourable, may be a more suitable time point for evaluating the potential benefits (and harms) of intravenous iron. Patients recovering from critical illness experience a substantial burden of morbidity that is characterised by poor physical function, weakness and high levels of fatigue. These are also the cardinal symptoms of untreated anaemia. A large, multicentre RCT is required to answer this question but there are uncertainties regarding patient selection, recruitment and follow-up rates, variability in Hb and iron profiles in the post-ICU period, and choice of patient-centred outcome measures.

In order to address these uncertainties, I designed the INTACT (INtravenous iron to Treat Anaemia following CriTical care) feasibility RCT. Patients being discharged from critical care with moderate-severe anaemia (Hb $\leq$100 g/l) were randomly allocated to either intravenous iron (ferric carboxymaltose 1000mg) or usual medical care. Primary feasibility outcomes included recruitment rates, protocol adherence and completeness of follow-up. Secondary outcomes included clinical outcomes (nosocomial infection, in-hospital mortality, hospital length of stay), changes in haemoglobin, iron profiles and health-related quality of life (HRQoL), and safety data. Participants were followed up at 28 and 90-days post-randomisation.

This study took place in four ICUs across three UK hospitals from September 2018 to December 2019 and 98 participants were randomised over 15 months, with 49 in each arm. The overall recruitment rate for the trial was 34% (95% CI: 28% to 40%) with 6.5 patients recruited per month. Forty-seven out of 49 participants (96%) received the intervention (95% CI: 86% to 100%). All HRQoL measures were collected for at least 79/93 (85%) (95% CI: 76% to 92%) survivors at the 90-day follow-up visit. Overall, INTACT satisfied the pre-defined criteria for demonstration of feasibility. Ferric carboxymaltose was well-tolerated during the study with no drug-related serious adverse events.

Intravenous iron resulted in a higher mean (SD) haemoglobin at 28 days (119.7 (13.3) vs. 106.7 (14.9) g/l) and 90 days (130.5 (15.1) vs. 122.7 (17.3), adjusted mean difference 11.0 (95% CI: 5.0 to 17.0) g/l, $p < 0.001$). Ferritin, iron and transferrin saturation concentrations were higher in the intravenous iron group at 90 days. Median (IQR) hepcidin concentrations were lower in the usual care group at 90 days when compared with intravenous iron (13.9 (3.6-33.3) vs. 35.0 (7.6-60.1) ng/ml, $p = 0.048$). These data support the biological efficacy of intravenous iron in a cohort of patients with significant inflammation. Exploratory analyses suggested that baseline inflammation and iron status, as defined by C-reactive protein and hepcidin respectively, may be potential effect modifiers.

There were no differences in infection and in-hospital mortality in both groups. The median (IQR) post-ICU length of stay was shorter in the intravenous iron group when compared with usual care, but this did not reach statistical significance (5.0 days (3.0-13.0) vs. 9.0 days (5.0-16.0), $p=0.15$). Hospital readmissions in the first 90 days following discharge from the ICU were also lower in the intravenous iron group when compared with usual care (7/40 (17.5%) vs. 15/39 (38.4%), Odds Ratio 0.34 (95% CI: 0.12 to 0.96)). There were no statistically significant differences in HRQoL and fatigue scores between both groups, but INTACT was not powered to detect such differences.

The programme of work arising from this research has the potential to benefit the large number of patients that survive a critical care admission each year by improving their recovery. The findings of the INTACT trial are hypothesis-testing and an important step towards a larger definitive trial.

Peer reviewed publications related to work presented in this thesis

Frost JN, Tan KT, Abbas M … **Shah A**, et al. Hepcidin-mediated hypoferremia disrupts immune responses to vaccination and infection. *Med (N.Y.)* 2021; 2(2): 164-179.

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Shah A, Frost JN, Aaron L, Donovan K, Drakesmith H. Systemic hypoferremia and severity of hypoxemic respiratory failure in COVID-19. *Critical Care* 2020; 24: 320

Boyd-Carson H, **Shah A***, Sugavanam A, Reid J, Stanworth SJ, Oliver CM. The association of pre-operative anaemia with morbidity and mortality after emergency laparotomy. *Anaesthesia* 2020; 75: 904-912 (*Joint first author)

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Shah A, Wray K, James T, et al. Serum hepcidin potentially identifies iron deficiency in survivors of critical illness at the time of hospital discharge. *British Journal of Haematology* 2019; 184: 279-81.

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Abbreviations

AE Adverse events

AI Anaemia of inflammation

APACHEII Acute Physiology and Chronic Health Evaluation II

ARDS Acute respiratory distress syndrome

ATP Adenosine triphosphate

AUC Area under curve

BFU-E Burst forming unit-erythroid

BMP Bone morphogenic protein

CDC Centers for Disease Control and Prevention

CENTRAL Cochrane Central Register of Controlled Trials

CFU-E Colony forming unit-erythroid

CI Confidence Interval

CKD Chronic kidney disease

CLP Common lymphoid progenitors

CMP Common myeloid progenitors

CONSORT Consolidated Standards of Reporting Trials

CRF Case report form

CRP C-reactive protein

CV Coefficient of variance

DMT Divalent metal transporter 1

DNA Deoxyribonucleic acid

DO_2 Global oxygen delivery

DVT Deep vein thrombosis

ECG Electrocardiogram

EPICS Erythroid progenitor defects in critical illness

EPO Erythropoietin

Epo-R Erythropoietin receptor

ELISA Enzyme-linked immunosorbent assay

ESAs Erythroid stimulating agents

ESICM European Society of Intensive Care Medicine

EQ-5D-5L EuroQol-5D-5L

FACIT Functional Assessment of Chronic Illness Therapy

FACIT-F Functional Assessment of Chronic Illness Therapy-Fatigue

FACT Functional Assessment of Cancer Therapy

FBC Full blood count

GCP Good Clinical Practice

GMP Granulocyte-macrophage progenitors

GRADE Grading of recommendations assessment, development and evaluation

HAI Healthcare-associated infection

Hb Haemoglobin

HDU High dependency unit

HIF Hypoxia inducible factor

HFrEF Heart failure with reduced ejection fraction

HR Hazard ratio

HSC Haematopoietic stem cells

HRQoL Health-related quality of life

IBD Inflammatory bowel disease

ICU Intensive Care Unit

IDA Iron deficiency anaemia

IDI Iron deficiency and inflammation

IFN Interferon

IL Interleukin

INTACT INtravenous iron to Treat Anaemia following CriTical care

IQR Interquartile range

ISRCTN International Standard Randomised Controlled Trial Number

IV Intravenous

JAK Janus Kinase

kDa Kilodaltons

LOS Length of stay

LMPP Lymphoid-primed multipotent progenitor

MCV Mean corpuscular volume

MD Mean difference

MEP Megakaryocytic erythroid progenitors

MK Megakaryocyte

MFI-20 Multidimensional Fatigue Inventory-20

MHRA Medicines and Healthcare products Regulatory Agency

MPP Multipotent progenitors

NHS National Health Service

NRS Non-randomised studies

OCTRU Oxford Clinical Trials Research Unit

OR Odds ratio

PaO_2 Arterial oxygen tension

PE Pulmonary embolism

PICS Post-intensive care syndrome

PIL Participant information leaflet

PRISMA Preferred reporting items for systematic reviews and meta-analysis

RBC Red blood cell

RCT Randomised controlled trial

RNA Ribonucleic acid
ROC Receiver Operator Characteristic

RR Relative risk/ratio

RRI Relative risk increase

RRR Relative risk reduction

SAE Serious Adverse Event

SaO_2 Arterial oxygen saturation

SD Standard deviation

SE Standard error

$ScvO_2$ Central venous oxygen saturation

SD Standard deviation

SmPC Summary of Product Characteristics

sTfR Soluble transferrin receptor

Tsat Transferrin saturation

TNF Tumour necrosis factor

TSA Trial sequential analysis

UK United Kingdom

WCC White cell count

WHO World Health Organization

WIMM Weatherall Institute of Molecular Medicine

Chapter 1 Introduction and literature review

The findings of this chapter have, in part, been published in:

(1) Shah A, Oczkowski S, Aubron C, et al. Transfusion in critical care: Past, present and future. *Transfusion Medicine* 2020; 30: 418-32.

(2) Benson CS, Shah A, Stanworth S.J., et al. The effect of iron deficiency and anaemia on women's health. *Anaesthesia* 2021; 76 (Suppl 4): 84-95.

1.1 Current clinical challenges

1.1.1 Life after critical illness and patterns of recovery

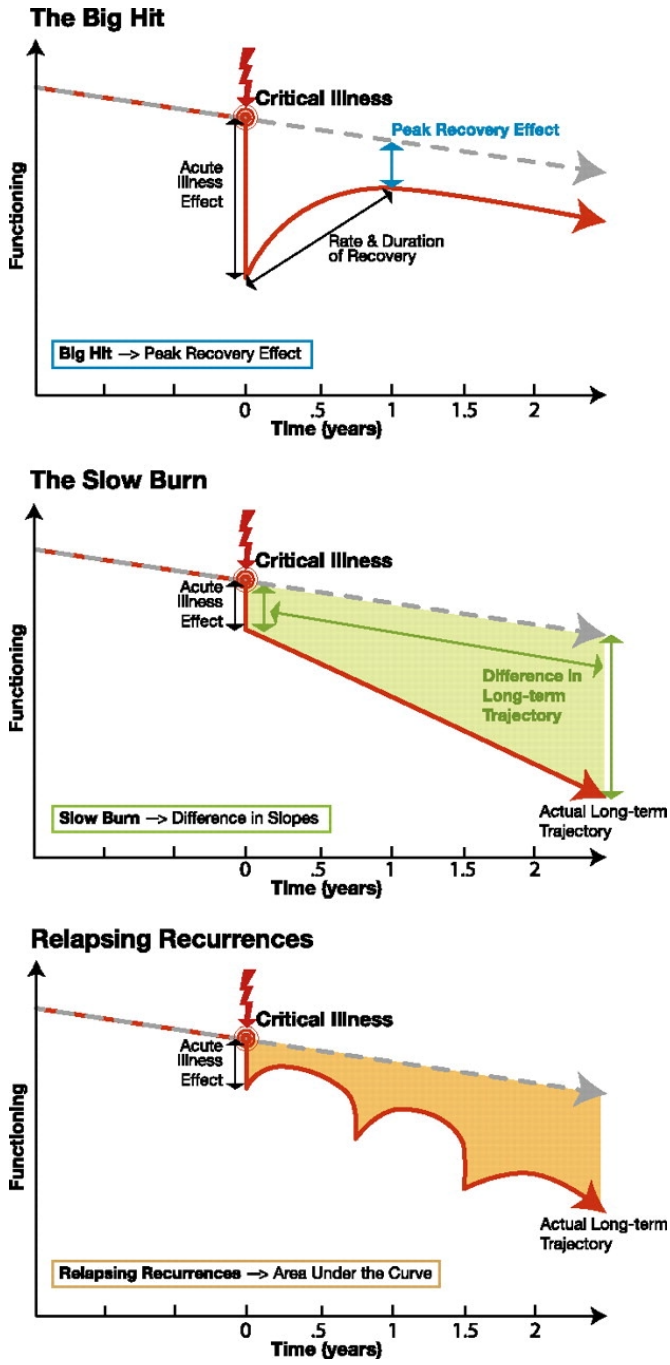
Approximately 135,000 patients survive an intensive care unit (ICU) admission each year in the UK. Survivors commonly report long-lasting physical, cognitive and psychosocial problems impacting their health-related quality of life (HRQoL), a combination termed post-intensive care syndrome (PICS) (1-3). In addition, many survivors and their families incur financial losses with decreased return to work, loss of earnings and increased healthcare costs (4, 5). Further, many ICU survivors are readmitted to hospital within the first year after discharge, often for potentially preventable complications (6). In a cohort study of just under 95,000 UK ICU sepsis survivors over a 5-year period, approximately 50% experienced an unplanned hospital readmission or died in the first year after hospital discharge (7). In another population-level study in Scotland, ICU survivors experienced increased 5-year mortality and hospital readmission rates, and incurred higher hospital costs when compared with hospital control subjects who did not require an ICU admission (8).

Improving outcomes following critical illness is now an international research priority. In order to address this, we need to better understand the factors that impact PICS and the trajectories of recovery after critical illness. This will help inform the design of future clinical trials by optimising patient selection, ensuring that interventions are delivered at the correct time-point in the disease process and that we are measuring outcomes that matter to patients (9). Recent evidence suggests that pre-ICU health status and comorbidities are key drivers of PICS (8, 10). Whilst these are largely unmodifiable,

other factors in the post-ICU period can be addressed and these include appropriate medication and chronic disease management support (11, 12), nutritional supplementation (13) and multidisciplinary recovery programs to aid cognition and physical recovery (14).

Iwashyna provides a conceptual framework for different recovery trajectories following ICU discharge (15). Traditional post-ICU trajectories have presumed a 'Big Hit' model, where patients have an acute loss of function as a result of their critical illness which is followed by a gradual recovery over the next 1–2 years. Two additional trajectories have also been defined – 'Slow Burn' and 'Relapsing Recurrences' (**Figure 1.1**) and all of these have implications for trial design.

Many of the features of PICS, such as fatigue and poor physical function, overlap with those of untreated anaemia. The overarching aim of the work to be presented in this thesis is to investigate whether intravenous iron is a potential therapeutic option for treating anaemia in survivors of critical illness and if this translates into improved recovery from critical illness.



Intervention targeted to increase the rate of recovery and change in absolute level of function at maximal recovery

Intervention to change the trajectory of decline

Intervention to maximise impairment-free months

Figure 1.1 Proposed trajectories of recovery following critical illness. The upper grey lines extending past critical illness are the trajectories that would have occurred if the patient had not experienced critical illness. Differences in trajectories require careful consideration for future trial design. (Adapted with permission from Iwashyna *Am J Resp Crit Care Med* 2012; 186: 302-4 (15)

1.1.2 Epidemiology and patterns of anaemia in critical care

Interpreting the prevalence of anaemia in critically ill patients is complicated by differences in definitions used. The World Health Organization (WHO) has defined anaemia as a haemoglobin (Hb) concentration of < 130 g/l for men, < 120 g/l for non-pregnant women, and < 110 g/l for pregnant women (16). These thresholds are now thought to be outdated and more recent expert consensus statement advocate a Hb <130 g/l for both men and women (16-18). Observational studies and randomised controlled trials (RCTs) in critically ill patients have used a threshold of <100 g/l, which has broadly been described as moderate anaemia.

Generally, anaemia can be divided into three time periods:

- Pre-ICU admission
- Acute phase (e.g. the first 3–7 days of ICU admission)
- ICU discharge

A large proportion of patients entering the ICU already have a low Hb concentration. Two large observational studies, enrolling approximately 9000 patients in total, found that the mean (SD) Hb on admission to ICU was 113 (23) g/l (19) and 110 (24) g/l (20), respectively. Approximately 39% present with Hb <100 g/l and 25% present with Hb <90 g/l.

Whilst on ICU, Hb falls by an average (SD) of 5.2 (6.9) g/l per day, but this decline is greatest in the first 3 days when compared with afterwards (6.6 g/l per day vs. 1.2 g/l) (21). This drop appears to be related to illness severity, commencing before ICU admission and continuing after. By day 8, 97% of critically ill patients are anaemic according to WHO definitions and 100% by day 3 (22). Approximately 40–50% of critically ill patients will have moderate to severe anaemia (Hb <90 g/l) at some point during their ICU stay (23).

Anaemia is also highly prevalent at ICU and hospital discharge, with one study showing that 77% of patients remained anaemic (WHO definition), 33% had Hb <100 g/l and 11.3% had Hb <90 g/l (24). A recent database analysis of 6901 patients surviving an ICU admission found that 80% were anaemic at hospital discharge, with 39% having moderate anaemia (Hb 80–100 g/l) and 3% with severe anaemia (Hb <89 g/l) (25). Rates of complete recovery of anaemia at 12 months post-discharge were <50% in those who were discharged with moderate or severe anaemia. Bateman et al. found that approximately half of ICU survivors still had Hb <100 g/l at 6 months post-ICU discharge and that this was associated with poor physical function and persistent inflammation (26). Another study of 107 ICU survivors found that 80% of patients were still anaemic at 28 days post-discharge and 25% at 6 months (27). With the widespread adoption of restrictive red blood cell (RBC) transfusion practices, it is likely that the numbers of patients being discharged with moderate to severe anaemia from ICU, and subsequently hospital, will increase (28).

1.1.3 Physiological effects of anaemia

There are three physiological variables that are necessary for adequate oxygen delivery to tissues in mammals which are: (i) an efficient pump – the heart; (ii) an efficient distribution system – the circulatory system; and (iii) an effective oxygen reservoir – Hb (29). Haemoglobin is a critical component for oxygen delivery and therefore RBCs are crucial for oxygen delivery. Acute isovolaemic reduction in Hb in resting healthy adults from 130 g/l to 50 g/l leads to progressive increases in heart rate, stroke volume, oxygen extraction and cardiac index, without any evidence of impaired oxygen delivery (30). The underlying mechanisms are described in **Table 1.1.**

Table 1.1 Systemic adaptations to anaemia

Adaptation	Underlying mechanism
Oxygen sensing	Decrease in PaO_2 in renal tissue triggers an increase in renal EPO production (31). Increase expression of hypoxia sensing molecules such as HIF (32).
Respiratory	Anaemia results in increased minute ventilation, whilst NO-mediated mechanisms improve ventilation-perfusion matching. These responses lead to an increase in PaO_2 and SaO_2 (33).
Cardiovascular	Activation of aortic chemoreceptors leads to increases in cardiac output (34). Acute vasoconstriction of the splanchnic circulation leads to an increase in venous return and preload observed during anaemia (35). Preferential vasodilatation and distribution of blood flow to vital organs such as the coronary circulation and the brain. A six-fold increase in coronary blood flow can occur to support the increase in myocardial oxygen consumption (36).
Increased tissue oxygen extraction	A right shift of the oxyhaemoglobin dissociation curve to reduce Hb oxygen affinity, which is mediated by increased 2,3-DPG, acidosis and NO-signalling (37). Increased tissue blood flow which favours oxygen diffusion into tissues. However, critical organs that already have a high oxygen-extraction ratio (brain, heart) are unable to compensate using this mechanism.

DPG, diphosphoglycerate; EPO, erythropoietin; HIF, hypoxia inducible factor; NO, nitric oxide; PaO_2, partial pressure of oxygen; SaO_2, oxygen saturation

During critical illness, these adaptive mechanisms may begin to fail, increasing the risk of organ ischaemia and injury. This can be as a direct result of illness severity (e.g. septic shock) or patient factors. Approximately 30% of patients admitted to ICU in the UK have co-existing cardiovascular disease and therefore may have limited reserve to cope with anaemia which will result in myocardial ischaemia (38). Due to critical illness, changes in red cell structure and function, such as reduced deformability and increased aggregation, may also impair oxygen delivery (39).

1.2 Clinical impact of anaemia

1.2.1 Short-term outcomes

Evaluating the impact of anaemia in critically ill patients on short-term outcomes such as organ injury and in-hospital morbidity and mortality is confounded by the impact of RBC. The Transfusion Requirements In Critical Care RCT provided early evidence that anaemia in critical illness may not necessarily be harmful (40). Non-bleeding critically ill adults (n = 838) with Hb <9 g/dl within 72 hours of ICU admission were randomised to a restrictive (transfusion trigger <7 g/dl, target Hb 7–9 g/dl) or liberal (transfusion trigger <10 g/dl, target 10–12 g/dl). The primary outcome was 30-day mortality. The authors found that a restrictive strategy of RBC transfusion was as least as effective as a liberal transfusion strategy, with no evidence of an effect on 30-day mortality (78/418 (18.7%) vs. 98/420 (23.3%), absolute difference (95% CI): 4.7 (-0.84–10.2), p = 0.11). Furthermore, a lower mortality rate was observed in patients who were less acutely ill and those who were < 55 years of age.

The study was not without limitations. Only 13% of eligible participants were enrolled, which raises questions about external validity of the findings. The study stopped earlier than scheduled due to slow recruitment and only enrolled 838 participants out of a planned 1620. The trial also did not use leukodepleted RBCs, which may have increased the rate of adverse events in the liberal transfusion arm. However, more recent RCTs in critically ill patients (41) have also demonstrated that a restrictive transfusion strategy is safe and effective when compared with liberal transfusion.

Anaemia may contribute to worsening organ injury and increased organ support requirements in critically patients. As discussed in Section 1.1.3, this may be more relevant for patients who are more severely ill and in whom oxygen supply and demand imbalance occurs more readily. In one study of 91 critically ill patients who required a median of 3 days of invasive mechanical ventilation, anaemia (defined as Hb <100 g/l) was associated with failure of liberation from mechanical ventilation (unadjusted risk ratio (95% CI): 5.3 (2.1–13.6)) (42). In critically ill patients with pre-existing cardiovascular disease, 27/68 (50%) patients who experienced a likely type 2 myocardial infarction

had Hb <9 g/dl (43). Nadir haemoglobin was also associated with higher 6-month mortality in all included patients (n=279). Preliminary data from a feasibility RCT comparing a restrictive (transfusion trigger <7 g/dl) vs. liberal (transfusion trigger <9 g/dl) transfusion strategy in patients with traumatic brain injury showed that a liberal transfusion strategy was associated with a lower incidence of post-traumatic vasospasm, lower hospital mortality and better neurological status at 6 months (44).

1.2.2 Outcomes after ICU discharge

Studies in ICU survivors have reported that anaemia, defined as Hb <100 g/l, is associated with increasing fatigue at 28 days post-ICU (27), and that anaemic survivors have reduced HRQoL scores when compared to a national cohort of unselected ICU survivors, particularly in the physical function and role physical domains of the SF-36 form (26). In one study of 1161 ICU survivors, iron deficiency (defined as hepcidin <20 ng/ml or ferritin <100 ng/l) was associated with increased one-year mortality and poor physical recovery (45). Patients with iron deficiency had lower median (IQR) haemoglobin concentrations at ICU discharge when compared with those without iron deficiency (9.8 (8.9–10.8) g/dl vs. 10.1 (9–11.3) g/dl, p = 0.011).

Higher hospital discharge Hb has been associated with reduced post-discharge mortality in general ICU survivors (25). In acute respiratory distress syndrome (ARDS) survivors, higher Hb at ICU discharge was associated with improved exercise capacity, as judged by a 6-minute walk test, and fewer activities of daily living dependencies at 3-month follow-up (46). These observational data support a causal pathway in which anaemia and/or iron deficiency may be a driver for increased morbidity and poor HRQoL in ICU survivors.

1.3 Aetiology

Critically ill patients display the hallmarks of the anaemia of inflammation (AI) that is characterised by disturbed iron homeostasis, impaired erythropoiesis and decreased erythrocyte survival. The severity and contributions of each of these processes will vary with the underlying causes of

inflammation, the clinical context, patient factors (e.g. comorbidities, medications, genetic background), and pre-existing iron stores.

1.3.1 Systemic iron homeostasis in health

Iron is essential for the synthesis of haemoglobin, cell growth and differentiation, neurotransmission, immunity and cardiopulmonary function (47). Many cellular proteins require iron to function. Examples include components of the mitochondrial electron transport chain. Enzymes involved in DNA metabolism, such as ribonucleotide reductase, DNA primase and DNA helicase bind iron (48, 49). The ATPase *ABCE1*, which is needed for mRNA translation, also binds iron (50). Iron also plays a crucial role in oxygen sensing as part of the hypoxia inducible factor (HIF) pathway. The degradation of HIFs, especially HIF-2α by prolyl hydroxylases, is dependent on both iron and oxygen (51) and studies in healthy volunteers have demonstrated that iron depletion augments the pulmonary hypertensive response to hypoxia, whilst iron loading attenuates this response (52). Iron is also essential for immunity and recent work has demonstrated that hypoferraemia impairs both T and B cell responses to infection and vaccination (53).

Iron deficiency is the most common cause of anaemia worldwide and affects approximately 1 billion people, whilst also being a leading cause of years lived with disability (54, 55). This problem becomes even more significant when we take AI into consideration.

In health, total body iron is approximately 3–4 g, most of which is in erythrocyte Hb (2–3 mg). Other iron-rich organs include the liver and spleen. One to two mg of iron is lost daily, which is balanced by an equivalent amount that is absorbed from the gut, but an additional 1 mg is lost during menstruation in women each month. Most iron (20–25 mg daily) is recycled by macrophages following erythrophagocytosis (47). An interesting and unusual aspect of iron homeostasis is that there is no mechanism for excretion of excess iron and yet iron deficiency is extremely common. Therefore, iron balance is tightly regulated at systemic, tissue and cellular levels.

Systemic iron homeostasis is controlled by the peptide hormone hepcidin, which is synthesised in the liver (47). Hepcidin transcription is regulated by hepatocytes in response to changes in circulating iron through the bone morphogenic protein (BMP)-6/SMAD pathway (56). An overview of iron homeostasis is provided in **Figure 1.2**. Hepcidin expression results in internalisation and degradation of cellular ferroportin, the sole mammalian exporter of iron, which impairs release of iron from macrophages and absorption of iron by duodenal enterocytes (57). Hepcidin levels are increased in response to inflammation and high circulating levels of free iron, and are decreased in iron deficiency, hypoxia, blood loss and by erythropoietin (EPO) (58).

1.3.2 Disturbed iron homeostasis

Critical illness precipitates an inflammatory response that results in early, profound, and sometimes persistent changes, in iron homeostasis. Ferritin and other iron-binding proteins, such as lactoferrin, are acute phase reactants that are upregulated during inflammation and serve to bind iron with a greater affinity than transferrin, the circulating iron transporter and negative acute phase reactant. This results in systemic hypoferraemia (58).

The profile of low serum iron, low transferrin and high serum ferritin concentrations develop within the first few hours of an inflammatory/infectious insult and are present in approximately 75% of patients within 3 days of an ICU admission (59, 60). These changes are also present before Hb starts to drop (59). Various definitions have been used to describe iron deficiency, with the two most common terms being functional iron deficiency and iron restricted erythropoiesis, but there is no agreed consensus on the thresholds used to differentiate between these states (see Section 1.4.1). These processes are part of an evolutionary response to limit free (or non-transferrin bound) iron for invading pathogens, particularly iron-dependent extracellular organisms that could otherwise cause overwhelming infection (61). Non-transferrin bound iron is a potent stimulus for the pathogenicity of gram-negative bacteria (62).

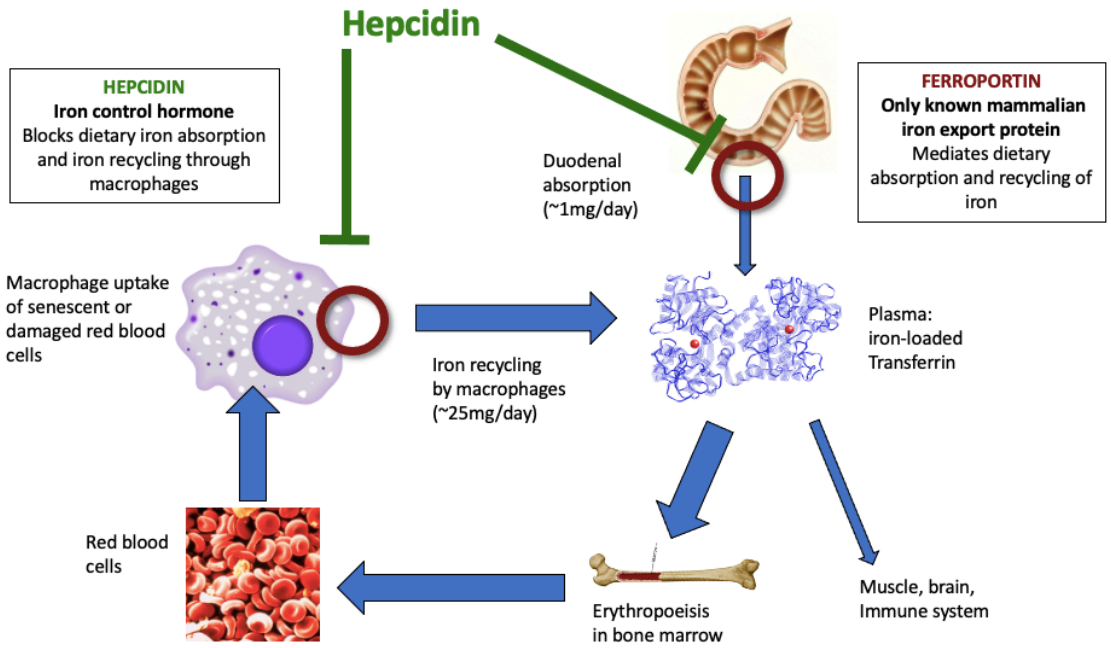


Figure 1.2 Hepcidin-ferroportin interaction and major systemic iron pathways Approximately 1 mg of iron is absorbed daily in the gut. Ferroportin is the sole mammalian exporter of iron and delivers stored, dietary or recycled iron to blood plasma. In plasma, iron is taken up by transferrin. A small proportion is used for muscle function and development of neural tissue, whilst the rest is used by the bone marrow for erythropoiesis. At the end of their life cycle, red blood cells are taken up by macrophages and approximately 25 mg of iron is recycled daily through this pathway. This is the major source of body iron. Hepcidin expression results in degradation of ferroportin which impairs release of iron from macrophages and duodenal enterocytes. Hepcidin levels are low in states of iron deficiency, which allows for increased absorption of iron through ferroportin.

Microbial molecules and inflammatory cytokines, in particular interleukin (IL)-6, stimulate hepatocytes to produce hepcidin (63, 64) which exerts its iron-regulatory effects through ferroportin as described previously. Recent data suggest that hepcidin, in high concentrations, may directly block iron export by occluding ferroportin (65). These processes result in iron retention in macrophages and reduced dietary absorption of iron ('hepcidin block'). In addition, various cytokines act through hepcidin-independent mechanisms, but these are poorly characterised to date. Tumour necrosis factor (TNF) reduces duodenal iron absorption whereas other cytokines such as IL-1, IL-10 and TNF-α facilitate iron acquisition by macrophages possibly through transferrin-receptor mediated endocytosis (66).

Recent data suggest that disturbed iron homeostasis is of prognostic significance in critically ill patients. Higher serum iron and transferrin saturations (Tsat) concentrations are associated with increased mortality in critically ill patients admitted with sepsis (67, 68). Conversely, systemic hypoferraemia at ICU admission is associated with severe hypoxaemia (69). A large population study in Norway found that severe iron deficiency (serum iron <6 mmol/l) was associated with increased risk of a future bloodstream infection (70).

These processes may be ameliorated by treatments that target the infectious or inflammatory process or by allowing the inflammatory process to resolve. In a prospective cohort study of 100 critically ill surgical patients, serum hepcidin concentrations were markedly increased on ICU admission but decreased significantly at 28 days post-admission. This decrease was associated with decreased IL-6 and C-reactive protein (CRP) concentrations and an accompanying increased reticulocyte response, although there was no resolution in anaemia (71).

1.3.3 Impaired erythropoiesis

Erythrocytes are generated from haematopoietic stem cells in the bone marrow through a series of stepwise differentiations. In brief, the development pathway of multipotent progenitors (MPPs) bifurcates into progenitor cells with myeloid potential (common myeloid progenitors (CMPs)) or lymphoid potential (common lymphoid progenitors (CLPs)). CMPs then give rise to a population of granulocyte-macrophage progenitors (GMPs) or megakaryocytic-erythroid progenitors (MEPs) (72, 73). The latter then differentiate into lineage-committed erythroid cells and platelet-producing megakaryocytes. The first precursor fully committed to erythroid differentiation is the burst forming unit-erythroid (BFU-E) and erythroid differentiation and division is controlled by concentrations of erythropoietin and iron (74).

Inflammation results in bone marrow reprogramming to promote myelopoiesis and lymphopoiesis at the expense of erythropoiesis. This process is mediated by cytokines such as Interferon (IFN)-γ and TNF-α and is described in greater detail in Chapter 4. These cytokines also inhibit the capacity of

BFU-Es to give rise to more differentiated erythroid cells. Impaired erythropoiesis also occurs due to reduced production, reduced biological efficacy of EPO secondary to inflammation, or receptor downregulation due to iron restriction (58). EPO exerts its effects by binding to its homodimeric erythroid receptor (Epo-R) via Janus kinases 2 (*JAK2*) signalling cascades. *JAK2* catalyses the phosphorylation of tyrosine residues of the Epo-R and various intracellular enzymes and transcription factors (75). Erythropoiesis, in health, is a slow process and becomes apparent 3–4 days after a rise in plasma EPO. EPO acts to increase iron availability by inhibiting hepcidin formation via induction of HIF-1, erythroferrone, and platelet-derived growth factor-BB (76, 77). Therefore, reduced availability and activity of EPO in AI exacerbates hepcidin-mediated erythroid iron restriction.

Mouse models have demonstrated that inflammation rapidly suppresses the EPO-producing potential of renal interstitial cells and that this may be reversed once inflammation resolves (78). Epo-R receptors do not appear to be reduced in some patients with AI and it is the efficacy of EPO-mediated signalling that is reduced and inversely linked to circulating concentrations of IL-1 and IL-6 (79). Recent evidence from mouse models suggests that erythroid iron deficiency results in Epo-R downregulation (74). Specifically, iron deficiency impairs transferrin-receptor-2-mediated iron sensing in erythroid cells (80) resulting in downregulation of *Scribble*, a master regulator of receptor trafficking and signalling (74). This in turn, results in reduced surface expression of Epo-R.

1.3.4 Decreased erythrocyte survival

Normal erythrocyte life span is 120 days and in patients with AI this is moderately reduced by approximately 25% to 90 days (81). This decrease is also seen in patients with inflammation who do not have anaemia (81). Therefore, anaemia is likely to develop when erythropoiesis is also impaired. The mechanisms contributing towards decreased erythrocyte survival include increased erythrophagocytosis by activated macrophages, bystander inflammatory damage from IFN-γ and TNF-α and mechanical damage from microvascular fibrin strands (82, 83).

1.3.5 Haemodilution

Non-bleeding critically ill patients are often prescribed large volumes of crystalloid fluids as part of an initial resuscitation phase (84), which causes haemodilution. There is no change in the Hb mass but there is an increased volume of distribution resulting in a low measured Hb. However, one study found that fluid balance during the first 3 days of an ICU admission did not significantly influence the fall in Hb. After the third day, changes in Hb were inversely related to illness severity (21).

The precise contribution of haemodilution towards anaemia has not yet been established because no studies have simultaneously measured Hb mass during the dynamic phases of fluid resuscitation and techniques for measuring haemoglobin mass, such as the optimised carbon monoxide rebreathing method (85), are not routinely available in clinical practice.

1.3.6 Blood loss

Blood loss through frequent sampling is a common cause of iatrogenic anaemia in ICU, where patients may lose between 41 and 70 ml of blood per day (19). In patients requiring a prolonged ICU stay, an average increase of only 3.5 ml/day from laboratory tests was associated with a doubling of the odds of receiving an RBC transfusion after 21 days (86). Renal replacement therapy, and its associated anticoagulation and filter circuits, is another important cause of iatrogenic anaemia with one study reporting a mean (SD) loss of up to 384.3 (291.7) ml over seven days.

Increasing numbers of patients are prescribed antiplatelet or anticoagulant therapy for the prevention or treatment of major cardiovascular and thrombotic events (87). Many medications given to critically ill patients have side effects than can cause haemolysis and thrombocytopaenia, and impair erythropoiesis and haemostasis. Examples include anticoagulants, antiplatelet agents, antibiotics (e.g. linezolid, nitrofurantoin, piperacillin), anticonvulsants and cytotoxic chemotherapy.

1.3.7 B_{12} and folate deficiency

There are limited data on the prevalence of vitamin B_{12} or folate deficiency in critically patients. Rodriguez et al. found evidence of these deficiencies in only 2% of patients (88). However, these may be overlooked as their diagnostic hallmark of megaloblastic anaemia may not develop in the context of iron-restriction and inflammation. Low levels may be common in critically ill patients with a background of alcoholism, malignancy, those requiring renal replacement therapy or prescribed drugs such as methotrexate and trimethoprim (89).

1.4 Approaches to investigation and management of anaemia in critically ill patients

1.4.1 Routine laboratory tests

Routine tests in the investigation of anaemia include full blood count (FBC), renal and liver function tests and CRP. FBC usually reveals normochromic and normocytic anaemia. Additional tests such as iron profiles and B_{12} and folate concentrations are seldom performed unless there is a strong clinical suspicion of one of these deficiencies. Similarly, tests such as lactate dehydrogenase, direct antiglobulin test and blood film should be performed if there is suspicion of haemolysis.

As described before, iron deficiency is the most common cause of anaemia but diagnosing in the context of AI is a challenge. Ferritin is the most frequently used test for iron deficiency but in the setting of inflammation, ferritin loses its diagnostic value and various thresholds ranging from 70 to 150 mcg/l have been proposed, often in combination with Tsat and CRP (90). The most recent guidance published in 2020 from WHO recommends that a ferritin cut-off of <70 mcg/l may be indicative of iron deficiency in adults with co-existing infection or inflammation, but this is a conditional recommendation based on low certainty of evidence (91). Between 20 and 85% of patients with AI may also suffer from true iron deficiency as a result of blood loss from chronic gastrointestinal bleeding, excessive phlebotomy and interventions such as haemodialysis (66). Newer markers such as hepcidin and soluble transferrin receptor (sTfR) have shown promise but are currently limited by availability of assays and lack of an international reference standard. In Chapter 4, I will investigate the diagnostic utility of hepcidin in diagnosing iron deficiency in ICU survivors

with AI. The definitions, laboratory characteristics and potential iron supplementation strategies of the different stages of iron deficiency are shown in **Table 1.2.** Iron supplementation will be discussed in greater detail in Section 1.4.

Table 1.2 Definitions, laboratory characteristics and potential treatment strategies of the various stages of iron deficiency

Iron status	Definition	Laboratory findings	Expected hepcidin levels	Iron therapy strategies
True/absolute iron deficiency	Depletion of body iron stores, which are inadequate to maintain erythropoiesis	Low ferritin and low Tsat	Low	Oral iron, IV iron if poorly tolerated
Iron-deficiency anaemia	Reduced Hb and erythrocytes due to insufficient iron availability	Low Hb, low ferritin and low Tsat	Low	Oral iron, IV iron if poorly tolerated or rapid correction required
Functional iron deficiency	Insufficient mobilisation of iron stores due to increased demands, despite adequate iron stores	Low Tsat, variable ferritin	Variable	IV iron, consider oral iron if low disease activity or inflammatory burden
Iron sequestration / iron-restricted erythropoiesis	Reduced supply of iron to meet erythropoietic requirements	Low Tsat, normal to elevated ferritin, elevated CRP	High	IV iron, consider erythropoietin

CRP, C-reactive protein; Hb, haemoglobin; IV, intravenous; Tsat, transferrin saturation

1.4.2 Physiological and microcirculatory indices

Currently, Hb is the main measurement used to guide anaemia management. A recent survey found that 87% of ICU clinicians use alternative transfusion triggers in some circumstances, and 27% always use them. The most common triggers were tachycardia, hypotension, significant ECG changes and lactate levels >2 mmol/l (92). Advanced measurements such as central or mixed venous oxygen saturation, arteriovenous difference, oxygen difference and cerebral oxygen are infrequently used.

To date, we have no accurate method of measuring oxygen delivery and tissue oxygenation. The critical Hb is the Hb concentration at which the compensatory mechanisms described previously in **Table 1.1** are exhausted and further reductions in Hb will lead to reductions in oxygen delivery. This point is known as the critical DO_2 (global oxygen delivery) and it is when oxygen consumption becomes supply dependent. The corresponding Hb at this point is the critical Hb. The critical DO_2 is not a fixed value but varies between organs, depending on their metabolic activity. The critical Hb will also be different in each critically ill patient and may also vary within the same patient over time. At present, we do not have the means to accurately detect tissue hypoxia, and clinicians will transfuse RBCs at certain Hb thresholds to prevent its consequences.

A variety of techniques have been developed to study tissue oxygenation in critically ill patients, including technology to study microcirculatory function (93). Global indices of tissue oxygenation, such as $ScvO_2$ and lactate clearance, can be useful to detect global decreases in perfusion, but normal values do not rule out inadequate oxygen delivery, and their sensitivity and specificity to detect microcirculatory impairments are poor (94). The use of near-infrared spectroscopy in critically ill patients has slowly been increasing. It is a non-invasive technique that monitors peripheral oxygenation of various tissues by comparing the ratio of oxygenated to deoxygenated Hb and providing an indirect measure of tissue oxygenation (95). Its signal is limited to vessels less than 1 mm in diameter, making it well suited to measure the microcirculation. Other techniques include sidestream dark field imaging, tissue microdialysis and transcutaneous oxygen tension (95, 96).

A recent systematic review evaluated these techniques and found that transfusion did not generally improve tissue oxygenation or the microcirculation, but many studies were single centre, observational and lacking a control group (93). A hypothesis generating finding of this review was that patients with abnormal tissue oxygenation or microcirculatory indices before RBC transfusion showed significant improvements in these indices following transfusion, irrespective of which measurement technique was used.

In summary, there is a limited body of evidence to support the use of alternative transfusion triggers in contrast to the large body of evidence evaluating Hb-based thresholds. Prospective trials evaluating Hb vs. alternative triggers are warranted.

1.4.3 Red blood cell transfusion

Allogeneic RBC transfusion is the current mainstay of treating anaemia in critically ill patients. One in four critically ill patients will receive a RBC transfusion during their ICU stay (97). As discussed previously, the primary purpose of RBC transfusion is to increase the haemoglobin concentration, thereby augmenting oxygen delivery and limiting tissue hypoxia. The design of RCTs has been based on this physiological principle, but evidence of biological efficacy is conflicting. Tissue hypoxia may also be related to poor cardiac output, abnormalities in oxyhaemoglobin curve dissociation and the ability of RBCs to traverse the microcirculation (98-101). The biochemical, metabolic and structural changes that occur in stored blood, collectively termed known as the 'storage lesion', are also thought to impair oxygen uptake and delivery but a Cochrane systematic review of all RCTs (22 RCTs, 42,835 participants) failed to demonstrate any evidence of an effect on clinical outcomes that are related to storage of RBCs (102). However, there are many clinical, methodological and statistical problems that may have affected the analysis and subsequent interpretation of results which are discussed in greater detail elsewhere (103).

The potential benefits of RBC transfusions must be considered alongside the associated harms. Important concerns include the risks of transfusion-related acute lung injury, transfusion associated circulatory overload, non-haemolytic febrile reactions, haemolytic reactions and immunomodulation (104-106). Some of these have been mitigated with the widespread introduction of leukoreduction which has been associated with decreased mortality, fewer fever episodes and less infection (107, 108). Observational studies suggest that patients who receive RBC transfusions are also at increased risk of death, ischaemic complications, thrombosis, delayed wound healing and increased hospital length of stay (109-111). However, causality cannot be established from these studies due to residual confounding and that sicker patients, in whom the occurrence of these events is common, also receive

more RBC transfusions.

Transfusion practice in critically ill patients has largely moved towards a more restrictive approach over the past two decades. This has, in part, been driven by the publication of large, pragmatic RCTs in general critically ill patients (40), septic shock (41), upper gastrointestinal bleeding (112), cardiac surgery (113) and hip fracture surgery (114). However, uncertainties still exist in particular subgroups such as critically ill elderly patients and those with cardiovascular disease, traumatic brain injury or solid organ malignancy. These patients may have greater degrees of oxygen supply and demand imbalance occurring more readily (115). A recent worldwide audit of RBC transfusion practice involving 10,069 patients from 730 ICUs across 84 countries, observed a stepwise decrease in the hazard ratio for in-hospital death after transfusion with increasing illness severity (97).

A detailed review of the evidence base is beyond the scope of this thesis. However, as part of the European Society of Intensive Care Medicine (ESICM) Transfusion Task Force, I was involved in the development of a clinical practice guideline on transfusion strategies in non-bleeding critically ill adults (116). The recommendations are summarised in **Table 1.3**.

1.4.4 Non-pharmacological strategies

Potential strategies to minimise blood loss from excessive blood sampling include:

- Limiting blood tests to only those likely to affect changes in clinical management
- Avoiding standing orders
- Use of small-volume blood collection tubes. Small observational studies in critically ill adults have demonstrated small reductions in blood loss and transfusion requirements (117, 118). Although these are already in routine use in paediatric care, implementation of small-volume tubes may be initially challenging due to the need to train staff, resampling in the event of insufficient sampling the first time and less opportunity to store blood for future testing. The ongoing Small-Volume Tubes to Reduce Anemia and Transfusion (STRATUS)

(NCT03578419) crossed over, stepped wedge cluster RCT, which aims to enrol 10,000 critically ill patients, will provide definitive evidence for inform future clinical practice and guideline recommendations.

- Closed-loop blood sampling devices that allow return of wasted blood back to the patient. Pooled evidence from small RCTs suggests that devices can reduce daily blood sampling volume by 24.6 ml (95% CI: 23.35–25.78 ml) which may translate to fewer patients being transfused (RR 0.72, 95%CI: 0.55–0.95) (116). These devices may be more effective in more severely ill patients in whom ICU stay is likely to be longer, and blood sampling and transfusion requirements higher (119).
- Appropriate management of medications likely to cause bleeding and/or anaemia

Table 1.3 Summary of recommendations related to transfusion of red blood cells in critically ill adults

	Recommendation	Strength of recommendation	Certainty of evidence
Liberal vs. restrictive red blood cell transfusion			
General critically ill patients, with or without ARDS	Restrictive transfusion threshold (7 g/dl)	Strong	Moderate
Sepsis and septic shock	Restrictive transfusion threshold (7 g/dl)	Conditional	Moderate
Critically ill patients with acute coronary syndrome	Liberal transfusion threshold (9–10 g/dl)	Conditional	Low
Prolonged weaning from mechanical ventilation	Restrictive transfusion threshold (7 g/dl)	Conditional	Low
Cardiac surgery	Restrictive transfusion threshold (7.5 g/dl)	Strong	Moderate
Acute neurological injury (TBI, SAH, stroke)	No recommendation Transfusion at either restrictive (7 g/dl) or liberal (9–11.5 g/dl) is appropriate	Research recommendation	Low
Veno-venous or veno-arterial ECMO	No recommendation Transfusion at either restrictive (7 g/dl) or liberal (9 g/dl) is appropriate	Research recommendation	Very low
Malignancy (haematologic or solid organ)	No recommendation Transfusion at either restrictive (7 g/dl) or liberal (9 g/dl) is appropriate	Research recommendation	Low
Elderly	No recommendation Transfusion at either restrictive (7 g/dl) or liberal (9 g/dl) is appropriate	Research recommendation	Low

ARDS, Acute respiratory distress syndrome; ECMO, Extracorporeal membrane oxygenation; SAH, Subarachnoid haemorrhage; TBI, Traumatic brain injury

1.5 Iron therapy

The focus of this thesis in on evaluating a potential role for iron therapy to treat anaemia in critical illness. Iron can be administered enterally (dietary modifications and/or iron supplements) or parenterally (intramuscular or intravenous).

1.5.1 Iron supplementation strategies

Dietary iron

Dietary iron is absorbed in two forms – non-haem (inorganic) and haem-bound iron. Non-haem iron is largely derived from plant-based foods whereas meat, fish and poultry are rich sources of haem-bound iron. Non-haem iron exists predominantly in the oxidised ferric (Fe^{3+}) form and needs to be reduced to the ferrous (Fe^{2+}) form in order to be absorbed. This process is facilitated by a brush-border ferrireductase which reduces ferric iron, allowing the resulting ferrous form to enter enterocytes through divalent metal transporter 1 (DMT1) (120). DMT1 is a transmembrane protein that can also transport other divalent cations such as manganese and cobalt (120).

Non-haem iron is also highly insoluble, and its bioavailability is influenced by various environmental and dietary factors. Gastric acid and ascorbic acid promote the reduction of ferric iron and thus improve absorption, whereas tannins found in tea and coffee, and phyate found in cereals and legumes, limit absorption (120). The combination of these factors may explain the high prevalence of iron deficiency in societies that consume vegetarian or vegan diets. In contrast, haem-bound iron is absorbed more efficiently and largely uninfluenced by other factors. The precise mechanisms through which haem-bound iron is absorbed are unclear and two transporters have been proposed – haem carrier protein 1 (121) and a folate transporter (122). In clinical practice, dietary absorption on its own will seldom correct iron deficiency and pharmacological interventions are usually required.

Oral iron

Oral iron is a cheap and effective way of treating true iron deficiency and/or AI with low-grade inflammation, with comparable efficacy to intravenous preparations (123). Oral iron preparations are

available as divalent iron salts or polysaccharide complexes. The most commonly used iron salts are ferrous sulphate, ferrous gluconate and ferrous fumarate. However, oral iron is absorbed through the same, less efficient pathways of non-haem iron. The key factor to consider in oral iron preparations is the content of elemental iron and most preparations do not contain more than 30% elemental iron.

Oral iron is frequently associated with gastrointestinal side-effects such as nausea, vomiting, metallic taste, constipation and epigastric pain. These are likely due to direct toxicity from unabsorbed iron in the intestinal mucosa. A recent meta-analysis, which included data from 43 RCTs and 6831 participants, found that ferrous sulphate was associated with an increased risk of gastrointestinal side-effects when compared with placebo (odds ratio (OR) (95% CI) 2.32 (1.74 to 3.08), I^2 = 53.6%) or intravenous iron (OR (95% CI) 3.05 (2.07 to 4.48), I^2 = 41.6%) (124). These side effects inevitably lead to a reduction in compliance, which coupled with the requirement for prolonged treatment, means that a significant proportion of patients will remain undertreated.

However, recent advances in our understanding of iron homeostasis have led to guidelines recommending dose regimes that may enhance the efficacy of oral iron and reduce the frequency of side effects. Studies in non-pregnant women measuring iron bioavailability using isotopic label abundance in erythrocytes have shown that once daily or alternate day dosing may be more efficacious and better tolerated when compared with higher total daily divided doses (125, 126). Oral ingestion of a single dose of ferrous sulphate results in a rapid rise in circulating hepcidin, which can take up to 48 hours to return to normal. Subsequent oral doses may not be absorbed during this period because of the ‘hepcidin block’ and may expose patients to side effects.

As a result, guidelines in non-critically ill patients recommend anywhere between 40–80 mg of elemental oral iron once daily each morning or intermittent doses of 100–120 mg (17, 127). Hepcidin levels are also lowest in the morning and therefore dosing at this time is advised (128). This differs significantly from previous guidelines recommending 100–200 mg daily. Oral iron is ideal for conditions where there is sufficient time for it to act effectively such as pregnancy (first trimester) or

elective surgery that is >6 weeks away. Co-ingestion of ascorbic acid with oral iron is also recommended to promote iron absorption. However, a recent RCT in otherwise healthy adults with iron deficiency anaemia found that oral iron supplements alone were equivalent to oral iron plus ascorbic acid in improving Hb recovery and iron absorption (129). An important limitation of this study was that the oral iron dosing schedule was 100 mg of oral iron (ferrous succinate) every 8 hours daily and therefore the results may not be applicable to currently recommended dosing schedules.

Intravenous iron

The focus of this thesis is on intravenous iron. Parenteral iron preparations have been available since the early 20th century, when ferric hydroxide solutions were administered subcutaneously or intramuscularly (130). The observed improvements in Hb were proportional to the amount of iron administered. These preparations lacked a carbohydrate shell which resulted in the immediate release of labile-free iron and severe haemodynamic instability in recipients. In 1954, high molecular weight iron dextran was introduced for intravenous or intramuscular use (131). The molecular weight was 265 kilodaltons (kDa). Dextran covered the iron oxide core which reduced the amount of free iron that was released, and bioavailability occurred through uptake of iron dextran via the reticuloendothelial system (liver macrophages, spleen, bone marrow). Rapid haematological responses occurred but cases of severe, and sometimes fatal, anaphylaxis were reported. This was thought to be due to patients developing or already have pre-existing antidextran antibodies, and the large dextran molecules as a result of their high molecular weight (132). The combination of these factors meant that parenteral preparations were largely reserved for exceptional cases when no other options for correcting iron deficiency anaemia were available and required the administration of a test-dose. Increased levels of circulating free iron may also promote the growth and virulence of many bacterial pathogens (133, 134).

In 1991, a low molecular weight iron dextran (165 kDa) preparation was manufactured as a safer alternative to high molecular weight dextran. Newer preparations, notably ferric gluconate and iron sucrose, were introduced in the late 1990s and early 2000s. These preparations lacked a dextran coat.

Iron sucrose is composed of a iron (III)-hydroxide core surrounded by non-covalently bound sucrose molecules, with a molecular weight of approximately 43 kDa (135). A retrospective review of over 30 million doses of intravenous iron found that the absolute rates of life-threatening adverse drug events were 0.6, 0.9, 3.3 and 11.3 per million for iron sucrose, ferric gluconate, low molecular weight iron dextran and high molecular weight dextran, respectively (136). Despite the relatively lower incidence of adverse reactions of these newer preparations, historical concerns about the dangers of parenteral iron still led many clinicians to avoid prescribing intravenous iron.

In the past decade, three new preparations have entered the market (ferric carboxymaltose (Ferinject), ferumoxytol, iron maltoside (Monofer)) and can be collectively termed as third generation compounds **(Table 1.4)**. These preparations have a more stable carbohydrate shell that permit a controlled delivery of larger doses of iron (1000–2000 mg) to the reticuloendothelial system (135). Iron is subsequently delivered in a slow and controlled manner to iron binding proteins such as ferritin. These mechanisms limit the amount of freely available and potentially toxic unbound iron circulating in the bloodstream, whilst also bypassing the 'hepcidin block' in inflammatory states (135). These preparations can also be administered over short time frames, typically 15–20 minutes, and are increasingly being offered in an outpatient setting.

Table 1.4 Characteristics of different intravenous iron preparations available in the UK

	Ferric carboxymaltose	**Iron isomaltoside**	**Iron (III) hydroxide sucrose**	**Iron (III) hydroxide dextran**
Brand name	Ferinject®	Monofer®	Venofer®	Cosmofer®
Iron content, mg/ml	50	100	20	50
Stability	High	High	Low-moderate	Low
Labile iron (% injected dose)	0.6	1.0	3.5	3.5
Route of administration	Intravenous, slow infusion	Intravenous, slow infusion	Intravenous, slow infusion	Intravenous, slow infusion; intramuscular (gluteal)
Test dose required	No	No	First dose for new patients	Yes, before every dose
Maximal single dose	20 mg/kg diluted in 100 ml 0.9% saline. Maximum weekly dose of 1000 mg.	20 mg/kg diluted in maximum 500 ml 0.9% saline.	200 mg, can be repeated up to 3 times in one week	20 mg/kg, diluted in maximum 500 ml 0.9% saline or 5% glucose
Half-life	7–12 h	5 h	6 h	20 h
Infusion time, minimum	15 minutes	15 minutes, Doses greater than 1000 mg should be administered over >30 min.	30 minutes	Over 4–6h
Use in pregnancy	Avoid in first trimester	Avoid in first trimester	Avoid in first trimester	Avoid in first trimester
Lactation	<1% iron passed into breastmilk	Low transfer into breastmilk; doubtful clinical significance	No available data	No available data
Adverse drug reactions	Common: nausea (2.9%) headache, dizziness, injection site reactions, transient hypophosphataemia	Common: nausea, injection site reactions	Common: nausea, injection site reactions, hypotension, hypertension	Approximately 5% will experience dose-dependent adverse reactions
Anaphylactic reactions	Rare (≥ 1/10,000 to <1/1000)	Rare (≥1/10,000 to <1/1000)	Rare (≥1/10,000 to <1/1000)	Rare (≥1/10,000 to <1/1000)
Product cost / 1000mg, BNF indicative price as per, £	154.23	169.50	109.09	159.40

BNF, British National Formulary

Current, broad indications for intravenous iron are when (137, 138):

- Oral iron treatment is insufficient to replenish iron stores, e.g. during slow ongoing blood loss) or the gastrointestinal side effects are intolerable;
- Rapid replenishment of iron stores is required, e.g. interval between diagnosis of iron deficiency anaemia and surgery is too short for oral iron to be effective;
- Oral iron is unlikely to be absorbed due to inflammation or malabsorption

Example conditions are listed below in **Table 1.5.**

Table 1.5 Current indications for intravenous iron therapy (66, 102, 137, 138)

Established indications	**Examples / comments**
Failure of oral iron / malabsorption	Non-adherence, gastrointestinal side effects, Coeliac disease, atrophic/autoimmune gastritis, bariatric surgery, genetic IRIDA, active IBD
End-stage CKD	Often in combination with ESAs
Pregnancy	Severe iron deficiency in second or third trimester
Heart failure	Symptomatic patents with reduced ejection fraction and iron deficiency
Perioperative anaemia	Preoperative: If interval between diagnosis of anaemia and surgery is too short for oral iron to be effective Postoperative: Hb <100 g/l with evidence of iron deficiency (ferritin <100 mcg/l) (138)
Restless leg syndrome	If oral iron not tolerated or moderate to severe symptoms with ferritin <100 mcg/l and Tsat <45%
Severe iron-deficiency anaemia	Hb <80 g/l, Secondary to menorrhagia, excessive exercise, vegan diets
Expanded / potential indications	**Examples / comments**
Elderly patients	If comorbidities/polypharmacy prevent adherence or effectiveness of long-term oral iron
Acute mountain sickness	Through attenuation of hypoxic pulmonary vasoconstriction
Inflammatory conditions	Anaemia of critical illness, malignancies, COPD, obesity

CKD, Chronic kidney disease; COPD, Chronic obstructive pulmonary disease; ESA, Erythroid stimulating agents; Hb, Haemoglobin; IBD, Inflammatory bowel disease; IRIDA, Iron-refractory iron deficiency anaemia; Tsat, transferrin saturation

1.5.2 Clinical effectiveness and safety of intravenous iron

Clinical effectiveness

Systematic reviews have demonstrated superior biological efficacy of intravenous iron for treating anaemia, improving iron stores and reducing allogeneic RBC transfusion when compared with oral iron or no iron/placebo (139, 140). These findings have also been consistent across specific subpopulations (e.g. pregnancy, chronic kidney disease, surgery) although with varying effect sizes (141-144).

Although evidence of efficacy is now generally accepted, there is a paucity of data on whether this translates into improvements in patient-centred outcomes such as HRQoL, fatigue and physical function. A recent scoping review on patient-centred outcomes in the management of anaemia, which included 130 studies, found that patient-centred outcomes were primary endpoints in 33 studies (24.5%) and secondary endpoints in 74 studies (56.9%) (145). The most common outcome evaluated was fatigue using the Functional Assessment of Cancer Therapy (FACT) and Functional Assessment of Chronic Illness Therapy (FACIT) scales (61 studies (46.9%)).

The best available evidence comes from the heart failure and chronic kidney disease populations, where functional iron deficiency is highly prevalent. In patients with heart failure and iron deficiency (with or without anaemia), intravenous iron results in improvements in exercise capacity, quality of life and fewer heart failure-related hospitalisations (146, 147). Similarly, treating anaemia in patients with dialysis-dependent chronic kidney disease with erythroid stimulating agents and/or iron therapy experienced a better HRQoL and less healthcare resource utilisation. Data from RCTs in patients undergoing major surgery suggest that intravenous iron may reduce postoperative length of stay and hospital readmissions (148-150). However, these findings were secondary outcomes and require confirmation in future adequately powered RCTs. It is unclear whether these findings also apply to critically ill patients and I systematically review this in Chapter 2.

It is worth highlighting that intravenous iron is considerably more expensive than oral iron, even without including the costs of nursing time and administration kits. Given the improved effectiveness and favourable side-effect profile of newer intravenous iron preparations, it remains possible that the overall economic cost might favour intravenous therapy, but no formal cost-effectiveness evaluations have been undertaken to date.

Safety

Common side effects of intravenous iron include nausea, headaches, hypertension, flushing and injection site reactions and these can be managed by providing symptomatic care and reducing the rate of infusion (**Table 1.6)**. True hypersensitivity (anaphylactic) reactions are very rare, especially with newer preparations (151). Intravenous iron is currently only licensed to be given in specific settings (e.g. hospital outpatients or wards) where there are trained staff and equipment for managing anaphylaxis. Monitoring during and for 15–30 minutes after administration is also recommended. Extravasation leading to permanent haemosiderin skin pigmentation has been reported and patients should be counselled to report any pain at the infusion site (152). Extra-vigilance may be required in patients who are unable to communicate (e.g. critically ill patients).

Table 1.6 Side effects associated with intravenous iron preparations

Side effect	Frequency
Nausea	Very common (in more than 1 in 10 participants)
Headache, dizziness, flushing, high blood pressure, injection site reactions (e.g. pain, irritation, discolouration)	Common (more than 1 in 100 but fewer than 1 in 10)
Allergic reaction (hypersensitivity)	Uncommon (more than 1 in 1000 but fewer than 1 in 100)

Transient hypophosphataemia has been reported in several studies, and this may be more pronounced with ferric carboxymaltose (153). Whether this hypophosphataemia has any clinically relevant consequences is uncertain but cases have been reported of symptomatic hypophosphataemia leading

to fractures. A Medicines and Healthcare products Regulatory Agency (MHRA) alert issued in November 2020 advises healthcare professionals to monitor serum phosphate levels in patients:

- Requiring multiple administrations of ferric carboxymaltose at higher doses
- On long-term treatment with ferric carboxymaltose
- With pre-existing risk factors for hypophostaemia such as vitamin D deficiency, inflammatory bowel disease, hyperparathyroidism and osteoporosis.

Intravenous iron increases the levels of circulating free iron, which may be detrimental to the host by promoting pathogen growth (154), and caution is advised in patients with acute or chronic infection. Pooled data from RCTs suggest that intravenous iron may result in an increased risk of infection but it is unclear to what extent and in which clinical settings. I will investigate the risk of infection associated with intravenous iron in greater detail in Chapter 3.

1.5.3 Iron therapy in clinical guidelines

The British Society of Haematology 2013 guidelines (155) recommend against routine iron supplementation in critical illness unless there is clear evidence of iron deficiency (weak recommendation, moderate certainty). More recent guidance from the ESICM 2019 guidelines (116) makes the following recommendations for iron therapy in critical illness:

- Iron therapy (oral or intravenous) should not be routinely used in critically ill patients with anaemia (conditional recommendation, low certainty)
- Future research should focus on the identification of patients most likely to develop an erythropoietic response to iron therapy along with the optimal route, dose, and timing of administration. Trials should be adequately powered to detect changes in patient-centred and functional outcomes, such as fatigue and quality of life, with adequate long-term follow-up and assessment of safety endpoints such as infection

However, surveys of clinical practice demonstrate discordance with these recommendations. A global survey of 947 critical care physicians found that 41% of respondents use iron to improve erythropoiesis and prevent RBC transfusion (92). The work undertaken as part of this thesis is aligned with the ESICM research agenda whilst also aiming to address the discrepancy between guideline recommendations and clinical practice.

1.6 Novel agents currently under development

New agents targeted towards the mechanisms that reverse hypoferraemia and reduce hepcidin concentrations are currently under development **(Table 1.7)**. Prolyl hydroxylase inhibitors stimulate production of EPO and may also act on intestinal mucosa to increase iron absorption.

Table 1.7 Novel agents under development for the treatment of anaemia of inflammation

Agent	Mechanism of action	Stage of development
Monoclonal antibodies		
LY2787106	Neutralises hepcidin, prevents ferroportin internalisation	Phase 1 (156)
LY3113593	Blocks BMP binding to its receptor which decreases hepcidin and increases iron	Phase 1 (157)
LY2928057	Binds ferroportin blocking interactions with hepcidin allowing iron efflux	Phase 1 (157)
Momelotinib	Blocks BMP receptor ACVR1 which inhibits hepatic hepcidin expression	Pre-clinical (158)
Lexaptepid	Nucleotide that binds and inactivates hepcidin	Phase 1 (159)
Prolyl hydroxylase inhibitors	HIF prolyl hydroxylase inhibitors that mimic natural response to hypoxia and decrease hepcidin	Phase 3 (160, 161)

ACVR1, activin A receptor kinase 1; BMP, Bone morphogenic protein; HIF, Hypoxia inducible factor

1.7 Summary and current gaps in knowledge

Improving recovery from critical illness is now an internationally recognised research priority. Available evidence from observational studies supports a causal pathway between untreated anaemia and poor outcomes following critical care. Newer markers of iron status may have a potential role in identifying iron deficiency in the context of AI. Intravenous iron is a biologically attractive treatment option to treat the AI that accompanies critical illness. There are three key areas of uncertainty, which I will aim to address in this thesis:

(1) Collation of high-quality data

To date, there have been few small trials of iron supplementation in critically ill patients. In Chapter 2, I will systematically review the available evidence of the safety and efficacy of iron therapy in critically ill patients. In Chapter 3, I will systematically review the evidence on the risk of infection associated with intravenous iron across all populations

(2) Diagnosis and characterisation of AI

There are limited data on the characterisation of iron status and erythropoiesis AI in patients recovering from an ICU stay. In Chapter 4, I will investigate the diagnostic utility of new markers of iron status such as hepcidin. In addition, I plan to characterise erythropoiesis using novel single-cell profiling techniques at two distinct time-points in a critically ill patient's pathway (acute phase and recovery)

(3) Treatment of AI

To date, there are no published trials evaluating the safety and efficacy of using intravenous iron to treat anaemia in ICU survivors. Conducting research in ICU survivors is challenging, especially in terms of patient selection, maximising retention and minimising loss to follow-up. In Chapters 5 and 6, I will discuss the rationale, methods and results of the INtravenous Iron to Treat Anaemia following criTical Care (INTACT) feasibility RCT.

1.8 Thesis objectives

The objectives of this thesis are:

(1) To systematically review the current evidence of the safety and efficacy of iron therapy in critically ill patients

(2) To systematically review the safety data for intravenous iron on the risk of infection across all patient groups

(3) To investigate if newer markers of iron status, such as hepcidin, can better identify iron deficiency (or systemic iron requirement) in the context of inflammation than current routine tests

(4) To explore the variability in subpopulations of erythroid progenitors, as a marker of erythropoiesis, obtained from peripheral blood in a heterogenous cohort of critically ill patients at two distinct time-points (acute illness and recovery).

(5) To conduct a feasibility RCT of intravenous iron for the treatment of anaemia in patients recovering from critical illness.

Chapter 2 What is the current evidence on the safety and efficacy of iron therapy in critically ill patients? A systematic review

The findings of this chapter have, in part, been published in:

(1) Shah A, Fisher SA, Wong H, et al. Safety and efficacy of iron therapy on reducing red blood cell transfusion requirements and treating anaemia in critically ill adults: A systematic review with meta-analysis and trial sequential analysis. *Journal of Critical Care* 2019; 49: 162-171.

(2) Vlaar APJ, Oczkowski S, de Bruin S, et al. Transfusion strategies in non-bleeding critically ill adults: a clinical practice guideline from the European Society of Intensive Care Medicine. *Intensive Care Medicine* 2020; 46: 673-696.

2.1 Background

2.1.1 Description of the condition

As described in Chapter 1 (Introduction and literature review), anaemia is common in critically ill patients and associated with poor clinical outcomes. At present, allogeneic RBC transfusion is the mainstay for treatment of anaemia in critically ill patients. Depending on the case mix, 30 – 70% of critically patients will receive a RBC transfusion (97, 162) and this equates to 10 - 20% of all RBC transfusions nationally in some countries (163, 164). Whilst transfusion may be lifesaving, balancing its benefits, harms (165, 166), costs and supply remains challenging (167). In view of these challenges, there has been increasing interest in the role of iron therapy as a safe and effective alternative intervention to treat anaemia in critically ill patients and subsequently reduce transfusion requirements. In particular, intravenous iron therapy decreases severity of anaemia and RBC transfusion requirements in non-critically ill patients, albeit with varying effect sizes (139, 140, 168).

2.1.2 Why is it important to do this review?

I have previously conducted a systematic review on iron supplementation, by any route, to treat anaemia in adult critically ill patients. This review found no evidence of an effect of iron therapy on

reducing RBC transfusion requirements (169), but it did not include the findings of the Intravenous Iron or Placebo for Anaemia in Intensive Care (IRONMAN) RCT (170), which was still ongoing at the time. IRONMAN subsequently demonstrated that intravenous iron did not result in a reduction in RBC transfusion requirements. A recent worldwide survey of ICU physicians (n = 947) found that 41% of respondents routinely use iron therapy as a strategy to reduce transfusion requirements (92). In the absence of any clear benefit, critically ill patients may risk being exposed to unnecessary harms of iron therapy, such as increased risk of infection. The cost effectiveness of iron therapy also warrants further investigation.

Current recommendations advocate updating systematic reviews when new evidence is available and when newer, relevant data synthesis methods can be applied (171). With the publication of the IRONMAN RCT, I decided to update my previous systematic review to investigate if this would alter the findings on clinically important efficacy and safety outcomes. To improve the quality of data analysis and clarity of findings, I applied trial sequential analysis (TSA) and assessed the quality of evidence using the grading of recommendations assessment, development and evaluation (GRADE) guidelines (172).

The main objective of this systematic review update was to evaluate the effect of iron therapy, by any route, on clinically important efficacy (transfusion requirements, changes in Hb concentration) and safety (infection, mortality) outcomes in critically ill patients.

