Title: Evaluation of oral iron supplementation and its effect on postoperative outcomes in non-anaemic iron deficient patients undergoing surgery, with a focus on lower limb arthroplasty

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Abstract

Aim

Non-anaemic iron deficiency although identified as a potential area for improving patient outcome in the literature, its effect on patient outcomes post-surgery is an under researched topic, with numerous gaps in the published literature. The aim of this project was to identify the prevalence of non-anaemic iron deficiency, and its effect on patient outcomes in patients undergoing surgery, with a focus on lower limb arthroplasty. Systematically reviewing the evidence on the effectiveness of treatment of non-anaemic iron deficiency and conducting a randomised controlled trial to explore if a measurable impact on patient outcome can be made.

Results

A retrospective cohort analysis performed on 956 non-anaemic iron deficient patients undergoing lower limb arthroplasty, 2214 in the control group, demonstrated a reduction in postoperative haemoglobin 0.96gdl p=0.007 (CI -1.66 to -0.26) day 1 after surgery in the non-anaemic iron deficient population and an increased length of stay (IRR) 1.08 CI 1.03-1.14 p=0.002. A systematic review of the treatment of non-anaemic iron deficiency demonstrated the lack of available evidence, a meta-analysis at day 1 demonstrated no statistical difference p=0.32 (mean 12.63 CI 9.75 to 15.52), however, narrative synthesis suggested treatment improved patients' haemoglobin postoperatively. A dual arm parallel randomised controlled trial (RCT) involving supplementation with oral Floradix against no intervention was performed. Unfortunately, the early cessation of the trial due to COVID 19 meant the planned statistical analysis was not able to be performed, the results were presented narratively, demonstrating a trend towards supplementation being beneficial, however, due to the early cessation of the trial, this could not be shown to be causative and may have been merely an association.

Conclusion.

Non-anaemic iron deficiency is a real phenomenon with an association of adverse post-operative haemoglobin and length of stay, in patients undergoing lower limb arthroplasty. Although the published literature is sparse, supplementation improves may improve patient outcomes. However, further research is recommended to fully explore the potential impact on treatment of non-anaemic iron deficiency in patients undergoing lower limb arthroplasty and the application to the wider patient population.

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PREFACE

I began my university education in 2002, quickly developing a thirst for knowledge and a love of further education. In the following twenty years, I have become the eternal student, constantly learning, evolving and educating myself and others, which culminated in the opportunity to undertake this PhD. Working as an anaesthesia associate within an organisation with a large orthopaedic department and an active research profile, I became involved in several projects aimed at improving care for patients. Improving anaemia screening and treatment within our organisation had been successfully implemented and discussions were held on extending that treatment to the non-anaemic iron deficient population, however the published evidence was sparse. I agreed to undertake this project to assess the need to extend the treatment and to undertake a trial to analyse the impact of iron supplementation in this iron replete group. I feel I have successfully completed this project, establishing the prevalence of non-anaemic iron deficiency and its effect on the surgical population, systematically reviewing the evidence on the effectiveness of treatment and performed a small clinical trial, which although halted early due to the COVID 19 pandemic, will hopefully add to the narrative and ultimately improve patient care.

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I would also like to acknowledge and thank my wife Candice and son Jack, whose unwavering support, care, love and understanding when things have been stressful or difficult. I truly could not have completed this project without your support and greatly appreciate all you have done to ease the burden. I am proud to be a positive role model for my son, showing what years of dedication and hard work can achieve.

AUTHOR DECLARATION

I hereby certify that this thesis has been composed by me and is based on my own work, unless stated otherwise. No other person's work has been used without due acknowledgement in this thesis.

CHAPTER 1: BACKGROUND AND ACADEMIC JUSTIFICATION

1. Introduction

The purpose of this chapter is to justify and describe the rationale for the proposed research topic, non-anaemic iron deficiency and surgery, with a focus on lower limb arthroplasty. Section 1.1 will explore the role of iron and iron deficiency; it will provide background information and provide context and place for the proposed research. In section 1.2, non-anaemic iron deficiency, its effect clinically, psychologically and sociologically on surgical and non-surgical patient populations will be investigated, with exploration of the relevant literature, forming a rationale for the proposed thesis. The focus on lower limb arthroplasty will be discussed, explained and justified. Patient reported outcome measures will be discussed, with differences in their measurement clinically, psychologically and sociologically from the clinical measures explored, justifying their role in this research. Aims and objectives will be identified and explained in section 1.2, with an outline of the structure and content of the thesis provided in section 1.3.

1.1 Research rationale

1.1.1 Background

Iron is an integral element in many bodily processes, including synthesis of haemoglobin and myoglobin (McDermid and Lönnerdal, 2012), it is essential to optimise highly metabolic cellular processes involving skeletal cells or cardiomyocytes (Anand and Gupta, 2018). Iron is absorbed as ferrous from diet or supplementation and regulated through homeostatic mechanisms to maintain an optimal level of iron (McDermid and Lönnerdal, 2012; Wessling-Resnick, 2017). The majority of iron is stored within the globin proteins, facilitating oxygen transport (Knovich et al., 2009). Like many nutrients the body utilises, the body requires an

optimum amount, too much or too little, leads to less ideal functioning (McDermid and Lönnerdal, 2012). Excess dietary iron is regulated through a number of mechanisms, including the hormone hepcidin, which limits entry of iron into the plasma (Britton et al., 1994; Camaschella, 2019), to maintain homeostasis and reduce the risks of iron toxicity, associated hepatic disease, diabetes and cardiac disease (Wessling-Resnick, 2017). Some genetic disorders, such as, haemochromatosis, make control of bodily iron more challenging and some can lead to excess iron or deficiencies in iron, require regular monitoring to reduce the risk of associated complications (Wessling-Resnick, 2017). Iron deficiency however can lead to a number of clinical issues, which will be elaborated in the following section.

1.1.2 Iron deficiency

Iron deficiency is described as a lack of overall iron stores within the body, to maintain optimal functioning (Camaschella, 2019). Iron deficiency can be present due to a variety of causes, including insufficient iron consumption, reduced absorption, chronic inflammation, blood loss or increased bodily demand (Al-Naseem et al., 2021b; Warner and Kamran, 2022). Insufficient dietary intake, blood loss and increased bodily demand are self-explanatory; not enough iron is being consumed, iron is lost through explained or unexplained blood loss, or increased demand due to exercise, such as in athletes haemoglobin can decrease, given aerobic exercise expands plasma volume, reducing the concentration of haemoglobin, leading to a dilutional anaemia (Al-Naseem et al., 2021b). However, the mechanism of other causes is more complex. Iron absorption occurs primarily in the small intestine and is influenced by medication, levels of stomach acid and bariatric surgery (Al-Naseem et al., 2021b). Chronic inflammation, for example, inflammatory bowel disease, increases the body's production of hepcidin, which reduces iron absorption. The causal condition is usually explored before treating iron deficiency, lack of nutrition and gastrointestinal bleeding are common causes,

the latter requiring further investigation to rule out malignancy (Warner and Kamran, 2022). However, in a third of cases explored for gastrointestinal causes, no source can be found (Warner and Kamran, 2022) which make decisions on possible treatment of the iron deficiency more complex, as the underlying cause is unknown. Symptoms of iron deficiency may vary depending on age, cause, severity and activity of the individual, they may include fatigue, shortness of breath, worsening heart failure or developmental, or cognitive delay in children (Camaschella, 2019; Warner and Kamran, 2022). Although the effect of iron on haemoglobin is well known, recent studies have also shown an impact on cellular respiration, with randomised controlled trials (RCTs) demonstrating the negative impact of iron deficiency on cellular metabolism (Hoes et al., 2018; Melenovsky et al., 2017). Lack of iron stores reduces the ability to synthesise haemoglobin, leading to diminished levels within the blood stream (Camaschella, 2019).

Iron deficiency is the most common cause of anaemia, a treatable condition defined as 'a deficiency of red cells or of haemoglobin in the blood' (Oxford Dictionary, 2016). Anaemia is described as a haemoglobin less than 120g/l in women or 130g/l in men (World Health Organisation, 2011). Complications associated with persistent, untreated anaemia include heart conditions, infections, depression, delayed development in children and complications during pregnancy (Warner and Kamran, 2022). Several systematic reviews on diagnosis, guidelines and treatment of anaemia have been performed, in a variety of patient settings, including gastrointestinal bleeding (Cotter et al., 2020), heart disease (Kansagara et al., 2011), and across multiple patient groups (De Franceschi et al., 2017). Oral supplementation of iron (Peyrin-Biroulet et al., 2015) and intravenous infusion are both advocated (De Franceschi et al., 2017) depending on the severity of the anaemia, the cause and the frailty of the patient (De Franceschi et al., 2017). The consensus of opinion from several systematic reviews

demonstrated benefits to patients of improved haemoglobin (Abdelsalam et al., 2021), and quality of life (Kansagara et al., 2011; Ross et al., 2003), through treatment and management of their condition.

1.1.3 Iron deficiency and surgery

Pre-operative anaemia is associated with increased post-operative morbidity and mortality as well as increased red blood cell transfusion rates, hospital readmission and length of stay (Wilson et al., 2008; Spahn, 2010; Saleh et al., 2007; Pujol-Nicolas et al., 2017; Muñoz et al., 2015; Kotze et al., 2012; Khan et al., 2012; Jans et al., 2014). Treatment of iron deficiency has been shown in systematic reviews of RCTs to improve haemoglobin and clinical outcomes in both the surgical and non-surgical population, with an associated improvement/reduction in the symptoms of iron deficiency anaemia (Elhenawy et al., 2021; Kansagara et al., 2011). As such, many hospitals have implemented successful screening and treatment processes (Banerjee and McCormack, 2019), with demonstrable benefits, for example rates of transfusion were significantly reduced.

Elhenawy et al. (2021), conducted a systematic review of 10 RCTs, which met their inclusion criteria preoperative iron administration, in patients undergoing surgery, demonstrating a significant increase in haemoglobin with treatment (7.15 g/L, 95% CI: 2.26, 12.04, $p = 0.004$), with an increase postoperatively also demonstrated (6.46 g/L, 95% CI: 3.10, 9.81, $p = 0.0002$). In a single centre study by Pujol-Nicolas et al. (2017) analysing the implementation of a screening and treatment program, in a lower limb arthroplasty population, demonstrated the number of transfusions was significantly reduced (108 vs. 63 [4.1%], $p = 0.005$, readmission rate decreased (81 [4.5%] vs. 48 [2.3%], $p= 0.020$) and critical care admission was also reduced (23 [1.3%] vs. 9 [0.5%], $p = 0.030$). Length of stay,

in the same patient population (LOS) was significantly reduced from 3.9 days to 3.6 days (p = 0.017). The cost saving for the cohort was £263,000 (Pujol-Nicolas et al., 2017). Although a single, before and after, centre study, the significant results and associated potential cost savings, suggest treatment may be beneficial. However, as a before and after study other explanations are available for the observed effect, such as regression to the mean, or temporal effects. In contrast, a recent randomised controlled in abdominal surgery trial suggested, no difference in risk of death (risk ratio 1·03, 95% CI 0·78–1·37; p=0·84), or transfusion rates in (rate ratio 0·98, 95% CI 0·68–1·43; p=0·93), patients who were randomised to treatment of iron deficiency preoperatively and concluded supplementation was not recommended (Richards et al., 2020b). However, Keegan et al. (2021) disagreed with the authors interpretation and whilst acknowledging their findings, suggested improved haemoglobin prior to surgery (4·7 g/L, 95% CI 2·7–6·8), demonstrated the treatment did have an effect. They also suggested a lack of differences in transfusion rate could be due to lack of consistency in transfusion thresholds and practice across multiple study centres, which may have affected the result. Supplementation on balance across the studies demonstrated measurable benefits.

1.1.4 Blood transfusion

Iron deficiency prior to surgery has been shown to increase the risk of blood transfusion. Blood transfusion is not a benign process, there are small but inherent risks of a transfusion reaction (Clevenger and Kelleher, 2014) and transfusion-related acute lung injury (Kilic and Whitman, 2014). It has also been associated with increased rates of postoperative infection (Kilic and Whitman, 2014), post-operative complications and increased length of stay (Pujol-Nicolas et al., 2017; Bower et al., 2010). The 2015 National Comparative Audit of Blood Transfusion was performed in 190 hospitals and concluded that hospitals should have a

preoperative management protocol, due to the wide variation in practice and management of blood transfusion across each hospital and across the cohort with the benefits demonstrating that when management of anaemia was performed preoperatively, patient outcomes improved (Allard et al., 2015; Klein, 2015). The audit concluded there is a need to increase the investigation and management of preoperative anaemia and iron deficiency in the UK, stating improvement in practice may help ensure the appropriate use of transfusion and alternatives, which would benefit patients and reduce healthcare costs (Allard et al., 2015; Klein, 2015).

The prevalence of blood transfusion in unselected patients undergoing lower limb arthroplasty has been reported as between 21% and 70%, although most of the studies report figures in the middle of the range (Browne et al., 2013; Rosencher et al., 2003; Vuille-Lessard et al., 2010). Joint replacement surgery is estimated to utilise approximately 10% of the blood supply available in the UK (Wells et al., 2002). Within a large NHS trust in England, unpublished retrospective data has shown 20% of anaemic patients receive blood transfusions, with an overall transfusion rate of approximately 5% (Khan et al., 2012). In an observational study Carling et al. (2015), reviewed 114 patients undergoing hip replacements and 79 undergoing knee replacements, demonstrating a transfusion rate overall of 16% (18% in total hip replacements and 11% in total knee replacements), preoperative haemoglobin concentration was a predictor blood transfusion in total hip and total knee replacement patients. Transfusion rates comparable in emergency general surgery 4.8% (Medvecz et al., 2020), with cardiac surgery accounting for 2.5 million or 20% of the blood transfusions in America. The National Institute for Clinical Excellence (NICE, 2015) , recommends a transfusion threshold 70gl, however differences in transfusion thresholds between institutions, both in the United Kingdom and internationally has been identified (De Franceschi et al.,

2017; Kilic and Whitman, 2014; Speiss, 2002), and may account for some of the differences in transfusion practice.

Whilst acknowledging the clinical evidence for the need to reduce blood transfusion, there are also significant potential economic benefits. Blood transfusion costs approximately £170 per unit (NICE, 2015). If iron supplementation were effective at reducing transfusions it has the potential to produce significant monetary savings, giving significant cost improvement to a National Health Service Trust of £263,000, up to £500,000 per annum depending on transfusion rate (NICE, 2015; Pujol-Nicolas et al., 2017). This is driven by the fact that iron supplementation has an estimated cost of £1.51 per person for a standard daily treatment dosage of 200mg (NICE, 2022) and has been shown to reduce transfusion rates (Pujol-Nicolas et al., 2017).When this cost improvement is added to the reduction in post-operative complications associated with blood transfusion, this demonstrates a significant potential service improvement.

1.2 Non-anaemic iron deficiency

The prevalence of iron deficiency and the potential risks and benefits of treatment have been demonstrated among the anaemic population. In this section, non-anaemic iron deficiency, its impact and treatment in the non-surgical and surgical patient populations will be explored, providing justification, context and placing the proposed research within the established knowledge base.

Non-anaemic iron deficiency has been described in a meta-analysis, as a related, but newly emerging disease process, worthy as a topic for exploration and research (Pratt and Khan, 2015). It is defined by a low ferritin (iron store) in the presence of normal haemoglobin

(Pratt and Khan, 2015). Ferritin is an iron storage protein which is found in both intracellular and extracellular compartments (Knovich et al., 2009). Iron binds to the ferritin protein storing the iron and buffering cellular level iron to maintain homeostasis (Knovich et al., 2009). It is used to measure the amount of bodily iron stored (Knovich et al., 2009). The normal ferritin range is 24-300 mcg/l, although it may slightly differ between laboratories (Koperdanova and Cullis, 2015). The ferritin level at which non-anaemic iron deficiency is identified in the general population is less than 15mcg/l (Al-Naseem et al., 2021b), with a suggested treatment level of 30ngl (Camaschella, 2015). However, while a higher serum ferritin may still indicate non-anaemic iron deficiency, the normal level for an individual differs, with patient history and clinical symptoms playing an important role in diagnosing an overall iron deficiency (NICE, 2021). The etiology and pathophysiology and symptomology of non-anaemic iron deficiency are equivalent to anaemic iron deficiency, requiring the same investigational considerations. However, the iron stores have not fallen sufficiently to affect the haemoglobin level, but it is possible that many, without treatment, may go on to develop anaemia (Al-Naseem et al., 2021b). It has been suggested, in non-anaemic iron deficient patients undergoing a surgical procedure, the threshold for treatment could be a ferritin below 50 to 100mcg/l (Munoz et al., 2016; Verdon et al., 2003). Symptoms of non-anaemic iron deficiency are thought similarly vague to anaemia, with fatigue, shortness of breath and reduced exercise tolerance (Al-Naseem et al., 2021b). It is unclear if cellular level decreases in functioning would be affected in less severe iron deficiency, with the symptoms thought to be difficult to differentiate, but likely to be less severe than in anaemic iron deficiency (Al-Naseem et al., 2021b).

Clinical implications of non-anaemic iron deficiency are also thought to be similar, with heart conditions, infections and depression, identified as associated complications (Al-Naseem et

al., 2021b; Warner and Kamran, 2022), therefore the treatment iron deficiency, may also benefit patients with non-anaemic iron deficiency.

Philip et al. (2020), demonstrated clinically an increase in mortality over a 14-year measuring period (1·58 95% CI 1·29-1·93), comparing an older adult non-anaemic iron deficient population (n=389) against a control of non-iron deficient people (n=4062) aged 50 and over, incorporating males and females in an even split, although more females were non-anaemic iron deficient (65%). They suggested it was a common phenomenon, which is detrimental to those with the condition, and is a population group which is comparable to the older surgical population.

Houston et al. (2018), conducted a systematic review of RCTs on treatment of non-anaemic iron deficiency incorporating all adults over 18, supplementation demonstrated a reduction in patient reported outcome of self-reported fatigue (-0.38; 95% CI −0.52 to −0.23; I2 0%; 4 trials; 714 participants), clinically, significant improvement in haemoglobin (4.01 g/L; 95% CI 1.22 to 6.81; I2 48%; 12 trials; 298 participants), however, it was not associated with improved physical capacity (0.11; 95% CI -0.15 to 0.37; I2 0%; 9 trials; 235 participants). Houston et al. (2018), demonstrated iron supplementation clinically improved haemoglobin concentration (4.01 g/L; 95% CI 1.22 to 6.81; I^2 48%; 12 trials; 298 participants), ferritin (9.23 μmol/L; 95% CI 6.48 to 11.97; I^2 58%; 14 trials; 616 participants), and was linked with reduction in the patient reported outcome of self-reported fatigue (-0.38; 95% CI -0.52 to -0.23; I^2 0%; 4 trials; 714 participants), however, it was not associated with differences in maximal oxygen consumption (0.11; 95% CI -0.15 to 0.37; I^2 0%; 9 trials; 235 participants). They acknowledged 15 of the 18 studies reviewed featured solely women, with an age range

across the studies from 18-50 (Houston et al., 2018) and therefore this lack of heterogeneity makes it difficult to reliably extrapolate a benefit to the older surgical population.

Miles et al. (2019b), reviewed non-anaemic iron deficient adults receiving intravenous iron therapy and demonstrated a clinical benefit, with a small increase in haemoglobin (3.04 g/L, 95% CI 0.65 to 5.42; I2 = 42%; 8 studies, 548 participants). The result was not clinically significant, and they deemed it to be low quality evidence, due to differences in reporting and measures used to identify and treat non-anaemic iron deficiency, as this varied greatly across the studies. Improvement of quality-of-life scores, reflecting patient focused outcomes, were demonstrated (Piper Fatigue Scale 0.73, 95% CI 0.29 to 1.18; I2 = 0%; studies = 3), and peak oxygen consumption improved (MD 2.77 mL/kg/min, 95% CI −0.89 to 6.43; I2 = 36%; 2 studies, 32 participants). Heterogeneity was demonstrated in the sample, study areas included heart failure, athletes, pre-menopausal women and restless leg syndrome, demonstrating generalisability across patient groups.

Clinical and patient reported outcomes have been shown to improve with supplementation. Although, issues with heterogeneity must be acknowledged, the body of evidence suggests treatment of non-anaemic iron deficiency may be beneficial to patients in a similar age bracket to the arthroplasty population.

1.2.1 Non-anaemic iron deficiency and surgery

The background literature has established the role of iron, the impact and benefit of treatment of iron deficiency in the surgical and non-surgical population. Non-anaemic iron deficiency has been identified as a treatable phenomenon in the non-surgical population. The aim of this

section is to identify and analyse the impact of non-anaemic iron deficiency in the surgical patient population

When researching non-anaemic iron deficiency in the surgical population, a newly emerging area (Pratt and Khan, 2015), it was evident there was not an enormous volume of literature. Although some literature recommend treatment of non-anaemic iron deficiency for patients undergoing surgery (Pratt and Khan, 2015; Munoz et al., 2016), with associated increases in serum ferritin and transferrin saturation (Munoz et al., 2016), and reduction in allogenic blood transfusion (Cuenca et al., 2007), they were small in number and in participants, with no systematic review on the topic previously performed. The most recent international consensus statement suggesting the need to treat preoperative anaemia was published in 2016 (Munoz et al., 2016) , it has not currently been further updated.. A workshop attended by experts within the field of perioperative anaemia management, was gathered to review the available evidence and provide recommendations for clinical practice (Muñoz et al., 2018). In their statement, they provided an expert opinion based on the evidence reviewed in the workshop, hence it was not a formal registered systematic review, more a published report based on the outcomes of the discussions and the expert opinion gleaned. Anaemic and non-anaemic iron deficient patients were identified as patient groups that would benefit from the introduction of preoperative anaemia screening, assessment and treatment. They suggested patients with low iron levels, defined as low ferritin, with or without anaemia may benefit from supplementation, to enable them to recover from surgery.

Munoz et al. (2016) identified the presence of non-anaemic iron deficiency using the meta-analysis by (Pratt and Khan, 2016), who defined non-anaemic iron deficiency as a new disease in the non-surgical population, worthy of further research in its own right. Analysis of

their systematic review on establishing non-anaemic iron deficiency, its impact on outcomes and the effect of treatment, demonstrated improvements in outcomes in individual RCTs, such as birth weight (p0.028), not comparable to the surgical population and self-rating of fatigue (p0.03), which could be more relevant. Meta-analysis was severely limited and where performed, not statistically significant (Pratt and Khan, 2016). Munoz et al. (2016) advocated treatment based on two papers. Firstly, Cuenca et al. (2007) in a before and after implementation of treatment study, who demonstrated an improvement in transfusion in the non-anaemic patient (2.4% vs. 26.1% for Hb ≥130 g/L (chi-squared = 28.9, $p < 0.001$)), which was statistically significant, and an increase in transferrin saturation. However, patients were not screened for iron deficiency prior to surgery. Therefore, it is difficult to reliably assess the impact of this research on non-anaemic iron deficiency, as a cohort analysis measuring the impact of an intervention against retrospective data, analysis and subsequent conclusion also have an inherent risk of selection bias (Friedman et al., 2015). The second paper a prospective observational cohort study of patients undergoing lower limb arthroplasty, Lachance et al. (2011), demonstrated an improvement in the ferritin (25.8 [38.6] ng/mL, $p < 0.001$), with supplementation, however there was insufficient separation for non-anaemic iron deficiency in the reported data. Although Munoz et al. (2016), recommend treatment of non-anaemic iron deficiency prior to surgery, this is primarily an expert consensus opinion, based on evidence from Pratt and Khan (2016), in the non-surgical patient population, with insufficient data explored to show benefits in the surgical population.

Several small studies have reported patient improvements in clinical outcomes, when treating non-anaemic iron deficient surgical patients, demonstrating improvements in postoperative haemoglobin (D'Amato et al., 2017; Spahn et al., 2019b), ferritin (Na et al., 2011), and length of stay (Sanchez et al., 2015). It must be acknowledged the numbers included are relatively

small, incorporating differing patient cohorts, types of surgery, approaches to treatment and control across the research papers, with differing measured endpoints, which makes comparability and reliability more difficult. However, it does support the notion that iron treatment may be beneficial, which when added to the body of evidence identified in the non-anaemic non-surgical patient and the anaemic surgical population, enhances reliability. All authors focused on iron deficiency initially, using subgroup analysis for non-anaemic iron deficiency, demonstrating a lack of studies solely of non-anaemic iron deficiency. The lack of published research focusing solely on non-anaemic iron deficiency, demonstrates a gap in the literature, which this thesis aims to explore.

1.2.2 Treatment of anaemia and non-anaemic iron deficiency

When reviewing the treatment of non-anaemic iron deficiency and surgery, it was clear there was a lack of national guidance on treatment of this newly emerging field. Therefore, guidance for treatment options was sought from the related field of anaemia and surgery. A systematic review of the treatment of non-anaemic iron deficiency and surgery proposed as a part of this thesis.

The National Institute for Clinical Excellence (NICE, 2020), produced guidance on the management of perioperative anaemia derived from reviews of clinical trials. They acknowledged treatment with oral or intravenous iron may be beneficial for patients, with the significantly greater cost of intravenous iron, needing to be weighed against the reduced cost of oral iron, as little clinical benefit was identified for one delivery route over the other. Numerous preparations of intravenous iron with or without erythropoietin, have been successfully utilised in clinical practice (Elhenawy et al., 2021; Keegan et al., 2021), with little commonality in approach. As discussed previously, although transfusion improvements

were not seen in a large RCT of intravenous iron vs placebo (Richards et al., 2020b), improvements in postoperative haemoglobin (Keegan et al., 2021), demonstrating a benefit of treatment. Common side effects of intravenous iron include, dizziness, headache, hypertension, GI upset, with uncommon side effects including blurred vision, anaphylaxis and vomiting (BNF, 2020). Oral iron has been demonstrated to be an efficient, cost-effective alternative to intravenous iron therapy (NICE, 2020) in the surgical patient population. It is subject to a similar side effect profile to intravenous iron, primarily GI upset, sickness and constipation (NICE, 2020).

The anaemia guidance published by NICE (2020) suggested smaller doses or alternative day oral iron (Düzen Oflas et al., 2020) may be beneficial, giving a similar result with improved compliance and reduced side effects (Düzen Oflas et al., 2020; NICE, 2020). Recent publications have suggested lower dose iron may be more effective and have less side effects than traditional treatment (Moretti et al., 2017) whilst achieving the same benefits. As a small-scale clinical trial with limited resources, it was decided that oral iron would be used in the clinical trial, primarily due to cost and the lack of evidence that intravenous iron is greater, provided the patients have sufficient time prior to surgery to begin the oral iron supplementation. Therefore, a lower dose iron food supplementation Floradix, was chosen to attempt to improve compliance and reduce side effects for the research population, whilst, receiving effective treatment.