2.2 Methods

This systematic review was conducting according to a predefined study protocol registered on PROSPERO (CRD42015016627) which was published online (http://www.crd.york.ac.uk/prospero). I followed the recommendations by the Cochrane Collaboration (173), the preferred reporting items for systematic reviews and meta-analysis (PRISMA) statement (174) and the GRADE guidelines (172).

2.2.1 Eligibility criteria

To be included in this systematic review, the following criteria had to be met: (i) RCTs; (ii) patients admitted to any ICU or high-dependency unit (HDU); (iii) iron supplementation by any route (oral, intravenous, intramuscular) versus placebo or no iron therapy. Co-interventions (e.g. erythropoietin, vitamin C) were only included if they were present in both or all study arms.

2.2.2 Outcomes

The pre-defined co-primary efficacy outcomes were:

- Requirement for allogeneic RBC transfusion
- Mean number of RBCs transfused
- Changes in Hb concentration

Secondary outcomes included:

- In-hospital mortality
- In-hospital infection (as defined by the study authors)
- Length of stay (LOS) – ICU and hospital
- Organ dysfunction scores
- Changes in HRQoL
- Adverse events

I defined two study time points: (i) short-term (≤10 days); and (ii) medium-term (>10 days, the last measured time point in hospital or end of the trial).

2.2.3 Search strategy

The following databases were searched: CENTRAL (Cochrane Central Register of Controlled Trials) in The Cochrane Library, MEDLINE, Embase, CINAHL, PubMed (e-publications ahead of print

only), Web of Science (1990 onwards) and the Transfusion Evidence Library (1950 onwards). Ongoing trials were sought from trial registration websites. The full search strategy is available in Appendix 1. The last search date was 15th January 2018. I augmented database searching by hand-searching reference lists of all included studies and relevant review articles.

2.2.4 Study selection and data extraction

Two review authors (AS, HW) independently screened all electronically derived citations and abstracts of papers from the systematic search. All trials that fulfilled the inclusion criteria were investigated in full text. Data were extracted by the same two review authors (AS, HW) onto a pre-piloted data collection form that was used for the previous version of this review. We were not blinded to the names of authors, institutions of journals of the trials. Extracted data included general information about study design, methodology, characteristics of study participants at baseline, intervention delivery (e.g. route, timing, dose), clinical outcomes, laboratory results pre- and post-intervention and information for risk of bias assessments. Disagreements were solved through discussion and if no resolution was found a third author (SJS) adjudicated. I sought unclear or missing data by contacting the authors of the individual trials and allowing them 28 days to respond.

2.2.5 Risk of bias assessment and grading the quality of evidence

Study quality was judged using the Cochrane Collaboration tool for assessing risk of bias (175). The following domains were assessed for each study: random sequence generation, allocation concealment, blinding of participants, personnel and outcome assessors, incomplete outcome data, selective reporting and other bias such as financial conflicts of interest. A three-point scale was used to determine low, high, or unclear risk of bias. Two review authors (AS, HW) assessed risk of bias independently. Any disagreement was resolved by discussion.

Two review authors (AS, HW) rated the overall quality of evidence for all primary outcomes and the secondary outcome of in-hospital infection using the GRADE approach (172). GRADE is a systematic and transparent framework for developing and presenting summaries of evidence and

providing recommendations for clinical practice. GRADE has four levels of evidence and the terms 'certainty of evidence' or 'quality of evidence' are often used interchangeably. It is important to note that GRADE is used to rate the body of evidence at an outcome level rather than the study level. The four levels are:

- High – We are very confident that the true effect is similar to the estimated effect
- Moderate – The true effect is probably close to the estimated effect
- Low – The true effect might be substantially different from the estimated effect
- Very low – We have little confidence in the results and the true effect is probably markedly different from the estimated effect

These ratings can be upgraded or downgraded based on the following considerations:

- Risk of bias (see Section 2.2.5)
- Imprecision – insufficient information size to make a judgement (e.g. low event rates or effect estimate comes from few small studies resulting in wide confidence intervals and lack of confidence in the true effect estimate)
- Inconsistency – significant and unexplained variability in results from different trials
- Indirectness – inclusion of studies with participants that are not reflective of daily clinical practice, outcomes measured are a surrogate for a different outcome, not using the intervention of interest
- Publication bias – systematic overestimation of treatment effects based on selective publication of studies, e.g. studies with 'negative' / no-effect findings are less likely to get published

An overview of the GRADE method is shown in **Figure 2.1**. I completed steps one to three, which is required for systematic reviews. Steps four and five are reserved for those developing healthcare recommendations, such as guideline panels. In keeping with the Methodological Expectations of

Cochrane Intervention Reviews, another author (HW) independently assessed the certainty of the evidence and disagreements were solved through discussion.

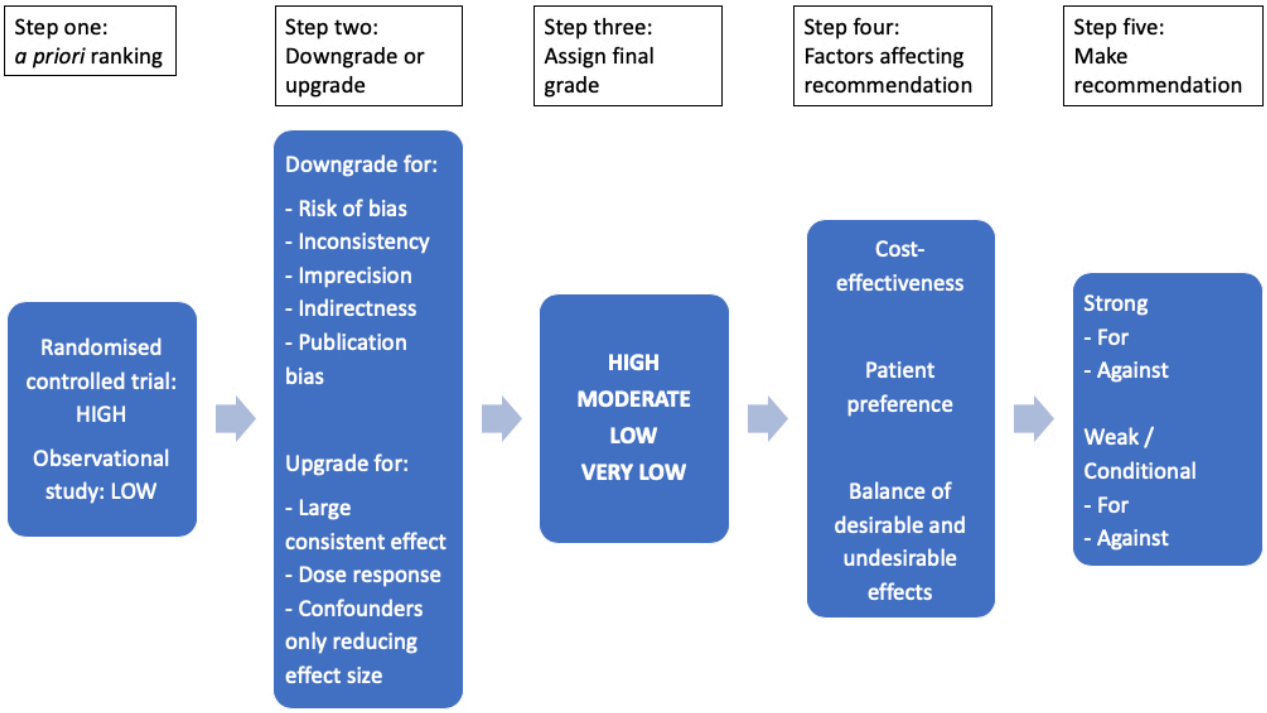


Figure 2.1 Flow diagram of the GRADE approach for synthesising evidence and developing recommendations

2.2.6 Data synthesis

Statistical analyses were performed using Review Manager software (RevMan, version 5.3) and TSA program version 0.9 beta (www.ctu.dk/tsa). I used random-effects methods for meta-analysis throughout. I anticipated that studies would include different patient populations with variation in delivery of intervention (e.g. route, dose, timing) and therefore would not equate to a common (or fixed) effect size. Where possible, continuous variables were reported as mean difference (MD) with 95% confidence interval (CI). For outcomes that were deemed likely to be skewed in distribution, namely number of RBC transfusions, reported mean and standard deviation (SD) were transformed to a natural logarithmic scale using the methods of Higgins et al. (176) Dichotomous variables were reported as relative risk (RR) with corresponding 95% CI. Heterogeneity was assessed using the I^2

statistic (177) and classified as moderate if >50%, or considerable if >80%.

2.2.7 Subgroup and sensitivity analyses

I planned to perform subgroup analyses if there were at least two RCTs with comparable subgroups reporting the primary outcomes: (i) different iron preparation (enteral vs. intravenous) and (ii) use of co-interventions e.g. ESAs. My rationale for performing these subgroup analyses was based on the biological plausibility that intravenous iron may be more efficacious than oral iron as it can bypass the 'hepcidin block', and that ESAs may work synergistically with iron. Combination therapy of iron and ESAs has shown to be effective in treating anaemia in mouse models of AI (178). I intended to perform sensitivity analysis by only including trials at low risk of bias (defined as trials with no high risk of bias domains and at least half of the remaining domains considered to be at low risk of bias).

2.2.8 Trial sequential analysis

Cumulative meta-analysis may result in type 1 errors due to repeated testing of accumulating data (179), and this is more likely with meta-analyses with sparse data (i.e. small numbers of trials or events) (180, 181). I was concerned about this occurring for this update in view of the limited number of trials I analysed in my previous review. Over the past decade, TSA has emerged as a useful statistical method to mitigate the likelihood of type 1 errors and I incorporated TSA this into this review to address this risk. TSA combines conventional meta-analytical techniques with statistical monitoring boundaries, that create thresholds for determining significance based on the impact of multiple testing and amount of information already available in the meta-analysis. TSA calculates a required information size IS (overall sample size to obtain the required level of statistical power) based on the available data by taking into the consideration the event rate in the control group, a plausible relative risk reduction (RRR) or relative risk increase (RRI) and the anticipated heterogeneity variance (D^2) of the meta-analysis (182). It is analogous to a sample size calculation for a randomised trial and like a clinical trial, these assumptions should be prespecified in a systematic review protocol.

I applied TSA to the following dichotomous outcomes, which I initially felt would be of importance to both patients and clinicians: (i) requirement for RBC transfusion (efficacy); (ii) in-hospital infection (safety). I estimated a baseline risk of 50% of requiring an allogeneic RBC transfusion based approximately on the proportion of participants receiving a transfusion in the control group on the trials included in this systematic review. I considered a 12% RRR as clinically meaningful. I estimated a baseline risk of developing an infection of 30%. This was based on the results of a large observational study (183). I considered a 17% RRI of developing an infection from receiving iron therapy as clinically relevant. These estimates are from a meta-analysis assessing the safety and efficacy of intravenous iron across a range of patient populations (139). Futility boundaries were produced such that if the cumulative Z-curve crossed the futility threshold, evidence showed that the treatment effect did not differ more than the anticipated effect size. I used the O'Brien Fleming alpha-spending function with an overall type 1 error rate of 5% and with 80% statistical power to derive two-sided sequential monitoring and futility boundaries.

2.3 Results

2.3.1 Search results

The database searched identified 1123 records, reduced to 964 after duplicates were removed. After exclusion of records that were clearly irrelevant, 33 full-text articles were assessed for eligibility. Twenty-seven studies were excluded (reasons summarised in **Figure 2.2)**, leaving six RCTs to be included in the quantitative synthesis (170, 184-188). One ongoing trial was identified (189).

2.3.2 Characteristics of included studies

The six RCTs included a total of 805 participants of which 428 were randomly allocated to receive iron therapy and 367 to receive no iron or placebo. Details of the included RCTs are shown in **Table 2.1**. Four trials were single centre, four were carried out in surgical ICUs (trauma, cardiothoracic, neurosurgery, burns and general surgical), and two were carried out in mixed ICUs that included medical, surgical and trauma patients.

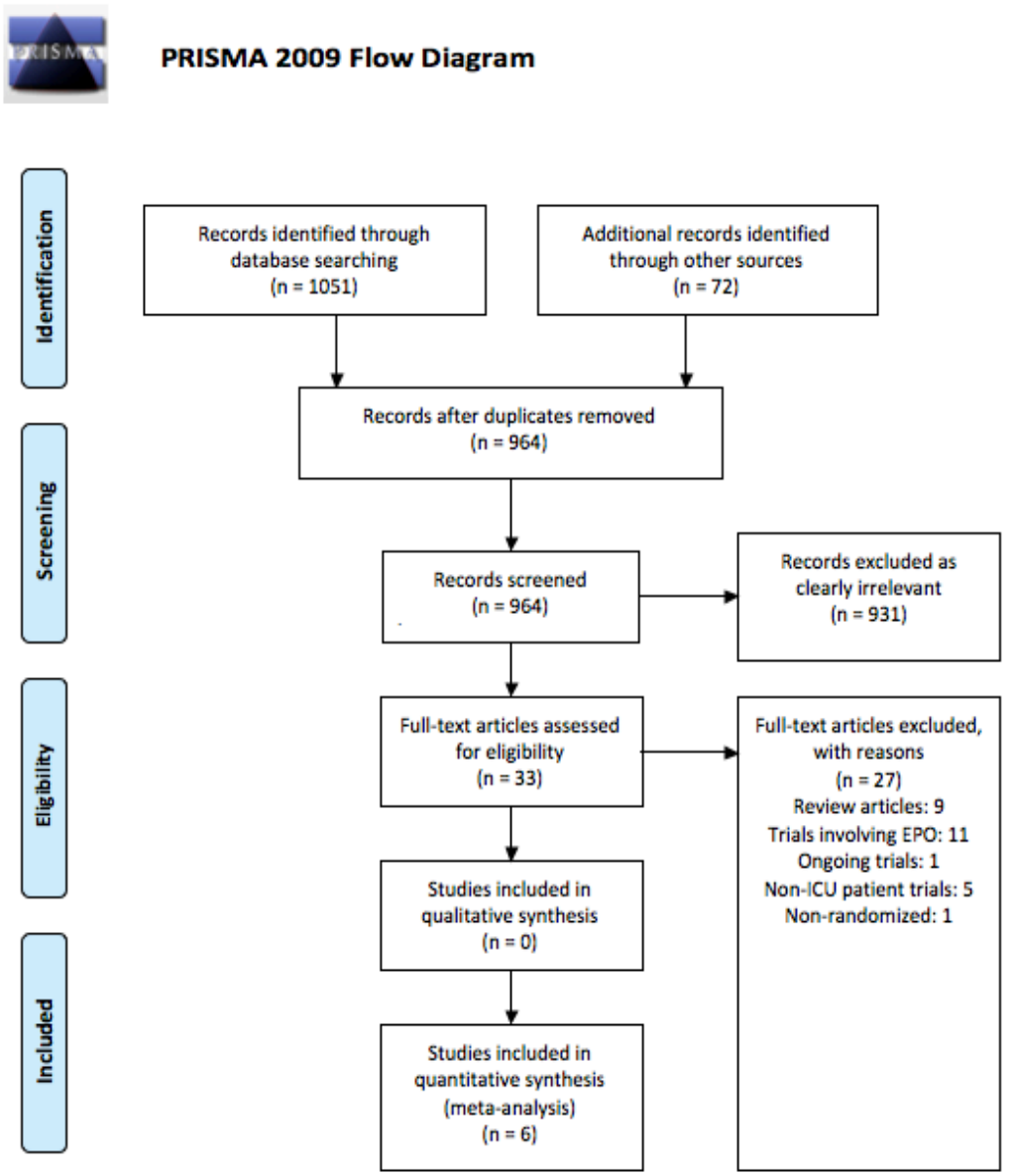


Figure 2.2 Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flow diagram

Table 2.1 Characteristics of the included studies evaluating the safety and efficacy of iron therapy in critically ill patients

Study:	**Garrido-Martin et al. (2012)**		**Madi-Jebara et al. (2004)**		**Pierraci et al. (2009)**		**Pierraci et al. (2014)**		**van Iperen et al. (2000)**		**Litton et al. (2016)**	
	Iron	**No iron**	**Iron**	**No iron**	**Iron**	**No iron**	**Iron**	**No iron**	**Iron**	**No iron**	**Iron**	**No iron**
No. randomised:	IV 71, Oral 73	66	40	40	97	103	75	76	12	12	70	70
No. analysed:	IV 54, Oral 53	52	40	40	97	103	75	75	12	12	70	70
Age (mean, SD/ range)	IV 65 (11) Oral 65 (10)	65 (12)	59.1 (9.1)	55.3 (9.5)	55.7 (1.9)	58.2 (1.7)	41.6 (18-83)	40.4 (18-87)	67 (49-89)	69 (45-80)	58.5 (18.8)	56.0 (21.1)
Sex (Male), %:	IV 70.3% Oral 71.7%	76.9%	90%	90%	50.5%	46.6%	77.3%	60.5%	66.6%	66.6%	63%	74%
ICU setting:	Cardiothoracic		Cardiothoracic		General surgical, burns, neurosurgical		Trauma		Mixed (surgical, medical, neurological, trauma)		Mixed (general surgical, cardiothoracic, neurosurgical, medical, trauma)	
No. of centres	One		One		One		Four		One		Four	
Inclusion criteria:	Age >18 years, elective cardiac surgery under extracorporeal circulation, no previous anaemia, susceptible to treatment, no preoperative blood		Elective cardiac surgery with CPB, Post-pump Hb 7-10 g/dl.		General surgical, burn, neurosurgical ICUs, Age >18 years, Hb <13 g/dl prior to enrolment, <72 hours from hospital admission, current		Admitted to ICU with trauma, Hb<12 g/dl, Age>18 years, <72 hours from ICU admission, expected ICU LOS >5 days.		Hb <11.2 g/dl, <12.1 g/dl if cardiac disease, age >18 years, expected ICU LOS >7 days, informed consent from patient or relative.		Admitted to an ICU for less than 48 hours, anticipated to require ICU care beyond the next calendar day, Hb <100 g/l at any time during the preceding	

Study:	Garrido-Martin et al. (2012)	Madi-Jebara et al. (2004)	Pierraci et al. (2009)	Pierraci et al. (2014)	van Iperen et al. (2000)	Litton et al. (2016)
	transfusion, able to complete all study visits are per protocol, able to provide written consent.		tolerance of enteral medication, expected ICU LOS >5 days.			24 hours, age 18 years or greater
Exclusion criteria:	Elective cardiac surgery without exclusion criteria, fibrinolytic therapy 48 hours prior to CPB, impaired renal function (CrCl<50 mls/min), previous surgery for IE, re-do surgery, pregnant or lactating, active gastrointestinal bleeding, B_{12} deficit, ferropenic anemia, asthma or allergy, active infection, included in another study, hepatic disease, history of allergy to iron, unlikely to adhere to protocol follow-up.	Intra-operative blood transfusion, post-operative hemodynamic instability, ejection fraction <40%, chronic kidney disease, hypothermic bypass, hypersensitivity to iron.	Active bleeding, chronic inflammatory conditions, end-stage renal disease, hematologic disorders, macrocytic anaemia, current use of EPO, pregnancy, prohibition of RBC transfusion, imminent death, co-enrolment in another trial.	Active haemorrhage, iron overload (serum ferritin>1000 ng/ml), conditions associated with iron overload e.g. haemachromatosis, active infection, chronic inflammatory conditions, pre-existing haematological disorders, macrocytic anaemia, current/recent (within 30days) use of immunosuppression, use of EPO within 30 days, pregnancy or lactation, prohibition of RBCs, imminent death, history of allergy to iron.	Pregnancy, iron deficiency anaemia (ferritin<50 ug/L), vitamin B_{12} deficiency (<160 pmol/l), recent use of cytostatic or recent radiotherapy, life expectancy <7 days, chronic renal failure, prior use of EPO.	Suspected or confirmed sepsis, serum ferritin >1200 ng/m; or Tsat >50%, history of haemochromatosis, known administration if IV iron in the preceding 3 months, Jehovah's witness or other documented exclusion to receiving blood products, receiving ESA in the 3 months prior to ICU admission, known hypersensitivity to intravenous iron, pregnancy, palliative treatment intent, death is deemed imminent and inevitable, weight less than 40 kg, participant in competing study
Intervention(s):	i) IV iron-hydroxide sucrose complex (Venofer; Uriach Lab) 3 doses of 100mg/24	i) IV iron (Venofer; Luitpold Pharmaceuticals)	Enteral ferrous sulphate 325 mg (oral solution or capsule) (Rockwell	IV Iron sucrose (Venofer; Luitpold Pharmaceuticals) 100mg thrice weekly	IV iron saccharate (Venofer; Vifor) 20mg	IV iron (ferric carboxymaltose) 500mg. Assessed for repeat dosing after 4

Study:	Garrido-Martin et al. (2012)	Madi-Jebara et al. (2004)	Pierraci et al. (2009)	Pierraci et al. (2014)	van Iperen et al. (2000)	Litton et al. (2016)
	hours during pre- and post-hospitalization, and 1 pill/24 h of oral placebo during the same period and during 1 month after discharge. ii) Ferrous fumarate (105 mg of iron) 1 pill/24h orally pre- and postoperatively and during 1 month after discharge, and intravenous placebo while hospitalised.	200mg/day to reach total iron deficit + s/c placebo [a]	Compounding Inc.) thrice daily until hospital discharge. Co-intervention: Ascorbic acid 500mg thrice daily, Cyanocobalamin 1mg, Folic acid 1mg.	for up to 6 doses or until ICU discharge.	and IV folic acid 1mg daily from D1-14 [b] Co-intervention: Folic acid 1mg daily	days if still in ICU and fulfilling all other eligibility criteria.
Comparator:	Oral and intravenous placebo pre- and postoperatively following same protocol.	Placebo – s/c and IV (0.9% Saline).	Oral placebo, same schedule as intervention protocol. Co-intervention: Ascorbic acid 500mg thrice daily, Cyanocobalamin 1mg, Folic acid 1mg.	IV placebo (100 mls of 0.9% saline) similar dosing schedule to intervention.	No iron Co-intervention: Folic acid 1mg daily.	IV placebo (100mls of 0.9% saline) similar dosing schedule to intervention
Reported outcomes	• Hb concentration (Baseline, operating room entry (D7), exit	• Hb concentration (D0, D1-5, D15, D30)	• Difference in Hct (Baseline, D7, D14,	• Number of total doses of study drug received	• Hb concentration (D0, D7, D14, D21)	• RBC transfusion (mean number of units)

Study:	Garrido-Martin et al. (2012)	Madi-Jebara et al. (2004)	Pierraci et al. (2009)	Pierraci et al. (2014)	van Iperen et al. (2000)	Litton et al. (2016)
(follow-up time points, days):	operating room, ICU admission, ICU discharge, postoperative D10 and D30 post-hospital hospital discharge) • Immature reticulocyte fraction, reticulocyte count, serum ferritin (D1, postoperative D10 and D30 post-hospital discharge) • RBC transfusion (no. patients transfused, location of transfusion, mean number of units)	• Reticulocyte counts (D1, D5, D15, D30) • Serum ferritin (D0, D5, D15) • RBC transfusion • Mortality	D21, D28) (primary outcome) • Serum iron, serum ferritin, eZPP (Baseline, D7, D14, D21, D28) • RBC transfusion • Estimated blood loss per study day • Nosocomial infection • Antibiotic days • Adverse outcomes – gastrointestinal upset • ICU & Hospital LOS • Mortality	• Hb concentration (daily) • Serum iron, serum ferritin, serum Tsat, eZPP (Baseline, D7, D14) • RBC transfusions • Transfusion-free days • Nosocomial infection & type • Antibiotic exposure • ICU & Hospital LOS • Mortality	• Reticulocyte count, sTfR (D0, D7, D14, D21) • Serum EPO (D0, D2, D6, D10, D21) • Serum iron, transferrin, Tsat, ferritin, eZPP, CRP (D0, D10, D21) • Mean blood loss (D0-D21) • RBC transfusion (mean number of units) (D0-D21) • ICU LOS • Mortality	(Randomisation to hospital discharge) • Hb at hospital discharge • Proportion of patients receiving RBC transfusion • ICU and hospital LOS • Nosocomial infection • Mortality

CBP = cardiopulmonary bypass; CrCl, creatinine clearance; EPO, erythropoietin; eZPP, erythrocyte zinc protoporphyrin; Hb, haemoglobin; Hct, Haematocrit; ICU, Intensive care unit; LOS, length of stay; RBC, Red blood cell; sTfR, soluble transferrin receptor; Tsat, transferrin saturation; IE, Infective endocarditis

(a) In a second intervention arm, patients received intravenous iron and recombinant-human EPO(300 IU/kg) subcutaneously on day 1; this treatment arm was not included in this review because the co-intervention was not matched in the control group.

(b) In a second intervention arm, patients received intravenous iron and EPO alfa (300 IU/kg) subcutaneously on days 1,3,5,7,9; this treatment arm was not included in this review because the co-intervention was not matched in the control group.

Three trials compared intravenous iron with intravenous placebo (170, 185, 188), one RCT compared intravenous iron with no iron (184) and one trial compared oral iron with oral placebo (184). One RCT included three arms (187): (i) intravenous iron and oral placebo; (ii) oral iron and intravenous placebo; and (iii) oral and intravenous placebo. Two trials included study groups that only received ESAs, and these were excluded for this analysis (184, 187). Two trials administered the following co-interventions – ascorbic acid, cyanocobalamin and folic acid (185) or folic acid only (184).

2.3.3 Risk of bias assessments

Assessment for individual domains is summarised in **Figure 2.3**. There were too few trials to assess publication bias. Two trials were judged as being low risk of bias across all domains (186, 189).

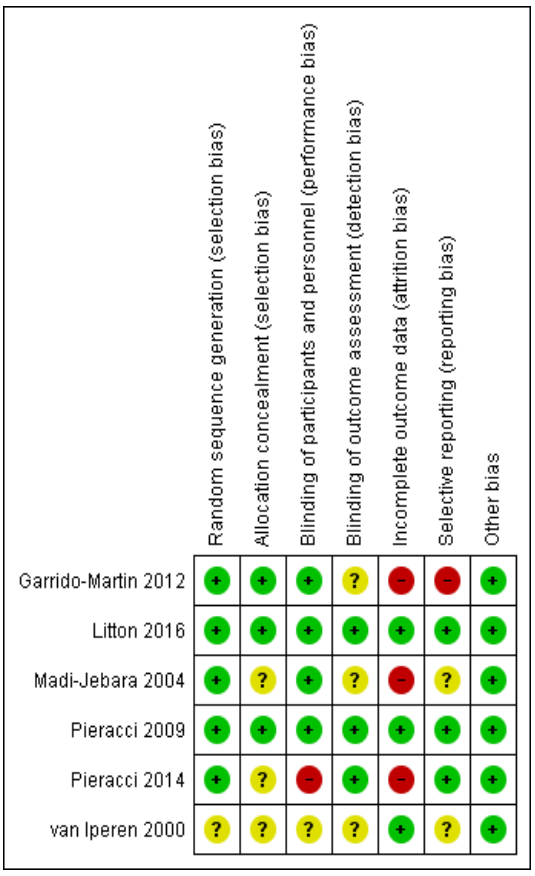


Figure 2.3 Risk of bias summary. Low risk of bias for a category is indicated by + , unclear by ? and high risk by −

Random sequence generation (selection bias)

Five trials were at low risk of bias for sequence generation because they used computer generated random number sequences (170, 185-188) and one trial did not provide sufficient details to make a judgement (184).

Allocation concealment (selection bias)

Three trials were at low risk of selection bias as they used sealed opaque envelopes, prepared the intervention in a disguised form or concealed the randomisation sequence until the intervention was assigned (186, 188, 189). The remaining three trials did not report details of treatment allocation (184, 185, 187).

Blinding (performance and detection bias)

Four trials managed to achieve adequate participant and personnel blinding (170, 186-188). Blinding of intravenous iron preparations, which are often reddish-brown in colour, was mainly achieved using opaque sleeves covering the study infusion. Drug blinding and handling were always performed by central pharmacy staff. One trial used oral placebo capsules or similar colour (186). One trial used 0.9% saline as a placebo but this was not blinded using an opaque sleeve infusion (185) and one trial did not provide sufficient details on blinding (184). Three trials were judged as having unclear risk of detection bias (184, 187, 188)

Incomplete outcome data (attrition bias)

Three trials were considered at low risk of attrition bias because participants were analysed on an intention to treat basis (170, 184, 186). Three trials reported high levels of attrition bias (185, 187, 188). One trial excluded 24 participants from analysis for unclear reasons (188), one excluded patients who received an RBC transfusion which was not specified *a priori* (187), and one only analysed patients who received all six doses of the study drug, which resulted in 56 participants being excluded (185). *Selective reporting (reporting bias)*

Three trials were considered to be at low risk of reporting bias (170, 185, 186) because they reported

all pre-specified outcomes, were prospectively registered or had a pre-published trial protocol (190). One trial was judged to be at high risk because of reporting outcomes that were not pre-specified (188). Insufficient information was provided to make a judgement for two trials (184, 187).

Other bias

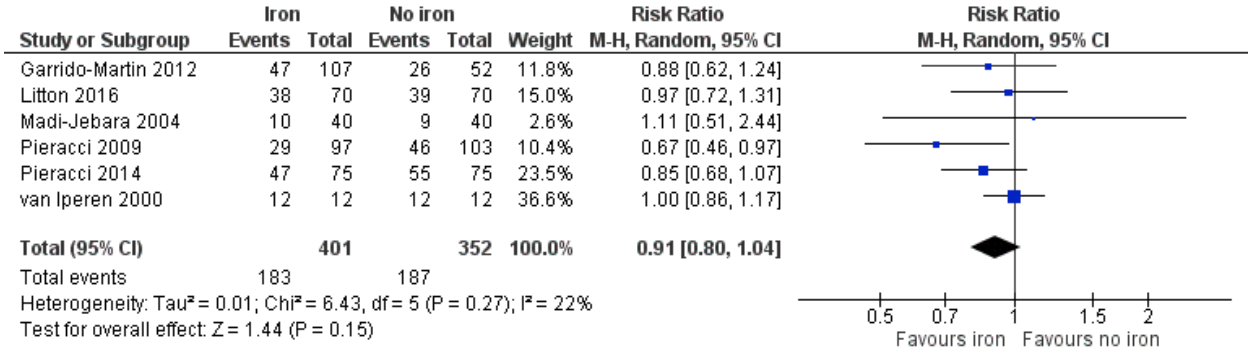
No significant sources of other bias were identified for all included trials.

2.3.4 Effect on outcomes

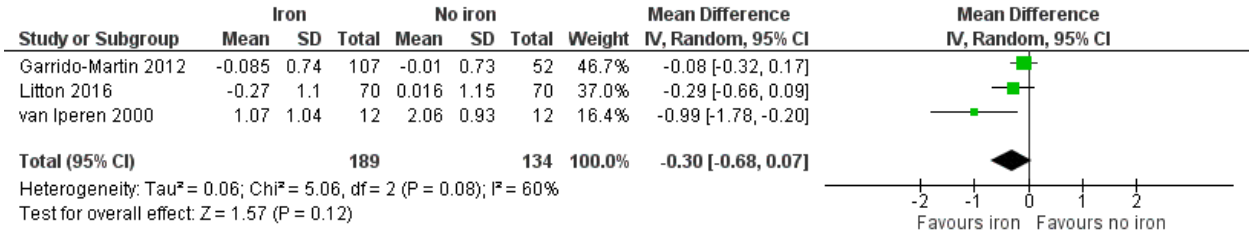
2.3.4.1 Requirement for RBC transfusion

All six trials provided data on the number of participants who received a RBC transfusion in a way that could be analysed by meta-analysis. Overall, there was no evidence of an effect of iron therapy on the number of participants requiring a RBC transfusion (RR (95% CI): 0.91 (0.80 to 1.04), p = 0.15, I^2 = 22%; 753 participants, 6 RCTs) (**Figure 2.4a**). The overall quality of evidence was judged to be *low*. Three trials reported mean number of RBCs received per participant in both groups (184, 188, 190). After transforming to a log scale, meta-analysis showed no evidence that iron therapy reduced the mean number of RBCs transfused (MD (95% CI): -0.30 (0.68 to 0.07) units, p = 0.15, I^2 = 60%; 323 participants, 3 RCTs) (**Figure 2.4b)**. The overall quality of evidence was judged to be *low*. Subgroup analysis by route of administration by either intravenous or oral routes showed no evidence of a difference (**Figure 2.5)**.

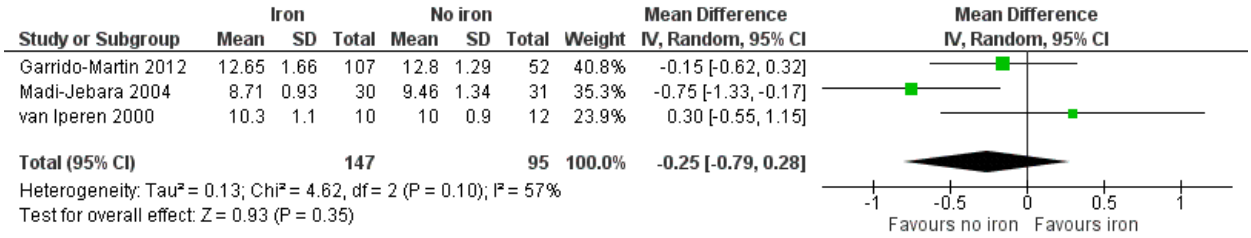
(a) Requirement for RBC transfusion



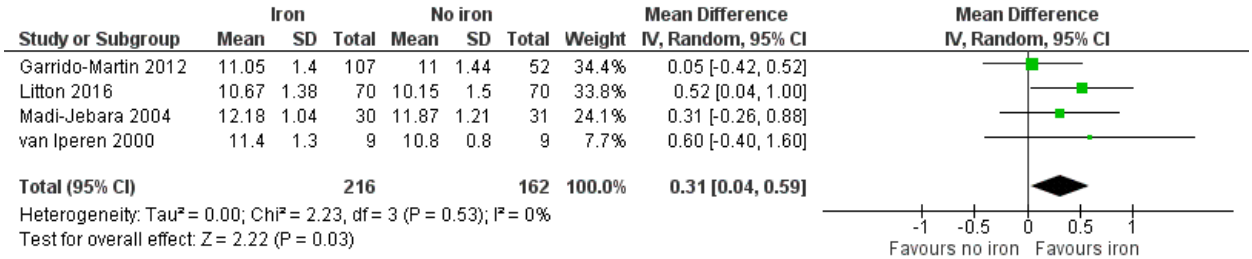
(b) Mean number of RBCs transfused



(c) Mean Hb concentration (g/dl) (≤10 days)



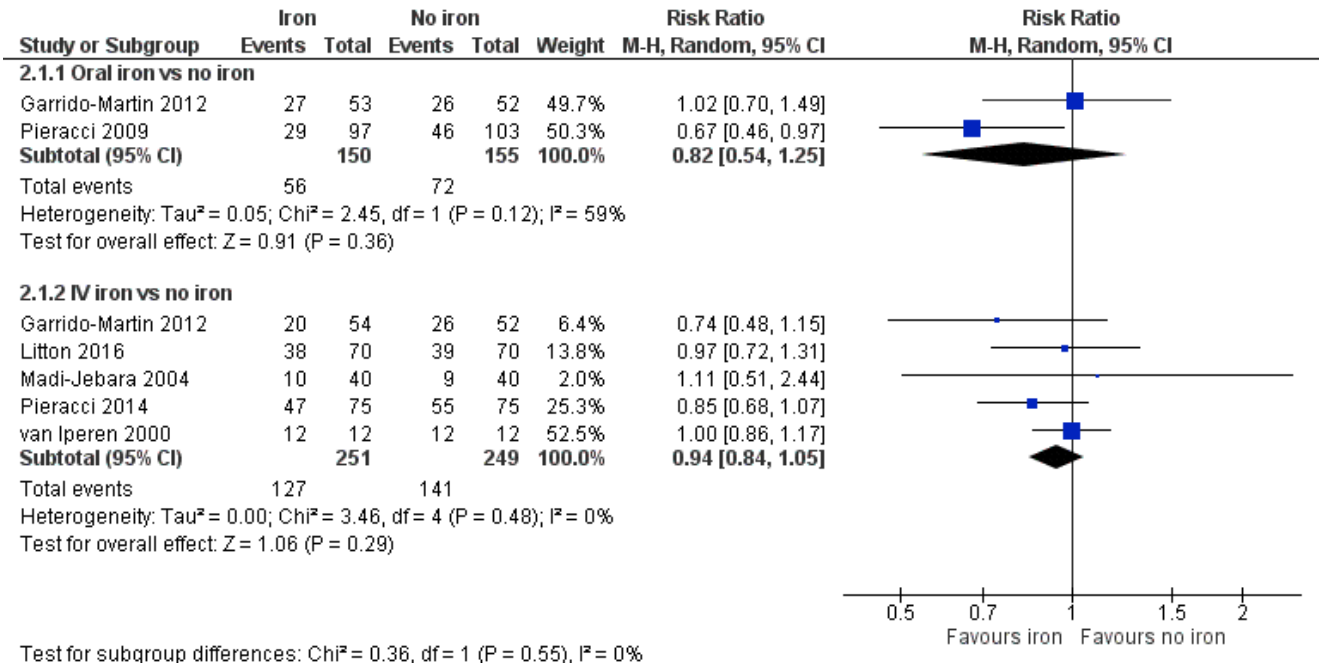
(d) Mean Hb concentration (g/dl) (>10 days)



CI, Confidence interval; Hb, haemoglobin; IV, Inverse variance; M-H, Mantel-Haenszel; RBC, Red blood cell

Figure 2.4 Forest plots of the effect of iron supplementation, by any route, on co-primary outcomes

(a) Requirement for RBC transfusion



CI, Confidence interval; Hb, haemoglobin; IV, Inverse variance; M-H, Mantel-Haenszel; RBC, Red blood cell

Figure 2.5 Subgroup analysis: forest plots of the effect of iron supplementation by either intravenous or oral iron administration on RBC transfusion requirements

2.3.4.2 Changes in haemoglobin concentration

Mean (SD) changes in Hb concentration were reported at short-term follow up (≤10 days) in three trials (184, 187, 188) and at medium-term follow up (>10 days) in four trials (170, 184, 187, 188). There was no significant difference in mean Hb concentrations between groups at short term follow up (MD (95% CI) -0.25 (-0.79 to 0.29) g/dl, p = 0.35, I^2 = 57%; 242 participants, 3 RCTs) (**Figure 2.4c)**. The overall quality of evidence was judged to be *very low*. Mean Hb at medium-term follow-up was higher in participants that received iron (MD (95% CI) 0.31 (0.04 to 0.59) g/dl, p = 0.003, I^2 = 0%; 378 participants, 4 trials) (**Figure 2.4d)**. The overall quality of evidence was judged to be *moderate*.

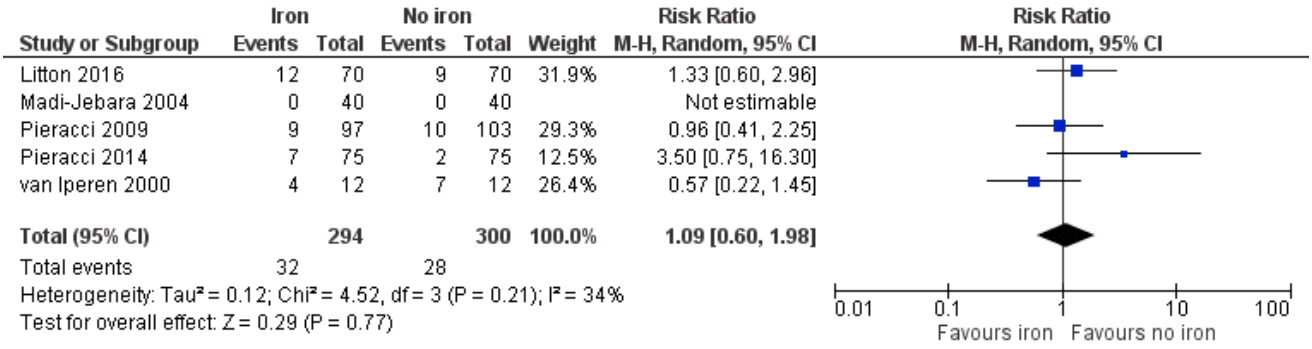
2.3.4.3 In-hospital mortality

Five trials provided data on in-hospital mortality (170, 184-187). There was no evidence of an effect of iron therapy on in-hospital mortality (RR (95% CI) 1.09 (0.60 to 1.98), p = 0.77, I^2 = 34%; 594 participants, 5 RCTs) (**Figure 2.6a)**. No trial reported data on causes of death.

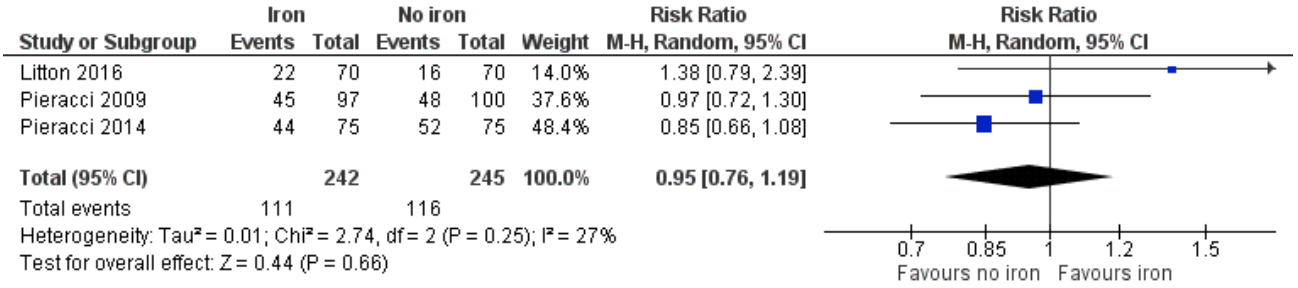
2.3.4.4 In-hospital infection

Three trials provided data on this outcome with varying definitions of infection (170, 185, 186). One trial used a pragmatic definition of 'commencement, escalation of change of i.v. antibiotics for a confirmed or strongly suspected infection and was adjudicated locally by blinded clinical staff' (170). Two trials used standardised definitions provided by the Centres for Disease Control and Prevention criteria (191). There was no evidence of an increased risk of infection association with iron therapy (RR (95% CI) 0.95 (0.76 to 1.19), p = 0.25, I^2 = 27%; 487 participants, 3 RCTs) (**Figure 2.6b)**. The overall quality of the evidence was judged to be *low*. Two trials reported antibiotic use and did not observe a significant difference in the mean number of antibiotic days between the intervention and control groups (14 vs. 16 days, p = 0.45 (186); 14 vs. 16 days, p = 0.64 (185)).

(a) Mortality



(b) In-hospital infection



CI, Confidence interval; Hb, haemoglobin; IV, Inverse variance; M-H, Mantel-Haenszel; RBC, Red blood cell

Figure 2.6 Forest plots of the effect of iron supplementation, by any route, on secondary outcomes

2.3.4.5 Length of stay, organ dysfunction scores and HRQoL

Three trials reported ICU and hospital LOS (170, 185, 186) whereas one only reported ICU LOS (184). We did not perform meta-analysis as LOS is often reported as median (with interquartile range) due to non-parametric distribution. One trial did report mean (SD) data and found a significantly longer ICU LOS in the control group compared with the intervention group (58 (31) vs. 29 (18) days, $p < 0.05$) (184). Of the remaining three trials, there was no difference in median ICU LOS (6 vs. 6 days, $p = 0.70$ (170); 14 vs. 16 days, $p = 0.69$ (186); 10 vs. 11 days, $p = 0.53$ (185)) and in median hospital LOS (15 vs. 18 days, $p = 0.75$ (170); 14 vs. 16 days, $p = 0.24$ (186); 14 vs. 16 days, $p = 0.50$ (185)). Only one trial reported on median ICU organ failure support-free days and found no difference between the intervention and control group (2 vs. 2 days, $p = 0.89$) (170). No trials reported on changes in HRQoL.

2.3.4.6 Adverse events

Adverse events were generally infrequent. One trial reported serious adverse events (SAE) and found no difference between the intravenous iron (pulmonary embolus (PE) = 2, deep vein thrombosis (DVT) = 2) and placebo groups (PE = 3, DVT = 1) (RR (95% CI): 1.0 (0.26 to 3.84), p = 1.0) (170). One trial of oral iron vs. oral placebo found no differences in adverse events between groups (12.4% vs. 8.7%, p = 0.62) (186). Other notable adverse events of such as anaphylaxis, hypersensitivity reactions, and extravasation were not reported.

2.3.5 GRADE quality of evidence

Overall GRADE quality of evidence ranged from very low to moderate (**Table 2.2)**. The evidence was predominantly downgraded for risk of bias, in particular attrition bias, and for imprecision due to the wide confidence intervals and the optimal sample size not being reached.

2.3.6 Trial sequential analysis

I applied TSA to two outcomes of interest – requirement for RBC transfusion and in-hospital infection. For the outcome of RBC transfusion, the TSA adjusted 95% CI was 0.69 to 1.20 with available data showing that only 27% of the required informed size to detect or reject a RRR of 12% had been reached (**Figure 2.7a)**. When adjusted for the two trials with overall low risk of bias (170, 186), the TSA adjusted 95% CI was 0.76 to 1.09, with the available data showing that 60% of the required information size had been reached (**Figure 2.7b)**.

For the outcome of in-hospital infection, the TSA adjusted CI was 0.39 to 2.33 with the cumulative Z-curve showing that only 12% of the required information for to detect or reject a relative risk increase of 17% had been reached (**Figure 2.8**).

Table 2.2 Summary of findings including GRADE quality assessment of evidence

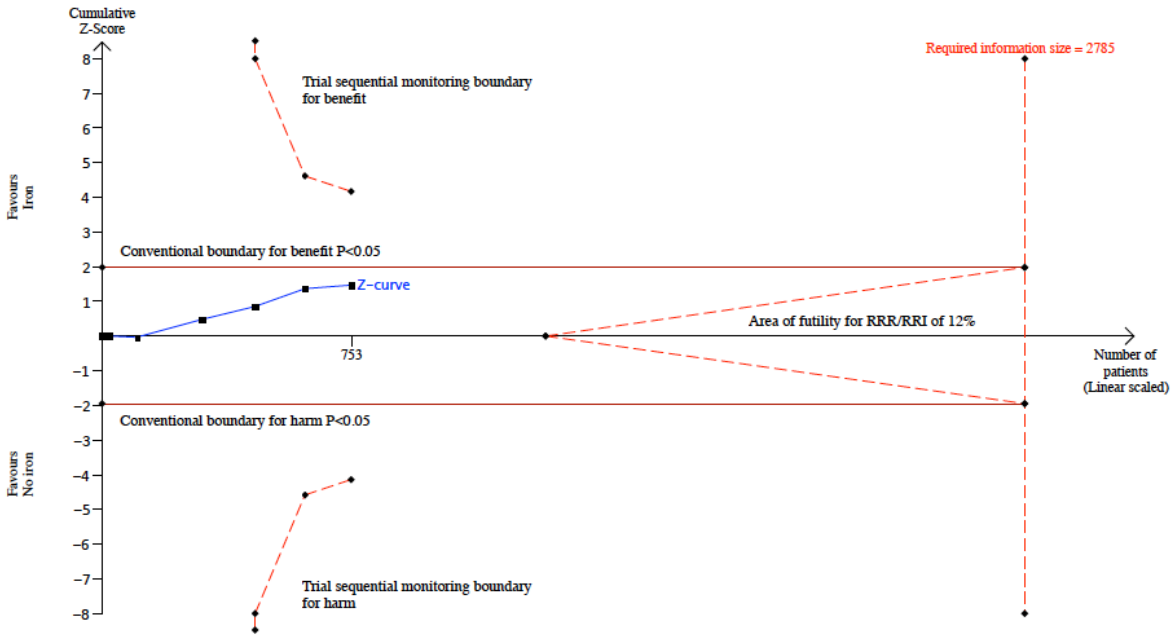
Certainty assessment							Summary of findings				
No. of participants (studies) Follow-up	**Risk of bias**	**Inconsistency**	**Indirectness**	**Imprecision**	**Publication bias**	**Overall certainty of evidence**	**Study event rates (%)**		**Relative effect (95% CI)**	**Anticipated absolute effects**	
							With no iron	**With Iron (by any route)**		**Risk with no iron**	**Risk difference with Iron (by any route)**
Blood transfusion											
753 (6 RCTs)	serious [a]	not serious	not serious	serious [b]	none	⨁⨁◯◯ LOW	187/352 (53.1%)	183/401 (45.6%)	RR 0.91 (0.80 to 1.04)	531 per 1,000	**48 fewer per 1,000** (106 fewer to 21 more)
Mean number of RBC units received											
323 (3 RCTs)	serious [c]	not serious	not serious	serious [b]	none	⨁⨁◯◯ LOW	134	189	-	The mean number of RBC units received ranged from **-0.01 to 2.06** mean (log no. of RBC units)	MD **0.3 mean (log no. of RBC units) lower** (0.67 lower to 0.08 higher)

Certainty assessment							Summary of findings				
Mean Hb (g/dl) at short-term follow-up											
242 (3 RCTs)	serious [c]	serious [d]	not serious	serious [b]	none	⊕◯◯◯ VERY LOW	95	147	-	The mean Hb (g/dl) at short-term follow-up ranged from **9.46 - 12.8** g/dl	MD **0.25 g/dl lower** (0.79 lower to 0.28 higher)
Mean Hb (g/dl) at mid-term follow-up											
378 (4 RCTs)	serious [c]	not serious	not serious	not serious	none	⊕⊕⊕◯ MODERATE	162	216	-	The mean Hb (g/dl) at mid-term follow-up ranged from **10.15 - 11.87** g/dl	MD **0.31 g/dl higher** (0.04 higher to 0.59 higher)
In-hospital infection											
487 (3 RCTs)	not serious	not serious	serious [e]	serious [d]	none	⊕⊕◯◯ LOW	116/245 (47.3%)	111/242 (45.9%)	RR 0.95 (0.76 to 1.19)	473 per 1,000	**24 fewer per 1,000** (114 fewer to 90 more)

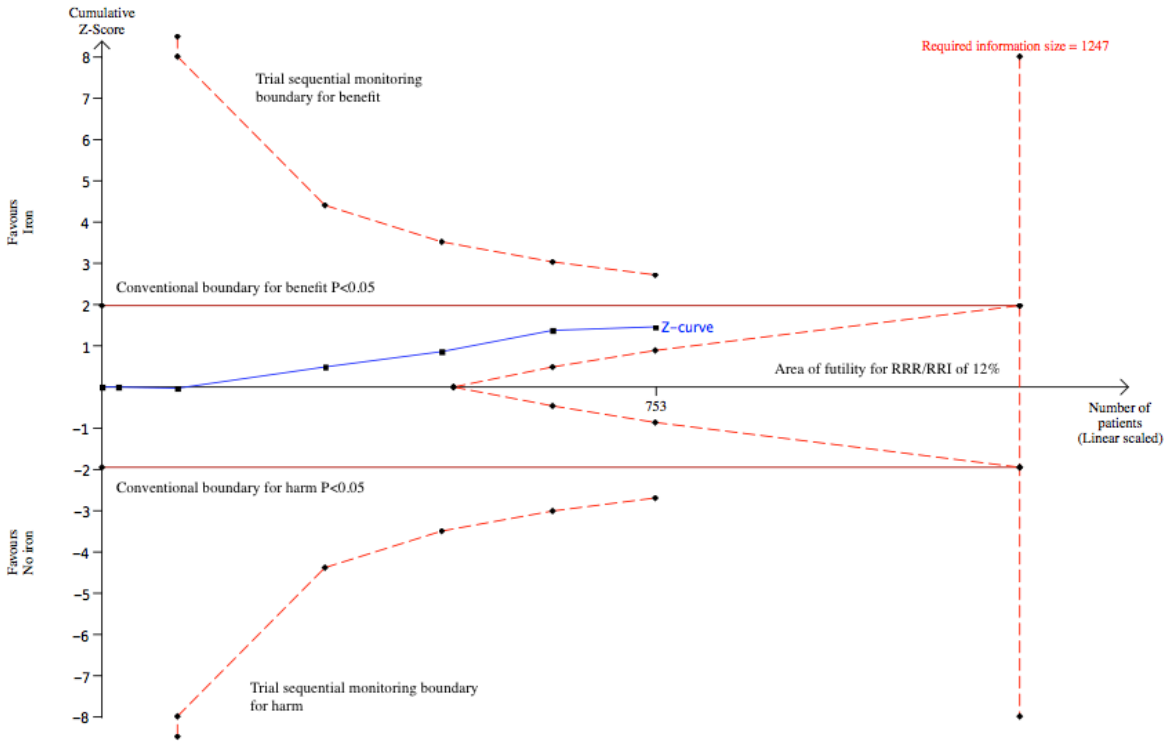
CI, Confidence interval; Hb, Haemoglobin; MD, Mean difference; RBC, Red blood cell; RCT, Randomised controlled trial; RR, Relative risk

a. Downgraded one level as 3 studies were at high risk of incomplete reporting; b. Rated down for imprecision as optimal information size not reached; c. Downgraded one level was 1 study was at high risk of reporting bias and 1 study was at unclear risk across multiple domains; d. Downgrade by one level due to wide variation in confidence intervals; e. Downgraded by one due to differences in outcome reporting

(a) All trials

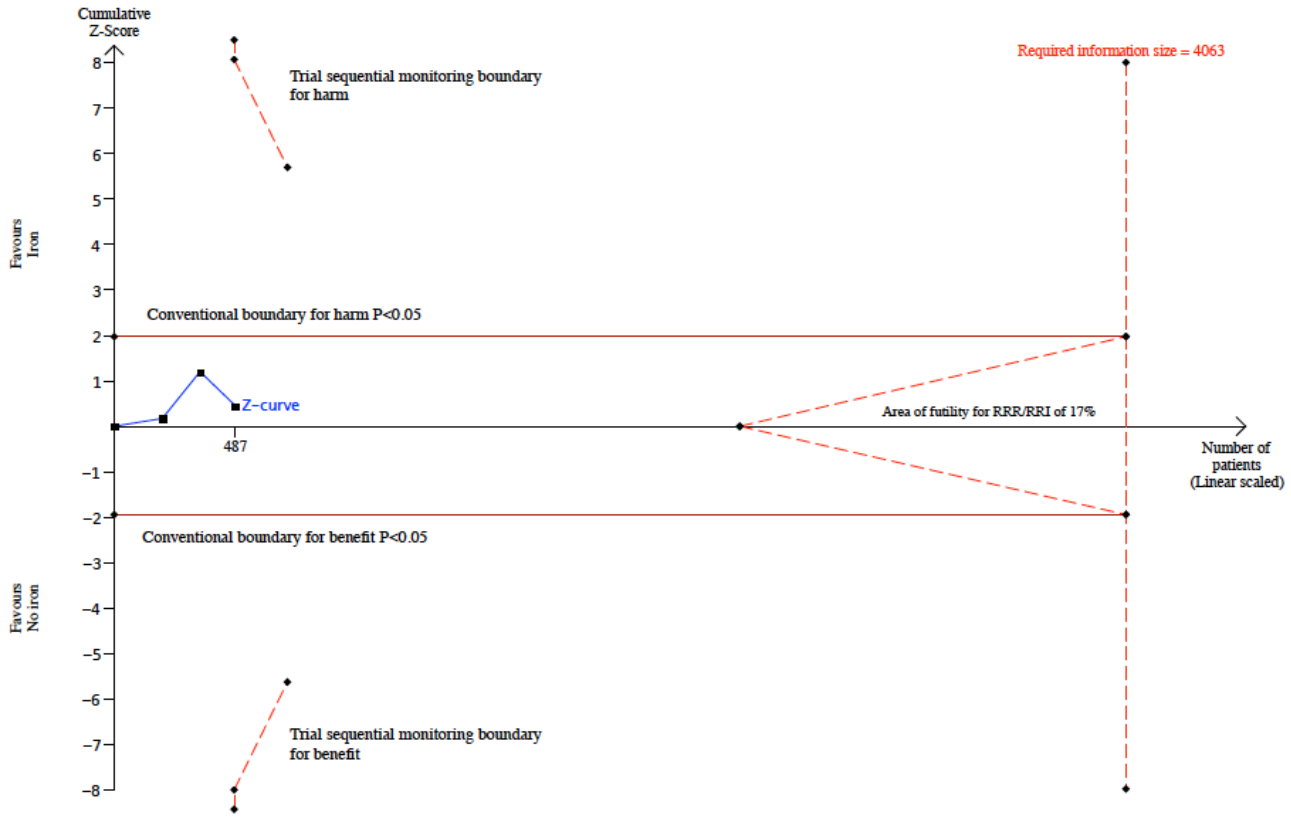


(b) Low risk of bias



RRI, relative risk increase; RRR, relative risk reduction

Figure 2.7 Trial sequential analysis of (a) all trials and (b) of two trials with overall low risk of bias of the effect of iron (by any route) on requiring RBC transfusion. The accrued sample size is significantly lower than the required information size in both scenarios.



RRI, relative risk increase; RRR, relative risk reduction

Figure 2.8 Trial sequential analysis of all trials of the effect of iron (by any route) on the risk of developing infection. The accrued sample size is significantly lower than the required information size.

2.4 Discussion

2.4.1 Summary of key results

This systematic review identified six RCTs with 805 participants where the efficacy and safety of iron therapy in critically ill patients was assessed. The main findings of this systematic review are:

- There was no evidence of an effect of iron on transfusion requirements, mortality or risk of infection
- The route of administration, in particular intravenous administration, did not appear to influence the risk of requiring a RBC transfusion
- Iron therapy, even when given to critically ill patients in the acute of phase of illness, results in a modest improvement in Hb at longer follow-up time points.

Critically ill patients display the hallmarks of the anaemia of inflammation as described in Chapter 1 (192). Iron restriction is predominantly driven by the effects of hepcidin and hepcidin expression upregulated by inflammation (47). Hepcidin also blocks absorption of iron from the gastrointestinal tract. These factors may, in part, contribute to the lack of efficacy of iron preparations, especially oral iron. Intravenous iron therapy may be indicated when rapid replenishment of iron stores is required or when oral iron therapy is unlikely to be efficacious (66). However, the peak effect of intravenous iron may not be observed for two to four weeks following administration (135). Data from the included RCTs in this review (170) and large observational studies have shown that the majority of transfusion episodes occur in the first 7 days of ICU admission (19). Therefore, it is unlikely that iron therapy, even if given intravenously, will work rapidly enough to affect transfusion requirements in critically ill patients.

My findings are also consistent with a systematic review of postoperative iron therapy following major elective surgery which also found that intravenous iron did not reduce transfusion requirements. Surgery also triggers an inflammatory process resulting in a state of functional iron deficiency and/or iron sequestration. In addition, blood loss may also be more common in surgical patients and they may require transfusion early. The majority of participants included in my systematic review were from surgical ICUs and the pre-randomisation iron profiles of participants randomly allocated to receive intravenous iron from the most

recent RCT in my review (190) are broadly similar to those of a large RCT of postoperative iron following major surgery (148) (**Table 2.3)**.

Table 2.3 Comparison of participant iron profiles between intravenous iron arms of two RCTs

	Litton et al. (n = 70)	**Khalafallah et al. (n = 103)**
Setting	Intensive care unit	Elective surgery (postoperative)
Haemoglobin (g/dl)	89 (81 – 94)	109 (99 – 120)
Ferritin (ng/ml)	317 (218)	1370 (108 – 284)
Tsat (%)	13 (10)	10 (7 – 15)
CRP (mg/l)	111 (83)	85 (17 – 150)

Data are median (IQR) or mean (SD).

Iron therapy was associated with an increase in Hb at longer follow-up periods. Unfortunately, the data do not allow for the potentially beneficial clinical consequences of a rise in Hb to be addressed. Persistence of anaemia beyond ICU discharge and its possible association with long-term morbidity and mortality is now increasingly being recognised as discussed in Chapter 1 (26, 27, 45). This is important because any therapeutic effect of iron may be missed in studies focusing on short-term outcomes such as transfusion.

Observational studies have shown that hepcidin levels are markedly increased on ICU admission but reduce significantly over time and this reduction is associated with resolution of inflammation as defined by decreased IL-6 and CRP concentrations (71). Mouse models of ICU anaemia have also shown an improvement in Hb when treatment is delayed (days 7 and 8 after illness onset) when compared with early treatment (days 1 and 2) (178). Therefore, it is plausible to consider administering iron during the latter / recovery phases of critical illness when inflammation is regressing. In non-critically ill patients, improvements in Hb have translated into meaningful improvements in fatigue and overall well-being (193, 194). No studies in this systematic review provided data on HRQoL outcomes or on fatigue – a cardinal symptom of anaemia and one which is becoming increasingly recognised as being the most distressing to ICU

survivors (195).

The risk of infection associated with iron therapy is an important concern in critically ill patients. The prevalence of healthcare-associated infections (HAIs) in the UK is thought to 6.4%, of which the three commonest types are respiratory tract infections (22.8%), urinary tract infections (17.2%) and surgical site infections (15.7%) (196). Estimates of HAIs in critically ill patients range from 14 – 30% (183, 197, 198). Intravenous iron can lead to increased levels of circulating free iron, which has been associated with impaired immunity and organ dysfunction in pre-clinical studies (134, 199). Data from clinical trials are conflicting with one systematic review (139) showing an increased risk of infection with intravenous iron when compared with oral iron (RR (95% CI) 1.34 (1.10 to 1.64), 24 RCTs, 4400 participants), whilst another more recent review demonstrated no increased risk, although the point estimate favoured infection (RR (95% CI) 1.17 (0.83 to 1.65) (168). Important considerations when interpreting these infection data are that infection is often not a prespecified outcome measure, many of the included trials were small and data were not consistently collected using standardised definitions. This review on critically ill patients found no evidence of an increased risk of infection but this should be interpreted with caution for these reasons. I explore these issues in greater detail in Chapter 3. Other adverse events were rare and events of such as anaphylaxis, hypersensitivity reactions, and extravasation were not reported.

The TSA results suggest that the required sample size to definitively answer the question of 'does iron reduce transfusion requirements in critically ill patients?' has not been met. Litton et al., in their supplementary material, provided a power calculation suggesting that 1572 participants would be required to detect a 0.5 unit decrease in RBC transfusion requirements. A projected sample size of approximately 8 times the number of participants included in this review would have been required to answer this question definitively. Putting it all together, I suggest against caution in using iron therapy, by any route, in critically ill patients with anaemia as a means to reducing RBC transfusion. In the absence of a clear benefit, the additional resources and financial costs of administering iron to all critically ill anaemic patients are also likely to be significant. It is likely that the relative risks and benefits of intravenous iron depend on the timing of administration (**Figure**

2.9). A wider therapeutic window may occur during the recovery phase of critical illness, when the benefits may outweigh the risks, and patient-centred outcomes can be prioritised.

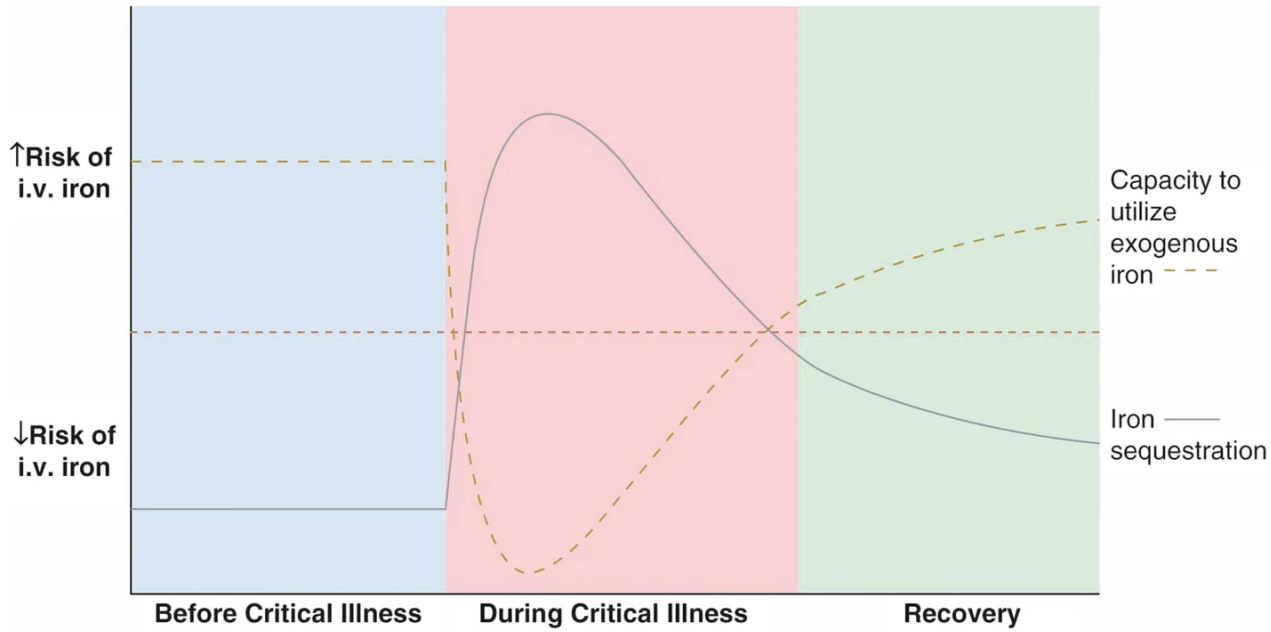


Figure 2.9 Temporal changes in iron status and response to intravenous iron. During critical illness, the capacity to utilise exogenous iron is likely to be impaired by inflammation. As inflammation resolves, the recovery phase may be a more suitable time point for intravenous iron administration. (Adapted with permission from Litton et al *Critical Care* 2019; 23: 18)

This is in keeping with guidance from ESICM that '*suggest against the routine use of iron therapy (oral or intravenous) in critically ill patients with anaemia'* (200). This was a conditional recommendation based on low certainty of evidence. The guidelines also recommended that future research should focus on the identification of patients most likely to develop an erythropoietic response to iron therapy and that future trials should focus on patient-centred and functional outcomes, with adequate long-term follow-up.

2.4.2 Strengths and limitations

The strength of the review is the strict methodological process; I followed Cochrane Collaboration and PRISMA recommendations, performed a comprehensive search (with the assistance of CD) and carried out duplicate data extraction and risk of bias assessments. Compared to the previous review, I used TSA to

increase the reliability of the meta-analysis and provide required sample sizes. A potential caveat to this would be our estimation of a 30% baseline risk of developing infection. This was based on a study published 12 years ago and it is reasonable to assume this risk will have reduced over time. A reduction in the baseline risk, assuming a 17% RRI remains clinically relevant, would influence the sample sizes calculated. I also performed GRADE evaluations on the quality of evidence for important efficacy and safety outcomes. The limitations to the conclusions of this systematic review can be attributed to the clinical and methodological differences between the trials. Outcomes and follow-up time points for certain outcomes, e.g. haemoglobin measurements, were not standardised across trials thereby limiting meta-analysis. The *a priori* anticipated intervention effects could be considered as being too conservative and therefore lead to larger than required information sizes – a recognised limitation of TSA (182).

2.5 Conclusion

The potential impact of iron therapy on RBC transfusion requirements in critically ill patients remains inconclusive. I consider there to be little value in conducting more trials targeted towards reducing RBC transfusion. Iron therapy is unlikely to work rapidly enough to reduce transfusion, most of which occurs early in critical illness. The finding of an improvement in Hb at follow-up periods of >10 days warrants further research, as there may be clinical benefits of improved functional recovery in a patient cohort that has a substantial burden of poor HRQoL after intensive care. The overall quality of evidence ranged from very low to moderate.

In view of these results and the literature review undertaken in Chapter 1, I suggest the following for future research and clinical trial design:

- Intravenous therapy may be more effective when given to patients recovering from critical illness at the point of discharge from ICU or whilst convalescing on the hospital ward
- There should a shift towards measuring patient-centred outcomes such as fatigue and HRQoL
- Newer markers of iron status (e.g. hepcidin) may identify patients who are more likely to develop an erythropoietic response

- Blinding of intravenous iron is possible but challenging and likely to be associated with additional trial costs. The decision to blind will depend on the choice of outcome measures but a recent meta-epidemiology study found that blinding may not be as important as residual confounding or imprecision (201).
- Data on infection should be collected in a standardised manner adhering to recognising definitions.

Chapter 3 Can we be certain of the effect of iron therapy on the risk of infection?

Sections of this chapter have, in part, been published in:

Shah A, Sugavanam A, Reid J, et al. Risk of infection associated with intravenous iron preparations: a protocol for updating a systematic review. *BMJ Open* 2019; 9: e024618

3.1 Background

3.1.1 Description of the condition

Intravenous iron is recommended for the treatment of iron deficiency, with or without anaemia, in many common conditions including CKD and heart failure. Over the past decade, the indications for intravenous iron have expanded into other clinical settings where rapid replenishment of iron stores is required or where oral iron may not be absorbed (e.g. inflammation) (17, 202-206). This may, in part, be driven by newer preparations being reported to be safer with regards to risks such as anaphylaxis when compared with older dextran-based formations (151). However, whether intravenous iron is associated with other harms, in particular the risk of infection, remains uncertain (139, 168).