1.2.3 Methodological decisions and placebo control.

The design of the RCT in Chapter 4, involved supplementation with oral iron measured against a control group of patients with no intervention. Whilst it is common to perform trials in this manner (Cheah et al., 2018), the potential benefits and limitations must be

acknowledged. For RCTs to produce reliable results, the choice and design of the control group is extremely important, to ensure both groups are comparable (Cheah et al., 2018). Proponents of placebo-controlled trials, see advantages in truly blinding patients, assessors and clinicians, utilizing the placebo to produce a reliable result, with a reduced risk of bias (Cheah et al., 2018; Hohenschurz-Schmidt et al., 2023). Placebo control reduces the risk of patients opinions and experiences being influenced by knowing whether or not they are receiving the intervention (Hohenschurz-Schmidt et al., 2023). Cheah et al. (2018) acknowledge the benefits of placebo controlled RCTs, however, they note considerable ethical debate on when it is appropriate to use placebo control, following controversial research on the Human Immunodeficiency Virus (HIV) in the 1990s. The Declaration of Helsinki, advises placebo control can be used when scientific justification can be demonstrated for the methodological approach (World Medical Association., 2008). Millum and Grady (2013) suggest four domains must be addressed to ensure a placebo control is the correct ethical approach:

1. No proven effective intervention for condition under study.
2. No or negligible harms from delaying or forgoing treatment.
3. Compelling methodological reasons for use of placebo; and Participants are not at risk of excessive harm.
4. Compelling methodological reasons for use of placebo; and Participants are not deprived of interventions they would otherwise receive.

The RCT in Chapter 4 on the effects of supplementation in patients undergoing lower limb arthroplasty, would meet the guidance to be appropriate for inclusion as a placebo-controlled trial. It was a new area, with no proven effective treatment, negligible known harms from

forgoing treatment, as the effectiveness of the treatment has not been ascertained, with the condition not normally treated. However, the major limiting factor in the decision to not utilize a placebo control was primarily due to the associated increased costs. As a newly emerging topic, it was decided the body of evidence could be improved without the need for a placebo control, the primary outcome measured a haematological factor, postoperative haemoglobin at three weeks, which was unlikely to be affected by the lack of placebo control. However, it must be acknowledged the lack of placebo control meant participants were told they may have a non-anaemic iron deficiency and potentially receive no treatment, as the trial was ascertaining if there was any potential benefit to supplementation. Although the patients were asked not to take any supplementation outside of the trial, it's impossible to ensure that the lack of placebo control did not alter patients' behavior or opinions depending on their randomization group, which is acknowledged as a potential risk and discussed further in Chapter 4.

1.2.4 Patient reported outcomes.

Patient reported outcomes are tools that assess from the patient's point of view, daily function health outcomes and quality of life (Quittner et al., 2019). They are generally more important measures of patient overall experience, which have been shown to improve the rigor of research, enhance economic value and improve patient outcomes (McGee, 2020). The aim of patient reported outcome measures are to assess physical, social, and emotional well-being associated with an illness or its treatment (Cella et al., 2015). Five main categories have been identified to measure performance. Quality of life measures are multidimensional questionnaires, incorporating physical, social, and emotional aspects to understand the impact of a treatment or illness (Cella et al., 2015). Functional status questionnaires, measure physical or cognitive function of a specific disease or problem, but does not look at the wider

patient impact (Quittner et al., 2019). Symptoms or symptom burden questionnaires utilize scales to identify the severity of a problem (Cella et al., 2015). Health behaviors questionnaires, often reported in other patient reported outcome measures, can be used as an identifier in the assessment of risk of disease (Cella et al., 2015). Finally patient experience of care questionnaires, incorporate patient satisfaction, patient motivation and activation, reporting their perception of their experiences (Cella et al., 2015).

Patient reported outcomes measure patient experience and aim to identify care from a patient's perspective, with regards to functionality or quality of life (Cella et al., 2015) and so differ from more objective 'clinical' outcomes such as mortality or infection (Krumholz et al., 2013), The question for patient reported outcomes becomes, what is recovery from a patient's perspective? These may involve a 'trade-off' between say enhanced mobility due to reduced pain from a pharmaceutical treatment with increased tiredness from the drug. Traditionally the focus is on medical recovery, however increasingly the social and psychological impact of recovery are becoming more important (Auais et al., 2019). From a clinical perspective, in the context of surgery, recovery is the point at which the problem or procedure has been successfully resolved, through surgery and or physiotherapy. Clinically a knee replacement may be judged to be successful if the arthritic joint has been replaced and pain has been reduced. The clinical approach may not capture extra benefits, such as a reduction in depression due to pain removal or an increase in social activities due to improved mobility. However, the overall impact that joint replacement (or other surgical procedures) has on a patient can be assessed clinically, psychologically, and sociologically. However, there are important psychological and sociological influences on whether surgery makes an impact on the patients quality of life and their recovery (Mavros et al., 2011). Quality of life issues such as pain and mobility impact recovery post-surgery (Ring, 2020). Recovery from a social

perspective involves an improvement in function, removal of some of the limitations the condition has imposed, and a return to function closer to the patients normal lifestyle (Auais et al., 2019). Psychologically patients previous experience, pain tolerances, sociological background, expectations and preoperative preparation, are all known to have an impact on recovery from surgery (Levett and Grimmett, 2019). This multi-focal approach demonstrates the benefit of quality of life measurements, which have been demonstrated to add context and enhance the robustness of clinical studies (McGee, 2020), justifying the use of quality-of-life studies within this research project.

Self-reported patient outcomes can be split into three main groups, generic quality of life, health utility and condition or disease specific (Meadows, 2011). Generic quality of life, for example, the short-form series (SF 36, SF 20 and SF 12), are used to identify the impact health has on everyday life (Meadows, 2011). The impact of health is measured across eight domains: limitations in physical activities, social activities, usual roles or activities, bodily pain, general mental health, emotional problems and fatigue (Crispin Jenkinson, 1997). Quality-of-life measures aid planning and measurement of the impact of clinical and social interventions (Burholt and Nash, 2011). Health related utility is a measure of health related quality-of-life, whereby patients attach a preferred value to their overall health status (Bakker and van der Linden, 1995). EQ-5D-5L is an example of health utility measurement, a five-domain survey measuring mobility, self-care, usual activities, pain/discomfort, and anxiety/depression, against a preset value which yields an index score anchored at 0 (dead) and 1 (full health) (McClure et al., 2017). Condition specific questionnaires aim to assess the impact of a specific condition on health (Meadows, 2011). For example, in anaemia management, Acaster et al. (2015), validated the use of FACIT-fatigue, a 13-item measure of

the impact of anaemia and daily functions, demonstrating it as an effective measure of functional assessment of the symptoms of anaemia.

Staibano et al. (2020) performed a systematic review of the use patient reported outcome measures in anaemia treatment for renal disease and found a lack of commonality in usage and reporting of patient reported outcome measures. Although SF36 was most the commonly used quality of life measure, there was great variation in the patient reported outcome measures and patient centered measures analysed in the systematic review. In their systematic review, Staibano et al. (2020) suggested greater use of condition specific patient reported outcome measures. The challenge with so many different patient reported outcome measure tools, is choosing the correct tool for the topic being studied (Churruca et al., 2021) and ensuring patient centered outcomes used are condition specific (Staibano et al., 2020).

There was no common patient reported outcome measures for non-anaemic iron deficiency and surgery identified, due to its emergence as a new area of study. In the related topic of anaemia after surgery, studies have utilised a variety of quality-of-life measurements to identify improvements in an anaemia after surgery (Ng et al., 2019a). Conlon et al. (2008), utilised the validated short form (SF) 36, to assess quality of life in patients undergoing hip arthroplasty. Keeler et al. (2019) chose EQ-5D-5L, to assess health utility in patients undergoing surgery for colorectal cancer. In their systematic review, Ng et al. (2019a), demonstrated no common approach to measuring quality of life across the surgical populations, with quality-of-life measures often not reported in the publications (Ng et al., 2019a).

Although a number of options were available to assess patient reported outcomes, for this thesis, I have chosen to use EQ-5D-5L as a patient reported outcome measure, due to its greater sensitivity as a measurement of quality-of-life index (Greene et al., 2015; Herdman et al., 2011). This is also partially due to EQ-5D-5L being present in the retrospective data to be analysed, therefore the decision was made to utilise the same measure in the RCT. A patient centered outcome measure, the FACIT-fatigue scale, will also be used in the prospective RCT, due to its validation as a disease specific functional assessment tool in the associated topic of anemia (Acaster et al., 2015).

1.2.5 Justification for the focus on lower-limb arthroplasty

The decision to focus on lower limb arthroplasty was taken for several reasons, incorporating clinical and logistical aspects, such as, prevalence of arthroplasty, lack of published research, access to patient outcome data, support for the research topic and time/researcher limitations which will be explained further and justified. The lack of guidance identified in the non-anaemic iron deficient surgical population, demonstrated the need to perform quality research to assess its impact and the impact of treatment.

As previously identified, there is a distinct lack of existing evidence on non-anaemic iron deficiency and surgery its impact on surgical outcomes and the effect of treatment. Where recommendations have been made (Munoz et al., 2016), a lack of evidence and quality in the surgical population has been identified. This demonstrates the need to conduct further research to ascertain the prevalence and treatment effect of non-anaemic iron deficiency in the surgical population. The prevalence of joint replacement within the UK is continually increasing due to an ageing population (Birrell et al., 1999), with 202,309 cases recorded for 2018/19 (National Joint Registry Editorial Board, 2019), the last year of data available which

is not affected by COVID. Therefore, potential improvement in patient outcomes in the non-anaemic iron deficient lower-limb arthroplasty population, as demonstrated in the anaemic arthroplasty population (Pujol-Nicolas et al., 2017) could be significant. Treatment of anaemia in the same patient population has demonstrated significant potential economic benefits (Pujol-Nicolas et al., 2017), allowing more appropriate use of resources. Further investigation would demonstrate if comparative improvements could produce substantial replicable benefits in the non-anaemic arthroplasty population.

Working in a large NHS Foundation Trust with an active orthopaedic research department, the decision to focus on lower limb arthroplasty was taken primarily due to access to a large volume of patient outcome data, which incorporated both clinical and patient reported outcomes. Analysis of this data would enable a thorough review of clinical and patient outcomes, analysed for the prevalence/impact of non-anaemic iron deficiency on the defined patient population. In our orthopaedic department, screening, assessment and treatment of anaemia was already being applied within the orthopaedic lower limb surgical population, with benefits of monitoring and intervention already demonstrated in the anaemic lower limb arthroplasty population (Pujol-Nicolas et al., 2017; Wilson et al., 2008; Spahn, 2010; Saleh et al., 2007; Muñoz et al., 2015; Kotze et al., 2012; Khan et al., 2012; Jans et al., 2014). Lack of engagement is a common problem in medical research (Gough et al., 2017), especially with newly emerging areas with a lack of published evidence, this can lead to a reduction in confidence and participation on a research project (Friedman et al., 2015). However, an active appetite prevailed within the speciality, to assess if similar differences in outcomes were demonstrated in the non-anaemic iron deficient population and whether treatment could affect patient outcomes, as demonstrated in the anaemic lower limb arthroplasty population.

Friedman et al. (2015), suggests decisions when choosing the scope of a research project are often complex, multifactorial and may require focusing on a specific area or subject. Although non-anaemic iron deficiency would be likely to have similar affects across a range of surgical interventions, to identify the prevalence and impact, with access to a large volume of lower limb arthroplasty patient outcome data, it seemed sensible to focus on this area for the research topic. This would enable identification of the scope of the problem within a defined patient population, where treatment could then be measured with generalisability to the wider surgical population. It is commonplace for researchers to focus on a speciality, to identify the prevalence and effectiveness of an intervention as the comparators are more easily controllable, with less variability between procedures such as blood loss, outcome measurements and complication rates (Friedman et al., 2015; Gough et al., 2017), which can make comparison across specialities more difficult (Friedman et al., 2015).

Finally, time and researcher constraints were further reasons to focus on lower limb arthroplasty. The primary researcher was a novice researcher, undertaking the research as a PhD project, with time limitations and limited research acumen or experience. Researchers must be aware of their limitations and use the wider research community to achieve reliable results (Friedman et al., 2015). Time and funding constraints often influence the scope of a project, with difficult practical decisions being made to successfully design and complete a research project (Friedman et al., 2015). Deciding to conduct this research in one speciality, with an active supportive research department, rather than across multiple specialities or centres, would make the workload and management of the project achievable.

Clinical and economic factors, with their effect on clinical services and service improvements have been explored. Potential benefits of supplementation in the non-anaemic patient

population have been explored and justified, including reduced cost and potential risk reduction with possible patient benefits. However, initial searches of the literature have demonstrated a lack of published research, a thorough examination of the relevant literature is required to address this potential gap in the research evidence, leading to an original contribution to the literature.

1.2.6 Summary

Upon reflection and exploration of the published literature, a distinct gap in the evidence was demonstrated relating to the prevalence and impact of treatment of non-anaemic iron deficiency. However, a lack of evidence does not suggest that treatment may not be beneficial, it simply means more research is required to assess the prevalence, impact and effectiveness of treatment. Correction of non-anaemic iron deficiency is not routinely performed in most hospitals prior to major surgery, although it is considered best practice (Muñoz et al., 2015), despite a clear lack of evidence in the surgical population. If improvements demonstrated in the anaemic surgical population and non-anaemic iron deficient non-surgical populations could be replicated, implementation of this best practice could provide an opportunity to improve patient care. Review of the relevant literature, defined two potential gaps in the research:

1. The prevalence of non-anaemic iron deficiency in the surgical population, its impact on clinical and patient reported outcomes.
2. The impact and effectiveness of treatment of non-anaemic iron deficiency in the lower limb arthroplasty population.

1.3 Research methodologies proposed

It is important for healthcare professionals to continually improve their knowledge and skills to ensure they are providing appropriate evidence-based care (Glasziou, Irwig, Bain and Colditz, 2001). Egger, Smith and Altman (2001) suggest in many specialisms within healthcare, it has become difficult to read and critically analyse current professional knowledge and almost impossible to continually update that knowledge on a regular basis. Healthcare professionals are faced with a dizzying array of types of literature review, 14 differing approaches have been identified (Grant and Booth, 2009). Researchers must identify the most appropriate research design based on numerous factors, such as, research topic, data available, funding, duration and impact of the study (Thiese, 2014). Quality, robustness and generalisabilty must be assessed to ensure the study findings were rigorously undertaken and accurate (Egger et al., 2001; Glasziou et al., 2001).

Clinical studies can largely be split into two categories, interventional, whereby the researcher intervenes as part of the study design and observational, where the researcher is not intervening with study participants, but instead observing relationships between outcomes (Thiese, 2014). Interventional studies are designed to assess the impact of a treatment or intervention, utilising a defined study design, such as, comparing patients from before and after the implementation of an intervention, or an RCT (Torgerson and Torgerson, 2008). RCTs vary greatly in design and must be assessed individually for quality, bias and rigour (Torgerson and Torgerson, 2008). A well-designed RCT is seen as the gold standard of interventional study (Torgerson and Torgerson, 2008).

Observational studies are an important study design, they can address questions where randomised controlled trials are not ethical, appropriate to conduct or as in addition to

randomised controlled trials and may be the most appropriate method to answer some questions (Song and Chung, 2010). Well-designed observational studies can be retrospective or prospective in design, demonstrating comparable results to randomised controlled trials in some areas and are justifiable to answer some research questions (Song and Chung, 2010). Observational research is often used to address clinical questions where randomised controlled trial data is lacking, however, it can also make potential contributions as an adjunct when randomised controlled trials have been conducted (Boyko, 2013).

Gough, Oliver and Thomas (2017) demonstrate a tiered approach to design, with randomised controlled trials the highest standard, followed by prospective observational studies, retrospective observational studies, with case studies at the lower end of the standard. Prospective studies are suggested to be more rigorous due to their structured nature, incorporating the required data points in a planned manner, with randomised controlled trials the uppermost form, as it can reduce the risk of bias due to the nature or its design (Boland, Cherry and Dickson, 2014; Granholm, Alhazzani and Moller, 2019). However, researchers have little control over data collection, data can only be extracted from what has already been collected, researchers have no control over the variables, which may be subject to missing data and reporting bias (Thiese, 2014). Unlike in prospective studies where researchers choose which variables they require, to assess the impact of a treatment or intervention results.

Systematic reviews are designed to look at all the evidence on a chosen topic, summarise narratively or perform meta-analysis, depending on the results gleaned, to answer the proposed hypothesis. Systematic review methodology has been designed to be quantitative in nature, enabling data comparison and meta-analyses, to establish the effectiveness of a given

treatment or event (Glasziou, Irwig, Bain and Colditz, 2001) Systematic reviews and meta-analysis are therefore becoming increasingly essential tools for individuals to remain current with accumulating evidence in a field (Egger, Smith and Altman, 2001; Chalmers and Altman, 1995).

In this thesis, a retrospective cohort analysis, a systematic review and a prospective randomised controlled trial will be utilised, to thoroughly assess the impact of non-anaemic iron deficiency in the lower limb arthroplasty and wider surgical population. These elements were chosen after reviewing the methodologies available, as they were most appropriate methods to thoroughly research the identified gap in the literature.

1.4 Aims and objectives

The overall aim of this thesis is to explore the gaps in the research identified, investigating the relationship between postoperative outcome in non-anaemic iron deficient patients undergoing surgery with a focus on lower limb arthroplasty. The main research objectives are to:

1. Identify the prevalence of non-anaemic iron deficiency and its effect on patient outcomes, through retrospective cohort analysis of a patient population, who have undergone lower limb arthroplasty.
    - A retrospective cohort study, reviewing clinical and patient outcomes of patients with non-anaemic iron deficiency who have undergone lower limb arthroplasty in a single centre against a similar normal patient population. Analysing haemoglobin, ferritin, transfusion rate, length of stay, infection, morbidity, mortality and patient reported outcome measures.

2. Systematic review of the literature on the treatment of non-anaemic deficiency in the surgical population including the following,
    - Identification and analysis of randomised controlled trials and non-randomised controlled studies in patients with non-anaemic iron deficiency undergoing surgery.
    - Analysing monitoring and/or treatment of outcomes - haemoglobin, ferritin, transfusion rate, length of stay, infection, morbidity, mortality and patient reported outcome measures.
3. Explore the effect of treatment of non-anaemic iron deficiency on clinical and patient reported outcomes for lower limb arthroplasty will be identified using the following parameters: population, interventions or exposures, comparisons (or control groups), outcomes, setting and study design (PICOS)
    - Population - patients with non-anaemic iron deficiency undergoing lower limb arthroplasty
    - Interventions - treatment of non-anaemic iron deficiency in patients
    - Comparisons - no treatment (usual care)
    - Outcomes – haemoglobin at 3 weeks postoperative, ferritin, transfusion rate, length of stay, infection, morbidity, mortality, patient reported outcome measures
    - Setting - patients undergoing surgery in a single centre
    - Study Designs - randomised controlled trials and non-randomised controlled studies

1.5 Structure and content of the thesis

This thesis contains five chapters, of which the introduction is the first chapter. Chapter 2 will present the findings from a retrospective analysis of patient data, exploring how non-anaemic iron deficiency affects a number of patient outcomes, which will be identified in the chapter. Chapter 3 will detail a systematic review of the treatment of non-anaemic iron deficiency before surgery and its subsequent impact on patient outcomes and will highlight any knowledge gaps. The final empirical element, Chapter 4, will detail a randomised controlled trial assessing the impact of iron supplementation on haemoglobin at 3 weeks postoperative and a number of secondary outcomes, identified in the chapter. Finally in Chapter 5, the research and its findings are discussed in relation to the overall research aims. This includes a summary of the limitations and areas for further research and the potential implications for clinical practice.

CHAPTER 2: A RETROSPECTIVE COHORT STUDY, ANALYSING RETROSPECTIVE PATIENT DATA, COMPARING POSTOPERATIVE OUTCOME MEASURES, FOR NON-ANAEMIC IRON DEFICIENT PATIENTS UNDERGOING ARTHROPLASTY, AGAINST A CONTROL GROUP:

2.1 Introduction

As discussed in Chapter 1, there is evidence to suggest that patients with low iron levels (low ferritin) with anaemia may benefit from supplementation preoperatively to help them recover from surgery. However, there is less evidence for patients without anaemia and this Chapter focuses on the rationale, methods, and findings from a retrospective cohort study to explore this further. The Chapter then concludes with a discussion of the limitations and recommendations for future practice.

2.2.1 Rationale

Routine data is often used for observational studies due to the large swathes of data collected in clinical practice (Boyko, 2013). Observational studies can be quicker and cheaper to perform than randomised controlled trials, whilst selection bias is a potential problem, a robustly conducted observational study can provide a signpost to where a randomised controlled trial may be beneficial to support the observational study (Thiese, 2014; Boyko, 2013). Armstrong (2017) suggests one of the biggest research assets the NHS has is the access to data on millions of patients, advocating the development or anonymized data analysis tools to enable further exploration of this large data cache. Lawrence and Bradley (2018) agree with the benefits of exploring the NHS data cache and believe that artificial intelligence may improve data capture and exploration, however, they caution that public perception of this type of data sharing is mixed, suggesting positive examples should be

shared to improve patient perceptions. Access to a wealth of patient outcome data, which included haemoglobin and ferritin levels, which had not previously been investigated for this purpose, enabled comparison of outcomes between non-anaemic iron deficient patients undergoing lower limb arthroplasty against a haematinically normal patient population.

In Chapter 1, the concept of non-anaemic iron deficiency and the potential patient benefits of addressing this were defined. Initial searches of the literature however, found no large scale prospective or retrospective studies that looked solely at the outcomes of non-anaemic iron deficient patients, in comparison to the normal surgical patient population.

Working within an NHS trust with a large orthopaedic department, access to trust patient postoperative outcome measures for all arthroplasty patients was granted. More specifically, the focus was on primary arthroplasty, which would include total knee replacement and total hip replacement surgery. Hemiarthroplasty and revision arthroplasty were not included in the retrospective cohort analysis or the RCT in Chapter 4. The reason for excluding hemiarthroplasty was that they have a different patient profile with patients tending to be older, frailer and have multiple comorbidities (Gallardo-Calero et al., 2016) than the general lower limb arthroplasty population. Revision surgery was excluded due to the differences in outcomes, with greater risk of infection, length of stay and morbidity and mortality in revision surgery (Lenguerrand et al., 2018), when compared to primary lower limb arthroplasty.

Retrospective cohort analysis is known to be shaped by the data available to be analysed. However, researchers have little control over data collection, data can only be extracted from what has already been collected, researchers have no control over the variables, which may

be subject to missing data, reporting and selection biases (Thiese, 2014), unlike in prospective studies where researchers choose which variables they require, to assess the impact of a treatment or intervention results (Thiese, 2014). Selection of demographic, clinical and patient reported outcome measures was influenced by the data pool available, assessed with variables chosen based on the data previously collated.

2.2.2 Hypothesis

The null hypothesis suggests non-anaemic iron deficiency will produce no difference in postoperative clinical and non-clinical outcomes in patients undergoing lower limb arthroplasty, when compared against a haematinically normal control group.

2.3 Methodology

When presenting observational studies, to enable critique of the research undertaken, a defined structure has been proposed incorporating a clear presentation of the work undertaken with appropriate information, consistent appropriate reporting and acknowledgment of strengths and weaknesses in the study design (Cuschieri, 2019). Consequently, the reporting of this observational study has followed the STROBE guidelines. STROBE was a collaborative group of researchers, statisticians and other contributors who created a set of 22 points that should be covered in presenting an observational study to ensure all important methodological elements are covered in the publication to enable transparent and consistent reporting of such studies (Cuschieri, 2019). Therefore, this retrospective cohort study was designed and reported utilising the STROBE guideline for reporting cohort studies (Cuschieri, 2019) (see appendix 1). A full retrospective analysis data protocol is detailed in appendix 2.

2.3.1 Study design

The study design proposed was a retrospective observational 2-arm cohort study.

Group 1: non-anaemic iron deficient patients (defined as: haemoglobin over 120g/l females, 130g/l males with a ferritin less than 50mcg/l) undergoing primary elective hip or knee arthroplasty

Group 2: patients undergoing primary elective hip or knee arthroplasty without anaemia or non-anaemic iron deficiency.

2.3.2 Setting

Four hospital sites within a single NHS Foundation Trust based in Northern England.

2.3.3 Participants

Patients who had undergone primary lower limb joint replacement surgery, had routine bloods taken including full blood count (to test for haemoglobin), serum ferritin, urea and electrolytes, liver function and estimated Glomerular Filtration Rate (EGFR), haemoglobin and serum ferritin. This facilitated identification of non-anemic iron deficient patients and a similar control group of haematologically normal patients.

Inclusion criteria

For the non-anaemic iron deficient group, the inclusion criteria were as follows:

- Haemoglobin greater than 120g/l in females and 130g/l in males
- Ferritin less than 50mcg/l
- Undergoing primary hip or knee arthroplasty

For the control group, the inclusion criteria were as follows:

- Haemoglobin greater than 120g/l in females and 130g/l in males

- Ferritin 50mcg/l and higher
- Undergoing primary hip or knee arthroplasty

Exclusion criteria

- Haemoglobin less than 120g/l in females and 130g/l in males

2.3.4 Variables

The variables chosen prospectively for selection incorporated patient demographic data, comorbidities, pre and postoperative clinical and patient reported outcome measures. Patient demographic data and comorbidity data are important to collect to allow adjustment for any 'confounding' factors such as age, racial group or previous risk associated with comorbidities. They enable further analysis and understanding of any potential differences in the data groups analysed (Friedman et al., 2015), aiding assessment for unintended biases in the data pools compared (Friedman et al., 2015). It also aids in assessing the generalisability of study results, with further understanding of the cohort population, such as, gender, age or race, which may affect the interpretation and generalisability of study results (Glasziou et al., 2001). Clinical patient data was used to assess for inclusion and exclusion criteria and to assess the clinical impact of non-anaemic iron deficiency, in the arthroplasty population. Patient reported outcome data was used to assess the impact from the patient's perspective.

The patient reported outcome measurement commonly collected and present in the retrospective data was EQ-5D-5L, a general health index generic instrument, defining health by answering questions across 5 dimensions: mobility, self-care, usual activities, pain/discomfort, and anxiety/depression and a visual analogue score (Herdman et al., 2011). It was developed to be more sensitive than the previously used EQ-5D-3L, of which repeated

use had demonstrated a ceiling effect (Herdman et al., 2011), scoring from 1 to 5, rather than 1 to 3 across the 5 dimensions, leading to more separation in the data and greater sensitivity to health index measurement (Greene et al., 2015; Herdman et al., 2011). Jin et al. (2019) in a retrospective analysis and Greene et al. (2015) in a prospective analysis, assessed the performance and validity of EQ-5D-5L against EQ-5D-3L, for use in total hip and total knee arthroplasty. EQ-5D-5L reduced ceiling effects by up to 30% and was deemed to be more sensitive (Greene et al., 2015) and allowed greater differentiation of mobility in patients awaiting total hip and total knee arthroplasty, its use in this context was primarily due to it being available in the data collected, unfortunately only the visual analogue score was available for comparison.

The following data were extracted:

- Age at surgery
- Gender
- Smoking status
- Comorbidities
- Hypertension
- Atrial Fibrillation (AF)
- Ischaemic Heart Disease (IHD)
- Type I diabetes
- Type II diabetes
- Chronic Obstructive Pulmonary Disease (COPD)
- Thyroid disease
- Type of surgery
- Haemoglobin (pre- and post-surgery at day 1)

- Ferritin (pre- and post-surgery at day 1)
- EQ-5D-5L (90 days post-surgery)
- Length of stay
- Complications (during and after surgery including myocardial infarction (MI), transient ischaemic attack (TIA) or cerebrovascular accident (CVA), acute kidney injury (AKI) within 30 days of surgery and deep vein thrombosis (DVT) or pulmonary embolism (PE) within 60 days.
- Blood transfusion (up to 30 days)
- Readmission

2.3.5 Quality and Confidentiality

All data were stored on an NHS password protected server, with electronic files created to analyse the data kept on the same NHS server. I performed audit and quality control of the data, randomly sample checking the patient dataset against the trust blood results system to ensure data accuracy.