3.1.2 Pre-clinical evidence of the association between iron availability and infection

As discussed in Chapter 1, iron is required for growth of almost all human pathogens and therefore there is fierce competition between the host's iron withholding mechanisms and invading pathogens. Intravenous iron administration can lead to increased levels of circulating free iron, which has been associated with impaired T-cell and neutrophil function, promotion of pathogen growth, lung injury and worsening of shock in *in vitro* and *in vivo* studies involving animals and humans (134, 199). However, the effect of exogenous iron on the virulence of pathogens may vary by subtype or strain. One example is the difference between intracellular and extracellular pathogens. Iron-withholding mechanisms that confer protection against extracellular bacteria (e.g. *Staphylococcus aureus*) may increase the virulence of intracellular pathogens (e.g. *Salmonella typhimurium*), which preferentially infect macrophages (207). Conversely, elevated concentrations of circulating non-transferrin bound iron increase the risk of infection from extracellular pathogens such as

S.aureus (134) and gram negative bacteria such as *Escherichia coli* and *Klebsiella pneumoniae* (208, 209).

Certain bacteria have also evolved to obtain iron through mechanisms such as secreting siderophores. These are small molecules which bind iron with higher affinity than transferrin, and thereby extract iron from the host (210). There are now more than 500 recognised bacterial siderophores (61). *Staphylococcus aureus*, in response to hypoferraemia, can increase the expression of several dedicated iron acquisition genes and synthesise two important siderophores - staphyloferrin A and staphyloferrin B, both of which are able to avoid detection by the host (211).

Compared with bacteria, relatively little is known about the interactions between iron homeostasis and common viral infections such as influenza, and whether iron therapy increases pathogenicity. Iron accumulation is associated with worse clinical outcomes in patients with infected with hepatitis C and Human Immunodeficiency Virus (212). In patients with hepatitis C, commencement of antiviral therapy was associated with increased expression of hepcidin. The resulting hypoferraemia was greatest in those with the significant decreases in viral load (213).

3.1.3 Why is it important to do this review?

Previous systematic reviews have demonstrated the efficacy of intravenous iron for treating anaemia and reducing RBC transfusion requirements, but safety data on the risk of infection are conflicting (139, 168). Recent trials have questioned the benefit of intravenous iron in patients undergoing major surgery (214).

Healthcare-associated infections are a common and important cause of morbidity and the prevalence in the UK is thought to be 6.4%. The three commonest types of infections are respiratory, urinary tract and surgical site infections. The organisms frequently implicated are *Staphylococcus aureus, Staphylococcus epidermidis* and *Escherichia coli*, which as described above, are iron-dependent pathogens. This coupled with evidence of increasing use of intravenous iron across various settings with its associated costs (215-217) raises concerns about whether it is being used appropriately. Indiscriminate use of iron supplementation has been shown to result in increased morbidity and mortality from serious infections in areas with a pre-existing high endemic

rate of infection (218, 219).

A recent systematic review of 35 international guidelines on the management of iron deficiency anaemia using intravenous iron across multiple indications found that approximately 60% of the guidelines have not been updated in more than 5 years and consequently do not reflect the current evidence on the safety and efficacy of intravenous iron (220). In view of these uncertainties, I aimed to primarily evaluate the safety of intravenous iron, focusing on the risk of infection.

3.2 Methods

This systematic review was conducted according to a predefined study protocol registered on PROSPERO (CRD42018096023) which also been published (221). I followed recommendations by the Cochrane Collaboration (173), PRISMA statement (174) and the GRADE guidelines (172).

3.2.1 Eligibility criteria

For this systematic review, I included RCTs comparing intravenous iron with either oral iron or no iron/placebo from 1966. I included studies examining all patient populations. I also decided to include non-randomised studies (NRS). The reasons for this were twofold. First, infection is an adverse event and therefore may not always be reported as an outcome measure in RCTs and if it is, the available evidence may be indirect or incomplete. Second, the findings of infection outcomes reported in NRS may be useful to inform the design of a subsequent RCT, for example, through identification of relevant subgroups or definitions of infection used.

I followed Cochrane recommendations in developing criteria for including NRS and sought to limit NRS to those that would provide reasonably valid effect estimates, subject to assessment of risk of bias. I undertook a confirmatory approach and pre-specified infection as my outcome of interest. Therefore, I included NRS that met all of the following criteria: (i) report data on infection; (ii) presence of at least two comparable groups; (iii) quasi-RCTs; (iv) published since 1st January 2007. This date was chosen as this was the year that newer

iron preparations (e.g. ferric carboxymaltose, iron isomaltoside) received or renewed their marketing authorisation. Therefore, any data extracted from these studies is likely to be reflective of current practice.

3.2.2 Outcomes

The primary outcome was the risk of infection. I also aimed to characterise how infection was defined and reported in all included studies.

Secondary outcomes were:

- Differences in Hb at study endpoint or treatment response, as defined by the study authors (e.g. proportion of patients with Hb rise of >20 g/l, achieved a target Hb or were non-anaemic at the end of the study)
- RBC transfusion requirements
- Hospital LOS
- Mortality: short term (<30 days) and long term (≥ 30 days) mortality.

3.2.3 Search strategy

The following databases were searched: CENTRAL (Cochrane Central Register of Controlled Trials) in The Cochrane Library, MEDLINE, Embase, CINAHL, PubMed (e-publications ahead of print only), Web of Science (1990 onwards) and the Transfusion Evidence Library (1950 onwards). Ongoing trials were sought from trial registration websites. Reference lists of relevant systematic and narrative reviews were also screened. The first search date was 2nd May 2018 and an updated search was undertaken on 29th September 2020. The full search strategy is available in the Appendix 2**.** I augmented database searching by hand-searching reference lists of all included studies and relevant review articles.

3.2.4 Study selection and data extraction

Literature searches were uploaded to Covidence, a web-based software platform, where two reviewers independently screened studies' titles and abstracts for eligibility. The same two reviewers then independently

screened the full text reports for inclusion. Disagreements were resolved through discussion.

Data were extracted onto standardised and pre-piloted forms for RCTs and NRS by two reviewers independently. The following data, if reported, on the outcome of infection were extracted: (i) definitions of infection used; (ii) anatomical site of infection; (iii) positive microbiology culture data; and (iv) antibiotic usage. Disagreements were resolved through discussion or referred to a third reviewer. I sought unclear or missing data by contacting the authors of the individual trials and allowing them 28 days to respond.

3.2.5 Risk of bias assessment and grading the quality of evidence

Risk of bias for RCTs was assessed using the Cochrane Risk of Bias tool (175). Due to the potential financial and / or logistical difficulties in blinding intravenous iron preparations because of their rusty brown appearance, we made an a priori decision to classify open-label designs as low risk for detection bias for objective outcomes such as changes in Hb, mortality and LOS in the absence of other concerns. For analysis and presentation purposes, a three-point scale ('low risk', 'some concerns', 'high risk') was used to determine overall risk of bias for each study. All assessments were performed in duplicate by two independent reviewers.

Publication bias, whereby negative results of RCTs are less likely to be published than positive results, remains a significant threat to the validity of systematic reviews evaluating adverse events (222). Efforts to mitigate against this include efforts to develop core outcomes sets and to promote transparency in reporting, through registration of studies on trial registers and encouraging to adhere to reporting standards such as CONSORT. Despite this, a recent systematic review found strong evidence of higher numbers and ranges of adverse events in unpublished than in published versions of the same study, and that the inclusion of unpublished data may increase the precision of pooled estimates (223).

The existence of publication bias can be examined graphically by the use of 'funnel' plots. These plots are based on the assumption that the precision in the estimate of the underlying treatment effect will increase as the sample size of included studies increases. Effect estimates from small studies will scatter more widely at

the bottom of the plot, with the spread narrowing amongst larger studies. In the absence of publication bias, a plot will visually resemble a symmetrical inverted funnel. Asymmetry will indicate publication bias but another important cause for asymmetry is over-estimation of treatment effects in smaller studies of lower methodological quality. Therefore, I deemed it important to assess for publication bias on the risk of infection with a funnel plot if >10 RCTs were available, plotting the OR for the proportion that develop infection against the standard error (SE) of the log OR. Where possible, I also actively sought to obtain unpublished data from trial authors.

Factors that affect the validity of findings from NRS when compared with RCTs include the presence of confounding in the absence of randomisation, issues in the analysis of routinely collected data and less consistent development of a protocol prior to the study starting. Evidence suggests that assessing risk of bias in NRS has not always been performed or performed well. A descriptive analysis of systematic reviews on adverse effects found that less than half (106/256 (41.4%)) of reviews assessed the quality of the studies that were included. Forty-nine of the 106 reviewers used existing quality assessment instruments, mostly to assess RCTs, but assessment of NRS was suboptimal. Therefore, I chose to assess risk of bias for NRS was assessed using the I-ROBINS tool developed by the Cochrane Bias Methods Group (224). The seven bias domains covered by this tool are:

Pre-intervention

- Bias due to confounding: when one or more prognostic variables also predicts intervention received at baseline
- Selection bias: when exclusion of some eligible participants is related to both the intervention or outcome (e.g. inclusion of prevalent users, rather than new users, of an intervention)

At intervention

- Bias in classification of intervention (information bias): introduced by differential or non-differential misclassification of intervention status.

Post-intervention

- Bias due to deviations from intended interventions (confounding): arises from systematic differences in the care between intervention and comparator groups
- Bias due to missing data (selection bias): differential loss to follow-up that is affected by prognostic factors
- Bias in outcome measurement (information bias): arises when outcome assessors are aware of intervention status or if different methods are used to assess outcomes in different intervention groups
- Bias in selection of reported result (reporting bias): selective reporting of results across multiple measurements of the outcome, analyses or subgroups in a way that depends on the findings

The GRADE (172) approach, which I described in Chapter 2, was used to assess the overall quality of evidence for the primary outcome of infection. In keeping with the Methodological Expectations of Cochrane Intervention Reviews, another author also independently assessed the certainty of the evidence and disagreements were solved through discussion.

3.2.6 Data synthesis

I analysed and reported data from RCTs and NRS separately. The primary endpoint was the proportion of patients who developed an infection. A narrative synthesis was performed to characterise the definitions of infection used, reporting of infection sources and pathogens, and antibiotic use.

Dichotomous outcomes (infection, mortality, requirement for RBC transfusion) were reported as RR with corresponding 95% CIs. For continuous measures, I reported MD with 95% CI. Data from RCTs fulfilling eligibility were pooled for meta-analysis with a random effects model. Statistical heterogeneity was measured using the I^2 statistic and I^2 >50% was considered as showing substantial heterogeneity (173). For NRS, I reported results on infection descriptively instead of pooling results due to heterogeneity in clinical conditions, study designs and differences in adjusting for confounders (224). This is in keeping with Cochrane recommendations. Statistical analyses were conducted on RevMan V5.3 and STATA (V14, Statacorp LP,

College Station, Texas, USA). Two-sided p values <0.05 were considered as statistically significant.

3.2.7 Subgroup, sensitivity and meta-regression analyses

The risk of developing an infection due to iron therapy is likely to be context-specific depending on the patient's pre-existing iron status, exposure to potential infections and genetic background, and type and cumulative dose of iron preparations.

If sufficient data were available, I planned a priori to perform subgroup analyses on the following: (i) clinical settings (surgery, obstetrics, critical illness, CKD, IBD, heart failure, paediatrics); (ii) iron profiles at enrolment; (iii) mode of administration (single dose, multiple doses); (iv) iron preparation used and cumulative dose of iron. I performed a sensitivity analysis for infection by only including trials that were judged to be at overall low risk of bias.

I also decided to perform meta-regression analyses to investigate the effect of baseline iron status (Hb, ferritin, Tsat) on the risk of developing infection. This would depend on the whether these variables were reported adequately in the included RCTs and if sufficient RCTs are available. Cochrane guidelines recommend against meta-regression when there are fewer than ten studies in a meta-analysis.

Meta-regression is similar to simple regression where an outcome variable is associated with the values of one or more explanatory variables. In the context of a meta-analysis, meta-regression can be considered as an extension to convention subgroup analyses that allow the effects of explanatory variables or effect modifiers be investigated on a categorical or continuous outcome. However, larger studies have more influence on the relationship than smaller studies. I performed a random-effects meta-regression to allow for residual heterogeneity amongst the intervention effect that may not be explained by the explanatory variables.

3.3 Results

3.3.1 Search results

The database search identified 7655 records and 311 full-text articles were assessed for exclusion by screening of titles, duplicates and abstracts **(Figure 3.1)**. Details on all included studies and 33 ongoing trials are displayed in Appendix 3 and 4, respectively.

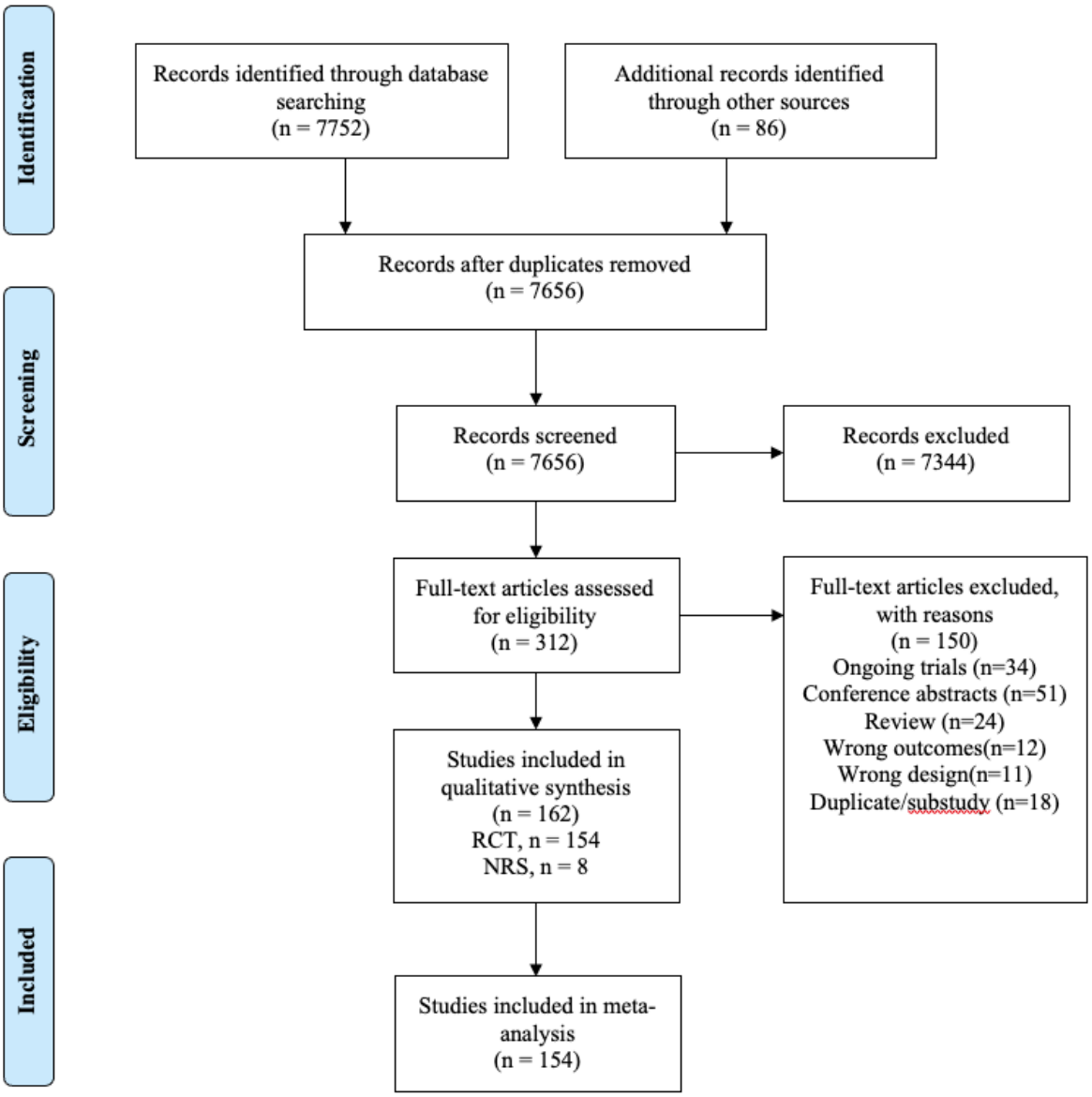


Figure 3.1 PRISMA flow diagram

3.3.2 Characteristics of included studies

A total of 162 studies including 40,066 participants were included in this systematic review, of which 154 were RCTs (32,920 participants) (123, 146-150, 170, 184, 185, 187, 188, 225-365) and 8 were NRS (7146 participants) (366-373).

Randomised controlled trials

The number of participants in the included RCTs ranged from fourteen to 2534. Seventy-four RCTs were multicentre and studies were carried out in a wide range of clinical specialties **(Table 3.1)**. The commonest settings were obstetrics (40 RCTs; 9993 participants), surgery (27 RCTs; 4223 participants) and CKD (22 RCTs; 6013 participants). Trials were conducted across all continents with the most common location being Europe (n = 56). The most common iron preparations evaluated were iron sucrose (n = 68) and ferric carboxymaltose (n = 51).

Dosing schedules varied between studies. Thirty-eight RCTs used either the Ganzoni formula (374) or a simplified method to calculate the required iron dose. Fifty RCTs used a single dose schedule with the commonest preparation being ferric carboxymaltose, with doses ranging from 500mg to 2000mg.

With regards to study outcomes, 102 RCTs reported a change in haemoglobin as their primary outcome. Definitions varied and included change in haemoglobin at the end of the study period, rise in haemoglobin by 10 or 20 g/l, proportion who achieved a particular target Hb and/or who remained non-anaemic at the end of the study period. The number of studies evaluating other outcomes as their primary outcome are displayed in **Table 3.2**.

Table 3.1 Summary characteristics of included randomised controlled trials (n = 154)

Characteristic	Intravenous iron vs. oral iron, *n*	Intravenous iron vs. no iron, *n*	Total
Single centre	51	30	81
Multicentre	37	36	73
Geographical location			
Australasia	5	4	9
Europe	25	31	56
Asia	28	14	42
Africa	4	0	4
North America	17	16	33
South America	2	1	3
Cross-continent	6	1	7
Clinical setting			
Blood donors	1	2	3
CCF	1	8	9
CKD	16	6	22
Critical care	-	5	5
Elite athletes	-	2	2
IBD	4	2	6
Mixed / general medical	2	7	9
Menorrhagia	1	2	3
Obstetrics	39	0	39
Oncology			
- Haematology	-	3	3
- Non-haematology	8	5	13
Paediatric	3	1	4
Restless legs	-	7	7
Surgery	10	17	27
Gastrointestinal bleeding	-	2	2
Inclusion criteria			
Iron deficiency anaemia	28	9	37
Functional iron deficiency	20	14	34
Hemoglobin only	31	25	56
Iron deficiency only	3	7	10
Non-anaemic	4	2	6
Unclear	2	9	11
Iron preparations evaluated			
Ferric carboxymaltose	21	30	51
Iron sucrose	43	25	68
Iron dextran	8	4	12
Iron isomaltoside	4	3	7
Iron polymaltose	3	-	3
Iron saccharate	2	-	2
Ferumoxytol	2	1	3
Ferric gluconate	3	4	7
Not reported	1	-	1

CCF, congestive cardiac failure; CKD, chronic kidney disease; IBD, inflammatory bowel disease

Table 3.2 Summary of primary outcomes measured in included trials (n = 154)

Primary outcome	n (%)
Haemoglobin-related	95 (61.7)
Red blood cell transfusion	14 (9.0)
Other efficacy (e.g. change in oxygen consumption)	11 (7.1)
Patient reported outcome	18 (11.8)
Safety/dosing-related	9 (5.9)
Unclear	6 (3.9)
Feasibility	1 (0.6)

Non-randomised studies

The number of participants in the included NRS ranged from 39 to 2547. The clinical settings included surgery (n = 5), CKD (n = 3) and digestive diseases (n=1). Three studies evaluated single dose infusions of ferric carboxymaltose, four studies evaluated iron sucrose or ferric gluconate given as multiple doses and one study was unrestricted evaluating any iron preparation with regards to type and dosing frequency (**Table 3.3).**

3.3.3 Risk of bias assessments

Overall risk of bias was low for 26 RCTs and high for 106 RCTs. Twenty-two RCTs had some concerns for bias. The overall high risk of bias was accounted for by most studies due to insufficient blinding of participants, study personnel or outcome assessors, or not providing sufficient information for a decision. More than half of included RCTs were also at high or unclear risk of bias for allocation concealment. An overall summary and assessments for individual domains for each included RCT are shown in **Figure 3.2** and **Appendix 5**, respectively. Visual inspection of funnel plots for publication bias did not demonstrate any asymmetry and therefore I do not consider there to be evidence of publication bias **(Figure 3.3).** However, visual assessments of funnel plots should be interpreted with caution. Apparently asymmetric funnel plots frequently occur by chance when there is no underlying asymmetry, unless the number of studies in a meta-analysis is large. Formal tests, such as Egger's test, are recommended and although they have appropriate type 1 error, they often lack statistical power (173).

Overall risk of bias was moderate for seven NRS and only one study was deemed to be at overall low risk of bias **(Appendix 6)**. All studies were at serious or moderate risk of bias due to confounding, mainly due to the absence of or lack of controlling for important confounders. Risk of bias in measurement of outcomes was moderate across all studies due to outcome assessors either being aware of the intervention status or insufficient information was available to make a decision.

Table 3.3 Characteristics of included non-randomised studies (n = 8)

Study ID	Study design	Participants	Exposure	Comparator	Key findings
Ikuta et al,(366) 2019	Prospective cohort study Multicentre (n = 12) (Japan)	Iron deficiency anaemia (Hb <12 g/dl in women and <13 g/dl in men, ferritin <12 ng/ml) secondary to digestive diseases n = 39	Ferric carboxymaltose 1000mg or 1500mg depending on weight	N/A	10/39 (25.6%) experienced an 'infection or infestation' MedDRA coding definition
Ishida et al,(367) 2015	Retrospective observational registry-based cohort Multicentre (USA)	CKD patients who had received IV iron in the 14 days preceding their first hospitalisation n = 2463	Receipt of IV iron of any dose, frequency, or type	No IV iron received	IV iron was not associated with hospital admission for infection or death within 30 days (OR (95% CI) 1.08 (0.96 to 1.22)) ICD-9 Classification used to define infection
Kim et al,(375) 2018	Retrospective propensity-matched cohort study Single centre (South Korea)	Elective hip arthroplasty and hip fracture with postoperative anaemia (Hb <10 g/dl) n = 300	Ferric carboxymaltose 1000mg	Usual medical care	No statistically significant differences in postoperative infections between those who received iron compared with usual care (7/150 (4%) vs. 6/150 (3%), p>0.05) Investigator defined – 'positive bacterial culture and the need for antibiotics'
Kuragano et al,(369) 2014	Prospective observational study Single centre (Japan)	Stable CKD on maintenance dialysis for a period of 1 year n = 1086	IV iron, high dose (>50mg per week) or low dose (<50mg per week)	No iron	IV iron (both cohorts) associated with increased risk of infection when compared with no iron (HR (95% CI) 1.78 (2.24 to 12.14)). High ferritin levels also associated with increased risk of infection when compared with low ferritin (HR (95% CI) 1.76 (1.29 to 2.4)) No definition provided
Munoz et al, 2014 (1)	Retrospective study, data obtained from previous publications,	Underwent either hip fracture surgery, total knee replacement or total hip replacement n = 2547	IV iron, either iron sucrose (100-200mg, 3 doses) or ferric	Oral iron or no iron	Lower infection rates in patients who received IV iron (+/- EPO) when compared with control

	doctorate theses and unpublished databases Multicentre (n = 4) (Spain)		carboxymaltose (600mg single dose) +/- EPO		(107/1000 (10.7%) vs. 97/361 (26.9%), p<0.01) CDC definition used
Munoz et al,(370) 2014 (2)	Retrospective matched cohort study Single centre (Spain)	Lower limb arthroplasty n = 394	IV iron, either iron sucrose (100-200mg, 3 doses) or ferric carboxymaltose (600mg single dose)	Usual medical care	No statistically significant differences in postoperative infection between those who received iron compared with usual care (3/182 (1.6%) vs. 6/182 (3.3%), p=0.502) No definition provided
Peters et al,(371) 2008	Retrospective, matched cohort study Multicentre (n = 5) (Germany)	Elective / emergency to perioperative ICU with anaemia n = 95	Ferric carboxymaltose 500mg	Usual medical care	No statistically significant differences in postoperative infections between those who received iron compared with usual care (n=78) (6/26 (23%) vs. 14/52 (27%), p>0.05) No definition provided
Zitt et al,(372) 2014	Prospective, observational cohort study Single centre (Austria)	CKD scheduled to start chronic dialysis treatment n = 222	IV iron, ferric gluconate weekly	No iron	Iron therapy was associated with reduced cardiovascular and sepsis-related mortality (HR (95% CI) 1.76 (1.29 to 2.4)) No definition provided

CDC, Centres for Disease Control and Prevention; CI, Confidence interval, CKD, Chronic Kidney Disease; EPO, Erythropoietin; HR, Hazard ratio; ICU, Intensive care unit; IV, Intravenous; OR, Odds ratio; RR, Risk ratio

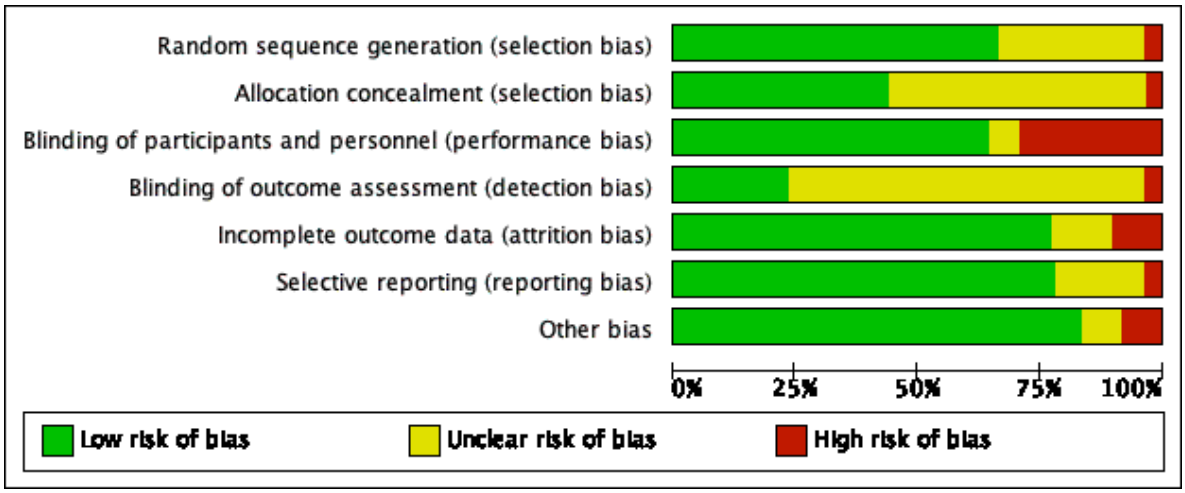
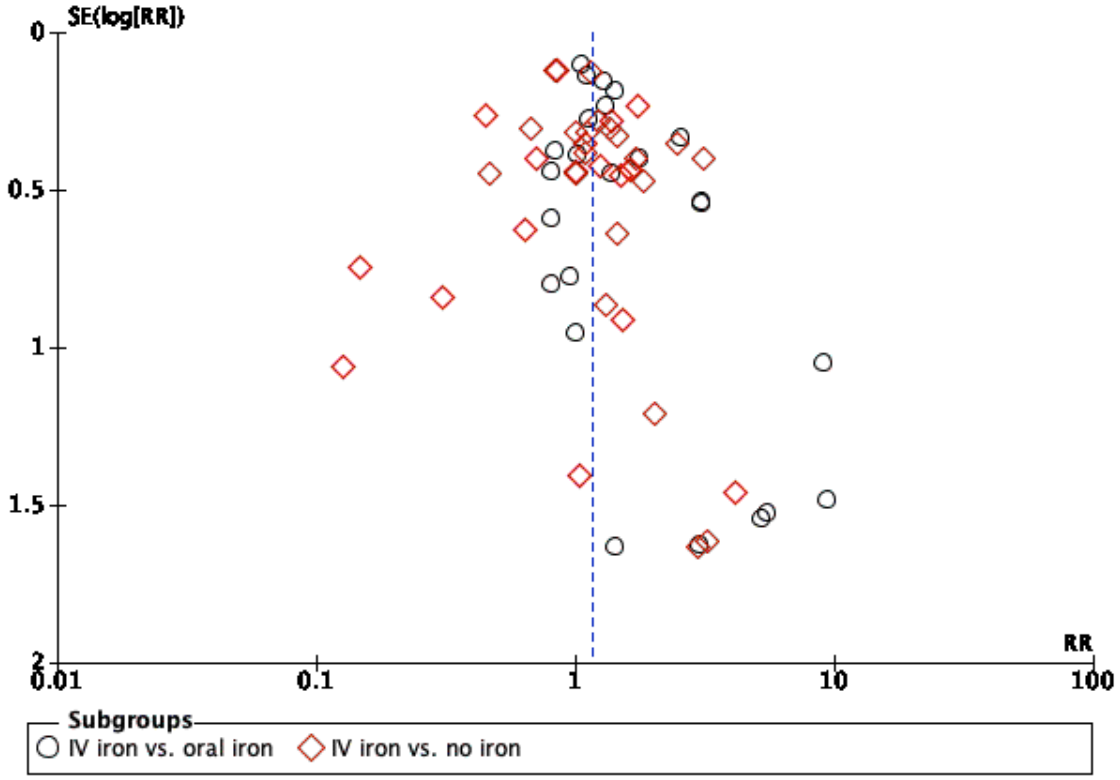


Figure 3.2 Risk of bias summary for included RCTs



IV, Intravenous; SE, Standard error; RR, Risk ratio

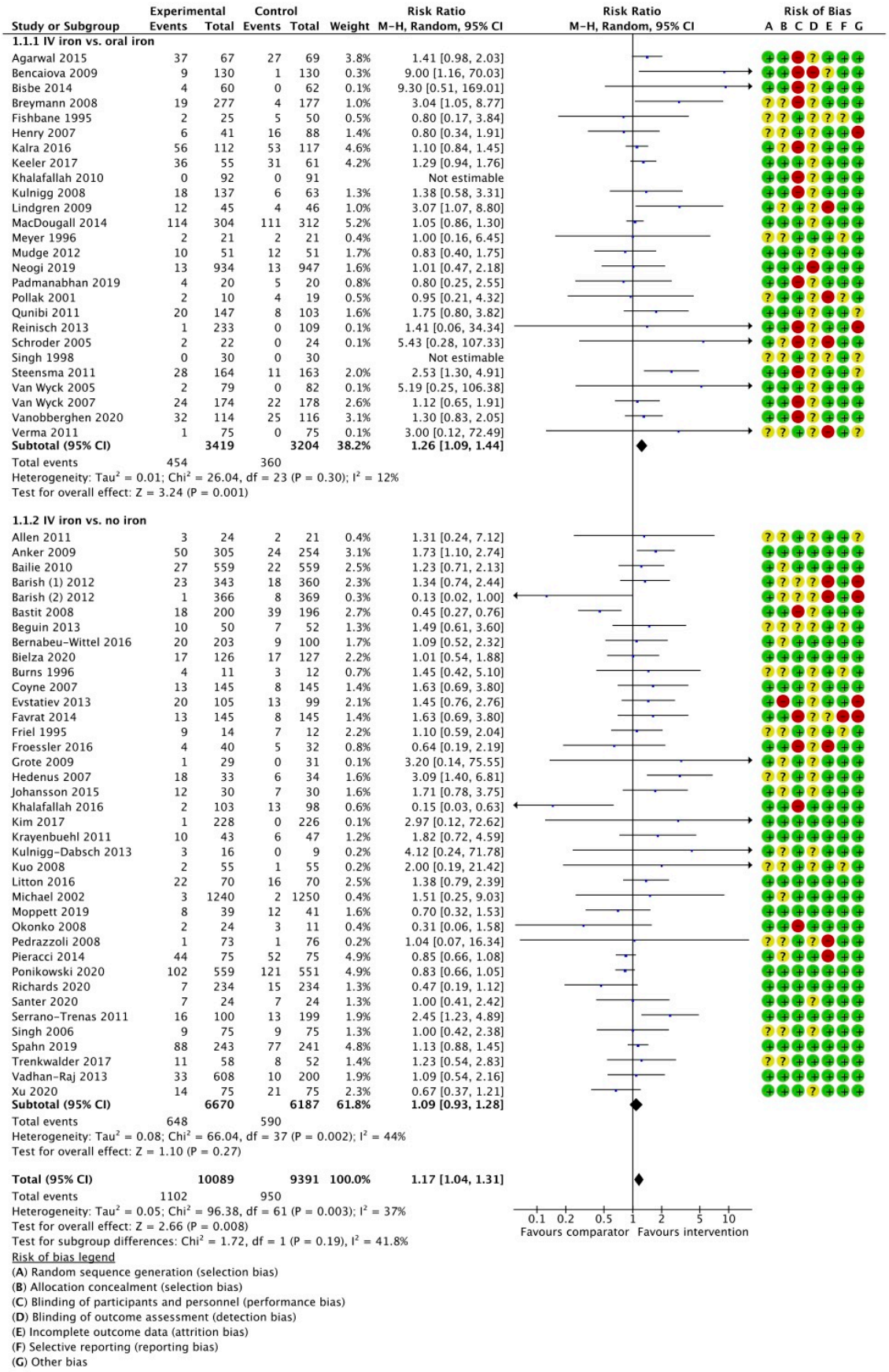
Figure 3.3 Funnel plot for primary outcome of infection.

3.3.4 Effect on outcomes

3.3.4.1 Infection

Sixty-four RCTs including 19,480 participants provided sufficient data for analysis. Intravenous iron was associated with a significant risk of infection when compared with oral iron or no iron (RR (95% CI): 1.17 (1.04 to 1.31), I^2 = 37%, p = 0.003)) **(Figure 3.4).** Based on the GRADE framework, I judged this to be moderate-quality evidence. In absolute terms, 17 more people per 1000 (95% CI: 4 to 31) will experience an infection when intravenous iron is used **(Table 3.4)**. In a pre-planned sensitivity analysis excluding studies at high risk of bias, the effect estimate was largely unchanged (RR (95% CI): 1.15 (0.98 to 1.35), I^2 = 37%, p = 0.07); 16 RCTs; 8719 participants).

Subgroup analysis by clinical setting showed evidence of a significant increased risk in patients with IBD (RR (95% CI): 1.73 (1.11 to 2.71), I^2 = 0%, p = 0.02); 6 RCTs; 908 participants). The direction of effect supported an increased risk of infection in almost all other subgroups although these was not statistically significant **(Table 3.5 and Figure 3.5)**. I found no evidence of an effect associated with iron or anaemia profiles at study enrolment (as defined by the study authors), or type of iron preparation used. Both single and multiple dose administration were associated with an increased risk of infection **(Table 3.6)**. Meta-regression found no significant interactions between baseline ferritin, haemoglobin, transferrin saturation and study year, and risk of infection (**Table 3.7)**. All the slopes (co-efficient) were not significant statistically.



CI, Confidence interval; IV, Inverse variance; M-H, Mantel-Haenszel

Figure 3.4 Forest plot of the effect of intravenous iron on the risk of infection

Table 3.4 Summary of findings and GRADE quality assessment of evidence for the primary outcome of infection

Certainty assessment							Summary of findings				
Participants (studies) Follow up	Risk of bias	Inconsistency	Indirectness	Imprecision	Publication bias	Overall certainty of evidence	Study event rates (%)		Relative effect (95% CI)	Anticipated absolute effects	
							With comparator	With IV iron		Risk with comparator	Risk difference with IV iron
Infection											
19480 (64 RCTs)	not serious	serious [a]	not serious	serious [b]	None	⨁⨁⨁◯ MODERATE	950/9391 (10.1%)	1102/10089 (10.9%)	RR 1.17 (1.04 to 1.31)	100 per 1,000	**17 more per 1,000** (from 4 more to 31 more)

CI, Confidence interval; RR, Risk ratio; OR, Odds ratio; MD, Mean difference; IV, Intravenous

Explanations

a. Downgraded for inconsistency due to moderate heterogeneity (41.4%)
b. Wide 95% CIs that do not exclude a benefit or harm of likely importance to patients and clinicians

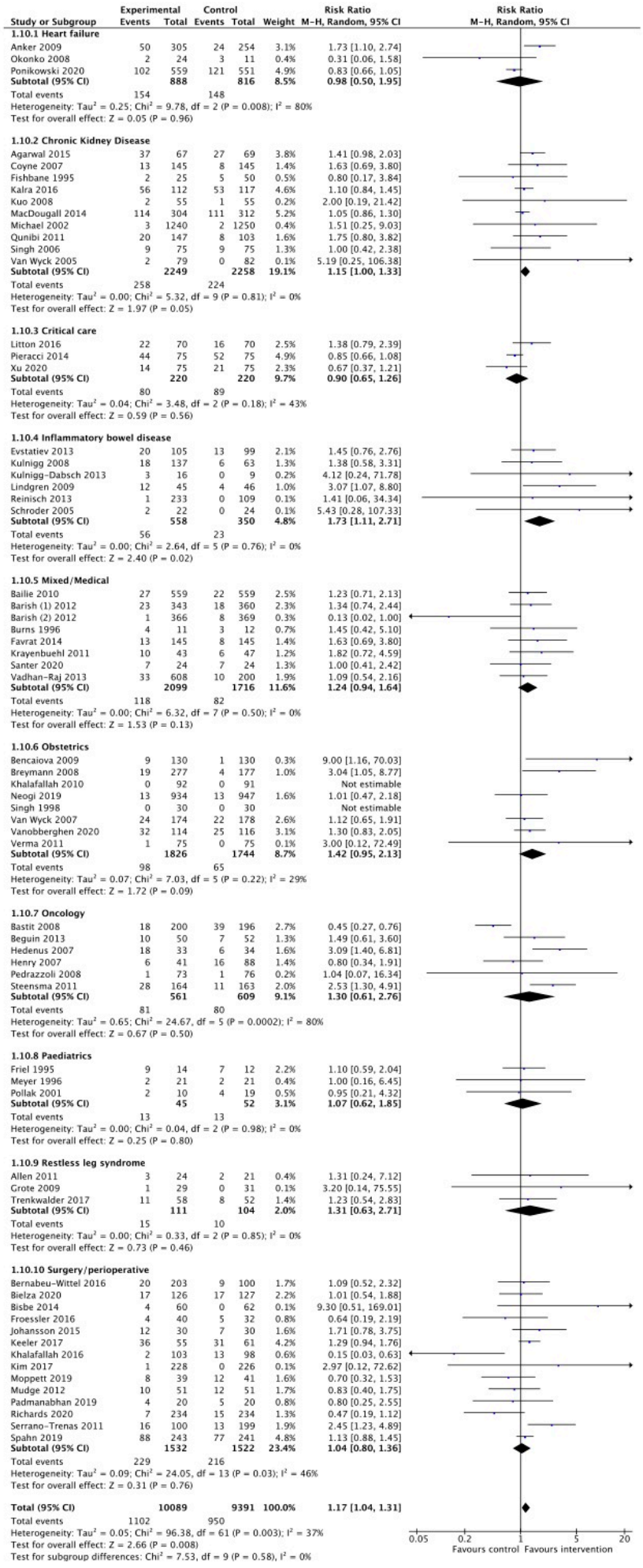


Figure 3.5 Forest plots of the risk of infection and intravenous iron by subgroups defined by clinical setting.

Table 3.5 Associations between intravenous iron and the risk of infection by clinical setting

Subgroup analysis	Number of participants [a]		Treatment effect (95% CI) [b]	p value	I^2
	Intravenous iron	Oral iron or no iron			
Heart failure	154/888	148/816	0.98 (0.50 to 1.95)	0.96	80%
Chronic Kidney Disease	258/2249	224/2258	1.15 (1.00 to 1.33)	0.05	0%
Critical care	80/220	89/220	0.90 (0.65 to 1.26)	0.56	43%
IBD	56/558	23/350	1.73 (1.11 to 2.71)	0.02	0%
Mixed/medical	118/2099	82/1716	1.24 (0.94 to 1.64)	0.13	0%
Obstetrics	98/1826	65/1744	1.42 (0.95 to 2.13)	0.09	29%
Oncology	81/561	80/609	1.30 (0.61 to 2.76)	0.50	80%
Paediatrics	13/45	13/52	1.07 (0.62 to 1.85)	0.80	0%
Restless leg syndrome	15/111	10/104	1.31 (0.63 to 2.71)	0.46	0%
Surgery/perioperative	229/1532	216/1522	1.04 (0.80 to 1.36)	0.76	46%

[a] Number indicates no. of events / total no. of participants

[b] Risk ratio with 95% CI

IBD, Inflammatory bowel disease

Table 3.6 Subgroup analyses of the effect of dosing schedules, type of iron preparation and participant baseline iron status on the risk of infection

Subgroup analysis	Number of participants [a]		Treatment effect (95% CI) [b]	p value	I^2
	Intravenous iron	Oral iron or no iron			
Dosing schedule					
Single dosing	393/4707	324/4319	1.15 (1.01 to 1.32)	0.04	4%
Multiple dosing	709/5382	626/5072	1.20 (1.02 to 1.40)	0.03	48%
Type of preparation					
Ferric carboxymaltose	689/4988	599/4611	1.13 (0.98 to 1.31)	0.09	34%
Iron sucrose	233/2222	212/2337	1.28 (0.95 to 1.74)	0.11	59%
Iron isomaltoside	69/375	60/256	1.16 (0.90 to 1.50)	0.26	0%
Ferric gluconate	51/1663	38/1722	1.59 (1.00 to 2.53)	0.05	9%
Ferumoxytol	33/608	10/200	1.09 (0.54 to 2.16)	0.82	-
Iron dextran	15/80	15/104	1.12 (0.66 to 1.88)	0.68	0%
Iron polymaltose	12/153	16/161	0.85 (0.44 to 1.67)	0.65	0%
Iron profile at enrolment	195/1808	152/1698	1.32 (0.98 to 1.77)	0.06	30%
Iron deficiency anaemia	485/4036	432/3872	1.15 (0.94 to 1.41)	0.19	51%
Functional iron deficiency	268/3235	234/2682	1.09 (0.85 to 1.39)	0.50	48%
Anaemia only	15/83	9/90	1.74 (0.84 to 1.39)	0.13	0%
Non-anaemic iron	50/345	38/374	1.29 (0.75 to 2.23)	0.35	35%
deficiency	65/408	59/507	1.24 (0.89 to 1.73)	0.20	10%
Non-anaemic					
Unclear					

a Number indicates no. of events / total no. of participants

b Risk ratio with 95% CI

Table 3.7 Meta-regression analyses of ferritin, transferrin saturation, haemoglobin and study year on log risk ratio on infection

Variable	No. of studies	Coefficient	95% Confidence Interval	p value
Ferritin	40	-.0000359	-0.0011785 to 0.0011067	0.950
Transferrin saturation	29	.0084419	-0.022902 to 0.0397858	0.585
Haemoglobin	36	-.0005157	-0.0054991 to 0.0044677	0.835
Study year	51	-.0083708	-0.0341953 to 0.0174536	0.518

I found significant variation in how infection was defined across included studies (**Table 3.8)**. Eight RCTs utilised an established classification system, the commonest one being Medical Dictionary for Regulatory Activities (MedDRA). Two trials used standardised CDC control definitions for nosocomial infections. Twenty-four trials reported the anatomical site of infection, with the lung being the most frequently reported. Seven RCTs had a pragmatic investigator-defined definition.

Table 3.8 Summary of reporting of infection in included randomised controlled trials.

Definition	Intravenous iron vs. oral iron, n	Intravenous iron vs. no iron, n	Total
Clinical discretion / not specified	23	26	49
Investigator defined	1	6	7
Classification system			
- Clavien Dindo	1		1
- MedDra	1	3	4
- CDC		2	2
- POMS		1	1
Characterisation			
- Anatomical sites	9	18	24
(any)	6	15	20
Lung	2	3	4
Gastrointestinal	6	7	35
Genitourinary	4	1	4
Skin	1	4	5
Surgical wound	4	3	6
Other		1	1
Line related			
- Pathogens	0		
- Antibiotic use	1		1

CDC, Centres for Disease Control and Prevention; MedDRA, Medical Dictionary for Regulatory Activities; POMS, Postoperative Morbidity Survey

Example definitions included:

"If clinical symptoms were evident, or positive blood CSF culture, or local lesions"

"Increase in body temperature to >38 degrees Celsius with a requirement for IV antibiotics"

"Commencement, escalation or change of IV antibiotics for a confirmed or strongly suspected infection"

"Antibiotic upgrade"

Whilst these definitions may be simple to use at the bedside, they may lack construct and content validity. Forty-nine RCTs did not provide a definition a priori. One RCT reported data on antibiotic use between groups. No RCTs reported data on positive microbiology cultures.

For the non-randomised studies, three studies reported no statistically significant increases in the risk of infection associated with intravenous iron. One study found an increased risk and two studies reported a lowering of risk. One study, which lacked a comparator group, only reported an incidence of 25.9% of infection in patients with underlying digestive diseases receiving intravenous iron. Four studies provided no definition of infection. Of the remaining four studies, three studies used recognised classification systems (CDC, MedDRA, International Classification of Diseases-9 classification) and one study used an investigator-led definition.

3.3.4.2 Haemoglobin

Data were available from 111 RCTs including 20,776 participants. When pooled, intravenous iron was associated with a significant increase in Hb at the end of the study period when compared with oral iron or no iron (MD (95% CI): 0.57 g/dl (0.50 to 0.64), I^2 = 94%, p < 0.0001)) **(Table 3.9)**. Intravenous iron was also associated with a significant treatment response, as defined by the study authors (e.g., rise in haemoglobin of > 2 g/dl), when compared with oral iron or no iron (RR (95% CI): 1.46 (1.32 to 1.60), I^2 = 92%, p < 0.0001)) **(Table 3.8).**

3.3.4.3 RBC transfusion requirements

RBC transfusion requirement data were available from 54 RCTs including 12,116 participants. Intravenous iron was associated with a significant reduction in the risk of requiring an RBC transfusion when compared with oral iron or no iron (RR (95% CI): 0.93 (0.76 to 0.89), I^2 = 15%, p < 0.0001)) **(Table 3.9)** and a lower number of mean RBCs transfused when compared with no iron (MD (95% CI): -0.20 (0.32 to -0.08, I^2 = 81%, p < 0.0001)) (**Table 3.9)**.

3.3.4.4 Mortality and hospital LOS

I observed no evidence of an effect of intravenous iron on short-term (RR 0.99 (95% CI: 0.69 to 1.42, I^2 = 0%, p = 0.73); 15 RCTs; 3445 participants) or long-term mortality (RR 0.94 (95% CI: 0.75 to 1.18, I^2 = 0%, p = 0.63); 12 RCTs; 5010 participants) when compared with oral iron or no iron (**Table 3.9).** I found no evidence of an effect of intravenous iron on hospital LOS when compared with oral iron or no iron (MD -0.43 days (95% CI: -1.10 to 0.24, I^2 = 50%, p = 0.05); 8 RCTs; 1690 participants) (**Table 3.9).** Only a few RCTs report mortality or LOS. The wide confidence intervals may encompass minimally clinically important differences.

Table 3.9 Association between intravenous iron and secondary outcomes

	Number of participants [c]		Treatment effect (95% CI) [a, b]	*P* value	I^2
Outcome	**IV iron**	**Oral/no iron**			
Continuous outcomes					
Haemoglobin (g/dl)	10816	9720	0.57 (0.50 to 0.64) [a]	<0.0001	94%
Mean number of RBCs transfused	998	956	-0.20 (-0.32 to -0.08) [a]	<0.0001	81%
Hospital LOS (days)	807	883	-0.43 (-1.10 to 0.24) [a]	0.05	50%
Dichotomous outcomes					
Treatment response [d]	4336/7137	2611/6165	1.46 (1.32 to 1.60) [b]	<0.0001	92%
Short-term mortality (<30 days)	40/1298	40/1292	0.99 (0.69 to 1.42) [b]	0.73	0%
Long term mortality (>30 days)	165/2752	161/2258	0.94 (0.75 to 1.18) [b]	0.63	0%
Requirement for RBC transfusion	802/6256	989/6040	0.83 (0.76 to 0.89) [b]	<0.0001	15%

[a] Mean difference with 95% CI [b] Risk ratio with 95% CI
[c] Number for dichotomous outcomes indicate no. of events / total no. of participants
[d] As defined by the study authors, e.g. rise in haemoglobin by 2 g/dl,

LOS, length of stay; RBC, red blood cell transfusion

3.4 Discussion

3.4.1 Key findings

This systematic review identified 154 RCTs and 8 NRS across a range of clinical settings. The main findings were:

- Intravenous iron is associated with an increased risk of infection (moderate certainty of evidence)
- There is substantial variation and inconsistency in how infection is defined and reported across included studies
- Intravenous iron remains efficacious in improving haemoglobin and reducing RBC transfusion requirements

Although I observed no differences in mortality or hospital length of stay, the CIs were wide and could encompass clinically important differences. The findings of this systematic review are consistent with two previous systematic reviews that have also investigated the safety of intravenous iron (139, 168). Both reviews identified and analysed a smaller number of trials. The first systematic review in 2013 identified an increased risk of infection (RR 1.34 (95% CI: 1.10 to 1.64); 24 RCTs; 4400 participants) (139). The second systematic review pooled data from 32 RCTs and showed a point estimate which favoured infection, although this was statistically non-significant (RR 1.17; 95% CI: 0.83 to 1.65)(168). Other reviews have demonstrated superior efficacy for intravenous iron over oral iron but did not evaluate the risk of infection (376). The risk of infection may be further increased in patients with disorders like IBD, many of whom who may be on immunosuppressive and biological drugs (377). Although there was no statistical evidence of an increased risk in other subgroups, the results favoured increased infection in nearly all groups, and the wide CIs could encompass clinically important differences.

I identified significant variation and inconsistency in the reporting of infection despite the availability of standardised definitions (191). Of the 33 ongoing RCTs, thirteen have pre-specified infection-

related outcomes. Of these, nine RCTs do not have a definition, two have an investigator-defined definition and two are using the Clavien-Dindo (378) Classification system. Reasons for this poor uptake are unclear but may include limitations with validity, reliability and practicality. Recent initiatives such as the updated Sepsis-3 definition (379) and the Standardised Endpoints in Perioperative Medicine initiative (380) have sought to address these limitations and bring consistency to reporting infection. No studies reported data on pathogens or positive microbiology results. This has important clinical and mechanistic implications on understanding whether exogenous iron can convert benign bacterial colonisation into virulent infection. Common commensal bacteria that are frequently implicated in nosocomial infection, such as *Staphylococcus aureus* and *Staphylococcus epidermidis*, have developed iron acquisition mechanisms and can become pathogenic under the right conditions (154, 381).

Imbalances of iron homeostasis, where both iron deficiency and overload, can affect the risk of developing infection but the impact of therapeutic iron on this remains unclear (53, 382, 383). I observed no evidence of an effect of an interaction between baseline iron status (as measured by ferritin, Tsat and Hb) and the risk of developing infection, although the number of studies reporting this information in a way that could be analysed for meta-regression ranged from 29 to 40. Trial data from the CKD population on maintenance haemodialysis (384) suggest that a high-dose intravenous iron regimen may be safe in patients with ferritin concentrations up to 700 mcg/l but data above this threshold are conflicting (209). The low free iron concentration associated with newer intravenous iron preparations is thought to be associated with a lower risk of infection. However, our subgroup analysis by type of preparation demonstrated no superiority of one preparation over another.

3.4.2 Implications for practice

The findings of this systematic review have broad applicability to many patients in whom iron deficiency and anaemia are common. Given the increasing use of intravenous iron globally, clinicians and policy makers should consider that the benefits of treating anaemia and reducing RBC transfusion requirements may have to be balanced against the risk of developing infection. These issues may

become more pertinent in some of the subgroups identified in this review and in settings with high endemic infection burdens where iron supplementation may neither be effective or not safe (385).

3.4.3 Implications for research

This review also has implications for ongoing and future research. Part of the uncertainty around the risk of infection arises from variation and inconsistency in reporting this outcome, despite the availability of standardised definitions. Ongoing and future trials should seek to implement these definitions in order to increase the reliability and validity of future meta-analyses. Future research should also focus on hypothesis testing mechanistic studies, such as the interactions between specific pathogens and host iron status on the risk of developing infection.

3.4.4 Strengths and limitations

The strength of this review is the strict methodological process. I followed Cochrane Collaboration, PRISMA and GRADE recommendations, performed two comprehensive searches and carried out duplicate data extraction and risk of bias assessments. Compared to the previous reviews, I sought to characterise the risk of infection further and performed clinically relevant subgroup analyses, extracted data on how infection was reported and also included non-randomised studies. The limitations to the conclusions of this systematic review can be attributed to the clinical and methodological differences, and variations in infection reporting between the included studies. Many studies did not report infection as an outcome measure and the doses and preparations used in the pooled studies varied. Although I identified an improvement in haemoglobin, I did not assess whether this translates into improved health-related quality of life although this has been investigated by others (386) The quality of the included studies was also variable. Nevertheless, a sensitivity analyses of only including trials at low risk of bias had minimal effect on our pooled estimate and heterogeneity for outcomes of infection and RBC transfusion was not substantial.

3.5 Conclusion

Intravenous iron use continues to be associated with an increased risk of infection. It is not clear how this risk is balanced by benefits such as a reduction in RBC transfusion requirements and improvement in haemoglobin. The findings of ongoing trials, when incorporated into future updates of this review, may reduce the imprecision of the pooled estimate.

Chapter 4 Can we use newer markers of iron status, such as hepcidin, and single-cell approaches to better characterise critical illness anaemia?

The findings of this chapter have, in part, been published in:

Shah A, Wray K, James T, et al. Serum hepcidin potentially identifies iron deficiency in survivors of critical illness at the time of hospital discharge. *Br J Haem* 2019; 184: 279-81

4.1 Introduction

As described in Chapter 1, the AI that accompanies critical illness, usually presents as a mild to moderate normochromic, normocytic anaemia. This is often associated with evidence of systemic inflammation (e.g. increased CRP). The diagnostic challenge lies in the identification of the co-existence of true iron deficiency (or requirement) and AI, which may allow for more targeted management of iron therapy. Estimates suggest that between 20-85% of patients with AI may also suffer from true ID either from disease-related bleeding from the gastrointestinal or urogenital tracts, excessive phlebotomy or losses from extracorporeal therapies such as renal replacement therapy (66).

4.1.1 Hepcidin structure, kinetics and analytical issues

There is growing interest in the use of hepcidin for the diagnosis and monitoring of iron disorders, and for guiding safe and effective iron therapies. Bioactive hepcidin is a 25 amino-acid hepatic peptide hormone (hepcidin-25). N-terminal degradation leads to smaller isoforms (hepcidin-24, -23, -22, and -20) which are of dubious significance, (387) but are present in conditions which increase hepcidin-25 concentrations e.g. CKD (388). Circulating hepcidin is bound to albumin and α-2-macroglobulin but the physiological impact of binding on hepcidin activity is unclear (389). In health, hepcidin-25 concentrations display a diurnal variation with concentrations being lowest in the morning, rising steadily throughout the day before declining in the evening (390). Hepcidin is excreted by the kidneys and urinary hepcidin concentrations correlate with those measured in serum hepcidin, with the

exception of CKD (391, 392). As discussed in Chapter 1, hepcidin synthesis is upregulated by iron overload, inflammation and infection and decreased by iron deficiency and hypoxia.

Hepcidin-25 can be measured from serum samples following centrifugation and storage. Concentrations decrease within 1-2 days when stored at room temperature but remain relatively stable at 4°C, -20°C, and -80°C for 1 week, 4 weeks and up to 2 years, respectively (393). Serum samples can be subjected to up to 3 freeze-thawing cycles without negatively affecting the concentrations (394). However, hepcidin is a 'sticky' peptide that will bind to laboratory plastics and other hydrophobic surfaces. Therefore, all measurements using immunoassays are likely to only measure circulating hepcidin (395).

Assays to measure hepcidin can be divided into two broad groups – mass spectrometry-based and immunoassays (radioimmunoassay and enzyme-linked immunosorbent assay (ELISA)) (393). Ideally all assays should satisfy the following minimum criteria (395):

- Specificity – the ability to measure the substance of interest, in the presence of other components
- Accuracy – agreement between expected value and observed value
- Precision – variability in the data from repeated experiments of the same sample under normal assay conditions. This is usually expressed as a coefficient of variance (CV). For enzyme assays, CV is usually <10%.
- Detection limit – lowest amount of analyte that can be detected. It is a low concentration that is statistically indistinguishable from a negative control, but it not sufficiently precise or accurate to be quantified
- Limit of quantitation – lowest and highest concentration of an analyte that can be quantified with suitable precision and accuracy
- Linearity – results that are directly proportional to the concentration of the target analyte in the sample

Mass spectrometry-based methods are highly specific for hepcidin-25 but are labour intensive and require expensive and complex equipment. Immunoassays, such as ELISA, are high throughput, relatively easy to perform and cheaper than mass-spectrometry based methods. However, they are less specific than mass spectrometry. In the absence of a primary reference material, absolute hepcidin concentrations can vary widely between assays by up to 10-fold, which in turn makes it difficult to determine limits of quantitation and interpret study results (396).

In order for hepcidin assays to be implemented into clinical practice, the equivalence between different measurement techniques needs to be improved. A recent exercise aimed to harmonize and assess the degree of equivalence between 11 most commonly used hepcidin measurement assays (5 mass spectrometry, 6 immunoassay) (396). The investigators found that absolute hepcidin concentrations and reproducibility differed significantly between all assays (intrameasurement procedure CV 2.9% to 8.9%). However, all were linear and correlated well. Equivalence however, measured as intermeasurement CV, was 28.6%. This was mostly attributed to differences in calibration. A candidate reference measure was identified – native, lyophilized plasma with cryolyoprotectant, which improved the equivalence from 28.6% to 7.7%. Until there is international agreement, harmonization and establishment of a universal reference range, each method and/or laboratory should aim to establish their own age- and sex-specific reference ranges.

Current evidence in critically ill patients

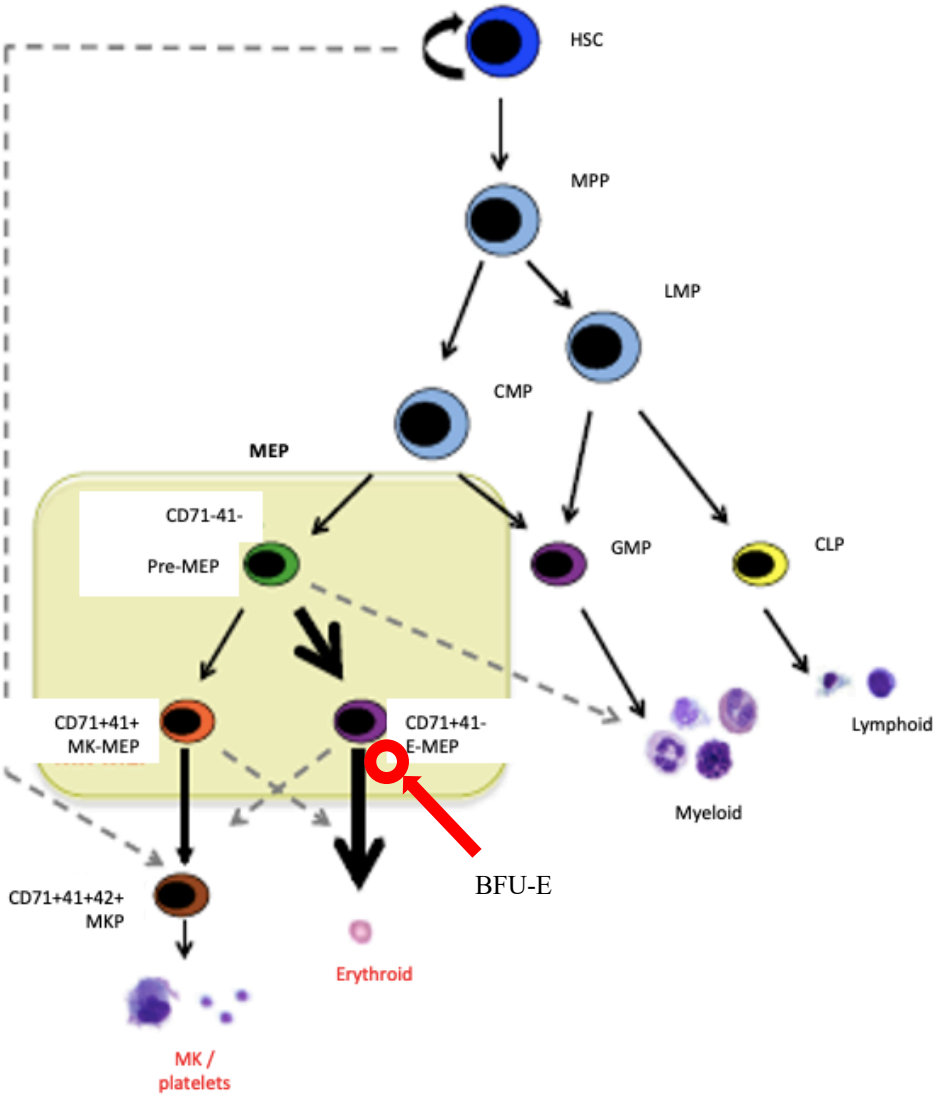
One study evaluated the diagnostic accuracy of hepcidin in critically ill patients and reported a threshold of <129.5 ng/ml to identify iron deficiency (397). In a prospective cohort study of one hundred critically ill surgical patients, serum hepcidin concentrations were markedly increased on ICU admission (median (IQR): 144 (100 to 218) ng/ml) but decreased significantly at 28 days post-admission (median (IQR): 36 (10 to 70) ng/ml). This decrease was associated with decreased IL-6 and CRP concentrations and an accompanying increased reticulocyte response, although there was no resolution in anaemia (71). Both these studies used a competitive ELISA method. Another study measured hepcidin at ICU discharge using mass spectrometry and found that iron deficiency, defined

as hepcidin <20 ng/ml, was associated with increased one-year mortality and poor physical recovery (45).

4.1.2 Single-cell profiling of erythroid differentiation

Mammalian production of blood and immune cells throughout life and during periods of stress is regulated by a small number of multipotent and self-renewing haematopoietic stem cells (HSCs) (398). As initially discussed in Chapter 1, HSCs undergo a series of stepwise differentiations becoming progressively more restricted in their lineage potential and finally producing unipotent or bipotent progenitors (399). In the traditional hierarchical model of HSC differentiation, the developmental pathway of multipotent progenitors (MPPs) bifurcates into myeloid potential (common myeloid progenitors (CMPs)) or lymphoid potential (common lymphoid progenitors (CLPs)). CMPs then give rise to a population of granulocyte-macrophage progenitors (GMPs) or megakaryocytic erythroid progenitors (MEPs) (72, 73). The latter then differentiate into lineage-committed erythroid cells and platelet-producing megakaryocytes.

Recent advances in transcriptional, phenotypic, functional single-cell sorting analyses and -omics based approaches have challenged this traditional hierarchical model and found that cellular heterogeneity and lineage-priming exists within the HSC compartments, including the existence of cells that are able to switch lineages (400-402). Progenitors may branch earlier than previously thought. Megakaryo-erythroid and myelo-lymphoid potential have been shown to diverge at a lymphoid-primed multipotent progenitor (LMPP) (403). This route bypasses the conventional lineage between MPP and CMP. Psalia et al. found that classically defined MEPs contain three distinct subpopulations: (i) “Pre-MEP”, enriched for megakaryocyte (MK)/erythroid progenitors but retaining some capacity for myeloid differentiation ($CD71^-$ $CD41^-$ MEP); (ii) “Erythroid-MEP”, strongly primed towards erythroid differentiation ($CD71^+$ $CD41^-$ MEP); and (iii) “MK-MEP” ($CD71^+$ $CD41^+$), a rare and previously unknown cell population that are bipotent (400). MK-MEPs are able to give rise to erythroid cells in vitro until they acquire CD42, which commits them to MK differentiation (400). These pathways are summarised in **Figure 4.1**.



BFU-E, Burst forming unit-erythroid; CLP, common lymphoid progenitor; CMP, Common myeloid progenitor; GMP, Granulocyte-macrophage progenitors ;HSC, Haematopoietic stem cells; LMP, Lymphoid multipotent progenitors; MEP, megakaryocytic erythroid progenitors; MK, Megakaryocyte; MPP, Multipotent progenitors.

Figure 4.1 Classical hierarchical model of haematopoiesis. HSCs are at the top of the hierarchy. Further downstream, CMPs give rise to a population of granulocyte-macrophage progenitors (GMPs) or megakaryocytic erythroid progenitors (MEPs). The first precursor fully committed to erythroid differentiation is the burst forming unit-erythroid (BFU-E) and erythroid differentiation and division is controlled by concentrations of erythropoietin and iron.

Differentiation of these progenitors into distinct lineages is dependent upon several transcription factors that are expressed by the CMP such as *PU.1*, *GATA-1*, *TAL1/SCL* and *c-Myb* (405, 406). *PU.1* is an essential factor for determining myeloid lineage (407) whereas *GATA-1* and *Klf1* are essential for erythrocyte lineage commitment (408). Inflammatory cytokines such as TNF-α, IL-1 and IFN-γ have been shown to have inhibitory effects on the development of BFU-Es and CFU-Es (409). Mouse models have demonstrated that IFN-γ can induce expression of *PU.1* resulting in a lineage switch towards increased myeloid differentiation and reduced erythropoiesis (83). In patients with rheumatoid arthritis, in vitro and in vivo studies have shown that increased TNF-α levels inhibit the production of BFU-Es and cause apoptosis in erythroid precursor populations (410, 411). These effects were reversed with the administration of anti-TNF-α antibodies (411). Survival of CFU-Es and early erythroblasts is determined by EPO and the differentiation and division of erythroblasts are controlled by concentrations of EPO and iron (74).

There are some experimental considerations that warrant discussion. Cells may behave differently depending on the experimental system being used. This becomes an issue when only a single assay is possible for any single cell. It is important to note that the absence of a particular lineage read-out should not be interpreted as lack of differentiation to that lineage ('absence of evidence is not evidence of absence') (73). In vitro assays are biased by selection of cytokines and culture mediums. For example, methylcellulose is an ideal in vitro culture solution for BFU-Es but not for other cells such as megakaryocytes.

Current evidence in critically patients

Data on erythropoietic pathways in critically ill patients are limited. One small study of 10 critically ill patients with septic shock examined erythropoiesis abnormalities and patterns of erythroid lineage through direct bone marrow sampling (412). The authors found that early onset anaemia is associated with defective erythropoiesis due to excessive apoptosis, which was potentially reversible *in vitro* by addition of recombinant EPO. In addition, BFU-E growth in bone marrow mononuclear cells was impaired in the presence of serum from patients with septic shock when compared with serum from

control subjects. Concentrations of IL-6 and TNF- α were higher in plasma from patients with septic shock when compared with controls.

A more recent study of 22 critically ill severely injured trauma patients also directly sampled the bone marrow (during time of trauma surgery) and found evidence of EPO dysfunction, erythroid progenitor growth suppression, reduced erythroid output and dysregulated iron homeostasis (413). Trauma patients had lower BFU-E and CFU-E growth when compared with patients undergoing elective hip arthroplasty and these levels were negatively correlated with plasma norepinephrine levels. The latter finding support a potential role for excessive catecholamines and neuroendocrine activation in the pathophysiology of critical illness anaemia (414).

Limitations of both these studies include the lack of external generalisability as they focused on a specific subgroup of critically ill patients. Samples were obtained through direct bone marrow sampling, which is invasive, painful and carries a small risk of major bleeding. Analyses were performed at one time point thereby providing only a single assessment of the erythropoietic response. How this response varies over time during the ICU stay and beyond, and any factors associated with improvement in erythropoiesis (or lack of) requires investigation. I am particularly interested in whether there are certain clinical and/or biological characteristics that commit cells to the erythroid or megakaryocytic lineage and if so, whether there is a link between this and recovery of erythropoiesis. It is also now possible to characteristic erythropoiesis from peripheral blood, but no study has been performed to date in critically ill patients.

4.2 The RECOVER trial

RECOVER was a RCT examining the effect of a complex rehabilitation package on physical outcome in ICU survivors (415). It included adult patients initially requiring >48 hours of continuous mechanical ventilation who were eventually deemed fit for ICU discharge by the treating physician. Patients were recruited at the point of ICU discharge and followed up for 3 months for a primary

functional outcome, and for 12 months for a range of secondary outcomes. The protocol, description of the intervention and trial results have been published previously. The trial demonstrated no effect of a rehabilitation package on physical recovery or HRQoL (415-417). The study was led by Professor Tim Walsh, University of Edinburgh.

4.2.1 Objectives

The primary objective of this study was to investigate the diagnostic utility of hepcidin as a marker of iron deficiency in patients recovering from critical illness. The secondary objective was to describe the prevalence and characteristics of anaemia in this patient cohort.

4.2.2 Ethical approval

The RECOVER study was funded by the Chief Scientists Office (CSO) (CZH/4/531, ETM/221). with ethical approval from the Scotland A Research Ethics Committee (10/MRE0018). Patients recruited to RECOVER, who consented to a biomarker sub-study and remained in hospital for ≥1 week after ICU discharge, were considered eligible for this secondary observational study of iron status.

4.2.3 Sample collection

Venous blood samples taken close to, or at the point, of hospital discharge were placed into pre-treated Serum Gel tubes (Starstedt AG and Company, Numbrecht, Germany), rested at room temperature for 30 minutes then centrifuged at 2500G for 10 minutes at room temperature before being transferred to storage tubes and frozen at -70°C until analysis.

4.2.4 Experimental methods

Haematological and biochemical variables already measured and recorded as part of the primary study included Hb concentration, mean corpuscular volume (MCV), mean cell Hb, mean cell haemoglobin concentration, white cell count (WCC), platelet count, liver function tests, serum electrolytes (sodium, potassium), urea & creatinine, albumin and CRP.

Routine tests of iron status, which included serum ferritin, serum iron, and Tsat erythropoietin, vitamin B_{12} and folate were measured by the Departments of Clinical Biochemistry and Haematology at the John Radcliffe Hospital, Oxford. Details of the assays and methods used are summarized in **Table 4.1**.

Table 4.1 Details of laboratory assays used by Oxford University Hospitals NHS Foundation Trust

Assay	Analyser	Method	Concentration			CV, %		
			Level 1	Level 2	Level 3	Level 1	Level 2	Level 3
B_{12} (ng/l)	Abbott Architect i2000	Indirect/ Competitive Immunoassay	270	534	997	7.6	5.7	6.4
Ferritin (mcg/l)		Direct/ Sandwich Immunoassay	29	176	379	5.2	4.2	4.6
Folate (ng/ml)		Indirect/ Competitive Immunoassay	3.7	9.1	14.8	9.9	7.2	7.6
Erythropoietin (IU/l)	Siemens Diagnostic Immulite 2000	Chemiluminescent immunoturbidimetric assay	10.9	42.9	124.9	6.4	8.0	7.4
Vitamin D (nmol/l)	Abbott Architect i2000	Indirect/ Competitive Immunoassay	48.5	61.6	133.6	4.5	4.5	4.5
Iron (mmol/l)	Abbott Architect c16000	Colorimetric – Ferene dye	15.60		28.70	2.8		2.7
Transferrin (g/l)		Immunoturbidimetric	1.77		3.29	1.7		1.6
CRP (mg/l)		Turbidimetric/ Immunoturbidimetric	3.4		30.9	3.5		2.1
Urea (mmol/l)		Enzymatic – kinetic urease	3.1		24.4	3.5		2.1
Sodium (mmol/l)		Ion-selective electrode (Indirect)	123.1		168.1	0.7		0.8
Potassium (mmol/l)		Ion-selective electrode	2.7		6.6	1.1		0.8
Creatinine (mmol/l)		Enzymatic – Creatininase and sarcosine oxidase	63		505	2.3		1.5
Albumin (g/l)		Colorimetric – Bromocresol Purple	28.4		50.3	1.6		1.6
Alk Phosphatase (U/l)		Enzymatic – *para*-nitrophenyl phosphate	68		362	2.4		1.8
Alanine Transaminase (U/l)		Enzymatic – nicotinamide adenine dinucleotide hydrogen	26		216	5.0		2.9
Bilirubin (Total) (mmol/l)		Colorimetric – diazonium salt with accelerator	13		96	6.4		3.2
Bilirubin (Conjugated) (mmol/l)		Colorimetric – Diazo reaction	7		41	2.8		1.5
Calcium (mmol/l)		Colorimetric – Arsenazo III	1.59		3.06	2.1		1.3
Phosphate (mmol/l)		Colorimetric - Phosphomolybdate	0.82		2.18	2.3		1.5

CRP, C-reactive protein; CV, Coefficient of variation

Serum hepcidin was measured by a competitive enzyme-linked immunosorbent assay (ELISA) (DRG Instruments, Marburg, Germany). Microtiter wells were coated with a monoclonal mouse antibody targeted towards an antigenic site of hepcidin-25. Endogenous hepcidin-25 in the patient's serum sample will complete with a hepcidin-25 biotin conjugate for binding to the coated antibody. Following incubation at room temperature for 60 minutes, the plate was washed to stop the competitive reaction. Streptavidin peroxidase (enzyme complex) was then added to detect the bound biotin molecules and the plate left to incubate for 30 minutes at room temperature. The plate was then washed for a second time and substrate solution is added. The intensity of the color developed is inversely proportional to the hepcidin-25 concentration in the sample. For my experiments, the absorbance of each well was read at 450 +/- 10 nm with a SPECTROstar^{Nano} plate reader. The performance characteristics of the assay I used are described below in **Table 4.2.** sTfR was also measured by competitive ELISA (R&D Systems, Abingdon, UK).

Table 4.2 Performance characteristics of the hepcidin-25 DRG ELISA assay

Characteristic	Value
Reference range	0.16 to 43.84 ng/ml
- Mean	11.02 ng/ml
- 2.5th to 97.5th centile	0.66 to 36.9 ng/ml
- Median	8.30 ng/ml
Limits of quantitation	0.153 ng/ml to 81 ng/ml
Specificity (analyte)	% Cross-reactivity
- Prohepcidin	< 0.001
- Insulin	< 0.001
- Hepcidin-22	24.2
- Hepcidin-20	87.7

4.2.5 Statistical analysis plan

First, I categorised patients into the following groups: iron deficiency anaemia (IDA), non-IDA, anaemia of iron deficiency and inflammation (IDI), and AI. Anaemia was defined according to WHO guidelines (males Hb <130 g/l and females Hb <120 g/l). A ferritin cut-off of <15 μg/l was used to diagnose iron deficiency anaemia (IDA). In the presence of inflammation (defined as CRP >8 mg/l), I opted to use a ferritin cut-off of 150 μg/l to differentiate between IDI (ferritin <150 μg/l) and AI

(ferritin >150 μg/l) (**Table 4.3**). This classification was based on a study assessing the diagnostic performance of ferritin in patients with chronic inflammatory conditions comparing CRP and ferritin levels to bone marrow iron staining - the accepted gold standard test of iron status (418).

Table 4.3 Study definitions of different types of anaemia

	Laboratory reference range	**Iron deficiency anaemia**	**Anaemia of inflammation**	**Anaemia of Iron deficiency & inflammation**
Haemoglobin (g/l)	M: 130 – 170 F: 120 - 160		M: Hb<130 F: Hb<120	
Ferritin (μg/l)	20 – 300	<15	>150	<150
C-reactive protein (mg/l)	0 – 8	<8	>8	>8

Hb, Haemoglobin; F, female; M, male

Next, I sought to compare basic clinical characteristics and laboratory data between AI and IDI groups. Non-parametric tests (CRP, Tsat, hepcidin, sTfR, EPO, vitamin B_{12}, folate) were logarithmically transformed to convert to a parametric distribution. Geometric means were calculated for logarithmically transformed data and mean values compared using the t-test. Univariate associations between hepcidin and ferritin and hepcidin and sTfR/log (ferritin) index (sTfR-F index) were estimated using linear regression on transformed variables. The sTfR-F index has been proposed as a good estimate of body iron status as it reflects both serum ferritin (storage iron compartment) and sTfR (functional iron compartment) (419). Statistical significance was defined as p value <0.05.

I calculated Receiver Operator Characteristic (ROC) curves for hepcidin as a diagnostic test of iron deficiency in the context of inflammation as compared with my pre-defined criteria: ferritin less than 150 μg/l. The sensitivity and specificity of hepcidin as an indicator of iron deficiency were determined for different cut-off values for hepcidin, and the area under the curve for ROC curves (AUC^{ROC}) was generated. The Youden index (also called Youden's J statistic) was calculated for each cut-off to assist in selection of an appropriate cut-off (420).

The Youden index provides a summary statistic of the performance of a diagnostic test with a value

ranging from 0 to 1. A zero value indicates that a diagnostic test gives the same proportion of positive results for groups with and without the disease. A value of 1 indicates that there a no false positives or false positives. The index is defined is defined for all points on the ROC curve and the maximum value may be used to determine the optimal cut-off for when a test provides a continuous instead a dichotomous result.

Data were entered into database software on a secure server (Microsoft Excel, Microsoft Corporation, Redmond, WA, USA) and transferred onto statistical software for analysis (STATA 14, College Station, Texas, USA).

4.2.6 Results

Clinical data and samples were available for one hundred and seventeen patients recruited between 1st December 2010 and 31st January 2013. The median (IQR) sample collection day was 3 (1-5.5) days prior to hospital discharge. Patient characteristics are displayed in **Table 4.4**.

Table 4.4 Characteristics of study population at time of hospital discharge (n = 117)

Variable	Value
Age (years), mean (SD)	61.1 (13.9)
Male sex, n (%)	75 (64)
APACHE II score, mean (SD)	21.6 (6.8)
Diagnosis category*, n (%):	
Cardiovascular	32 (27)
Respiratory	36 (30)
Trauma	3 (3)
Gastrointestinal	37 (32)
Neurological	5 (4)
Renal	2 (2)
Miscellaneous	2 (2)
ICU length of stay (days), mean (SD)	17.6 (15.9)
No. of ventilator days, mean (SD)	12.8 (12.6)
Vasopressor requirement, n (%)	89 (76)
Renal replacement therapy requirement, n (%)	35 (30)
Overall Hb (g/l), mean (SD)	98.5 (15.9)
Hb in anaemic men (71/75)	100.0 (16.7)
Hb in anaemic women (39/42)	94.6 (13.7)
No. of patients with Hb <90 g/l, n (%)	36 (31)
No. of patients with Hb 9-10 g/, n (%)	24 (24)
Ferritin (ug/l) (NR: 20 – 300), mean (SD)	502.6 ±360.9
Iron (um/l) (NR: 14 – 31), mean (SD)	8.5 ±5.1
Tsat (%) (NR: 16 – 50%), mean (SD)	22.5 ±12.3
C-reactive protein (mg/l), mean (SD)	51.0 ±49.8
Soluble transferrin receptor (sTfR) (ng/ml), mean (SD)	26.6 ±13.7
sTfR/log(ferritin) ratio, mean (SD)	10.8 ±6.8
Hepcidin (ng/ml), mean (SD)	32.0 ±21.1

APACHE, Acute Physiology and Chronic Health Evaluation; Hb, Haemoglobin; ICU, Intensive care unit; SD, Standard deviation; sTfR, soluble transferrin receptor; Tsat, Transferrin saturation

* Patients may have more than one diagnostic category and therefore total percentage may not equal 100%

Prevalence and characteristics of anaemia

The prevalence of anaemia & the classification of the types of anaemia observed are outlined in **Figure 4.2** below. Of the included patients, 110/117 (94%) patients were anaemic prior to hospital discharge. Of these, 89/110 (81%) had anaemia of inflammation (AI), and 11/110 (10%) combined iron deficiency and inflammation anaemia (IDI). Ten patients developed anaemia that appeared unrelated to iron deficiency, reduced B_{12} and/or folate levels, renal function or EPO levels.

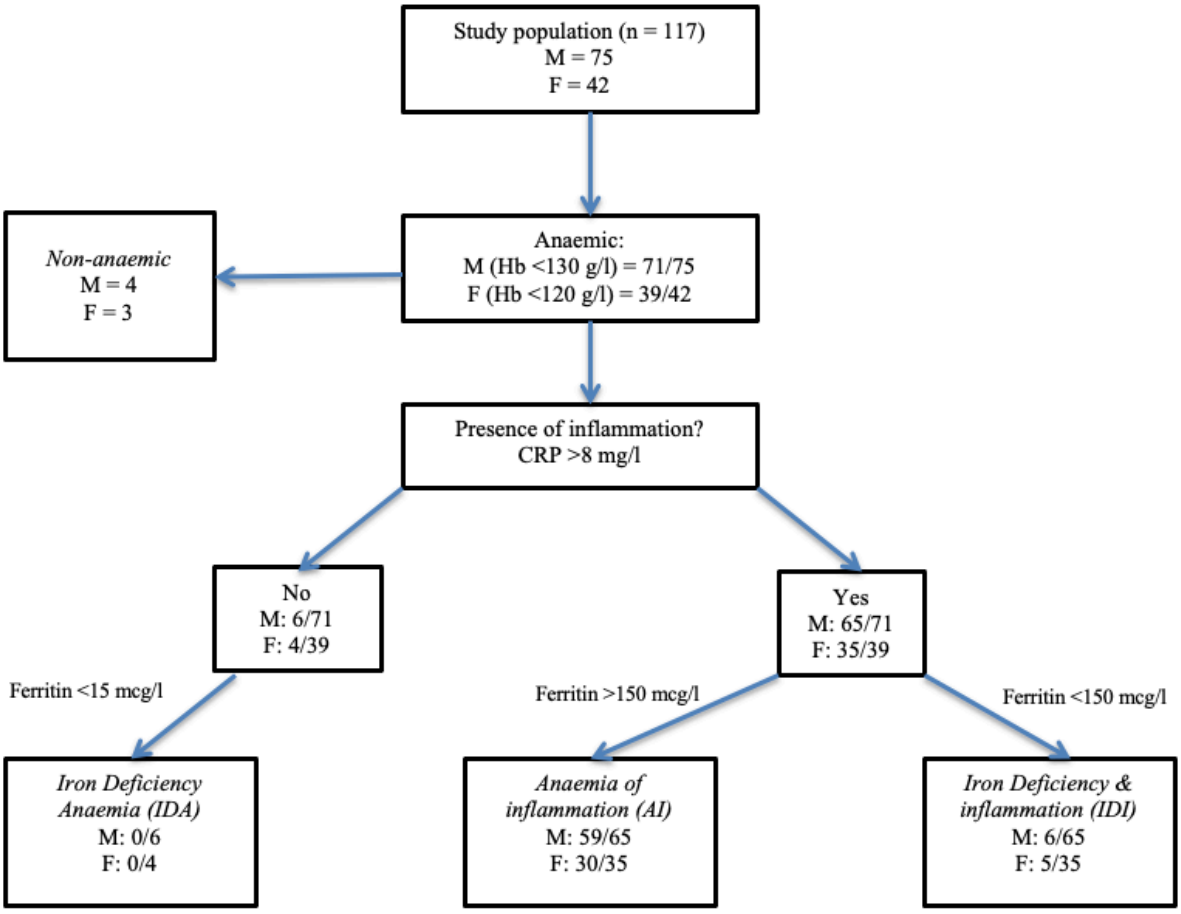


Figure 4.2 Flow of classification of different types of anaemia classified by haemoglobin (Hb), C-reactive protein (CRP) and ferritin cut-offs. By these definitions, 110/117 (94%) of patients are anaemic at point of hospital discharge and the vast majority of these patients have an anaemia of inflammation (89/110).

Patient characteristics, such as age and APACHE II scores, were similar between AI and IDI groups (**Table 4.5**). Geometric mean (SD) serum hepcidin concentration was significantly lower in the IDI group compared to the AI group (9.13 (16.7) ng/ml vs. 28.6 (19.3) ng/ml, p=0.002) as shown in **Figure 4.2**. There was no statistical difference in CRP concentrations between groups, which is suggestive that hepcidin concentrations were not necessarily reflecting a higher level of inflammation in the AI group.

Table 4.5 Patient characteristics and haematological parameters according to anaemic status

Variable	**Anaemia of inflammation (AI) (n = 83)**	**Anaemia of Iron deficiency *and* Inflammation (IDI) (n = 11)**	**p value[a]**
Age (years)	62.4 (14.1)	61 (14.1)	0.75
APACHEII[1]	20.7 (6.9)	22.8 (7.4)	0.34
Hb (g/l)[1]	95.4 (14.8)	95.4 (10.3)	1.00
CRP (mg/l)[2]	42.6 (37.8)	24.2 (19.5)	0.12
Tsat (%)[2]	18.2 (11.1)	13.6 (10.0)	0.2
Hepcidin(ng/ml)[2]	28.6 (19.3)	9.13 (16.7)	<0.05
sTfR (nmol/l)[2]	22.7 (12.8)	29.9 (24.1)	0.12
sTfR-F index[2]	8.5 (5.1)	15.9 (14.9)	<0.05
EPO (Miu /Ml)[2]	15.3 (10.7)	24.2 (38.5)	0.09
Creatinine (umol/l)[2]	76.2 (42.1)	64.9 (15.4)	0.38
Albumin (g/l)	22.7 (7.0)	24.7 (6.7)	0.37
Vitamin B_{12}[2] (180 – 900 pg/ml)	545.1 (276.5)	581.9 (243.9)	0.68
Folate[2] (3-20 ng/ml)	5.9 (3.3)	6.9 (5.4)	0.39

[1] Arithmetic mean; [2] Geometric mean; All values are expressed as mean (SD). APACHEII, Acute Physiology and Chronic Health Evaluation II; Hb, Haemoglobin; MCV, Mean Corpuscular Volume; CRP, C-reactive protein; Tsat, Transferrin saturation; sTfR, soluble transferrin receptor; sTfR-F, soluble transferrin/log (ferritin); EPO, erythropoietin

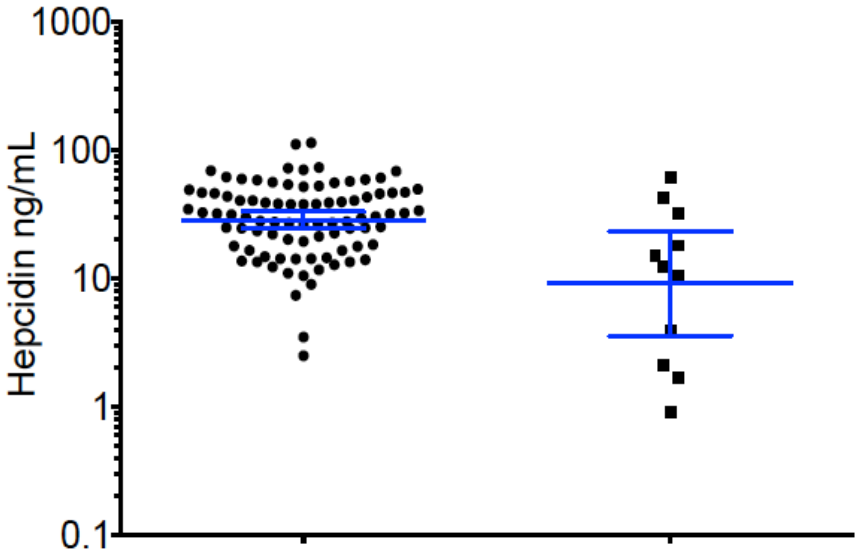


Figure 4.2. Scatterplot of hepcidin concentrations in AI and IDI groups. Hepcidin concentration was significantly lower in the IDI (geometric mean (95% CI): 9.1 (3.5 to 23.3) ng/ml) group compared to the AI group (geometric mean (95% CI): 28.6 (24.8 to 33.1) ng/mL, p = 0.002).

I tested for associations between serum hepcidin and serum ferritin – the most commonly used routine marker of iron status at present. By linear regression, log (hepcidin) was associated with log (ferritin) (co-efficient +0.6, p <0.0001) (**Figure 4.3a**). The association between log (hepcidin) and log (sTfR-f index) appeared stronger than that for log (ferritin) (co-efficient -0.8, p <0.0001) as shown in **Figure 4.3b**. There was also a statistically significant association between log (sTfR-F index) and log (ferritin) (co-efficient -0.4, p <0.001) (**Figure 4.3c**).

Hepcidin as a diagnostic test of iron deficiency

For the overall population, the mean (SD) hepcidin concentration was 32 (21.1) ng/ml. I found that a hepcidin cut-off of <19 ng/ml achieved the maximum AUC^{ROC} (0.72) and Youden index (0.47) with a sensitivity of 73% and specificity of 74%. Results for various different cut-offs are shown in **Figure 4.4**.

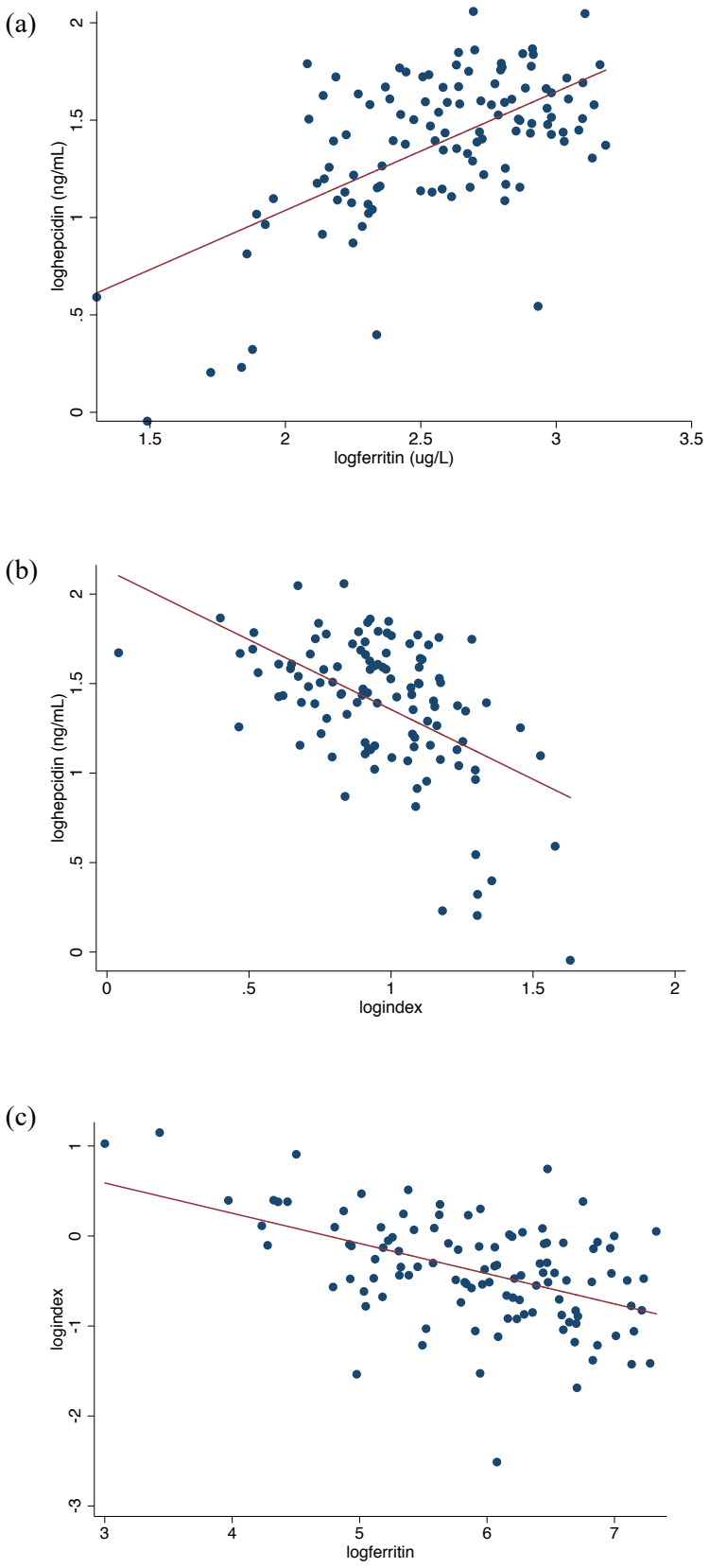
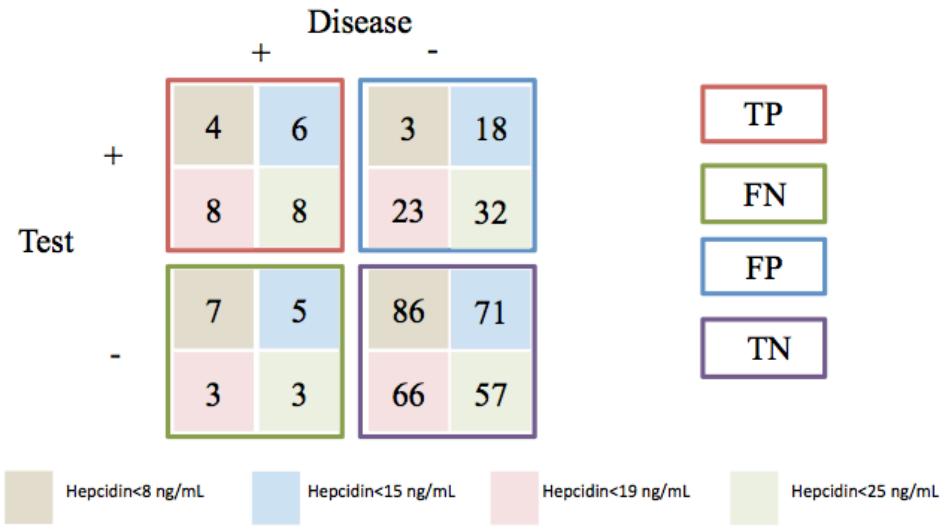


Figure 4.3. Linear regressions between hepcidin, ferritin and soluble transferrin receptor / log (ferritin) index



Hepcidin cut-off (ng/mL)	Sensitivity (%)	Specificity (%)	AUC^{ROC}	Youden Index
<8	36	97	0.65	0.33
<15	55	80	0.65	0.34
<19	73	74	0.72	0.47
<25	73	64	0.66	0.37

AUC^{ROC}, Area under curve for Receiver Operator Characteristic (ROC) curves

Figure 4.4 2 x 2 contingency tables for difference hepcidin cut-offs as a diagnostic test of iron deficiency in inflammation (defined by ferritin <150 ug/l and C-reactive protein >8 mg/l). Numbers represent patients from both AI and IDI groups. A hepcidin cut-off of <19 ng/ml achieved the highest combined sensitivity, specificity, AUC^{ROC} and Youden Index. The tan boxes show the number of patients allocated to each category when a cut-off of 8 ng/ml is used, the blue boxes when a cut-off of 15 ng/ml is used, the pink boxes a cut-off of 19 ng/ml and the green boxes a cut-off of 25 ng/ml. The bottom table sets out the sensitivity, specificity, AUC and Youden for each of these individual cut-offs. The value with the highest combined sensitivity and specificity is 19ng/ml.

4.2.7 Interpretation of findings

This study confirms the previously high documented prevalence of anaemia, and in particular the anaemia of inflammation, at hospital discharge in survivors of critical illness. I assessed the potential role of hepcidin as a discriminatory marker of iron deficiency during convalescence from critical illness when compared with currently available tests, such as ferritin. I found that hepcidin does not demonstrate any superiority over current tests in attempting to identify iron deficiency in a cohort of patients recovering from critical illness as evidenced by a maximal AUC^{ROC} of 0.72. However, this was

a secondary post hoc analysis, and we did not perform a sample size calculation. On balance, I would not currently recommend its use as a solitary diagnostic test against the complex multi-parameter definition of iron deficiency in inflammation.

However, measurement of hepcidin integrates multiple processes involved in iron homeostasis and therefore may still have a potential role in the identification of patients who may benefit from iron therapy (421, 422). In a large cohort of African children with malaria, single hepcidin cut-offs identified which children would incorporate >20% of an oral iron dose into their red blood cells (8). One study screened hepcidin levels in an attempt to identify which patients with IDA in non-dialysis dependent chronic kidney disease would respond appropriately (>10 g/l increase in Hb over 14 days) to oral iron (8). The authors showed that a hepcidin concentration of >20 ng/ml had specificity of 84.4% and positive predictive value of 81.6% for predicting non-responsiveness to oral iron and that approximately two-thirds of these 'nonresponders' subsequently responded to intravenous iron (421). In critically ill patients, Litton et al. recently found that a low hepcidin concentration (<53 mcg/l) within 48 hours of ICU admission is able to identify a group of patients in whom intravenous iron may result in a significant decrease in blood transfusion requirements (Risk ratio 0.48 (95% CI: 0.26 to 0.86), p = 0.013) (423).

From the results of this study, prospective research investigating a cut-off of 19 ng/mL to guide treatment with iron would be reasonable, as this achieved the highest combined sensitivity and specificity. Within this categorisation will be patients with low hepcidin concentrations and no evidence of iron deficiency by current parameters, but who could still have possible iron-*responsive* anaemia due to their low hepcidin concentration and ability to absorb oral iron.

Using this assay to identify patients eligible for iron therapy in our patient cohort would greatly increase the number of patients treated from 11 (using current definition of iron deficiency) to 45 (the 11 patients using current definition plus the 34 patients who do not match the current definition but have hepcidin <19 ng/ml). Undoubtedly, using this approach would result in patients who receive iron therapy without any benefit. The implications of false positive misclassification include over treatment with iron, which

would need to be balanced with the potential risk of increased infection as discussed in Chapters 2 and 3. However, if a significant proportion of patients with hepcidin <19 ng/ml respond to iron therapy, the potential improvement in anaemia could translate into significant improvements in quality of life.

I observed a relationship between hepcidin and ferritin, however the strength of this correlation was moderate rather than strong, which suggests that hepcidin may be providing an additional signal on iron requirement beyond that reported by ferritin alone in critical illness. There was a stronger association between hepcidin and sTfR-F index. The sTfR-F index (sTfR/log ferritin) incorporates the reciprocal relationship between sTfR and ferritin potentially increasing the diagnostic accuracy of sTfR alone. However, evidence of the superiority of the sTfR-F index is conflicting (424). Similar associations between hepcidin, ferritin and sTfR-F index have been reported in non-critically ill patients (425).

4.3 Study two: Identification of Erythroid Progenitor Defects in Critical Illness (EPICS)

At time of writing this thesis, the progress of this study had been interrupted due to the COVID-19 pandemic. The sections below will describe the methods, planned experiments and preliminary results obtained to date.

4.3.1 Study design

EPICS is a prospective cohort study aiming to assess sub-populations of erythroid progenitors at different stages of differentiation from peripheral blood using multi-colour flow cytometry. I planned on recruiting 30 critically ill patients who had been requiring ICU care for at least 72 hours. Enrolled participants would be followed up 28 (± 7) days later. A study flow chart is shown in **Figure 4.5**.

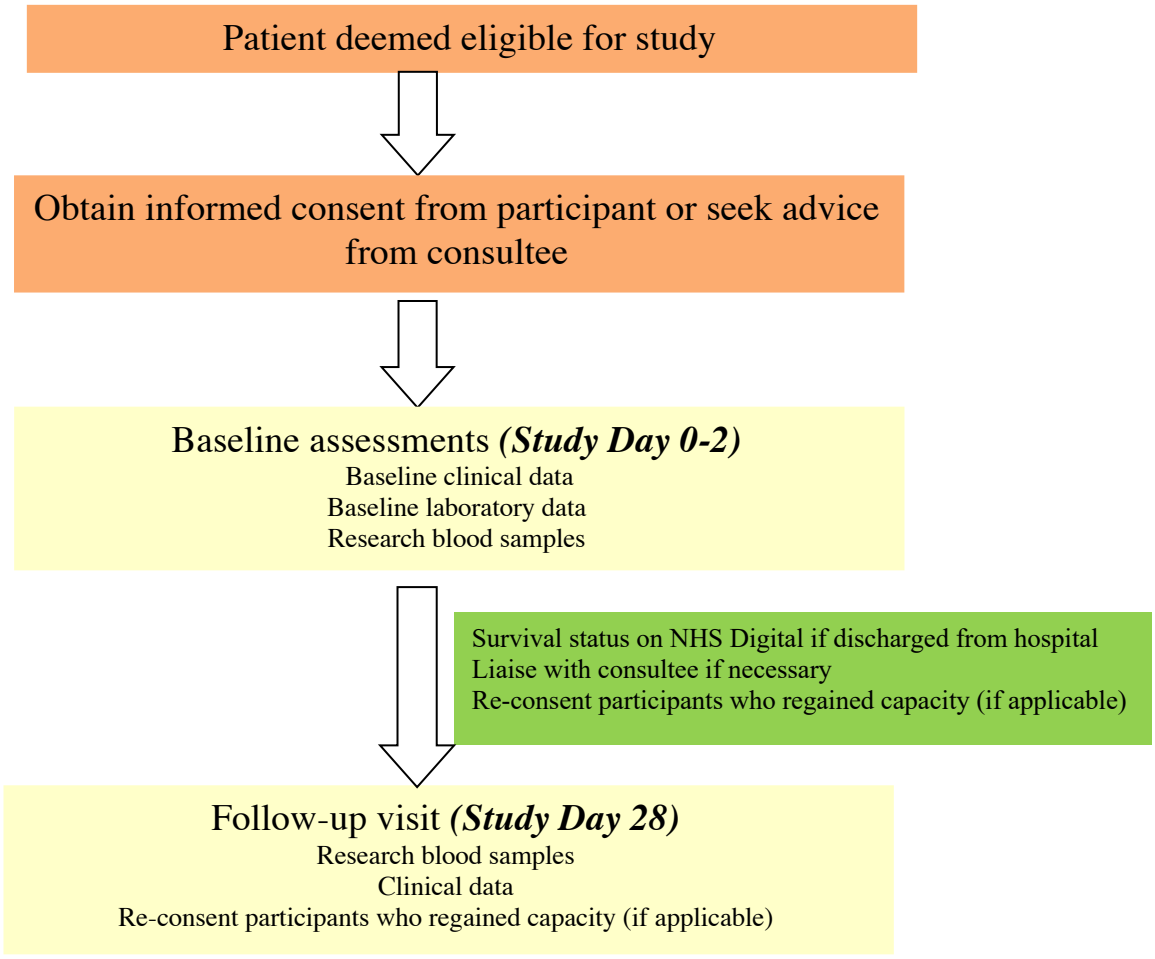


Figure 4.5. EPICS study flow chart.

4.3.2 Objectives

A tabular summary of the study objectives and outcome measures is displayed below in **Table 4.6.**

Table 4.6 EPICS study objectives and outcome measures

Objectives	Outcome Measures
Primary objective	
To explore the variability in the number of CD34+ cells, as a marker of bone marrow activity, that can be obtained from participants across a spectrum of illnesses present in intensive care.	Number of CD34+ cells extracted per participant & proportion of participants with >1 x 10^5 CD34+ cells extracted.
Secondary objectives	
To establish the colony forming potential of the MEPs, with a particular focus on the erythroid output (as a marker of erythropoiesis).	Erythroid output will be measured by the number of BFU-Es
To investigate the variability in the number of CD34+ cells over two time (enrolment and 28 days after).	Number of CD34+ cells extracted per participant, proportion of participants with >1 x 10^5 CD34+ cells extracted.
To explore what patient-related (e.g. illness severity score) and biological factors (e.g. iron status) are associated with impaired erythropoiesis (as measured by erythroid output) over two time points (enrolment and 28 days after).	Baseline clinical data and changes in laboratory profiles (FBC, iron profile etc) and number of BFU-Es

BFU-E, Burst forming units-erythroid; FBC, Full Blood Count; MEP, Megakaryocyte Erythroid Progenitor;

4.3.3 Ethical approval

EPICS was given a favourable opinion by South Central – Oxford C Research Ethics Committee (Ethics Ref: 18/SC/0545) on 2nd November 2018.

4.3.4 Inclusion and exclusion criteria

Inclusion criteria were:

- Critically ill patients (≥16 years old), both with capacity and without capacity (for medically induced reasons such as therapeutic sedation), who have required at least 72 hours of adult ICU care

Exclusion criteria were:

- Active haematological malignancy
- Chemotherapy or myelosuppressive treatment within last 30 days
- Documented or suspected HIV infection
- Received massive transfusion during index admission defined as 'replacement of >1 blood volume in 24 hours or >50% of blood volume in 4 hours'.
- Palliative care intent
- Participants who are lacking capacity for non-medically induced reasons e.g. dementia
- Death is imminent or likely during this admission
- Patients residing outside a reasonable geographic follow-up area (defined as within 30 miles of the John Radcliffe Hospital)

I decided on these criteria as it would capture a cohort that would be representative of a sick, general ICU patient population. The first three exclusion criteria were determined on the basis that these conditions do not represent homeostatic erythropoiesis and /or there may not be sufficient CD34+ cells present in the circulation either as result of the condition or because of the treatment. Participants who required a massive transfusion were excluded because the results would be confounded by donor CD34+ cells from all the transfused blood products.

4.3.5 Data collection

Clinical data at baseline

The following clinical data were collected at enrolment following informed consent:

- Participant demographics – age, sex
- Pre-existing comorbidities using the Charlson Comorbidity Index
- ICU admission data – APACHEII score, ICU admission category
- ICU resource use – requirement of any organ support (advanced respiratory, cardiovascular and renal support using Critical Care Minimum Dataset definitions), blood product requirements

Clinical data at day 28 follow-up visit

The following data were collected from the participant's medical records:

- Survival status
- ICU and hospital length of stay
- Blood product requirements since enrolment

Laboratory data at baseline

The following data were collected from routine medical records and the result closest to the point of enrolment was used:

- Full blood count (FBC), Urea and electrolytes (U&Es), Liver Function Tests (LFTs), bone profile (calcium, phosphate), CRP
- Arterial oxygen tension (PaO_2) and oxygen saturation (SaO_2) with corresponding FiO_2

Research samples

Peripheral blood was collected at baseline and at the follow up visit for progenitor research assays and measurement of reticulocyte count, erythropoietin (EPO) and serum ferritin, iron and transferrin saturation. Samples for FBC, CRP, and U&Es were collected at the day 28 visit as these tests would not have been part of standard care. These were processed at OUH Biochemistry laboratories. In total, approximately 60 ml of peripheral blood was required at both these time points. Three samples from 4 healthy controls were also obtained at 4-weekly intervals.

4.3.6 Analysis of blood samples

An overview of the proposed experimental workflow is shown in **Figure 4.6.** At time of writing this thesis, extraction of CD34+ progenitor cells is complete. The next steps, which I intend to undertake in my postdoctoral training, are to isolate MEP cells. An established 8-colour fluorescent assisted cell-sorting (FACS) panel will be used to separate the CMP (common myeloid progenitor, CD123+ CD45RA-), MEP (megakaryocytic erythroid progenitor, CD45RA- CD123-) and GMP (granulocyte-monocyte progenitor, CD123+ CD45RA+) sub-populations from the CD34+ enriched cells. Single MEPs will then be index sorted into 96-well plates and cultured in MethoCult, a semi-solid methylcellulose-based growth and differentiation medium. Colony type can be identified visually at 14 days and the proportions of erythroid (BFU-E) and myeloid colonies (CFU-GM and GEMM) will be scored per individual.

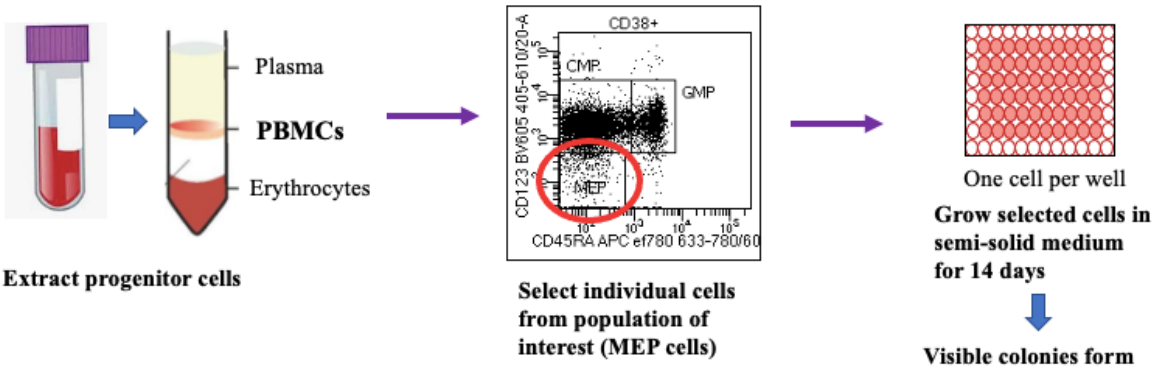


Figure 4.6 Current experimental plan. Progenitor cells are extracted from peripheral blood first. These then undergo single cell sorting with FACS with the target population being MEPs. These are then cultured in methylcellulose.

4.3.7 Statistical analysis plan

The primary outcome measures are:

(a) the number of CD34+ cells extracted per participant and;

(b) the proportion of participants with >1 x 10^5 CD34+ cells extracted from peripheral blood.

Previous work using the progenitor research assay indicates that this is the minimum number of cells sufficient to allow the distribution of cells in the sub-compartments giving rise to erythroid and megakaryotic lineages to be analysed.

The secondary objective, to determine the frequency and function of the megakaryocyte erythroid progenitor (MEP), will be evaluated by observing the colony forming potential of the MEP, with a focus on erythroid output. BFU-E colonies will be scored per individual according to established criteria and presented as mean (standard error of the mean (SEM)) (426). Where possible, complete case analysis will be carried out at the two time points. Reasons for missing data, particularly at the second time point, will be explored and reported in the analysis.

Descriptive characteristics were used to describe the demographic and clinical characteristics using mean and standard deviations (or medians and interquartile ranges) for continuous variables and numbers and percentages for binary or categorical variables. The paired t-test or Wilcoxon Signed Rank test were used as appropriate. Statistical significance was defined as $p < 0.05$. Data were entered into database software on a secure server (Microsoft Excel, Microsoft Corporation, Redmond, WA, USA) and transferred onto statistical software for analysis (STATA 14, College Station, Texas, USA).

4.3.8 Results

Thirty participants were enrolled between 19th March 2019 and 26th February 2020. Paired samples were available for 17 participants. The last follow-up visit occurred on 16th March 2020. Four participants were not followed up due to social distancing restrictions that were in place during the first wave of COVID-19. CD34+ cells from all peripheral samples have been extracted and are currently stored in liquid nitrogen in the WIMM.

Clinical characteristics and laboratory data at enrolment for all included patients (n = 30) and for whom paired data were available (n = 17) are displayed in **Tables 4.6 and 4.7**, respectively. Reasons for a missing paired sample at the follow-up time point were: died before follow-up visit (n = 4); participant declined (n = 4); not followed due to COVID-19 restrictions (n = 4); repatriated out of area (n = 1)

Table 4.7 Baseline characteristics of study cohort (n = 30)

Characteristic	Value
Age (years), median (IQR)	55 (46 - 60)
Sex, n (%)	
Male / Female	18 (60) / 12 (40)
BMI (kg/m^2), median (IQR)	26.1 (23.1 - 33.0)
Charlson Comorbidity Index, n (%)	
0	10 (33.3)
1	7 (23.3)
>2	13 (43.4)
APACHE II score, mean (SD)	21.2 (7.1)
Admission diagnoses, n (%)	
Elective operation	2 (6.7)
Emergency operation	13 (43.3)
Medical	15 (50)
No. with septic shock, n (%)	12 (40)
Organ support requirements, n (%)	
Advanced respiratory support	28 (93.3)
Advanced cardiovascular support	12 (40)
Advanced renal support	5 (16.7)
PaO_2 / FiO_2 ratio (mmHg)	159.5 (128 – 233)
Baseline laboratory parameters (Normal range), median (IQR)	
Haemoglobin (120- 170), g/l	90 (76 – 103)
White cell count (4.0 – 11.0), x 10^9/l	13.9 (11.4 – 17.58)
Platelet count (150 – 400), x 10^9/l	219 (128 – 296)
Reticulocyte A (0.04 – 0.12), x 10^{12}/l	0.04 (0.02 – 0.06)
Reticulocyte % (0.5 – 2.5)	1.45 (1.05 – 1.95)
Creatinine (49.0 – 104), umol/l	87 (64 – 152)
Albumin (32 – 50), g/l	19 (16 – 22)
CRP (0 – 5), mg/l	226.3 (144 – 364.4)
Ferritin (10 – 300), mcg/l	642.3 (393.2 – 2057.5)
Iron (11 – 31), mmol/l	3.65 (2.6 – 5.55)
Tsat (16 – 50), %	15 (9.5 – 25)
Transferrin (1.8 – 3.6), g/l	0.99 (1.1 – 1.4)
EPO (5.0 – 25.0), IU/l	44.6 (22.2 – 65.4)
B_{12} (180 – 900), ng/l	820 (556 – 1895)
Folate (3.0 – 20.0), mcg/l	4.35 (3 – 5.4)
Vitamin D (50 – 250), nmol/l	13 (20.5 – 35)
ICU mortality, n (%)	2 (6.7%)
ICU length of stay (days), median (IQR)	11.5 (7 – 18)
Time between ICU admission and 1st sample (days), median (IQR)	3 (3 – 6)
Time between 1st sample and follow-up sample (days), median (IQR)	26 (20 – 68)

APACHEII, Acute Physiology and Chronic Health Evaluation; BMI, Body mass index; CRP, C-reactive protein; EPO, erythropoietin; ICU, Intensive care unit; IQR, interquartile range; SD, standard deviation

Table 4.8 Baseline clinical characteristics of study cohort with paired samples (n = 17)

Characteristic	Value
Age (years), median (IQR)	58 (50 – 63)
Sex, n (%)	
Male	8 (47.0)
Female	9 (53.0)
BMI (kg/m^2)	27.0 (23.5 – 35.9)
Charlson Comorbidity Index, n (%)	
0	5 (29.5)
1	4 (23.5)
>2	8 (47)
APACHEII score, mean (SD)	21.8 (6.1)
Admission diagnoses, n (%)	
Elective operation	0(0.0)
Emergency operation	9 (53.0)
Medical	8 (47.0)
No. with septic shock, n (%)	8 (47.0)
Organ support requirements, n (%)	
Advanced respiratory support	16 (94.1)
Advanced cardiovascular support	9 (53.0)
Advanced renal support	13 (76.4)
PaO_2 / FiO_2 ratio (mmHg), median (IQR)	160 (128 – 248)
ICU length of stay (days), median (IQR)	9 (6 – 17)

APACHEII, Acute Physiology and Chronic Health Evaluation; BMI, Body mass index; ICU, Intensive care unit; IQR, interquartile range; SD, standard deviation

The median (IQR) age was 55 (46-60) years, and 18 (60%) participants were male. Thirteen participants had 2 or more co-morbidities. Mean (SD) APACHEII score was 21.1 (7.1) suggestive of high illness severity. Nearly all participants were receiving invasive mechanical ventilation at the time of enrolment. Median (IQR) time between ICU admission and collection of first sample was 3 (3-6) days and median (IQR) time between first sample and follow-up sample was 26 (20-28) days. Participants displayed the hallmarks of the anaemia of inflammation as evidenced by high CRP and ferritin concentrations, coupled with low serum iron and Tsat concentrations (**Table 4.7**).

Differences in haematological, inflammatory and iron markers between enrolment and follow-up time points are displayed in **Table 4.9.** Reticulocyte counts and serum iron, transferrin and vitamin D concentrations were statistically significantly higher at follow-up, whereas CRP, creatinine and white cell count concentrations were statistically significantly lower. Median (IQR) Hb was 82.5 (73-106.5) g/l at follow-up.

Table 4.9 Laboratory data at baseline and follow-up for participants with complete data (n = 17). All values are median (IQR).

Laboratory parameters (Normal range)	Baseline	Day 28 follow-up	p value
Haemoglobin (120- 170), g/l	95 (76 – 117)	82.5 (73 – 106.5)	0.2406
White cell count (4.0 – 11.0), x 10^9/l	13.96 (11.56 – 16.1)	10.5 (7.9 – 12.7)	0.02
Platelet count (150 – 400), x 10^9/l	195 (134 – 273)	349 (246 – 490)	0.007
Reticulocyte A (0.04 – 0.12), x 10^{12}/l	0.039 (0.027 – 0.06)	0.05 (0.03 – 0.14)	0.02
Reticulocyte % (0.5 – 2.5)	1.2 (0.8 – 1.5)	2.5 (1.3 – 4.7)	0.02
Creatinine (49.0 – 104), umol/l	111 (71 – 158)	73 (56 – 113)	0.04
Albumin (32 – 50), g/l	19 (16 – 23)	24 (22 – 34)	0.18
CRP (0 – 5), mg/l	274.5 (144 – 382.2)	26.2 (7.9 – 66.1)	0.0002
Ferritin (10 – 300), mcg/l	675.9 (400.6 – 3416.5)	1073.1 (416.9 – 1737.9)	0.567
Iron (11 – 31), mmol/l	4.3 (2.5 – 5.6)	8.2 (3.7 – 11.05)	0.026
Tsat (16 – 50), %	15 (12 - 30)	19 (14 – 28)	0.634
Transferrin (1.8 – 3.6), g/l	1.06 (0.99 – 1.28)	1.6 (1.3 – 2.2)	0.001
EPO (5.0 – 25.0), IU/l	28.4 (20.2 – 63.8)	18.8 (11.2 – 58.3)	0.521
B_{12} (180 – 900), ng/l	783 (602 – 2000)	650 (447 – 1137.5)	0.2391
Folate (3.0 – 20.0), mcg/l	4.6 (3.2 – 5.4)	8.9 (4.7 – 11.8)	0.112
Vitamin D (50 – 250), nmol/l	20 (13 – 33)	32.5 (29 – 46)	0.015

CRP, C-reactive protein; EPO, erythropoietin

4.3.9 Preliminary interpretation of findings

Taken together, these preliminary data support the internal validity of the study as I have captured a sick cohort of patients whilst also ensuring that samples were collected within the pre-specified time frames. The remaining work will be undertaken as part of my postdoctoral training.

Chapter 5 The INtravenous Iron to Treat Anaemia following CriTical care (INTACT) trial: Introduction and Methods

Sections this chapter have, in part, been published in:

Shah A, Marian I, Dutton SJ, et al. INtravenous Iron to Treat Anaemia following CriTical care (INTACT): A protocol for a feasibility randomised controlled trial. *Journal of the Intensive Care Society* 2019; https://doi.org/10.1177%2F1751143719870080

5.1 Introduction

This chapter covers the design and justification for an open-label, feasibility, parallel group, RCT of intravenous iron to treat moderate and severe anaemia in ICU survivors.

5.1.1 The need for a trial

As described in the previous chapters of this thesis, anaemia is common in survivors of critical illness and associated with poor HRQoL. There are no high-quality data on whether treating anaemia improves HRQoL and other patient-centred outcomes in this patient population. A large multicentre RCT is needed to answer this question but the potential benefits and risks of iron supplementation remain unclear. The findings of Chapters 2, 3 and 4 support the hypothesis that the recovery phase of critical illness may be a more suitable time point to investigate the efficacy and safety of intravenous iron

The European Society of Intensive Care Medicine has highlighted this area as a research priority (427) and stated that, *"future research should focus on the identification of patients most likely to develop an erythropoietic response to iron therapy along with the optimal route, dose, and timing of administration. Trials should be adequately powered to detect changes in patient-centred and functional outcomes, such as fatigue and quality of life, with adequate long-term follow-up and assessment of safety end points such as infection."*

The aims of INTACT were to address some of these uncertainties and to collect feasibility data to help inform the design and sample size calculations for a future definitive RCT. Feasibility was chosen because there are distinct challenges to conducting trials in survivors of critical illness (428).

My main concerns were:

- Loss to follow-up – this may arise from death or from difficulties in contacting participants. One-year mortality in ICU survivors ranges from 10.9% (8) to 21% (429). Retention rates in ICU survivors studies range from 32% to 100% (430) and participants lost to follow-up are likely to have important cognitive and/or physical impairments (428). Feasibility data on loss to follow up rates would help inform the sample size calculations of any future trial to ensure that there will be adequate numbers of patients to assess at relevant follow-up timepoints.
- Missing data points – as highlighted previously, many survivors of critical illness experience new or worse physical, psychological and cognitive impairments after hospitalisation. Whilst these patients may not necessarily be lost to follow-up, they may find it difficult to participate in longitudinal studies involving multiple evaluations. Missing data from incomplete study visits may lead to decreased precision and statistical power.
- Organisational factors – recent qualitative research has found that the most commonly cited barrier to conducting critical care research was insufficient human and financial resources, leading to difficulties in recruitment (431). Clinical uncertainty, lack of equipoise and engaging clinical teams were also identified as potential barriers to recruitment.

A feasibility study asks ‘whether something can be done, should we proceed with it, and if so, how’ (432). A recent analysis found that feasibility studies can be useful at avoiding waste and minimise of the risk of funding of more expensive definitive trials (433). However, this was offset by a time delay of approximately 10 months between completion of the feasibility study and application for a full trial.

5.1.2 Objectives

The primary objective of INTACT was to assess the feasibility of a future, adequately powered, multicentre RCT of using intravenous iron to treat anaemia in patients recovering from critical illness.

The secondary objective was to collect clinical, laboratory and HRQoL data to inform the sample size calculations and outcome measures for any future RCT. These data would also help to understand changes in Hb, iron profiles and inflammation during the recovery phase of critical illness.

5.2 Methods

5.2.1 Trial design

An open-label, feasibility, parallel group RCT with 1:1 randomisation to either the intravenous iron, in addition to usual medical care, or usual medical care alone. The RCT was co-ordinated by the Oxford Clinical Trials Research Unit (OCTRU) and sponsored by The University of Oxford. The study flow chart is outlined in **Figure 5.1** and the trial was conducted across four ICUs at three NHS sites (Adult and Churchill Intensive Care Units, Oxford University Hospitals NHS Foundation Trust; Critical Care Unit, Royal Infirmary Edinburgh NHS Lothian; Intensive Care Unit, Royal Berkshire Hospital NHS Foundation Trust).

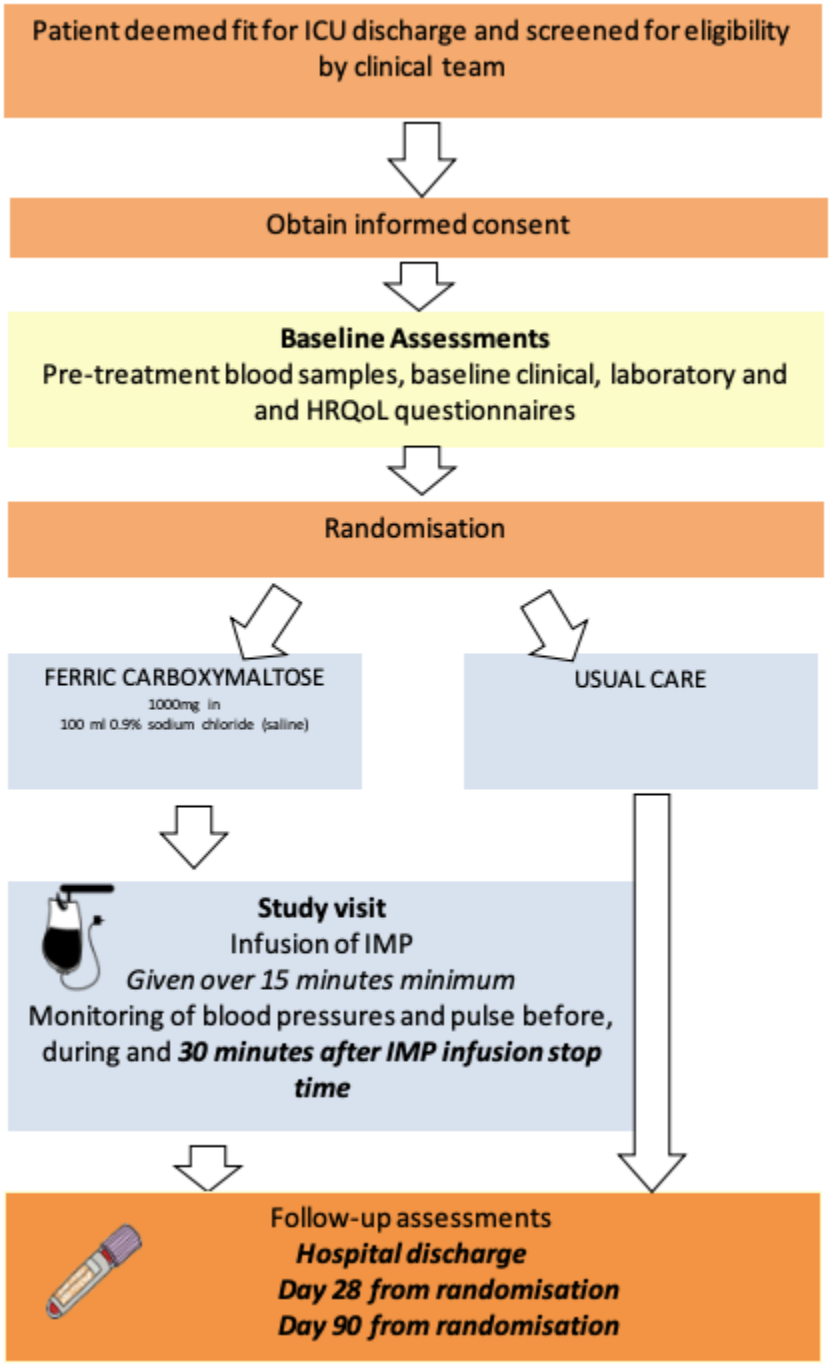


Figure 5.1 INTACT Study flow chart

HRQoL, Health-related quality of life; ICU, Intensive care unit; IMP, Investigative Medicinal Product

5.2.2 Patient and public involvement

I presented the INTACT trial proposal to our local Critical Care Patient Forum Group which consists of ICU survivors. They were a diverse group, with ages ranging from 21 to 75 years, both sexes, and with a broad range of medical problems causing their ICU stay. I explained the prevalence and causes of

anaemia during critical illness and highlighted the evidence gap in management of anaemia after intensive care. All of them complained about excessive blood sampling whilst they were in ICU. They all mentioned the overwhelming sense of exhaustion and fatigue they felt in the weeks after discharge from ICU. None were aware of being told that they were anaemic when they eventually went home, or that it was a possible cause of their fatigue. A particular point they raised was that if there is a potentially simple treatment option like intravenous iron, why doesn't everyone get it? Hence, they were strongly supportive of my proposed research. The patient forum group helped with the development of participant information leaflets, consent forms and advised on certain aspects of the design of INTACT such as blinding (Section 5.2.6), questionnaire burden and study follow-up procedures (Section 5.2.9).

5.2.3 Ethical and regulatory approval

A favourable ethical opinion was obtained from the South Central – Berkshire B Research Ethics Committee on 07 July 2018 (18/SC/0308). According to the Medicines and Healthcare products Regulatory Agency (MHRA), the study was categorised as type A (no higher than the risk of standard care). Clinical Trial Authorisation from the MHRA was received on 17 July 2018. Health Research Authority approval was obtained on 20 July 2018. INTACT required one non-substantial and two substantial amendments. These are summarised in **Table 5.1**.

The study protocol has been published previously (434) and I prospectively registered the trial on:

- International Standard Randomised Controlled Trial Number (ISRCTN): ISRCTN13721808
- European Clinical Trials Database (EudraCT): 2018-000767-91

INTACT was funded by an NIHR Doctoral Research Fellowship (NIHR-DRF-2017-10-094). There was no external industry involvement, and all study drugs were procured through local NHS commissioning arrangements.

Table 5.1 History of protocol amendments

Amendment No	Protocol version	Date issued by HRA/MHRA	Details of changes made
1	V2.0	30 Jul 2018	Addition of pregnancy testing to the protocol and PIL, specification of timeframe (24 hours) for notification of SAE to CI, and mention of heart rate and blood pressure monitoring before, during and after drug administration
2	V3.0	18 Apr 2019	Revision of inclusion and exclusion criteria, including definition of active infection. Greater clarity on recruitment process. Dose adjustment for participants weighing less than 50 kg. Addition of telephone follow-up. Addition of Royal Berkshire Hospital as a recruitment site.
3	V4.0	09 Dec 2019	Changes in the wording in Section 4.8 of the Investigative Medicinal Product Summary of Product Characteristics. These did not affect the conduct or safety of the trial.

CI, Chief Investigator; HRA, Health Research Authority; MHRA, Medicines and Healthcare products Regulatory Agency; PIL, Participant Information Leaflet; SAE, Serious Adverse Event

5.2.4 Participant selection

The study inclusion and exclusion criteria were designed to include participants who reflect a general population of survivors of critical illness with moderate (Hb 80–100 g/l) and severe (Hb <80 g/l) anaemia. I aimed to enrich the trial population by opting for a Hb threshold of ≤100 g/l. This was based on previous research, in both critically and non-critically ill patients, which has found associations between this threshold and persisting anaemia (23), high levels of fatigue (27), poor mobility (435) and increased hospital length of stay. In addition, intravenous iron is likely to be more efficacious when the Hb is being corrected from lower concentrations when compared with higher and/or normal Hb concentrations (436).

The study inclusion criteria at the start of the trial were:

- Adult ICU/HDU (Level 2 or 3) stay for ≥48 hours and now deemed fit for discharge from the ICU/HDU by the attending physician
- Last measured laboratory haemoglobin ≤100 g/l prior to being deemed fit for discharge from the ICU/HDU
- Participant is willing and able to provide written informed consent

Study exclusion criteria were:

- Planned palliative care or home ventilation
- Primary neurological diagnosis
- Known hypersensitivity to iron
- Acute infection - non-resolving temperature ≥38°C within the past 24 hours or patient on non-prophylactic antibiotics
- Patients residing outside a reasonable geographic follow-up area
- Immunosuppressive therapy for organ transplant
- Intravenous iron or erythropoietin in the previous 4 weeks
- Weight ≤50kg
- Pregnancy (however, breastfeeding is not an exclusion criteria)
- Personal or family history of iron overload disorders such as haemachromatosis or previously documented ferritin >1200 ng/ml and/or transferrin saturation >50%
- History of severe asthma, eczema, or other atopic allergy
- Chronic liver disease and/or screening Alanine Transferase / Aspartate Transferase x3 above upper limit of normal range
- Haemodialysis dependent chronic kidney disease
- Already enrolled into another trial where the trial protocol explicitly prohibits co-enrolment
- Requirement for English translation

The exclusion criteria were designed to exclude patients who would be unlikely to benefit or would be at increased risk of adverse events from intravenous iron.

Co-enrolment

As this trial was being undertaken in three research-active institutions, I anticipated that co-enrolment with other research studies may impact recruitment. I was concerned that patients may have already been enrolled into studies whilst being acutely unwell in the ICU, and that this may negatively impact recruitment into INTACT.

Critical care research activity has increased significantly over the past decade. In 2018/19, 83% of NHS trusts with ICUs recruited into studies on the NIHR Clinical Research Network portfolio. This expansion in activity may lead to research saturation, competition between studies and enrolment bias – all of which may have detrimental effects on the validity of findings. Other potential risks of co-enrolment include effects of potential biological interactions, adverse events rates, protocol compliance, and potentially increased burdens for patients or their surrogate decision-makers. However, there are many advantages for co-enrolment as evidenced by the Coronavirus Disease-19 RECOVERY (437) and REMAP-CAP trials (438). Advantages include:

- Improved study efficiency by allowing research to complete to time and target
- Opportunities for participants to be exposed to multiple novel treatments and procedures
- Development and maintenance of network collaborations

Published evidence from critical care trials suggests that co-enrolment has no negative impact on trial results and is safe, acceptable and feasible (439). I decided that participants enrolled into INTACT could be co-enrolled into or from other trials and that this would be reviewed in an individual participant basis by a member of the research team. At site initiation visits, I worked with the local research teams to identify the studies that were already recruiting at their sites and to set up co-enrolment agreements. I adhered to guidance from the UK Intensive Care Society (440) and used the criteria outlined in **Table 5.2** when considering co-enrolment (441):

Table 5.2 Criteria to be used when assessing whether co-enrolment in a proposed trial (Trial B) should be allowed for patients already enrolled in a trial that is underway (Trial A)

Criterion	Description
Biological interaction between intervention	There should be little or no plausible biological interaction between the experimental interventions in the two trials
Effects of protocol compliance and concurrent care	There should be little likely influence on Trial A protocol compliance or intercurrent care resulting from enrolment in Trial B
Equal allocation to trial groups	The treatment groups in Trial A should have equal chances of being allocated to the treatment groups in Trial B.
Treatment restrictions	Any protocol-mandated treatment restrictions in the intervention or control group of Trial B should not alter the treatment of either group in Trial A.
Mandated intercurrent treatments	Intercurrent treatment requirements in the control or intervention groups of Trial B should not substantially alter the treatments of patients in Trial A.
Outcome ascertainment	Ascertainment of outcomes in Trial B should not affect the outcomes in Trial A.
Clinical decision making	Information collected in Trial B that would not otherwise have been collected, and that is contemporaneously available, should not alter clinician decision making in any way that might affect the interventions, intercurrent care or outcomes of Trial A.
Adverse event procedures	The procedure for dealing with an adverse event in Trial B should not have an impact on the conduct of Trial A.

Using these criteria, I successfully set up agreements and agreed a policy for co-enrolment with the following NIHR-funded multicentre studies:

- Vaccination for immune recovery following sepsis (ISRCTN11401066)
- Biomarker-guided duration of antibiotic treatment for sepsis (ISRCTN47473244)
- Desmopressin for procedures or radiological interventions (ISRCTN12845429)

Changes to participant inclusion and exclusion criteria

Recruitment to the trial was initially slow and following review of the study screening logs, the Trial Management Group was felt that some of the inclusion and exclusion criteria were overly restrictive.

These were modified with input from the Trial Oversight Group, without affecting the scientific validity of the trial, and submitted as part of a substantial amendment in April 2019. This was approved in May 2019. The changes are described below in **Table 5.3**.

Table 5.3 Summary of changes to eligibility criteria following implementation of a substantial amendment in May 2019

Original Inclusion criteria	**Update post-amendment**
Adult ICU/HDU (Level 2 or 3) for ≥48 hours and now deemed fit for discharge from the ICU/HDU by the attending physician	Adult ICU/HDU (Level 2 or 3) reduced to for ≥24 hours

Original Exclusion criteria	**Update post-amendment**
Acute infection – non-resolving temperature ≥ 38°C within the past 24 hours or patient on non-prophylactic antibiotics	Acute infection – as judged by the treating clinician (e.g. ongoing bacteraemia or non-resolving sepsis) or patient expected to be on non-prophylactic antibiotics for greater than 14 days
Patients residing outside a reasonable geographic follow-up area	Removed to explore feasibility of telephone-only follow-up
Weight ≤ 50 kg	Dose adjusted to weight-based formula.
Chronic liver disease and/or screening Alanine Transferase / Aspartate Transferase x3 above upper limit of normal range	Known severe chronic liver disease (e.g. Child-Pugh C or above)

HDU, High dependency unit; ICU, Intensive care unit

5.2.5 Consent

The consent process for any clinical research study requires careful consideration and accordance with the applicable regulatory requirements from the following bodies:

- International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use – Good Clinical Practice
- The Declaration of Helsinki

- Medicines for Human Use (Clinical Trials) Regulations 2004
- Mental Capacity Act

Many critical care trials will enrol patients that are likely to be incapacitated at the time of screening for reasons such as mechanical ventilation and/or sedation. However, I anticipated that most potentially eligible participants would have capacity at the time of enrolment. Capacity would be assessed an appropriate member of the patient's clinical team who will have had previous experience and training in making such assessments. A participant who has capacity must be able to:

- Understand the information relevant to the decision
- Retain that information
- Use or weigh up that information as part of the process of making the decision

For consent to be considered both legal and ethical it must be:

- Given by a person with capacity
- Given voluntarily, with no undue influence
- Given by someone who has been adequately informed
- A fair choice

Any participants that were deemed potentially suitable were asked by a member of the clinical team if a member of the research team could discuss study participation with them. The clinical team would also provide the participant with a PIL. Potential participants could be approached anytime between ICU and hospital discharge. This would minimise loss of potential participants who may been discharged out of hours or during the weekend. Potential participants may also wish to take time to review the PIL and discuss the study with their family or carers. All potentially eligible participants were entered into a screening log which was kept in the investigator site file.

5.2.6 Randomisation and blinding

Randomisation aims to create two groups which are comparable for known, or unknown confounding factors, to allow for a reliable assessment of a treatment effect (442). Eligible participants were randomised to a 1:1 ratio to receive either the intravenous iron or usual medical care using minimisation on a secure web-based system controlled and managed by OCTRU.

Minimisation is a method of adaptive stratified sampling used in clinical trials to ensure excellent balance between study groups for many prognostic factors, even in small studies (443). The decision to include minimisation into the study protocol ensures that investigators have thought about prognostic factors from the outset. Examples of minimisation variables include age, sex, ethnicity, smoking status, disease severity and study site. The treatment allocated to the next participant enrolled in the trial depends on the characteristics of the participants already enrolled, with the overall aim of ensuring that each allocation should minimise any imbalance across multiple factors. However, one of the limitations of this process is that the allocation is not random and can therefore be predicted. This can be addressed by weighting the randomisation (e.g. by 80%) with a random element towards the minimisation favoured treatment group for that participant. This involves a trade-off because the random element may worsen the overall imbalance between the groups, but the balance for the chose variables will be better than with simple randomisation. The random element also makes the next allocation less unpredictable (444).

I decided to include the following minimisation variables for INTACT - anaemia severity (Hb <80 g/l vs. 80–100 g/l) and ICU LOS (<7 days vs. ≥7 days). ICU LOS was determined as the day of being medically stable for discharge from critical care as it is not uncommon for patients to spend an extra day or two whilst awaiting a bed on the medical or surgical wards. These were chosen on the basis of the findings of previous observational studies which have identified anaemia severity at ICU discharge and prolonged ICU LOS as predictors of persisting anaemia following critical care discharge (23, 25, 26, 46). The number of participants required prior to start of the minimisation procedure was set at 10, and the random element was set at 80% (443). I opted not to minimise for study site as I considered clinical care in all three sites to be similar. This decision would be revisited for any future multicentre trial.

INTACT was an open-label trial with a usual care arm and no participant or study personnel blinding. Intravenous iron preparations can be challenging to blind due to their rusty brown colour. This can be overcome with the use of opaque infusion kits and barrier shields between the patient and the infusion kit, but these measures can significantly increase the costs of running a trial. In addition, it is not uncommon for patients to also experience a transient metallic taste during the course of an infusion and makes it impossible to blind patients to their allocated treatment.