2.3.6 Statistical analysis plan

Analyses were conducted in Stata 17 statistical analysis package. Significance tests were two-sided at the 5% significance levels unless otherwise stated. Parameter estimates were presented with associated 95% confidence intervals and p-values as appropriate. The retrospective data analysis plan is documented in appendix 3.

2.3.6.1 Baseline data

Baseline data were summarised using descriptive statistics overall and as analysed in the primary analysis model. No formal comparisons were made between the groups.

2.3.6.2 Primary analysis

The primary analysis compared haemoglobin postoperatively using a linear regression model adjusting for baseline Haemoglobin, age, gender, smoking status, number of comorbidities and type of arthroplasty (hip or knee). Model assumptions were checked prior to analysis including checking for independence, by ensuring the residuals are independent, with no correlation between consecutive residuals, homoscedasticity, ensuring the residuals have constant variance at every level of x, normality, ensuring the residuals of the model were normally distributed and multicollinearity, ensuring variables were not highly correlated and therefore not independent (Bland, 2015).

Regression model assumptions were analysed and addressed for each model used, (see appendix 4). Linear regression assumptions that were addressed (see appendix 4) independence was achieved by trial design and lack of crossover, homoscedasticity was assessed using a fitted vs residual scatterplot, normality was checked visually using a Q-Q plot and multicollinearity was assessed by using the variance inflation factor (VIF) to measures the correlation between the variables in the regression model (Bland, 2015).

Model estimates and associated 95% confidence intervals and p-values were reported. The main focus of the statistical analysis plan was to compare the outcomes in the non-anaemic iron deficient group to those in the haematinically normal control group. Adjustments for baseline haemoglobin, age, gender, smoking status, number of comorbidities and type of arthroplasty (hip or knee) were performed to control for any differences, hence the focus was not on the individual covariate parameter estimates.

2.3.6.3 Secondary analyses

Binary outcomes up to 60 days, were analysed using logistic regression, including, infection rate, transfusion rate, readmission rate, deep vein thrombosis, pulmonary embolism, pneumonia, cerebrovascular incident, myocardial infarction and mortality. Logistic regression model assumptions that were addressed (see appendix 4) including that variables were binary, independent by trial design, presence of multicollinearity was assessed using VIF, sufficient sample size was assessed by variable, with 10 instances per variable the suggested threshold (Bland, 2015). The continuous health-related quality of life outcome, EQ-5D-5L scores, were analysed using a linear regression, assumptions were checked, independence was achieved by trial design and lack of crossover, homoscedasticity was assessed using a fitted vs residual scatterplot, normality was checked visually using a Q-Q plot and multicollinearity was assessed by using the variance inflation factor (VIF). Length of hospital stay (in days) was analysed using a poisson regression model. The Poisson regression model assumptions that were analysed (see appendix 4) included the number of event counted, independence, time interval, whether two events could not occur simultaneously and assessing for overdispersion, the presence of greater variability than would be expected (Bland, 2015). If overdispersion was present, a negative binomial would be performed on the relevant data presented.

All secondary outcomes were adjusted for baseline haemoglobin, age, gender, smoking status, number of comorbidities and type of arthroplasty as fixed effects.

2.3.6.4 Sensitivity analyses

Analysis was planned to focus on comparing outcomes between patients identified as non-anaemic iron deficient and those in the haematinically normal group.

Blood transfusion was identified a potential outcome that required sensitivity analysis. The threshold for blood transfusion is identified as a haemoglobin level of less than 70g/l (NICE, 2015). In practice this differs greatly between organisations and clinicians, with more liberal transfusion policies quite common (Brown, 2019). The administration of a unit of blood can raise the haemoglobin by 10g/l (Campbell-Lee and Ness, 2007). Therefore, administration of blood products needed to be assessed in the analysis, to ensure it did not impact the reliability of the results.

It was possible that some patients required a blood transfusion with the time point for requiring transfusion expected to be <5 days. Clinical intervention for transfusions occurs if the patient has Haemoglobin (Hb)<80g/l. The number of patients this affected by group, was reported. For these patients, Hb levels was likely influenced by the transfusion. While it is important to investigate how this might have influenced the primary analysis, we expect <1% of patients to be affected (Pujol-Nicolas, et al 2017). We undertook a sensitivity analysis excluding these patients to explore the impact on the overall conclusions.

2.4 Results

2.4.1 Flow of participants

Retrospective patient data from 2011 to 2018 was assessed for eligibility, 3,172 patients met the eligibility criteria, with 1,638 excluded. This was either because they did not have a preoperative ferritin or because their data was insufficiently complete to be included in the study. 956 non-anaemic iron deficient patients were identified for comparison with a control

sample of 2,214 haematinically normal patients. For the primary analysis, there were five patients excluded from the non-anaemic iron deficient group and seven from the control group, due to missing haemoglobin data postoperatively for the primary outcome. There were no further amendments to the data, hence the study included 2,207 participants in the control group and 951 in the non-anaemic iron deficient group for the primary analysis and 2,214 and 956 respectively for the secondary analyses. This difference in numbers for the primary and secondary outcomes is due to missing postoperative haemoglobin for the primary outcome.

When making decisions on excluding patients from the study, care was taken to robustly apply the eligibility criteria to ensure the data were accurate. Removing the anaemic patients was assessed to have no impact on the reliability of the study, as this was not the patient group targeted. for patients who were missing a preoperative ferritin it would be impossible to establish if a patient had non-anaemic iron deficiency or was haematinically normal. In addition, these patients would drop out of the statistical analysis without the use of imputation of the baseline values. However, it must be acknowledged there was selection bias, due to missing data, meaning the full data pool was not available for inclusion. This results in a potential risk of bias, which could have affected the outcome. It is acknowledged that retrospective cohort data is prone to the risk of selection bias, reporting bias and missing data (Thiese, 2014), unfortunately, without the required information, eligibility could not be assessed and therefore there was no choice, but to exclude those patients from the cohort and acknowledge the risk associated.

Retrospective data flow chart (Figure 1)

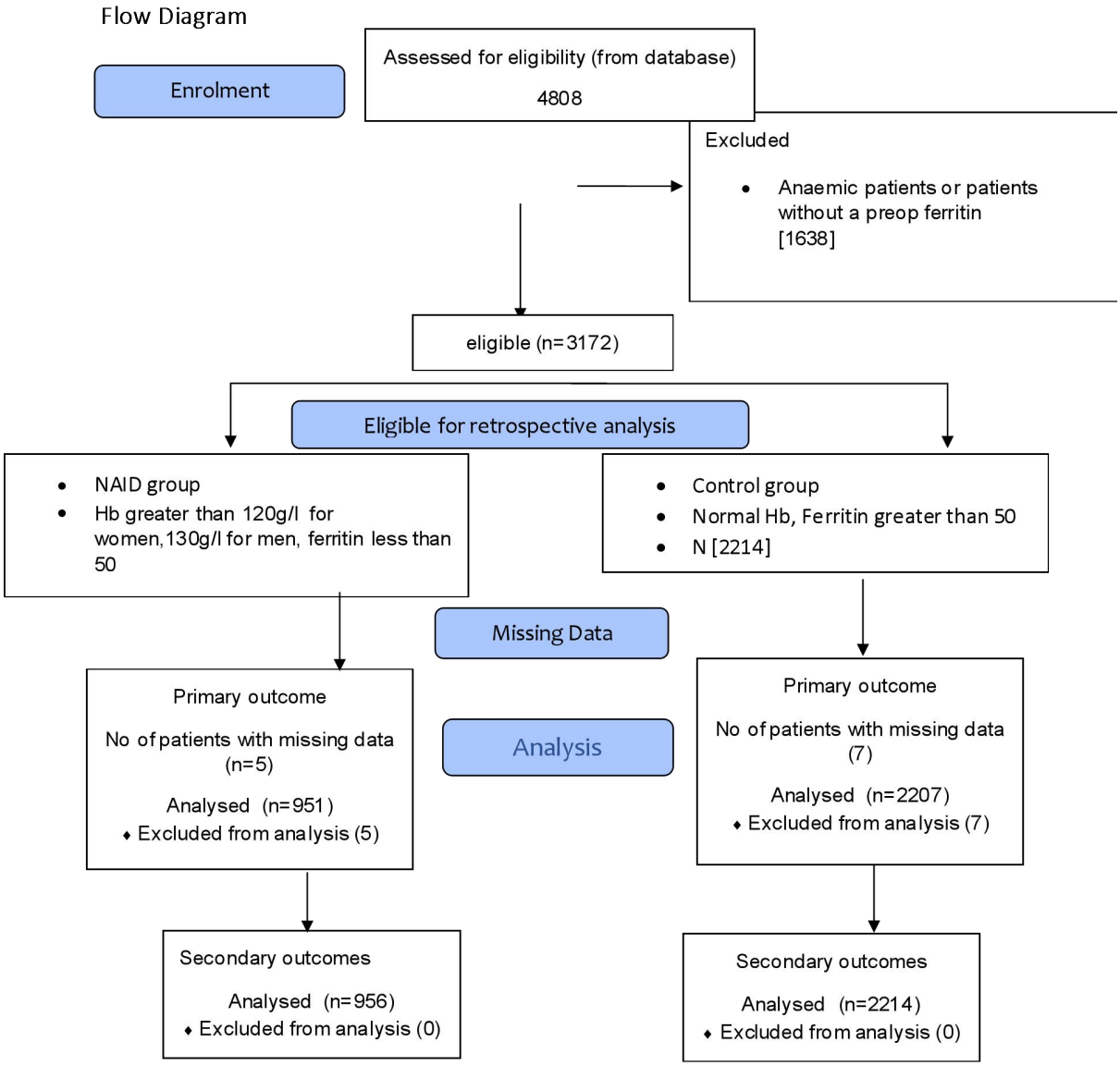


Figure 1: A flow diagram of patients through the study

2.4.2 Descriptive data

Baseline data and patient characteristics are tabulated below in Table 1. Overall, 57% of patients were female, on average they were 69 years old (SD 8.84) and 95% were non-smokers. There were 10 (0.3%) individuals with Type 1 diabetes and 320 (10%) with Type 2 diabetes. Overall, 50% (1590) had hypertension, 8% (246) had ischaemic heart disease, 4%

(n=140) had COPD, 8% (258) were hypothyroid and 0.4% (14) were hyperthyroid and 5% (164) had atrial fibrillation. The average Hb was 140.8 (SD 10.8) and the median ferritin was 78 (min 5 and max 1,705). There was a roughly equal split between knee (48%) and hip (52%) surgery patients included. Compared to the control group, the non-anaemic iron deficient group included more females, more patients with hypothyroidism, Type 2 diabetes, ischaemic heart disease, COPD and had slightly lower Hb (136.95 (SD 9.84) vs. 142.40 (SD10.85)).

Retrospective data baseline characteristics (Table 1)

Patient Characteristics	**Non anaemic iron deficiency (NAID) (n=956)**	**Control (n=2214)**	**Total (n=3170)**
Gender			
Male, n(%)	270 (28)	1093 (49)	1363 (43)
Female n(%)	686 (72)	1121 (51)	1807 (57)
Age (years)			
Mean (sd)	69.28 (9.20)	68.87 (8.67)	68.99 (8.84)
Median (min, max)	69 (36-91)	69 (37-96)	69 (36-96)
Smoking status			
Current smoker(%)	51 (5)	115 (5)	166 (5)
Non-smoker(%)	905 (95)	2099 (95)	3004 (95)
Diabetes 1			
Yes(%)	4 (0.4)	6 (0.3)	10 (0.3)
No (%)	952 (99.6)	2208 (99.7)	3160 (99.7)
Diabetes 2			
Yes(%)	113 (12)	207 (9)	320 (10)
No(%)	843 (88)	2007 (91)	2850 (90)
Hypertension			
Yes(%)	484 (50.6)	1106 (50)	1590 (50.1)
No(%)	472 (49.4)	1108 (50)	1580 (49.9)
Ischaemic Heart Disease			
Yes(%)	92 (9.6)	154 (7)	246 (7.8)

No(%)	864 (90.4)	2060 (93)	2924 (92.2)
COPD			
Yes(%)	56 (5.9)	84 (3.8)	140 (4.4)
No(%)	900 (94.1)	2130 (96.2)	3030 (95.6)
Hypothyroid			
Yes(%)	94 (9.8)	64 (2.9)	258 (8)
No(%)	862 (90.2)	2050 (97.1)	2912 (92)
Hyperthyroid			
Yes(%)	1 (0.1)	13 (0.6)	14 (0.4)
No(%)	995 (99.9)	2201 (99.4)	3156 (99.6)
Atrial fibrillation			
Yes(%)	52 (5.4)	112 (5)	164 (5.2)
No(%)	904 (94.6%)	2102 (95)	3006 (94.8)
Hb (g/dl)			
Mean (sd)	136.95 (9.84)	142.40 (10.85)	140.76 (10.84)
Median (min, max)	136 (120-171)	142 (120-194)	140 (120-194)
Ferritin			
Mean (sd)	29.16 (12.01)	138.59 (113.22)	105.59 (107.32)
Median (min, max)	30 (5-49)	105 (50-1705)	78 (5-1705)
Type of surgery			
Knee	549 (57.4)	1109 (50.1)	1512 (47.7)
Hip	407 (42.6)	1105 (49.9)	1658 (52.3)

Table 1: Patient demographics and comorbidities overall and by group.

2.4.3 Primary outcome analyses

The average haemoglobin level 1 day post operatively was 117.38 (12.86 SD) in the non-anaemic iron deficient group and was 123.04 (13.63 SD) in the control group. The results from the linear regression model (stata data commands in appendix 4), demonstrated a small statistically significant decrease of -0.96 (95%CI -1.66 to -0.26, p=0.007) in post operative haemoglobin in the non-anaemic iron deficient group, compared to the control group.

2.4.4 Secondary outcome analysis

The median length of hospital stay was 2 days (range 0 to 26) in the non-anaemic iron deficient group and was 2 (range 1 to 49) in the control group. There was evidence of a difference in the number of days in hospital between the two groups, model assumptions, suggested overdispersion, as the variance was greater than the mean in the poisson analysis (see appendix 2), therefore a negative binomial regression was performed as per protocol; however, the results were very similar incidence rate ratio (IRR) 1.08 CI 1.03-1.14 P=0.002 using the binomial regression.

There were 127 patients who were readmitted within 30 days of surgery: 42 (4.4%) NAID and 85 (3.8%) control. There were 3 transfusions: 1 (0.001%) NAID and 2 (0.0009%) control. There was one myocardial infarction in each group (0.001% NAID; 0.0004% control) and 8 DVTs; 2 (0.12%) NAID; 6 (0.003%) control. Overall, there were 5 cases of pneumonia with 1 (0.001%) in the NAID group and 4 (0.002%) in the control, 2 (0.0009%) cerebrovascular incidents in the control group but none in the NAID group and 19 pulmonary embolisms (8 (0.008%) NAID; 11 (0.005%) control). There were no deaths or TIAs attacks. There was no evidence of a difference in the number of patients readmitted (OR 0.898, 95% CI -0.498 - 0.285, p0.593), rate of transfusion (OR 0.40, 95% CI -0.003 to 0.001, p0.390), rate of MI (OR 0.33, 95% CI -0.003 to 0.001, p0.475), DVT (OR 0.94, 95% CI -0.0041 to 0.0037, p0.938), cases of pneumonia (OR 1.71, 95% CI -0.003-0.004, p0.70), cerebrovascular incidents (OR 1.12, p0.34 95%CI -0.001 to 0.003) or pulmonary embolism (OR 0.99, 95% CI -0.01 to 0.0016, p0.149).

The EQ-5D-5L was measured 3 months after surgery and was available for 943 (98.6%) in the NAID group and 2,199 (99.3%) in the control group. The five-dimensional scores were comparable between groups average mobility score was 1.55 (1.09 SD) vs 1.46 (1.05 SD),

the average self-care score was 1.30 (1.07 SD) vs 1.23 (0.97 SD), the average activity score was 1.65 (1.16 SD) vs 1.55 (1.08 SD), the average discomfort score was 1.75 (1.27 SD) vs 1.63 (1.12 SD), the average anxiety score was 1.38 (1.2 SD) vs (1.03 SD), the average EQ-5D-5L VAS score was 76.9 (17.1 SD) vs 76.3 (17.8 SD), the average health index score was 0.75 (0.11 SD) vs 0.75 .(0.11 SD). The results from the linear regression model demonstrated a an insignificant change of 0.001 p= 0.80 (95%CI -0.009-0.012) in the health index score and -0.75 p=0.35 (95%CI -2.34-0.83) in the visual analogue score, in the non-anaemic iron deficient group, when compared to the control group.

2.4.5 Sensitivity analyses

There were 3 people who received a blood transfusion, these were removed from the data. Given the small number of participants excluded the results from the primary analysis reduction in postoperative haemoglobin remained unchanged (OR -0.96 95% CI -1.66 to -0.26).

2.5 Discussion

In the non-anaemic iron deficient patient group, it was found there was a reduction in the overall iron stores, which was associated with a reduction in the haemoglobin post-surgery and allied with an increased length of stay. There was no evidence of differences in readmission, complications or rate of transfusions. Analysis of length of stay using a poisson regression model demonstrated the variance was greater than the mean, when reviewed using the negative binomial, it demonstrated a statistically significant increase in length of stay in the non-anaemic iron deficient group. This highlights the importance of using the correct statistical analysis, which may differ depending the data being analysed (Bland, 2015). Overdispersion is present when the variability in the data is greater than would be expected

for the model chosen. A poisson model does not allow variance to be assessed independent of the mean, therefore a negative binomial regression was more appropriate as it accounted for the overdispersion in the data (Bland, 2015).The risk of not correcting for overdispersion, is a potential to have inaccurate coefficients and overall results (Bland, 2015). All other model assumptions were proven to be appropriate, except for the sample size for the secondary binary outcomes, it is suggested it should be greater than 10 incidences per variable (Bland, 2015; van Smeden et al., 2016), there were 127 patients who were readmitted within 30 days of surgery: 42 (4.4%) NAID and 85 (3.8%) control. There were 3 transfusions 1 NAID, 1 control: There was one myocardial infarction in each group and 8 DVTs; 2 NAID; 6 control. Overall, there were 5 cases of pneumonia with 1 in the NAID group and 4 in the control, 2 cerebrovascular incidents in the control group but none in the NAID group and 19. pulmonary embolisms 8 NAID, 11. There were no deaths or TIAs attacks. Therefore, this assumption [10 per variable] was met in the readmission and PE was only. However, van Smeden et al. (2016) performed a simulation to evaluate small sample bias and found the evidence of requiring greater than 10 instances was weak and needed further research to provide guidelines for sample sizes in binary regressions. Ultimately, all other assumptions were met, suggesting the statistical models chosen were appropriate.

The patient reported outcome measure analysed, EQ-5D-5L, demonstrated no difference in outcome measures in the health index score and a statistically insignificant change in the visual analogue score. The symptoms of low haemoglobin, described in the anaemia research (Foss et al., 2008; Frank and Cushing, 2021) include tiredness, fatigue, low mood and difficulty in performing the postoperative physiotherapy (Foss et al., 2008). Although reduction in postoperative haemoglobin may cause difficulties in the postoperative period, this was not demonstrated in the analysis of the patient reported outcome measure of EQ-5D-

5L when comparing a control sample to the non-anaemic iron deficient group. There was no causal link demonstrating a reduction in EQ-5D-5L VAS score, patient satisfaction is often reduced when patients' mood is lower (Eyers et al., 1994), with the associated fatigue and tiredness of the reduction in haemoglobin, making the patients experience different. The reduced length of stay demonstrated in the haematinically normal population may also explain the reduction in self-related health scores in the non-anaemic iron deficient group, as reduced length of stay is associated with improved satisfaction (Diwan et al., 2020). Morbidity analysis demonstrated no difference in any of the areas investigated between the two groups. The number of patients who had a morbidity event post-surgery was low in the retrospective sample, which corresponds to the prevailing literature (Belmont et al., 2014; Gutiérrez Rodríguez et al., 2021). A vastly inflated patient population would be required to accurately demonstrate whether a difference in morbidity occurs, due to the relative rarity of post-operative morbidity in arthroplasty. There were no deaths within 90 days of surgery, therefore no assertion on the impact of mortality or non-anaemic iron deficiency can be made. Transfusion rate post arthroplasty differs greatly in the literature (Browne et al., 2013; Komnos et al., 2021; Prasad et al., 2007). Our organization already has an active patient blood management program, with a low rate of overall transfusion (Pujol-Nicolas et al., 2017), therefore a greater sample may be required to demonstrate any potential difference.

2.5.1 Limitations

Retrospective studies, due to their design, have several limitations given they depend on already collected data not designed for research and are therefore prone to missing information or incomplete records (Talari and Goyal, 2020). There are several limitations that need to be highlighted, firstly the retrospective analysis used data from 2011 to 2018. The cohort was collected over a seven year period, which adds the risk that procedures or

techniques may have improved over time (Talari and Goyal, 2020) or that the healthcare environment may have changed (Anthonisen, 2009; Sedgwick, 2014). Although this risk and potential limitation must be acknowledged, its overall impact was deemed low, elements such as postoperative haemoglobin were haematological values and unlikely to be greatly affected over time. The organization the retrospective analysis was conducted in implemented a patient blood management and enhanced orthopaedic program prior to 2011 which is still in place today, therefore the duration of the cohort was unlikely to have affected the outcomes, although its potential is acknowledged.

Selection bias is described as an inaccuracy in the measure of association, which occurs due to the sample selection not reflecting the intended population accurately (Geneletti et al., 2008). Selection bias can be introduced in the design of a study due to the sampling methods, or the recruitment of participants (Talari and Goyal, 2020). Selection bias can occur in observational studies where the selection of participants is from a defined cohort, rather than a random sample, such as cohort studies (Talari and Goyal, 2020). It can also occur in prospective studies or clinical trials due to poor randomization (Phillips et al., 2022; Talari and Goyal, 2020). Selection bias may explain some of the differences demonstrated. Ferritin alone cannot be proved to be causal for the differences demonstrated, for example, it could be associated with unmeasured characteristics, such as socioeconomic group, wealth or poor diet which could demonstrate potential confounding factors. Therefore, it is impossible to suggest a causal link, merely that there was an association. This further justifies the need for an RCT.

Further data on preoperative and postoperative haemoglobin and preoperative ferritin were available, however, patient demographic information to identify non-anaemic iron deficiency was required to assess for non-anaemic iron deficiency, unfortunately this was missing

therefore further potentially relevant data was not included, thus reducing the size of the sample, which would reduce the instance of capturing rare events. The potential risk of this decision is the unintended risk associated with selection bias, which limited the data to be analysed, and therefore may not have been fully representative of the full sample, with potential to affect the data pool analysed. As a retrospective study, data collated was previously reported which meant I had little control over the data pool and could only ensure the eligibility criteria were robustly applied to optimize the data to be analysed, whilst acknowledging the potential risk. However, without the preoperative ferritin data to establish non-anaemic iron deficiency, there was no choice but to exclude the patients from the analysis. Once optimized, outcomes and analyses were planned appropriately, full datasets were utilised and incomplete datasets were not included in the cohort, therefore the risk of selection bias was present, but deemed low.

Recall bias can occur when participants allow the disease, condition or outcome to affect their response, or when there is a time lapse between an event and their response (Khare and Vedel, 2019), limiting their recall of the event. It is a common problem with patient reported outcomes, which can be influenced by the patients treatment success or failure and the overall patients experience (Khare and Vedel, 2019).

For most primary and secondary outcomes recall bias was a low risk, postoperative haemoglobin, length of stay, readmission and postoperative morbidity and mortality were all derived from patients' admission notes and not affected by recall bias. For the primary outcome postoperative haemoglobin, selection and recall biases were not deemed to be a significant factor, bloods were taken as required, are subjective and not subject to inherent bias. Selection and recall biases may affect the results, differences in treatment between

patients due to time elapsed and lost follow-up may lead to bias (Sedgwick, 2014; Talari and Goyal, 2020). As a retrospective study utilizing patient reported outcome measures, there was a small risk of recall bias, although patients are asked to complete how they feel that day, the patients experience and recovery may alter how they score but it is likely this would be present in both groups (Manary et al., 2013; Tsai et al., 2015).

Analysis of the morbidity data showed no statistical difference between non-anaemic iron deficient patients and the control group. However, the number of incidents were low across all morbidity elements potentially limiting the usefulness of these results, as its difficult to interpret whether there was no difference or whether a greater sample, with more patients, potentially demonstrating a different outcome.

Retrospective studies by design use retrospective convenience sampling, popular in clinical studies due to associated ethical issues and a reduction in cost (Hu and Qin, 2018). This purposive sampling can make generalisation more difficult and requires careful attention to the study design to ensure the capture of appropriate data, which enables robust interpretation and generalisability (Schulz and Grimes, 2019). Overgeneralisation in retrospective studies is cautioned, with cause-effect relationship questioned (Talari and Goyal, 2020). Before implementing any changes based on a retrospective study, critical analysis of the methodology and detailed analysis of the results must be undertaken (Hu and Qin, 2018).

Generalisability 2.5.2

It must be acknowledged that this retrospective cohort study was performed using data from at a single site, with a predominantly white patient population. Whilst single centre studies have their benefits, care, procedures and intervention thresholds are more likely to be

comparable between patient groups than in a multi-centered study, the generalisability across the wider patient population is more difficult to assess (Seifirad and Alquran, 2021). Just because a difference was found in this patient population, does not automatically mean that it is transferrable to a patient group from a different region, with different demographics or undergoing a different procedure (Schulz and Grimes, 2019; Seifirad and Alquran, 2021). Burchett et al. (2020) suggests processes and mechanisms should be the starting point rather than population and place, suggesting whilst contextual considerations must be analysed to see if a result is generalisable, much shared learning can be achieved by shifting focus to the process rather than population. Although this must be balanced with the caveat that some single centre studies which have shown a statistical difference, have not been demonstrated when expanded to other centres (Bellomo et al., 2009).

From the retrospective analysis, the primary outcome, demonstrated non-anaemic iron deficiency affects postoperative haemoglobin in lower limb arthroplasty, in theory, this could be applied across similar patient populations in other regions or undergoing other procedures. With the caution to over generalise acknowledged, non-anaemic iron patients undergoing any surgical procedure with a significantly associated blood loss, would be expected to behave in a similar manner. Their iron stores would be replete preoperatively, as such, their postoperative haemoglobin would be expected to drop similarly. The associated increase in length of stay, may be associated with a reduction in postoperative haemoglobin, and would be expected to behave similarly and therefore the results are transferable. However, further research across a more diverse patient population, with a multi-centered design, would allow the results to be explored across a more diverse patient population and a wider range of procedures with a similar blood loss profile.

2.6 Conclusion

The objective of this retrospective data analysis was to explore if there was a link between poorer outcomes and non-anaemic iron deficiency in patients undergoing lower-limb arthroplasty. The hypothesis suggested patients with non-anaemic iron deficiency would have worse outcomes than those in the normal patient population. The analysis of the retrospective data demonstrated non-anaemic iron deficient patients had lower haemoglobin and an increased length of stay. Although the reduction in haemoglobin did not translate to reduced transfusion, reduced readmission, or improved morbidity or mortality, it demonstrates an association between poorer outcomes of patients undergoing arthroplasty with non-anaemic iron deficiency. Demonstrating the potential to affect post-operative recovery, due to the reduction in post-op haemoglobin and length of stay. Retrospective studies are an important tool to study clinical and patient outcomes, findings from these studies may be used to inform prospective studies (Talari and Goyal, 2020).

As an association between non-anaemic iron deficiency, lower haemoglobin postoperatively and an increased length of stay was found, therefore a systematic review of the literature on treatment of non-anaemic iron deficiency for patients undergoing surgery was performed and is detailed in Chapter 3.

CHAPTER 3: A SYSTEMATIC REVIEW TO INVESTIGATE NON-ANAEMIC IRON DEFICIENCY IN THE SURGICAL PATIENT POPULATION

Introduction

As discussed in Chapter 2, patients with non-anaemic iron deficiency have reduced postoperative haemoglobin and patient reported outcome measures when compared to a control group of patients, with supplementation advocated (Munoz et al., 2016). To assess the available research on this issue a systematic review was undertaken to investigate the impact of treatment or monitoring of non-anaemic iron deficiency in patients undergoing surgery on haemoglobin, ferritin levels and transfusion rates (primary outcomes). Searches of relevant medical databases, grey literature and trials databases were undertaken, to obtain the appropriate literature to conduct a robust systematic review. This Chapter outlines the methods and findings of the systematic review and are then discussed before concluding the Chapter.

3.1 Scope

Initial scoping searches of non-anaemic iron deficiency and lower limb arthroplasty highlighted a limited amount of evidence. Two papers were initially identified; hence a decision was made to expand the scope of the review and explore non-anaemic iron deficiency and surgery, rather than focus solely on lower limb arthroplasty. This decision was undertaken as studies from other clinical areas may inform/enhance treatment during arthroplasty. Although the decision to expand to all surgery could be criticized, as comparison and synthesis could be more difficult, there are many examples of surgical specialties being compared in systematic reviews (Ng et al., 2019b; Henry et al., 2001), with examples of the benefit of pooled learning from other surgical specialties (Maruthappu et al.,

2015; Catchpole et al., 2008). This, therefore, justifies the decision to include all surgery and non-anaemic iron deficiency in this systematic review.

3.1.1 Objectives of the review

This systematic review aims to investigate the impact of treatment or monitoring of non-anaemic iron deficiency in patients undergoing surgery on patient outcomes. The research questions to be explored are:

- What is the impact of treatment or monitoring of non-anaemic iron deficiency in patients undergoing surgery on haemoglobin postoperatively up to 30 days?
- What is the impact of treatment or monitoring of non-anaemic iron deficiency in patients undergoing surgery on ferritin levels, transfusion rates, length of stay, infection, morbidity, mortality and patient reported outcome measures (PROMs)?

(Protocol for the systematic review is documented and located in appendix 4).

3.2 Methods

Following accepted good practice the protocol was registered *a priori* on PROSPERO (Randall et al., 2020). The final reporting follows the PRISMA reporting guidelines (Page et al., 2021), (see appendix 5).

3.2.1 Eligibility criteria

Primary quantitative studies monitoring or receiving treatment of non-anaemic iron deficiency in patients undergoing any type of surgery were considered eligible for this review. The population, interventions (or exposures), comparisons (or control groups), outcomes, setting and study design of included studies are outlined in Table 2.

Non-Anaemic Iron Deficiency and Surgery PICOS (Table 2)

Criteria for including studies in the review	
Population, or participants and conditions of interest	Patients with Non-Anaemic Iron Deficiency undergoing surgery
Interventions or exposures	Monitoring and/or treatment of non-anaemic iron deficiency in patients undergoing surgery
Comparisons or control groups	No treatment (usual care, no intervention, placebo)
Outcomes of interest	Haemoglobin, ferritin, transfusion rate, length of stay, infection, morbidity, mortality, patient reported outcome measures.
Setting	Patients undergoing surgery, including private patients
Study designs	Randomised controlled trials and non-randomised controlled studies

Exclusion criteria

Paediatric and pregnant patient populations, although unlikely in this patient population, were excluded. Joint replacements are predominantly performed in the aging population; therefore, they are extremely rarely performed in paediatric and pregnant patient populations, whose altered physiology and risk profile, led them to be excluded from the review process. When selecting the studies, it was noted that some studies reported both non-anaemic and anaemic patient data in the same study, it is not uncommon for studies to address similar populations in the same study. Higgins et al. (2019b) suggest the reviewer may have to carefully extract

the data that meets their eligibility criteria from within a study, with clarification from the author a useful tool. Where data was sufficiently reported separately for anaemic and non-anaemic patients, the studies were included in the systematic review, however, if clarity could not be obtained, then the study was excluded from the meta-analysis.

3.2.2 Information sources

Searches of relevant medical databases, grey literature and trials databases were undertaken, to obtain the appropriate literature to conduct a robust systematic review. Secondary referencing, reviewing the references lists in the identified papers to identify any further papers, and contacting the study authors were performed where relevant. The criteria for contacting the authors were as follows: when data was insufficiently separated for non-anaemic iron deficiency, when data needed clarification or to access unpublished data.

3.2.3 Search strategy

The search strategy for the systematic review was designed to access both unpublished and published reports and incorporated two stages:

1. Search terms and any synonyms utilised by respective databases, were used in an extensive literature search, using the following search terms: ‘iron deficiency’, ‘non-anaemic iron deficiency’, ‘low ferritin’ and ‘surgery’, utilising the synonyms and key words identified in table 3.
2. Bibliographies and reference lists of the articles collected in stage one were searched and further scrutinised to enhance literature capture.

Saltikov and McSherry (2016) advocate the use of a search strategy list to identify any synonyms or spelling discrepancies, this they suggest, allows the researchers to explore the

topic thoroughly, demonstrating breadth of scope, whilst documenting the design thoroughly to create easily reproduceable results.

Search terms synonyms (Table 3)

Search term	Potential spelling or synonyms
Iron deficiency	Deficiency, deficiencies
Non-Anaemic Iron deficiency	Anaemic, Anemic, Anaemia, Anemia,
Low Ferritin	Ferritin, No synonyms
Surgery	Surgery, surgeries, surgical procedures
Intervention	Treatment, intervention, Iron, iron compounds, ferric, ferrous, preoperative, before surgery
Clinical trial	Randomised controlled trial, clinical trial
Adults	Not children, not pregnant

The search strategy focused on articles published in English up to and including March 2020, with the following databases used: CENTRAL (Cochrane Library), OVID (Embase/Medline), PubMed, WHO International Clinical Trials Registry Platform (ICTRP) Search Portal, Clinicaltrials.gov, CINAHL and Web of Science. Electronic databases were searched in their entirety, with no date restrictions were applied. The individual search strategy for each database are outlined in appendix 5. The search was performed utilising search strategy, with two reviewers independently assessing papers for inclusion.

3.2.4 Selection Process

From the papers identified, titles and abstracts were screened against the PICOS described in Table 2. Two reviewers independently assessed studies for inclusion at first and second screening with arbitration by a third reviewer if necessary. Full texts were sought for eligible papers through the National Health Service and University library resources and were independently reviewed by both reviewers. Once the relevant studies were selected, the references were checked for inclusion of further studies and agreed between the reviewers.

3.2.5 Data collection process

To collect the correct data for this systematic review, an electronic data extraction tool was developed as a Google form (see appendix 7). The first and second reviewer independently screened identified full texts, extracting the required data, utilising the data extraction form. A third reviewer was used to resolve any disparities between the two reviewers after discussion. Efforts were made to contact individual study investigators to obtain or confirm relevant data where necessary.

3.2.6 Data items

Preselected criteria defined which data items were assessed systematically from the results of each study. The following data items were extracted from all studies.

Data Extraction (Table 4)

Study characteristics	Patient characteristics	Interventions	Outcome measures
Author identification	Age	Drugs utilized	Length of stay

Year of publication	Sex	Dose	infection
Language of publication	Ethnicity	Route of administration	Transfusion rate
Source of study funding	Country of residence	Comparator	Morbidity
Study design			Mortality
Study population			Patient reported outcome measures (PROMs)
Sample size			
Inclusion and exclusion criteria			

3.2.7 Risk of bias and certainty assessment

For this systematic review, the Cochrane risk of bias tool (ROB 2), created by Higgins et al. (2019a) was used to assess the risk of bias for randomised controlled trials and Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) (Cuschieri, 2019) was used for non-randomised and observational studies. Assessments were undertaken for individual outcome effects and the completed study to ensure reliable results. Risk of study bias, consistency and publication bias were explored utilising a GRADE approach (Granholm et al., 2019) GRADE is systematic clear method of assessing certainty of evidence in systematic reviews, clinical practice developments and clinical guideline creation, ensuring

effect estimates represent a true effect, leading to more reliable results (Granholm et al., 2019).

3.2.8 Effect measures

The primary outcome was post-operative haemoglobin. The following secondary outcomes were assessed: length of hospital stay, transfusion rate, ferritin, infection, morbidity, mortality and patient reported outcome measures. The following data were extracted for each study: the number of participants in each group, means, standard deviations for continuous outcomes and raw data of the number of individuals experiencing the event and the number in the group. If raw data were not presented or if adjusted analyses were reported, then mean differences or odds ratios/relative risks with 95% confidence intervals were extracted as appropriate. Where studies reported medians and interquartile ranges as opposed to means and standard deviations then standard formula were used to convert the values to a consistent metric, taken from Hozo et al. (2005);

Mean

$$\bar{x} \approx \frac{a + 2m + b}{4} \qquad (5)$$

Standard Deviation

$$S^2 = \frac{1}{12}\left(\frac{(a - 2m + b)^2}{4} + (b - a)^2\right).$$

m = Median

a = The smallest value (minimum)

b = The largest value (maximum)

3.2.9 Data synthesis

Rev Man 5 was used to pool studies in a meta-analysis (Charrois, 2015). It was decided that synthesis of studies would be divided by study design, for example, randomised controlled

trials, quasi-controlled studies and observational studies. The protocol (Randall et al., 2020) planned for data extracted to be pooled using a random effects meta-analysis model, based on the assumption that clinical and methodological heterogeneity were likely to exist and to influence the results (Gough et al., 2017; Littell et al., 2008).

Heterogeneity was explored using chi-squared tests and quantified using the I^2 statistic (Higgins et al., 2019b; Hozo et al., 2005). Meta-analyses were planned to be conducted if comparable outcome data from two or more studies were available. Providing sufficient data were available, then the following subgroup analyses were to be undertaken: gender (male vs. female), mode of iron therapy, and ferritin level a measurement used for identifying fatigue. If quantitative synthesis was not possible, then a narrative synthesis would be undertaken, describing the included studies and commenting on the methodological quality (risk of bias) of each study utilising the Synthesis Without Meta-analysis (SWiM) guidelines (Campbell et al., 2020). SWiM guidelines aim to summarise and collate results where meta-analysis is not able to be performed, either due to missing or incomplete statistics, wide variation in methodology or data collected (Campbell et al., 2020). SWiM provides a framework to standardise the narrative synthesis of quantitative data, grouping studies and comparing outcomes to further understand the pooled effect of the synthesis undertaken (Campbell et al., 2020).

Missing data was mitigated where possible, the search strategy was designed to include unpublished material, grey literature and multiple databases, with justification provided for decisions made when analysis was undertaken on the synthesis of outcomes of interest.

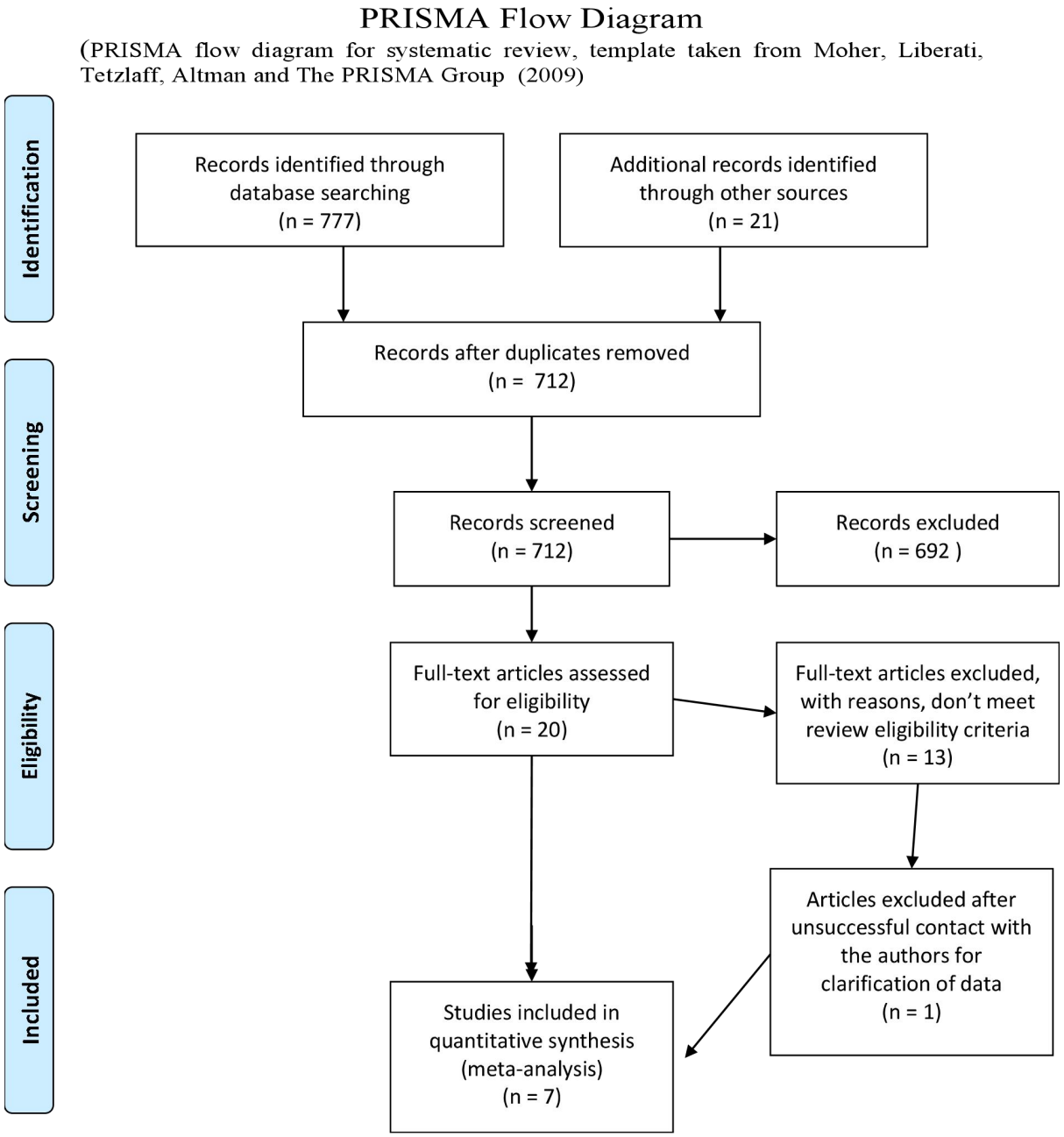
3.3 Results

3.3.1 Study selection

Figure 2 shows the flow of studies through the review including identification, screening, eligibility and analysis.

PRISMA flow diagram Figure

2



There were 712 studies screened for inclusion in the review, 20 full text articles were assessed for eligibility and 7 of those were included in the review. Efforts were made to contact all study authors to gain further information via the corresponding author information in the publication. Seven authors were contacted utilizing the corresponding information via letter or email by the lead reviewer, this was ultimately unsuccessful, as none of the authors responded, which has reduced the potential data available for comparison in the review.

3.3.2 Study characteristics

Table 5 demonstrates the multiple study designs included in the systematic review, which included two randomised controlled trials and five observational studies, three of which were retrospective and two prospective.

Incorporated Studies (Table 5)

Author	Publication	Year	Language	Study funding	Study design
Spahn et al	Lancet	2019	English	Multiple sources	Prospective Randomised Controlled Trial
Sanchez et al	Nutricion Hospitalaria	2015	Spanish (Abstract in English)	not reported	Prospective Observational study
D'Amato et al	Transfusion Medicine	2017	English	not reported	observational cohort study
Na et al	Transfusion	2011	English	not reported	Randomised Controlled Trial

Scardino et al	International orthopaedics	2018	English	Pharmanutra	Observational retrospective study
omelanczuk et al	Obesity surgery	2017	English	not reported	Observational prospective study
M Scardino	Conference Abstract	2017	English	not reported	Retrospective Observational study

3.3.3 Patient characteristics

Patient characteristics are detailed in the study characteristics table (appendix 8). There were large differences in the participant characteristics reported across studies, three studies reported age, four reported sex and no study reported ethnicity. Among those reporting age, data between intervention and control, results were similar 69 +/- 11, 69.4 +/-4.1 and 68.8 +/- 9.4 in the intervention group and 67 +/- 12, 67.9+/-5.2 and 68.4 +/-9.5. respectively, in the control groups. One study included only female patients, with the other two reporting similar gender distributions across the intervention 60%, 44% and 6% male, respectively, with control group 57%, 43% and 14% male.

3.3.4 Risk of study biases

For the two randomised controlled trials, a Cochrane risk of bias [ROB 2] (Higgins et al., 2019a) assessment was completed (see appendix 9), whilst for the four observational cohort studies, including those presented as conference abstracts, a STROBE risk analysis (Cuschieri, 2019) was performed located in appendix 9, the results of which are tabulated in Tables 6 and 7.

Risk of Bias Assessment RCTs (Table 6)

Study	Risk of bias tool used	Assessed risk
Spahn et al	RoB 2	Low risk of bias: This study was judged to be at low risk of bias for all domains. Funding reported
Na et al	RoB 2	Some concerns in the following domains: Domain 2: Randomization was not concealed, staff and patients aware of the randomization, adherence to supplementation not published Domain 3: Not enough separation in the data for non-anaemic iron deficiency Domain 4: Not enough information on whether assessors were aware of allocation Overall risk of bias assessed as some concern Funding reported

The study reviewed by Spahn et al (Spahn et al., 2019a) was deemed to be of low risk of bias, due to it being a double blinded randomised controlled trial, with a reported supplementary appendix clarifying further data. The randomised controlled trial by Na et al (Na et al., 2011) was deemed to have some concerns for risk of bias, this was mainly due to the lack of

blinding for the participants and staff involved, a lack with the associated potential for bias, leading to the difference in outcome of their risk of bias assessment.

Risk of bias assessment in non-randomised studies (Table 7)

Study	Risk of bias tool used	Assessed risk
Sanchez et al	STROBE	Prospective and observational in nature, although method not identified. Limitations on assessing risk of bias, due to the lack of reported evidence and the lack of blinding in the study design led to the risk of bias being judged as high concern. Funding not reported
D'Amato et al	STROBE	Prospective and observational in nature although method not identified. Limitations on assessing risk of bias due to the lack of reported evidence and the lack of concealment in the study design, led to the risk of bias being judged as high concern Funding not reported
Scardino et al	STROBE	Comparing a quality improvement with retrospective data, leading to a judgement of some risk of concern, observational in nature and not a concealed study.
Omelanczuk et al	STROBE	Limitations on assessing risk of bias due to the lack of reported evidence, the lack of blinding

		in the study design, led to the risk of bias being judged as high concern. Funding not reported
M Scardino	STROBE	Observational in nature, comparing a quality improvement with retrospective data. Limitations on assessing risk of bias due to the lack of reported evidence and the study design led to the risk of bias being judged as high concern. Funding not reported

In the observational study conducted by Scardino et al (Scardino et al., 2019), risk of bias was addressed by adherence to STROBE and making a judgement on the study design. Overall, the study was reported well, although funding was not documented. The risk of reporting bias was deemed to be low, however, the observational retrospective nature of the study design and the potential risk of inherent bias associated with that design (Higgins et al., 2019b), led to an overall judgement of some concerns for potential bias. As a design, observational studies are associated with more potential for risk that randomised controlled trials (Littell et al., 2008; Higgins et al., 2019b; Gough et al., 2017). The studies reported as conference extracts by Sanchez et al (Sanchez et al., 2015), D'Amato et al (D'Amato et al., 2017), Omelanczuk et al (Omelanczuk et al., 2013) and Scardino (Scardino et al., 2017) were all assessed for risk of bias by assessing the adherence to STROBE, conference extract version and making a judgement on the study design, information reported and the likelihood of bias. The common problem in the reporting of all of these studies, was the lack of information in the reporting including leaving large gaps in information on study design,

eligibility criteria, and funding and merely summarizing the studies, rather than describing the analysis undertaken. Most studies had some or high risk of bias; there was only one conference abstract that was considered low risk of bias.

3.3.5 Funding Sources

Funding sources were identified in only two of the seven studies included. One study was sponsored by a pharmaceutical company and one reported multiple sources of funding, including grants and a pharmaceutical company.

3.3.6 Results of individual studies

The results of the individual studies are tabulated in Table 8. The common issue within data gathered was a lack of common reporting, across all studies. Efforts were made to contact all authors, this was ultimately unsuccessful, and the reported data was collected as published. Patient reported outcome measures were sought but none were reported in any study.

Review results (Table 8)

Author identification	Population	Age and Sex	Ethnicity	sample size	Intervention	Results
Spahn et al	Patients with iron deficiency or anaemia undergoing cardiac surgery	Age Intervention arm 69 +/- 11 Sex Intervention arm 40% female 60% male Control arm 67 +/- 12	Not reported	Overall 240 Intervention arm 121 Control arm 119	ferric carboxymaltose 20mg/kg Intravenous + Erythropoetin 1mg subcuticular Control group (placebo)	<u>Intervention arm (post-op hb is median)</u> Length of Stay (Mean no of Days) 10.5 +/- 8 Hb preop 140 +/- 10, Hb postop (day 1) 102 SD not reported IQR 89-102, Hb postop (day 3) 89 SD not reported IQR 79-104, Hb postop (day 5) 96 SD not reported IQR 86-110, Hb 4 postop day 7 96 SD not reported IQR 84-110. Ferritin pre-op 65 +/- 23, Ferritin post-op not reported. Infection rate (days 90) 31% Transfusion rate (No of days 7) 32%, Number of units (7 days) 1 +/- 2.2 (90 days) 1.1 +/- 2.5, Mortality 3%, Morbidity MI 0%, Stroke 2%, Acute kidney injury 5%, Bleeding 3%, Blood clots/thromboembolic events 0%, Serious adverse events 22% <u>Control group</u> (placebo) Length of stay (mean no of days) 12.3 +/- 12.2 Hb preop Mean 140 +/- 10, Hb postop (day 1) 94 SD not reported, IQR 85-107, Hb postop (day 3) 86 SD not reported, IQR 78-98, Hb postop (day 5) 89 SD not reported, IQR 82-101, Hb postop (day 7) 85 SD not reported IQR 80-95. Ferritin preop 61 +/- 25, Ferritin postop not reported. Infection rate (90 days) 26%,

		Control arm 43% female 57% male				Transfusion rate 37%Number of units (7 days) 1.3 +/- 2.8 (90 days) 1.7 +/- 3.1, Mortality 5%, Morbidity MI 3%, Stroke 3%, Acute kidney injury 6%, Bleeding 1%, Blood clots 2% Serious adverse events 35%
Sanchez et al	Morbidly obese patients undergoing bariatric surgery	Not Reported	Not reported	Overall 24 Intervention 9 Control 15	Ferric Carboxymaltose 500mg Intravenous	<u>Intervention arm</u> Hb preop 12.8 +/- 1.2 Hb postop (30 days) 13.5 +/- 0.7 Control arm Hb preop 13.7 +/- 0.9 Hb postop (30 days) 12.3 SD not reported No other collectable data published
D'Amato et al	iron deficient patients undergoing knee/hip arthroplasty	Age Not reported Sex Intervention Female 47 Male 3	Not reported	100 Overall Intervention 50 Control 50	Ferric Carboxymaltose 1g Intravenous	Intervention arm Hb preop 125 SD not reported Hb postop (30days) 122 Control Arm Hb preop 131 SD not reported Hb postop (30 days) 112 SD not reported

		Control Female 43 Male 7				No other collectable data published
Na et al	Iron deficient patients scheduled for primary knee arthroplasty	Intervention Age 69.4 +/- 4.1 Female 100% Control arm Age 67.9+/- 5.2 Female 100%	Not reported	108 Overall Intervention 54 Control 54	Iron Succrose 200mg Intravenous Erythropoetin 3000 Subcuticular	<u>Intervention arm</u> Preop Hb 121 +/- 13, postop Hb not reported Ferritin preop 80.2 +/- 40, Ferritin postop (day 1) 195 +/- 57.4, Transfusion rate (day1) 20.4 %, Number of Units 0.2 +/- 0.5 <u>Control Arm</u> Hb preop 121 +/- 12, postop Hb not reported. Ferritin preop 68.7 +/- 31.9, Ferritin postop (day 1) 39.5 +/- 32.2, Transfusion Rate (day 1) 53.7%, Number of Units 0.8 +/- 0.8 No other collectable data published
Scardino et al	patients admitted for elective hip surgery	Intervention Age 68.8 +/- 9.4 Sex Female 56 Male 44	Not reported	Overall 200 Intervention 100 Control 100	Succrosomial Iron 30mg oral Daily for for weeks pre-op	<u>Intervention Arm</u> Length of Stay (no of days) 4 Hb preop 134.5 +/- 26, Hb postop (day 1) 97 +/- 12.4, Hb postop (on discharge) 112 +/- 13.7, Hb postop (day 30) 133 +/- 15.4, preop Ferritin 65.4 +/- 12.37, postop Ferritin not reported

		Control Age 68.4 +/- 9.5 Sex Female 57 Male 43				<u>Control Arm</u> Length of stay (no of days) 6.5 Hb preop 135 +/- 21, postop (day 1) 84 +/- 8.2, Hb postop (on discharge) 96 +/-11.6, Hb postop (day 30) 102 +/- 11.9, Ferritin preop 66 +/- 10.25, postop ferritin not reported No other collectable data published
Omelanczuk et al	Morbidly obese patients undergoing bariatric surgery	Not reported	Not reported	Overall 13 Intervention 5 Control 7	Ferric Carboxymaltose 500mg Intravenous	<u>Intervention Arm</u> Hb preop 124.6 +/- 12.7, Hb postop (|Unknown no of days) 119.2 +/- 12.4, Ferritin preop 70.64 +/- 84.79 Ferritin postop (unknown no of days) 136 +/- 157.96 <u>Control Arm</u> No other collectable data published
M Scardino	Prosthetic Hip Replacement	Not reported	Not reported	Overall 648 not reported per arm	Succrosomial iron 1 capsule Oral administered daily	<u>Intervention arm</u> Length of stay (no of days) 10, Transfusion rate 10% <u>Control Arm</u> Length of stay (no of days) 5, Transfusion rate 0%

Spahn et al. (2019a), published results of a randomised controlled trial conducted in iron deficient and anaemic patients undergoing cardiac surgery, the data in the supplement identified the data of the non-anaemic and iron deficient patients separately, which enabled inclusion. Inclusion and exclusion criteria were clearly defined, (presented in the study characteristics table in appendix 8). Overall, 240 patients were recruited, evenly distributed, age ranges were similar, Intervention arm, Age 69 +/- 11, Control arm 67 +/- 12, with a fairly even sex distribution, Intervention arm 40% female 60% male, Control arm 43% female 57% male. The authors used a combination therapy of ferric carboxymaltose and Erythropoetin in comparison to placebo. They observed a reduction in length of stay of 12.3 +/- 12.2 to 10.5 +/- 8 (Mean no of Days) and a reduction in the median haemoglobin from day 1 to 7, from 102 (IQR 89=102) to day 7 96 (IQR 84-110), in the intervention group, and 94 (IQR 85-107) to 85 (IQR 80-95). The data demonstrated a continuing trend for haemoglobin postoperatively to be higher in the intervention group when compared with the placebo group, day 1 102 (IQR 89-102) to 94 (IQR 85-107), day 3, 89 (IQR 79-104) to 86 (IQR 78-98), day 5 96 (IQR 86-110) to 89, (IQR 82-101), day 7 96 (IQR 84-110) to day 7 85 (IQR 80-95). Ferritin preoperatively was comparable pre-op 65 +/- 23, intervention group and 61 +/- 25 in the control group, ferritin postop not reported and therefore no comparison between groups can be made. Infection rate at 90 days reported a reduction in infection in the placebo group, however statistical significance was not reported for this sub-group. Transfusion rate at day seven demonstrated in decrease in transfusion in the intervention group with a reduction in the number of units administered at 7 days. Mortality was less in the intervention group in the intervention group. Morbidity in the intervention group was reduced by comparison for myocardial infarction, stroke, acute kidney injury, bleeding, blood clots/thromboembolic events and serious adverse events.