A usual care arm could negatively impact participant recruitment and retention whilst also resulting in performance bias and reporting bias, particularly in the reporting of HRQoL measures. Changes in practice may occur through the Hawthorne effect. The Hawthorne effect refers to a tendency for people to change their behaviour in response to their awareness of being observed (445). Some of these limitations can be offset by ensuring outcome assessors are blinded to study allocation and to offer participants incentives to maintain study retention. Examples of incentives with some supporting evidence include financial incentives (e.g. gift vouchers) (446) or tokens such as study pens (447). The potential advantages of a usual care arm include minimising unnecessary procedures (e.g. cannulation and placebo administration), a control arm representative of 'real-world' practice, thereby increasing generalisability, and lower costs (448, 449). A recent meta-epidemiological study of 1153 trials found no evidence of an average difference in estimated treatment effects between trials with and without blinded patients, healthcare staff or outcome assessors (201). Other options include protocolised usual care or a specific standard care.

I discussed these issues with patient forum group. They didn't perceive the open-label design as a barrier to recruitment and stressed that many patients take part in research on the grounds of altruism. For potential participants who may be concerned about being randomised to the usual medical care arm, the group suggested to mention that 'this is what would have happened anyway' but to also stress the potential benefits of taking part in research in general. These may include extra monitoring and follow-up visits, that they may not necessarily get as part of usual care. It is now well-recognised that patients who are treated in research-active hospitals have better clinical outcomes (450).

The group also shared their own personal experiences of poor cognition, 'brain fog' and impaired memory in the early days and weeks after leaving the ICU. They had little/no recollection of what medications they received after being stepped down from critical care. Therefore, they felt that blinding wouldn't make a significant difference to many patients in terms of recollection and reporting HRQoL measures. A useful suggestion they had was to ask participants whether they could remember their study group allocation at the last follow-up visit of the study. Therefore, on balance, I opted to pursue an open-label design for the purposes of this feasibility study. This decision would be kept under review by the Trial Management Group during the course of the study and for any future RCT.

5.2.7 Intervention and comparator arms

Intervention

Participants randomised to the intervention arm received 1000mg of ferric carboxymaltose diluted in 100ml of 0.9% saline. This was administered as an intravenous infusion over a minimum of 15 minutes. Participants were closely monitored for signs of hypersensitivity during the infusion and for at least 30 minutes after the treatment by a trained member of the research team. The intervention could be administered at any time between ICU discharge and hospital discharge. I recorded the time frame between randomisation and administration of the intervention.

I opted for the intravenous route as it would bypass the hepcidin and inflammation-mediated restriction of enteral absorption of iron as described in Chapter 1 and allow for a more rapid correction of iron stores and/or anaemia. This has the potential to increase the speed of recovery from critical illness. Whilst oral iron is inexpensive, its gastrointestinal side effects such as constipation, nausea and vomiting would limit patient compliance.

I chose the dose of 1000mg for the following reasons:

- Safety – this is the maximal recommended dose within the current licensing indication, as listed in the Summary of Product Characteristics (SmPC). The risk of anaphylaxis is also rare.

- Ease of administration – one 20ml vial of ferric carboxymaltose contains 1000mg of ferric carboxymaltose. This minimises the risk of drug calculation errors.
- Single dosing – this dose is higher than that of other intravenous iron preparations (e.g. iron sucrose) and would avoid multiple dosing (135). This may be also be more preferable to patients.
- Supporting evidence – Published studies have shown this dose to be safely tolerated and more efficacious in treating anaemia, when compared with oral iron or no iron (148, 299, 451).

Usual medical care

Usual medical care consisted of routine ward-based care including observation with monitoring and RBC transfusion when required. Current UK guidelines recommend a restrictive transfusion policy, with the exception of certain groups of patients such as those with ischaemic heart disease (137). It is not routine practice to prescribe intravenous iron for patients recovering from critical illness at present. Any decision to commence iron therapy in this group would be at the discretion of the treating physician and independent of the study and would be recorded in the study case report forms (CRFs).

5.2.8 Study outcomes

The primary feasibility outcome measures for INTACT were:

- Recruitment and randomisation rates – overall and by study site
- Protocol adherence – defined as the number of participants allocated to the intervention who go on to receive the study drug
- Completion rates of HRQoL questionnaires

Secondary outcomes included:

- New nosocomial infection
- Mortality – in-hospital and at 90 days
- Post-ICU hospital LOS

- Changes in laboratory haematological and iron profiles from baseline to 28 and 90-days post-randomisation
- Changes in Multidimensional Fatigue Inventory-20 (MFI-20), Functional Assessment of Chronic Illness Therapy-Fatigue (FACIT-F) and EuroQol-5D-5L (EQ-5D-5L) questionnaire scores from baseline to 28 and 90-days post-randomisation.
- Healthcare resource use, including hospital readmissions

I defined nosocomial infection as 'commencement of new antibiotic therapy or escalation from prophylactic antibiotics for a confirmed or strongly suspected new infection'. This definition was felt to be pragmatic, easy to use at the bedside and has been used before in trials evaluating intravenous iron (170).

5.2.9 Data collection and follow-up procedures

Data that were collected as part of INTACT are listed in **Table 5.4**. Participants were followed up at two time points (28 and 90-days post-randomisation). This would provide an optimal balance between obtaining reliable scientific data on efficacy and safety, minimising participant burden and completing the study within a relevant time frame. Data were collected using paper CRFs and then transferred to a password-protected electronic data entry system managed by OCTRU. Paper CRFs were stored in the Investigator Site File under secure conditions.

Study-specific research blood samples were taken at three different study time points (baseline, 28- and 90-days post-randomisation). The volume of blood required was approximately 15ml. Samples were stored in the relevant local hospital's laboratories. On arrival at the laboratory, 600 µl of serum was aliquoted from the serum samples for non-standard assays (hepcidin, soluble transferrin receptor (sTfR)). These were frozen immediately at -80ºC for collection and further analysis at the Weatherall Institute of Molecular Medicine, using the methods I have previously described in Chapter 4. Standard assays (e.g. FBC, urea and electrolytes) were run according to usual laboratory processes.

I opted for three HRQoL questionnaires – one generic and two evaluating fatigue. I chose to focus on

fatigue as it is one of the cardinal symptoms of untreated anaemia and may be a barrier to recovery from critical illness. Survivors of critical illness have listed fatigue, along with lack of physical strength and decreased walking distance, as the three most important outcomes following critical illness (195). The ESICM have also called for more research into the epidemiology of fatigue and potential treatments in ICU survivors (452). These themes resonated with my conversations with the patient forum group who highlighted the overwhelming sense of fatigue they felt in the early days and weeks after being discharge from critical care and subsequently hospital. Whilst this fatigue is likely to be multifactorial, untreated anaemia may be a potentially modifiable factor. The questionnaires used were:

- EQ-5D-5L – this is a generic preference-based measure of health that is recommended by the National Institute for Health and Care Excellence (NICE) for health economic evaluations. It is also part of the core outcome measurement set for clinical research in acute respiratory failure survivors (453).
- FACIT-F – this is a subscale of a questionnaire originally developed to assess anaemia-related symptoms in patients with cancer (454). It consists of thirteen items and each item allows for five response options from 'Not at all' (score 4) to 'Very much' (scored 0). The scores are added, multiplied by 13 and then divided by the number of items answered to adjusted for missing data points. The final (raw) scores range from 0-52 with higher scores representing less fatigue. FACIT-F has been validated to assess fatigue in ICU survivors and may be better at capturing the negative aspects of fatigue (455). It has also been demonstrated to have adequate psychometric properties in assessing fatigue in other groups of patients with iron deficiency anaemia and various underlying conditions (456). Minimal important differences for fatigue for FACIT-F are also available and these may be used to derive the sample size calculations for a future definitive study, should the primary outcome be improvement in fatigue (457).
- MFI-20 – this is a 20-item self-reporting questionnaire designed to measure fatigue across five domains – general fatigue, physical fatigue, mental fatigue, reduced motivation and reduced activity. High scores indicate high levels of fatigue. It has been used previously in ICU survivors but has not been formally validated (27).

I piloted these questionnaires with the patient forum group and we found that approximately 15-20 minutes would be required to complete all three questionnaires. I proposed that participants could ask for help completing the questionnaires from relatives or the bedside nurse. The group felt that this was reasonable as many patients experience short-term problems with concentration and attention and that it may help minimise participant burden.

An important consideration was on determining a suitable method for assessing baseline scores of these HRQoL measures. As discussed previously in Chapter 1, baseline (or pre-ICU admission) HRQoL is now recognised an important determinant of post-ICU outcomes (10, 458). A reliable assessment of baseline HRQoL would be useful for understanding if this is a potential effect modifier and for informing health economic evaluations. In general, asking critically ill patients to complete such questionnaires can be problematic as they may be incapacitated or unable to provide a self-assessment of their health status at the time of randomisation. Potential solutions to this problem have included waiting until patients are well enough to complete HRQoL questionnaires, asking patients to retrospectively recall their HRQoL and the use of proxies such as next of kin or carers (459). However, these are heavily influenced by recall bias and the longer the length of the recall period, the more difficult it will be for patients to accurately recall their HRQoL.

I sought advice from a senior health economist, adhered to consensus recommendations (459) and decided on the following:

- The inclusion of participants who had capacity to consent into the trial does in part assume that they are relatively well enough or lucid to complete HRQoL questions
- Assessment of HRQoL would occur at the earliest time possible during the randomisation process, ideally pre-randomisation before the participant knew their allocation status as this was an unblinded study
- Baseline HRQoL would be deemed as on the day of enrolment/randomisation. I decided that the risk of recall bias was high in this group of patients and would lead to unreliable results about

pre-ICU HRQoL. For the purposes of INTACT, HRQoL would be affected by the reason for ICU admission but also by any beneficial effects of intravenous iron. Evaluating the change in HRQoL between randomisation and follow-up would more likely reflect the effect of intravenous iron than comparing with pre-ICU levels – although this would not pose a problem since participants are being randomised.

- Follow-up assessments would occur at 28 and 90 days

Follow-up procedures

In order to minimise loss to follow-up, I used the following strategies that have been used previously to achieve follow-up rates of over 80% (415, 428):

- All participants were invited to return to hospital or the ICU follow-up clinics that were part of routine care at the participating sites. The clinic visits usually coincided with the 90-day follow-up timepoint. Participants would be fully re-imbursed for travel and inconvenience. Where possible, either of these of follow-up visits could also be planned with any other outpatient appointments that the participant may have. A ±7-day window around each study timepoint was permitted to allow a degree of flexibility for participants and the research team.
- Home visits by a member of the research team for participants who were unable to attend follow-up visits. All institutions had lone working policies in place. Research staff who conducted these visits would be reimbursed for follow-up visits.
- Telephone only follow-up visits for participants that did not wish to attend clinic or have a home visit and for those who lived outside a reasonable geographic follow-up area. This was broadly determined as being more than 30 miles from the base hospital.

In addition, I set up multiple strategies for maintaining patient contact including regular reminders on the study database for when study visits were due, obtaining multiple contacts (e.g. email addresses) for each participant and one additional contact point (e.g. next of kin or close friend/relative).

Table 5.4. Timing of visits and data collection

Baseline (pre-randomisation)	• Confirmation with inclusion and exclusion criteria • Patient demographics (age, sex, weight) • Documented history of pre-existing anaemia prior to ICU admission • Charlson Co-morbidity Index • ICU admission – date and time of admission, ICU admission category, APACHE II score • ICU organ support – duration and requirement (advanced respiratory, cardiovascular and renal support using the Critical Care Minimum Dataset definitions) • Date and time of ICU discharge, ICU length of stay • Blood product administration (red cells, platelets, fresh frozen plasma, cryoprecipitate) • Hb – ICU admission and ICU discharge • Research blood samples (FBC, U&Es, LFTs, calcium, phosphate, iron profiles*, Vitamin D, EPO, hepcidin, sTfR • Last recorded PaO_2 and SaO_2 with corresponding FiO_2 time point closest to ICU discharge • Pregnancy test result (if indicated) • HRQoL questionnaires (EQ-5D-5L, MFI-20, FACIT-F)
Hospital discharge	• Nosocomial infection • Survival status • ICU readmissions (if any) • Total hospital length of stay • Post-ICU blood product administration • Adverse events
Day 28	• Research blood samples (FBC, U&Es, LFTs, calcium, phosphate, iron profiles*, Vitamin D, EPO, hepcidin, sTfR • HRQoL questionnaires (EQ-5D-5L, MFI-20, FACIT-F) • Bespoke questionnaires for participants use of healthcare resources • Adverse events
Day 90	• Research blood samples (same as day 28) • HRQoL questionnaires (EQ-5D-5L, MFI-20, FACIT-F) • Bespoke questionnaires for participants use of healthcare • Adverse events

*includes ferritin, serum iron, transferrin and transferrin saturation (%)

APACHEII, Acute Physiology and Chronic Health Evaluation II; EQ-5D-5L, EuroQol-5Dimension-5Level; EPO, erythropoietin; FACIT-F, Functional Assessment of Chronic Illness Therapy-Fatigue; FBC, Full Blood Count; Hb, Haemoglobin; LFTs; HRQoL, Health-related quality of life; ICU, Intensive Care Unit, Liver Function Tests; MFI-20, Multidimensional Fatigue Inventory-20; sTfR, soluble transferrin receptor; U&Es, Urea and Electrolytes

5.2.10 Safety reporting

Ferric carboxymaltose is a drug with a well-known safety profile. Adverse reactions that are common ((≥1/100 to <1/10) and may be expected to occur include headache, dizziness, hypertension, nausea and metallic taste. If these were not serious, I decided that they should not be reported as part of the trial as they will not improve the knowledge of the safety profile of the drug. They would still be recorded in the medical notes. Survivors of critical illness are at high risk of developing adverse events (AEs) or serious AEs (SAEs) secondary to their underlying disease process, the expected complications of critical illness (e.g., hospital readmission) and pre-existing health conditions.

Overall, I decided that only SAEs that may reasonably be a consequence of participation in the trial and were judged by a member of the research team to be not due to the underlying disease or expected complications of critical illness would be reported. In addition, the following rare events, if serious would also be reported on a trial specific SAE form:

- Anaphylactic/anaphylactoid reactions
- Loss of consciousness or syncope
- Bronchospasm
- Angioedema and facial oedema
- Severe anaemia (Hb <80 g/l) at either study follow-up time points

I did not include infection in this as it is already being reported as a secondary outcome measure. In the event of an SAE, an SAE form would be scanned and emailed within 24 hours to the trial specific inbox. I would then review the SAE and raise any queries as appropriate. The SAE would also be reported to the Chair of the Trial Oversight Group within 24 hours of the trial team being aware. Causality between adverse events and the administration of ferric carboxymaltose would be a clinical

decision made by the local PI based on all available information. The following definitions would be used:

- Related - the adverse event follows a reasonable temporal sequence from trial medication administration. It cannot reasonably be attributed to any other cause
- Unrelated - the adverse event is probably produced by the participant's clinical state or by other modes of therapy administered to the participant

5.2.11 Trial monitoring

I developed a trial monitoring plan with OCTRU which complied with the principles of Good Clinical Practice. As this was small study, my supervisors, OCTRU and I decided that there was no need for a formal Data Monitoring Committee. Instead, we opted for a Trial Oversight Group which would include independent clinicians experienced in the fields of clinical trials, intensive care and haematology and an independent statistician. The oversight group would work closely with the Trial Management Group and provide overall supervision of the study to ensure it is being conducted in accordance with GCP. I devised charters for both groups which included details of the members, roles, responsibilities and planned methods of functioning.

The Trial Management Group would be responsible for the day to day running and management of the trial. A core team, which included two other senior investigators (SS, SM) and myself, aimed to meet at least two weekly during the planning and conduct of the trial. The wider team aimed to meet initially 3 months after trial commencement and less frequently after depending on the progress of the trial. OCTRU conducted a one-off audit of the study from in December 2019 in accordance with the trial monitoring plan.

5.3 Statistical analysis plan

I developed a statistical analysis plan with OCTRU statisticians (IM, SD). The key considerations are described in this section. Recruitment and follow-up rates were the main drivers for the feasibility design on the basis that unless reasonable rates can be achieved no definitive trial will be possible. As this was a feasibility study not aiming to assess treatment effects, we did not undertake a formal power

calculation. However, I aimed to recruit a sufficient number of participants to provide reasonable estimates of the standard deviation that would be required for the sample size estimations for a definitive trial.

Using local case mix data for the John Radcliffe Hospital, which would be the main recruitment centre), approximately 800 patients are admitted annually. One hundred and twenty patients would be elective postoperative admissions who would be expected to stay less than 48 hours. Of the remaining 680 patients, assuming unit mortality of 14%, 584 would be expected to survive ICU. Based on pretrial work and previous studies, 50% (292 patients) would leave ICU with a Hb of ≤100 g.l-1. Assuming a conservative estimate of 30-40% enrolment, we expected to enrol on average 100 participants/year (approximately 2 participants per week) at this site. In addition, I also anticipated recruiting an additional 20 participants from the other two sites (Edinburgh Royal Infirmary, Royal Berkshire Hospital). The planned recruitment period was 52 weeks. Therefore, I aimed to enrol up to 130 participants, which would also ensure that I could obtain outcome data for a minimum of 70 participants in order to provide a reasonable estimate of the standard deviation for the sample size estimation for a definitive trial (460).

Recruitment rate was calculated by the number of participants randomised as a proportion of the total number of eligible patients. The protocol adherence rate was calculated as the number of participants allocated to the intervention who received the study drug over the number of participants allocated to the intervention. HRQoL questionnaire completion rates were calculated as the number completed at each time point over the number expected (total randomised less those participants who died). Rates, together with 95% CIs were estimated based on data collected are reported.

I prespecified a 'traffic light' system (**Table 5.5)** that would be used to guide progression for a definitive trial (461). I chose a minimum threshold of 60% for completion of outcome data. This is based on data from the social science literature (462), but no such guidance exists for critical care trials.

Table 5.5 Traffic light system to determine progression to a definitive trial.

	Recruitment	Protocol adherence	Outcome data
Green (Go)	8-11 participants per month across two centres	< 5% failure to receive allocated intervention in the IV iron group	At least 80% of survivors will provide follow-up data at 90 days
Amber (Amend)	4-8 participants per month	5-10% failure to receive allocated intervention in the IV iron group	60-80% of survivors will provide follow-up data at 90 days
Red (Stop)	Less than 4 patients per month	>10% failure to receive allocated intervention in the IV iron group	50-60% of survivors will provide follow-up data at 90 days

IV, intravenous

Descriptive characteristics were used to describe the demographic and clinical characteristics between the two groups at baseline using mean and standard deviations (or medians and interquartile ranges) for continuous variables and numbers and percentages for binary or categorical variables. All clinical and laboratory outcomes were analysed on the intention-to-treat population on available data. No missing data were imputed. The models adjusted for the stratification factors - haemoglobin and ICU LOS at time of randomisation, as continuous variables. Treatment estimates, 95% CIs and p-values are reported. No sensitivity analysis was undertaken for any of the clinical or laboratory outcomes. Analyses were undertaken using Stata version 15.1 (www.stata.com).

A multilevel mixed-effects model was undertaken to analyse the repeated key secondary HRQoL (EQ-5D-5L Visual Analogue Scale and Utilities, FACIT-F, MFI-20) and the laboratory (haemoglobin, ferritin, serum iron, Tsat, transferrin, erythropoietin, phosphate, CRP, vitamin D, hepcidin and sTfR)) outcomes at 28- and 90-days post-randomisation. The model included robust standard errors based on hospitals as

clusters, a time-treatment interaction with time as a categorical variable and was adjusted for the baseline values of the outcome (if appropriate) and stratification factors (Hb and ICU LOS at randomisation as continuous variables).

A multilevel model was chosen to analyse the clustered data that would arise from this study. Clustered data violate the assumption of independence, that is the value of one observation could be influenced by the value of another. In other words, observations in a cluster tend to be more similar to each other than to individuals in the rest of the sample. Clustered data can arise from three sources:

- Repeated measures in longitudinal studies where the clusters are the subjects. Repeated measures on the same participants will be more similar to each other than to measures on another participant
- Multiple measures on the same participant
- Studies where the participants are grouped such as cluster RCTs, family studies and cluster sampling surveys.

It is important to account for clustering otherwise the standard errors will be too small. This will lead to very narrow CIs and very small p-values. Multilevel (or random effects) models allow the average response to vary between clusters by modifying the linear predictors to include an amount that varies randomly between clusters. Multilevel modelling is the generally accepted method for analysing clustered data, but other methods include calculating summary measures and analysing these measures using conventional statistics, using robust standard errors to correct standard errors for the clustering and using generalised estimating equations.

I decided to undertake post-hoc exploratory, hypothesis-generating analyses to investigate the relationship between hepcidin and CRP concentrations at baseline (pre-randomisation), and Hb response at 28 days post-randomisation. I chose these two parameters as they may be two potential determinants of a treatment response. I used similar methodology to Steensma et al. (463) and hepcidin values were

divided into tertiles and compared with haemoglobin responses. I also compared clinical and biological characteristics at baseline in responders versus non-responders. This may help with the identification of clinically important subgroups. Biological treatment response was defined as a rise in Hb of >20 g/l. All statistical tests were 2-sided and statistical significance was set at <0.01 for this post-hoc analysis.

5.4 Conclusion

Improving recovery from critical illness is a recognised research priority. Whether or not correcting anaemia, with intravenous iron, improves health-related quality of life and recovery after critical illness requires further investigation. If so, it has the potential to become a rapidly translatable intervention. Prior to embarking on a phase III, multicentre trial, a carefully designed and implemented feasibility trial is essential.

Chapter 6 The INTACT trial: Results and Discussion

6.1 Introduction

This chapter covers the overall progress, results and interpretation of the findings of the INTACT trial.

6.2 Trial progress

INTACT opened for participant recruitment on 18th September 2018, with the John Radcliffe Hospital being the first site. The first participant was enrolled and randomised on 19th September 2018. Recruitment for the first three months was slower than expected, which is not an uncommon scenario in the early stages of clinical trials. Changes of subsequent protocol amendments have been discussed in Chapter 5.

Due to severe delays in obtaining regulatory approvals, the Royal Berkshire site did not officially open for recruitment until 7th September 2019, which was less than two weeks before the planned overall recruitment end date (18th September 2019). I did not see much value in opening this site for only a very brief period. Therefore, following discussions with the Trial Management Group, it was agreed that the trial would be extended briefly up to 20th December 2019.

Demonstrating 'feasibility' at an additional site would also help with the design and funding applications for any future definitive trial. This extra time period would also allow for additional recruitment at the remaining sites, whilst also being within a window that would enable me to complete my fellowship in a timely manner. There were no costs implications associated with this brief extension.

INTACT closed to recruitment on 20th December 2019 with the final follow-up visit of the last participant occurring on 13th March 2020. Unfortunately, subsequent data cleaning, statistical analysis and laboratory were delayed until July 2020 due to the COVID-19 pandemic. I was redeployed to a full-time clinical rota on the ICU at the John Radcliffe Hospital from March to June 2020. Laboratory work did not commence until September 2020 due to the social distancing restrictions in the place at the WIMM.

6.3. Results

6.3.1 Characteristics of included participants

A total of 606 patients were screened, and of the 290 that were eligible for recruitment, 98 were randomised over 15 months. Patients were enrolled from four ICUs across three sites (Oxford University Hospitals NHS Foundation Trust, Royal Infirmary of Edinburgh NHS Lothian, Royal Berkshire Hospital NHS Foundation Trust) from 17th September 2018 to 20th December 2019 with final patient follow-up completed on 13th March 2020. A CONSORT flowchart summarising patient recruitment is shown in **Figure 6.1**. Reasons for ineligibility are detailed below in **Table 6.1**.

Table 6.1 Breakdown of reasons for ineligibility during screening

Reason for ineligibility	n (%)
Acute infection – non-resolving temperature ≥ 38°C within the past 24 hours or patient on non-prophylactic antibiotics[1]	102 (32%)
Chronic liver disease and/or screening Alanine Transferase / Aspartate Transferase x3 above upper limit of normal range [4]	49 (16%)
Immunosuppressive therapy for organ transplant	38 (12%)
Patients residing outside a reasonable geographic follow-up area[2]	22 (7%)
Planned palliative care, Planned home ventilation, Primary neurological diagnosis	19 (6%)
Intravenous iron or erythropoietin in the previous 4 weeks	19 (6%)
Haemodialysis dependent chronic kidney disease	17 (5%)
Requirement for English translation	14 (4%)
Already enrolled into another trial where the trial protocol explicitly prohibits co-enrolment	13 (4%)
Weight ≤ 50kg[3]	8 (3%)
Personal or family history of iron overload disorders such as haemochromatosis or previously documented ferritin >1200 ng/ml and/or transferrin saturation >50%.	6 (2%)
History of severe asthma, eczema, or other atopic allergy	7 (2%)
Pregnancy (however, breastfeeding is not an exclusion criteria)	2 (1%)
Total	**316**

1 Updated Post-Amendment May 2019 to: Acute infection – as judged by the treating clinician (e.g. ongoing bacteraemia or non-resolving sepsis) or patient expected to be on non-prophylactic antibiotics for greater than 14 days

2 Removed Post-Amendment April 2019

3 Removed Post-Amendment April 2019 but dose for these patients reduced

4 Updated Post-Amendment April 2019 to: Known sever chronic liver disease (e.g. Child-Pugh C or above)

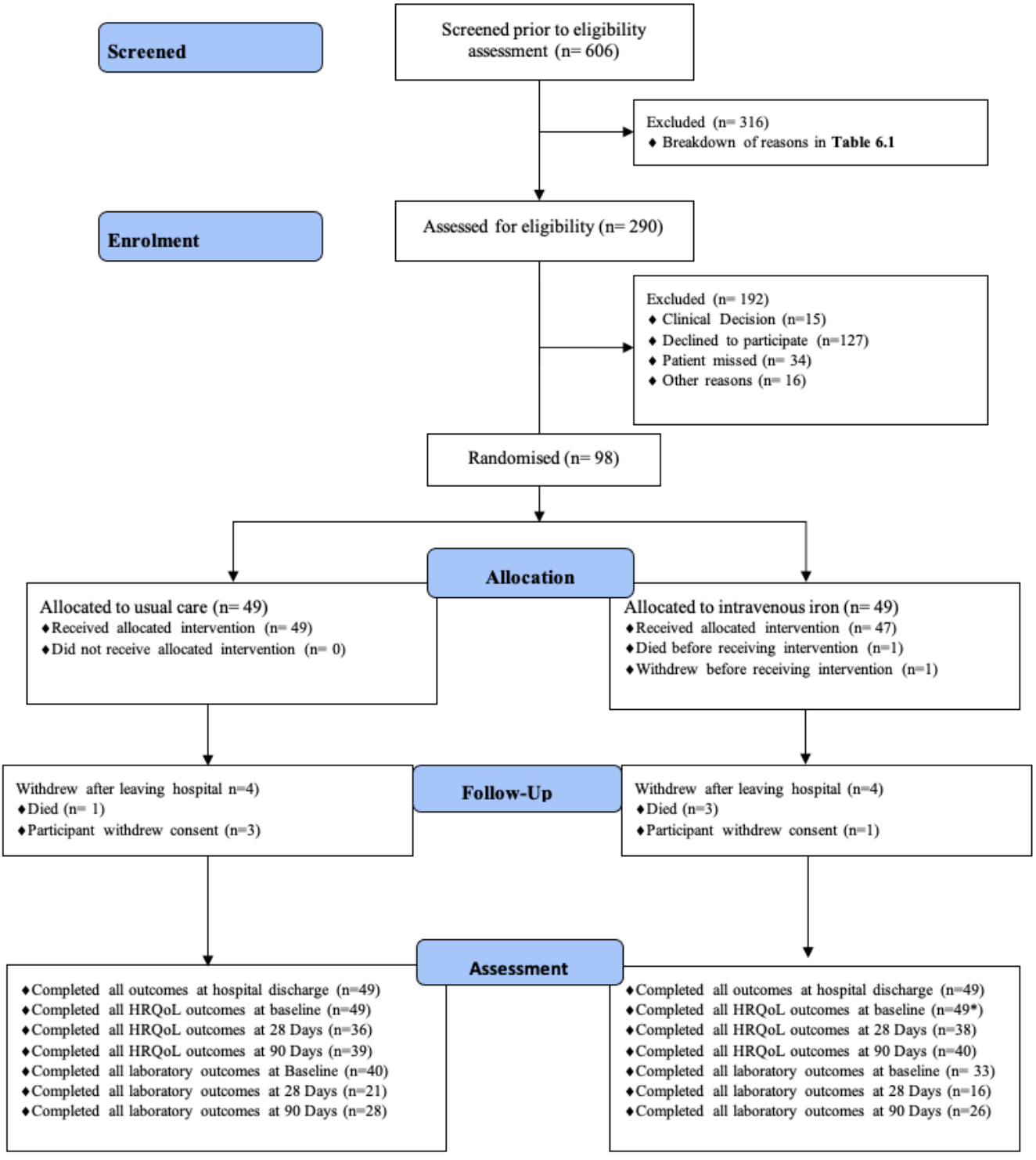


Figure 6.1 CONSORT flow diagram

As shown in **Table 6.1**, acute infection, deranged liver function and residence outside a reasonable geographical follow-up area were the leading reasons for ineligibility. The relatively large number of participants excluded because of organ transplant therapy is likely to be a reflection of two of the hospitals being regional organ transplant centres (Oxford, Edinburgh). Nineteen patients had received intravenous iron or erythropoietin in the previous 4 weeks. Some of these patients may have received intravenous iron for the treatment of anaemia prior to major surgery. However, there were also a few cases where clinicians lacked equipoise and administered intravenous iron as part of routine care.

Fourteen participants were ineligible due to the requirement for English translation and include important underserved groups such as those from Black, Asian and ethnic minorities. This is an important consideration for a future trial where I would seek to obtain additional funding for translation services to include these groups in keeping with the NIHR INCLUDE framework (464). The sponsor of one trial (NCT03653832) explicitly prohibited co-enrolment. Participants for this study were enrolled whilst acutely unwell in ICU and were therefore ineligible for INTACT. I have discussed some of the issues related to co-enrolment in Chapter 6.

Of those that were potentially eligible, 192 participants were excluded. A breakdown of reasons is provided below in **Table 6.2**.

Table 6.2 Breakdown of reasons, where available, for exclusion for potentially eligible participants

Reason	n (%)
Patient did not want to participate	127 (66.1)
Where reasons provided	
-Too much on	7
-Too tired to participate in research	8
-No further venepuncture	10
-Travel inconvenience	3
-Did not want to be randomised	1
-Not interested in research	1
Patient missed	34 (17.8)
Where reasons provided	
-Discharged home prior to being approached by research team	17
-Research staff unavailable	5
-Transferred out of area (e.g. repatriation to another hospital)	7
Other	16 (8.3)
Where reasons provided	
-Fluctuating capacity	6
-Family decision	1
Clinician decision	15 (7.8)
Where reasons provided	
-Complex case	5
-Lack of equipoise	1

Clinical and laboratory characteristics of enrolled participants in both groups were similar at baseline, suggestive of an adequate randomisation strategy **(Table 6.5)**.

Table 6.3 Patient characteristics at baseline

	Usual care (n = 49)	IV iron (n = 49)
Age, years, mean (SD)	58.9 (17.7)	61.4 (17.9)
Sex, n (%)		
Male	30 (61%)	28 (57%)
Female	19 (39%)	21 (43%)
APACHE II Score, mean (SD)	17.4 (7.5)	17.3 (7.3)
Weight, kg, mean (SD)	76.7 (16.5)	79.8 (19.6)
No. of Charlson Comorbidity Index categories, n (%)		
0	9 (18%)	10 (20%)
1	14 (29%)	11 (22%)
2 or more	26 (53%)	28 (57%)
ICU admission type, n (%)		
Elective operation	16 (33%)	18 (37%)
Emergency operation	18 (37%)	18 (37%)
Medical	15 (31%)	13 (27%)
ICU organ support requirements, n (%)		
Advanced respiratory support	26 (53%)	19 (39%)
Advanced cardiovascular support	17 (35%)	17 (35%)
Advanced renal support	1 (2%)	4 (8%)
ICU red blood cell transfusion requirements		
No. of patients transfused, n (%)	15 (31%)	21 (43%)
No. of RBC units per patient, mean (SD)	2.89 (2.7)	2.52 (1.5)
ICU length of stay at randomisation*, days		
< 7 days	37 (76%)	36 (73%)
≥7 days	12 (24%)	13 (27%)
Mean (SD)	5.1 (4.8)	5.6 (4.7)
Median (IQR)	3 (3 to 6)	4 (2 to 7)
History of anaemia prior to ICU admission, n (%)	2 (4%)	8 (16%)
Anaemia severity at randomisation*		
<80 g/l	11 (22%)	11 (22%)
80-100 g/l	38 (78%)	38 (78%)
Laboratory parameters ǂ		
Haemoglobin*, g/l	85.8 (9.8)	86.4 (7.7)
Serum ferritin, µg/l, median (IQR)	358.1 (246.0 to 640.0)	370.6 (197.2 to 548.8)
Transferrin saturation, %, median (IQR)	12.0 (9.0 to 16.0)	11.0 (8.0 to 15.0)
Serum iron, µm/l, median (IQR)	4.3 (3.1 to 6.1)	4.0 (3.0 to 6.0)
CRP, mg/l	153.4 (103.0)	140.1 (85.7)
Erythropoietin, Miu/ml	55.9 (70.9)	67.1 (61.8)
Serum hepcidin‡, ng/ml, median (IQR)	36.8 (18.2 to >81)	39.7 (17.4 to 72.8)
Soluble transferrin receptor, nmol/l	28.6 (15.0)	29.2 (14.0)
Serum creatinine, mmol/L, median (IQR)	65.0 (56.0 to 89.0)	65.0 (47.0 to 82.0)
Serum albumin, g/l	21.9 (4.4)	22.6 (4.8)
Phosphate, mmol/l	0.98 (0.30)	0.97 (0.2)

* Minimisation factors for the randomisation were anaemia severity and participant ICU length of stay

ǂ Mean (SD) unless otherwise indicated

‡ The hepcidin assay was unable to record measurements higher than 81, all participants with >81 were given a value of 85 and the median, IQR calculated these are included as >81 for the IQR if relevant

APACHE, Acute Physiology and Chronic Health Evaluation; ICU, Intensive Care Unit; IQR, Interquartile range; IV, intravenous; RBC, Red blood cell; SD, standard deviation

6.3.2 Primary outcomes

Feasibility outcomes are displayed in **Table 6.4**. The overall recruitment rate for the trial was 34% (95% CI: 28% to 40%) with 6.5 patients recruited per month. Individual site recruitment rates ranged from 0.5 to 5.2 participants per month (**Table 6.5**). Forty-seven out of 49 participants received the intervention (96% (95% CI: 86% to 100%)) and no patients in the usual care arm received intravenous iron during the course of the study. One participant died and one participant withdrew from the study prior to receiving intravenous iron. All HRQoL measures were collected for at least 79/93 (85%; 95% CI: 76% to 92%) survivors at 90 days. Overall, INTACT met the amber category for recruitment, and green categories for protocol adherence and HRQoL outcome data collection (**Figure 6.3)**.

Table 6.4 Primary feasibility outcomes

Feasibility outcome	**Eligible**	**Randomised**	**Rate (95% CI)**
Recruitment	290	98	34% (28%, 40%)
	Expected*	**Completed**	
Protocol adherence (intervention arm)			
Intervention arm	49	47†	96% (86%, 100%)
Usual care arm	49	49	100% (93%, 100%)
Completion of 90-day HRQoL questionnaires			
EQ-5D-5L	93‡	81	87% (79%, 93%)
FACIT-F	93	81	87% (79%, 93%)
MFI-20	93	79	85% (76%, 92%)

*Number expected = number randomised minus number who died before each time point

†49 participants were randomised to the intervention arm, one participant died and one withdrew before receiving the intervention

‡ Five participants had died (four whilst in-hospital and one in the community) and four withdrew from the study before their 90-day timepoint

CI, Confidence Interval; EQ-5D-5L, EuroQol-5D-5L; FACIT, Functional Assessment of Chronic Illness Therapy-Fatigue; HRQoL, Health-related quality of life; MFI-20, Multidimensional Fatigue Inventory-20

Table 6.5 Recruitment details by activated site

Site	**Date site opened**	**Date site closed**	**Months Open**	**Total screened**	**Total Eligible**	**Total Randomised**	**Recruitment per month (randomised/ months open)**
Oxford University Hospitals NHS Foundation Trust*	12Sep2018	20Dec2019	15	269	140	76	5.1
Edinburgh Royal Infirmary	17Dec2018	20Dec2019	12	324	143	20	1.7
Royal Berkshire Hospital	16Aug2019	20Dec2019	4	13	7	2	0.5
Total			15	606	290	98	6.5

*John Radcliffe ICU and Churchill ICU

	Recruitment	Protocol adherence	Outcome data
Green (Go)	8-11 participants per month across two centres	< 5% failure to receive allocated intervention in the IV iron group	At least 80% of survivors will provide follow-up data at 90 days
Amber (Amend)	4-8 participants per month	5-10% failure to receive allocated intervention in the IV iron group	60-80% of survivors will provide follow-up data at 90 days
Red (Stop)	Less than 4 patients per month	>10% failure to receive allocated intervention in the IV iron group	50-60% of survivors will provide follow-up data at 90 days

IV, intravenous

Figure 6.2 Results of traffic light system to determine progression to a definitive trial

Reasons for missing laboratory and HRQoL data are shown below in **Table 6.8**. Common reasons included difficult venepuncture and telephone only follow-up for missing laboratory data and being unable to contact participants for HRQoL data. More laboratory data were missing at the 28-day follow up time point compared with 90-days. This was largely due to more telephone only follow-ups occurring.

Table 6.6 Reasons for missing data at study time points

Reason laboratory sample not taken	Participant Refusal	Sample forgotten	Difficult venepuncture	Sample haemolysed	Other	Total
Baseline						
Usual care	1	0	1	0	8	10
Intravenous iron	1	1	1	1	12	16
Total	2	1	2	1	20	26
	Participant Refusal	Sample forgotten	Difficult venepuncture	Telephone follow-up	Other	Total
28-day						
Usual care	4	0	1	8	7	20
Intravenous iron	0	0	3	12	7	22
Total	4	0	4	20	14	42
90-day						
Usual care	2	0	1	5	2	10
Intravenous iron	1	0	2	7	6	16
Total	3	0	3	12	8	26
Reason HRQoL not completed *	Participant not alive	Withdrawal of consent	Unable to contact participant	Other	Unknown	Total
28-day						
Usual care	1	2	8	2	0	13
Intravenous iron	3	1	6	0	0	10
Total	4	3	14	2†	0	23v
90-day						
Usual care	1	2	3	3	0	9
Intravenous iron	4	1	0	2	1	7
Total	5	3	3	5‡	1	17‡

* All participants completed HRQoL at baseline although one participant missed an anxiety item on EQ-5D-5L questionnaire

†Excludes one participant missing an MFI questionnaire at 28 days for reason "Other"

‡ Excludes two participants with missing MFI questionnaires at 90 days for reason "Other"

HRQoL, Health-related quality of life

6.3.3 Secondary outcomes

Clinical and HRQoL outcomes are displayed in **Table 6.7** There were no statistically significant differences in in-hospital mortality and nosocomial infection rates between both study groups. The median (IQR) post-ICU LOS was shorter in the intravenous iron group when compared with usual care, but this did not reach statistical significance (5.0 days (3.0-13.0) vs. 9.0 days (5.0-16.0), p=0.15). Hospital readmissions in the first 90 days following ICU discharge were also lower in the intravenous iron group. Overall HRQoL scores did not show a statistically significant difference by treatment assignment.

Participants allocated to the intravenous iron group had higher Hb, ferritin, iron and Tsat concentrations, and lower transferrin concentrations throughout the follow-up period when compared with usual care (**Figure 6.3 and Table 6.8).** EPO concentrations were lower in the IV iron group during the study follow up period but there was no evidence of an effect on serum phosphate and sTfR concentrations (**Figure 6.4** and **Table 6.8**). There was no evidence of a treatment difference in hepcidin concentrations at 28 days but median (IQR) hepcidin concentrations were lower in the usual care arm at 90 days when compared with the intervention arm (13.9 (3.6-33.3) ng/ml vs. 35.0 (7.6-60.1) ng/ml, p=0.048) (**Figure 6.4 and Table 6.9**).

The upper limit of detection for the hepcidin assay kit was 81 ng/ml. Therefore, any measurements above this are likely to be unreliable. This occurred for 30 samples and was most prominent at baseline / pre-randomisation (19 samples). Both groups were affected evenly. These high concentrations could be due to hepcidin being upregulated by coexisting inflammation. Following discussions with the trial statisticians, hepcidin concentrations above 81 ng/ml would be given a value of 85 ng/ml. A Mann-Whitney rank test was used for the analysis which gives all participants with concentrations >81 ng/ml the same rank. These samples are still stored in the WIMM and may be reanalysed at dilution at a later date in order to obtain specific values.

Vitamin D concentrations were lower at 90-days post-randomisation in participants allocated to receive intravenous iron when compared with usual care. There was no evidence of a treatment difference in serum phosphate levels during the study period (**Table 6.8)**. C-reactive protein concentrations were higher in the intravenous iron group at 90-days, but this was of borderline statistical significance (adjusted difference (95% CI) -19.62 (-39.16 to -0.09) mg/l, p = 0.05) **(Figure 6.4 and Table 6.8)**. Ferric carboxymaltose was well tolerated by all patients and there were no study drug-related serious adverse events or infusion reactions.

Table 6.7 Secondary clinical and health-related quality of life outcomes

Outcome	Usual care (n = 49)	Intravenous iron (n = 49)	Effect estimate (95% CI)	p value
Clinical outcomes				
In-hospital mortality, n (%)	1 (2%)	3 (6%)	3.00 (0.32 to 27.9) [2]	0.31
Nosocomial infection, n (%)	13 (27%)	13 (27%)	1.00 (0.52 to 1.93) [2]	1.00
Infection site, n				
Lung	5	7		
Gastrointestinal tract	4	4		
Skin	1	1		
Invasive device	0	0		
Bone/joint	0	0		
Other, unspecified	3	2		
Post-ICU LOS, days[1]				
Mean (SD)	12.94 (12.63)	11.86 (19.24)		
Median (IQR)	9.0 (5.0, 16.0)	5.0 (3.0, 13.0)		0.15
Hospital readmission post-randomisation				
28 days	6/36 (16.6%)	5/38 (13.1%)	0.76 (0.21 to 2.74) [3]	0.67
90 days	15/39 (38.4%)	7/40 (17.5%)	0.34 (0.12 to 0.96) [3]	0.037

1 Post-ICU hospital length stay calculated as the time from randomisation until hospital discharge

2 Relative Risk (95% CI)

3 Odd Ratio (95% CI)

Health-related quality of life outcomes							
	n	**Mean (SD)**	**n**	**Mean (SD)**	**Unadjusted diff (95% CI)[1]**	**Adjusted diff (95% CI)[2]**	
EQ-5D-5L Utilities							
Baseline	49	0.17 (0.37)	48	0.22 (0.38)			
28 Days	37	0.53 (0.31)	42	0.53 (0.33)			
90 Days	41	0.59 (0.29)	45	0.56 (0.37)	0.001 (-0.10, 0.10)	-0.001 (-0.12, 0.12)	0.98
EQ-5D-5L Visual Analogue Scale							
Baseline	49	41.78 (23.08)	49	44.14 (24.31)			
28 Days	36	62.22 (16.81)	39	60.08 (21.25)			
90 Days	40	64.43 (18.32)	41	70.00 (18.84)	-2.02 (-4.44, 0.40)	-2.31 (-5.55, 0.93)	0.16

FACIT-F							
Baseline	49	16.86 (12.54)	49	16.69 (12.73)			
28 Days	36	28.22 (10.28)	39	28.18 (11.19)			
90 Days	40	30.90 (13.16)	41	34.37 (12.26)	-0.16 (-4.80, 4.49)	-0.18 (-4.89, 4.53)	0.94
MFI-20 Physical							
Baseline	49	13.49 (2.96)	49	13.29 (3.08)			
28 Days	36	14.06 (2.03)	38	13.71 (2.35)			
90 Days	39	13.54 (1.88)	40	13.33 (2.44)	-0.30 (-2.83, 2.22)	-0.26 (-2.56, 2.04)	0.82
MFI-20 Mental							
Baseline	49	11.78 (3.89)	49	12.10 (2.93)			
28 Days	36	11.31 (2.86)	38	11.16 (2.09)			
90 Days	39	11.54 (2.51)	40	11.13 (2.29)	-0.13 (-1.09, 0.83)	-0.10 (-1.10, 0.90)	0.85
MFI-20 Reduced Activity							
Baseline	49	13.76 (3.00)	49	13.33 (3.11)			
28 Days	36	13.75 (2.02)	38	13.39 (2.25)			
90 Days	39	12.87 (1.89)	40	12.75 (2.62)	-0.28 (-1.70, 1.13)	-0.27 (-1.64, 1.11)	0.70
MFI-20 General							
Baseline	49	12.02 (3.38)	49	12.24 (3.21)			
28 Days	36	11.14 (2.51)	38	10.95 (2.14)			
90 Days	39	10.87 (2.14)	40	11.20 (2.00)	-0.25 (-0.76, 0.26)	-0.25 (-0.77, 0.26)	0.34
MFI-20 Reduced motivation							
Baseline	49	12.47 (3.67)	49	12.43 (3.30)			
28 Days	36	12.28 (2.94)	38	12.37 (2.73)			
90 Days	39	12.69 (2.57)	40	12.40 (2.75)	0.15 (-0.14, 0.44)	0.18 (-0.15, 0.51)	0.28

1 Modelled with time treatment interaction, adjusted for time as categorical and baseline values with cluster robust standard errors for sites

2 Modelled with time treatment interaction, adjusted for time as categorical, baseline values, randomisation variables (haemoglobin and length of stay in ICU at randomisation as continuous)

EuroQol-5D-5L; FACIT, Functional Assessment of Chronic Illness Therapy-Fatigue; HRQoL, Health-related quality of life; ICU, Intensive Care Unit; LOS, Length of stay; MFI-20, Multidimensional Fatigue Inventory-20

Table 6.8 Changes in laboratory outcomes

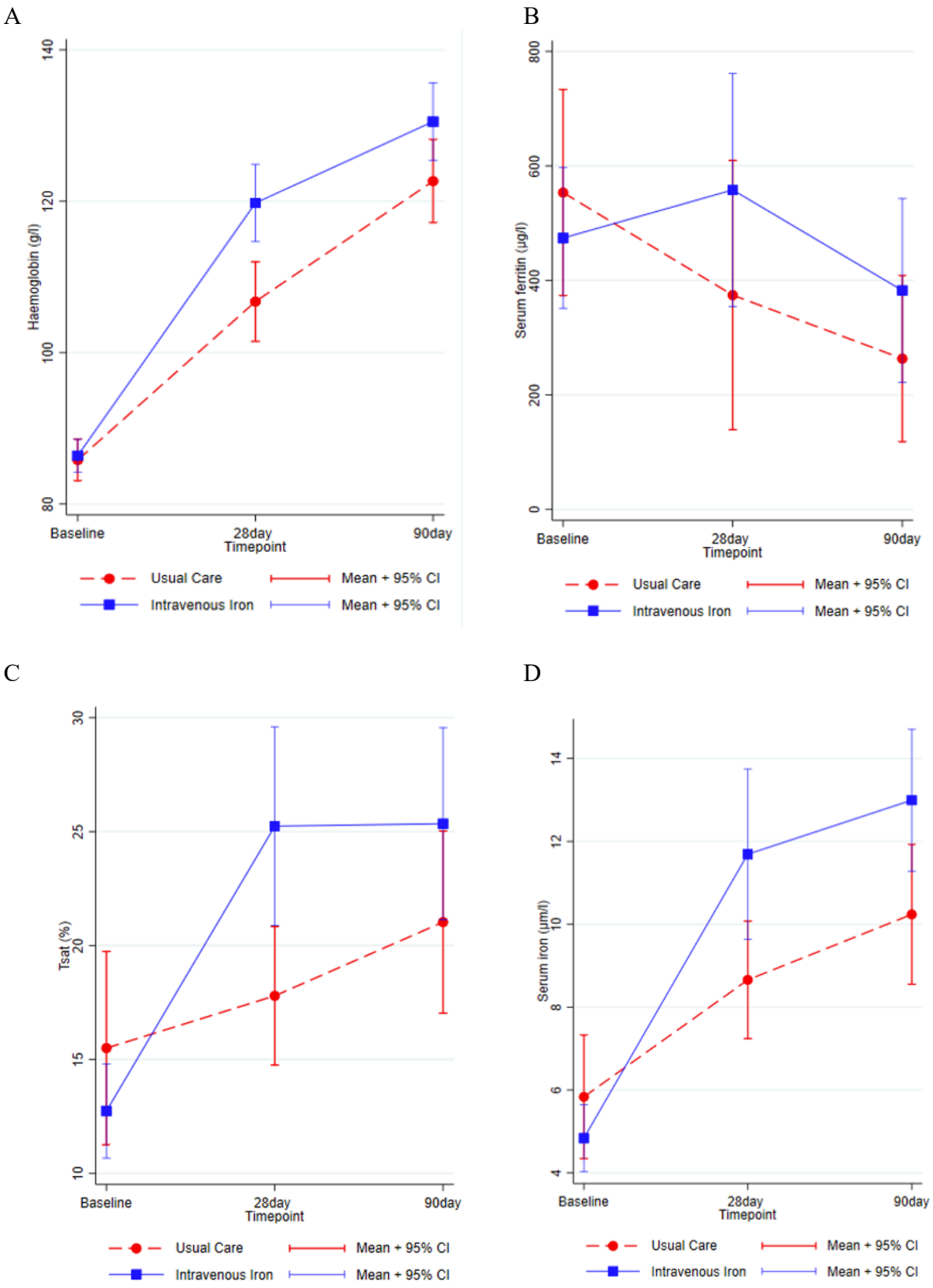
	Usual Care		Intravenous Iron		Unadjusted diff	Adjusted diff	p value
	n	Mean (SD)	n	Mean (SD)	(95% CI) *	(95% CI) †	
Haemoglobin (g/l)§							
Baseline	49	85.82 (9.82)	49	86.39 (7.74)			
28 Days	31	106.74 (14.93)	26	119.77 (13.28)			
90 Days	38	122.66 (17.26)	33	130.52 (15.07)	10.92 (4.62, 17.22)	10.98 (4.96, 17.01)	<0.001
Serum ferritin (µg/l)							
Baseline	46	553.38 (622.50)	47	473.96 (429.83)			
28 Days	29	374.28 (646.20)	21	558.06 (476.47)			
90 Days	35	263.22 (437.99)	32	382.36 (463.57)	311.22 (146.97, 475.47)	311.91 (158.14, 465.69)	<0.001
Serum iron (µm/l)							
Baseline	48	5.84 (5.27)	46	4.84 (2.78)			
28 Days	29	8.66 (3.90)	21	11.69 (4.81)			
90 Days	36	10.24 (5.16)	31	12.99 (4.86)	3.48 (3.12, 3.84)	3.41 (2.98, 3.84)	<0.001
Tsat (%)							
Baseline	48	15.50 (15.00)	46	12.74 (7.15)			
28 Days	29	17.79 (8.35)	21	25.24 (10.19)			
90 Days	36	21.03 (12.23)	31	25.35 (11.96)	8.87 (7.30, 10.44)	8.81 (7.05, 10.57)	<0.001
Transferrin (g/l)							
Baseline	48	1.58 (0.38)	46	1.64 (0.49)			
28 Days	29	2.18 (0.58)	21	2.03 (0.35)			
90 Days	36	2.36 (0.54)	31	2.18 (0.52)	-0.29 (-0.38, -0.20)	-0.28 (-0.36, -0.20)	<0.001
Erythropoietin (Miu/ml)							
Baseline	47	55.93 (70.93)	45	67.13 (61.78)			
28 Days	28	30.87 (26.70)	20	17.17 (11.31)			
90 Days	32	15.88 (11.39)	31	14.59 (10.07)	-9.11 (-11.43, -6.79)	-9.02 (-10.73, -7.32)	<0.001
Phosphate (mmol/l)							
Baseline	49	0.98 (0.30)	49	0.97 (0.30)			
28 Days	29	1.12 (0.28)	24	1.01 (0.23)			
90 Days	37	1.08 (0.25)	31	1.02 (0.18)	-0.11 (-0.23, 0.02)	-0.10 (-0.23, 0.03)	0.12
C-reactive protein (mg/l)							
Baseline	48	153.41 (103.03)	49	140.08 (85.74)			
28 Days	30	32.80 (38.36)	24	13.46 (20.69)			
90 Days	38	12.98 (19.71)	31	22.08 (53.04)	-18.61 (-43.39, 6.18)	-19.57 (-42.07, 2.93)	0.09
Vitamin D (nmol/l)							
Baseline	47	41.15 (22.29)	44	36.80 (22.02)			
28 Days	26	52.50 (26.57)	21	43.86 (24.57)			
90 Days	33	57.02 (26.05)	31	53.87 (28.32)	-8.60 (-14.86, -2.35)	-9.03 (-16.94, -1.12)	0.03
Soluble transferrin receptor (nmol/l)							
Baseline	46	28.60 (15.05)	43	29.17 (13.97)			
28 Days	27	39.76 (13.89)	18	34.64 (11.23)			
90 Days	31	31.17 (13.03)	27	24.93 (7.46)	-3.88 (-12.04, 4.28)	-3.63 (-12.33, 5.07)	0.41

* Modelled with time treatment interaction, adjusted for time as categorical and baseline values with cluster robust standard errors for sites

† Modelled with time treatment interaction, adjusted for time as categorical, baseline values and randomisation variables (haemoglobin and ICU LOS at randomisation) as continuous variables, with cluster robust standard errors for sites

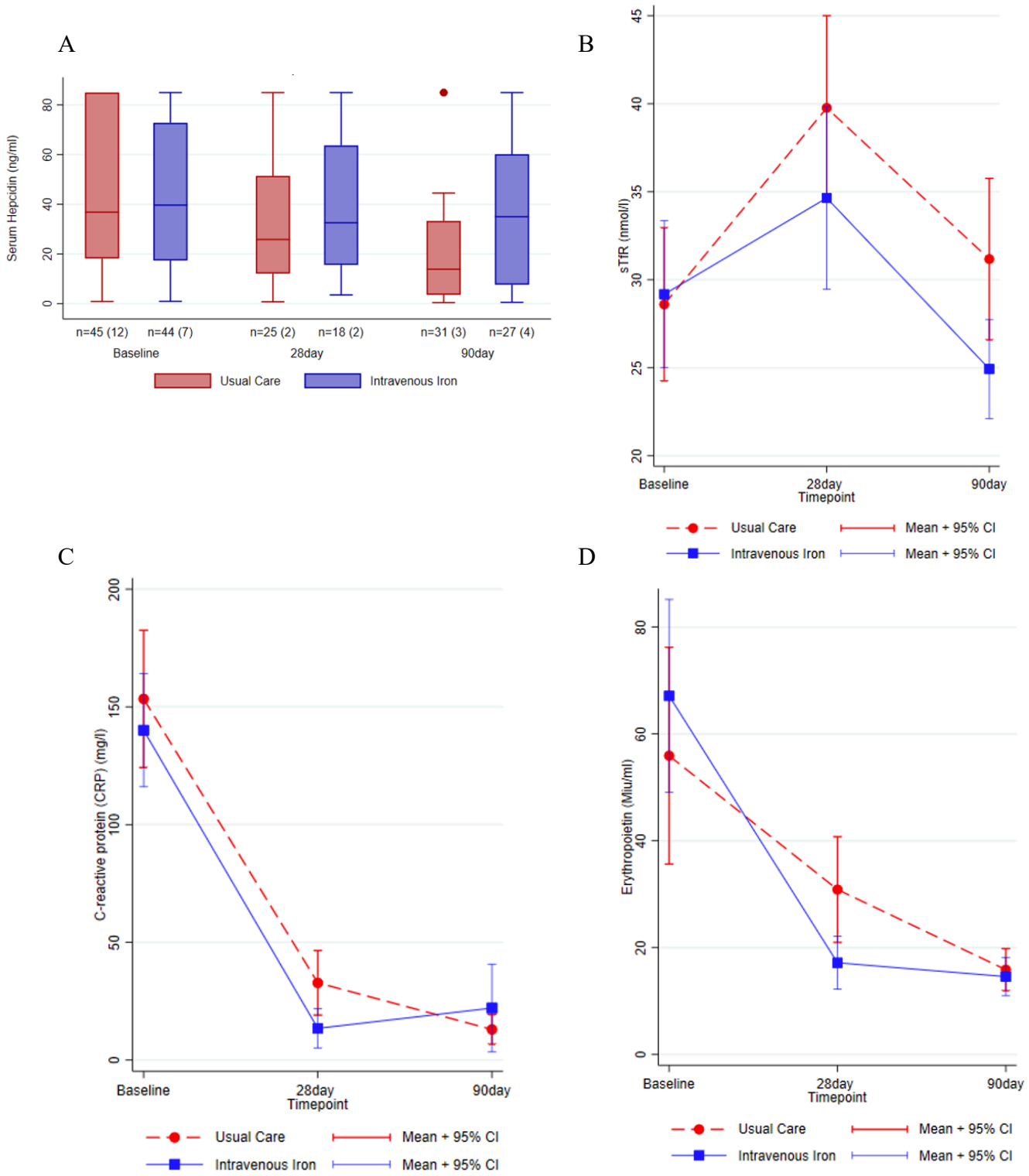
§ Baseline value for haemoglobin outcome was haemoglobin at randomisation

Figure 6.3 Changes in (A) haemoglobin, (B) ferritin, (C) transferrin saturation and (D) serum iron concentrations at randomisation and 28 and 90-days post-randomisation.



Bars indicate ± mean and 95% confidence intervals (CI)

Figure 6.4 Changes in (A) hepcidin, (B) soluble transferrin receptor, (C) C-reactive protein and (D) EPO at randomisation and 28 and 90-days post-randomisation



Bars indicate ± mean and 95% confidence intervals (CI)

Table 6.9 Changes in serum hepcidin concentrations at randomisation and 28 and 90-days post-randomisation

	Usual care			Intravenous iron			
	Total (n>81) *	Median (IQR)	Min,Max ǂ	Total (n>81) *	Median (IQR)	Min/Max ǂ	p value ǂ
Serum hepcidin (ng/ml)							
Baseline	45 (12)	36.8 (18.2, >81)	0.88, >81	44 (7)	39.6 (17.4,72.8)	0.93, >81	-
28 days	25 (2)	25.8 (12.1, 51.4)	0.77, >81	18 (2)	32.6 (15.6, 63.6)	3.5, >81	0.38
90 days	31 (3)	13.9 (3.6, 33.3)	0.41, >81	27 (4)	35.0 (7.6, 60.1)	0.55, >81	0.048

* Total includes all participants with available data at each timepoint (the number of participants with hepcidin >81)

ǂ The assay was unable to record measurements higher than 81 and therefore the max is >81 ng/ml.

ǂ Calculated with all available data. Participants with >81 were replaced with 85 ng/ml and therefore given the same rank on each treatment arm.

IQR, Interquartile Range

6.3.3 Exploratory analyses

Relationships between iron status, inflammation and persisting anaemia

As described in Chapter 4, I decided to undertake exploratory, hypothesis-generating analyses to investigate the relationships between iron status (as defined by hepcidin), inflammation and haemoglobin recovery at 28 days. I chose this relatively early time point based on the above data which shows that haemoglobin separation is greater at 28-days when compared with 90-days.

Study participants with serum hepcidin levels in the lowest tertile, and who received intravenous iron, experienced a greater change in haemoglobin and had a higher median haemoglobin at 28 days when compared with the usual care arm **(Figure 6.5)**. These effects were more marked in participants with hepcidin levels in the third tertile (>64.3 ng/ml). Similar effects were also observed when participants were stratified by baseline CRP levels **(Figure 6.6)**.

Using previously accepted definitions of systemic inflammation (465), 12/38 (31.6%) participants in the usual care group and 8/31 (25.8%) in the IV group had a CRP concentration >10 mg/L at 90 days. Numerically, more participants had Hb ≤100 g/L in the usual care group at 28- and 90-days post-randomisation as shown below in **Table 6.10.**

When compared with non-responders, hepcidin concentrations at baseline were significantly associated with a haemoglobin response at 28 days **(Table 6.11)**. Responders were also younger, surgical patients, with lower haemoglobin, ferritin and CRP concentrations although these were statistically not significant (**Table 6.13**).

Table 6.10 Prevalence of different categories of anaemia at 28 and 90 days post-randomisation

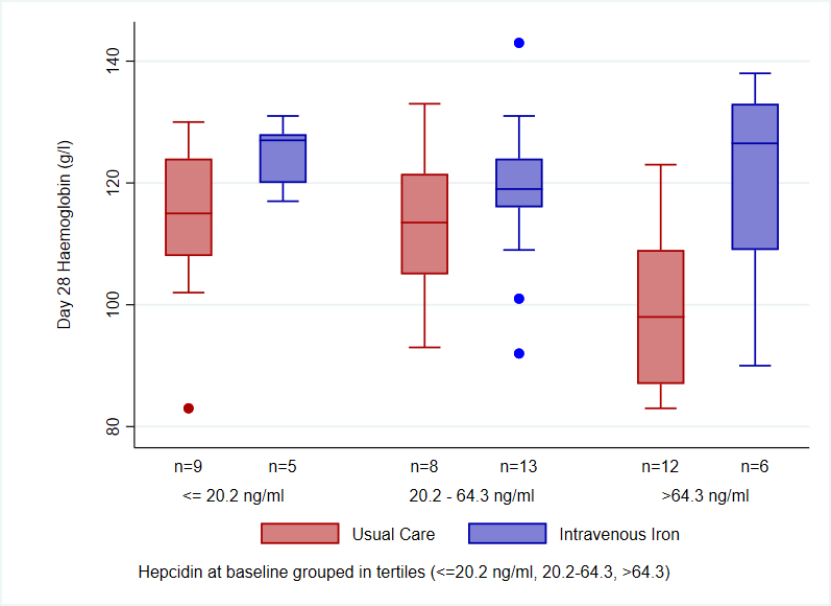
Study time point	Usual Care, n (%)	Intravenous iron, n (%)	p value*
28 days			
Moderate and severe anaemia (Hb ≤100 g/l) (n=13)	10 (32%)	3 (12%)	
Mild anaemia (Hb 101-129 g/l) (n=36)	19 (61%)	17 (65%)	
Not anaemic (Hb ≥130 g/l) (n=8)	2 (6%)	6 (23%)	0.07
90 days			
Moderate and severe anaemia (Hb ≤100 g/l) (n=6)	5 (13%)	1 (3%)	
Mild anaemia (Hb 101-129 g/l) (n=33)	20 (53%)	13 (39%)	
Not anaemic (Hb ≥130 g/;) (n=32)	13 (34%)	19 (58%)	0.09

ŧ Fisher's exact

Hb, Haemoglobin

Figure 6.5 Distributions of haemoglobin at day 28 by treatment and baseline hepcidin

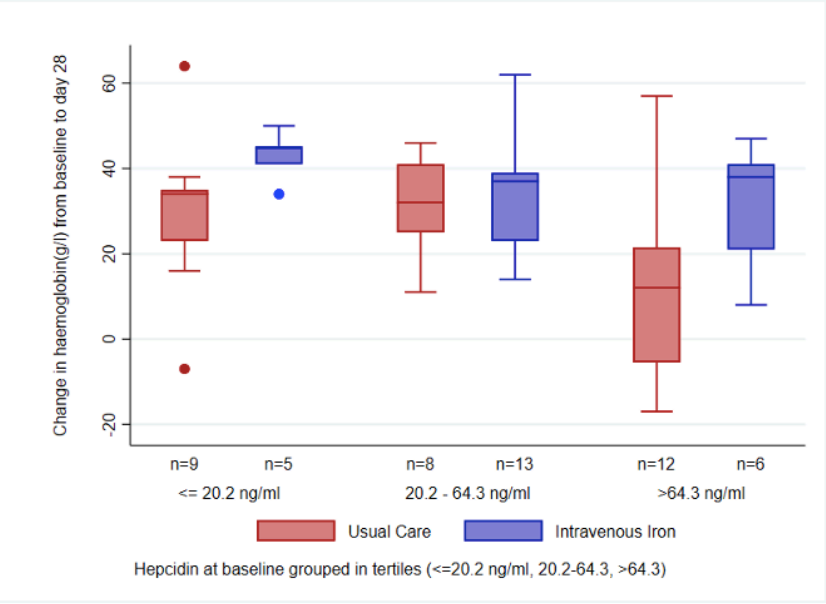
(A)



		Distribution of haemoglobin (g/l) at day 28				
	Usual care			Intravenous iron		
Hepcidin baseline tertile	n*	Median (IQR)	Min, Max	n*	Median (IQR)	Min, Max
≤ 20.2 ng/ml	9	115 (108, 124)	83, 130	5	127 (120, 128)	117, 131
20.2 – 64.3 ng/ml	8	114 (105, 122)	93, 133	13	119 (116, 124)	92, 143
>64.3 ng/ml	12	98 (87, 109)	83, 123	6	127 (109, 133)	90, 138

* Total with available haemoglobin results at day 28 by hepcidin tertile

(B)

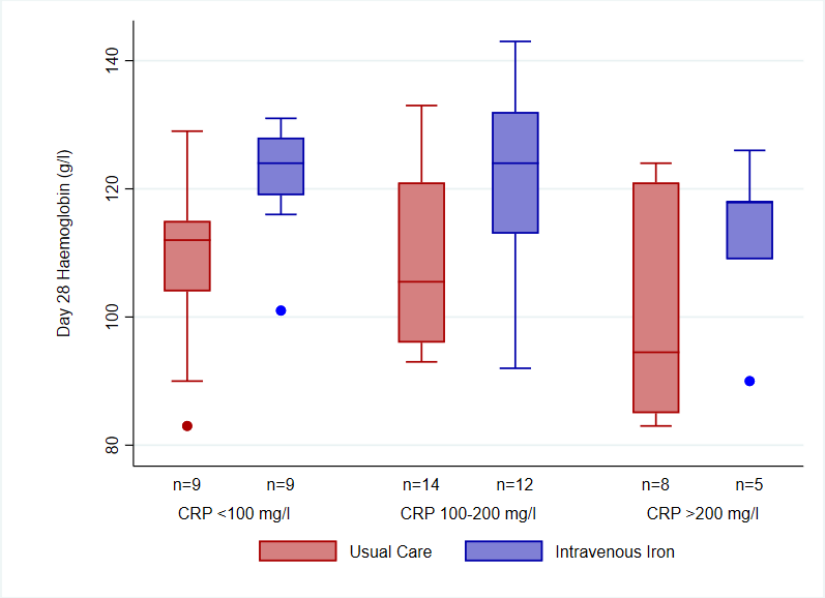


		Change in haemoglobin from baseline to day 28 (g/l)				
	Usual care			Intravenous iron		
Hepcidin baseline tertile	n*	Median (IQR)	Min, Max	n*	Median (IQR)	Min, Max
≤ 20.2 ng/ml	9	34 (23, 35)	-7, 64	5	45 (41, 45)	34, 50
20.2 – 64.3 ng/ml	8	32 (25, 41)	11, 46	13	37 (23, 39)	14, 62
>64.3 ng/ml	12	12 (-5.5, 21.5)	-17, 57	6	38 (21, 41)	8, 47

* Total with available haemoglobin results at day 28 by hepcidin tertile

Figure 6.6 Distributions of haemoglobin at day 28 by treatment and baseline CRP

(A)



		Distribution of haemoglobin (g/l) at day 28				
		Usual Care			Intravenous iron	
CRP baseline tertile	n*	Median (IQR)	Min, Max	n*	Median (IQR)	Min, Max
<100 mg/l	9	112 (104, 115)	83, 129	9	124, (119, 128)	101, 127
100-200 mg/l	14	105.5 (96, 121)	93, 133	12	124 (113, 132)	92, 143
>200mg/l	8	94.5 (85, 121)	83, 124	5	118 (109, 118)	90, 126

* Total with available haemoglobin results at day 28 by CRP tertile

Table 6.11 Clinical and laboratory characteristics between responders and non-responders at 28-days post-randomisation.