The utilisation of a placebo reduces the risk of patient or clinician bias. This study, with a risk of bias rated as low seems to suggest an improvement in haemoglobin postoperatively, length of stay, infection rate, transfusion rate, morbidity, and mortality.

Sanchez et al. (2015) conducted a prospective observational study of morbidly obese patients undergoing bariatric surgery. No patient demographics were reported for this study. Overall, 24 patients were included, unevenly distributed, Intervention 9
Control 15. The authors used a treatment of Ferric Carboxymaltose versus no intervention. The results were published as follows; haemoglobin preoperatively was comparative, intervention arm 12.8 +/- 1.2, control arm 13.7 +/- 0.9. No other collectable data published with supplementation improving haemoglobin postoperatively at 30 days in the intervention group 13.5 +/- 0.7 compared to the control group 12.3. Standard Deviation was not reported, and no other collectable data was published.

D'Amato et al. (2017) conducted an observational cohort study of 100 iron deficient patients undergoing knee/hip arthroplasty. Inclusion and exclusion criteria were reported (documented in study characteristic table in appendix 8), age was not reported, sex was evenly distributed between groups, however the majority of patients in both groups' were female, evenly distributed between intervention and control. The authors used a treatment of Ferric Carboxymaltose compared to no intervention. The average haemoglobin levels preoperatively were 125 in the intervention group and 131 in the control group and at 30 days was 122 and 112 respectively; no measures of dispersion were reported. This study had a risk of bias judged to be of high concern (Table 6) and it is difficult to assess the reliability and validity of this study and its application to the wider patient population.

Na et al. (2011) conducted a randomised controlled trial of 108 iron deficient patients scheduled for primary knee arthroplasty. Inclusion and exclusion criteria were defined and reported (study characteristics table in appendix 8), age in the two groups was comparable, intervention 69.4 +/-4.1, control group 67.9+/-5.2, all patients were female and evenly distributed between intervention and control, 54 in each group. The authors choosing treatment of Iron Succrose and Erythropoetin compared to no treatment. Haemoglobin preoperatively was comparable 121 +/- 13 to 121 +/- 12 respectively, however postoperative haemoglobin was not reported. Ferritin preoperatively was lower in the control group 68.7 +/- 31.9 compared to the intervention group 80.2 +/- 40. Ferritin was improved postoperatively in the intervention group 195 +/- 57.4, compared to the control group 39.5 +/- 32.2. Transfusion rate at day one was improved in the intervention group 20.4 % compared with 53.7% in the control group, with a reduction in the number of units transfused 0.2 +/- 0.5 when compared to the control group 0.8 +/- 0.8. No other collectable data published.

Analysing this study, with a risk of bias of some concern (Table 6), in context of other studies was difficult, the authors chose to collect serum iron postoperatively rather than haemoglobin, leading to difficulties in comparison, with other studies, however, an increased ferritin in the intervention group postoperatively and the reduction in blood transfusion rate was demonstrated.

Scardino et al. (2019) conducted a retrospective observational study involving 200 patients admitted for elective hip surgery. Inclusion and exclusion criteria were defined and reported (study characteristics table in appendix 8), age and sex distribution were comparable intervention, 68.8 +/-9.4 female 56 male 44 and control, 68.4 +/-9.5, female 57 male 43, with patients evenly distributed between intervention and control 100 per arm. The authors

compared Succrosomial Iron to no treatment. The results were reported as follows; haemoglobin preoperatively was comparable, intervention 134.5 +/- 26, control 135 +/- 21, whereas haemoglobin postoperatively on day one 97 +/- 12.4 and control 84 +/- 8.2, and day thirty intervention 133 +/- 15.4, control 102 +/- 11.9 demonstrated a consistent improvement in the intervention group. Preoperative ferritin was comparable intervention 65.4 +/- 12.37, control 66 +/- 10.25, postop ferritin not reported. A reduction in length of stay (no of days) was demonstrated in the intervention group, 4 compared to 6.5 in the control group. No other collectable data published.

Analysing the results of this study with a risk bias of 'some concern' (Table 6), with a well distributed patient population, a trend in improvement in length of stay and haemoglobin from day one to thirty postoperatively were demonstrated with the prescribed intervention, there was a lack of reporting in statistical analysis and confidence intervals which were not identified, made establishing the reliability of the study more difficult.

Omelanczuk et al. (2013) conducted a retrospective observational study incorporating 13 morbidly obese patients undergoing bariatric surgery, inclusion/exclusion criteria age and sex distribution were not reported. The authors chose a treatment of Ferric Carboxymaltose. The results were reported as follows; haemoglobin preoperatively was reported across all iron deficient patients 124.6 +/- 12.7, haemoglobin postoperatively was reported in the intervention group 119.2 +/- 12.4 at an unspecified number of days, however it was not reported in the control group. Ferritin was collected in the intervention group preoperatively 70.64 +/- 84.79 and postoperatively 136 +/- 157.96, ferritin in the control group wasn't published. No other collectable data published.

Analysing this study, with a risk bias of high concern and presented as a conference abstract, there was insufficient data to assess anything meaningful, with a lack of consistent reporting and no statistical analysis performed relating to the systematic review outcomes and no reported data on confidence intervals, making it difficult to assess this study as valid or reliable based on the reported data.

Scardino et al. (2017) conducted a retrospective observational study involving 648 patients undergoing prosthetic hip replacement. Inclusion/exclusion, age and sex demographics were not reported. The authors chose Succrosomial iron supplementation as treatment. The report does not identify the figures of patients in the intervention versus no intervention groups, but merely states the supplement was introduced over a period of time. A reduction in length of stay in number of days from 10 to 5 and transfusion rate 10% to 0% was reported, however insufficient detail on how this reduction was distributed and what proportion of patients were prescribed the intervention, and which were not was reported.

Analysing this study, with a risk of bias of 'high concern', there as a lack of distinction in the data from the beginning, it is difficult to sort the data by intervention versus control, as the numbers are not reported in sufficient detail, this meant the reported reduction in length of stay and transfusion, although quantified by the authors, is impossible to analyse in greater detail due to a lack of reporting information.

3.3.7 Results of synthesis

3.3.7.1 Primary outcome - Haemoglobin

Haemoglobin levels were reported in six studies preoperatively and in four studies postoperatively, ranging from day one to discharge up to day thirty post-operatively (Table 9).

One study reported haemoglobin data postoperatively without a specific date and one, measured at discharge. Commonality of reporting occurred twice, two studies reported on day one (Spahn et al and Scardino et al) and two on day thirty (Sanchez et al and Damato et al).

Haemoglobin Data (Table 9) (Intervention = (I), Control = (C)

Study	Pre-op Hb (Mean)	Postop Hb (Mean)	SD and IQR
Sanchez et al	Hb preop 128 +/- 12 Control arm Hb preop 137 +/- 9	Hb postop (30 days) 135 +/- 7 Hb postop (30 days) 123	SD not reported
Spahn et al	(I) 140 +/-10 (C) 140 +/- 10	(Median not mean) Day 1 (I) 102, (C) 94 Day 3 (I) 89, (C) 86 Day 5 (I) 96, (C) 89 Day 7 (I) 96, (C) 85	Day 1 IQR (I) 89-114, (C) 85-107 Day 3 IQR (I) 89-102 (C) 78-98 Day 5 IQR (I) 86-110, (C) 82-101 Day 7 IQR (I) 84-110, (C) 80-95
Damato et al	(I) 125 SD not reported (C) 131 SD not reported	Day 30 – (I) 122, (C) 112	not reported
Na et al	(I)121 +/-13 (C) 121	not reported	not reported
Scardino et al	(I) 134.5 +/- 26 (C) 135 +/- 21	Day 1 – (I) 97, (C) 84 Discharge (I)– 112 (C) 96 Day 30 (I) – 133 (C)102	Day 1 - SD (I) 12.4, (C) 8.2 Discharge - SD (I) 11.2 (C) 9.5 Day 30 - SD (I) 15.4, (C) 11.9
Olmeanzuk et al	(I) 124.6 +/- 12.7 (C) not reported	Day not reported (I) 119.2 (C) not reported	(I)124 (C) not reported
Scardino	not reported	not reported	not reported

Spahn et al, reported median and interquartile range, as opposed to mean and standard deviation, therefore, mean and standard deviation were derived for the purpose of synthesis (Table 10).

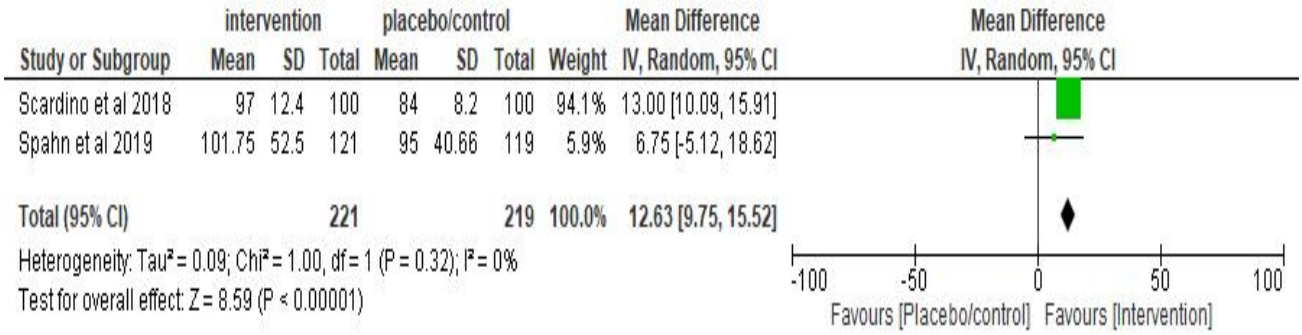
Derived statistics (Table 10)

	Day 1	Day 2	Day 5	Day 7
Derived Mean	(I) 101.75 (C) 95	(I) 92.5 (C) 87	(I) 97 (C) 90.25	(I) 96.5 (C) 86.25
Derived SD	(I) 52.1 (C) 40.66	(I) 17.6 (C) 33.6	(I) 48.8 (C) 30.6	(I) 56.4 (C) 22.3

Unfortunately, missing data was present in both 30-day reporting studies, both were missing standard deviation and not enough information was published for a standard deviation to be derived, hence it could not be included in a meta-analysis. The study reporting on discharge is not a fixed end point and therefore was not pooled for analysis, while the study reporting no date for the postoperative sample was missing data and therefore was excluded from meta-analysis.

The meta-analysis on haemoglobin postoperatively at day 1 in the two studies identified were pooled, all other haemoglobin results are presented narratively. It demonstrated a mean difference of 12.63 and a 95% confidence interval [9.75 - 15.52] in favour of the intervention. The difference in standard deviation between the two studies was noted, with calculations checked to ensure derived statistics were correct. This wide standard deviation suggests greater dispersion of the data, which may explain why median averages were reported in the original publication.

Haemoglobin day 1 postoperative meta-analysis Figure 3)



Spahn et al, demonstrated a small improvement in median haemoglobin in the intervention group at day three, intervention 89 (IQR 89-102), control 86 (IQR 78-98), day five intervention 96 (IQR 86-110), control 89 (IQR 82-101) and day seven intervention 96 (IQR 84-110), control 85 (IQR 80-95). Scardino et al, demonstrated an improved haemoglobin at discharge 112+/-11.2 intervention, 96+/-9.5 control and at day 30, 133+/-15.4 intervention, 102+/-11.9 control. Damato et al, day demonstrated an improvement in mean haemoglobin with intervention at day 30, intervention 122, control 112. Sanchez et al, reported in improved haemoglobin with intervention at 30 days 135, intervention compared to 123 control, standard deviation was not reported. Olmeanzuk et al reported haemoglobin ti the intervention group, but not in the control group and didn't specify the dat the haemoglobin was taken, therefore further inference was impossible. Na et al and Scardino, did not report postoperative haemoglobin. Narratively, the trend in studies seemed to demonstrate treatment of non-anaemic iron deficiency improved postoperative haemoglobin postoperatively, however there was no further commonality in the studies for days postoperatively analysed.

3.3.7.2 Secondary outcome - Ferritin

Ferritin was reported preoperatively in four studies (see Table 11) and postoperatively in two studies (Na et al and Omelanczuk et al), but only one had sufficient data for analysis (Na et al).

Ferritin data (Table 11)

Author	Preop ferritin	Postop ferritin	SD + IQR	Change
Spahn et al	(I) 65 +/¬- 23, (C) 61 +/- 25,	Not reported	Not reported	Not reported
Sanchez et al	Not reported	Not reported	Not reported	Not reported
Damato et al	Not reported	Not reported	Not reported	Not reported
Na et al	(I) 80.2 +/- 40 (C) 68.7 +/- 31.9	Day 1 (I) 195 (C) 39.5,	(I) +/- 57.4 (C) +/- 32.2	Not reported
Scardino et al	(I) 65.4 +/- 12.37, (C) 66 +/- 10.25	Not reported	Not reported	Not reported
Omelanczuk et al	(I) 70.64 +/- 84.79	(I) 136 +/- 157.96 (unknown no of days)	Not reported	Not reported

Na et al. (2011) demonstrated an improvement in postoperative ferritin in patients who had received iron therapy, however it was reported in only one study, which incorporated only

female patients. Omelanczuk et al demonstrated an improvement in ferritin with intervention from preoperative 70.64 +/- 84.79 to postoperative 136 +/- 157.96, however the timepoint is not documented, neither is the ferritin reported in the control sample. Due to poor reporting, there was insufficient data available to pool the studies. The general findings from the studies which analysed ferritin, suggested there was an improvement in postoperative ferritin with intervention compared to a control group. However collectively, with missing data, there is not enough evidence to rule out confounding factors, with more research required to ascertain the true impact.

3.3.7.3 Secondary outcome – Length of stay

Length of stay was reported in three studies (Table 12), one reported mean and standard deviation (Spahn et al), one reported mean only and no standard deviation (Scardino et al) and one reported the results but the reporting data was not described sufficiently by trial arm to pool the data. The general findings across these studies were a reduced length of stay in the intervention compared to control group.

Length of stay (Table 12)

Spahn et al	Scardino et al	Scardino
(I) 10.5+/-8 (C) 12.3+/12.2	(I) 4 (C) 6.5 SD not reported	10 to 15 Not reported by trial arm

3.3.7.4 Secondary outcome – Infection control

Infection rate was reported in only one study (Spahn et al), which reported a lower infection rate, 25% in the placebo group, 31% in the intervention group at 90 days. Narratively

infection seemed to increase with intervention, however this is based on one study and further synthesis was not possible, with insufficient data to draw firm conclusions.

3.3.7.5 Secondary outcome – Transfusion rate

Transfusion rate was reported by three studies (Spahn et al, Na et al and Scardino) Spahn et al. (2019a) reported at day 7, Na et al. (2011) at day one and Scardino et al. (2017) reported as pre and post implementation of iron therapy not by trial arm, therefore there was insufficient separation in the data reporting to further analyse these studies.

Transfusion rate (Table 13)

Spahn et al	Na et al	Scardino
7 days (I) 1 +/- 2.2 32% (C) 1.3 +/-2.8 37%	1 day (I) 0.2 +/-0.5 20.4% (C) 0.8+/-0.8 53.7%	Day not reported 0% - 10% Not reported by trial arm

Due to poor reporting, there was insufficient data available to pool the studies. Narratively, the data would suggest that iron therapy decreases the number of units and percentage of patients transfused, however there was insufficient data to draw firm conclusions.

3.3.7.6 Secondary outcome – Morbidity and Mortality

Morbidity (MI, Stroke Aki, Bleeding, Thromboembolic events SAE) and Mortality were reported in one study, Spahn et al. (2019a) reported better outcomes across all elements of morbidity and mortality measured with iron supplementation compared to a control group MI

0% - 3%, Stroke 2% - 3%, acute kidney injury 5% - 6%, blood clots/thromboembolic events 0%- 2% respectively. Mortality was improved with intervention 3% compared to 5% for the control group and serious adverse events reduced to 22% in the intervention group compared with 35% in the control group. Narratively it would seem intervention reduces morbidity and mortality.

3.3.8 Subgroup analysis

There were too few studies to undertake any of the planned subgroup analyses.

3.4 Discussion

3.4.1 Review findings

The limited data available suggest a beneficial impact of iron therapy, improving postoperative haemoglobin, ferritin, length of stay, transfusion rate and morbidity/mortality, however, caution must be noted, as this is primarily based on single studies, with small pockets of support from the other included studies. Hence there is limited robust evidence to be able to firmly conclude that the treatment of non-anaemic iron deficiency is effective at improving clinical outcomes.

Data provided by Spahn et al. (2019a) unexpectedly demonstrated a lower infection rate in those not receiving iron therapy. However, this is only a single study hence validity and reliability are difficult to measure.

Whilst this review provides some data on elements, there is a lack of information from a patient perspective, as patient reported outcome measures were not reported in any of the studies reviewed. Patient reported outcome measures could add context and situational

understanding and would be an important outcome to measure in the future (Boland et al., 2014).

3.4.2 Comparison to other literature

Treatment of non-anaemic iron deficiency in the general population has undergone a systematic review (Miles et al., 2019a), which was inconclusive and suggested more high quality studies needed to be undertaken to analyse the effectiveness of treatment. Non-anaemic iron deficiency in the surgical patient population guidance suggests patients may benefit from supplementation (Munoz et al., 2016). The consensus of this review, although demonstrating a potential benefit of treatment, supporting the established premise that treatment may aid patients with non-anaemic iron deficiency undergoing surgery, it was not conclusive, supporting the need for further in-depth studies. When this review was undertaken six of the seven papers were more than five years old.

3.4.3 Review Strengths

This systematic review conducted, identified an appropriate research question and scope, utilizing a defined six-step approach. The systematic review targeted the appropriate literature, although it is acknowledged the inclusion criteria had to be expanded to all surgery due to the lack of studies focused on lower limb arthroplasty. The required participants, outcomes of interest and identification of the study designs, enabled a robust, thorough and defined review to be undertaken (Higgins et al., 2019b). Identifying both inclusion and exclusion criteria, enabled irrelevant studies to be removed and streamlined and focused the review on the defined research question and objectives (Higgins et al., 2019b; Gough et al., 2017; Boland et al., 2014). Care and attention were taken to ensure all elements of the PRISMA checklist were addressed and implemented to create a robust review process, with a

considered and planned robust search strategy, independent reviewers and data collection, risk of bias, certainty of evidence with narrative and statistical synthesis appropriately managed. The papers included in the review came from several sources, incorporating both academic journals and conference presentations, this demonstrated the breadth of information sources utilised in the review. The search included trial registries and grey literature, which ultimately was unsuccessful, but was necessary to reduce the risk of missing unpublished studies (Higgins et al., 2019b).

3.4.4 Limitations

The decision to include all surgery, was justified as demonstrating comparable outcomes within the differing surgical specialties (Ng et al., 2019b; Henry et al., 2001), with examples of the benefit of pooled learning from other surgical specialties (Maruthappu et al., 2015; Catchpole et al., 2008). However, it must also be acknowledged as a potential design limitation. Different types of surgery have diverse risk profiles, with variability in blood loss, infection rates and outcomes measures (Messano et al., 2013; Chand et al., 2007). All RCTs should be reported in accordance with CONSORT/STROBE and therefore should be consistent irrespective of discipline. However, harmony in reporting was a common problem, which may have been exacerbated by incorporating differing surgical specialties, whose focus, data collection and style of reporting may differ (Maruthappu et al., 2015). Heterogeneity was further affected by a lack of standardised reporting outcome measures in the anaemic or non-anaemic iron deficient patient populations.

Due to the COVID 19 pandemic, I was returned to full time clinical practice, this meant a delay between the literature review being performed and the data collection and analysis of nine months. Gough et al. (2017), suggest reviews should be completed in a timely manner

as they can quickly be out of date, however they acknowledge complexities mean this is not always possible. It must be acknowledged that this delay may mean subsequent publications may have been missed in the nine months between literature review and analysis, it is well established that a literature review performed as part of a systematic review is only accurate on the day it is undertaken (Boland et al., 2014; Gough et al., 2017) and the researcher acknowledges this potential limitation. The delay from selection to analysis is the greatest potential design limitation, as papers published in the interim were not included, which could reduce how current the review is.

It must be acknowledged there was an overall lack of studies, with inconsistencies in reporting, which potentially limit the generalisability of this review. Seven studies matched the criteria accurately but there was a lack of consistency in mode of treatment, commonality of reporting and study design. Although efforts were made to contact the authors for clarification and further data by email or post to the corresponding author, this was unfortunately unsuccessful.

Prespecified inclusion criteria were reported in five of the seven studies and collectively required non-anaemic iron deficiency and to be undergoing one of the procedures specified, depending on the surgical specialty involved. Exclusion criteria were reported in four of the seven studies, including patients undergoing emergency procedures, relevant medical conditions/ comorbidities, such as heart disease, liver disease and kidney disease and sensitivities to the medication utilised, located in the study characteristics table in appendix 8. Although most studies reported a prespecified inclusion criteria adding strength and reliability and that the correct patients have been included, unfortunately, not all studies have reported the inclusion and exclusion criteria. This makes it difficult assessing whether

decisions made deciding if the participant population were addressed appropriately in the remaining studies, risking potential bias being introduced.

Bias in study design and reporting was assessed using STROBE and the ROB 2 risk bias tool (Ranholm et al., 2019; Higgins et al., 2019a), which demonstrated the potential for bias and must be acknowledged as a potential limitation. The common problem in the reporting of all of studies was lack of information published, one randomised controlled trial published detailed demographics and trial data, whereas the other reported differing data, the lack of commonality in reporting is a recurring theme. The quality of reporting of observational studies and conference extracts was poor. This led to the potential risk of bias in these studies being deemed high, as there was often insufficient information presented to assess otherwise, which limits the reliability of the review. This is by no means a judgment on the quality, design, or integrity of the relevant studies, simply a risk assessment made on the evidence available when conducting the review. Risk of bias assessment was undertaken on individual studies and suggested high concern in four studies, some concern in two studies and low concern in one study (Table 6). These ratings are mainly due to study design and lack of reporting information. Non-reporting of funding commonly occurred, only two of the seven studies included reported funding source. One was sponsored by a pharmaceutical company and one reported multiple sources of funding, including grants and a pharmaceutical company, the others did not report their funding. It is not un-common for those likely to benefit from an improvement, for example, a pharmaceutical company, to provide finance towards the trial.

Missing data can be split into two main categories, unreported data, more likely in studies which produced little or no effect, leading to potential bias and missing outcome data, where

data outcomes required by the review have not been reported, leading to difficulties in performing meta-analysis (Higgins et al., 2019b; Gough et al., 2017). In this systematic review, a wide review was undertaken to glean studies form as many sources as possible, the problem of missing data was not reporting bias, but more likely missing outcome data leading to potential bias. Limitations in this review are acknowledged due to the overall lack of studies, missing data and a lack of commonality in the study reporting.

3.5 Conclusion

Narrative synthesis combined with a meta-analysis of two studies suggests that treatment of non-anaemic iron deficiency undergoing surgery may be beneficial. However, due to limitations acknowledged in design, reporting and volume of studies, with the inherent risk of potential bias, the test of validity and reliability has not been fully met. Further research is needed to explore the impact of treatment of non-anaemic iron deficiency in patients undergoing surgery on postoperative outcomes. The next Chapter will use the information gleaned from the retrospective data analysis in Chapter 2 and the systematic review in Chapter 3, to inform a clinical trial on non-anaemic iron deficiency and lower limb arthroplasty.

CHAPTER 4: A RANDOMISED CONTROLLED TRIAL TO INVESTIGATE THE EFFECTIVENESS OF AN ORAL IRON SUPPLEMENT IN THE NON-ANAEMIC IRON DEFICIENT PATIENT POPULATION UNDERGOING LOWER LIMB ARTHROPLASTY POSTOPERATIVE HAEMOGLOBIN, LENGTH OF STAY, TRANSFUSION RATE, MORBIDITY AND MORTALITY AND PATIENT REPORTED OUTCOME MEASURES.

4.1.1 Overview of the Chapter

As discussed in Chapter 3, the systematic review suggests treatment of non-anaemic iron deficiency may be beneficial, supported by the general professional consensus (Munoz et al., 2016). This Chapter outlines the importance of randomised controlled trials and the trial methodology. The results and analysis of primary and secondary outcomes are then presented, followed by discussion of the topics raised by the trial and acknowledgement of potential limitations.

4.1.2 Introduction

Randomised controlled trials are the gold standard single study design for demonstrating effectiveness in medicine (Nardini, 2014). The impact of clinical trials benefits the wider patient population by enabling evidence-based medicine to improve treatments and services (Umscheid et al., 2011; Armijo-Olivo, 2018). A clinical trial protocol summarises the design, methodology, objectives, ethical considerations and statistical analysis to be undertaken in advance of the study starting (Cipriani and Barbui, 2010). Trial protocols must meet stringent standards of good clinical practice and are used in the ethical approval process with the ethical review committee to document the process the trial will undertake (Cipriani and Barbui, 2010).

Ethics is essential in medical research and used to ensure the patients involved are treated appropriately and research is conducted in the safest manner for the research participants (Nardini, 2014; Goldstein et al., 2018). Modern medical ethics are influenced by research conducted previously without appropriate care and standards and are inspired by three important documents, the Nuremberg code (Nuremburg, 1996), the declaration of Helsinki (Association, 2013) and the Belmont report (Department of Health, 1979), with entrenched concepts of human rights, beneficence and the safety of research participants. In the United Kingdom, all clinical trials must undergo an independent research process involving approval from an ethical committee, to ensure the research undertaken meets the required standards of patient safety and has appropriate contingencies to investigate potential problems that arise and are subject to Good Clinical Practice (GCP) guidelines (MHRA., 2014).

4.1.3 Background

In Chapter 1, the concept of non-anaemic iron deficiency was identified as a treatable phenomenon. The retrospective data analysis in Chapter 2, demonstrated non-anaemic iron deficient patients were adversely affected by non-anaemic iron deficiency. The systematic review in Chapter 3, demonstrated a narrative which supported supplementation in the non-anaemic iron deficient population, however it was not conclusive and there was no consensus in the treatment regime. Supplementation with iron has shown to improve Hb recovery in patients with low ferritin (Zhou et al., 2015). The findings of the systematic review in Chapter 3 suggest the potential for treatment of non-anaemic iron deficiency to improve haemoglobin postoperatively. Patients with a low ferritin below 50, but not anaemic have been shown to benefit from supplementation (Verdon et al., 2003) in quality of life studies, with improvement of symptoms.

Previous research has shown patients Hb continues to drop following arthroplasty until day five, before naturally improving as the patient recovers from surgery (Rimon et al., 2005). The drop between day 0 and 5 is quite pronounced (Zhou et al., 2015), with improvement beginning at day five. The impact of oral iron on this sudden surgical insult may be difficult to detect at day five, as the body is still recovering.

Iron absorption between patient populations has been shown to vary between research participants (Banerjee and McCormack, 2019). Inflammation is known to effect iron absorption by the over production of Interleukin 6, which elevates Hepcidin levels outside of their homeostatic range, contributing to a decrease in iron absorption (Nemeth et al., 2004; Moretti et al., 2017). CRP levels will be monitored to assess for potential effects on iron absorption.

The proposed randomised controlled trial was designed to assess the impact of supplementation with oral iron against a control group of non-anaemic iron deficient patients undergoing lower limb arthroplasty.

4.1.4 Objectives and Hypothesis

The objective of this randomised controlled trial was to ascertain the effect of iron supplementation in patients undergoing lower limb arthroplasty against a set of predefined parameters.

The hypothesis of this randomised controlled trial was that patients with reduced overall iron stores would benefit from iron supplementation, to enable their haemoglobin to recover after lower limb arthroplasty, when compared to no intervention. Therefore, the primary endpoint

was haemoglobin at 30 days, with secondary outcome measures at 30 days, 4 weeks and 3 months postoperatively.

It may also reduce length of stay, readmission rate at 30 days, complication rates, transfusion rates post-surgery and reduce the symptoms of anaemia (FACIT-Fatigue), whilst improving patient reported outcome measures (EQ-5D-5L), although these are secondary outcome measures, which the trial was not powered for.

4.2 Methods

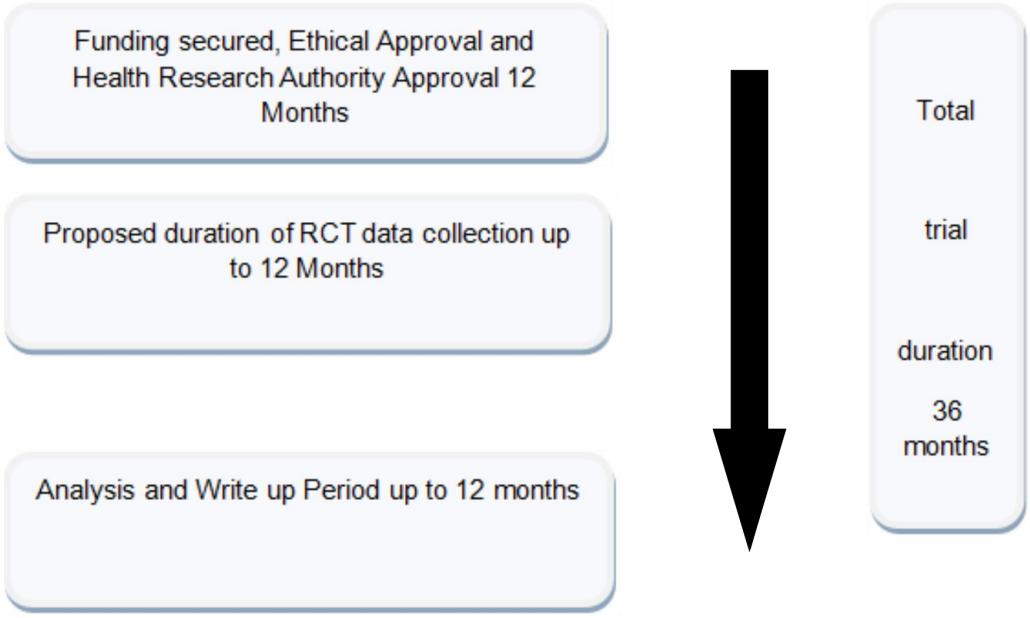
A randomised controlled trial protocol (appendix 10), designed in accordance with CONSORT guidelines (CONSORT, 2010) (see appendix 11) was used for this clinical trial with ethical approvals submitted and gained. The design is a pragmatic two-arm parallel randomised controlled trial, comparing treatment with oral iron with no intervention in non-anaemic iron deficient patients undergoing lower limb arthroplasty.

4.2.1 Participants

The trial was conducted utilising patients identified as non-anaemic but iron deficient undergoing lower limb arthroplasty.

Trial timeline [Figure

4]



4.2.2 Setting

This clinical trial collected data across four hospital sites within a single NHS Foundation Trust based in England.

4.2.3 Ethics and ethical review

This clinical trial was completed within the principles of the Declaration of Helsinki (World Medical Association, 2013) and complied with the approved protocol ISRCTN 48118194 (Randall, 2021), the principles of GCP (Medical Health Research Authority, 2014). Formal NHS Research Ethics Committee (REC) approval was sought and obtained (North East Research Ethics Committee (York) Ref: 18/NE/0371) via the Health Research Authority (HRA), university and local R&D, with further amendments submitted and approved by REC. The trial was assessed as Non-CTIMP (studies which do not use Investigational Medicinal

Products) by the HRA, as the intervention was a food supplement, not an investigational medical product, therefore it did not need MHRA approval.

4.2.4 Eligibility criteria

The eligibility criteria were carefully considered and used to ensure both the safety of participants and that the validity of the trial results could be appropriately used to make future treatment decisions for other people with similar disease or medical condition. Patients eligible for joint replacement surgery had routine bloods taken full blood count (to test for haemoglobin), serum ferritin, CRP (C-Reactive Protein), urea and electrolytes, liver function and estimated Glomerular Filtration Rate (EGFR) and were checked as per standard practice (baseline).

4.2.5 Inclusion/exclusion criteria

A Good Clinical Practice trained researcher screened laboratory results to identify potential participants. All patients were provided with a patient information sheet prior to screening by the clinical team and were informed their details would be passed on to the research team if they met the study criteria. Lower limb arthroplasty patients identified as non-anaemic iron deficiency (defined as a haemoglobin >120g/l in women and 130g/l in Men (World Health Organisation, 2011) with a ferritin below 50ng/ml) and who fulfilled all other eligibility criteria were contacted by the research team, which consisted of research nurses and trained research assistant practitioners. If the patient agreed to participate, consent was obtained in person at a research clinic prior to recruitment to the study.

Participant inclusion criteria

- Patients must be undergoing primary knee or hip replacement surgery.
- Patients aged over 18 years

- Non anaemic iron deficiency: Haemoglobin >120 g/l for women 130g/l for men and ferritin < 50ng/ml.
- Patients must not already be taking regular oral iron
- Patients must provide informed consent.

Participant exclusion criteria

- Patients who lack capacity to consent to inclusion in the trial.
- Patients with a known allergy/intolerance to Floradix
- Pregnant
- Patients listed for surgery within four weeks of commencing iron supplementation.
- Patients with a history of Haemochromatosis
- Patients with a history of Thalassemia
- Patients with a ferritin less than 15ng/ml, who have not had it investigated

Trial flow chart Figure 5

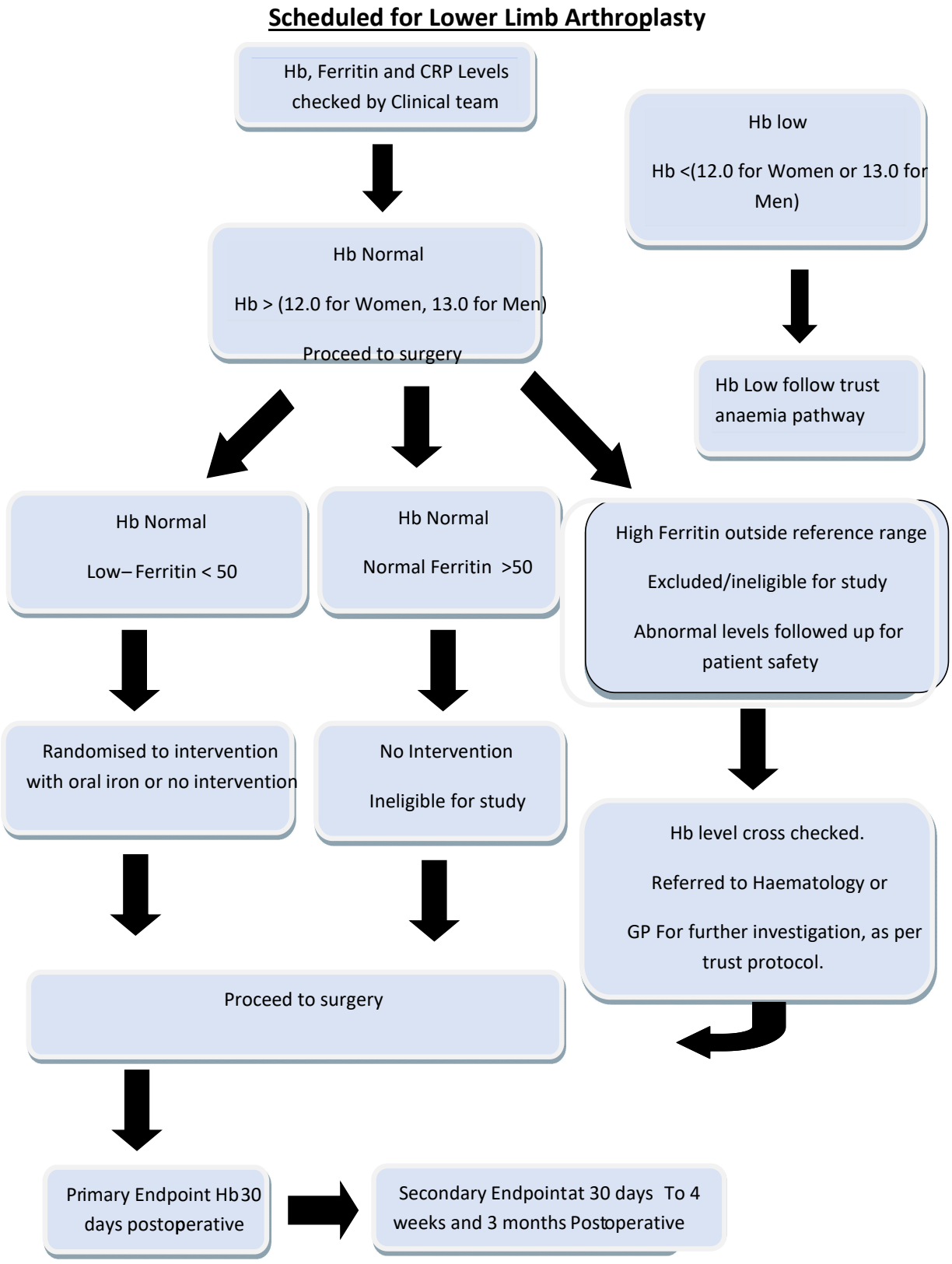


Figure 5: Flow chart from screening to primary and secondary end point

4.2.6 Risks and benefits for patient

Risks associated with trial participation were discussed at length and explored by the research team, with fully informed consent undertaken. Screening for anaemia or iron deficiency was not associated with any greater risk as it was already standard practice and part of patient's routine bloods through the arthroplasty clinic. However, additional blood tests were required for research purposes, out with standard practice, which exposed the patient to additional blood testing and associated risks (Omiepirisa, 2013). Common risks included discomfort, bleeding and bruising have little overall lasting impact on the patient (Omiepirisa, 2013). Infection as an extremely rare complication of phlebotomy (Omiepirisa, 2013), all samples were obtained using aseptic technique.

Oral iron treatments are usually well tolerated but have well documented gastrointestinal symptoms including abdominal pain, constipation and diarrhoea, nausea, and dark stools (Tolkien et al., 2015; Bries et al., 2019; Allen, 2002). There are few significant risks with oral iron supplements (Tolkien et al., 2015), any side effects or adverse events were documented and supplementation discontinued if deemed appropriate. Patients full blood count, CRP and Ferritin were monitored every four weeks during the trial to monitor the effect of the supplementation.

The potential risk of the proposed treatment and the follow-up required were assessed by the trial team as of low risk, although the patient burden, regularity of testing and the number of appointments were discussed and highlighted as a potential conflict. The patients randomised to supplementation, if the hypothesis was proven would benefit from the supplementation, with minimal risk. However, those in the control group, would receive no benefit, with a low

risk of multiple testing. This is common in clinical research, with the greater good and development of evidence-based medicine established principles (Goldstein et al., 2018), with informed consent the key to successfully navigating this process (Umscheid et al., 2011; Fogel, 2018; Farrell et al., 2010).

4.2.7 Consent

Informed consent is a key principle in research to ensure participants are fully informed, can make an informed decision, whilst understanding and weighing up the risks and benefits of participation (Nijhawan et al., 2013; Kadam, 2017; Grady et al., 2017). A detailed Patient Information Sheet (PIS) (see appendix 12), which clearly explained the risks and benefits of trial participation was produced and given to all patients in clinic, to allow them time to digest the information. Patients who met the inclusion criteria were approached for recruitment, with written consent obtained by the research team (see appendix 13) from all the patients who were eligible, had received the PIS with sufficient time to consider and were willing to participate. Patients were informed that their participation was not a requirement and refusal would not interfere with any kind of treatment the patients are already receiving. No financial incentive was offered to the patients, although transport was available, if required to attend the hospital as per routine care.

4.2.8 Interventions

Oral supplementation with 36.8 mg of Floradix with iron (also known as Floradix mit Eisen) was given daily, for six months from diagnosis of non-anaemic iron deficiency, which aimed to encompass both the pre-operative and post-operative phases until the final outcome measures at 90 days. The control group received usual care without supplementation. The Floradix was supplied by the trial funder SALUS (SALUS, 2018) .

This project was planned for 36-months in length which included 12 months for study setup funding, trial design and ethical approval, 12-month clinical trial duration (including 3 months follow up) and 12 months for analysis and write-up see figure 5).

4.2.9 Outcomes

4.2.9.1 Primary Outcome

The primary outcome was Haemoglobin at 3-weeks post-operatively. Research has demonstrated patients Hb continues to fall following arthroplasty until day five, before naturally improving as the patient recovers from surgery (Rimon et al., 2005). The drop between surgery and day five is quite pronounced (Zhou et al., 2015), with improvement beginning at day five. The impact of oral iron on this sudden surgical insult may be difficult to detect at day five, as the body is still recovering. The researcher has chosen to analyse at three weeks postoperatively, allowing time for natural recruitment of haemoglobin as demonstrated by Zhou et al. (2015) and the effectiveness of the iron supplementation to be analysed.

.4.2.9.2 Secondary Outcomes

- Length of hospital stay (midnights in hospital).
- Transfusion Rate up to 30 days
- Number of units transfused up to 30 days.
- 30-day readmission rate
- FBC, CRP and Ferritin at 4, 8, 12, 16, 20 and 24 weeks
- Adverse events (including all cause morbidity and mortality at 30 and 90 days)
- Pneumonia within 30 days
- Inpatient DVT within 30 days of surgery

- Inpatient PE within 30 days of surgery
- Cerebrovascular incident within 30 days of surgery
- Myocardial infarction within 30 days of surgery
- FACIT-Fatigue 4 weeks post-surgery
- Health related quality of life (EQ-5D-5L) at 90 days

4.2.10 Assessment schedule (Table 14)

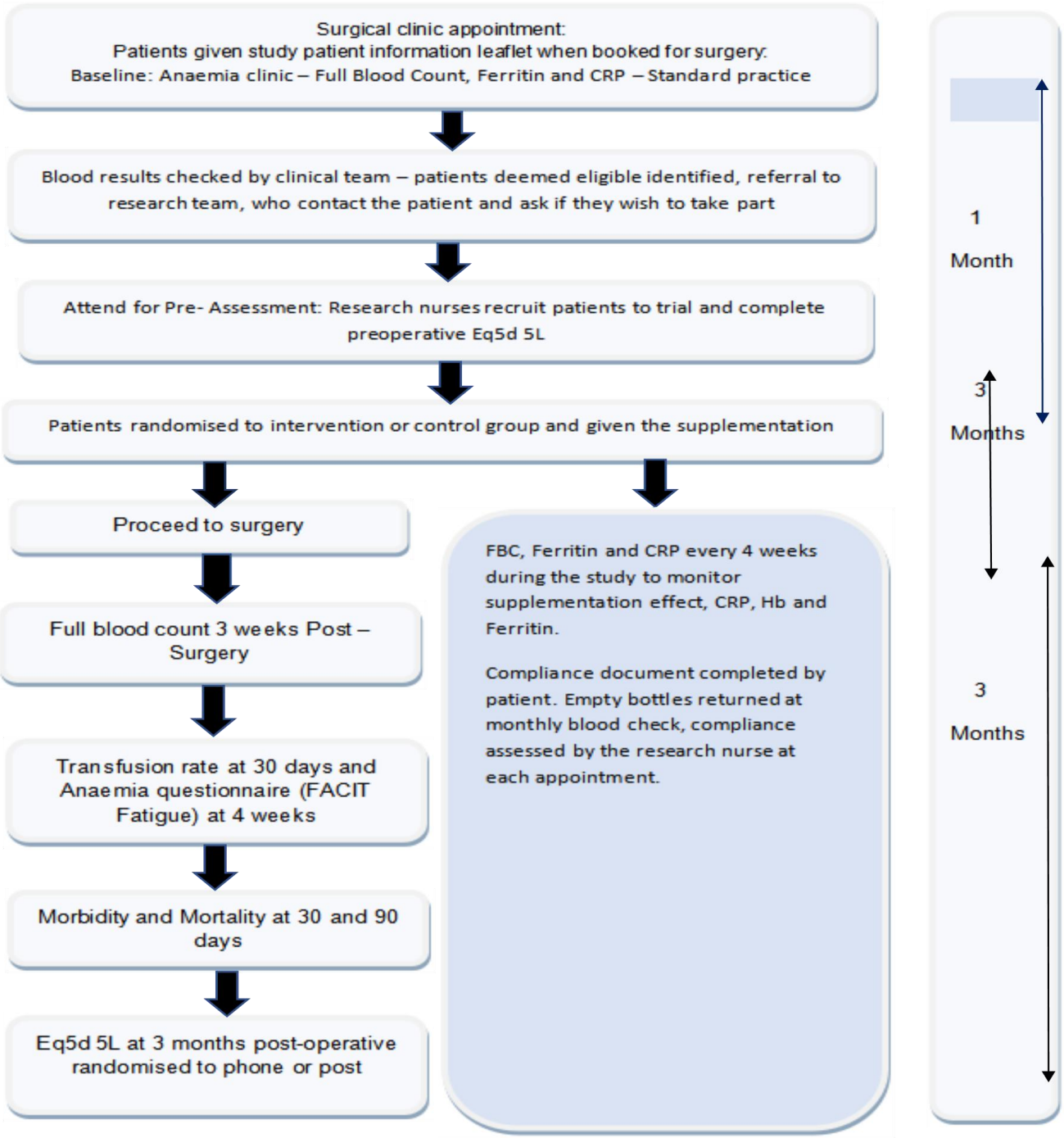
Assessment (D=day, W=week, M=month)	**Routine practice**	**PRE-CLINIC SCREENING**	**D0**	**WK 3**	**WK4**	**30 and 90 days postop**	**4 weekly blood tests**
Allowed variation in days							
Eligibility screen		X					
Blood Test	X						X
Screen for anaemia	X						
Consent		X					
Randomise		X					
Commence oral supplement		X					
Repeat blood screen		X		X			
Symptom Questionnaire and EQ-5D-5l pre-op and 3 months post-op		X			X	X	
Routine data use including transfusion requirement up to 30 days post-operative, and mortality at 30 and 90 days	X					X	
Morbidity 30 days	X					X	

including: Infection rate Myocardial Infarction (heart attack), Transient ischaemic attack (TIA) Cerebrovascular accident (stroke/ CVA) Acute Kidney Injury (AKI) Deep vein thrombosis (DVT) or pulmonary embolism (PE) within 60-days of surgery							

4.2.11 Participant Flow

Figure 6 below demonstrates the participant journey from screening to end of follow up.

Participant Flow chart Figure 6



4.2.12 Sample size

Previous research in patients undergoing lower limb arthroplasty have reported a mean drop in haemoglobin levels of -18.1 g/dl, with Standard Deviation (SD) of 6.6 in patients at 3 weeks (Zhou et al., 2015). Therefore, assuming similar variation in haemoglobin levels (SD 6.6) and to detect a 3.3 difference in haemoglobin, with 90% power and 2-sided testing at 5% significance level, it was estimated that a total of 168 participants would be required. Adjusting for 10% attrition required 188 participants to be randomised (Torgerson and Torgerson, 2008).

4.2.12.1 Randomisation

Randomisation was undertaken at the research clinic more than 30 days prior to surgery, to allow sufficient time for the supplementation to begin using the website: www.randomization.com, which generated a simple (without blocking or stratification) randomisation sequence for 188 patients in a random sequence, with a ratio of 1 to 1. Following patient consent and completion of baseline data, individual patients were randomly allocated to treatment and control. The allocation was undertaken centrally by the lead sites research and development team. A telephone call was made by the recruiting research nurse or clinician to the research and development administrator who informed them of the patient group allocation. The unpredictability of surgical planning meant that the time from randomisation to surgery varied between participants, which was beyond the control of the research team.

4.2.12.2 Blinding

The clinician managing each patient and General Practitioner were informed of trial participation, so patient care was not negatively impacted. Patients were also informed of

their allocations after randomisation verbally. Laboratory staff sampling bloods and all clinical teams caring for the patient were not aware of the trial specific blood results. Access to these was restricted to the chief investigator and principal investigator having access to all of the participant's routine bloods. Trial specific bloods were stored on a minimal access required system to reduce the risk of biasing the study or altering the patient's normal pathway, however all standard care blood results were available for clinicians for patient safety.

4.2.13 Statistical methods

Analyses were conducted following the principles of intention-to-treat with patients analysed according to their original, randomised group irrespective of deviations based on non-compliance, unless otherwise specified. For full information see ISIDA statistical analysis plan appendix 14. Analyses were conducted in Stata (version 17), (see appendix 15 for Stata commands). Significance tests were two-sided tests at the 5% significance levels unless otherwise stated.

4.2.13.1 Baseline data

Demographic data was summarised using descriptive statistics overall and by randomised group. Continuous measures were reported using descriptive statistics (e.g., mean, standard deviation, median, minimum and maximum) while categorical data were reported as counts and percentages.

Demographic data collected included:

- Age at surgery
- Type of surgery

- Gender
- Smoking status

Comorbidities:

- Hypertension
- Atrial Fibrillation
- Ischaemic Heart Disease
- Type I diabetes and Type II diabetes
- Chronic Obstructive Pulmonary Disease.

4.2.13.2 Primary Outcome analysis

A linear regression model was used to compare Haemoglobin at 3 weeks post-surgery adjusting for baseline Haemoglobin, age, gender and type of arthroplasty (hip or knee). Model estimates and associated 95% confidence intervals and p-values were reported.

4.2.13.3 Secondary Outcome analysis

The number of days in hospital was analysed using Poisson regression. Transfusion, readmission, inpatient deep vein thrombosis, inpatient pulmonary embolism, pneumonia, cerebrovascular incident, and myocardial infarction were all analysed using logistic regression. The number of units transfused, EQ-5D-5L and FACIT-fatigue scores were analysed using linear regression. All secondary outcome regression models adjusted for baseline haemoglobin, age, gender, and type of arthroplasty.

4.2.13.4 Haemoglobin over time repeated measure analysis

Analysis of haemoglobin between trial arms was performed, using a mixed model incorporating all time points where effects of interest and baseline covariates (haemoglobin, age, gender and type of arthroplasty), time from randomisation to surgery was specified as fixed effects, and the correlation of observations within patients over time modelled by a covariance structure.

4.2.13.5 Sensitivity analysis

It was possible that some patients required blood transfusion between pre-operation and the 3 weeks post-surgery time points. The expected time point for requiring transfusion would usually be expected to be soon after surgery, expected to be <5 days. Clinical intervention for transfusions occurred if the patient had haemoglobin <8g/dl. The number of patients affected by trial arm was measured and a brief description given of when this occurred by patient. For those patients' haemoglobin levels at the 3-week post-operative time point was more likely related to transfusion levels than trial arm, whilst it was important to investigate how this might have influenced the primary analysis, we expected that a small minority were affected. Therefore, a sensitivity analysis excluding these patients was planned.

4.2.13.6 Missing data

Some patients may not provide the post-operative, or four weekly haemoglobins, because (a) they did end up having the operation within the timeframe of study, (b) they died, (c) or they decided to withdraw completely. Withdrawals were summarised by treatment arm and type of withdrawal, with reasons and timings (in terms of length of follow-up completed) where possible. Losses to follow up, for example, patients not returning questionnaires or not responding to telephone follow up were logged and described in the CONSORT diagram

(Figure 7) and text where appropriate. There was no plan to adjust the statistical comparison based on missing data.

4.2.14 Adverse Events

Safety reporting incorporated serious adverse events, complications and adverse reaction related to the treatment proposed and applies to all trial participants.

A serious adverse event was defined as the following:

- Death
- Life-threatening event (that is it placed the participant, in the view of the Investigator, at immediate risk of death)
- Required unplanned hospitalisation or prolongation of existing hospitalisation (Unplanned refers to emergency hospitalisation resulting in an inpatient stay.
- Prolonged hospitalisation was deemed to be where a patient's stay was longer than expected (patient was operated on as day-case but remained in hospital overnight)
- Resulted in persistent or significant disability or incapacity (substantial disruption of one's ability to conduct normal life functions)
- Was another important medical condition

An adverse reaction was defined as the following: untoward and unintended response in a subject which was caused by or related to the research treatment or procedure. Such as an allergic reaction, gastrointestinal upset, abdominal pain, constipation and diarrhoea and nausea.

Adverse Events that were expected as part of surgical interventions and were deemed unlikely to be related, included: wound infection, venous thrombo-embolic phenomena, pneumonia, blood transfusion, cerebrovascular accident, myocardial infarction, deep vein thrombosis, readmission. These were recorded as complications and monitored as secondary outcomes.

All participants experiencing serious adverse events were followed-up as per protocol until the end of the trial. Serious adverse events were fully investigated if they appeared to be related to an aspect of taking part in the study and it was an unexpected occurrence. The research team recorded all adverse events related to the proposed intervention, whether expected or not, in the patient's medical notes and on the trial adverse events form and sent to data manager within an agreed timescale (usually five days). Serious adverse events were notified to the Chief Investigator and the Sponsor within 24 hours of the clinical research team becoming aware of the event.

At the time of reporting the investigator was asked to record an assessment of causality (to trial treatment) selecting an option from the list below:

- Definitely related —there was clear evidence to suggest a causal relationship, and other possible contributing factors can be ruled out.
- Probably related —there was evidence to suggest a causal relationship, and the influence of other factors is unlikely.
- Possibly related —there was some evidence to suggest a causal relationship (e.g. the event occurred within a reasonable time after administration of the trial medication). However, the influence of other factors may have contributed to the event (i.e. the patient's clinical condition, other concomitant events).

- Unlikely to be related —there was little evidence to suggest there is a causal relationship (e.g. the event did not occur within a reasonable time after administration of the trial medication). There is another reasonable explanation for the event (e.g. the participant's clinical condition, or other concomitant treatments).
- Unrelated—there is no evidence of any causal relationship.

An event was defined as 'related' if the event was due to the administration of any research procedure. Whereas an 'unexpected event' was defined as a type of event not listed in the protocol as an expected occurrence. The relatedness of an event was then reviewed by the Chief Investigator. Salus were informed of all SAEs deemed related to the supplementation, so that they could be reported to the Medicines and Healthcare products Regulatory Agency.