Baseline Characteristic	Overall (n = 57)	Responder at 28 days (n = 39)	Non-responder at 28 days (n = 18)	p value
	Mean (SD)	**Mean (SD)**	**Mean (SD)**	
Age	62.1 (17.4)	59.0 (19.2)	68.6 (10.8)	0.02*
APACHEII Score	18.5 (7.9)	19.1 (8.4)	17.4 (6.4)	0.46
Sex, n (%)				
Female	25 (44%)	20 (51%)	5 (28%)	
Male	32 (56%)	19 (49%)	13 (72%)	0.1
Advanced respiratory support, n (%)				
No	32 (56%)	20 (51%)	12 (67%)	
Yes	25 (44%)	19 (49%)	6 (33%)	0.28
Treatment Allocated				
Usual Care	31 (54%)	17 (44%)	14 (78%)	
Intravenous Iron	26 (46%)	22 (56%)	4 (22%)	0.02
Categories of CCI, n (%)				
0	10 (18%)	8 (21%)	2 (11%)	
1	17 (30%)	11 (28%)	6 (33%)	
2 or more	30 (53%)	20 (51%)	10 (56%)	0.68
ICU Admission Type, n (%)				
Elective operation	16 (28%)	8 (21%)	8 (44%)	
Emergency Operation	24 (42%)	20 (51%)	4 (22%)	
Medical	17 (30%)	11 (28%)	6 (33%)	0.08
ICU LOS at randomisation, days				
Mean (SD)	5.1 (4.2)	5.0 (4.3)	5.1 (4.2)	
Median (IQR)	3.0 (2.0, 6.0)	4.0 (2.0, 6.0)	3.0 (2.0, 7.0)	0.93‡
Hepcidin, ng/ml				
N (%), Missing	53 (93%), 4	38 (97%), 1	15 (83%), 1	
Median (IQR)	40.2 (19.3, 77.8)	29.3 (18.3, 56.3)	80.4 (54.0, 85.0)	<0.001
Hepcidin tertiles				
<=20.2 ng/ml	14 (25%)	12 (31%)	2 (11%)	
20.2-64.3 ng/ml	21 (37%)	18 (46%)	3 (17%)	
>64.3 ng/ml	18 (32%)	8 (21%)	10 (56%)	
Unknown	4 (7%)	1 (3%)	3 (17%)	0.007
Haemoglobin at randomisation, g/l				
Mean (SD)	85.2 (8.8)	83.3 (8.9)	89.3 (7.2)	
Median (IQR)	86.0 (81.0, 92.0)	83.0 (78.0, 90.0)	90.0 (82.0, 96.0)	0.02
Serum ferritin, µg/l				
N (%), Missing	55 (96%), 2	38 (97%), 1	17 (94%), 1	
Mean (SD)	516.0 (591.0)	438.5 (507.2)	689.0 (733.4)	
Median (IQR)	341.5 (197.2, 609.1)	304.0 (166.8, 475.4)	514.5 (312.0, 866.7)	0.03‡
Ferritin tertiles				
<=100 µg/l	1 (2%)	0 (0%)	1 (6%)	
100-300 µg/l	20 (35%)	18 (46%)	2 (11%)	
>300 µg/l	34 (60%)	20 (51%)	14 (78%)	
Unknown	2 (4%)	1 (3%)	1 (6%)	0.02
CRP, mg/l				
Mean (SD)	149.9 (94.1)	133.6 (86.3)	185.2 (102.9)	

Median (IQR)	137.5 (64.7, 186.8)	132.7 (61.4, 178.3)	171.8 (119.0, 260.9)	0.08‡
Tsat, %				
N (%), Missing	55 (96%), 2	38 (97%), 1	17 (94%), 1	
Mean (SD)	15.3 (13.9)	16.5 (16.4)	12.7 (4.3)	
Median (IQR)	13.0 (9.0, 17.0)	13.0 (9.0, 17.0)	13.0 (10.0, 17.0)	0.83‡
FACIT-F Baseline				
Mean (SD)	16.7 (12.4)	16.0 (12.6)	18.2 (12.1)	
Median (IQR)	13.0 (7.0, 28.0)	11.0 (6.0, 23.0)	14.0 (8.0, 31.0)	0.42‡
FACIT-F 28 Days				
N (%), Missing	55 (96%), 2	38 (97%), 1	17 (94%), 1	
Mean (SD)	28.7 (10.8)	27.2 (11.2)	32.1 (9.1)	
Median (IQR)	28.0 (23.0, 38.0)	27.0 (22.0, 34.0)	31.0 (25.0, 40.0)	0.18‡

* There was evidence against the null hypothesis of equal variance in age by responder status (p<0.001), therefore an unequal t-test was performed.

I No p-value was calculated

‡ Mann-Whitney Rank test p-value

APACHE II, Acute Physiology and Chronic Health Evaluation II; CCI, Charlson Comorbidity Index; FACIT-F, Functional Assessment of Chronic Illness Therapy-Fatigue; Hb, Haemoglobin; ICU, Intensive Care Unit; LOS, Length of stay

Participant recall

Participants who completed the 90-day HRQoL assessment were asked if they could recall which treatment arm they were allocated to (**Table 6.12**). Overall, approximately one-third (27/79 (34.1%)) of participants were either unsure or incorrectly remembered which treatment arm they were randomised to.

Table 6.12 Participants' recall of allocation at 90-day follow-up.

Allocation answer	Usual care (n=40)	Intravenous iron (n=39)
Correct	26	26
Incorrect	1	3
Unsure	13	10

6.4 Discussion

6.4.1 Summary of results

INTACT confirms the feasibility of conducting a large, multicentre trial of intravenous iron in patients recovering from critical illness with moderate and severe anaemia. Overall, the study protocol compliance was excellent, and patient reported HRQoL outcome data were available for over 80% of surviving patients at 90-days post-randomisation. Intravenous iron did result in improvements in haemoglobin concentrations and iron stores, which persisted up to 90-days post-randomisation, when compared with usual care. The greatest degree of separation occurred at 28-days post-randomisation. In-hospital mortality and nosocomial infection rates were not different between both groups and there were no study drug-related serious adverse events or infusion reactions. Participants in the intravenous iron group experienced a shorter post-ICU hospital length of stay and fewer hospital readmissions. There was no evidence of an effect on quality of life and fatigue scores, but INTACT was not powered to detect such differences.

6.4.2 Interpretation of results

Higher hospital discharge haemoglobin has been associated with reduced post-discharge mortality in general ICU survivors (25). In ARDS survivors, higher haemoglobin at ICU discharge was associated with improved exercise capacity, as judged by 6-minute walk test, and fewer activities of daily living dependencies at 3-month follow-up (46). Uncontrolled data from postoperative rehabilitation studies suggests that untreated anaemia may impair recovery and prolong hospital stay through increased fatigue, reduced exercise capacity and impaired muscle function (466-468). Other studies have also demonstrated an association between anaemia at hospital discharge and increase risk of hospital re-admission (467, 469). To my knowledge, INTACT will be the first piece of randomised evidence in critical care survivors to support some of the associations reported from these observational studies.

My findings are consistent with two RCTs in patients undergoing major abdominal surgery, where giving intravenous iron pre-operatively was associated with higher postoperative haemoglobin

concentrations, a decrease in hospital LOS (150) and reduced readmissions to hospital (470). Taken together, these data suggest that correcting anaemia with intravenous iron may have a role in aiding the recovery of patients recovering from critical illness and merits further investigation.

Any potential benefits of intravenous iron would have to be balanced against risks such as infection (382), as discussed in Chapters 2 and 3. I found no association between intravenous iron and nosocomial infection, although INTACT was not sufficiently powered for this. The definition of infection I opted for was pragmatic and reflective of clinical practice. Iron-induced hypophosphataemia is now a well-recognised complication associated with iron preparations such as ferric carboxymaltose (153). The clinical significance of this still uncertain but I observed no long-term changes in phosphate levels between both groups. A recent MHRA alert advised monitoring serum phosphate levels in patients:

- requiring multiple doses of ferric carboxymaltose at higher doses
- on long-term treatment with ferric carboxymaltose
- with pre-existing risk factors for hypophosphatemia such as pre-existing calcium and phosphate malabsorption, osteoporosis, hyperparathyroidism and osteoporosis.

Such patients would have been excluded as part of the eligibility criteria. Critically ill patients during the course of their ICU stay will have their calcium and phosphate levels regularly monitored and supplemented as required. Therefore, I do not perceive this to be a significant risk, especially with a single dose infusion.

I found no differences in HRQoL and fatigue scores, although INTACT was not powered to detect such differences. I evaluated fatigue using a validated tool (FACIT-F) (455). The mean score at baseline was approximately 16, indicating high levels of fatigue. Although fatigue scores improved in both groups at 90-days, values were lower than population norms and more similar to patients with other inflammatory conditions such as rheumatoid arthritis (471) and cancer (472). The estimates

provided can be used to calculate power calculations for a future definitive trial and minimally important differences of greater than 3 points have been defined previously (457).

As discussed in this thesis, currently available tests of iron status, such as ferritin, are also acute phase proteins and have limited utility in characterising iron status in the context of inflammation. In Chapter 4, I found that hepcidin may have a potential role in guiding iron therapy (397, 473). Inflammation may also be a barrier to haemoglobin recovery. The exploratory analyses that I conducted support these hypotheses. Participants with high hepcidin and CRP concentrations in the usual care arm demonstrated suboptimal haemoglobin recovery when compared with those who received intravenous iron. This may be a group of patients who may benefit from more aggressive management of anaemia in the post-ICU period. However, hepcidin is not routinely available in hospitals and it may be that other clinical and laboratory markers that are readily available (e.g. CRP) may be useful for guiding therapy. When I compared the clinical and laboratory characteristics between 'responders' and 'non-responders', responders were younger, more likely to be surgical patients, and had lower haemoglobin, CRP and ferritin concentrations at randomisation. However, these were not statistically significant.

6.4.3 Strengths and limitations

Strengths of this multicentre trial include a published study protocol (434), predefined trial progression criteria, central randomisation, and high protocol adherence and follow-up completion rates. I utilised a multimodal approach to minimise missing data and selection bias from loss to follow-up. I obtained outcome data for a minimum of 70 participants in order to provide a reasonable estimate of the standard deviations for the sample size estimation of a definitive trial (460).

INTACT also has limitations. It was open label, which may have introduced performance bias, particularly for HRQoL measures, and attrition bias. Ferric carboxymaltose is a challenging and costly substance to blind due to its rusty brown colour. However, I observed no differences in HRQoL scores or completion rates between groups. Although the participants enrolled into INTACT were

broadly representative of the UK national cohort, they were also a heterogenous group and this may have diluted any potential benefits of IV iron. In Chapter 7, I discuss the concept of enrichment which may be a useful strategy for addressing the heterogeneity encountered in critical care trials. The exploratory outcome results should be considered hypothesis-generating but serve as a platform for testing in future clinical trials.

6.5 Conclusion

In summary, INTACT confirmed the feasibility of conducting a large, multicentre trial of intravenous iron in patients recovering from ICU stay with moderate and severe anaemia whilst also demonstrating early evidence of biological efficacy and possible improvements in clinical outcomes. Some of the earlier difficulties with recruitment were addressed with modifications to the study inclusion and exclusion criteria. There was a high level of adherence to the study protocol and completeness of outcome data collection. The next steps include the optimising the design of a future trial, particularly with regards to patient selection and choice of outcome measures. These are discussed in Chapter 7.

Chapter 7 Discussion and future directions

7.1 Discussion

The aim of this thesis was to investigate the role intravenous iron as a therapeutic option for treating anaemia in ICU survivors through different study designs including systematic reviews, laboratory work and a feasibility RCT. This work has generated new data and identified remaining uncertainties to inform the directions for future research. The main findings and implications are summarised below.

Objective one: To systematically review the current evidence of the safety and efficacy of iron therapy in critically ill patients

The rationale for conducting this systematic review was to provide a contemporary summary of the evidence on the safety and efficacy of iron therapy, by any route, in critically ill patients. This evidence would then in turn be used to inform guideline recommendations, whilst also identifying gaps for future research.

Following a comprehensive search, I identified six RCTs comparing iron therapy with either no iron or placebo across a range of ICU settings. A total of 805 participants were included. Overall, there was no evidence of an effect of iron therapy, by any route, on reducing RBC transfusion requirements. Iron therapy did result in higher Hb concentrations at medium-term follow-up. I found no evidence of an effect of iron on the risk of infection. The findings of trial sequential analysis showed that the required sample size to answer some of these questions, in particular whether iron therapy reduces RBC transfusion requirements or increases the risk of infection, has not been met.

None of the included studies in this review provided data on patient-centred outcomes such as physical function or HRQoL. Intravenous iron will simply not work rapidly enough to increase Hb in critically ill patients. Therefore, I do not consider there to be any value in conducting further RCTs to

determine if intravenous iron reduces RBC transfusion requirements in critically ill patients. In the absence of any clear patient-centred benefits, it should not be routinely used to treat anaemia in critically ill patients. This view is now supported by guideline recommendations from ESICM. A more suitable time point, which is supported by some experimental data, would be during recovery from critical illness. This would also allow for an assessment of its effect on patient-centred outcomes.

I believe that data on infection need to be more comprehensively collected and published in order to gain a better understanding of the balance between the risks and benefits of intravenous iron. This becomes more relevant in the context of patient populations at risk of developing a new or worsening infection. These considerations led to the evolutions of the next research project (Objective two).

Objective two: To systematically review the safety data for intravenous iron on the risk of infection across all patient groups

Thinking beyond critically ill patients, many clinical practice guidelines across a range of clinical conditions advocate the use of intravenous iron for treating anaemia. In certain conditions, such as HFrEF and CKD, these recommendations are supported by large, well-conducted RCTs. There is evidence that global use of intravenous iron is increasing. My concern is that with this increased use, there may be associated harm, and in particular the risk of infection. This provided the rationale for conducting this systematic review.

I found that intravenous iron was associated with an increased risk of infection when compared with oral iron or no iron (RR (95% CI) 1.17 (1.04 to 1.31), p = 0.003, I^2 = 37%; 19480 participants, 64 RCTs; *moderate* certainty of evidence). The estimate was largely unchanged in a pre-planned sensitivity analysis that excluded studies at high risk of bias. In a subgroup analysis, the highest risk was in patients with IBD but the direction of effect favoured infection in almost all other subgroups. It remains unclear so whether this increased risk translates into worse outcomes such as prolonged hospitalisation or death.

Close scrutiny of the definitions of infection used found that 49 RCTs did not provide a definition of infection. The remaining RCTs used a range of definitions, including pragmatic investigator defined definitions and various classification systems. Only two RCTs used a standardised definition from the CDC. Of the 33 ongoing RCTs that I identified, thirteen have infection listed as a pre-specified outcome measure. Rather worryingly, only four of these RCTs have provided a definition of infection. These issues limit the understanding of the nature and true extent of the risk of infection, and the risk may be higher than that reported in this systematic review.

To my knowledge, this is the largest and most comprehensive meta-analysis evaluating the risk of infection associated with intravenous preparations across all populations. The findings of this work will help inform clinical practice by encouraging clinicians to weight up the benefits and the risks of intravenous iron more carefully. Researchers involved in the design of ongoing and future trials should seek to incorporate standardised definitions of infection to increase the reliability of future meta-analyses.

Objective three: To investigate if newer markers of iron status, such as hepcidin, can better identify iron deficiency than current routine tests in the context of inflammation

Running parallel to the systematic reviews, I sought to better characterise AI and understand the underlying mechanisms through two laboratory studies. The first study investigated the diagnostic utility of hepcidin to identify iron deficiency in AI. I analysed blood samples taken from a RCT evaluating the effect of a rehabilitation programme delivered to patients discharged from ICU. These samples were taken as close as possible to hospital discharge and were available for 117 participants. I measured hepcidin and sTfR by ELISA, along with other routine markers of iron status such as ferritin, serum iron and Tsat.

Approximately 80% of included ICU survivors were discharged with AI from hospital. Using linear regression and receiver operator characteristic (ROC) analysis, I found that a hepcidin concentration of <19 ng/ml achieved the maximum AUC^{ROC} (0.72) and Youden index (0.47) with a sensitivity of

73% and specificity of 74%, for identifying true iron deficiency in the context of inflammation. Using this threshold, approximately one-third of ICU survivors would be identified as potentially having true iron deficiency compared with only 10% using routine tests of iron status. However, based on the AUC^{ROC} value, I would not recommend its use as a solitary diagnostic test for iron status in the setting of AI.

These findings add further weight to the hypothesis that the recovery phase of critical illness may be more suitable timepoint for intravenous iron therapy. As the evidence base for the role of hepcidin grows, studies investigating its association with responses to intravenous iron and functional outcomes after ICU would be of substantial interest.

Objective four: To explore the variability in subpopulations of erythroid progenitors, as a marker of erythropoiesis, in a heterogeneous cohort of critically ill patients

I planned to undertake a prospective cohort study aiming to assess sub-populations of erythroid progenitors at different stages of differentiation from peripheral blood using multicolour flow cytometry. Erythroid output would be measured at two distinct illness timepoints – acute illness and 28 days later when participants are likely to be convalescing. This would allow for an assessment of the temporal changes in erythropoiesis whilst also exploring if there are any person-specific or illness-specific factors associated with impaired erythropoiesis. The planned sample size was 30 participants.

At the time of writing this thesis, the study has been interrupted by the COVID-19 pandemic. However, I managed to recruit and collect peripheral blood samples from 30 participants within 72 hours of ICU admission, with a follow-up sample available for 17 patients. Eight participants were lost to follow-up due to death (n=4) or because of social distancing restrictions (n=4) that were in place during the first wave of COVID-19. We have completed the first phase of the study which was extracting CD34+ cells from peripheral blood and these are currently stored in liquid nitrogen.

Preliminary data analysis supports the internal validity of the study as I have captured a sick cohort of

patients, whilst also ensuring that all samples were collected within the pre-specified time frames. The next steps include isolation of MEP cells using a FACS panel and to visually quantify the proportion of erythroid and myeloid colonies.

Objective five: To conduct a feasibility RCT of intravenous iron for the treatment of anaemia in patients recovering from critical illness

The findings of the work conducted in Chapters 2, 3 and 4 provided the platform and rationale for conducting the INTACT trial. It became increasingly apparent that the recovery phase may be a more suitable time point for intravenous iron administration, and that there is an urgent need for interventions that may improve recovery from critical illness. However, as discussed in Chapter 5, there are challenges in conducting research in ICU survivors particularly around recruitment and retention. Critically ill patients are also a heterogenous population with differences in illness diagnoses and characteristics, premorbid health states and clinical outcomes. This will inevitably result in differential responses to interventions.

INTACT was a multicentre, feasibility RCT conducted across 4 UK ICUs. The aims were to collect feasibility data to help inform the design and sample size calculations for a future definitive RCT. Patients being discharge from critical care with moderate anaemia (Hb ≤100 g/l) were eligible. Ninety-eight participants were randomised over 15 months to either intravenous iron or usual care (49 in each arm). Overall, INTACT satisfied the pre-defined criteria for demonstration of feasibility.

Participants allocated to the intravenous iron group had significantly higher haemoglobin, serum ferritin, serum iron and transferrin saturation concentrations and lower transferrin concentrations throughout the study follow-up period when compared with participants receiving usual care. These data support the biological efficacy of intravenous iron in a cohort of patients with significant inflammation. Exploratory analyses suggested that baseline inflammation and iron status, as defined by C-reactive protein and hepcidin respectively, may be potential effect modifiers. There were no differences in infection and in-hospital mortality in both groups. The directions of effect were

encouraging for intravenous iron in reducing post-ICU length of stay and hospital readmissions. There were no statistically significant differences in HRQoL and fatigue scores between both groups, but INTACT was not powered to detect such differences.

The feasibility, biological and clinical findings of INTACT are supportive of a future definitive RCT. In the next section, I will discuss the implications of these findings for planning such a trial.

7.2 Future directions arising from this thesis

7.2.1 A definitive trial (INTACT-2)

INTACT highlighted many of the challenges of conducting RCTs in general, but also of conducting RCTs in this patient population. In this section, I will discuss these challenges and potential strategies to inform the design of a future trial. The following aspects will be covered: (i) trial design, conduct and processes (e.g. recruitment, retention and follow-up); (ii) participant selection and sample enrichment; (iii) delivery of intervention or comparator; and (iv) choice and timing of outcome measures. Where possible, I will adhere to recent recommendations from the First Critical Care Clinical Trialists Workshop that were published in February 2020 (474).

Trial design, conduct and processes

Recruitment

A review of the recruitment and retention of participants in single or multicentre RCTs funded by the NIHR Health Technology Assessment (HTA) found that (475):

- The final recruitment target sample size was only achieved in 56% (85/151) RCTs
- More than 80% of the final target sample size was achieved for 79% of the RCTs (119/151)
- The median (IQR) recruitment rate (participants per centre per month) was 0.92 (0.43 to 2.79)
- The median (IQR) retention rate with valid primary outcome data at follow-up was estimated at 89% (79 to 97%)

As highlighted in Chapter 5, recruitment into the study was slow. It could be argued that I was perhaps overly optimistic in my initial recruitment projections, aiming for 8-10 participants per month. However, I regularly evaluated the inclusion and exclusion criteria to see if adjustments could be made to improve recruitment whilst also ensuring the safety of trial participants and maintaining the scientific validity of the trial.

I have carefully considered the reasons for non-participation that were listed in **Table 6.4** in Chapter 6. A recent systematic review on methods for improving recruitment to RCTs found that the following techniques may be useful (476):

- Open label design – although this may improvement recruitment, it will involve a trade-off with compromising the internal validity of the study design. Blinding may not be as crucial a factor as discussed in Chapter 5 and as shown in Chapter 6, where one-third of participants were unsure or incorrectly remembered what treatment arm they were allocated to.
- Using a telephone reminder to contact non-responders to a postal invitation – this would not be applicable for INTACT.
- Optimising PILs through bespoke development with the target population plus formal user-testing – I had originally devised the PIL with the Oxford ICU Patient Form. For a future trial, I will work with this group again and explore alternative approaches such as one-page PILs and/or infographics. This may minimise the cognitive burden at the initial approach, particularly for those potential participants who are already concerned about 'having too much on'.
- Offering financial incentives – I offered participants full reimbursement for any travel costs. Other financial incentives include offering participants monetary gift vouchers. There is evidence that participants consider this practice acceptable, but this would have to be balanced against potential ethical concerns and increased trial funding requirements (477, 478). This is something I will consider for a future study.

- Incorporating qualitative research and mixed methods process evaluations into RCTs are also useful methods for highlighting reasons for poor recruitment and for finding solutions that increase recruitment (479).

Retention and follow-up

Retention rates for INTACT achieved the 'green' category for our stop-go criteria. This could be attributed to the multi-modal strategy described in Chapter 5. Although successful, this strategy was resource intensive in terms of staff and finances. The ICUs participating in INTACT all had established post-ICU services. My concerns are whether this would be scalable on a national scale to 50+ ICUs.

I observed that the 28-day follow-up time point was the most challenging in terms of contacting participants. Although I did not systematically collect data on this, potential reasons include participants being discharged to short-term rehabilitation facilities, briefly residing with others (e.g., close family or friends) instead of in their own residence, or they may be experiencing extremely poor HRQoL. Published data suggests that HRQoL scores in ICU survivors reach a nadir at approximately 1 month before slowly improving over time (3). Some or all these issues may resolve over time and this may reflect the better participant contact rates at 90-days post-randomisation.

For a future trial, I would consider the following changes which are based on the findings of INTACT, discussions with experienced clinical trialists and published evidence on strategies to improve retention in randomised trials (446):

- Use of monetary and financial incentives in the form a gift voucher
- An open label design
- Giving participants a study pen and/or fridge magnets with the trial name and logo. These may serve as a reminder for follow-up visits and increase retention and response rates by

1.9% (0.0% to 3.7%). (https://www.trialforge.org/resource/evidence-pack-retention-adding-a-pen-ret3/)

- Electronic prompts via text message or emails. These may increase retention by 6.3% (95%CI: 0.5% to 12.2%). (https://www.trialforge.org/resource/evidence-pack-retention-electronic-prompts-id-ret2/)
- Undertaking detailed assessments, such as laboratory and physical function tests, whilst participants are still in hospital. This may become more relevant in an era of social distancing due to COVID-19, where undertaking face-to-face visits in participants' home may be challenging.
- Inclusion of postal questionnaires with recorded delivery

Trial processes

I have demonstrated a viable approach for managing anaemia in patients recovering from critical illness. Patients are already in hospital, being nursed and monitored in a hospital bed, and therefore the logistics of applying an intervention and obtaining blood tests are easier without additional burden on the healthcare system. This was viewed as a positive aspect from informal discussions with research team members at participating sites and trial participants themselves.

Participant selection and sample enrichment

Over the past decade there have been numerous 'negative' or 'no effect' RCTs that have been published in critical care. There is considerable debate as to whether these RCTs have improved the care of critically ill patients (480, 481). An important point raised in all these debates is the heterogeneity of the included patient populations and how best to optimise patient selection.

Heterogeneity is the variability in the risk of a patient developing the outcome of interest and the variability in the likelihood of benefit or harm from the intervention being evaluated. Enrichment has been proposed as a method to reduce population heterogeneity in order to identify participants who

are most likely to benefit (and less likely to be harmed) by the intervention (482). It can be applied to the formula of physiological statistics (483) where enrichment increases the signal and reduces the noise:

$$\textbf{Confidence in trial result} = \frac{\textbf{Signal}}{\textbf{Noise}} \textbf{X} \sqrt{\textbf{Samplesize}}$$

Much of the work so far in enrichment in critical care has been conducted in sepsis and ARDS, where attempts have been made to characterise particular clinical phenotypes that could be used as trial eligibility criteria. Examples include the Sepsis 3.0 (379) and Berlin ARDS definitions (484), where septic shock and severe ARDS are associated with higher risk of death. Other methods for refining the population include using illness severity scores or clinical prediction models for the outcome of interest, molecular phenotyping (485) and determining endotypes (486). In brief, there are three different types of enrichment methods that are possible before randomisation – practical, predictive and prognostic (487). These are described in **Table 7.1.**

As part of the design of INTACT, I considered a form of prognostic enrichment and chose a Hb threshold of ≤100 g/l. This was based on research, in both critically ill and non-critically ill patients, which found associations between this threshold and persisting anaemia (23), high levels of fatigue (27) and poor mobility (435). In addition, intravenous iron is likely to be more efficacious when the haemoglobin is being corrected from lower concentrations when compared with higher and/or normal haemoglobin concentrations (436). This threshold has been supported by further database analysis work that I, and others, have undertaken which has demonstrated that a Hb ≤100 g/l is associated with prolonged post-ICU hospitalisation and increased hospital readmissions after ICU discharge.

Table 7.1 Types of enrichment

Type of enrichment	Description	Examples
Practical	Tests or biomarkers to reduce illness level heterogeneity (i.e. increasing diagnostic specificity of illness)	Chest X-rays, echocardiograms, confirmation of infection using rapid molecular testing techniques
Prognostic	Patient or illness characteristics that increases the risk of *outcome*. Generates high risk of outcome population characteristics.	Use of risk scores to identify population at high risk of outcome e.g. APACHE II, SOFA, MAKE30
Predictive	Patient or illness characteristics that increase or predict response to treatment. Generates treatment specific responder characteristics within heterogenous illnesses.	Test-doses of interventions e.g. titrating PEEP to identify PEEP-responsive (or unresponsive) subgroup. Biomarkers e.g. CRP, IL-6, Troponin

APACHEII, Acute Physiology and Chronic Health Evaluation II; CRP, C-reactive protein, IL; Interleukin; MAKE30, Major Adverse Kidney Events within 30 days; PEEP, Positive End Expiratory Pressure; SOFA, Sequential Organ Failure Assessment.

The exploratory analyses that I undertook aimed to identify particular clinical and biological characteristics that may be useful for prognostic and predictive enrichment for a future trial. Participants with elevated hepcidin and CRP concentrations in the usual care arm at baseline demonstrated lower haemoglobin increments at 28 days post-randomisation when compared with those who received intravenous iron. Using haemoglobin response as an outcome measure, I found that younger age, and lower haemoglobin, ferritin and CRP concentrations, were associated with the outcome, although these were not statistically significant. Therefore, anaemia severity, and hepcidin and CRP concentrations, could all be considered as prognostic and predictive markers for intravenous iron therapy.

Another option to improve prognostic enrichment would be the use of a risk prediction model for post intensive care syndrome (PICS) following critical illness. However, a recent systematic review found only three studies that developed a model for any post-ICU impairment (488). All models were at

high risk of bias and none are externally validated as yet. Two of these studies evaluated physical function and are summarised below (**Table 7.2)**. Some of the risk factors identified in these studies (e.g. age, being a medical patient) are similar to those observed in the exploratory analyses of INTACT.

Table 7.2 Description of studies evaluating physical function.

	Outcome measures	**Timing of measurements**	**Risk factors identified**	**Outcome measure score direction**
Detsky et al (489)	Independent ambulation up to 10 stairs and independent toileting	6 months post-ICU discharge	Higher APACHE III score, medical patient, older age, non-white race, recent hospitalisation, prior transplantation, history of cancer or of neurologic or liver disease	Not applicable
Schandl et al (490)	Katz ADL Index	2 months post-ICU discharge	Low educational level, impaired core stability, fractures, ICU length of stay >2 days	Score out of 180. Each predictor renders a risk score. Higher scores equate to worse outcomes

ADL, Activities of Daily Living; APACHEII, Acute Physiology and Chronic Health Evaluation II; ICU, Intensive Care Unit

Despite the perceived benefits of enrichment, it may not necessarily create better or more efficient RCTs when compared with large, simple, pragmatic RCTs for the following reasons:

- Criteria to determine particular phenotypes requires consensus which can cause disagreements
- Use of novel biomarkers may not always be deliverable at the bedside or produce results within a reasonable timeframe. This may negatively impact recruitment.
- It reduces the potential population that may be eligible for a trial, which may increase the duration of the trial and associated costs

- Limited applicability of any positive results to patients who do not meet the enrichment criteria during routine clinical care

Of relevance to INTACT, measuring hepcidin and other biomarkers of inflammation e.g. IL-6 are not readily available at the bedside currently, although haemoglobin and CRP are. A potential solution could include collecting and storing samples for later analysis. However, at the design stage of a future trial I would ensure that adequately powered, *a priori* subgroup analyses of these biomarkers are included (491). This may allow for a more credible examination, in the event of a no-effect trial, of subsets of patients who may benefit from intervention.

Delivery of intervention or comparator

As discussed earlier, there were advantages of delivering the intervention whilst participants were still in hospital. I opted for a pragmatic one-off dose of 1000mg ferric carboxymaltose (492). Although there was evidence of biological efficacy, others have criticised this dose as it may be under-dosing patients (493). According to the SmPC, higher doses of up to 2000mg can be safely given but this would have to be balanced against the costs and slightly increased complexity of administration. I did not evaluate EPO. EPO, in combination with iron therapy, has been advocated for the treatment of anaemia of inflammation (178, 494) and is a standard of care in patients with chronic kidney disease. Low quality evidence also suggests a possible mortality benefit in critically ill patients (495). However, due to safety concerns regarding thrombosis and lack of robust cost-effectiveness data, it is not currently licensed for this group of patients in the UK (496).

For a future trial, the decision between using a placebo or a usual care arm would require careful consideration. The experience from INTACT was that it had no effect on participant response and retention rates. Although it may influence reporting of subjective outcome measures, one-third of participants were unable to correctly recall which arm they were allocated to. As discussed earlier, open label designs may also be beneficial for increasing participant recruitment and retention. A usual care arm may also result in lower costs and trial complexity, particularly as intravenous iron is a

challenging substance to blind. However, inadequate blinding may result in performance and detection bias may prejudice the effect estimates of the study outcomes. Potential strategies for blinding in a future trial could include: (i) concealing the infusion and the arm into which it is being administered behind a barrier, which would be relatively cheap; (ii) wrapping the infusion tubing in black plastic or aluminium foil; and (iii) covering the infusion bag itself. Given the increasing feasibility, experience and potential low cost options of being able to blind IV iron with the methods listed above, I would opt for using a placebo. In order to minimise bias in outcome measurements, particularly where a study outcome requires some judgement (e.g. infection) outcome assessors could be also blinded to the study allocation.

Choice and timing of outcome measures

The primary outcome for a phase III/IV trial should ideally be one that is important to patients and provide convincing evidence directly related to the objectives of the trial. Secondary outcomes can help support the objectives of the trial and evaluate additional effects of the interventions (e.g. safety). Through INTACT, I obtained preliminary data on a range of subjective and objective outcome measures. At the time of writing this thesis, I have not selected a primary outcome for the definitive trial. The outcome I choose will largely be influenced by the patient and public involvement work and clinician surveys that I will undertake. In addition, there must also be sound biological plausibility on how intravenous iron may influence the chosen outcome. I have summarised the potential outcome measures for a definitive trial, along with their advantages and disadvantages in **Table 7.1**.

Mortality is the most frequent outcome measure used in critical care trials, however the duration for mortality (e.g. ICU, hospital, 28-day, 90-day) remains unclear. Although survival is important, it is increasingly being recognised that patients may place greater value on other outcomes such as quality of life, quicker return to their usual residence, return to employment and avoiding hospital readmissions (195, 474).

Using a patient-reported outcome measure is another suitable option. For INTACT, I focused on

fatigue. The FACIT-F is a validated tool used to assess fatigue in survivors of critical illness. It was relatively straight forward and quick to administer in person or over the telephone. It also appeared to be sensitive to the intervention, when compared with the MFI-20 score and I observed no 'ceiling' effect. An important issue which I discussed in Chapter 5, is how to account for pre-existing HRQoL and one approach would be to use pre-existing comorbidity burden (10).

Minimal important differences for fatigue for FACIT-F are also available and these may be used to derive the sample size calculations for a future definitive study, should the primary outcome be improvement in fatigue (457). Assuming the mean (SD) FACIT-F score of 16.86 (12.54) in the usual care arm, to detect an improvement from intravenous iron to a mean FACIT-F score of 19.86 (difference of 3 points) at 90% power, allowing a conservative 10% loss to follow-up, the required sample size for a definitive trial would be 830 patients (415 participants per group).

Measures of physical function such as 6-minute walk test (6-MWT) are also increasingly becoming popular in critical care. A recent study validated its use in survivors of ARDS (497). However, limitations include its applicability to other conditions requiring critical care, effects of pre-existing comorbidity and newly acquired musculoskeletal impairments as a result of critical illness (498), and patient preferences.

Table 7.3 Potential outcome measures for a future definitive trial of intravenous iron in survivors of critical illness

Measure	Advantages	Disadvantages	Supporting data and Comments
Functional / patient-centred outcomes			
Fatigue - FACIT-F	Important outcome for patients FACIT-F is a validated for use in critically ill patients Easy to complete	'So what' risk from potential funders i.e. is a change in fatigue score by few points worth the costs? May be counter-balanced if patients think it is an important research priority	Preliminary data from INTACT suggest that FACIT-F may be sensitive to intervention MCIDs are available for sample size calculations.
EQ-5D	Ties in well with HEE Generic instrument – good evidence for phone use with available translations	Risk of insensitivity to intervention	Preliminary data available from INTACT
SF-36 / SF-6D VT domain	Similar arguments for EQ-5D	Takes longer to complete	Wealth of data available in the post-ICU literature to use for sample size calculations
'Hard' clinical outcomes			
Death	Easy to measure Directly applicable to HEE	May be irrelevant to survivors No clear biological plausibility between intravenous iron and mortality	Can be obtained through data linkage
Length of acute hospital stay	Easy to measure Important to patients Directly applicable to HEE	Influenced by local discharge capabilities Skewed outcome measure	Can be obtained through data linkage
Days alive and out of hospital (DAOH)	Relatively easy to measure but requires a defined time point (e.g. DAOH-30, DAOH-60). Important to patients	Provided little information about the *quality* of home stay Early discharge from hospital may not necessarily be beneficial	Sensitive measure in surgical setting Limited validation data in ICU setting Preliminary data available from INTACT but would require further PPI input
Healthcare resource use	Easy to measure Easy link to HE	Uncertainty regarding sensitivity to intervention System oriented	Aspects (e.g. readmissions) can be obtained through data linkage Challenging to derive sample size calculations

Laboratory outcomes			
Haemoglobin	Easy to measure Evidence of biological efficacy of intervention	Largely irrelevant to patient unless on causal pathway to better clinical outcomes Adds data collection and patient burden (e.g. additional blood tests)	Timing of Hb important – effect of intervention may be greatest when Hb separation is greatest.
Markers of iron status	Easy to measure. Evidence of biological efficacy of intervention	Largely irrelevant to patient unless on causal pathway to better clinical outcomes Adds data collection and patient burden (e.g. additional blood tests)	
Blood transfusion	Easy to measure	Not important to patients Unlikely to be sensitive to intervention (most transfusion will be pre-randomisation on ICU)	Little transfusion occurs in the post-ICU period as demonstrated by INTACT
'Safety' outcomes			
Infection	Important for patients, clinicians and HEE Remains a clinical uncertainty as discussed in Chapter 3	Definition requires consideration	Standardised definition, which may involve more data collection, vs. pragmatic definition used in INTACT
Hypophosphatemia	Easy to measure Remains a safety concern	Dubious importance to patients.	Clinical significance of single dose uncertain No evidence of differences in phosphate levels in INTACT
DVT/PE	Safety concern with if considering use of EPO Potentially easy to measure	Adds to data collection burden	Can be obtained through data linkage

DVT, Deep vein thrombosis; EPO, erythropoietin; FACIT, Functional Assessment of Chronic Illness Therapy-Fatigue; HEE, health economic evaluation; ICU, intensive care unit; MCID, minimal clinically important difference; SF-36, Short Form-36, VT, Vitality

Composite outcomes that combine morbidity and mortality, such as ventilator-free days and days-alive-at-home (DAH) (or out of hospital), are increasingly becoming popular to most stakeholders. For example, DAH can be measured at 30 days and 90 days post-randomisation and incorporates mortality, hospital length of stay and readmissions (499). Although DAH is now a validated and recommend outcome measure for perioperative trials (500), its use in the critical care setting requires further evaluation. An advantage of this outcome is that many of the data can be obtained through data linkage with various databases such as Hospital Episodes for Statistics (HES) and ICNARC Case Mix Programme, which minimises ascertainment bias. A limitation of this outcome measure is the competing risk of mortality, which is not insubstantial in the post-ICU period, although advanced statistical approaches are available to account for death and analyse non-mortality outcome measures (501).

It is important to ensure that the outcomes I choose for a future trial adhere to any core outcome sets. A core outcome set is an agreed, standardised collection of outcomes that would be measured and reported, as a minimum, in all clinical trials for a defined field of interest (502). This would allow for more reliable comparisons between individual studies and increase the certainty of evidence in meta-analyses. Core outcome sets have been developed, or are in the process of being developed, for studies evaluating intervention such as mechanical ventilation (503), acute respiratory failure (453) and rehabilitation following critical illness (504).

7.2.2 Characterising inflammatory phenotypes post-critical care

A recurring theme throughout this thesis has been on the role of inflammation in anaemia. Data on the presence of persisting inflammation after ICU after sparse. Griffith et al, in a cohort of 184 patients, found that many patients had persisting inflammation at 3 months following ICU discharge (CRP >3 mg/l in 59% and >10 mg/l in 28%). This was predominantly a proinflammatory phenotype characterised by elevated human neutrophil elastase, IL-6 and IL-8 concentrations.

A better understanding of inflammation in the post-ICU period may be helpful for determining recovery from anaemia, responses to interventions such as intravenous iron and associations with HRQoL

outcomes such as fatigue. Post-hoc analyses of trials in sepsis and ARDS have identified subphenotypes with differential responses to interventions that can be used for future biomarker-guided trials.

I intend on undertaking a secondary analysis of samples that were taken and stored as part of INTACT. The aim of this study would be to characterise inflammatory profiles and subphenotypes in ICU survivors. One possible method would be to use Olink Target96 proteomics (Inflammation panel, https://www.olink.com/products/inflammation) panel. The Olink inflammation panel encompasses 92 key inflammatory cytokines, chemokines and modulators implicated in initiation, propagation and resolution phases of inflammation, activation and regulation of the immune response and apoptosis. It includes, but extends far beyond, markers previously implicated in ARDS and sepsis subphenotypes. A proposed study outline and how it would fit in with INTACT and INTACT-2 are displayed below in **Figure 7.1**.

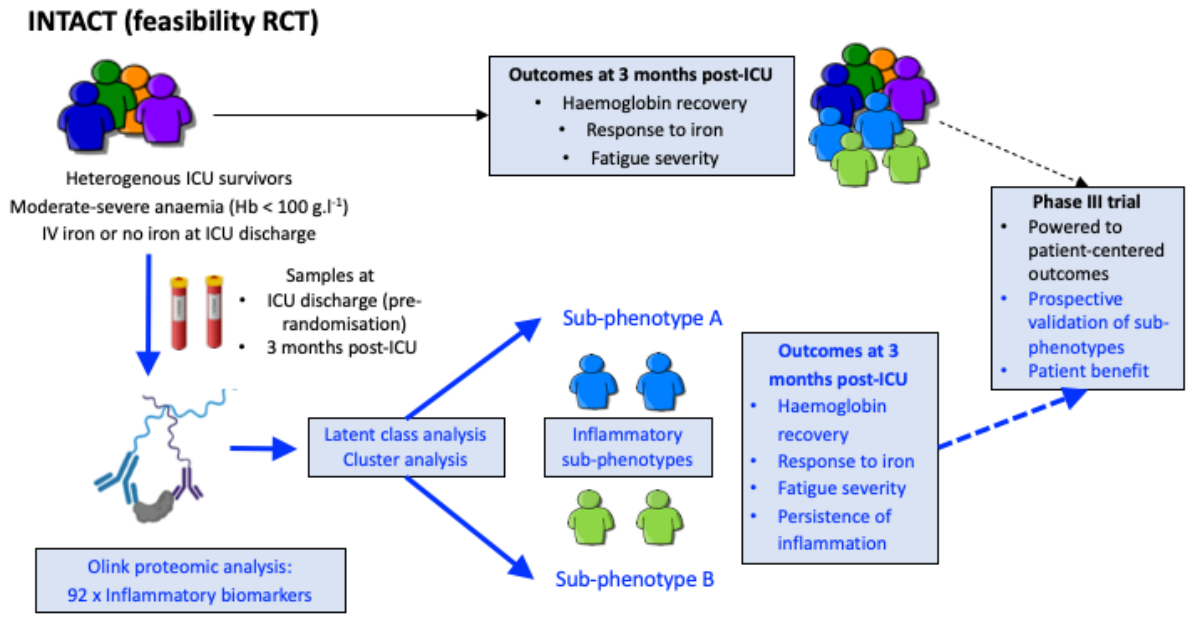


Figure 7.1 Proposed outline of aims and objectives for a secondary analysis of samples collected as part of INTACT

Using such panels would allow for simultaneous multiplex quantification of serum inflammatory biomarker proteins in paired samples (<20µl required) taken pre-randomisation (ICU discharge) and 3 months post-ICU discharge. In brief, Olink uses pairs of oligonucleotide-labelled antibodies to bind specific target proteins. Binding brings complementary oligonucleotide pairs into close proximity, allowing annealing and extension to create PCR targets that are quantified by microfluidic real-time PCR. Data are expressed as NPX (Normalised Protein eXpression) values, which can be used to identify subphenotypes.

A statistical approach would involve using latent class analysis - a validated method to identify distinct subgroups defined by a combination of variables, without mandating consideration of outcome. Baseline (pre-randomisation) clinical and biomarker data would be considered as class-defining variables. Any previously identified biomarkers associated with persisting inflammation in ICU survivors will be included as inputs. Identifying the number of classes and the association between different classes and outcomes will performed using the approach taken by Calfee et al. I would seek the relevant training and support required for such a study from the WIMM Centre for Computational Biology.

7.2.3 Exploring similar relationships in other acutely unwell patients

During the period of this research, I considered the potential implications of anaemia in other groups of acutely unwell / hospitalised patients who may not always require critical care. Based on my clinical experience of undertaking emergency theatre lists, two groups sprang to mind – patients undergoing hip fracture surgery and emergency laparotomy. Each year, approximately 70,000 and 30,000 UK patients undergo these operations respectively.

Perioperative anaemia has been extensively studied in patients undergoing elective surgery, but I suspected that there were would be limited data in patients undergoing emergency surgery. I undertook two separate pieces of work – a systematic review and a database analysis of data submitted to the National Emergency Laparotomy Audit (NELA), to better understand the epidemiology and relationships between anaemia and perioperative outcomes in patients undergoing emergency surgery.

Both these pieces of work have been published in peer-reviewed journals (505, 506).

The aim of the systematic review was to investigate the safety and efficacy of intravenous iron in patients undergoing non-elective/emergency surgery (505). I found only 3 RCTs (605 participants), two of which were conducted in hip fracture surgery and one in patients undergoing living-donor or deceased-donor kidney transplantation. There was no conclusive evidence of an effect of intravenous iron on changes in Hb, RBC transfusion requirements, hospital LOS, infection rates and mortality. The sample sizes of the included RCTs were small and the CIs for all outcomes were wide. These limits could include clinically important differences. Observational data from the hip fracture population suggests that anaemia impeded functional recovery and increased hospital LOS and re-admission rates (435, 507).

I worked with the NELA group for the database analysis study. We hypothesised that pre-operative anaemia was common and associated with poor clinical outcomes after emergency laparotomy. The primary outcome was 90-day mortality. Secondary outcomes included 30-day mortality, return to theatre and postoperative LOS. Primary outcome data were available for 86,763 patients over a 4-year period. Over half of patients (45,306/86,763) were anaemic (Hb <130 g/l) prior to surgery, of which 21% (n=18,626) had evidence of moderate anaemia (Hb 80-109 g/l). In multivariate models, pre-operative anaemia, at all severities, was associated with increased 90 and 30-day mortality, and prolonged hospital stay. Moderate and severe anaemia were associated with increased risk of returning to theatre (**Table 8.1**).

Although one could argue that the findings are not that surprising, this is the first detailed description of anaemia and its clinical impact in patients undergoing emergency surgery. The key question is whether these association are causal or not and anaemia may just be marker of illness severity. I suspect that the association of anaemia with 30-day mortality is more likely to be causative than the association with 90-days, where it is likely to be associated with underlying diseases such as cancer.

Table 7.4 Association between preoperative anaemia and clinical outcomes in patients undergoing emergency laparotomy

Outcome	Effect estimates (95% CI)
90-day mortality*	
Hb >129 g/l	Reference
Mild anaemia	1.15 (1.09 to 1.21)
Moderate anaemia	1.44 (1.36 to 1.52)
Severe anaemia	1.42 (1.24 to 1.63)
30-day mortality*	
Hb >129 g/l	Reference
Mild anaemia	1.07 (1.00 to 1.12)
Moderate anaemia	1.30 (1.21 to 1.38)
Severe anaemia	1.22 (1.05 to 1.43)
Return to theatre*	
Hb >129 g/l	Reference
Mild anaemia	1.02 (0.96 to 1.09)
Moderate anaemia	1.13 (1.06 to 1.21)
Severe anaemia	1.23 (1.06 to 1.43)
Hospital length of stay+	
Hb >129 g/l	Reference
Mild anaemia	1.31 (1.01 to 1.62)
Moderate anaemia	3.41 (3.04 to 3.77)
Severe anaemia	2.80 (1.83 to 3.77)

* Odds ratio

+ Linear regression coefficient

CI, Confidence interval; Hb, Haemoglobin

The findings of both these pieces of work have been used to inform the design of a phase IV, adaptive RCT aiming to evaluate the clinical and cost-effectiveness and safety of haematopoietic interventions administered to recovering patients with anaemia following emergency surgery. A brief study outline is detailed below in **Table 8.2.** At the time of writing this thesis, I am a co-applicant on a funding application for this RCT from the NIHR HTA panel.

Table 7.5 Proposed study outline for an anaemia intervention trial in patients undergoing emergency surgery

Participants	Age >60 years following emergency laparotomy or fragility hip fracture surgery Mild to moderate anaemia (Hb 80-110 g/l) measured postoperatively between days 2-7	
Intervention	Arm A: Iron isomaltoside Arm B: Iron isomaltoside and ESA	
Comparator	Usual medical care	
Outcomes	Primary: DAH30	Secondary: HRQoL using EQ-5D Mortality DAH120 Complications of surgery
Planned sample size	2400 participants 792 patients per group would be required to achieve 90% power, to detect a difference of 2 days in DAH30 between an active treatment versus standard care, with a 2-sided 2.5% level of statistical significance (adjusted for 2 principal comparisons), allowing for 5% non-collection of primary outcome data and a conservative estimate of 11 for standard deviation (SD).	

DAH, Days-alive-at-home; ESA, Erythroid stimulating agents; EQ, EuroQol; HRQoL, Health-related quality of life; Hb, Haemoglobin

7.4 Conclusion

This thesis has taken an in-depth look at the potential role of iron therapy in treating anaemia in ICU survivors. The work has confirmed that:

- Anaemia of inflammation is common in ICU survivors and associated with poor clinical outcomes
- Iron therapy may be efficacious in treating anaemia in patients recovering from an ICU stay but any benefits would have to be weighed against risks such as infection
- Newer markers of iron status, such as hepcidin, may be useful in stratifying patients who may be more responsive to iron therapy
- A large RCT to test these hypotheses is feasible and warranted

Many questions remain unanswered but ongoing work will address these areas in the coming years.

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Appendix 1: Search strategy for systematic review in Chapter 2

The following databases were searched on 15.1.18 for RCTs and SRs:

CENTRAL (*The Cochrane Library* Issue 12, 2017)
MEDLINE (1946 onwards)
EMBASE (1974 onwards)
CINAHL (1982 onwards)
PUBMED (epublications ahead of print only)
TRANSFUSION EVIDENCE LIBRARY (1950 onwards)
WEB OF SCIENCE (ISI Conference Proceedings, 1990 onwards)

Ongoing Trial Databases:
ClinicalTrials.gov
WHO International Clinical Trials Registry Platform (ICTRP)

Searches retrieved 2,190 references plus 115 ongoing trials before duplicates were removed, and 1,592 references plus 95 ongoing trials with duplicates removed. Previously screened references were then removed, leaving 195 references plus 34 ongoing trials for screening.

SEARCH STRATEGIES

CENTRAL
#1 MeSH descriptor: [Intensive Care Units] explode all trees
#2 MeSH descriptor: [Critical Care] explode all trees
#3 MeSH descriptor: (508) this term only
#4 MeSH descriptor: [Critical Care Nursing] this term only
#5 MeSH descriptor: [Trauma Centres] this term only
#6 ((critical* or serious* or severe*) near/2 (ill* or injur* or wound*))
#7 ((intensive* or critical* or neurointensive* or neurocritical*) near/3 (care or therap* or treatment*))
#8 (critical* or intensive* or trauma*):so
#9 (ICU or PICU or MICU or CICU or CVICU or CCU or NICU or SICU or POCCU or ITU or HDU)
#10 "high dependency" or "coronary care unit*"
#11 #1 or #2 or #3 or #4 or #5 or #6 or #7 or #8 or #9 or #10
#12 MeSH descriptor: [Iron] this term only
#13 MeSH descriptor: [Iron Compounds] this term only
#14 MeSH descriptor: [Ferric Compounds] explode all trees
#15 MeSH descriptor: [Ferrous Compounds] explode all trees
#16 MeSH descriptor: [Iron Carbonyl Compounds] this term only
#17 (iron or ferritin or ferrous):ti
#18 (ferrous next (sulfate* or sulphate* or saccharate* or succinate* or fumarate* or fumerate* or gluconate*)):ab
#19 "ferric gluconate" or Fersaday* or Galfer* or Ironorm* or Feospsan* or Ferrograd* or "sodium feredetate" or Sytron* or Niferex*
#20 (iron near/3 (oral* or supplement* or salt* or complex or inject* or infus* or intravenous* or IV or parenteral* or replac* or product* or tablet* or pill* or capsule* or sulphate* or sulfate* or saccharate* or gluconate* or therap*)):ab
#21 "iron sucrose" or venofer* or "iron dextran" or cosmofer* or "iron isomaltoside" or monofer* or ferumoxytol or rienso* or "ferric carboxymaltose" or ferinject*
#22 (dextriferron or fedex or "ferrum lek" or injectafer or "sodium ferrigluconate" or "ferric gluconate" or ferlecit or ferlixit or (ferric near/2 mattol))
#23 dextrofer or hematran or imposil or norferan or imferon or imperon or dexferrum or feosol or imfergen or ferridextran
#24 #12 or #13 or #14 or #15 or #16 or #17 or #18 or #19 or #20 or #21 or #22 or #23
#25 #11 and #24

#26 MeSH descriptor: [Perioperative Care] explode all trees
#27 MeSH descriptor: [Perioperative Period] explode all trees
#28 MeSH descriptor: [Specialties, Surgical] explode all trees
#29 MeSH descriptor: [Surgical Procedures, Operative] explode all trees
#30 (preoperat* or postoperat* or perioperat* or operati* or surg* or presurg* or postsurg* or perisurg*)
#31 #26 or #27 or #28 or #29 or #30
#32 MeSH descriptor: [Anemia] explode all trees
#33 (anemi* or anaemi* or (iron near/2 (status or deficien* or sufficien* or count*)) or low hemoglobin or low haemoglobin or low blood count* or low red cell count* or low red blood cell count* or low serum ferritin or low serum iron or low transferrin saturation or low hematocrit or low haematocrit)
#34 #32 or #33
#35 #31 and #34
#36 #11 or #35
#37 #36 and #24

MEDLINE (OvidSP)
1. exp Anemia/
2. (anemi* or anaemi* or (iron adj2 (status or deficien* or sufficien* or count*)) or low h?emoglobin or low blood count* or low red cell count* or low red blood cell count* or low serum ferritin or low serum iron or low transferrin saturation or low h?ematocrit).tw,kf.
3. 1 or 2
4. exp Perioperative Care/
5. exp Perioperative Period/
6. exp Specialties, Surgical/
7. (preoperat* or postoperat* or perioperat* or operati* or surg* or presurg* or postsurg* or perisurg*).mp.
8. 4 or 5 or 6 or 7
9. 3 and 8
10. exp Intensive Care Units/
11. exp Critical Care/
12. Critical Illness/
13. exp Perioperative Care/
14. Critical Care Nursing/
15. Trauma Centres/
16. (((critical* or serious* or severe*) adj2 (ill* or injur* or wound*)) or trauma*).tw.
17. ((intensive* or critical* or neurointensive* or neurocritical*) adj3 (care or therap* or treatment*)).tw.
18. (critical* or intensive* or trauma*).jn.
19. (ICU or PICU or MICU or CICU or CVICU or CCU or NICU or SICU or POCCU or ITU or HDU).tw.~
20. (high dependency or coronary care unit*).tw.
21. or/10-20
22. 9 or 21
23. Iron/
24. Iron Compounds/
25. exp Ferric Compounds/
26. exp Ferrous Compounds/
27. Iron Carbonyl Compounds/
28. (iron or ferritin or ferrous or ferric).ti.
29. (ferrous adj (sulfate* or sulphate* or saccharate* or succinate* or fumarate* or fumerate* or gluconate*)).ab.
30. (ferric gluconate or Fersaday* or Galfer* or Ironorm* or Feospsan* or Ferrograd* or sodium feredetate or Sytron* or Niferex*).tw.
31. (iron adj3 (oral* or supplement* or salt* or complex or inject* or infus* or intravenous* or IV or parenteral* or replac* or product* or tablet* or pill* or capsule* or sulphate* or sulfate* or saccharate* or gluconate* or therap*)).ab.

32. (iron sucrose or venofer* or iron dextran or cosmofer* or iron isomaltoside or monofer* or ferumoxytol or rienso* or ferric carboxymaltose or ferinject*).tw.
33. (dextriferron or fedex or ferrum lek or injectafer or sodium ferrigluconate or ferric gluconate or ferlecit or ferrlecit or ferlixit or (ferric adj2 mattol)).tw.
34. (dextrofer or hematran or imposil or norferan or imferon or imperon or dexferrum or feosol or imfergen or ferridextran).tw.
35. or/23-34
36. 22 and 35

EMBASE (OvidSP)

1. Anemia/ or Iron Deficiency Anemia/
2. (anemi* or anaemi* or (iron adj2 (status or deficien* or sufficien* or count*)) or low h?emoglobin or low blood count* or low red cell count* or low red blood cell count* or low serum ferritin or low serum iron or low transferrin saturation or low h?ematocrit).tw.
3. 1 or 2
4. exp Surgery/
5. (preoperat* or postoperat* or perioperat* or operati* or surg* or presurg* or postsurg* or perisurg*).mp.
6. 4 or 5
7. 3 and 6
8. exp Intensive Care/
9. Intensive Care Unit/
10. Coronary Care Unit/
11. Burn Unit/
12. Stroke Unit/
13. Critical Illness/
14. ((critical* or serious* or severe*) adj2 (ill* or injur* or wound*)).tw.
15. ((intensive* or critical* or neurointensive* or neurocritical*) adj3 (care or therap* or treatment*)).tw.
16. (critical* or intensive* or trauma*).jn.
17. (ICU or PICU or MICU or CICU or CVICU or CCU or NICU or SICU or POCCU or ITU or HDU).tw.
18. (high dependency or coronary care unit*).tw.
19. or/7-18
20. antianemic agent/ or chondroitin sulfate iron/ or dextriferron/ or ferric carboxymaltose/ or ferric gluconate/ or ferric maltol/ or ferric pyrophosphate/ or ferrocholinate/ or ferrous fumarate/ or ferrous gluconate/ or ferrous succinate/ or ferrous sulfate/ or ferrous sulfate plus folic acid/ or ferrous sulfate plus multivitamin/ or ferumoxytol/ or iron dextran/ or iron glycinate/ or iron polymaltose/ or iron polysaccharide/ or iron protein succinylate/ or iron saccharate/ or iron salt/ or iron sorbitex/ or resoferon/
21. *Iron/
22. Iron Therapy/
23. Ferric Ion/
24. Ferrous Iron/
25. (iron or ferritin or ferrous or ferric).ti.
26. (ferrous adj (sulfate* or sulphate* or saccharate* or succinate* or fumarate* or fumerate* or gluconate*)).ab.
27. (ferric gluconate or Fersaday* or Galfer* or Ironorm* or Feospsan* or Ferrograd* or sodium feredetate or Sytron* or Niferex*).tw.

28. (iron adj3 (oral* or supplement* or salt* or complex or inject* or infus* or intravenous* or IV or parenteral* or replac* or product* or tablet* or pill* or capsule* or sulphate* or sulfate* or saccharate* or gluconate* or therap*)).ab.
29. (iron sucrose or venofer* or iron dextran or cosmofer* or iron isomaltoside or monofer* or ferumoxytol or rienso* or ferric carboxymaltose or ferinject*).tw.

30. (dextriferron or fedex or ferrum lek or injectafer or sodium ferrigluconate or ferric gluconate or ferlecit or ferrlecit or ferlixit or (ferric adj2 mattol)).tw.
31. (dextrofer or hematran or imposil or norferan or imferon or imperon or dexferrum or feosol or imfergen or ferridextran).tw.
32. or/20-31
33. 19 and 32

CINAHL (EBSCOHost)
S1 (MH "Anemia+")
S2 TI ((anemi* or anaemi* or (iron N2 (status or deficien* or sufficien* or count*)) or low hemoglobin or low haemoglobin or low blood count* or low red cell count* or low red blood cell count* or low serum ferritin or low serum iron or low transferrin saturation or low hematocrit or low haematocrit)) OR AB ((anemi* or anaemi* or (iron N2 (status or deficien* or sufficien* or count*)) or low hemoglobin or low haemoglobin or low blood count* or low red cell count* or low red blood cell count* or low serum ferritin or low serum iron or low transferrin saturation or low hematocrit or low haematocrit))

S3 S1 AND S2
S4 (MH "Surgery, Operative+")
S5 TX preoperat* or postoperat* or perioperat* or operati* or surg* or presurg* or postsurg* or perisurg*
S6 S4 OR S5
S7 S3 AND S6
S8 (MH "Critical Care+")
S9 (MH "Intensive Care Units+")
S10 (MH "Critical Illness")
S11 (MH "Catastrophic Illness")
S12 (MH "Critically Ill Patients")
S13 (MH "Critical Care Nursing+")
S14 (MH "Trauma Centres")
S15 TX ((critical* or serious* or severe*) N2 (ill* or injur* or wound*))
S16 TX ((intensive* or critical* or neurointensive* or neurocritical*) N3 (care or therap* or treatment*))
S17 JN (critical* or intensive* or trauma*)
S18 TX (ICU or PICU or MICU or CICU or CVICU or CCU or NICU or SICU or POCCU or ITU or HDU) S19 TX ("high dependency" or "coronary care unit*")
S20 S8 OR S9 OR S10 OR S11 OR S12 OR S13 OR S14 OR S15 OR S16 OR S17 OR S18 OR S19
S21 S7 OR S20
S22 (MH "Iron")
S23 (MH "Iron Compounds+")
S24 TI (iron or ferritin or ferrous or ferric)
S25 AB (ferrous W1 (sulfate* or sulphate* or saccharate* or succinate* or fumarate* or fumerate* or gluconate*))
S26 TX ("ferric gluconate" or Fersaday* or Galfer* or Ironorm* or Feospsan* or Ferrograd* or sodium feredetate or Sytron* or Niferex)
S27 TX (iron N3 (oral* or supplement* or salt* or complex or inject* or infus* or intravenous* or IV or parenteral* or replac* or product* or tablet* or pill* or capsule* or sulphate* or sulfate* or saccharate* or gluconate* or therap*))
S28 TX ("iron sucrose" or venofer* or "iron dextran" or cosmofer* or "iron isomaltoside" or monofer* or ferumoxytol or rienso* or "ferric carboxymaltose" or ferinject*)
S29 TX (dextriferron or fedex or "ferrum lek" or injectafer or "sodium ferrigluconate" or "ferric gluconate" or ferlecit ferrlecit or ferlixit or (ferric W2 mattol))
S30 TX (dextrofer or hematran or imposil or norferan or imferon or imperon or dexferrum or feosol or imfergen or ferridextran)
S31 S22 or S23 or S24 or S25 or S26 or S27 or S28 or S29 or S30
S32 S21 AND S31

PubMed (epublications)

#1 ((critical* OR serious* OR severe*) AND (ill OR illness* OR injur* OR wound*))

#2 ((intensive* OR critical* OR neurointensive* OR neurocritical*) AND (care OR therapy OR therapies OR treatment*))

#3 (ICU OR PICU OR MICU OR CICU OR CVICU OR CCU OR NICU OR SICU OR POCCU OR ITU OR HDU)

#4 "high dependency" OR "coronary care unit"

#5 critical*[TI] OR seriously[TI] OR severely[TI] OR intensive*[TI] OR neurointensive*[TI] OR neurocritical*[TI]

#6 ((anaemi* OR anemi*) AND (preoperat* OR postoperat* OR perioperat* OR operated OR operating OR operation OR operations OR surgery OR surgeries OR surgical* OR presurg* OR postsurg* OR perisurg*))

#7 #1 OR #2 OR #3 OR #4 OR #5 OR #6

#8 iron[TI] OR ferritin[TI] OR ferrous[TI] OR ferric[TI]

#9 ("ferrous sulfate" OR "ferrous sulphate" OR "ferrous saccharate" OR "ferrous fumarate" OR "ferrous fumerate" OR "ferrous gluconate" OR "ferrous succinate")

#10 ("ferric gluconate" OR Fersaday* OR Galfer* OR Ironorm* OR Feospsan* OR Ferrograd* OR "sodium feredetate" OR Sytron* OR Niferex*)

#11 ("oral iron" OR "iron supplement" OR "iron supplements" OR "iron supplementation" OR "iron salt" OR "iron salts" OR "iron complex" OR "injectable iron" OR "iron infusion" OR "iron infusions" OR "infused iron" OR "intravenous iron" OR "IV iron" OR "parenteral iron" OR "iron replacement" OR "iron product" OR "iron products" OR "iron tablet" OR "iron tablets" OR "iron pill" OR "iron pills" OR "iron capsule" OR "iron capsules" OR "iron sulphate" OR "iron sulfate" OR "iron saccharate" OR "iron gluconate" OR "iron therapy")

#12 ("iron sucrose" OR venofer* OR "iron dextran" OR cosmofer* OR "iron isomaltoside" OR monofer* OR ferumoxytol OR rienso* OR ferric carboxymaltose OR ferinject*)

#13 (dextriferron OR fedex OR ferrum lek OR injectafer OR "sodium ferrigluconate" OR "ferric gluconate" OR ferlecit OR ferrlecit OR ferlixit OR "ferric mattol")
#14 (dextrofer or hematran or imposil or norferan or imferon or imperon or dexferrum or feosol or imfergen or ferridextran)

#15 #8 OR #9 OR #10 OR #11 OR #12 OR #13 OR #14

#16 #7 AND #15

#17 (random* OR blind* OR "control group" OR placebo* OR "controlled trial" OR "controlled study" OR groups OR trials OR studies OR "systematic review" OR meta-analysis OR metaanalysis OR "literature search*" OR medline OR cochrane OR embase) AND (publisher[sb] OR inprocess[sb] OR pubmednotmedline[sb])
#18 #16 AND #17

TRANSFUSION EVIDENCE LIBRARY
Clinical Specialty: Critical Care OR Surgery

Subject Area: Management of Anaemia
AND
iron OR ferrous OR ferritin OR ferric

WEB OF SCIENCE – Conference Proceedings Citation Index (CPCI-S)
#1 TS=(critical care OR intensive care OR critically ill OR critical illness OR trauma) OR TS=(anemia OR anemic OR anaemia OR anemia) AND (preoperat* OR postoperat* OR perioperat* OR operated OR operating OR operation OR operations OR surgery OR surgeries OR surgical* OR presurg* OR postsurg* OR perisurg*))

#2 TS=(iron OR ferritin OR ferrous OR ferric)

#3 TS=(randomized OR randomised OR randomly OR controlled trial OR controlled study OR control group OR control groups OR double blind OR blind study OR blind trial)
#4 #1 AND #42 AND #3

WHO ICTRP
Title: critical care OR intensive care OR ICU OR PICU OR ITU OR critically ill OR critical illness OR trauma OR high dependency OR preoperative OR postoperative OR perioperative OR operation OR surgery OR surgical OR presurgery OR postsurgery OR perisurgery
Condition: iron deficiency OR iron deficient OR low iron OR anemia OR anaemia OR low hemoglobin OR low haemoglobin OR low red cell count
Intervention: iron OR ferritin OR ferrous OR ferric
Recruitment: ALL

ClinicalTrials.gov
Search Terms: critical care OR intensive care OR ICU OR PICU OR ITU OR critically ill OR critical illness OR trauma OR high dependency OR preoperative OR postoperative OR perioperative OR operation OR surgery OR surgical OR presurgery OR postsurgery OR perisurgery
Condition: iron deficiency OR iron deficient OR low iron OR anemia OR anaemia OR low hemoglobin OR low haemoglobin OR low red cell count
Intervention: iron OR ferritin OR ferrous OR ferric
Type of Studies: Interventional

Appendix 2: Search strategy for systematic review in Chapter 3

The following databases were searched for randomised controlled trials (from 2013 to present), systematic reviews and non-randomised trials with a comparator group (from 2007 to present) on 28.9.20:

Cochrane Central Register of Controlled Trials (CENTRAL, *The Cochrane Library*) & CDSR 2020, Issue 9
MEDLINE (OvidSP, 1946 to present)

Embase (OvidSP, 1974 to present)
CINAHL (EBSCOHost) (1937 to present)
PubMed (e-publications ahead of print only)
Transfusion Evidence Library (1950 to present)
LILACS (1982 to present)
Web of Science Conference Proceedings Citation Index-Science (CPCI-S) (Thomson Reuters, 1990 to present)

Ongoing trial databases:
CENTRAL
ClinicalTrials.gov (clinicaltrials.gov)
WHO International Clinical Trials Registry Search Platform (ICTRP)

SEARCH STRATEGIES

CENTRAL (*The Cochrane Library*)
#1 MeSH descriptor: [Ferric Compounds] explode all trees
#2 MeSH descriptor: [Ferrous Compounds] explode all trees
#3 MeSH descriptor: [Iron] explode all trees
#4 (alvofer or colliron or faremio or ferion or feriv or fermed or ferri saccharate or ferric hydroxide sucrose or ferric oxide saccharate or saccharated ferric oxide or ferric saccharate or ferrinemia or ferrisaccharate or ferrivenin or ferrologic or ferrous saccharate or ferrovin or fesin or hemafer s or hemafer-s or idafer or (iron near/2 hydroxide sucrose complex) or iron saccharate or iron sucrose or ironcrose or iviron or nefro-fer or nefrofer or neo ferrum or nephroferol or proferrin or referen or reoxyl or saccharate ferric or saccharate iron or saccharated ferric oxide or saccharated iron oxide or sucro fer or sucrofer or sucroven or veniron or venofer or venotrix)
#5 #1 or #2 or #3 or #4
#6 MeSH descriptor: [Administration, Intravenous] explode all trees
#7 (intravenous* or IV or "IV" or infus* or inject* or parenteral*)
#8 #6 or #7
#9 #5 and #8
#10 (ferric carboxymaltose or Ferinject or Injectafer or Iroprem or ferlecit or ferlixit or ferric gluconate or ferrigluconate or ferrlecit or gluconate ferric sodium or (iron near/2 gluconate) or iron isomaltoside or intravenous iron sucrose or iron sucrose injection* or sodium ferrigluconate or diafer or ferric derisomaltose or venofer or monofer or monafer or monoferro or monover or ferumoxytol or feraheme or rienso or "IV iron" or "IV iron" or "iron therapy" or ((intravenous* or inject* or infus* or parenteral) near/3 iron))
#11 (anaemex or cosmofer or dexferrum or dexiron or dextrafer or dextran fe or dextran ferrous or dextran iron or driken or fenate or fer dextran or ferric dextran or ferridex or tranferrisat or ferrodex or ferrodextran or ferrous dextran or ferrum lek or fervetag or hibiron or imferdex or imferon or impheron or imposil or infed or infufer or iron dextran or ironate or monofar or proferdex or uniferon or uniferon or uniferron)
#12 ferumoxytol or injectafer or infed or cosmofer or iron polymaltose or triferic or ferric pyrophosphate citrate
#13 #4 or #9 or #10 or #11 or #12 with Publication Year from 2013 to 2020, in Trials

#14 #4 or #9 or #10 or #11 or #12 with Cochrane Library publication date Between Jan 2007 and Dec 2020, in Cochrane Reviews, Cochrane Protocols
#15 #13 or #14

MEDLINE (OvidSP)
1. exp Ferric Compounds/
2. exp Ferrous Compounds/
3. exp Iron/
4. (alvofer or colliron or faremio or ferion or feriv or fermed or ferri saccharate or ferric hydroxide sucrose or ferric oxide saccharate or ferric oxide,saccharated or ferric saccharate or ferrinemia or ferrisaccharate or ferrivenin or ferrologic or ferrous saccharate or ferrovin or fesin or hemafer s or hemafer-s or idafer or (iron adj2 hydroxide sucrose complex) or iron saccharate or iron sucrose or ironcrose or iviron or nefro-fer or nefrofer or neo ferrum or nephroferol or proferrin or referen or reoxyl or saccharate ferric or saccharate iron or saccharated ferric oxide or saccharated iron oxide or sucro fer or sucrofer or sucroven or veniron or venofer or venotrix).tw,kf.
5. or/1-3
6. exp Administration, Intravenous/
7. (intravenous* or IV or "IV" or infus* or inject* or parenteral*).tw,kf.
8. 6 or 7
9. 5 and 8
10. (ferric carboxymaltose or Ferinject or Injectafer or Iroprem).tw,kf.
11. (ferlecit or ferlixit or ferric gluconate or ferrigluconate or ferrlecit or gluconate ferric sodium or (iron adj2 gluconate) or sodium ferrigluconate or intravenous iron sucrose or iron sucrose injection* or venofer).tw,kf.
12. (diafer or ferric derisomaltose or iron isomaltoside or monofer or monafer or monoferro or monover or ferumoxytol or feraheme or rienso).tw,kf.
13. (IV iron or "IV iron" or iron therapy or ((intravenous* or inject* or infus* or parenteral) adj3 iron)).tw,kf.
14. (ferumoxytol or injectafer or infed or cosmofer or iron polymaltose or triferic or ferric pyrophosphate citrate).mp.
15. (anaemex or cosmofer or dexferrum or dexiron or dextrafer or dextran fe or dextran ferrous or dextran iron or driken or fenate or fer dextran or ferric dextran or ferridex or tranferrisat or ferrodex or ferrodextran or ferrous dextran or ferrum lek or fervetag or hibiron or imferdex or imferon or impheron or imposil or infed or infufer or iron dextran complex or ironate or monofar or proferdex or uniferon or uniferon or uniferron).mp.
16. 4 or 9 or 10 or 11 or 12 or 13 or 14 or 15
17. RANDOMIZED CONTROLLED TRIAL.pt.
18. CONTROLLED CLINICAL TRIAL.pt.
19. (randomi* or trial*).tw,kf.
20. (placebo* or randomly or groups).ab.
21. CLINICAL TRIALS AS TOPIC.sh.
22. or/17-21
23. exp animals/ not humans/
24. 22 not 23
25. 16 and 24
26. limit 25 to yr="2013 -Current"
27. exp COHORT STUDIES/
28. (cohort* or controlled trial* or controlled stud* or comparative trial* or comparative stud* or comparison group* or comparator group* or control group*).tw,kf.
29. ((follow up or observational) adj (study or studies)).tw,kf.
30. (longitudinal* or retrospective* or prospective* or cross sectional*).mp.
31. CROSS-SECTIONAL STUDIES/
32. CONTROLLED BEFORE-AFTER STUDIES/
33. OBSERVATIONAL STUDY/
34. HISTORICALLY CONTROLLED STUDY/
35. INTERRUPTED TIME SERIES ANALYSIS/
36. (nonrandom* or non random*).tw,kf.