4.2.15 Trial Management

For this randomised controlled trial, as per protocol, a trial management group was created, involving the chief and principal investigators, the trust research lead and allocated research nurse meeting annually or more frequently as required. Due to the low-risk nature of this study, one independent steering and monitoring committee to undertake the roles traditionally undertaken separately by the trial steering committee and the data monitoring ethics committee was created. These comprised of an independent chair, a surgeon with expertise in lower limb arthroplasty surgery, a member of the patient group, the chief investigator and trial principal investigator, the research lead, and the nurse in charge of recruitment. Other study collaborators also attended meetings when required and provided advisory assistance as a part of the PhD supervision. The trial steering and monitoring committee were informed of all adverse events and serious adverse events, where required, as per protocol.

4.2.16 Trial registry

The trial has been registered for an ISRCTN Number www.isrctn.com.

ISRCTN NUMBER: 48118194

4.2.17 Amendment to Methodology due to COVID 19 pandemic

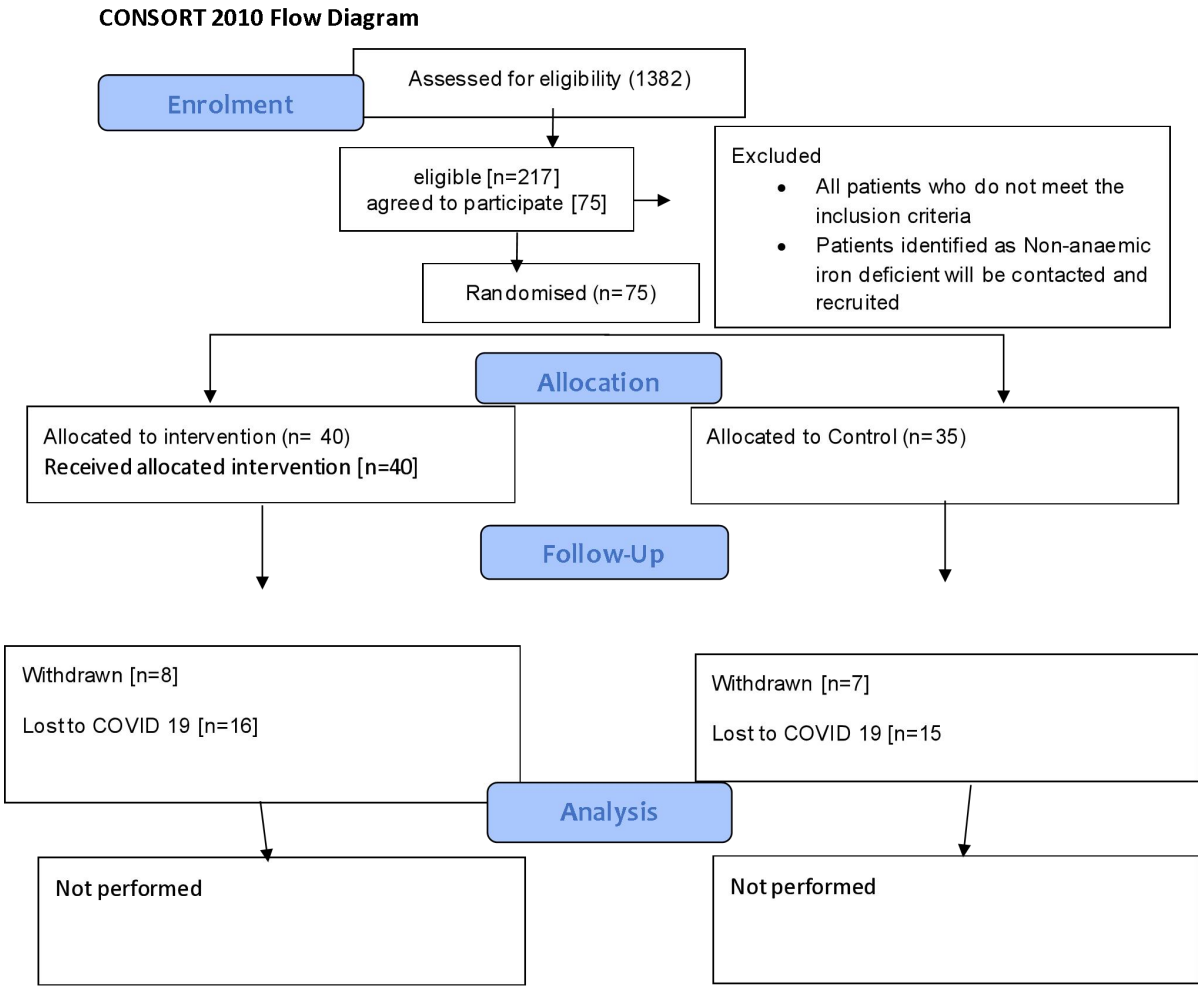
In the first quarter of 2020, the World Health Organisation (WHO) declared COVID 19 as a global pandemic (Kunz et al., 2020). The UK government called a national lockdown to slow the spread of the virus, in doing so, suspending all elective surgery to focus on the issues arising from the pandemic. Unfortunately, due to the suspension of elective surgery and the slow progress in restarting surgery through subsequent waves, the trial was temporarily paused. The research team were unable to follow-up all the recruited patients, who were at different stages in their participant timeline, leading to a significant loss of trial data. Guidance was published to aid researchers to manage their trials (European Medicines Agency, 2020a; European Medicines Agency, 2020b), enabling the pausing and restarting of trials, as deemed appropriate by REC submission, with significant leeway on altering timelines to maximise recruitment. A trial management meeting was convened in the trust by the trial sponsor, where it was decided that patients who had not proceeded to surgery would not be used in the final analysis. Recruitment recommenced at the end of 2020 and remained difficult and comparatively slow, primarily due to the significantly reduced elective surgery programme and because of patient reluctance to attend multiple appointments for blood tests or to have the research team in their home. Due to the time constraints of this thesis, it was decided that the trial results collected prior to the pause would be present descriptively, and no formal statistical analyses would not be performed.

Results 4.3

Recruitment began in June 2019 and the trial was paused between March 2020 and December 2020 due to the COVID 19 pandemic. All patients undergoing lower limb arthroplasty were assessed for eligibility, 76 consented, 76 were randomised, although one file was destroyed, leaving 75 patients (40 to receive iron supplementation and 35 to receive no iron supplementation). The 1382 figure represented all patients scheduled to undergo arthroplasty, those anaemic and those with no iron deficiency were excluded and not contacted. Two hundred and seventeen patients were eligible, 75 agreed to participate, a recruitment rate of 35%.

4.3.1 Participant flow

CONSORT flow diagram for clinical trial figure 7



Of the 75 participants randomised all presented in the baseline data. However, 31 were excluded from the narrative summary due to the covid 19 pandemic (16 intervention and 15 control) and 14 withdrew from the study after surgery (8 intervention and 7 control). For the primary outcome, one additional person withdrew from the study before the primary outcome assessment leaving 29 participants included in the narrative summary (16 intervention and 13 control).

4.3.2 Baseline Data

Baseline data and patient characteristics for the recruited 75 patients are tabulated below in Table 14. Approximately, three-quarters of participants were female, they were on average 68 years old (SD 8.9) and were all white (100%). Comorbidities were similar across both arms. Mean preoperative haemoglobin was comparable between groups (supplementation 137.7 SD 12.46, control 135.74 SD 10.17).

Baseline characteristics data (Table 15).

Patient Characteristics	**Iron supplementation (n= 40)**	**Control (n=35)**	**Total (n=75)**
Gender Male, n(%)	14 (35)	4 (11)	18 (24)
Age (years) Mean (sd) Median (min, max)	 66.4 (7.2) 66.5 (50, 80)	 68.9 (10.4) 71 (38, 83)	 67.6 (8.9) 69 (38, 83)
Ethnicity White	 40 (100)	 35 (100)	 75 (100)
Smoking status Current Ex-smoker Never smoked	 3 (8) 16 (40) 21 (53)	 2 (6) 14 (40) 19 (54)	 5 (7) 30 (40) 40 (53)
Diabetes 1	1(3)	1 (3)	2 (3)
Diabetes 2	8 (20)	4 (11)	12 (16)
IHD	3 (8)	0 (0)	3 (4)
Hypertension	17 (43)	16 (46)	33 (44)
TIA/CVA	2 (5)	3 (9)	5 (7)
COPD	1 (3)	4 (11)	5 (7)
Asthma	5 (13)	8 (23)	13 (17)
Thyroid Disease	2 (5)	2 (6)	4 (5)
AF	2 (5)	2 (6)	4 (5)
Liver Disease	0 (0)	0 (0)	0 (0)
Renal Disease	0 (0)	0 (0)	0 (0)
Hb (g/dl) Mean (sd) Median (min, max)	 137.7 (12.46) 138.5 (120, 165)	 135.74 (10.17) 135 (122, 169)	 136.79 (11.41) 136 (120, 169)
Ferritin Mean (sd) Median (min, max)	 33.88 (10.55) 35.5 (13, 49)	 32.03 (11.26) 33 (12, 49)	 33.01 (10.85) 35 (12, 49)
CRP Mean (sd) Median (min, max)	 5.03 (6.49) 3 (1, 39)	 3.74 (2.59) 3 (1, 11)	 4.43 (5.07) 3 (1, 39)

Type of surgery			
Knee	30 (75)	25 (71)	55 (73)
Hip	10 (25)	10 (29)	20 (27)

The time from randomisation to surgery varied among patients (tabulated in Table 15) but the mean time to surgery was comparable between supplementation 69.61(SD 26.25) and control 68.42(SD 30.37).

Time of surgery (Table 16)

Supplementation yes/no/total (n)	Yes	No	Difference
N	18	14	32
Mean(SD)	69.61(26.25)	68.42(30.37)	-1.19(4.12)
Median(min-max)	68(32-113)	74(23-116)	6

4.3.3 Numbers achieved

The level of attrition was collated to identify both those who had withdrawn from the trial and been lost due to the COVID19 trial pause, in total, for the primary outcome and by trial arm (see Table 16). Of the 76 patients who were recruited prior to the trial pause, 31 were lost to COVID 19 delay, one file had missing data and hence 29 patients were included in the narrative summary for the primary outcome. Secondary outcomes were measured using the completed data collected where possible.

Attrition primary outcome by trial arm (Table 17)

Supplementation	Analysed	Withdrawn	Covid 19	Total	% lost to attrition	%lost prior to COVID 19 pause
Yes	16	8	16	40	60%	33%
No	13	7	15	35	62%	35%
Total	29	15	23	75	61%	34%
Missing data	N/A	N/A	N/A	1		

4.3.4.1 Statistical Analyses

The trial was incomplete due to the early cessation relating to the COVID 19 pandemic. Therefore, the primary statistical analysis could not be undertaken, the date has been presented narratively.

4.3.4.2 Primary outcome

The mean haemoglobin levels at 3 weeks in the supplementation group was 124.12 (SD 11.5) and in the no supplementation group was 126.38 (11.3), demonstrating little difference in mean (2.36) or median (-1.5) haemoglobin at 3 weeks post op Thus signifying comparable levels of post-operative haemoglobin levels between groups at this time point.

Primary outcome data (Table 18)

Supplementation yes/no/difference (n)	Yes	No	Difference
	16	13	29
Hb basesline			
Mean (sd)	136.6(12.01)	135.7(5.6)	-0.9(-6.5)
Median (min, max)	136(121, 159)	135(128, 146)	-1

Supplementation yes/no/difference (n)	Yes	No	Difference
	16	13	
Hb 3 weeks postop			
Mean (sd)	124.12 (11.5)	126.38 (11.3)	2.36 (0.2)
Median (min, max)	127.5 (92, 138)	126 (113, 156)	-1.5

4.3.4.3 Secondary outcomes

The results at each individual time point are documented in Table 18. Mean differences were demonstrated between supplementation and control from preoperative to repeated measures at each monthly measurement, baseline Hb 137.7 SD 12.46 vs 134.74 SD 10.17, Hb1 136.20 SD 12.34 vs 134.65 SD 8.37, Hb2 131.27 SD 13.85 vs 137.22 SD 11.02, Hb3 130.05 SD 10.07 vs 131.38 SD 12.22, Hb4 130.62 SD 15.55 vs 129.4 SD 10.12, Hb5 132.33 SD 14.33 vs 128.6 SD 10.12, Hb 6 139.9 SD 10.37 vs 135.22 SD 7.99. Thus, demonstrating a trend that haemoglobin improves with supplementation across the repeated measures.

Haemoglobin over time repeated measure analysis (Table 19)

Supplementation yes/no/difference (n) **Hb basesline** Mean (sd) Median (min, max)	Yes(40) 137.7(12.46) 138.5(120-165)	No(35) 134.74(10.17) 135(122-169)	Difference(75) -2.96(-2.29) -3.5
Supplementation (n) **Hb 1** Mean (sd) Median (min, max)	Yes(29) 136.20(12.34) 134(114-164)	No(23) 134.65(8.37) 133 (119-152)	Difference(52) -1.55(-3.97) -3
Supplementation (n) **Hb 2** Mean (sd) Median (min, max)	Yes(22) 131.27(13.85) 134(92-151)	No(18) 137.22(11.02) 139(113-156)	Difference(40) 5.95(-2.83) 5
Supplementation (n) **Hb 3** Mean (sd) Median (min, max)	Yes(19) 130.05(10.07) 128(103-146)	No(13) 131.38(12.22) 132(113-147)	Difference(32) 1.33(2.15) 4
Supplementation (n) **Hb 4** Mean (sd) Median (min, max)	Yes(16) 130.62(15.55) 131.5(100-155)	No(10) 129.4(10.12) 128(120-141)	Difference(26) -1.12(-5.43) -3.5
Supplementation (n) **Hb 5** Mean (sd) Median (min, max)	Yes(12) 132.33(14.33) 132.5(102-152)	No(10) 128.6(10.12) 129(108-143)	Difference(22) -3.73(-4.21) 131(102-152)
Supplementation (n) **Hb 6** Mean (sd) Median (min, max)	Yes(10) 139.9(10.37) 140.5(123-157)	No(9) 135.22(7.99) 136(121-143)	Difference(19) -4.68(-2.38) 140.5(123-157)

Haemoglobin over time repeated measure analysis (Table 18)

Length of hospital stay (in days) was measured for 30 patients and the median was 1 (0 to 3) in the supplementation group and was 1 (1 to 3) in the no supplementation group. There were no readmissions, infections, transfusions, DVT, PE, pneumonia, cerebrovascular incident, or MI in either of the study arms. Hence, no descriptive summary of these variables was undertaken.

Patient reported outcome measures were collected as planned, using EQ-5D-5L and FACIT-fatigue, pre an post-operatively and are tabulated descriptively in Tables 19 and 20, preoperative samples were comparative by trial arm supplementation 55.7 SD 22.58, control 56.97 SD 21.19.

EQ-5D-5L Preoperative (Table 20)

Supplementation yes/no/total (n)	Yes(40)	No(35)	Difference(75)
Mobility score Mean(SD) Median(min-max)	 2.92(0.99) 3(1-4)	 2.91(0.95) 3(1-5)	 -0.1(-0.4) 0
Self care score Mean(SD) Median(min-max)	 1.57(0.84) 1(1-4)	 1.77(1.03) 1(1-4)	 0.2(0.93) 0
Usual activities score Mean(SD) Median(min-max)	 2.9(1.17) 3(1-5)	 3(1.03) 3(1-5)	 0.1(0.19) 0
Pain score Mean(SD) Median(min-max)	 3.35(0.95) 3(1-5)	 3.43(0.78) 3(2-5)	 0.8(-0.17) 3(1-5)
Anxiety score Mean(SD) Median(min-max)	 1.98(0.89) 2(1-3)	 2.06(1.03) 2(1-5)	 0.6(0.14) 0
Your health score Mean(SD) Median(min-max)	 55.7(22.58) 55.5 (0-100)	 56.97(21.19) 60(10-95)	 1.27(-1.39) 57(0-100)

EQ-5D-5L postoperative scores (Table 20)

Supplementation yes/no/total (n)	Yes(3)	No(1)	Difference(4)
Mobility score Mean(SD) Median(min-max)	 2.67(1.53) 3(1-4)	 1(1) 1(1)	 -1.67(-0.53) -2
Self care score Mean(SD) Median(min-max)	 2.33(1.15) 3(1-3)	 1(1) 1(1)	 -1.33(-0.15) -2
Usual activities score Mean(SD) Median(min-max)	 3(1) 3(1-3)	 1(1) 1(1)	 2(0) 2.5(1-4)

Pain score Mean(SD) Median(min-max)	3.33(1.15) 4(2-4)	1(1) 1(1)	2.33(0.15) -3
Anxiety score Mean(SD) Median(min-max)	1.33(0.57) 1(1-2)	1(1) 1(1)	-033(0.43) 0
Your health score Mean(SD) Median(min-max)	76.67(5.77) 80(70-80)	80(N/A) 80(80)	3.33(N/A) 0
EQ-5D-5L Index score Mean(SD) Median(min-max)	0.46(0.29) 0.30(0.29-0.80)	0.84(N/A) 0.84(0.84-0.84)	0.38(N/A) 0.54

FACIT-fatigue scores pre and postoperatively (Table 21)

Supplementation yes/no total	Yes(40)	No(35)	Difference(75)
FACIT-fatigue preop Mean(SD) Median(min-max)	40 31(12.14) 31(0-49)	35 29.26(13.29) 32(3-55)	75 -1.74(1.15) 1
FACIT-fatigue postop Mean(SD) Median(min-max)	5 34.86(14.89) 42(14-49)	3 24.33(4.93) 22(21-30)	8 -10.53(-9.96) -20

The average EQ-5D-5L and FACIT-fatigue scores, were assessed preoperatively as tabulated and three months and four weeks post-operatively respectively. FACIT-fatigue and EQ-5D-5L scores were comparative preoperatively, Unfortunately, a massively reduced sample and lack of completion by patients make comparison between mean values of postoperative questionnaires unwise.

4.3.4 Harms

There were no reported serious adverse events during the study period. Reasons for withdrawal were compiled along with the side effect profile of the supplementation. There were eight people in the iron supplementation group who withdrew due to known side effects

of iron supplementation and seven people who withdrew in the no supplementation group due to time commitments. Of those who were randomised to the iron supplementation group, 23 (58%) reported no side effects. The most common side effect was gastrointestinal issues (loose stools n=2, upset stomach n=7; constipation n=1) (Table 22). The patient who reported a loose tooth was followed up; upon investigation it was deemed that the supplement was not causative of an already damaged tooth. One patient reported teeth discoloration and one reported a metallic taste.

Side effect profile (Table 22)

Side effect profile	Number	Percentage
No side effects	23	57.5
Loose stools	2	5
Upset stomach	7	17.5
Constipation	1	2.5
Dental Damage	1	2.5
Tooth discolouration	1	2.5
Metallic taste	1	2.5
Missing data	4	10
Totals	40	100%

4.4 Discussion

Early cessation of this trial during COVID 19 was pragmatic decision taken by the trial management group in April of 2020. This decision was made primarily due to the cancellation of elective surgery and outpatient appointments in response to the pandemic which made it impossible to continue the trial at that point or follow-up the participants. Paperwork was filed and the trial was paused at that point, with the potential to re-start the trial when things became more normal. The collected data prior to that point was collated and filtered for withdrawal and loss to COVID 19. Initially it was discussed with my supervisors that as a PhD student, I could perform the analysis as a technical examination, to demonstrate for the thesis, that I could implement the statistical analysis plan as per protocol. However, as the resulting analysis would be unreliable and inaccurate against the proposed outcomes due to the reduced power associated with a drop in numbers recruited. Early interpretation is associated with an increase the number of statistical analyses completed, with potential for a false positive being declared or may affect the future conduct of the trial (Law, 2000). Interim analysis performed prior to completion of the trial may overestimate benefits, leading to inaccurate or unreliable conclusions (Liu and Garrison, 2022). It was decided ultimately that the data would be summarised narratively where possible from the data collected, to remove the risk of unintended bias from an early analysis.

The primary outcome compared the haemoglobin at 3 weeks post-surgery by trial arm. It was not possible to do a comparative analysis due to the early cessation of the trial, therefore no data comparison could be performed. The published guidance by Munoz et al. (2016), which advocated the potential benefits of treatment of non-anaemic iron deficiency was not able to be assessed, the early suspension of the trial and the resulting loss of existing recruits and inability to recruit replacements meant that the study did not have the power to identify a

clinically important difference if one existed. The narrative synthesis and small meta-analysis from the systematic review undertaken in Chapter 3, both indicated the potential benefit of treatment of non-anaemic iron deficiency, however the lack of analysis of the randomised controlled trial meant this could not be further assessed. The treatment of non-anaemic iron deficiency is advocated in the published literature in treatment of both the in the surgical and non-surgical patient populations (Spahn et al., 2019a) (Sharma et al., 2014; Muñoz et al., 2017; Al-Naseem et al., 2021a) and are supported by current National Institute for Clinical Excellence guidelines (Excellence., 2020), it is difficult to add any conclusions of the benefit of treatment of non-anaemic iron deficiency to the published guidance from this research.

Data on haemoglobin measured over time between trial arms was collated. Review of the literature did not identify repeated measures analysis for monitoring haemoglobin during supplementation for surgical patients. Although, Spahn et al. (2019a), did perform repeated measures analysis in the non-anaemic iron deficient patient, it was measured at baseline and then multiple times postoperatively without intervention. However, improvement of haemoglobin over time was demonstrated in the non-surgical population (Sharma et al., 2016; Pittori et al., 2011; Gera et al., 2007).

Length of stay data was collated with a mean length of stay of 1 day in both trial arms, however further statistical analysis was not undertaken. Although the clinical trial undertaken did not demonstrate a difference between supplementation and control for length of stay, this may be due to the early summation and reduced cohort reviewed. It must also be acknowledged that the study was not powered for length of stay, length of stay is multifactorial, may be algorithm based and can be impacted by many external factors, frequently unexplainable (Liu et al., 2001), requiring greater numbers and scrutiny to ensure

interpretations are correct. Sanoufa et al. (2015) demonstrated a reduction in length of stay with treatment of anaemic patients undergoing orthopaedic surgery, which is supported by further studies (Khan et al., 2012; Pujol-Nicolas et al., 2017). If the benefit demonstrated in anaemic patients can be demonstrated in the non-anaemic iron deficient patient, further justification could be provided for treatment, due the potential cost savings associated with a reduced length of stay.

No participants had received a blood transfusion in this study. Evidence on the treatment of anaemia and reduction in blood transfusion has been demonstrated in the orthopaedic surgical population (Wainwright and Middleton, 2010; Pujol-Nicolas et al., 2017) and the non-anaemic iron deficient population (Spahn et al., 2019a), supported by the findings from the systematic review in Chapter 3. However, a recent multi-centre randomised controlled trial demonstrated no difference in transfusion in patients undergoing general surgery (Richards et al., 2020a). Blood loss in major abdominal surgery is associated with between 200 -6000ml (Anant M et al., 2021; Zhao et al., 2019; Ho et al., 2004), depending on the procedure and circumstances, with associated transfusion rate of 44% (Richards et al., 2020a), whereas, arthroplasty is associated with a blood loss between 600 (Prasad et al., 2007) and (Park et al., 2013) 1500ml and a transfusion rate of up to 10% (Komnos et al., 2021). This difference in blood loss and transfusion rate may demonstrate why marginal gains are not demonstrated in differing specialities, representing different risk of blood loss profiles.

No patients had been readmitted within 30 days of surgery, although with a massively reduced sample size, it is difficult to glean anything from this. Reasons for readmission are multifactorial (Jacobs B et al., 2018) and may often be for more than one issue. Anaemia is known to increase postoperative complications (Wilson et al., 2008), which may lead to

increased readmission rates. Pujol-Nicolas et al. (2017), have demonstrated an improvement in readmission with treatment of anaemia with a p-value of 0.02. However, a literature search of the non-anaemic patient population did not find any literature which measured readmission in that patient population. It must be acknowledged that this study was not powered to measure readmission, and was analysed prematurely, with greatly reduced numbers which may have contributed to a lack of data collated.

Morbidity and mortality were planned to be analysed for deep vein thrombosis, pulmonary embolism, pneumonia, cerebrovascular incident, myocardial infarction. However, no patients had suffered any morbidity and mortality issues within 30 days of surgery, therefore no statistical analysis was undertaken.

EQ-5D-5L and FACIT-fatigue scores, were collated preoperatively and three months and four weeks post-operatively as per trial schedule. Statistical analysis could not be undertaken due to the early cessation of the randomised controlled trial, however, treatment with oral or intravenous iron of patients in the non-surgical population have demonstrated improvement in patient reported outcome measures (Sharma et al., 2014; Al-Naseem et al., 2021a).

Although early cessation of the trial was unavoidable, it has led to an opportunity to improve recruitment and retention of participants when the trial restarted after the COVID 19 pandemic had subsided. With greater understanding of the reasons for attrition, there is the potential to improve patient contacts, using home visits or remote testing to improve the participation and retention rate.

4.4.1 Limitations

Randomisation was performed at the first patient appointment, however the time between randomisation and surgery was beyond the control of the trial team, as surgical planning is complicated, unpredictable and subject to changes. However, there is no reason to suspect that these differences will be related to group allocation. Indeed, from the data obtained, there was no difference in the time from randomisation to surgery between the groups 69.61(68.42). Therefore, data would be comparable, however, the longer the time between randomisation and measurement of the primary outcome, the greater the risk of practices changing within that time period (Caruana et al., 2015). This may make comparison difficult leading to potential biases (Torgerson and Torgerson, 2008), although it would likely affect both trial arms equally and therefore any potential bias would be minimal. The time between randomisation and surgery was not within the control of the research team and was comparable between the trial arms, however, it is acknowledged as a potential limitation in the trial design and would be monitored upon completion of the trial.

Using the risk of bias assessment tool as a guide, an assessment was performed on the ISIDA trial design (see appendix 18), demonstrating some limitations in trial design, firstly, the lack of blinding after allocation of treatment and secondly, the lack of blinding post randomisation i.e the use of placebo control, a decision chosen early by the trial sponsor. It was decided the expense associated with a double blinded randomised controlled trial using placebo control, was not appropriate. Changes in haemoglobin, the primary outcome, was unlikely to be affected by the lack of blinding, although it must be acknowledged, patients were informed they were iron deficient, but not allocated to treatment, may supplement outside of the trial. This was mitigated by counselling patients not to take supplements if not allocated or to inform us if they were taking any relevant over the counter medications, however it is

impossible to assure that this did not happen. Human behaviour is not always predictable (Day and Altman, 2000), participants in clinical trials are known to not always participate in the manner requested (Day and Altman, 2000). It was decided that all elements of surgical intervention, follow-up and postoperative care would be unified across the trial, to reduce the further risk of bias.

Participant attrition in clinical trials is a common problem which can reduce the statistical power of the trial, with the potential risk of introduction of bias (Siddiqi et al., 2008). Identification of early causes of attrition may help to reduce patient withdrawal and improve rates of attrition (Siddiqi et al., 2008). Reasons for withdrawal may be individual, multifactorial or trial specific, including elements such as, time commitment, side effects of the intervention and inconvenience (Schulz and Grimes, 2019; Silverman D, 2014).