37. ((before adj15 (after or during)) or "before-after" or time series or time point* or repeated measur*).tw,kf.
38. (pre-post or pre-test* or pretest* or posttest* or post-test* or (pre adj5 post)).tw,kf.
39. or/27-38
40. Meta-Analysis.pt.
41. Systematic Review.pt.
42. (meta analy$ or metaanaly$).ti.
43. (systematic* adj2 (review* or overview*)).tw,kf.
44. (evidence synthes* or cochrane or medline or pubmed or embase or cinahl or cinhal or lilacs or "web of science" or science citation index or search terms or literature search or published articles or search strateg* or reference list* or bibliograph* or handsearch* or hand search* or manual* search*).ab.
45. (additional adj (papers or articles or sources)).ab.
46. ((electronic or online) adj (sources or resources or databases)).ab.
47. (relevant adj (journals or articles)).ab.
48. "REVIEW LITERATURE AS TOPIC"/
49. "Systematic Reviews as Topic"/
50. META-ANALYSIS AS TOPIC/
51. or/40-50
52. Review.pt.
53. exp CLINICAL TRIALS AS TOPIC/
54. selection criteria.ab. or critical appraisal.ti.
55. (data adj (extraction or analys*)).ab.
56. RANDOMIZED CONTROLLED TRIALS/
57. OBSERVATIONAL STUDY/
58. ((cohort* or observational or retrospective*) adj1 (trial* or stud*)).tw,kf.
59. or/53-58
60. 52 and 59
61. 51 or 60
62. (Comment or Letter or Editorial).pt.
63. 61 not 62
64. 39 or 63
65. exp animals/ not humans/
66. 64 not 65
67. 16 and 66
68. limit 67 to yr="2007 -Current"
69. 26 or 68

EMBASE (OvidSP)
1. ferric carboxymaltose/ or ferric gluconate/ or iron dextran/ or iron isomaltose/ or iron saccharate/

2. (alvofer or colliron or faremio or ferion or feriv or fermed or ferri saccharate or ferric hydroxide sucrose or ferric oxide saccharate or ferric oxide,saccharated or ferric saccharate or ferrinemia or ferrisaccharate or ferrivenin or ferrologic or ferrous saccharate or ferrovin or fesin or hemafer s or hemafer-s or idafer or (iron adj2 hydroxide sucrose complex) or iron saccharate or iron sucrose or ironcrose or iviron or nefro-fer or nefrofer or neo ferrum or nephroferol or proferrin or referen or reoxyl or saccharate ferric or saccharate iron or saccharated ferric oxide or saccharated iron oxide or sucro fer or sucrofer or sucroven or veniron or venofer or venotrix).tw.
3. (anaemex or cosmofer or dexferrum or dexiron or dextrafer or dextran fe or dextran ferrous or dextran iron or driken or fenate or fer dextran or ferric dextran or ferridex or tranferrisat or ferrodex or ferrodextran or ferrous dextran or ferrum lek or fervetag or hibiron or imferdex or imferon or impheron or imposil or infed or infufer or iron dextran complex or ironate or monofar or proferdex or uniferon or uniferon or uniferron).tw.
4. (Ferric carboxymaltose or Ferinject or Injectafer or Iroprem).tw.
5. (ferlecit or ferlixit or ferric gluconate or ferrigluconate or ferrlecit or gluconate ferric sodium or (iron adj2 gluconate) or sodium ferrigluconate or intravenous iron sucrose or iron sucrose injection* or venofer).tw,kf.

6. (diafer or ferric derisomaltose or iron isomaltoside or monofer or monafer or monoferro or monover or ferumoxytol or feraheme or rienso).tw,kf.
7. ferumoxytol/
8. (IV iron or "IV iron" or iron therapy or ((intravenous* or inject* or infus* or parenteral) adj3 iron)).tw.
9. (ferumoxytol or injectafer or infed or cosmofer or iron polymaltose or triferic or ferric pyrophosphate citrate or feraheme or rienso).mp.
10. 1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9
11. Meta Analysis/
12. Systematic Review/
13. (meta analy* or metaanalys*).tw.
14. ((systematic* or literature) adj2 (review* or overview* or search*)).tw.
15. (cochrane or embase or cinahl or cinhal or lilacs or BIDS or science citation index or psyclit or psychlit or psycinfo or psychinfo or cancerlit).ti,ab.
16. ((electronic* or online) adj (sources or resources or databases)).ab.
17. (additional adj (articles or papers or sources)).ab.
18. (reference lists or bibliograph* or handsearch* or hand search* or manual* search*).ab.
19. (relevant adj (journals or articles)).ab.
20. (search term* or published articles or search strateg*).ab.
21. or/11-20
22. (data extraction or selection criteria or inclusion criteria).ab.
23. review.pt.
24. 21 or (22 and 23)
25. editorial.pt.
26. 24 not 25
27. crossover-procedure/ or double-blind procedure/ or randomized controlled trial/ or single-blind procedure/
28. (random* or factorial* or crossover* or cross over* or cross-over* or placebo* or doubl* blind* or singl* blind* or assign* or allocat* or volunteer*).mp.
29. 27 or 28
30. MAJOR CLINICAL STUDY/
31. LONGITUDINAL STUDY/
32. RETROSPECTIVE STUDY/
33. OBSERVATIONAL STUDY/
34. INTERVENTION STUDY/
35. PROSPECTIVE STUDY/ not RANDOMIZED CONTROLLED TRIAL/
36. COHORT ANALYSIS/
37. COMPARATIVE STUDY/
38. (cohort* or controlled trial* or controlled stud* or comparative trial* or comparative stud* or comparison group* or comparator group* or control group*).tw.
39. ((follow up or observational) adj (study or studies)).tw.
40. (longitudinal* or retrospective* or prospective* or cross sectional*).mp.
41. (nonrandom* or non random*).tw.
42. ((before adj15 (after or during)) or "before-after" or time series or time point* or repeated measur*).tw.
43. (pre-post or pre-test* or pretest* or posttest* or post-test* or (pre adj5 post)).tw.
44. or/30-43
45. 26 or 44
46. 10 and 29
47. limit 46 to yr="2013 -Current"
48. 10 and 45
49. limit 48 to yr="2007 -Current"
50. 47 or 49

CINAHL (EBSCOHoST)

S1 (MH "Ferric Compounds+")
S2 (MH "Ferrous Compounds")

S3 (MH "Iron")
S4 S1 OR S2 OR S3
S5 (MH "Administration, Intravenous+")
S6 TX (intravenous* or IV or "IV" or infus* or inject* or parenteral)
S7 S5 OR S6
S8 S4 AND S7
S9 TX (alvofer or colliron or faremio or ferion or feriv or fermed or ferri saccharate or ferric hydroxide sucrose or ferric oxide saccharate or ferric oxide,saccharated or ferric saccharate or ferrinemia or ferrisaccharate or ferrivenin or ferrologic or ferrous saccharate or ferrovin or fesin or hemafer s or hemafer-s or idafer or (iron N2 hydroxide sucrose complex) or iron saccharate or iron sucrose or ironcrose or iviron or nefro-fer or nefrofer or neo ferrum or nephroferol or proferrin or referen or reoxyl or saccharate ferric or saccharate iron or saccharated ferric oxide or saccharated iron oxide or sucro fer or sucrofer or sucroven or veniron or venotrix)
S10 TX (anaemex or cosmofer or dexferrum or dexiron or dextrafer or dextran fe or dextran ferrous or dextran iron or driken or fenate or fer dextran or ferric dextran or ferridex or tranferrisat or ferrodex or ferrodextran or ferrous dextran or ferrum lek or fervetag or hibiron or imferdex or imferon or impheron or imposil or infed or infufer or iron dextran complex or ironate or monofar or proferdex or uniferon or uniferon or uniferron)
S11 TX (ferric carboxymaltose or Ferinject or Injectafer or Iroprem or venofer or ferlecit or ferlixit or ferric gluconate or ferrigluconate or ferrlecit or gluconate ferric sodium or (iron N2 gluconate) or sodium ferrigluconate or diafer or ferric derisomaltose or iron isomaltoside or monofer or monafer or monoferro or monover or ferumoxytol or feraheme or rienso or "IV iron" or "IV iron" or "iron therapy" or ((intravenous* or inject* or infus* or parenteral) N3 iron))
S12 S8 OR S9 OR S10 OR S11
S13 (MH "Prospective Studies+")
S14 (MH "Case Control Studies+")
S15 (MH "Correlational Studies") OR (MH "Cross Sectional Studies")
S16 TI ((cohort study or cohort studies)) OR AB ((cohort study or cohort studies))
S17 TI ((observational stud* or retrospective stud*)) OR AB ((observational stud* or retrospective stud*))
S18 S13 OR S14 OR S15 OR S16 OR S17
S19 (MH Clinical Trials+)
S20 PT Clinical Trial
S21 TI ((controlled trial*) or (clinical trial*)) OR AB ((controlled trial*) or (clinical trial*))
S22 TI ((singl* blind*) OR (doubl* blind*) OR (trebl* blind*) OR (tripl* blind*) OR (singl* mask*) OR (doubl* mask*) OR (tripl* mask*)) OR AB ((singl* blind*) OR (doubl* blind*) OR (trebl* blind*) OR (tripl* blind*) OR (singl* mask*) OR (doubl* mask*) OR (tripl* mask*))
S23 TI randomi* OR AB randomi*
S24 MH RANDOM ASSIGNMENT
S25 TI ((phase three) or (phase III) or (phase three)) or AB ((phase three) or (phase III) or (phase three))
S26 (TI (random* N2 (assign* or allocat*))) OR (AB (random* N2 (assign* or allocat*)))
S27 MH PLACEBOS OR (TI placebo* OR AB placebo*)
S28 S19 OR S20 OR S21 OR S22 OR S23 OR S24 OR S25 OR S26 OR S27
S29 MH META ANALYSIS
S30 MH SYSTEMATIC REVIEW
S31 TI ("meta analys*" OR metaanalys* OR "systematic review" OR "systematic overview" OR "systematic search*") OR AB ("meta analys*" OR metaanalys* OR "systematic review" OR "systematic overview" OR "systematic search*")
S32 TI ("literature review" OR "literature overview" OR "literature search*") OR AB ("literature review" OR "literature overview" OR "literature search*")
S33 TI (cochrane OR embase OR cinahl OR cinhal OR lilacs OR BIDS OR science AND citation AND index OR cancerlit) OR AB (cochrane OR embase OR cinahl OR cinhal OR lilacs OR BIDS OR science AND citation AND index OR cancerlit)
S34 MH QUANTITATIVE STUDIES

S35 (MH "Controlled Before-After Studies") OR (MH "Historically Controlled Study") OR (MH "Interrupted Time Series Analysis") OR (MH "Nonrandomized Trials") OR (MH "Pretest-Posttest Design+") OR (MH "Static Group Comparison") OR (MH "Solomon Four-Group Design")
S36 TX nonrandom* or non random*
S37 TX ((before N15 (after or during)) or "before-after" or time series or time point* or repeated measur*)
S38 TX (pre-post or pre-test* or pretest* or posttest* or post-test* or (pre N5 post))
S39 TX TI ((controlled stud*) or (clinical stud*)) OR AB ((controlled stud*) or (clinical stud*))

S40 S18 OR S29 OR S30 OR S31 OR S32 OR S33 OR S34 OR S35 OR S36 OR S37 OR S38 OR S39
S41 S12 AND S28
S42 S12 AND S28 Limiters - Published Date: 20130101-20201231
S43 S12 AND S40
S44 S12 AND S40 Limiters - Published Date: 20070101-20201231
S45 S42 OR S44

PubMed

#1 (alvofer[TIAB] OR colliron[TIAB] OR faremio[TIAB] OR ferion[TIAB] OR feriv[TIAB] OR fermed[TIAB] OR "ferri saccharate"[TIAB] OR "ferric hydroxide sucrose"[TIAB] OR "ferric oxide saccharate" [TIAB] OR "ferric saccharate"[TIAB] OR ferrinemia[TIAB] OR ferrisaccharate[TIAB] OR ferrivenin[TIAB] OR ferrologic[TIAB] OR "ferrous saccharate"[TIAB] OR ferrovin[TIAB] OR fesin[TIAB] OR "hemafer-s"[TIAB] OR "hemafer-s"[TIAB] OR idafer[TIAB] OR "iron hydroxide sucrose complex"[TIAB] OR "iron saccharate"[TIAB] OR "iron sucrose"[TIAB] OR ironcrose[TIAB] OR iviron[TIAB] OR "nefro-fer"[TIAB] OR nefrofer[TIAB] OR "neo ferrum"[TIAB] OR nephroferol[TIAB] OR proferrin[TIAB] OR referen[TIAB] OR reoxyl[TIAB] OR "saccharate ferric"[TIAB] OR "saccharate iron"[TIAB] OR "saccharated ferric oxide"[TIAB] OR "saccharated iron oxide"[TIAB] OR "sucro fer"[TIAB] OR sucrofer[TIAB] OR sucroven[TIAB] OR veniron[TIAB] OR venofer[TIAB] OR venotrix[TIAB] OR anaemex[TIAB] OR cosmofer[TIAB] OR dexferrum[TIAB] OR dexiron[TIAB] OR dextrafer[TIAB] OR "dextran fe"[TIAB] OR "dextran ferrous"[TIAB] OR "dextran iron"[TIAB] OR driken[TIAB] OR fenate[TIAB] OR "fer dextran"[TIAB] OR "ferric dextran"[TIAB] OR ferridex[TIAB] OR tranferrisat[TIAB] OR ferrodex[TIAB] OR ferrodextran[TIAB] OR "ferrous dextran"[TIAB] OR "ferrum lek"[TIAB] OR fervetag[TIAB] OR hibiron[TIAB] OR imferdex[TIAB] OR imferon[TIAB] OR impheron[TIAB] OR imposil[TIAB] OR infed[TIAB] OR infufer[TIAB] OR "iron dextran"[TIAB] OR ironate[TIAB] OR monofar[TIAB] OR proferdex[TIAB])
#2 (intravenous*[TIAB] OR IV[TIAB] OR "IV"[TIAB] OR infus*[TIAB] OR inject*[TIAB] OR parenteral*[TIAB])
#3 #1 AND #2
#4 ("ferric carboxymaltose"[TIAB] OR Ferinject[TIAB] OR Injectafer[TIAB] OR Iroprem[TIAB] OR venofer[TIAB] OR ferlecit[TIAB] OR ferlixit[TIAB] OR "ferric gluconate"[TIAB] OR ferrigluconate[TIAB] OR ferrlecit[TIAB] OR "gluconate ferric sodium"[TIAB] OR " iron gluconate"[TIAB] OR "sodium ferrigluconate"[TIAB] OR diafer[TIAB] OR "ferric derisomaltose"[TIAB] OR "iron isomaltoside"[TIAB] OR monofer[TIAB] OR monafer[TIAB] OR monoferro[TIAB] OR monover[TIAB] OR ferumoxytol[TIAB] OR feraheme[TIAB] OR rienso[TIAB] OR "IV iron"[TIAB] OR "IV iron"[TIAB] OR "intravenous iron"[TIAB] OR "iron injection"[TIAB] OR "injectable iron"[TIAB] OR "iron infusion"[TIAB] OR "iron infusions"[TIAB] OR "infused iron"[TIAB] OR "parenteral iron"[TIAB] OR "iron therapy"[TIAB])
#5 #3 OR #4
#6 (random* OR blind* OR "control group" OR placebo* OR controlled OR groups OR trial* OR "systematic review" OR "meta-analysis" OR metaanalysis OR "literature search" OR medline OR pubmed OR cochrane OR embase OR case control* OR "case series" OR cohort* OR comparative OR comparison OR comparator OR "follow-up study" OR "follow-up studies" OR observational* OR retrospective* or non-random* OR pre-post or pre-test* or pretest* or

posttest* or post-test* OR before-after) AND (publisher[sb] OR inprocess[sb] OR pubmednotmedline[sb])

#7 #5 AND #6

TRANSFUSION EVIDENCE LIBRARY

Subject Area: Clinical Practice – Management of Anaemia
Date Limit: 2013-2020

LILACS

tw:((ferric carboxymaltose OR ferinject OR injectafer OR iroprem OR iron sucrose OR venofer OR ferlecit OR ferlixit OR ferric gluconate OR ferrigluconate OR ferrlecit OR gluconate ferric sodium OR iron gluconate OR sodium ferrigluconate OR diafer OR ferric derisomaltose OR iron isomaltoside OR monofer OR monafer OR monoferro OR monover OR ferumoxytol OR feraheme OR rienso OR iv iron OR IV iron OR iron therapy OR intravenous iron OR injectable iron OR iron infusion OR iron infusions OR infused iron OR parenteral iron)) AND (instance:"regional") AND (db:("LILACS") AND year_cluster:("2007"-"2020"))

WEB OF SCIENCE

#1 TS=(alvofer or colliron or faremio or ferion or feriv or fermed or ferri saccharate or ferric hydroxide sucrose or ferric oxide saccharate or ferric saccharate or ferrinemia or ferrisaccharate or ferrivenin or ferrologic or ferrous saccharate or ferrovin or fesin or hemafer s or hemafer-s or idafer or iron hydroxide sucrose or iron saccharate or iron sucrose or ironcrose or iviron or nefro-fer or nefrofer or neo ferrum or nephroferol or proferrin or referen or reoxyl or saccharate ferric or saccharate iron or saccharated ferric oxide or saccharated iron oxide or sucro fer or sucrofer or sucroven or veniron or venofer or venotrix)

#2 TS=(anaemex or cosmofer or dexferrum or dexiron or dextrafer or dextran fe or dextran ferrous or dextran iron or driken or fenate or fer dextran or ferric dextran or ferridex or tranferrisat or ferrodex or ferrodextran or ferrous dextran or ferrum lek or fervetag or hibiron or imferdex or imferon or impheron or imposil or infed or infufer or iron dextran or ironate or monofar or proferdex)

#3 #1 or #2

#4 TS=(intravenous* or IV or "IV" or infus* or inject* or parenteral*)

#5 #3 and #4

#6 TS=(ferric carboxymaltose or Ferinject or Injectafer or Iroprem or venofer or ferlecit or ferlixit or ferric gluconate or ferrigluconate or ferrlecit or gluconate ferric sodium or (iron near/2 gluconate) or sodium ferrigluconate or diafer or ferric derisomaltose or iron isomaltoside or monofer or monafer or monoferro or monover or ferumoxytol or feraheme or rienso or "IV iron" or "IV iron" or "iron therapy" or ((intravenous* or inject* or infus* or parenteral) NEAR/3 iron))

#7 #5 OR #6

#8 TS=(random* OR blind* OR "control group" OR placebo* OR controlled OR groups OR trial* OR "systematic review" OR "meta-analysis" OR metaanalysis OR "literature search" OR medline OR pubmed OR cochrane OR embase OR case control* OR "case series" OR cohort* OR comparative OR comparison OR comparator OR "follow-up study" OR "follow-up studies" OR observational* OR retrospective* or non-random* OR pre-post or pre-test* or pretest* or posttest* or post-test* OR before-after)

#9 #7 AND #8 [Indexes=CPCI-S, BKCI-S Timespan=2007-2020]

ClinicalTrials.gov
Intervention or Title: (ferric carboxymaltose OR ferinject OR injectafer OR iroprem OR iron sucrose OR venofer OR iron isomaltoside OR monofer OR ferumoxytol OR feraheme OR ferrlecit or rienso OR IV iron OR iron th
erapy OR intravenous iron OR iron infusion)

ICTRP
Single Search Box: (ferric carboxymaltose OR ferinject OR injectafer OR iroprem OR iron sucrose OR venofer OR iron isomaltoside OR monofer OR ferumoxytol OR feraheme OR ferrlecit or rienso OR IV iron OR iron therapy OR intravenous iron OR iron infusion

Appendix 3: Characteristics of included studies incorporated into meta-analysis in Chapter 3

Study ID	Methods	Participants	Interventions (Intravenous iron)	Comparator	Primary outcome	Secondary outcomes
Abdelazim et al,(225) 2017	Multicentre RCT (n = 3) (Kuwait)	Pregnant women >18 years, 24-30 weeks' gestation, Hb 8-10 g/dl due to iron deficiency anaemia n = 260	Iron sucrose 200mg doses - calculated to total iron deficit	Oral iron	Treatment efficacy - changes in haemoglobin, serum ferritin, MCV at 3-months post-treatment	
Abhilashini et al,(226) 2014	Single centre RCT (India)	Pregnant women, 30-34 weeks' gestation, with established iron deficiency anaemia with Hb 6-8 g/dl n = 100	Iron sucrose according to Ganzoni formula, given over multiple dose	Ferrous sulphate 200mg x3/day	Change in Hb up to 37 weeks' gestation	
Adhikary et al,(227) 2011	Single centre RCT (Nepal)	CKD established on EPO, Hb <11 g/dl, Tsat <25%, Ferritin <300 ng/ml n = 90	Iron sucrose (Total 1000mg over 5 weeks)	Ferrous fumarate 152mg t.d.s. Both groups: 4000 IU EPO weekly	Improvement in Hb 30 days after therapy	
Agarwal et al,(229) 2006	Multicentre RCT (n = 26) (USA)	Age >18 years, CKD (eGFR between 10 and 59 ml/min), Hb <12 g/dl, serum ferritin <100 ng/ml, Tsat <20% n = 75	Ferric gluconate 250mg on 4 separate days	Ferrous sulfate 325mg x3/day for 6 weeks	Change in haemoglobin from baseline to end point (day 43)	(i) Changes in iron parameters; (ii) HRQoL
Agarwal et al,(228) 2015	Multicentre RCT (n = 2) (USA)	CKD Stage 3, Hb <120 g/lL, Serum ferritin <100 ng/ml or Tsat	Iron sucrose 200mg weekly for 5 weeks	Ferrous sulfate 325mg x3/day for 8 weeks	Between group difference in slope of	(i) Changes in haemoglobin; (ii) Changes in KDQOL.

		<25% n = 136			mGFR from baseline to two years	
Aggarwal et al,(230) 2003	Single centre RCT (India)	Established CKD on conservative treatment, age >15 years, Hb 5-8 g/dl n = 40	Iron dextran 200 mg	Oral iron sulphate 200mg t.d.s.	Not specified, but measurements included changes in Hb and iron profiles, and adverse events	
Akhtar et al,(231) 2018	Single centre RCT (Pakistan)	Postpartum anaemia within 24-48 hours of delivery, Hb 7-10 g/dl and ferritin <15 mcg/l n = 60	Iron sucrose 400mg (two doses)	Ferrous sulphate 200mg x3/day for 6 weeks	Changes in Hb and ferritin from baseline until 40 days postpartum	
Al et al,(232) 2005	Single centre RCT (Turkey)	Pregnant women between 26-34 weeks' gestation, Hb 8 to 10.5 g/dl, ferritin <13 ng/ml n = 90	Iron sucrose calculated to target haemoglobin (divided doses)	Oral iron 100mg x3/day	Hb concentration at day 28 post-treatment and at delivery	(i) Changes in ferritin levels; (ii) adverse effects; and (iii) fetal birth weight
Al-Momen et al,(233) 2006	Single centre RCT (Saudi Arabia)	Pregnant women gestational age <32 weeks, Hb <9 g/dl, ferritin <20 ng/ml n = 111	Iron sucrose calculated to target Hb	Ferrous sulphate 300 mg x3/day	Not specified, but measurements included changes in Hb and iron profiles, and safety monitoring	
Allen et al,(234) 2015	Multicentre RCT (n = 15) (USA)	Age >18 years, Restless leg syndrome with baseline IRLS score >1 n = 46	Ferric carboxymaltose 500mg given twice	0.9% saline i.v. placebo	Changes in IRLS scores	(i) Changes in CG1-1 and PG-1; (ii) safety
Anker et al,(146) 2009	Multicentre RCT (n = 75) (Europe)	Adults >18 years, NYHA class II or III, LVEF <45%, Hb 95 - 135 g/dl, ferritin <100 ng/ml or 100 - 299 ng/ml with Tsat <20% n = 459	Ferric carboxymaltose 200mg doses calculated to iron deficit	0.9% saline i.v. placebo (opaque infusion kits)	Self-reported Patient Global Assessment and NYHA functional class at week 24	(i) Self-reported PGA and NYHA class at weeks 4 and 12; (ii) distance on 6-minute walk-test; (iii) EQ-5D-5L; (iv) adverse events;

						(v) changes in Hb and iron profiles
Athibovonsuk et al,(235) 2013	Single centre (Thailand)	Age 20-70 years, Gynaecological malignancy receiving first-line platinum based chemotherapy n = 64	Iron sucrose 200mg after each cycle of chemotherapy	Ferrous fumarate 200mg x3/day throughout course of chemotherapy	Requirement for blood transfusion	(i) Total number of transfusion units; (ii) number of cycles requiring blood transfusion; (iii) changes in haemoglobin; (iv) serious adverse events
Auerbach et al,(236) 2004	Multicentre RCT (n = 4) (USA)	Histologic diagnosis of cancer, Hb <105 g/dl, ferritin <450 ng/ml, Tsat <19% n = 157	Intervention 1: Iron dextran 100mg boluses Intervention 2: Iron dextran total dose infusion	Comparator 1: No iron Comparator 2: Ferrous sulphate 325mg daily	Change in Hb from baseline to end point (6 weeks)	Haematopoeitic response; (ii) time to Hb response; (iii) QoL
Auerbach et al,(237) 2010	Multicentre RCT (Europe, USA, Russia)	Age >18 years, active nonmyeloid malignancies, Hb <10 g/dl related to cancer and chemotherapy n = 243	Iron dextran 400mg	No iron All groups received darbopoeitin 300-500 mcg	Achievement of target Hb (>11 g/dl)	(i) Time to acheive target Hb; (ii) proportion of patients with an Hb rise >2 g/dL; (iii) change in FACT-F fatigue scale; (iv) safety
Bager et al,(238) 2014	Single centre RCT (Denmark)	Age >18 years admitted with non-variceal upper GI bleeding; ~48h post stabilisation of bleeding source and endoscopy; Anaemia according WHO definitions (Hb <120 g/dl in women; <130 g/dl in men) n = 97	Ferric carboxymaltose 1000mg	Oral ferrous sulphate 100mg x2/day for 3 months	Difference in Hb between both groups at 3 months post-treatment	(i) Proportion of patients with normal Hb at end of study; (ii) proportion of patients with Hb increased >2 g/dl; (iii) restoration of iron stores

Bailie et al,(239) 2010	Multicentre RCT (n=62) (USA), cross-over trial	Adults with anaemia (WHO definition) with evidence of iron deficiency into one of the following cohorts: (i) secondary to CKD (Tsat <25% and ferritin <300 ng/ml) (ii) secondary to IBD (Tsat <25% and ferritin <300 ng/ml) (iii) other conditions (Tsat <25% and ferritin <100 ng/ml) n = 559	Ferric carboxymaltose 15mg/kg	0.9% saline i.v. placebo	Treatment-related adverse events within first 7 days	(i) Treatment-related adverse events during first 24 hours; (ii) all adverse events
Barish et al,(240) 2012 (1)*	Multicentre RCT (n = 95) (USA)	Age 18-85 years old, Diagnosis of iron deficiency anaemia (Hb <11g/dl), Ferritin <100 ng/ml, Tsat <30% (mixed population included CKD, Postpartum, GI bleeding, other) n = 708	Intravenous ferric carboxymaltose 750mg weekly until calculated iron deficit met (Ganzoni formula)	Standard medical care	Evaluate safety of maximal dose of ferric carboxymaltose	Changes in haemoglobin and iron status
Barish et al,(240) 2012 (2)*	Multicentre RCT (n = 95) (USA)	Age 18-85 years, Diagnosis of iron deficiency anaemia (Hb <11g/dl), Ferritin <100 ng/ml, Tsat <30% (mixed population included CKD, Postpartum, GI bleeding, other) n = 738	Intravenous ferric carboxymaltose 750mg or 15 mg/kg, whichever was smaller, one-off dosing	Standard medical care	Evaluate safety of maximal dose of ferric carboxymaltose	Changes in haemoglobin and iron status
Bastit et al,(241) 2008	Multicentre RCT (Europe)	Age >18 years, Hb <11 g/dl, non-myeloid malignancy n = 396	Iron sucrose 200mg at each study visit (weeks	Usual care	Proportion of patients achieving Hb >12 g/dl or rise of >2 g/dl	(i) Time to achieving response; (ii) transfusion requirements; (iii)

			1, 4, 7, 10, 13 and 16)			HRQoL measured by FACT-F; (iv) adverse events
Batool et al,(242) 2018	Single centre RCT (Pakistan)	Postpartum anaemia (Hb <10 g/dl) n = 82	Iron sucrose 200mg weekly for six weeks	Ferrous sulphate 200mg x3/day for 6 weeks	Hb response, defined as Hb rise of >3.5 g/dl, after 6 weeks of therapy	
Bayoumeu et al,(243) 2002	Single centre RCT (France)	Age >18 years, Pregnant women at 6 months gestation, Hb 8 - 10 g/dl, ferritin <50 mcg/l n = 50	Iron sucrose calculated to target Hb given over 6 slow i.v. injections	Oral iron 80 md x3/day for 4 weeks	Primary outcome not specified, but outcome measures included changes in Hb, iron profiles up to day 30, neonatal iron status	
Beck-da-Silva et al,(244) 2013	Multicentre RCT (Brazil)	Age <18 years, Heart failure NYHA functional class II, LVEF <40% within previous six months, Hb 9-12g/dl, Tsat <20%, ferritin <500 mcg/l n = 23	Iron sucrose 200mg once a week for 5 weeks + oral placebo t.d.s. for 8 weeks	Comparator 1: Ferrous sulphate 200mg t.d.s. for 8 weeks and i.v. placebo for 5 weeks Comparator 2: Oral and i.v. placebo	Changes in maximal oxygen consumption	Changes in haemoglobin and iron stores
Beguin et al,(245) 2013	Multicentre RCT (Belgium)	Age 16-70 years, received autologous stem cell transplant for multiple myeloma or lymphoma, ferritin >100 ng/ml n = 72	Iron sucose 200mg on days 28, 42 and 56 after stem cell transplant + EPO	Comparator 1: EPO 300 mcg given at day 28 post-stem cell transplant followed by 2-weekly intervals until day 112 post-transplant	Proportion with complete correction of anaemia (Hb >13g/dl) before day 126 post-transplant	(i) Median time to increase Hb level by >2 g/dL; (ii) proportion of responders (>2 g/dl) before day 126; (iii) transfusion requirements; (iv) adverse events; (v) quality of life using

				Comparator 2: no i.v or EPO		FACIT-F questionnaires
Bencaiova et al,(246) 2009	Single centre RCT (Switzerland)	Non-anaemic pregnant women between 15 and 20 weeks' gestation n = 260	Iron sucrose (1st phase - 400mg, 2nd phase - 600mg)	Ferrous sulphate 80mg daily until delivery	Percentage of pregnant women prior to delivery with Hb <11 g/dl	(i) Changes in Hb and ferritin; (ii) birth weight; (iii) gestational age at birth; (iv) transfusion requirements; (v) hospital LOS after delivery
Bernabeu-Wittel et al,(247) 2016	Multicentre RCT (n = 13) (Spain)	Age >65 year, requiring hip fracture surgery with Hb 9-12 g/dl n = 306	Intervention 1: Ferric carboxymaltose 1000mg and EPO Intervention 2: Ferric carboxymaltose 1000mg + s.c placebo	s.c. placebo and 0.9% saline i.v. placebo (opaque infusion kits)	RBC transfusion requirements within 60 days	(i) Changes in Hb levels 24 and 72 hours after surgery; (ii) mortality; (iii) adverse events; (iv) HRQoL; (iv) medical complications (MI, DVT, stroke, infection, delirium)
Bhandal et al,(248) 2006	Single centre RCT (UK)	Postpartum IDA, defined as Hb <9 g/dl and ferritin <15 mcg/l at 24-48 hours post delivery) n = 44	Iron sucrose 400mg split over 2 doses	Ferrous sulphate 200mg x2/day over 6 weeks	Primary outcome not specified but study outcomes included changes in Hb, ferritin and adverse events	
Bhavi et al,(249) 2017	Single centre RCT (India)	Pregnant women between 14 and 34 weeks' gestation, Hb 7-11 g/dl, ferritin <15ng/ml n = 112	Iron sucrose 200mg - total administered over multiple doses as per Ganzoni formula	Ferrous fumarate 100mg daily for 4 weeks	Changes in haemoglobin and iron stores	Safety and tolerability

Biboulet et al,(250) 2018	Single centre RCT (France)	Scheduled for elective hip or knee arthroplasty with Hb between 10 and 13 g/dl n = 100	Ferric carboxymaltose 1000mg (single dose) and 40,000 IU Eprex weekly for 3 weeks prior to surgery	Ferrous sulphate 160mg x2/day until day of surgery and 40,000 IU Eprex weekly for 3 weeks prior to surgery	Hb the day before surgery	(i) Changes in ferritin; (ii) erythrocyte mass the day before surgery; (iii) postoperative Hb
Bielza et al,(251) 2020	Single centre RCT (Spain)	Age >70 years scheduled to undergo hip fracture surgery n = 153	Iron sucrose 200mg on days 1, 3 and 5 of admission	0.9% saline i.v. placebo (opaque infusion kits)	Difference in absolute functional gain defined as Barthel's Index at discharge minus BI on admission	(i) Functional recovery at 3 months; (ii) mortality at 3,6 and 12 months; (iii) transfusion requirements; (iv) Hb at 3 months; (v) nosocomial infection; (vi) postoperative delirium
Birgegard et al,(253) 2010	Single centre RCT (Sweden)	Blood donors with at least 5 previous blood donations, n = 120	Iron sucrose 200mg after each donation	Iron sulphate 100mg once daily for 20 days after each donation	Iron status at the end of the study	(i) iron status during the study; (ii) RLS frequency and severity
Birgegard et al,(252) 2016	Multicentre RCT (n = 47) (Asia, USA, Europe)	Age >18 years, Non-myeloid malignancies on chemotherapy, Hb <120 g/dl, Tsat <50%, ferritin <800 mcg/l n = 350	Iron isomaltoside (amount as per Ganzoni formula) single dose	Iron sulfate 100mg x2/day for 12 weeks	Change in haemoglobin from baseline to 4 weeks post-treatment	(i) Changes in haemoglobin, serum ferritin, TIBC, Tsat and iron levels; (ii) Changes in quality of life from baseline; (iii) adverse drug reactions
Bisbe et al,(254) 2014	Single centre RCT (Spain)	Adults >18 years, undergoing total knee arthroplasty,	Ferric carboxymaltose (amount as per	Ferrous sulphate 100mg once daily for 21 days	(i) Change in haemoglobin from post-operative day 4 to day 30; (ii) percentage of	(ii) Hb at day 30; (ii) changes in quality of life (EQ-5D and BI); (iii) distance in 6-

		postoperative Hb <12g/dl or Tsat <20% n = 122	Ganzoni formula) single dose		patients without anaemia (Hb >12g/dl)	MWT; (iv) percentage of transfused patients; (v) adverse events (including infection)
Boomershine et al,(255) 2016	Single centre RCT (USA)	Age >18 years with fibromyalgia, Hb <130 g/dl n = 81	Ferric carboxymaltose (15mg/kg), given over two doses	0.9% saline i.v. placebo (opaque infusion kits)	Proportion of patients with >13-point improvement from baseline to day 42 in FIQR score	(i) Changes in Hb and iron stores; (ii) changes in vital signs; (iii) adverse events
Breymann et al,(256) 2008	Multicentre RCT (n = 20) (Europe)	Postpartum anaemia with Hb <10.5 g/dL n = 349	Ferric carboxymaltose 1000mg	Ferrous sulfate 100mg x2/day for 12 weeks	Change from baseline Hb to week 12 post-treatment	(i) Changes in iron profiles; (ii) transfusion requirements; (iii) proportion of patients who achieved Hb levels of 12-16 g/d; (iv) safety
Breymann et al,(257) 2017	Multicentre RCT (n = 29) (International)	Age >18 years, 2nd or 3rd trimester pregnancy with iron deficiency anaemia (Hb <11 g/dl, ferritin <20 ng/ml) n = 252	Ferric carboxymaltose 1000-1500mg	Oral ferrous sulphate	Change in Hb from baseline to 3 weeks post-treatment	(i) Changes in Hb and iron profiles; (b) proportion of women who achieved anaemia correction; (c) time to anaemia correction; (d) changes in HRQoL (SF-36)
Burden et al,(258) 2015	Single centre RCT (UK)	Elite athletes with non-anaemic iron deficiency (ferritin <30 mcg/l for females; <40 mcg/l for men) n = 15	Ferric carboxymaltose 500mg single dose	0.9 saline i.v. placebo (opaque infusion kits)	Changes in hepcidin and iron profiles in response to iron treatment up to 4 weeks post-treatment	(i) changes in Hb and t-Hb mass; (ii) changes in aerobic capacity
Burns et al,(259) 1996	Single centre RCT (USA)	Hospitalised patients requiring total parenteral nutrition, iron deficiency defined as Fe-to-TIBC ratio <10% n = 23	Iron dextran 10mg supplemented to TPN for 7 days	No iron	Not specified. Outcome measurements included changes in Hb, iron profiles, transfusion requirements and infection	

Charles-Edwards et al,(260) 2019	Single centre RCT (UK)	Age >30, Symptomatic chronic stable heart failure, ferritin <100 mcg/l or 100-300 mcg/l with Tsat <20% n = 40	Iron isomaltoside 1000mg	0.9% saline i.v. placebo (opaque infusion kits)	Change in skeletal muscle energetics two weeks post-treatment	(i) Changes in 6MWT; (ii) peak oxygen consumption; (iii) Hb and iron profiles; (iv) NYHA symptoms; (v) LVEF
Charytan et al,(262) 2005	Multicentre RCT (n = 16) (USA)	Age >18 years, non-dialysis dependent CKD, Hb <10.5 g/dl, Tsat <15%, ferritin <300 ng/ml n = 96	Iron sucrose 200mg on days 1, 8, 15, 22 and 29	Ferrous sulfate 325mg x3/day	Mean changes in Hb and ferritin from baseline to day 43	(i) changes in Tsat; (ii) number of patients achieving Hb >11 g/dl; (iii) adverse events
Charytan et al,(261) 2012	Multicentre RCT (n = 56) (USA)	Adults aged 18-85, 3/12 history non-dialysis dependent CKD (Hb <11.5 g/dl) or dialysis dependent CKD (Hb <12.5 g/dl) n = 254	Ferric carboxymaltose 15mg/kg (max 1g) in non-dialysis dependent CKD; 200mg in dialysis dependent CKD	Usual medical care Both groups received EPO 2000 U	Safety of maximum administered dose	(a) Proportion of patient receiving Hb increase of >1.0 g/dl; (b) proportion of patients achieving an Hb >12.0 g/dl ; (b) Mean changes in Hb, ferritin, Tsat
Cho et al,(263) 2016	Single centre RCT (Korea) Crossover trial	Age >18 years, Primary restless legs syndrome, no co-morbidities n = 65	Ferric carboxymaltose 1000mg	0.9% saline i.v. placebo (opaque infusion kits)	Change from baseline in the International RLS Severity scale at week 6 post-treatment	(i) Change in sleep (Pittsburg Sleep Quality Index) and health-related quality of life (SF-36)
Cho et al,(264) 2018	Single centre RCT (Korea)	Restless leg syndrome, International RLS score >15, ferritin <300 ng/ml, Hb >12 g/dl, Tsat <45% n = 65	Ferric carboxymaltose 500mg	0.9% saline i.v. placebo (opaque infusion kits)	Change from baseline in IRLS score at week six post-randomisation	(i) Changes in SF-36 and sleep quality
Coyne et al,(265) 2007	Multicentre RCT (n = 37) (USA)	Age >18 years, CKD on HD for >90 days, ferritin <1200 ng/ml,	Ferric gluconate 1g adminstered over 8 consecutive doses of 125mg	Usual care	Change in Hb from baseline to week 6	(i) Percentage of with Hb response >2 g/dl; (ii) changes in iron profiles; (iii) safety

		Tsat <25%, Hb <11 g/dl, established on EPO n = 134				
Dalal et al,(266) 2008	Single centre RCT (India)	Pregnant women (26-34 weeks' gestation) with anaemia, Hb 7-11 g/dl N = 150	Iron sucrose 200mg weekly - calculated to match iron deficit	Ferrous sulphate 100mg x2/day until delivery	Changes in Hb from baseline to 2 weeks, 4 weeks and delivery	(i) Safety; (ii) maternal and fetal outcomes
Damineni et al,(267) 2016	Single centre RCT (India)	Postpartum anaemia, defined as Hb 7-10 g/dl n = 90	Ferric carboxymaltose 1000mg	Ferrous ascorbate 100mg x2/day for 6 weeks	Change in Hb at 6 weeks post-treatment	(i) Proportion of patients with rise in Hb >3 g/l; (ii) tolerability and compliance
Dangsuwan et al,(268) 2010	Single centre RCT (Thailand)	Age 20-65 years, gynaecology malignancy who have undergoing surgery and receiving chemotherapy, Hb <10 g/dl n = 44	Iron sucrose 200mg one dose	Ferrous sulphate 200mg x3/day	Incidence of RBC transfusion in subsequent chemotherapy cycle	(i) changes in Hb; (ii) no. of RBC units; (iii) adverse events; (iv) QoL scores measured by FACT-An
Darwish et al,(269) 2019	Single centre RCT (Egypt)	Pregnant women gestational >14 weeks, Hb 7-10 g/dl, ferritin <12mg/l n = 66	Iron dextran, amount as per Ganzoni formula over multiple doses	Ferrous fumarate 60 m.g. x3/day for 4 weeks	Change in Hb from baseline to 4 weeks	(i) change in iron stores; (ii) adverse effects
Deeba Shafi et al,(276) 2012	Single centre RCT (India)	Pregnant women 28-37 weeks' gestation with established iron deficiency anaemia (Hb 6-10 g/dl and Ferritin <15ng/ml) n = 200	Iron sucrose 200mg on alternate days, calculated to achieve Hb of 12 g/dl	Ferrous ascorbate 200mg daily	Hb at 2, 4 and 6 weeks post-treatment	Ferritin at 2, 4 and 6 weeks post-treatment

Deng et al,(270) 2017	Single centre RCT (China)	CKD patients on haemodialysis with RLS, ferritin <200 ng/ml, Tsat <20% n = 32	Iron sucrose 100mg x3/week until total dose of 1000mg	0.9% saline i.v. placebo (opaque infusion kits) Both groups received EPO	Changes in International RLS scores at two weeks post-treatment	(i) Changes in Hb and iron status; (ii) safety and adverse events
Drexler et al,(271) 2020	Single centre RCT (Austria)	Iron deficient (ferritin <30 mcg/l) blood donors n = 176	Ferric carboxymaltose 1000mg	Ferrous sulphate 100mg (x3 per week) for 10 weeks	Change in Tsat (%) at 8-12 weeks post-randomisation	(i)Changes in other markers of iron status and Hb; (ii) adverse events; (iii) tolerance; (iv) symptoms of restless leg syndrome, HRQoL and fatigue
Dubey et al,(272) 2016	Single centre RCT (India)	Pregnant women gestation age 20-34 weeks, Hb 7-9 g/dl, Ferritin <15 ng/ml n = 200	Iron sucrose calculated to target Hb of 11 g/dl	Oral iron 100mg x3/day throughout pregnancy	Changes in Hb on day 14 and day 28 post-randomisation	(i) Changes in reticulocyte count and ferritin; (ii) side effects; (iii) proportion of women achieving Hb >11 g/dl; (iv) neonatal outcomes; (v) transfusion requirements
Edwards et al,(273) 2009	Single centre RCT (UK)	Adults scheduled to undergo bowel resection for colorectal cancer n = 62	Iron sucrose 600mg split over 2 doses	0.9% saline i.v. placebo (opaque infusion kits)	Change in Hb between recruitment and day of admission	(i) RBC transfusion rate; (ii) changes in iron profiles; (iii) hospital LOS; (iv) adverse events
El Khouly et al,(274) 2017	Single centre RCT (Egypt)	Postpartum iron deficiency anaemia, Hb <9 g/dl and ferritin < mcg/l n = 352	Iron sucrose 200mg x3/week	Oral ferrous sulphate 150mg x2/day for 6 weeks	Changes in Hb and ferritin up to day 60 postpartum	Safety, tolerability and compliance

Evstatiev et al,(275) 2013	Multicentre RCT (n = 69) (Europe)	Age >18 years, Non-anaemic patients with IBD n = 206	Ferric carboxymaltose 500mg	0.9% saline i.v. placebo (opaque infusion kits)	Time to occurrence of anaemia (Hb <12 g/dl in females and 13 g/dl in males)	(i) Proportion of patients with anaemia at 2,4,6 and 8 months post-treatment; (ii) changes in HRQoL (SF-36); (iii) changes in Crohn's disease activity
Favrat et al,(277) 2014	Multicentre RCT (n = 21) (Europe)	Women aged>18 years (premenopausal), symptomatic fatigue, borderline anaemia (Hb >11.5 g/dl) with evidence of iron deficiency (ferritin <15 mcg/l or ferritin <50 mcg/l and Tsat <20%) n = 290	Ferric carboxymaltose 1000mg	0.9% saline i.v. placebo (opaque infusion kits)	Proportion of patients who improved Piper Fatigue Score by >1 from baseline to day 56	(i) changes in PFS total and subscale score and SF-12 QoL score; (ii) correction of anaemia and iron deficiency; (iii) adverse events
Ferrer-Barcelo et al,(278) 2019	Single centre RCT (Spain)	Age >18 years, non-variceal acute upper gastointestinal bleed (Hb <10 g/dl on day of hospital discharge) n = 61	Ferric carboxymaltose 1500-2000mg	Ferrous sulphate 325mg x2/day for 6 weeks	Resolution of anaemia at day 42 (men, Hb>13 g/dl; women, Hb>12 g/dl)	(i) Partial response (Hb rise >2 g/dl); (ii) changes in iron profiles; (iii) adverse events
Fishbane et al,(279) 1995	Single centre RCT (USA)	Adults with CKD on haemodialysis for atleast 3 months, receiving EPO, serum ferritin >100 ng/ml, Tsat >15% n = 28	Iron dextran 200mg twice weekly	Ferrous sulphate (325mg x3/day) or iron polysaccharide (150mg x2/day)	Not specified. Outcome measurements included haematocrit, ferritin and Tsat	
Friel et al,(280) 1995	Single centre RCT (Canada)	Low birth weight newborns n = 26	Iron dextran 250 mcg/kg/day	No iron	Not specified. Outcome measurements included haematologic and biochemical parameters, infection rates, red blood	

					cell haemolysis and iron balance	
Froessler et al,(281) 2013	Single centre RCT (Australia)	Women with iron deficiency anaemia (Hb <110 g/l and ferritin <12 mcg/l), antenatal between 28-36 weeks' gestation, or within 72 hours of birth or delivery with blood loss >500 mls. n = 271	Iron sucrose 400mg (divided into 2 doses)	Ferrous sulphate 160mg daily for 6 weeks postpartum or until delivery (if started antenatal)	Incidence of severe anaemia (Hb<8 g/dl) prior to hospital discharge	RBC transfusion requirements
Froessler et al,(150) 2016	Single centre RCT (Australia)	Age >18 years scheduled for abdominal surgery, Hb <12 g/dl (females) and <13 g/d; (males), ferritin <300 mcg/l, Tsat <25% n = 72	Ferric carboxymaltose 15mg/kg pre-operatively, and 0.5 mg per 1mL of blood loss postoperatively	Usual medical care	Incidence of RBC transfusion	(i) Changes in Hb from randomisation to admission; (ii) ICU admission; (iii) perioperative morbidity (infection, respiratory failure, renal impairment, DVT); (iv) discharge Hb; (v) length of stay; (vi) 30-day mortality; (vii) HRQoL scores
Garrido-Martin et al,(188) 2012	Single centre RCT (Spain)	Age >18 years, elective cardiac surgical ICU (postoperative) n = 159	Iron sucrose 300mg (given over 3 separate doses) and oral placebo	Comparator 1: Ferrous fumarate 105mg daily for 1 month and i.v. placebo Comparator 2: i.v. and oral placebo	Change in Hb from baseline up to day 30 post-hospital discharge	(i)Changes in iron profiles; (ii) postoperative transfusion requirements

Grote et al,(509) 2009	Multicentre RCT (n = 3) (Sweden)	Age 18-70 years, Restless leg syndrome, ferritin <45 mcg/l n = 60	Iron sucrose 200mg (x5 doses over 3 weeks)	0.9% saline i.v. placebo (opaque infusion kits)	Between group difference in International RLS score at the 11-week follow-up visit	(i) Treatment effects on daytime sleepiness; (ii) adverse events; (iii) changes in Hb and iron status
Gupta et al,(282) 2014	Single centre RCT (India)	Singleton pregnancy between 24-34 weeks' gestation, Hb 7 - 9 g/dl and ferritin <15 ng/ml n = 100	Iron sucrose calculated to target Hb of 11 g/dl	Ferrous sulphate 200 mg x3/day for 4 weeks	Increase in haemoglobin and ferritin over 4 weeks post-treatment	(i) Reticulocyte count rise; (ii) mean Hb at the time of delivery; (iii) side effect; (iv) perinatal outcomes
Hedenus et al,(283) 2007	Multicentre RCT (n = 15) (Sweden)	Adults with Non-Hodgkin's Lymphoma, Chronic Lymphocytic Leukaemia or myeloma, Hb 9-11 g/dl, evidence of stainable iron on bone marrow aspirate within 1 month prior to inclusion n = 67	Iron sucrose 100mg weekly from weeks 0 to 6, every second week from weeks 8 to 14	No iron	Mean change in Hb from baseline to end of treatment	(i) Hb response (increase >2 g/dl); (ii) time to Hb response; (iii) changes in iron profiles; (iv) EPO requirements
Hedenus et al,(284) 2014	Multicentre RCT (n = 11) (Europe)	Adults with lymphoid malignancies, Hb 8.5-10.5 g/dl, Tsat <20% and ferritin >30 ng/ml, received chemotherapy for >8 weeks prior to inclusion n = 17	Ferric carboxymaltose 1000mg	Usual medical care	Mean change in Hb from baseline to week 8 post-treatment	(i)Safety; (ii) Hb response (increase >1 g/dl) and correction (Hb >11 g/dl)
Henry et al,(285) 2007	Multicentre study (USA)	Age >18 years, due to start chemotherapy for a non-myeloid malignancy, Hb <11 g/dl, ferritin >100 ng/ml or Tsat >15%. n = 187	Ferrous gluconate i.v. 125mg once weekly for 8 weeks	Comparator 1: Ferrous sulphate 325mg t.d.s Comparator 2: No iron	Mean change in Hb from baseline to endpoint, first RBC transfusion or study withdrawal	(i)Hb response (>2 g/dl); (ii) safety

Holm et al,(286) 2017	Single centre RCT (Denmark)	Postpartum haemorrhage >700 ml and <1000 ml, or PPH >1000ml and Hb >6.5 g/dl at least 12 hours after delivery n = 196	Iron isomaltoside 1200mg, single dose	Oral iron 100mg daily	Change in physical fatigue scores of MFI questionnaire within 12 weeks postpartum	(i) changes in Hb, ferritin, iron; (ii) changes in other dimensions of MFI and symptoms of depression; (iii) time to lactogenesis; (iv) time to discontinuation of breastfeeding; (v) transfusion
Iyoke et al,(287) 2017	Multicentre RCT (n = 2) (Nigeria)	Postpartum anaemia Hb 6 - 7.9 g/dl, red cell features of iron deficiency at 48 hours or later following vaginal delivery n = 284	Iron dextran, calculated as haemoglobin required to reach 10 g/dl multiplied by 250 mg of parenteral iron	100mg oral elemental iron for 6 weeks	Proportion of women who attained a Hb of at least 10 g/dl by 6 weeks postpartum	
Jain et al,(288) 2013	Single centre RCT (India)	Postpartum Hb <8 g/dl within 48h of delivery n = 40	Iron sucrose calculated to total iron deficit, given over three separate doses in one week	Ferrous fumarate 300 mg daily for 14 days	Change in Hb at14 days postpartum	Safety and tolerability
Johansson et al,(289) 2015	Single centre RCT (Denmark)	Age >18 years, undergoing elective or subacute CABG, valve or combination without anaemia (Hb >12 g/dl for women, Hb >13 g/dl for men) n = 60	Iron isomaltoside 1000mg once only	0.9% saline i.v. placebo (opaque infusion kits)	Less decrease in postoperative Hb level in non-anaemic patients undergoing cardiac surgery	(i)RBC transfusion requirements; (ii) changes in iron profiles; (iii) safety
Kalra et al,(290) 2016	Multicentre RCT (n = 67) (Europa, Asia, USA)	Age >18 years, Hb <11 g/dl, serum ferritin mcg/l, Tsat <20%, not received EPO in previous 8 weeks	Iron isomaltoside calculated according to Ganzoni formula	Iron sulphate 100 mg x2/day for 8 weeks	Change in Hb from baseline from baseline to week 4 post-randomisation	(i)Change in Hb from weeks 2 to 8; (ii) changes in iron profiles; (iii) changes in HRQoL from baseline

		n = 351				to weeks 4 and 8; (iv) safety
Karkouti et al,(291) 2006	Single centre RCT (Canada)	Adults >18 years, postoperative open-heart surgery, total hip arthroplasty or spinal fusion, Hb 70-90 g/dl n = 38	Intervention 1: Iron sucrose 600mg (given over 3 split doses) Intervention 2: Iron sucrose 600mg (given over 3 split doses) and EPO (1200 IU/kg)	0.9% saline i.v. placebo (opaque infusion kits)	Change in Hb from postoperative day 1 to day 7	(i) Hb up to 6 weeks postoperatively; (ii) changes in iron profiles on day 7; (iii) RBC transfusion requirements; (iv) hospital length of stay; (v) changes in HRQoL (SF-36)
Kasper et al,(292) 1998	Single centre RCT (Germany)	Age >18 years, Hb <12.0 g/dl, scheduled to undergo major surgery and eligible for autologous blood donation n = 108	Ferric gluconate 102.5 mg i.v. (once) and oral iron (ferric succinate) 92.5mg x3/day for 21 days	Oral iron (ferric succinate) 92.5mg x3/day for 21 days and i.v. placebo	Improvement in Hb during the study period	(i)Changes in iron profiles; (ii) volume of blood donated; (iii) adverse events
Keeler et al,(293) 2017	Multicentre RCT (n = 7) (UK)	Scheduled to undergo colorectal cancer excision with anaemia, defined as Hb <11 g/dl in women and <12 g/dl in men n = 116	Ferric carboxymaltose 1000mg, stratified on Hb (if Hb <10 g/dl, 2000mg could be given	Ferrous sulphate 200mg x2/day until surgery	Transfusion requirements from preoperative recruitment until postoperative outpatient review	(i) Changes in haemoglobin and haematinic profiles; (ii) postoperative complications; (iii) RBC transfusion requirements
Keller et al,(294) 2020	Single centre RCT (Switzerland)	Blood donors with evidence of non-anaemic iron deficiency (ferritin <50 mcg/l) n = 203	Ferric carboxymaltose 800mg	0.9% saline i.v. placebo (opaque infusion kits)	Self-rated average fatigue during the past 7 days, assessed 6-8 weeks post-randomisation	(i) Changes in other self-reported fatigue and HRQoL measures; (ii) changes in Hb and ferritin; (iii) safety reporting

Khalafallah et al,(295) 2010	Single centre RCT (Australia)	Pregnant women <28 weeks' gestation with Hb 8.5 – 11.5 g/dl and ferritin <30 mcg/l n = 200	Iron polymaltose, dose calculated according to weight and target Hb	Ferrous sulphate 80mg daily until delivery	Predelivery Hb and iron stores	Safety, tolerability and compliance
Khalafallah et al,(148) 2016	Multicentre RCT (n = 2) (Australia)	Age >18 years, requiring major elective surgery with at least planned hospital stay of 2 nights, Hb 70-120 g/L, ferritin <100 or Tsat 20% n = 201	Ferric carboxymaltose 1000mg	Usual medical care	Changes in Hb and iron stores at 4 weeks postoperatively	(i) Safety and tolerability; (ii) length of hospital stay; (iii) infections; (iv) HRQoL at 4 and 12 weeks postoperatively
Khalafallah et al,(296) 2018*	Single centre RCT (Australia)	Pregnant women in second/third trimester of pregnancy, Hb 8.5-12 g/dl, ferritin <100 mcg/l or Tsat <20% n = 166	Intervention 1: Ferric carboxymaltose 1000mg Intervention 2: Iron polymaltose 1000mg	Ferrous sulphate 105mg daily until delivery	Change in Hb and ferritin from baseline to 4 weeks post-randomisation	(i) Tolerability and adverse events; (ii) transfusion requirements; (iii) changes in HRQoL; (iv) fetal outcomes
Kim et al,(298) 2007	Single centre RCT (Korea)	Age>18 years, Hb <12 g/dl, undergoing chemotherapy for cervical cancer n = 75	Iron sucrose 200mg at each chemotherapy cycle	Usual medical care	RBC transfusion requirements	(i) Changes in Hb; (ii) proportion of patients with resolution of anaemia (defined as Hb >12 g/dl)
Kim et al,(297) 2009	Multicentre RCT (n = 3) (South Korea)	Women experiencing menorrhagia with Hb <9 g/dl, scheduled to undergo elective surgery n = 76	Iron sucrose 200mg 3 days a week, for 3 weeks prior to surgery	Iron succinylate 80mg once daily for 3 weeks prior to surgery	Changes in Hb preoperatively	Safety

Kim et al,(299) 2017	Multicentre RCT (n = 7) (South Korea)	Adults (age >20 years) with acute isovolaemic anaemia (Hb 7-10 g/dl) at 5-7 days post-gastrectomy n = 454	Ferric carboxymaltose 1000mg	0.9% saline i.v. placebo (opaque infusion kits)	No. of haemoglobin responders (Hb rise >2 g/dl) at 12 weeks post-randomisation	(i) Percentage of patients with Hb >10 g/dl, 11 g/dl and 12 g/dL by weeks 3 and 12; (ii) self-reported HRQoL; (iii) changes in Hb and iron profiles over the study duration
Kochhar et al,(300) 2013	Single centre RCT (India)	Singleton pregnant women between 24-34 weeks' gestation, moderate iron deficiency anaemia (Hb 7 -9 g/dL, MCV <85 fl, ferritin <15 ng/ml) n = 100	Iron sucrose, calculated to total iron deficit and targeted to Hb >12 g/dl, given over three separate doses	Ferrous sulphate 200 mg x3/day for 4 weeks	Change in Hb from baseline up to day 30 post-randomisation	(i) Requirement for RBC transfusion; (ii) adverse events; (iii) neonatal outcomes
Krayenbuehl et al,(301) 2020	Multicentre RCT (n = 4) (Switzerland)	Premenopausal, menstruating women aged 18 years with fatigue and non-anaemic iron deficiency (Hb >12 g/dL, ferritin <50 mcg/l) n = 90	Iron sucrose 800 mg over multiple doses)	0.9% saline i.v. placebo (opaque infusion kits)	Changes in fatigue scores 6 weeks after treatment initiation	(i) Changes in Hb and ferritin; (ii) Safety
Kulnigg et al,(302) 2008	Multicentre RCT (n = 36) (Europe and South America)	IBD with iron deficiency anaemia (Hb<10 g/dl and Tsat <20% or ferritin <100 mcg/l) n = 200	Ferric carboxymaltose, dose calculated according to Ganzoni formula	Ferrous sulphate 100mg x2/day for 12 weeks	Change in Hb from baseline to week 12	(i) Changes in Hb and iron profiles from baseline to weeks 2, 4 and 8; (ii) treatment responses, defined as Hb rise >2 g/dL and/or proportion of participants with resolution of anaemia; (iii) tolerability

Kulnigg-Dabsch et al,(303) 2013	Multicentre RCT (Austria and Germany)	IBD associated thrombocytosis and Hb >10.5 g/dl, age 18 and 60 years, ferritin <100 ng/ml, Tsat <30% n = 26	Intervention 1: Ferric carboxymaltose 500mg single dose Intervention 2: Ferric carboxymaltose 1000mg single dose	0.9% saline i.v. placebo (opaque infusion kits)	Decrease in platelet count of at least 25% at 6 weeks	(i) Effect on ferric carboxymaltose on platelet count, disease activity and HRQoL; (ii) safety at week 6
Kuo et al,(304) 2008	Multicentre RCT (n = 2) (Taiwan)	CKD on long-term haemodialysis, ferritin <800 mcg/l n = 110	Iron sucrose 100mg once weekly for 12 weeks	0.9% saline i.v. placebo	Not specified. Outcomes measurements included: (i) oxidative damage to peripheral blood lymphocytes; (ii) changes in ferritin	
Li et al,(305) 2008	Single centre RCT (China)	CKD on maintenance haemodialysis, Hb 6-9 g/dl, ferritin <500 mcg/l, Tsat <30% n = 46	Iron sucrose 100mg twice a week for 8 weeks, once a week for 4 weeks after	Ferrous succinate 200mg x3/day for 12 weeks	Increase in Hb >3g/dl or Hct >10% during therapy	
Lindgren et al,(306) 2009	Multicentre RCT (n = 11) (Sweden)	Age 18-85, IBD, Hb <11.5 g/dl, ferritin <300 mcg/l n = 91	Iron sucrose 1000mg split over 200mg doses on alternate days	Ferrous sulphate 200mg x2/day over 20 weeks	Hb response (>2 g/dl) at 20 weeks post-treatment	
Litton et al,(170) 2016	Multicentre RCT (n = 4) (Australia)	Age >18 years, within 48hrs of ICU admission, anticipated to require ICU beyond next calendar day, Hb <100 g/l at any time in the preceding 24 hours n = 140	Ferric carboxymaltose 500mg, redosing 4 days later if still eligible (max 4 doses allowed)	0.9% saline i.v. placebo (opaque infusion kits)	Number of RBCs transfused per patient between randomisation and hospital discharge	(i) Hb at hospital discharge; (ii) ICU and hospital LOS; (iii) proportion of patients receiving a transfusion; (iv) mortality; (v) infection

Maccio et al,(307) 2010	Single centre RCT (Italy)	Age >18 years, advanced cancer, Hb <10 g/dl, ferritin 100-800 ng/ml, Tsat >15% n = 148	Ferric gluconate 125mg once weekly for 12 weeks	Oral lactoferrin 200mg daily for 12 weeks	Change in Hb from baseline until end of study period	(i) Hb response (Hb rise of >2 g/dl or Hb >12 g/dl); (ii) changes in iron profiles, CRP and ESR
Macdougall et al,(309) 1996	Single centre RCT (UK)	CKD on regular haemodialysis, Hb <8.5 g/dl, ferritin 100-800 mcg/l n = 37	Iron dextran 250mg every two weeks	Comparator 1: Ferrous sulphate 200mg x3/day Comparator 2: No iron	Changes in Hb, iron status and EPO dosage requirements from baseline until end of study period (16 weeks)	
Macdougall et al,(308) 2014	Multicentre RCT (n = 193) (USA, Europe)	Age >18 years, non-dialysis CKD, Hb 9-11 g/dl, Ferritin <100 ng/ml or <200 ng/ml with Tsat 20%, eGFR <60 n = 626	Ferric carboxymaltose adjusted to target ferritin in two groups: (i) High: 400-600; (ii) Low: 100-20	Ferrous sulfate 304mg x2/day for 52 week	Time to initiation of other anaemia management of occurrence of an Hb trigger (Hb <10 g/dl during weeks 8-52)	(i) Changes in Hb and iron profiles; (ii) transfusion requirements (iii) safety
Madi-Jebara et al,(187) 2004	Single centre RCT (Lebanon)	Postoperative cardiac surgery critical care, Hb 7-10 g/dl n = 120	Iron sucrose 200mg daily until calculated total iron deficit reached	0.9% saline i.v. placebo (opaque infusion kits)	Change in Hb from baseline until postoperative day 30	(i) Changes in iron profiles; (ii) RBC transfusion requirements; (iii) In-hospital mortality
McMahon et al,(310) 2010	Multicentre RCT (n = 6) (Australia)	Age 18-80 years, CKD stages 3-5, Hb >11 g/dL, ferritin <300 mcg/l, Tsat <25% n = 100	Iron sucrose 200mg every 2 months to maintain ferritin between 300-500 mcg/l	Ferrous sulphate 105mg daily to maintain ferritin between 100-150 mcg/l	Changes in Hb at 12 months or at termination (e.g. starting dialysis, adverse event, starting ESA)	(i) Changes in renal function; (ii) HRQoL; (iii) time to commencement of dialysis/ESA
Meyer et al,(311) 1996	Single centre RCT (South Africa)	Pre-term infants (<33 weeks' gestation, <1.5kg), venous Hct <0.38	Iron sucrose 6mg/kg per week	Ferrous sulphate 12 mg/kg/day	Specific a priori endpoints not reported	

		n = 42				
Michael et al,(312) 2002	Multicentre RCT (n = 69) (USA)	Adults with CKD on chronic haemodialysis, established on EPO, Hb <12.3 g/dl, ferritin <800 ng/ml, Tsat <50% n = 2534	Ferric gluconate 125mg	0.9% saline i.v. placebo (opaque infusion kits)	Drug intolerance and life-threatening adverse events	(i) rate of all adverse events; (ii) cross-reactivity to previously exposed iron dextran
Montano-Pedroso et al,(313) 2018	Multicentre RCT (n = 2) (Brazil)	Adult women (18-55 years), post-bariatric surgery with a BMI <32 kg/m^2 and stabilised weight loss for at least 6 months n = 56	Iron sucrose 400mg (given over two doses)	Iron hydroxide 100mg x2/day for 8 weeks	Hb at postoperative day 56	(i) changes in iron profiles; (ii) adverse effects; (iii) postoperative complications on days 7,28 and 56; (iv) changes in HRQoL (SF-36 and FACIT-F)
Moppett et al,(314) 2019	Multicentre RCT (n = 2) (UK)	Age >70 years, emergency admission with hip fracture n = 80	Iron sucrose 200mg on three separate occasions perioperatively	Usual medical care	Difference in mean reticulocyte count at day 7 after randomization	(i) cardiovascular or infective complications; (ii) changes in Hb and iron parameters; (iii) length of stay; (iv) mortality; (v) transfusion requirements; (vi) postoperative mobility
Mudge et al,(315) 2012	Single centre RCT (Australia)	Adults (age >18 years) scheduled to undergo living-donor or deceased donor kidney transplant surgery n = 104	Iron polymaltose 500mg single dose on postoperative day 4	Ferrous sulphate 210 mg x2/day until Hb >11 g/dl	Time to Hb more than or equal to 11 g/dl	(i) adverse events; (ii) acute rejection episodes; (iii) infections; (iv) blood transfusions or EPO

Na et al,(316) 2011	Single centre RCT (South Korea)	Scheduled for bilateral total knee arthroplasty, Hb >10 g/dl, ferritin 100-300 mcg/l and Tsat <20% n = 108	Iron sucrose 200mg – one dose intraoperatively, followed by single dose on post-op days 1,2,3 and 5 if postoperative Hb was between 7-8 g/dl	No iron	Changes in postoperative Hb and RBC transfusion requirements up to 6 weeks postoperatively	Changes in iron profiles
Nanthini et al,(317) 2017	Single centre RCT (India)	Age 18-45 years, singleton pregnancy 12-32 weeks' gestation, Hb 7-9 g/dl n = 127	Iron sucrose 48 hourly, dose calculated to iron deficit	Intramuscular iron every 8 weeks	Proportion achieving target Hb of 11 g/dl up to 8 weeks post-treatment	Safety and tolerability
Neeru et al,(318) 2012	Single centre RCT (India)	Pregnant women 14-36 weeks' gestation, Hb 6.5 - 10.9 g/dl, ferritin <27 ng/dl n = 100	Iron sucrose on alternate days, dose calculated to iron deficit	Ferrous fumarate 300mg	Efficacy defined as improvement in Hb by 1 g/dl at 4 weeks post-treatment	Tolerability
Neogi et al,(319) 2019	Multicentre RCT (n = 4) (India)	Pregnant women age >18 years, Hb 5-8 g/dl if gestation 20 and 28 weeks, Hb 5-9 g/dl if gestation >28 weeks n = 2018	Iron sucrose on alternate days, dose calculated to target Hb	Oral iron 100mg x2/day until 6 weeks postpartum	Composite of postpartum haemorrhage, puerperal sepsis, shock, prolonged hospital LOS, need for transfusion, ICU admission and referral to another centre	(i) Components of maternal primary outcomes; (ii) composite fetal outcome (low birthweight, preterm labour, perinatal death); (iii) changes in Hb and ferritin
Ng et al,(320) 2018	Multicentre RCT (n = 2) (UK)	Adult with oesophageal cancer and treatment decision for palliative chemotherapy (Hb<12 g/dl in females, <13 g/dl in men)	Iron maltoside - dose calculated according to Ganzoni formula	Usual medical care	Feasibility (screen failure rates, acceptability, non-concordance, retention rates)	(i) Changes in Hb and iron profiles post-chemotherapy; (ii) transfusion requirements; (iii)

		n = 27				mortality; (iv) HRQoL scores
Noronha et al,(321) 2018	Single centre RCT (India)	Age >18 years, malignancy requiring chemotherapy, Hb <12 g/dl, ferritin <100 ng/ml or Tsat <20% n = 192	Iron sucrose every 3 weeks, calculated as per iron deficit formula	Ferrous sulfate 100mg x3/day for 6 weeks	Change in haemoglobin from baseline to 6 weeks post-treatment	(i) Proportion of patients with Hb rise >1g/dl; (ii) RBC transfusion requirements; (iii) quality of life (FACT-An questionnaires); (iv) response to cancer therapy and overall survival; (v) safety profile of iron
Okonko et al,(322) 2008	Multicentre RCT (n = 2) (UK and Poland)	Age >21 years, NYHA Class II or III, Hb <12.5 g/dl (anaemic group) or Hb 12.5 to 14.5 g/dl (non-anaemic group), ferritin <100 mcg/l or 100-300 mcg/l if Tsat <20%, LVEF <45% n = 35	Iron sucrose administered every 4 weeks up to 16 weeks, calculated to iron deficit	Usual medical care	Change in absolute pVO2 from baseline to week 18 post-treatment	(i) Changes in Hb, iron profiles; (ii) changes in LVEF, NYHA class; (iii) changes in global HRQoL and fatigue
Olijhoek et al,(323) 2001	Multicentre RCT (n = 18) (Europe)	Age >18 years, scheduled to undergo orthopaedic surgery that was estimated to require 2 to 4 units of blood, Hb 10-13 g/dl, ferritin >50 mcg/l n = 110	Intervention 1: EPO and iron saccharate 200mg on days 1 and 8 Intervention 2: Placebo and iron saccharate 200mg on days 1 and 8	Comparator 1: EPO + oral iron 200mg daily for 14 days Comparator 2: Placebo + oral iron 200mg daily for 14 days	Total RBC production from day 1 to day 14 (day before scheduled surgery)	Changes in Hb

Onken et al,(324) 2014#	Multicentre RCT (n = 84) (USA)	Age >18 years, iron deficiency anaemia of varying aetiology (heavy uterine bleeding, GI disorders, nutritional deficiencies), Hb <11 g/dL, ferritin <100 ng/ml or ferritin <300 ng/ml with Tsat <30%, who did not respond to a two-week run-in with oral iron n = 507	Ferric carboxymaltose 30 mg/kg (max 1.5 kg)	Ferrous sulphate 325 mg x3/day for two weeks	Mean change from baseline to highest observed Hb	(i) Proportion of patients achieving Hb >12 g/dl; (ii) proportion of patients achieving an increase of at least 2 g/dl at any point (iii) changes in ferritin; (iv) adverse events
Padmanabhan et al,(325) 2018	Single centre RCT (UK)	Scheduled for open heart surgery with WHO definition of anaemia (male, Hb <13 g/dl; female, Hb <12 g/dl) n = 50	Ferric carboxymaltose 1000mg	Ferrous sulphate 200mg daily until surgery	Change in Hb 3 weeks after iron therapy	(i) Changes in iron profiles and CRP; (ii) transfusion requirements; (iii) postoperative complications (atrial fibrillation, renal failure, infection); (iv) HRQoL (EQ-5D-5L, SF-36)
Park et al,(326) 2019	Single centre RCT (South Korea)	Scheduled to undergo elective hip or knee arthroplasty, Hb >10 g/dl within 4 weeks of surgery n = 58	Ferric carboxymaltose 1000mg (following induction of anaesthesia)	0.9% saline i.v. placebo (opaque infusion kits)	Change in Hb from preoperative value to 1 month after surgery	(i) Changes in Hb and iron profiles from preoperative values to postoperative day 5; (ii) RBC transfusion requirements; (iii) changes in perioperative oxygenation; (iv) adverse events

Pedrazolli et al,(327) 2008	Multicentre RCT (n = 33) (Italy)	Adults with solid organ malignancy planned to have chemotherapy for 12 weeks, Hb <11 g/dl, ferritin>100 mcg/l and Tsat >40% n = 149	Ferric gluconate 125mg per week for 6 weeks	No iron	Proportion of patients with response at 12 weeks (Hb rise >2 g/dl or Hb >12 g/dl)	(i) Time to response; (ii) transfusion requirements; (iii) changes in Hb over time; (iv) safety
Pieracci et al,(185) 2014	Multicentre RCT (n = 4) (USA)	Adults >18 years, critically ill trauma with Hb <12 g/dl, expected ICU length of stay >5 days n = 150	Iron sucrose 100mg thrice weekly for up to six doses or until ICU discharge	0.9% saline i.v. placebo (opaque infusion kits)	Number of participants requiring an RBC transfusion	(i) Changes in iron profiles and markers of erythropoiesis; (ii) infection at 28 days; (iii) 28-day mortality
Perello et al,(328) 2014	Single centre RCT (Spain)	Postpartum anaemia within 48 hours of delivery, Hb 6-8 g/dl n = 72	Iron sucrose 200mg on two consecutive days and ferrous sulphate 105mg x2/day for 30 days	0.9% saline on two consecutive days (opaque infusion kits) and ferrous sulphate 105mg x2/day for 30 days	Changes in Hb at one, two and six weeks postpartum	(i) Changes in iron profiles (ferritin, TIBC, retic count); (ii) Symptoms of anaemia, depression and anxiety; (iii) tolerability
Pollak et al,(329) 2001	Single centre RCT (Austria)	Preterm infants gestational age <31 weeks, Hct >30%, phlebotomy <1 ml/day n = 29	Iron sucrose 2mg/kg/day	Comparator 1: Oral iron Comparator 2: Oral iron + i.v. EPO	Unclear	
Ponikowski et al,(330) 2015	Multicentre RCT (n = 41) (Europe)	Age >18 years, Stable NYHA II-III congestive heart failure, LVEF < 45%, BNP > 100 pg/ml, able to perform 6-MWT, ferritin < 100 ng/ml or < 300 ng/ml with Tsat < 20% n = 304	Ferric carboxymaltose, calculated on Hb and body weight (administered 6-weekly for three doses, then 12	0.9% saline i.v. placebo (opaque infusion kits)	Change in 6-MWT from baseline to week 24	(i) Patient global assessments regularly up to week 52; (ii) changes in NYHA class; (iii) changes in fatigue scores and HRQoL (EQ-5D; (iv) adverse events; (v)

			weekly for 36 weeks			hospitalisation rate; (vi) mortality; (v) changes in laboratory markers (Hb, iron status, cardiac biomarkers); (vi) adverse events
Ponikowski et al,(147) 2020	Multicentre RCT (n = 121) (Europe, South America, Singapore)	Age >18 years, hospitalised for acute heart failure with LVEF <50%, evidence of iron deficiency defined as ferritin <100 ng/ml or 100-299 ng/ml with Tsat <20% n = 1132	Ferric carboxymaltose 500mg, two doses (repletion and maintenance), subsequent doses based if ID persisted	0.9% saline i.v. placebo (opaque infusion kits)	Composite of recurrent heart failure hospitalisations and cardiovascular death up to 52 weeks post-randomisation	(i) Composite of recurrent cardiovascular hospitalisations and cardiovascular death; (ii) heart failure hospitalisation; (iii) cardiovascular mortality; (iv) days lost due to heart failure hospitalisations or cardiovascular death; (v) safety
Price et al,(331) 2014	Multicentre RCT (USA)	Age >65 year, Hb 9.0 - 11.5 g/dl (females), Hb 9.0 - 12.7 g/dl (males), unexplained anaemia, serum ferritin between 20 and 200 ng/ml n = 19	Iron sucrose 200mg per week for five 5 weeks	No iron	Change in 6-MWT from baseline to 12 weeks post-randomisation	(i) Change in Hb; (ii) changes in Geriatric Evaluation Panel
Provenzano et al,(332) 2009	Multicentre RCT (n = 50) (USA)	Adults with CKD requiring haemodialysis for at least 90 days, Hb <11.5 g/dl, Tsat <30% n = 230	Ferumoxytol 510mg, two infusions	Ferrous fumarate 50mg daily for 21 days	Change in Hb from baseline to day 35	(i) Proportion of patients achieving Hb >1 g/dl; (ii) changes in iron profiles

Quinibi et al,(333) 2011	Multicentre RCT (n = 47) (USA, Australia)	Age >12 years, non-dialysis dependent CKD, Hb <11 g/dl, ferritin <300 mcg/l, Tsat <25% n = 255	Ferric carboxymaltose 1000mg (further 500mg doses on day 17 and day 31 if Tsat <30% and ferritin <500 mcg/l)	Ferrous sulphate 195 mg x3/day for 56 days	Percentage of patients with Hb response >1 g/dl	(i) Changes in Hb during the course of the study; (ii) adverse events
Rathod et al,(334) 2015^	Single centre RCT (India)	Postpartum anaemia, Hb <10 g/dl n = 366	Intervention 1: Iron sucrose, dose calculated for total iron deficit using Ganzoni formula Intervention 2: Ferric carboxymaltose 1000mg single dose	Ferrous ascorbate 100mg daily for six weeks	Changes in Hb and ferritin at two and six weeks	Adverse effects
Razzaq et al,(351) 2017	Single centre RCT (Pakistan)	Postpartum anaemia, Hb <10 g/dl n = 82	Formulation not reported, up to 1000mg weekly	Ferrous sulfate 325mg x3/day for 6 weeks	Proportion of participants with rise in Hb >3.5 g/dl	
Reinisch et al,(123) 2013	Multicentre RCT (n = 36) (Europe and Asia)	Age >18 years with IBD, Hb <12 g/dl, Tsat <20% n = 338	Iron isomaltoside, dose calculated according to Ganzoni formula	Iron sulfate 200 m.g. once daily for 8 weeks	Change in Hb from baseline to week 8	(i) Change in Hb at weeks 2 and 4; (ii) changes in ferritin and Tsat at week 8; (iii) HRQoL (IBD-Q); (iv) safety and adverse events
Richards et al,(149) 2020	Multicentre RCT (n = 46) (UK)	Age >18 years scheduled to undergo major abdominal surgery with WHO definition of anaemia	Ferric carboxymaltose 1000mg (between	0.9% saline i.v. placebo (opaque infusion kits)	Composite of RBC transfusion or death 30	(i)) Total number of RBC units transfused; (ii) changes in Hb from

		(Hb <12 g/dl for females, <13 g/dl for males) n = 487	10 – 42 days before surgery)		days after the index operation	randomisation to day of surgery and 8 weeks and 6 months postoperatively; (iii) ICU and hospital LOS; (iv) postoperative complications; (v) readmissions; (vi) HRQoL
Rudra et al,(335) 2016	Single centre RCT (India)	Pregnant women 24 to 34 weeks' gestation, Hb 7-9 g/dl n = 200	Iron sucrose, dose calculated to achieve target Hb of 11 g/dl	Ferrous ascorbate 100mg x2/day until 6 weeks postpartum	Efficacy, safety and acceptability of iron sucrose	
Santer et al,(336) 2020	Single centre RCT (UK)	Age >18 years, COPD with no exacerbation in previous 4 weeks n = 48	Ferric carboxymaltose 1000mg	0.9% saline i.v. placebo (opaque infusion kits)	Change in resting peripheral oxygen saturations from baseline to 1 week after infusion	(i) changes from baseline to follow-up visits in oxygenation; (ii) 6-MWT; (iii) laboratory parameters (Hb, CRP, iron profiles); (iv) COPD exacerbations; (v) HRQoL (MRC Dyspnoea, Fatigue Severity); (vii) echocardiography changes
Schijns et al,(337) 2020	Multicentre RCT (n = 3) (Netherlands)	Women aged 18-65 years, preoperative ferritin >20 mcg/l and then developed iron deficiency post-gastric bypass n = 120	Ferric carboxymaltose 1000mg	Comparator 1: Ferrous fumarate 200mg x3/day Comparator: Ferrous	Percentage of patients with ferritin >20 mcg/l at 3- and 12-months following treatment	

				gluconate 695mg x2/day		
Schroder et al,(338) 2005	Multicentre RCT (Germany)	Adult patients aged 18-85 years with IBD, iron deficiency anaemia defined as Hb <10.5 g/dl (females) or <11 g/dl (males) and Tsat <20% or ferritin <20 mcg/l n = 46	Iron sucrose 7 mg/kg loading dose, followed by 200mg weekly for 5 weeks	Iron sulfate 100-200mg daily for 6 weeks	Maximal change in Hb from baseline until 6 weeks	(i) Adverse events; (ii) changes in HRQoL; (iii) changes in Tsat or ferritin
Sedighi et al,(339) 2013	Multicentre RCT (n = 3) (Iran)	Age >18 years, CKD established on haemodialysis for more than 3 months, Hb <11 g/dL, ferritin >500 ng/ml, Tsat <25% N = 40	Iron sucrose 100mg twice weekly for 5 weeks	Ascorbic Acid for 12 weeks	Change in Hb from baseline to week 12	Changes in ferritin, serum iron, TIBC and Tsat
Seid et al,(341) 2008	Multicentre RCT (n = 28) (USA)	Postpartum anaemia (Hb <10 g/dl) within 10 days of delivery n = 291	Ferric carboxymaltose, dose calculated according to Ganzoni formula	Ferrous sulphate 65mg x3/day for 6 weeks	Percentage of participants with Hb >12g/dl between baseline and end of study	(i) Percentage of participants with Hb rise >3 g/dl; (ii) time taken to achieve Hb rise >3 g/dl; (iii) changes in Hb, ferritin, and Tsat; (iv) Safety
Seid et al,(340) 2017	Multicentre RCT (n = 130)	Heavy menstrual bleeding or post-partum anaemia, Hb <11 g/dL, iron deficiency (definition not provided) n = 2045	Ferric carboxymaltose 15mg/kg once only	Usual medical care	Safety (Incidence of AEs, death, hospitalisation, disability, congenital anomaly/birth defect, life-threatening events)	Changes in Hb and ferritin from baseline to highest post-randomisation value
Serrano-Trenas et al,(342) 2011	Single centre RCT (Spain)	Age >65 years, scheduled to undergo hip fracture surgery n = 200	Iron sucrose 600mg - given in 200mg every 48 hours (first dose pre-operatively)	Usual medical care	RBC transfusion requirements	(i) Changes in iron profiles; (ii) hospital length of stay; (iii) infection; (iv) 30-day

						mortality; (v) side-effects
Singh et al,(344) 1998	Single centre RCT (Singapore)	Pregnant women age >16 years, 20-28 weeks' gestation, Hb <9 g/dl, ferritin <20 mg/l n = 100	Iron polymaltose, dose calculated to Hb deficit	Iron fumarate 200mg x3/day for 12 weeks	Changes in Hb at 36 weeks, delivery and 6 weeks postpartum	Changes in iron profiles
Singh et al,(343) 2006	Multicentre RCT (n = 21) (USA & Mexico)	Age >18 years, CKD on peritoneal dialysis, Hb 9.5 to 11.5 g/dl, established on EPO, Tsat <25%, ferritin <500 ng/ml n = 121	Iron sucrose 1000mg, divided over a 28-day period	No iron	Change from baseline to highest Hb during study period	(i) Changes in Tsat and ferritin; (ii) time to anaemia intervention; (iii) safety events
Sloand et al,(345) 2004	Single centre RCT (USA)	CKD (end stage) and Restless leg syndrome n = 25	Iron dextran 1000mg (over multiple doses)	0.9% saline i.v. placebo (opaque infusion kits)	Changes in International RLS score from baseline to 4 weeks post-infusion	Adverse events
Spahn et al,(346) 2019	Single centre RCT (Switzerland)	Age >18 years, undergoing CABG and/or valve surgery, Hb <130 g/l (males) or <120 g/l (females) or ferritin <100 mcg/l n = 1006	Ferric carboxymaltose 1000mg once only and Eprex 40,000 units (preoperatively) B_{12} and folate also supplemented	0.9% saline i.v. placebo (opaque infusion kits)	Numbers of RBCs transfused within 7 days	(i) Percentage with any RBC transfusion in 90 days; (ii) duration of mechanical ventilation; (iii) acute kidney injury; (iv) infection (7 days); (v) VTE (90 days); (vi) 30 and 90-day mortality; (vii) hospital LOS; (viii) changes in peri-operative Hb concentrations
Spinowitz et al,(347) 2008	Multicentre RCT (n = 13) (USA)	Age >18 years, CKD stages 1-5, Hb <11 g/dl	Ferumoxytol 510mg – two doses	Ferrous fumarate 200mg daily for 21 days	Changes in Hb from baseline to day 35	(i) Changes in iron profiles; (ii) proportion

		n = 304				of patients increasing Hb by >1 g/dl
Steensma et al,(348) 2011	Multicentre RCT (USA)	Age >18 years, receiving chemotherapy for non-myeloid neoplasm, Hb <11 g/dl, ferritin >20 mcg/l, Tsat <60% n = 502	Ferric gluconate 187.mg every 3 weeks (for 5 doses)	Comparator 1: Ferrous sulphate 325mg for 16 weeks Comparator 2: Oral placebo for 16 weeks	Proportion of patients with Hb response >2 g/dl from baseline	(i) Transfusion requirements; (ii) ESA dose; (iii) changes in HRQoL; (iv) adverse events
Stoves et al,(510) 2001	Single centre RCT (UK)	Adults with progressive CKD and worsening anaemia Hb <11 g/dl n = 45	Iron sucrose 300mg - repeated monthly according to serum ferritin	Ferrous sulphate 200mg x3/day	Changes in ferritin and EPO dose requirements Average follow-up: 5.2 months	
Toblli et al,(349) 2007	Single centre RCT (Argentina)	Adults with LVEF <35%, anaemia (Hb <12.5 g/dl for men, <11.5 g/dl for women) with ferritin <100 ng/ml and Tsat <20% n = 40	Iron sucrose 200mg weekly for 5 weeks	0.9% saline i.v. placebo (opaque infusion kits)	Improvement in Hb and renal function	(i) Changes in BNP and inflammation; (ii) no. of hospitalisations; (iii) changes in exercise tolerance and changes in HRQoL
Trenkwalder et al,(350) 2017	Multicentre RCT (n = 13) (Finland, Germany, Switzerland)	Adults with restless leg syndrome, International RLS >15, Hb <11.5 g/dl (females); <12.5 g/dl (males) n = 110	Ferric carboxymaltose 1000mg	0.9% saline i.v. placebo (opaque infusion kits)	Mean change in International RLS score from baseline to week 4 post-randomisation	(i) Changes in IRLS score from baseline to week 12; (ii) changes in ferritin, Tsat; (iii) Adverse events
Vadhan-Raj et al,(352) 2013	Multicentre RCT (n = 192) (Europe, Asia, North America)	Anaemia (Hb 7-10 g/dl) secondary to any underlying condition (gastric bleed, cancer,	Ferumoxytol 510mg - two doses	0.9% saline i.v. placebo (opaque infusion kits)	Proportion of patients achieving Hb rise of >2 g/dl at any point from baseline to week 5	(i) Proportion of patients with Hb >12 g/dl; (ii) changes in iron profiles; (iii) changes in fatigue

		postpartum anaemia, heart failure, rheumatoid arthritis) n = 812				scales and SF-36; (iv) safety
Van Iperen et al,(184) 2000	Single centre RCT (Netherlands)	Age >18 years, expected ICU length of stay >7 days, Hb <11.2 g/dl (or 12.1 g/dl if ischaemic heart disease) n = 36	Iron sucrose 20mg daily from day 1-14	No iron	Changes in Hb and reticulocyte counts at 14 days	(i) Changes in EPO and iron profiles; (ii) blood loss; (iii) RBC transfusion requirements; (iv) ICU length of stay; (v) mortality
van Veldhuisen et al,(353) 2017	Single centre RCT (n = 28) (Europe)	Age >18 years, clinically stable heart failure NYHA II to III, LVEF <45%, Ferritin <100 ng/ml or <300 ng/ml with Tsat <20% n = 172	Ferric carboxymaltose, administered at week 0 and week 6, based on screening Hb and weight	Usual medical care	Change in peak oxygen consumption from baseline to week 24	(i) Effect on haematinic and cardiac biomarkers; (ii) HRQoL; (iii) safety
Van Wyck et al,(356) 2005	Multicentre RCT (n = 35) (USA)	Adults, Stage 3-5 CKD, Hb <11 g/dl, Tsat <25%, ferritin <300 ng/ml n = 188	Iron sucrose 1000mg in divided doses over 14 days	Ferrous sulphate 195mg x3/day for 56 days	Proportion of patients with an Hb increase of 1 g/dl	Changes in Hb and iron profiles
Van Wyck et al,(355) 2007	Multicentre RCT (n = 43) (USA and Mexico)	Postpartum anaemia, Hb <10 g/dl within 10 days of delivery n = 361	Ferric carboxymaltose, dose calculated to target Hb	Ferrous sulphate 65mg x3/day for 42 days	Change in Hb by >2g/dl after treatment	(i) Proportion of patients with Hb >12 g/dl; (ii) time to achieve to primary outcome; (iii) changes in iron profiles; (iv) transfusion requirements; (v) HRQoL

Van Wyck et al,(354) 2009	Multicentre RCT (n = 79) (USA and Mexico)	Adult females age >18 years, Hb <11 g/dl due to heavy uterine bleeding, Tsat <25%, ferritin <100 ng/ml n = 477	Ferric carboxymaltose, dose calculated to target Hb	Ferrous sulphate 65mg x3/day for 42 days	Proportion of patients with Hb increase >2 g/dl	i) Proportion of patients with Hb >12 g/dl; (ii) time to achieve to primary outcome; (iii) changes in iron profiles; (iv) RBC transfusion requirements; (v) HRQoL
Vanobberghen et al,(357) 2020	Multicentre RCT (n = 2) (Tanzania)	Pregnant women within 14 days of delivery with IDA (Hb <11 g/dL, ferritin <50 mcg/l) n = 230	Ferric carboxymaltose, doses based on weight and Hb	Ferrous sulphate 60mg once daily for 6 weeks	Proportion of patients with normalisation of Hb (>11.5 g/dL)	(i) Proportion of patients with corrected ID (raised serum ferritin >100 mcg/l); (ii) adverse events; (iii) SF-36; (iv) adherence to study medication
Verma et al,(365) 2011	Single centre RCT (India)	Postpartum anaemia Hb <8 g/dl, within 24 hours of delivery n = 150	Iron sucrose 200mg every 48 hours (3 doses)	Oral iron 200 mg ferrous sulphate x2/day for one month	Changes in Hb for the first 4 weeks postpartum	
Warady et al,(358) 2004	Multicentre RCT (n = 5) (USA)	Paediatric patients with ESRD (age 1-20 years), haemodialysis for >2 months, Tsat >20% n = 35	Iron dextran, (weight based) weekly for 6 weeks	Ferrous fumarate 4-6 mg/kg/day for 6 weeks	Maintenance of target Hct between 33% and 36%, reduction of EPO dose and prevention of iron deficiency	(i) Changes in iron status; (ii) parathyroid hormone levels and blood urea nitrogen levels during the study
Weisbach et al,(359) 1999	Single centre RCT (Germany)	Age >18 years, scheduled for autologous blood donation (minimum of 3 units) prior to major orthopaedic or cardiovascular surgery n = 123	Iron sucrose 200mg after each donation	Comparator 1: Ferrous fumarate 100mg x3/day up to day before surgery Comparator 2: No iron	Net RBC production during the donation period	(i) Changes in Hb values and iron profiles; (ii) postoperative erythropoiesis; (iii) adverse events

Westad et al,(360) 2008	Multicentre RCT (n = 5) (Norway)	Postpartum anaemia, Hb 6.5-8.5 g/dl within 48 hours after delivery n = 129	Iron sucrose 600mg (split over 3 doses)	Ferrous sulphate 100 mg x2/day from inclusion until 12 weeks	Change in Hb at 4 weeks postpartum	(i) Changes in Hb and iron profiles at 8 and 12 weeks postpartum; (ii) HRQoL (SF-36) after four, eight and 12 weeks
Woods et al,(361) 2014	Single centre RCT (Australia)	Elite runners with non-anaemic iron deficiency (Hb >12 g/dl, ferritin 30-100 mcg/l) n = 14	Ferric carboxymaltose 100mg twice a week for 4 weeks	0.9% saline i.v. placebo (opaque infusion kits)	Changes in fatigue (Brief Fatigue Inventory) and mood (Brunel Mood Scale)	(i) Changes in exercise performance; (ii) changes in Hb and iron profiles
Xu et al,(362) 2020	Single centre RCT (China)	Age 20-70 years, postoperative anaemia following cardiac surgery WHO criteria (male, Hb <13 g/dl; female, Hb <12 g/dL), ferritin 30-100 mcg/l or Tsat <20% N = 150	Iron sucrose 200mg on alternate days until calculated total iron deficit met, started day after surgery on cardiac ICU	0.9% saline i.v. placebo (opaque infusion kits)	Changes in Hb on postoperative day 7 and 14	(i) Changes in iron profiles; (ii) clinical – infection, length of ventilation, ICU and hospital stay; (iii) adverse events
Yeo et al,(363) 2018	Multicentre RCT (n = 2) (Singapore)	Adults admitted with decompensated heart failure, ferritin <100 ng/ml, Tsat <20%, Hb <14 g/dl n = 50	Ferric carboxymaltose 1000mg	0.9% saline (unblinded)	Change in 6-MWT from baseline to 12 weeks	(i) Changes in NYHA class and Kansas City Cardiomyopathy Questionnaire (KCCQ) (ii) hospital admission for heart failure; (iii) serious adverse events
Yoo et al,(364) 2011	Single centre RCT (South Korea)	Adults scheduled for valvular heart surgery, preoperative anaemia according WHO definition n = 74	Iron sucrose 200mg and EPO (500 IU/kg) 16-24 prior to surgery	0.9% saline i.v. placebo (opaque infusion kits)	RBC transfusion requirements during surgery and for first 4 postoperative days	(i) Changes in Hb and iron profiles; (ii) postoperative complications

Abbreviations: 6-MWT, six minute walk test; BI, Barthel's index; CRP, C-reactive protein; COPD, Chronic Obstructive Pulmonary Disease; DVT, deep vein thrombosis; CKD, chronic kidney disease; EPO, erythropoietin; ESA, erythropoiesis-stimulating agent; ESR, Erythrocyte sedimentation rate; ESRD, End-stage renal disease; FIQR, fibromyalgia impact questionnaire; Hb, haemoglobin; HD, haemodialysis; HRQoL, health-related quality of life; IBD, inflammatory bowel disease; ICU, intensive care unit; i.v., intravenous; LOS, length of stay; LVEF, left ventricular ejection fraction; MFI, Multidimensional fatigue inventory; MI, myocardial infarction; s.c., subcutaneous; NYHA, New York Heart Association; PFS, Piper Fatigue Scale; RBC, red blood cell; RLS, restless leg syndrome; SF-36, Short-Form 36; Tsat, transferrin saturation; VTE, venous thromboembolism

*Trial analysed as two separate studies

^ Data for both i.v. iron groups were combined

This trial had an oral iron run-in period with two parallel randomisations after this based on tolerance of oral therapy -- in the results we have only included the participants in the randomisation of oral-tolerant participants (FCM vs oral iron) and not the other randomisation (FCM vs other IV standard of care)

Appendix 4: Characteristics of ongoing trials discussed in Chapter 3

Study details and status	Planned sample size	Participants	Intervention(s)			Primary outcome	Secondary outcome	Infection reporting
			Arm 1	Arm 2	Arm 3			
Surgery								
NCT03817957; IDA-II; Germany (Recruiting)	407	Age >18 years; following major surgery (orthopaedic/trauma vascular, visceral, cardiac), Hb <12 g/dL for females and <13 g/dL for males or non-anaemic ID (ferritin <100 mcg/l, confirmed 28 days before surgery)	Ferric carboxymaltose 1000mg	Polyglucoferron 500-2000 mg (i.v.)	Ferrous sulphate 50-200mg b.d. for 28 days	Proportion of patients who achieve normal Hb or Hb rise >1.5 g/dL at 30 days; pre-and post-volume-corrected urine iron levels	Changes in Hb and iron profiles; adverse events and tolerability; all-cause mortality; changes in SF-36	Not specified
NCT02972294; HiFIT; France (Recruiting)	780	Age >18 years, NOF fracture; Hb 9.5-13 g/dL	Iron isomaltoside 1000mg + tranexamic acid (TXA)	Iron isomaltoside + i.v. placebo	I.v placebo + TXA **Arm 4**: I.v. placebo x2	Peri-operative transfusion requirements	No. of blood products per patients; changes in Hb up to day 30 post-op; peri-operative blood loss; proportion of patients home at day 30; all-cause mortality; postoperative complications (cardiovascular,	Yes – 'infectious complications'

						infection, transfusion-related, anaphylaxis); QoL (EQ-5D-5L, PQOL, IADL); all-cause mortality	
KCT0004020; South Korea; (Recruiting)	80	Age >60, scheduled to undergo total shoulder arthroplasty; post-op Hb <10 g/dL or fall greater than 3 g/dL from pre-operative value.	Ferric carboxymaltose 1000mg	I.v. placebo (0.9% saline)	Changes in Hb up to 3 months postoperatively	Changes in ferritin, iron, TIBC, Tsat	Not specified
NCT04083755 IRONPAD; Spain (Recruiting)	230	Age >18 years; Hb <130 g/L (males); Hb <120 (females), symptomatic lower limb ischaemia; >1 week prior to surgery	Ferric carboxymaltose 1000mg	No treatment or oral iron	Transfusion requirements	Changes in Hb; length of stay; hospital mortality; adverse events; SF-36 at day 30 post-op	Not specified
NCT03528564; HOPE-Hb; Canada (Recruiting)	74	Age >18 years, scheduled to undergo primary total hip or knee arthroplasty; Hb <12 g/dL	Iron sucrose 900mg + EPO 40000 IU	Iron sucrose 900mg + s.c. placebo	Feasibility and pre-operative Hb on day of surgery	Postoperative Hb; changes in Hb and iron profiles; transfusion requirements; DVT; postoperative morbidity; surgical wound infection; HADS; cognitive	Yes – part of composite of postoperative morbidity up to 3 months post-surgery and surgical wound infection (superficial and

						assessments; cost analysis	deep) 6 weeks from surgery
EudraCT 2018-004213-41; RIPAC; Belgium (Recruiting)	60	Age >18 years, scheduled to undergo colonic cancer resection; Hb <12 g/dL in women and <13 g/dL in men; Tsat <20%	Ferric carboxymaltose 1000mg	Usual care	Change in Hb at time of surgery	Peri-operative transfusion requirements; change in Hb at 1 month postoperative; postoperative complications; length of stay; QoL (EORTC QLQ-C30)	Yes – as part of postoperative morbidity assessment using Clavien-Dindo classification
NCT03565354; Hong Kong (Completed – results awaited)	40	Age >18 years, scheduled to undergo colorectal cancer surgery; Hb <13 g/dL, ferritin <30 mcg/l or ferritin 100-300 mcg/l with Tsat <20%	Iron isomaltoside 1000mg	Usual care	Changes in pre-operative Hb and ferritin	Peri-operative transfusion requirements; hospital length of stay, quality of recovery; surgical complication; DAOH-30	Yes – as part of postoperative morbidity assessment using Clavien-Dindo classification
NCT03574311; PREFER-CABG; Finland (Recruiting)	210	Age >18 years; open heart surgery	Ferric carboxymaltose 1000mg pre-operatively	I.v. placebo (0.9% saline)	Composite of incidence of RBC transfusion and/or nosocomial infection	Mortality at 90-days; length of stay; peri-operative MI; organ support requirements; HRQoL at one year	Yes – part of composite of primary outcome (incidence of RBC transfusion and/or nosocomial infection)

ACTRN12618001437257; ADEPT; Australia (Recruiting)	40	Scheduled to undergo elective open/laparoscopic abdominal surgery; Non-anaemic iron deficiency	Ferric carboxymaltose 1000mg pre-operatively	I.v. placebo (0.9% saline)	Changes in peak VO_2 measured by CPEX	Changes in AT; changes in Hb; changes in EQ-5D-5L and WHODAS V2.0 (30 days post-operatively); peri-operative transfusion requirements; post-operative complications (including infection); DAOH-90	Yes – part of postoperative complication assessment
ICTRP EUCTR2018-002571-18-NL; EFFECT KTx; Netherlands (Recruiting)	158	Age >18 years; Kidney transplant recipients (at least 4 months after transplant); ID (defined as ferritin < 100µg/l or ferritin between 100 and 300 µg/l in combination with a transferrin saturation < 20%);	Ferric carboxymaltose 1000mg	I.v. placebo (0.9% saline)	Changes in 6MWT at 24 weeks post-intervention	Changes in Hb and iron profiles; changes in LVEF; HRQoL (SF-36, EQ-6D, KDQOL-SF); infection; cardiovascular events; graft rejection; mortality	Yes – defined as 'occurrence of infectious diseases'
NCT03662789; IronIC; Norway (Complete – results awaited)	102	Age >18 years, presentation at least one year post-cardiac transplant; ID (ID (defined at Tsat <20% with	Iron isomaltoside 20mg/kg	I.v placebo (0.9% saline)	Peak oxygen consumption at 6 months	Changes in iron profiles; muscle strength (hand-grip dynamometer); changes in HRQoL (SF-36); changes	Not specified

		ferritin 100-300 µg/l or ferritin <100 µg/l)				in laboratory CRP, troponin and NT-proBNP	
NCT04268849; USA (Recruiting)	104	Age >18 years, undergone bariatric surgery >3 months ago, IDA (ferritin<30 mcg/l, Tsat <20%, Hb <13 g/dl)	Ferumoxytol 1020mg + oral placebo	Ferrous sulphate 60mg and vitamin C for 6 weeks + i.v. placebo (0.9% saline)	Changes in Clinical Global Impression at 6 weeks post-treatment; Changes in Hb	Changes in ferritin and Tsat; QoL measured by visual linear analog scale	Not specified
Oncology							
ICTRP KCT0004311; South Korea (Recruiting)	341	Adults >19 years, solid cancer or lymphoma, Hb 8.0-10.5 g/dL, recent chemotherapy	Ferric carboxymaltose 1000mg	Usual care	Change in Hb	Adverse events, quality of life with EORTC QLQ-C30	Not specified
ISRCTN13370767 ICARAS; UK(Recruiting)	40	Age ≥ 18 years; Solid tumours not amenable to curative treatment; Hb <130 g/L (males) / <120 g/L (females)' moderate-severe fatigue	Iron isomaltoside (20 mg/kg)	I.v placebo (0.9% saline)	Feasibility (recruitment, randomisation, protocol adherence, retention)	Quality of life (FACIT-F, EQ-5D, EORTC QOQ-C30 and baseline, 4 and 8 weeks post-infusion); transfusion requirements; changes in Hb, iron profiles, cytokine activity; gut microbiome; daily steps	Not specified

Eudra CT 2014-000246-30; FERINJECT; Germany (Complete – results awaited)	64	Age >18 years; inoperable colorectal cancer; iron deficiency anaemia: Hb ≤ 10.5 g/dl and transferrin saturation < 20 % and/or serum ferritin < 20 ng/ml	Ferric carboxymaltose 1000mg	Ferrous sulphate 100mg	Rise in Hb by 2 g/dL at 12 weeks	QoL (EORTC-QLQ-C30), hand grip via dynamometer; transfusion requirements; changes in iron profiles; adverse events; overall survival	Not specified
NCT04206228 IIISAS; Norway (Recruiting)	100 participants	Age>18 years, Aortic stenosis and scheduled for TAVI, ID (ferritin < 100 µg/l or ferritin between 100 and 300 µg/l in combination with a transferrin saturation < 20 %)	Iron isomaltoside (20mg/kg)	I.v. placebo (0.9% saline)	Baseline-adjusted 6-minute walk test (6 months after intervention)	Changes in iron status, NT-proBNP, and Troponin; muscle strength; SF-36; EQ-VAS, HADS, Kansas City Cardiomyopathy Questionnaire; adverse events	Not specified
Obstetrics / Women's health							
NCT03188445; Denmark (Recruiting)	200	Women aged >18 years; Pregnant >14 weeks gestation; Ferritin <30 µg/l after 4 weeks of oral iron	Iron isomaltoside 1000mg	Ferrous fumarate + ascorbic acid	Time to achieve Hb >11 g/dL (baseline to 18 weeks after treatment)	Changes in Hb and iron biomarkers	Not specified
ACTRN12619000283178p; IRONWOMAN;	50	Pregnant women aged >18 years; Hb 80-105 g/dL and	Ferric carboxymaltose 1000mg + oral	Oral iron 80 mg once	Feasibility – adequacy of blinding	Patient and clinician acceptability;	Not specified

Australia (Not yet open)		ferritin <30 µg/l; between 26-33 weeks' gestation	placebo capsules	daily + i.v. placebo		changes in SF-36 from baseline to 4 weeks; treatment side effects; oral iron compliance; proportion of women with Hb <105 g/dL at 4 weeks; changes in Hb and iron profiles; maternal outcomes (PPH, mode of delivery, transfusion requirements); fetal outcomes (SGA, birth weight, preterm labour, death, NICU admission)	
ACTRN12618001268235; REVAMP; Malawi (Recruiting)	862 participants	Pregnant women 13-26 weeks gestation; Hb <10 g/dL; negative malaria test	Ferric carboxymaltose 1000mg	Ferrous sulphate 200mg b.d. for 90 days	Prevalence of Hb <10 g/dL at timepoint closest to delivery	Maternal fatigue (1 month postpartum); changes in Hb and iron profiles; incidence of PPH, length of stay post-delivery; maternal cognitive function; transfusion requirements; adverse events; neonatal outcomes	Yes – incidence of malaria and /or parasitaemia

							(birth weight, SGA, preterm birth, still birth)	
NCT04505514; IVIronPPH; Malaysia (Recruiting)	60	Age >18 years; PPH >500 mls; Hb <10 g/dL	Iron isomaltoside 1000mg + oral iron preparation (Iberet-Folic 500)	I.v placebo + oral iron preparation (Iberet-Folic 500)		Changes in Hb, serum iron and ferritin at 6 weeks	General fatigue score; adverse events; transfusion requirements	No
NCT04205266; USA (Not yet recruiting)	76	Women aged 18-50; heavy menstrual bleeding; Hb <11 g/dL	Ferumoxytol 510 mg (2 infusion)	Ferrous sulphate 325mg o.d. for 60 days		Changes in Hb at 60 points	Patient satisfaction with treatment; quality of life score	No
NCT03957057; Slovenia (Recruiting)	300	PPH with Hb 7-10 g/dL within 48 hours after delivery	Ferric carboxymaltose 1000mg	Iron isomaltoside 1500mg	Ferrous sulphate 160 mg o.d. for 6 weeks	MFI score at 6 weeks postpartum	Edinburgh Postnatal Depression score; changes in Hb and iron profiles; side effects; compliance with oral iron	Yes – part of composite of adverse events listed as 'upper respiratory tract infection'
CTRI/2020/02/023125; India (Recruiting)	80	Women age 18-45 years, postpartum Hb 8-10 g/dL	Ferric carboxymaltose 1000mg	Ferrous fumarate 152mg b.d. for 6 weeks		Changes in Hb at 2 and 6 weeks postpartum	Safety and acceptability	No
Cardiology / heart failure								
ISRCTN16403302; IRONMAN;	1300	Age >18 years, LVEF<45%; NYHA class II-IV; ID	Iron isomaltoside	Usual care		Cardiovascular mortality or hospitalisation for	All-cause mortality; all-cause hospitalisation;	Yes - Hospitalisation primarily for

UK (Recruiting)		(defined at Tsat <20% with ferritin 100-300 µg/l or ferritin <100 µg/l)	(calculated to body weight)			heart failure throughout follow-up period (approx. 3 years)	HRQoL (Minnesota Living with Heart Failure; EQ-5D) at 4 and 20 months; QALY at 2.5 years; Death / hospitalisation primarily due to infection; 6MWT	infection is assessed through review of case notes and reporting of patient, relative, carer or clinician throughout the follow up period
EudraCT 2019-002912-25; HEART-FID; USA (Recruiting)	1508	Age >18 years, reduced LVEF; ID ((defined at Tsat <20% and/or ferritin <100 µg/l); Hb between 9 g/dl and 13.5 g/dL, able to perform 6MWT	Ferric carboxymaltose 1000mg	I.v. placebo (0.9% saline)		Composite of death, hospitalisation for heart failure (1 year) or change in 6MWT (6 months)	Time to first composite event; cardiovascular death; time to cardiovascular death; cardiovascular hospitalization; urgent heart failure visits	No
NCT03833336; PREFER-HF; Spain (Recruiting)	72	Stable, chronic HF; LVEF >45%; ID (defined at Tsat <20% with ferritin 100-300 µg/l or ferritin <100 µg/l), able to perform 6MWT	Ferric carboxymaltose 500-1000 mg at 0,6,12 and 24 weeks	Oral iron: Ferrous sulphate 100mg b.d. until week 24 OR Sucrosomial iron 30mg until week 24	I.v and oral placebo	Changes in 6MWT at 24 weeks	Changes in NYHA; hospitalisation; mortality; QoL (Kansas City Cardiomyopathy Questionnaire) – all at 24 weeks	No

ACTRN126200000285954; IRON-AF; Australia (Recruiting)	84	Age >18 years, persistent or paroxysmal AF, Hb <15 g/dL, ID (defined at Tsat <20% with ferritin 100-300 µg/l or ferritin <100 µg/l)	Ferric carboxymaltose 1000mg at weeks 0,1,4 and 8	I.v. placebo (0.9% saline)	Changes in VO_2 at 12 weeks	Changes in QoL (SF-36, AF Effect of QoL, Patient Global Assessment, AF severity scale); changes in 6MWT; changes in NYHA and echocardiography; mortality; hospitalisations	No
NCT04291690; TARGET-EFT; Canada (Recruiting)	144	Age >65, frail or pre-frail defined by EFT score >1, admission to cardiovascular unit	Multi-component- I.v. iron (Iron sucrose 300mg daily for 3 doses) if presence of IDA (Hb<13 g/dL, ferritin <100 µg/l or ferritin <300 µg/l and Tsat <20%) physical training; cognitive stimulation; nutritional support	Usual care	Changes in EQ-5D-5L from randomisation to 6 weeks	Changes in hospital-acquired disability; all-cause mortality; delirium; length of stay, mobility, step counts; HADS	Yes – part of composite of adverse events assessed up to 6 weeks post treatment

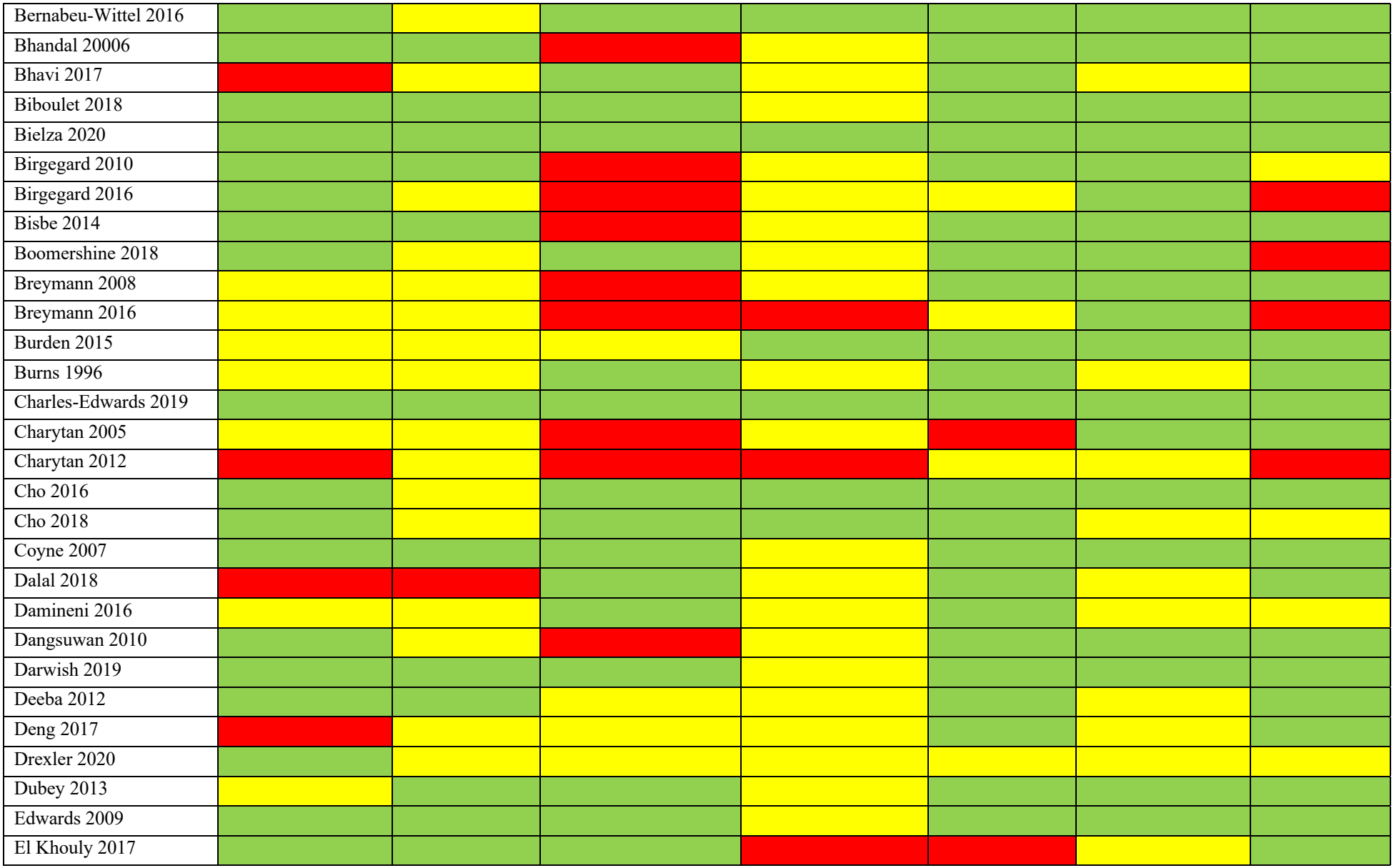
Paediatrics							
NCT03523117; USA (Recruiting)	72	Age 1-17 years; Hb <11 g/dL; ferritin <300 ng/mL, Tsat <30%, inadequate response to oral iron	Ferric carboxymaltose 15mg/kg (max 750 mg) at day 0 and day 7	Ferrous sulphate dose adjusted for age, for 28 days	Changes in Hb at day 35	Changes in ferritin, Tsat and Retic Hb	No
ChiCTR18000019815; China (Recruiting)	268	Children aged <14 years, scheduled to undergo major abdominal surgery; ferritin <15 mcg/l, Tsat <15%	I.v iron infusion (no further details)	Usual care	Incidence of RBC transfusion		No
Chronic Kidney Disease							
NCT04464850; IVO-IRON Thailand (Recruiting)	124	Age >18 years; Haemodialysis for at least 3 months; Hb 8-11.5 g/dL; Tsat <50% and ferritin <800 mg/dL	Iron sucrose 200mg every 2 weeks for 24 weeks	Ferrous fumarate 600 mg/day for 24 weeks	Change / reduction in EPO dose at 24 weeks	Changes in Hb; MACE; hospitalisations due to infection; Changes in HRQoL (KDQOL, EQ-5D-5L), cost-effectiveness of oral	Yes – secondary outcome of hospitalizations due to infection
Critical care							
NCT02276690; HEPCIDANE France (Completed – results awaited)	408	Age >18 years, required ICU care for at least 5 days, anaemia as per WHO definition	Intravenous iron +/- EPO according ferritin and hepcidin levels	Usual care	Hospital costs	Hospital and ICU LOS; fatigue; changes in Hb and iron profiles; 90-day mortality	No

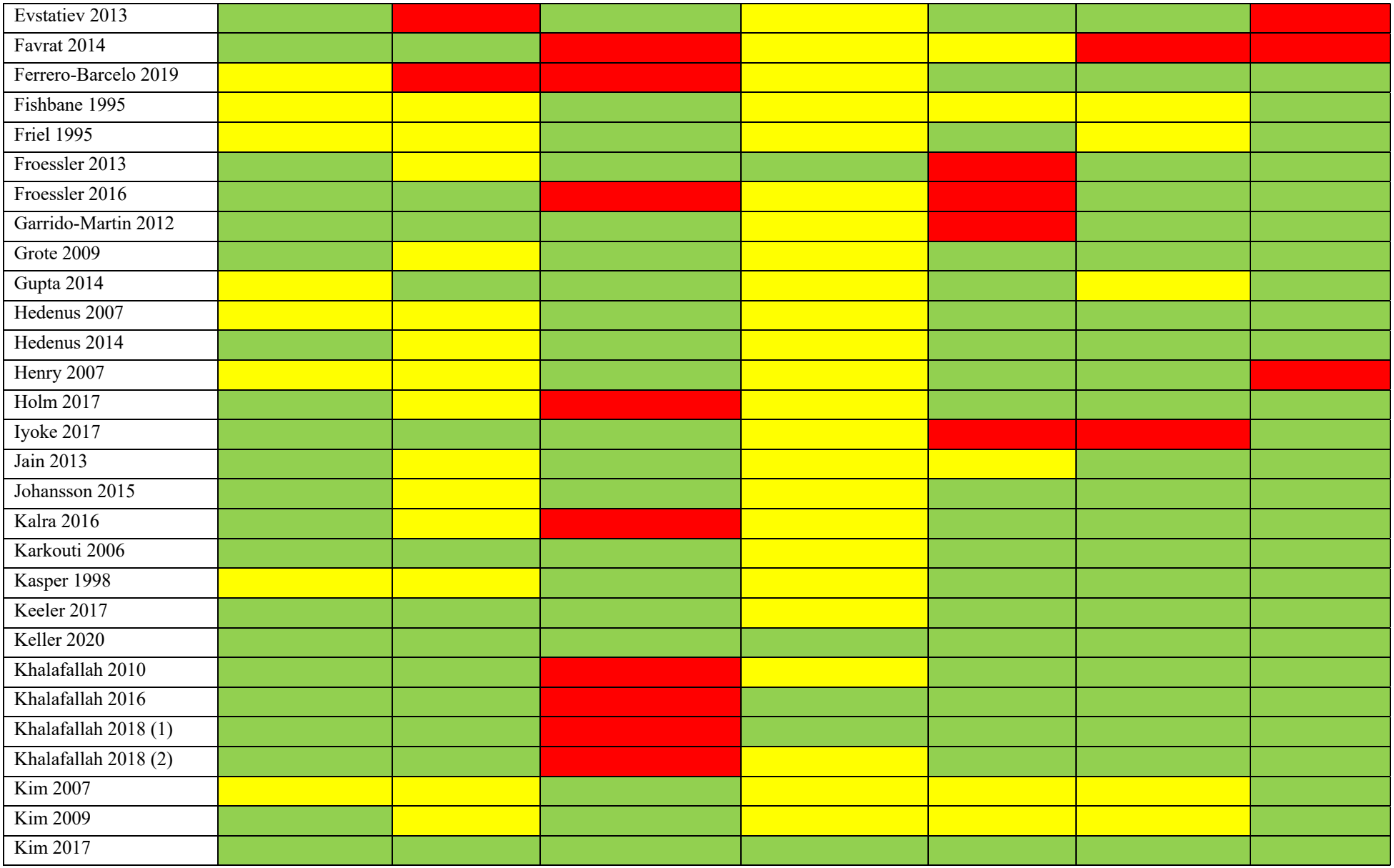
ISRCTN13721808; INTACT UK(Completed – results awaited)	130	Age >18 years, required ICU care for at least 24 hours, Hb <100 g/l prior to hospital discharge	Ferric carboxymaltose 1000mg	Usual care	Feasibility – recruitment, randomisation and follow-up rates	Changes in Hb and iron profiles; nosocomial infection, in-hospital mortality; changes in fatigue and HRQoL up to 90 days post-randomisation; readmissions	Yes – secondary outcome defined as 'commencement of new antibiotic therapy or escalation from prophylactic antibiotics for a confirmed or strongly suspected new infection.

Abbreviations: 6-MWT, 6-minute walk test; CPEX, Cardiopulmonary Exercise Testing; CRP, C-reactive protein; DAOH, Days Alive and Out of Hospital; DVT, Deep Vein Thrombosis; EFT, Essential Frailty Toolset; EORTC, European Organisation for Research and Treatment of Cancer; EPO, erythropoietin; EQ-5D-5L, Euroqol-5D-5L; EQ-VAS, EuroQol Visual Analogue Scale; Hb, HADS, Hospital Anxiety and Depression Scale; Haemoglobin; HRQoL, Health-related quality of life; IADL, Instrumental Activities of Daily Living; ICU, Intensive Care Unit; ID, Iron deficiency; IDA, Iron deficiency anaemia; IV, intravenous; KDQOL, Kidney Disease Quality of Life Instrument; LOS, Length of stay; LVEF, left ventricular ejection fraction; MACE, Major adverse cardiovascular events; MFI; Multidimensional Fatigue Inventory; MI, myocardial infarction; NICU, Neonatal Intensive Care Unit; NOF, Neck of femur; NYHA, New York Heart Association; PPH, Postpartum Haemorrhage; PQOL, Perceived Quality of Life; QALY, Quality adjusted life year; RBC, red blood cell; SF-36, Short Form-26; TAVI, Transcatheter Aortic Valve Implantation; Tsat, Transferrin saturation; WHO, World Health Organization; WHODAS, WHO Disability Assessment Schedule.

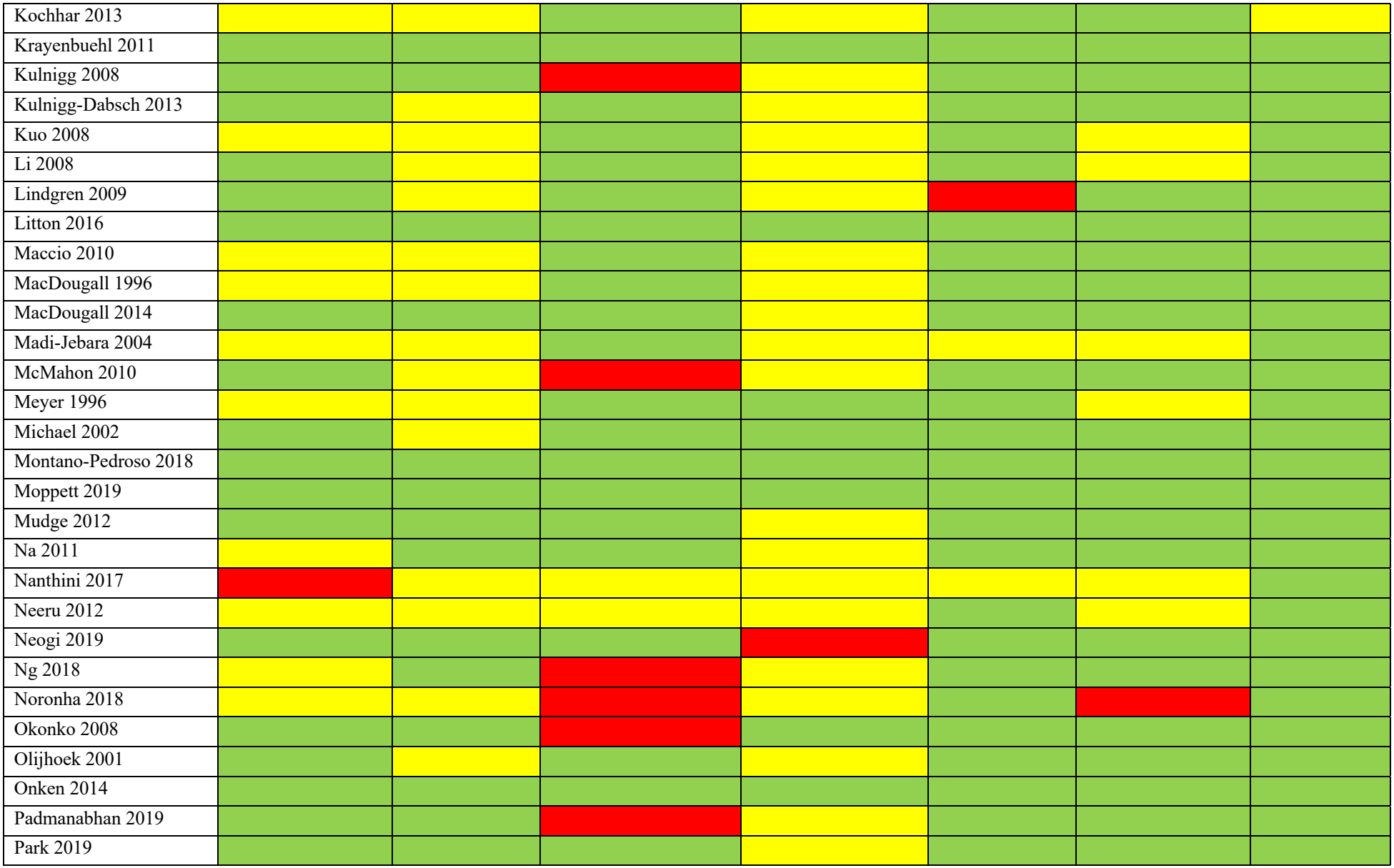
Appendix 5: Risk of bias assessments for trials incorporated into meta-analysis in Chapter 3

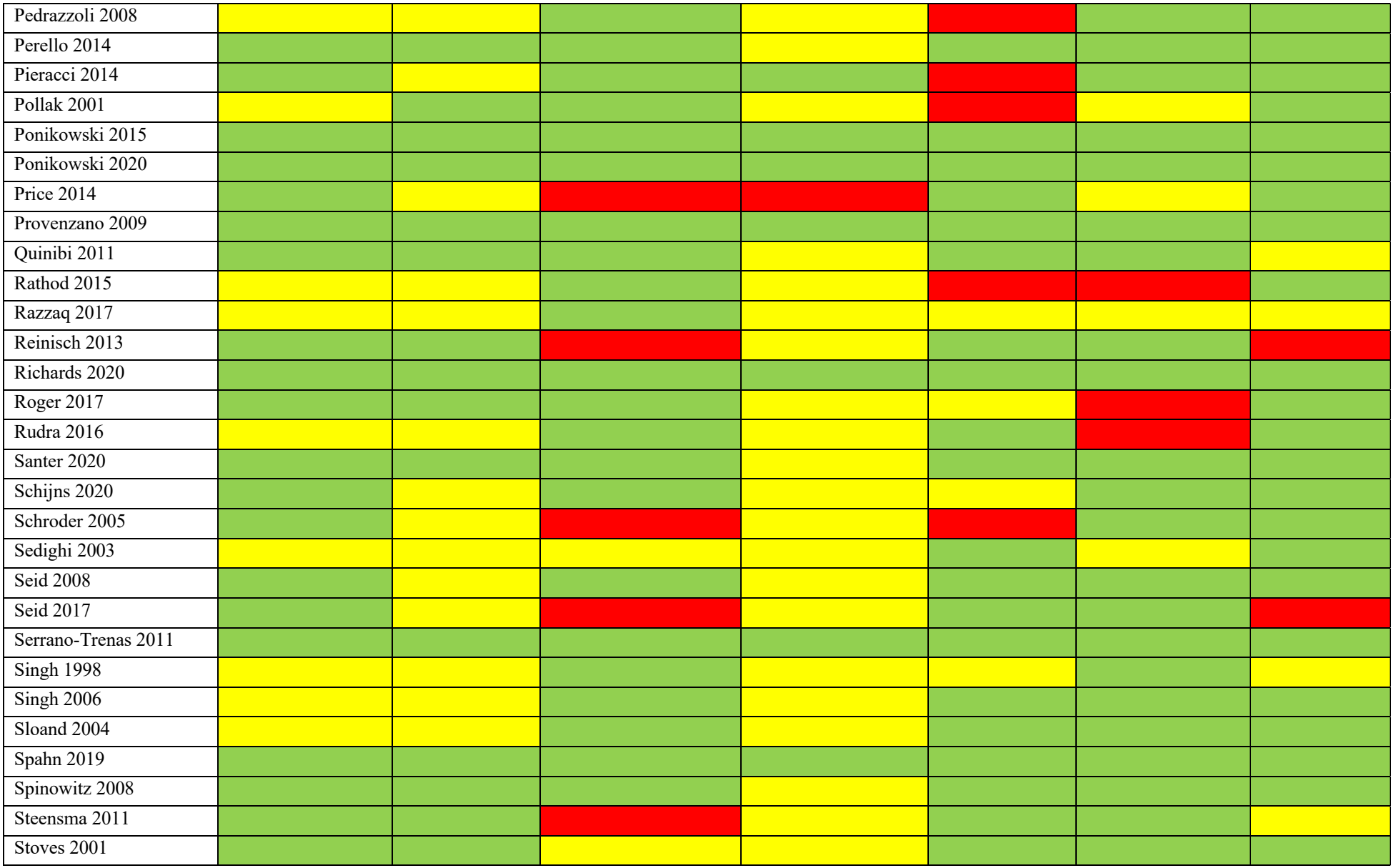
Study ID	Random sequence generation	Allocation concealment	Blinding of participants and personnel	Blinding of outcome assessment	Incomplete outcome data	Selective reporting	Other
Abdelazim 2017							
Abhilashini 2014							
Adhikary 2011							
Agarwal 2006							
Agarwal 2015							
Aggarwal 2003							
Akhtar 2018							
Al 2005							
Allen 2011							
Al-Momen 1996							
Anker 2009							
Athibovonsuk 2013							
Auerbach 2004							
Auerbach 2010							
Bager 2014							
Baillie 2010							
Barish (1) 2012							
Barish (2) 2012							
Bastit 2008							
Batool 2018							
Bayoumeu 2002							
Beck-da-Silva 2013							
Beguin 2013							
Bencaiova 2009							


Bernabeu-Wittel 2016
Bhandal 20006
Bhavi 2017
Biboulet 2018
Bielza 2020
Birgegard 2010
Birgegard 2016
Bisbe 2014
Boomershine 2018
Breymann 2008
Breymann 2016
Burden 2015
Burns 1996
Charles-Edwards 2019
Charytan 2005
Charytan 2012
Cho 2016
Cho 2018
Coyne 2007
Dalal 2018
Damineni 2016
Dangsuwan 2010
Darwish 2019
Deeba 2012
Deng 2017
Drexler 2020
Dubey 2013
Edwards 2009
El Khouly 2017


Evstatiev 2013
Favrat 2014
Ferrero-Barcelo 2019
Fishbane 1995
Friel 1995
Froessler 2013
Froessler 2016
Garrido-Martin 2012
Grote 2009
Gupta 2014
Hedenus 2007
Hedenus 2014
Henry 2007
Holm 2017
Iyoke 2017
Jain 2013
Johansson 2015
Kalra 2016
Karkouti 2006
Kasper 1998
Keeler 2017
Keller 2020
Khalafallah 2010
Khalafallah 2016
Khalafallah 2018 (1)
Khalafallah 2018 (2)
Kim 2007
Kim 2009
Kim 2017

L


Kochhar 2013
Krayenbuehl 2011
Kulnigg 2008
Kulnigg-Dabsch 2013
Kuo 2008
Li 2008
Lindgren 2009
Litton 2016
Maccio 2010
MacDougall 1996
MacDougall 2014
Madi-Jebara 2004
McMahon 2010
Meyer 1996
Michael 2002
Montano-Pedroso 2018
Moppett 2019
Mudge 2012
Na 2011
Nanthini 2017
Neeru 2012
Neogi 2019
Ng 2018
Noronha 2018
Okonko 2008
Olijhoek 2001
Onken 2014
Padmanabhan 2019
Park 2019


Pedrazzoli 2008
Perello 2014
Pieracci 2014
Pollak 2001
Ponikowski 2015
Ponikowski 2020
Price 2014
Provenzano 2009
Quinibi 2011
Rathod 2015
Razzaq 2017
Reinisch 2013
Richards 2020
Roger 2017
Rudra 2016
Santer 2020
Schijns 2020
Schroder 2005
Sedighi 2003
Seid 2008
Seid 2017
Serrano-Trenas 2011
Singh 1998
Singh 2006
Sloand 2004
Spahn 2019
Spinowitz 2008
Steensma 2011
Stoves 2001

Toblli 2007							
Trenkwalder 2017							
Vadhan-Raj 2013							
Van Iperen 2000							
Vanobbergen 2020							
Van Veldhuisen 2017							
Van Wyck 2005							
Van Wyck 2007							
Van Wyck 2009							
Verma 2011							
Warady 2004							
Weisbach 1999							
Westad 2008							
Woods 2014							
Xu 2020							
Yeo 2018							
Yoo 2011							

Colours indicate:
Green = Low risk of bias
Yellow = Unclear risk of bias
Red = High risk of bias

Appendix 6: Risk of bias assessments for non-randomised studies discussed in Chapter 3

Study ID	Confounding	Selection	Intervention classification	Deviation from intervention	Missing data	Measurement of outcome	Selection of reported result	Overall
Munoz 2014 (1)	Serious	Moderate	Low	Low	Low	Moderate	Low	Moderate
Munoz 2014 (2)	Serious	Moderate	Low	Low	Low	Moderate	Low	Moderate
Zitt 2014	Moderate	Low	Low	Low	Low	Moderate	Low	Moderate
Kurugano 2014	Moderate	Low	Moderate	Low	Low	Moderate	Low	Moderate
Ishida 2015	Moderate	Low	Low	Low	Low	Low	Low	Low
Kim 2018	Moderate	Low	Low	Low	Low	Moderate	Low	Moderate
Peters 2018	Moderate	Low	Low	Moderate	Low	Moderate	Low	Moderate
Ikuta 2019	Serious	Moderate	Low	Low	Low	Moderate	Low	Moderate