Attrition pre and post-trial pause was calculated and represented in table 16). Prior to the COVID pause, withdrawal was 33% in the intervention group and 35% in the control group, primarily due to the time commitment associated with participation.
This attrition rate is significantly higher than the 10% attrition accounted for when estimating the trial sample size, with potential to reduce the statistical power further. The potential impact of a high attrition rate is not achieving the number of outcome points the trial is powered for, which could therefore produce inaccurate, unreliable results. Although this attrition value may be artificially high, due to the early cessation and the impact of the COVID pause, it must be acknowledged it was significant, with common themes in the two groups. Gastrointestinal side effects from iron supplementation are not uncommon (Rimon et al., 2005) and are to be expected, little can be done to reduce these in the supplementation group. Attrition in the control group was solely due to time commitment, the trial design

limitation noted, not blinding post-randomisation, therefore not using placebo control, may have influenced the decision of participants to withdraw from the control group. With little perceived personal benefit from participating in the trial, in addition to the trial design, incorporating multiple bloods tests at specific timepoints, a burden may have placed a on patients not present in a placebo-controlled trial. Attrition must therefore be acknowledged as a potential limitation due to trial design, with risk of bias due to higher attrition in the control group. Attrition rates, though high, seem very similar between the two groups (35% vs 33%), which reduces the risk of attrition being a source of bias. Although it may be numerically balanced, reasons for attrition appear to differ, those with sensitive gastrointestinal problems dropped out of the intervention and those who find the time commitment too onerous dropped out of the control and this can introduce bias as the remaining participants differ by characteristic within each trial arm.

Recruitment of participants can be a difficult task, when conducting a clinical trial, with elements such as, screening, time commitment, follow-up schedule and patient engagement all acknowledged as potential challenges for the researcher (Schulz and Grimes, 2019). This was demonstrated in the difficulties recruiting patients to the trial and in the attrition rate. Identifying and recruiting trial participants is time consuming for participants and researchers and can take longer than planned (Jadad and Enkin, 2007), it is not uncommon for clinical trials to be extended to recruit the required numbers (Jadad and Enkin, 2007), achieving the required statistical power. Recruitment to the ISIDA trial initially progressed slower than planned, mainly due to patients not wanting to attend multiple appointments for the repeated blood measures to monitor the supplementation against the control group. The time commitment and the travel required to attend appointments, were common issues which challenged patient participation. Geography may have also played a role in recruitment, the

study was conducted in one of the largest geographical trusts in the National Health Service, with patients having to travel to repeated appointments for blood tests. This would increase cost and time commitment required to participate, which may appeal to the intervention group to get their supplement, however, is likely to be a barrier for the control group, with the associated attrition.

The early cessation of the randomised controlled trial could inform potential changes in design to the clinical trial protocol when the trial re-starts, aiming to reduce patient burden and therefore attrition. The time commitment and vast geographical area could be mitigated, home visits or postal blood tests are areas that could be implemented to reduce patient burden, therefore improving recruitment and attrition.

As the randomised controlled trial could not be analysed as planned, there can be no inference taken from the collated results due to the trial being incomplete.

4.4.2 Interpretation

Interpretation of the results of the ISIDA trial were incomplete due to the early cessation and lack of analysis undertaken. For the reasons discussed earlier, analysis could not be undertaken due the reduced sample size and the associated biases. Narratively a trend towards improved haemoglobin across the repeated measures analysis was demonstrated, however, due to the reduced sample size it's difficult to reliably say this is an accurate trend. No significant adverse events or serious adverse events were noted, with side effects from the proposed treatment, mainly gastrointestinal upset, consistent with other forms of iron supplementation (Allen, 2002; Moretti et al., 2017). Therefore, the risk versus harm

assessment, would suggest if any perceived benefit in the proposed intervention was seen, it does not require mitigation against harms from the data collated.

A review of the literature was performed to ascertain if further research had been undertaken after the systematic review was conducted using the same terms as the original search strategy. Further research was not available to support or disprove the effects of treatment of non-anaemic iron deficiency and surgery. However, an observational study of the adverse effects of non-anaemic iron deficiency, and its impact following cardiac surgery was found. Miles et al. (2018) analysed data on 277 patients, 109 iron deficient and 168 iron replete patients, with iron deficient patients shown to have an increased length of stay (7 (6-9 [2-40]) vs. 7 (5-8 [4-23]) days; p = 0.013) with fewer days reported of patients being alive and out of hospital at day 90 postoperative (83 (80-84 [0-87]) vs. 83 (81-85 [34-86]), p = 0.009). However, when pre-operative age, sex, renal function, EuroSCORE and haemoglobin, was adjusted for the mean increase in length of stay was 0.86 days (95%CI -0.37 to 2.22, p = 0.098), not statistically significant, for the iron replete group when compared to the iron deficient group and therefore deemed weak evidence of an association. Miles et al. (2019c) conducted follow up research to assess what outcome measures would be appropriate, in an exploratory manner for future research, suggesting days alive and out of hospital at day 90, postoperative re-admission and postoperative infection may be meaningful outcome measures. Although these measures will not be suitable across all specialties, creating a core set of common outcome measures, would improve the interpretation and generalisability of future studies and aid with future systematic reviews. These additions to the body of evidence support the trend that non-anaemic iron deficiency may adversely affect patient outcomes, however, more research would need to be conducted to ascertain if it was causative or merely an association.

4.4.3 Generalisability

It can be difficult to generalize the results of a clinical trial, as the studied population may differ from the general population (Collet, 2000). The ISIDA trial had a defined patient population, with a stringent inclusion and exclusion criteria, analysed against confounding factors, with reproduceable results, strictly adhering to a defined trial protocol and defined statistical analysis plan. Unfortunately, the statistical analysis plan was not implemented due to the early cessation of the trial and therefore the benefit of generalising a narrative trend towards the merits of supplementation in the non-anaemic iron deficient surgical population would seem premature. Although heterogeneity was demonstrated between trial arms, with similar patient characteristics in both trial arms (see patient characteristic Table 14), it should be acknowledged all participants had the same ethnicity, which could affect generalising across the entire population and may not be generalisable across alternative populations, without further investigation in a more diverse setting.

4.4.4 Disseminating results

Results from the retrospective data analysis and the systematic review have been shared via the regional research network. They have been presented to the orthopaedic and anaesthetic directorate and a poster presentation was performed at a local research event for the retrospective data analysis. Due to COVID 19 delays between beginning and completing the research, it has been difficult to have them published in peer review journals, I am now looking at research square or F1000, as a means of sharing the results further. Disseminating results of the randomised controlled trial would not be appropriate with or without an interim analysis, although the trend suggested haemoglobin improved with time in the repeated measures analysis, there is an inherent risk of bias or overestimation in sharing results for

which the study is not powered (Law, 2000; Liu and Garrison, 2022). Therefore, the results will not be shared until the trial has completed.

4.4.4.1 Trial restart

Following the COVID 19 pause, the trial was restarted, with recruitment numbers increased to cover the patients lost from the trial to COVID 19. Unfortunately, recruitment issues became apparent post-pause, as patients attitudes to committing to the repeated blood tests and trips to hospital had changed. The patient population for arthroplasty as a whole and in the region the research was taking place was primarily older patients with comorbidities (Nham et al., 2023). These patients were identified as at greater risk of adverse outcomes if infected with COVID 19 (Mueller et al., 2020), and subsequently, during and post-pandemic seem to have become more cautious, leading to a reluctance to unnecessarily, in their view, to participate in research (Abdulhussein et al., 2022; Tuttle, 2020), attend the repeated appointments, especially if they were not going to receive the intervention. The trial management group, met twice to discuss potential alternatives. The original trial protocol allowed for home appointments; therefore, the decision was taken to attempt to offer patients appointments at home. However, some patients were still weary of repeatedly allowing people from the hospital to enter their home multiple times, due to their perceived increased risk of participating in the research post COVID 19 (Abdulhussein et al., 2022). The research was also being conducted in one of the largest geographical trusts in the UK, which meant logistical, geographical and financial implications of implementing this home visit plan (Ninnis et al., 2019), with the time commitment proving an inefficient use of staffing (Ninnis et al., 2019). When the trial management group met the second time, discussions were held on reducing the number of repeated measures, which seemed a pragmatic solution, which would mean altering the trial protocol and applying to REC for an amendment. An alternative

suggestion of a company that would post the tests to patients, who would perform a home test and post it back, thus reducing the multiple attendances to hospital. Tidy et al. (2018) identified efficiency, reduced patient time commitment and patient empowerment as advantages of at home testing kits, however, they acknowledge the risk that patients may not accurately perform the test and suggest it should be validated with patient groups before use. The test chosen has been validated, however for quality control and patient education purposes, it was suggested each patient should have a baseline test, via the lab and the home kit at recruitment, to enable analysis of the reliability and validity of the home test. These changes have been discussed with the patient advocate and experience group, who deemed it suitable. The changes to the protocol and submission to REC will be completed soon, which will hopefully mean the trial can continue and be completed successfully. Randomised controlled trials are sometimes unpredictable, with changes to protocol to maximize recruitment can be justifiable to produce valid (Joshi, 2023; Getz et al., 2016), reliable results, providing the changes do not affect the quality or reliability of the results (Getz et al., 2016).

4.4.4.2 Trial Funding and conflicts declaration

For full disclosure, the financial arrangements for the study were contractually agreed between the funder (a pharmaceutical company) and the Sponsor (an NHS Foundation Trust). The funder provided the required funds to complete the research and the food supplement, they had no responsibilities, input or access to the clinical management of the trial, to reduce the risk of bias and will only have access to the data when the trial has concluded. The clinical trial principal investigator was undertaking the clinical trial as part of their PhD, with no further conflicts declared.

4.5 Conclusion

The trial hypothesis suggesting iron supplementation, with Floradix mit Eisen, improved patient haemoglobin 3 weeks postoperatively, was not able to be accurately assessed, as the planned analysis could not be performed. Early analysis can lead to unreliable and inaccurate results and therefore statistical analysis will be undertaken when the trial has completed. Narrative synthesis supports a trend towards supplementation being beneficial, however the results as they stand are incomplete and cannot be reliably assumed to be causative.

Chapter 5: Conclusion and recommendations for further study

5.1 Overview of the chapter

This Chapter will conclude the thesis by offering a summary of the research and exploration of the topic undertaken, including, conduct, ethics and justification. Exploration of the thesis objectives will be summarised, whilst acknowledging difficulties and limitations identified, with recommendations for further research.

5.2 PhD thesis objectives

The main research objectives of this topic were:

- Investigate the prevalence of non-anaemic iron deficiency and its effect on patient outcomes for lower limb arthroplasty.
- Review the evidence for treatment of non-anaemic deficiency in the surgical population.
- Explore the effect of treatment of non-anaemic iron deficiency in lower limb arthroplasty on a patient population.

Non-anaemic iron deficiency was established as a phenomenon with a proven association in decline of postoperative haemoglobin and associated patient reported outcome measures. The systematic review of the evidence on treatment of non-anaemic iron deficiency was sparse, although the narrative synthesis suggested treatment would be beneficial. The randomised controlled trial investigating supplementation of non-anaemic iron deficient in patients undergoing lower limb arthroplasty, was unfortunately ended early due to the COVID 19 pandemic, with statistical analysis being not able to be performed a narrative towards an associated trend was shown but could not be proven causative.

5.3 Research conduct, difficulties and limitations

The retrospective analysis was designed and implemented using a defined protocol and conducted within the expected parameters. Limitations in exploring the retrospective data were explored, with associated design and implementation justified. Despite the potential limitations, the conclusions derived on the prevalence and effect of non-anaemic iron deficiency in the lower limb arthroplasty population was appropriate, robust and accurate.

The systematic review identified a small number of studies that lacked commonality in reporting and the vastly differing treatment methods made comparison difficult and were acknowledged as limitations. Thus, the resulting narrative synthesis, although demonstrating a trend, that supports the general consensus regarding supplementation, further research was recommended to ensure this is a reliable narrative.

Although the clinical trial was robustly designed, the acknowledged limitations, lack of placebo control, high rate of attrition and the premature pause of the trial, impacted the reliability of the results. The resulting early cessation of the trial due to the COVID 19 pandemic was problematic and unfortunate and beyond the control of the research team. The trial restart demonstrated a change in patient participation post COVID 19, due to the multiple appointments for blood sampling and the patient's perception of a greater risk of contact with people. However, it has enabled a greater discussion between the trial management group to improve recruitment and reduce attrition by amending the trial protocol to include a home testing kit and a subsequent participant training package. A process which is ongoing.

5.4 Recommendations and Priorities for future research

Chapter 2 demonstrated an association between a reduction in postoperative haemoglobin and length of stay in the presence of non-anaemic iron deficiency. Further research was recommended in larger populations to ascertain the effect on further postoperative outcomes and haemoglobin at different time points post-surgery. Research conducted in other patient populations are recommended to assess the impact on related outcomes and improve generalisability.

Chapter 3 demonstrated a lack of overall research on treatment of non-anaemic iron deficiency and a lack of commonality in reporting, which impacted the reliability, further research is recommended, preferably with commonality in treatment and outcome measures, to better understand the impact of supplementation and its effect on non-anaemic iron deficient patients undergoing surgery.

Chapter 4 incorporated a clinical trial which found no evidence of a difference in haemoglobin with supplementation. However, the early conclusion of the trial due to the pandemic means further research is recommended to fully ascertain the impact of supplementation on non-anaemic iron deficiency in the surgical patient population.

5.5 Final conclusions

Further research is recommended to fully explore the potential impact of treatment of non-anaemic iron deficiency on postoperative haemoglobin in patients undergoing lower limb arthroplasty in a fully powered randomised controlled trial.

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Appendix 1: STOBE checklist

STROBE Statement—Checklist of items that should be included in reports of ***cohort studies***

	Item No	Recommendation
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported
Objectives	3	State specific objectives, including any prespecified hypotheses
Methods		
Study design	4	Present key elements of study design early in the paper
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group
Bias	9	Describe any efforts to address potential sources of bias
Study size	10	Explain how the study size was arrived at
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding
		(*b*) Describe any methods used to examine subgroups and interactions
		(*c*) Explain how missing data were addressed
		(*d*) If applicable, explain how loss to follow-up was addressed
		(*e*) Describe any sensitivity analyses
Results		
Participants	13*	(a) Report numbers of individuals at each stage of study—

		eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed
		(b) Give reasons for non-participation at each stage
		(c) Consider use of a flow diagram
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders
		(b) Indicate number of participants with missing data for each variable of interest
		(c) Summarise follow-up time (eg, average and total amount)
Outcome data	15*	Report numbers of outcome events or summary measures over time
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included
		(*b*) Report category boundaries when continuous variables were categorized
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses
Discussion		
Key results	18	Summarise key results with reference to study objectives
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence
Generalisability	21	Discuss the generalisability (external validity) of the study results
Other information		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at

http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.

Appendix 2: SOP Retrospective analysis protocol

PROTOCOL TITLE:
A retrospective cohort study analysing retrospective patient data, comparing postoperative outcome measures, for non-anaemic iron deficient patients undergoing arthroplasty, against a control group.

SHORT TITLE:
NAIDALLA

PROTOCOL VERSION: 1.0
PROTOCOL DATE: 12th September 2021

SPONSOR: Northumbria Healthcare NHS Foundation Trust

CORRESPONDING CHIEF INVESTIGATOR:
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Consultant Orthopaedic Surgeon
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Data manager:
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SUMMARY OF STUDY

Acronym	NAIDALLA (Non-Anaemic Iron Deficiency And Lower Limb Arthroplasty
Long title	A retrospective cohort study analysing retrospective patient data, comparing postoperative outcome measures, for non-anaemic iron deficient patients undergoing arthroplasty, against a control group.
Study design	Two-arm, retrospective analysis of NAID versus control
Type of participants to be studied	Non-anaemic Iron deficient patients, undergoing lower limb arthroplasty against a control group of haemodynamically normal patients.
Setting	Northumbria Healthcare NHS Foundation Trust
Primary Objective	To assess the impact of NAID on postoperative patient outcomes for patients undergoing lower limb arthroplasty.
Primary Outcome	Postoperative haemoglobin compared to baseline
Secondary Outcomes	Length of hospital stay (midnights in hospital). Infection rate Transfusion Rate and number of units transfused up to 30 days 30 day readmission rate. Readmission within 30 days of surgery Inpatient DVT within 30 days of surgery Inpatient PE within 30 days of surgery Pneumonia Cerebrovascular incident Myocardial infarction
Planned trial sites	Wansbeck General Hospital, North Tyneside General hospital, Hexham General hospital, Northumbria Specialist Emergency Care Centre
Number of patients	#######
Sponsor	Northumbria Healthcare NHS Foundation Trust
Chief Investigator	Professor Mike Reed

Summary of study

Retrospective data analysis to analyse the effect of non-anaemic iron deficiency on patients undergoing arthroplasty. Patients with a normal haemoglobin (greater than 12 in women and 13 in men) (World Health Organisation 2011), and a ferritin level of below 50 will be investigated using regression analysis, using patients without anaemia or iron deficiency as a control, against specific outcome measures, utilising retrospective data.

Contents

Background and rationale
An international consensus statement suggesting the need to treat preoperative anaemia was published in 2016 (1). In this statement anaemia and non-anaemic iron deficiency were identified as patient groups that would benefit from the introduction of preoperative anaemia screening, assessment and treatment (1). They suggest patients with low iron levels, with or without anaemia, should be given supplementation to enable them to recover from surgery. Benefits of monitoring and intervention have been demonstrated in the anaemic (2) and non-anaemic iron deficient (3), lower limb arthroplasty population population. However, there is limited published research in the Non-anaemic iron deficient population. However there are limited published studies in the literature which prompted this retrospective analysis

Participant Population
Non-anaemic iron deficient patients undergoing arthroplasty
Control group of haemodynamically normal patient undergoing arthroplasty

Hypothesis
Patients with non-anaemic iron deficiency will have worse outcomes than those in the normal patient population.
·

Objective
The aim of this retrospective data analysis was to measure the effect of non-anaemic iron deficiency on patients undergoing arthroplasty. Patients with a normal haemoglobin (greater than 12 in women and 13 in men and a ferritin level of below 50 were investigated using regression analysis. Patients without anaemia or iron deficiency were used as a control, against specific outcome measures, utilising retrospective data.

- Assess the retrospective data, optimizing the patient cohort
- Analyse the effect of non-anaemic iron deficiency on patient postoperative outcomes

Study design
For this retrospective data analysis, a two-armed model was designed
Arm 1 non-anaemic iron deficient patients (haemoglobin over 12 females, 13 females with a ferritin less than 50) undergoing hip or knee arthroplasty
Arm 2, normal patient cohort of patients undergoing hip or knee arthroplasty (anaemic patients excluded).

Study Setting
This study will collect data across four hospital sites within a single NHS Foundation Trust based in England. The research sponsor Northumbria Healthcare NHS Foundation Trust.

Selection of patients
Patients who had undergone lower limb joint replacement surgery, had routine bloods taken full blood count (to test for haemoglobin), serum ferritin, CRP (C-Reactive Protein), urea and electrolytes, liver function and estimated Glomerular Filtration Rate (EGFR) which were checked to identify non-anemic iron deficient patients and a similar control group of haematologically normal patients, providing a baseline. Eligibility was assessed by reviewing the dataset for patient's preoperative standard blood tests of haemoglobin and ferritin.

Eligibility assessment
The eligibility criteria have been carefully considered. It is therefore vital that exceptions are not made to the following detailed selection criteria. Deviations from the eligibility criteria are considered to be protocol violations.

Participant inclusion criteria
In the non-anaemic iron deficient group, the recruitment criteria were as follows:
Haemoglobin greater than 12 in females and 13 in males
Ferritin less than 50
Undergoing primary hip or knee arthroplasty

In the control group, the recruitment criteria were as follows:
Haemoglobin greater than 12 in females and 13 in males
Ferritin greater than 50
Undergoing primary hip or knee arthroplasty

Participant exclusion criteria
Haemoglobin less than 12 in females and 13 in males

Study groups
NAID group
Non-anaemic iron deficient patients undergoing hip or knee arthroplasty

Control Group

Haemodynamically normal patient cohort of patients undergoing hip or knee arthroplasty (anaemic patients excluded).

Rehabilitation
For both the NAID and control groups, patients received standard physiotherapy and rehabilitation, in-line with current trust protocols.

Primary Outcome assessment
Postoperative haemoglobin compared to baseline

Secondary Outcome Assessment
- Length of hospital stay (midnights in hospital).
- Transfusion Rate
- Readmission rate.
- Mortality
- Readmission
- DVT
- PE
- CVA
- TIA
- Pneumonia
- Myocardial infarction
- PROMs EQ3D

Quality Assurance and Quality Control
Northumbria Healthcare NHS Foundation Trust has agreed to be the lead sponsor for this project and take overall responsibility for the quality of study conduct. This study will be fully compliant with the Research Governance Framework and MRC Good Clinical Practice Guidance. A study specific data management plan agreed by the Chief Investigator, Sponsor, and other study investigators will be drafted to provide detailed instructions and guidance, quality control processes involving data access and transfer of data.
A rigorous programme of quality control will be undertaken. The day-to-day management of the trial will be the responsibility of the principal investigator based at Northumbria Healthcare NHS Foundation Trust.

Direct access to source data/documents
In principle, anonymised data will be made available for meta-analysis and where requested by other authorised researchers and journals for publication purposes. Requests for access to data will be reviewed by the Chief Investigator and study Sponsor.

Data management
All records will be kept in locked locations. Clinical information will not be released without written permission. Any raw data shared for future studies or will be anonymised.

At the end of the study, data will be securely archived for a minimum of five years.
The PI will archive the trial essential documents generated at the site for the agreed archiving period in accordance with the signed Clinical Trial Site agreement.
The Investigator/institution will permit authorised representatives of the Sponsor and applicable regulatory agencies direct access to source data/documents to conduct trial-related monitoring, audits and regulatory inspection. Trial participants are informed of this during the informed consent discussion.

Data entry
Data will be stored and transferred following Health Insurance Portability and Accountability Act (HIPAA) protocol. The staff involved in the trial will receive training on data protection. The staff will be monitored to ensure compliance with privacy standards.
Data will be checked according to procedures detailed in the trial specific Data Management Plan.

Data storage
Data will be held according to the Data Protection Act 1998 and data will be collated in CRFs identified by a unique identification number (i.e. the Trial number) only. A Trial Enrolment Log at the site will list the ID numbers.
All essential documents, including source documents, will be retained for a minimum period of five years after study completion. The separate archival of electronic data will performed at the end of the trial, to safeguard the data indefinitely.

Statistical Analysis Plan
Analyses will be conducted in STATA 17 statistical analysis package. Significance tests will be two-sided at the 5% significance levels unless otherwise stated. Parameter estimates will be presented with associated 95% confidence intervals and p-values as appropriate.

Baseline data

Baseline data will be summarised using descriptive statistics overall and as analysed in the primary analysis model. No formal comparisons were made between the groups.

Primary analysis
The primary analysis will compare the haemoglobin postoperatively adjusting for baseline Haemoglobin, age, gender, smoking status and type of arthroplasty (hip or knee).

Secondary analyses
Length of hospital stay (in days), number of units transfused and EQ3D3L. infection rate, transfusion rate, readmission rate, deep vein thrombosis, pulmonary embolism, pneumonia, cerebrovascular incident, myocardial infarction and mortality.

Sensitivity analyses
It is possible that some patients required a blood transfusion. The number of patients this affected by group, was reported Therefore, for these patients Hb levels was likely influenced by the transfusion. While it is important to investigate how this might influence the primary analysis, we expect <1% of patients to be affected (4) We will therefore, undertake a sensitivity analysis excluding these patients.

Co-morbidities were a potential confounding factor, therefore analyse were planned to assess for the effect on the primary outcome, adjusted for 1 co-morbidity, 2 co-morbidities and 3 or more co-morbidities using a linear regression.

Missing Data
There is no plan to adjust the statistical comparison based on missing data**.**

Publication policy
The results will be disseminated in international, open-access peer-reviewed journals, through the local networks and at national and international meetings in surgical care within 12 months of trial completion in line with FDA rules. A dissemination and publication policy will be developed with an agreement between partners including ownership and exploitation of intellectual property, and publication rights. The publication policy and the agreement will ensure that any intellectual property generated during the project is protected and that the publication process is organised in a fair, balanced and transparent manner.

References

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2. Khan S, Jameson S, Fishley W, Tate R, Petheram T, Partington P.F, Reed M.R, (2012) The influence of pre-operative anaemia on outcomes after primary hip and knee arthroplasty under an Enhanced Recovery programme Podium presentation at the North East Surgical Society Meeting. Hexham.

3. Cuenca J, García-Erce J.A, Martínez F, Cardona R, Pérez-Serrano L, Muñoz M (2007) Preoperative haematinics and transfusion protocol reduce the need for transfusion after total knee replacement. *International Journal of Surgery*. Apr;5(2):89-94.

4. PUJOL-NICOLAS, A., MORRISON, R., CASSON, C., KHAN, S., MARRIOTT, A., TIPLADY, C., KOTZE, A., GRAY, W. & REED, M. 2017. Preoperative screening and intervention for mild anemia with low iron stores in elective hip and knee arthroplasty. Transfusion, 57, 3049-3057.

Appendix 3: Retrospective data statistical analysis plan

1 Objectives

1.1 Primary objective

The aim of this retrospective data analysis is to analyse the effect of non-anaemic iron deficiency on patients undergoing arthroplasty. Patients with a normal haemoglobin (greater than 12 in women and 13 in men) (World Health Organisation 2011), and a ferritin level of below 50 will be investigated using regression analysis, using patients without anaemia or iron deficiency as a control, against specific outcome measures, utilising retrospective data.

2 Design

Retrospective data analysis incorporating a two armed model using regression analysis.

Arm 1 non-anaemic iron deficient patients (haemoglobin over 12 females, 13 males with a ferritin less than 50) undergoing hip or knee arthroplasty

Arm 2 normal patient cohort of patients undergoing hip or knee arthroplasty (anaemic patients excluded).

This analysis will utilise data collected across four hospital sites within a single NHS Foundation Trust based in England and will be performed using data collected over a number of years.

3 Sample Size

Number of patients 4808

Total eligible 3172
NAID 956
Control 2214

4 Definition of terms

Provide a definition of any terms which require explanation. If a more elaborate definition is required, include this in the Appendix.

5 Outcomes

5.1 Primary outcome

Postoperative haemoglobin compared to baseline

5.2 secondary outcome data analysis

- Length of hospital stay (midnights in hospital).
- Transfusion Rate up to 30 days
- 30 day readmission rate.
- Adverse events (including all cause morbidity and mortality at 30 and 90 days)
- Readmission within 30 days of surgery
- DVT within 30 days of surgery
- PE within 30 days of surgery
- Blood transfusion rate and number of units
- Pneumonia
- Cerebrovascular incident CVA, TIA
- PNEUEMONIA
- Myocardial infarction

transfusion rate, morbidity and mortality, PROMs Eq5D 30/90 day

5.3 Other important Information

A report generated from the Trust information data will provide documentation from the episode of care. Data will be supplied by the Trust at the end of recruitment.

Demographic Data to be collected will include:

Age at surgery, Gender, Smoking status, Comorbidities, Hypertension, Atrial Fibrillation, Ischaemic Heart Disease, Type I diabetes, Type II diabetes, Chronic Obstructive Pulmonary Disease.

This section should cover details of data collection, monitoring and validation

Following surgery:

Complications during and after surgery are recorded as standard. Patients included in the study will be followed up for a further 30 days after their surgery to check if they required a blood transfusion.

Hospital episode statistics data:

Length of stay, Readmission within 30-days of surgery,

Complication data:

Infection rate (superficial and deep) Public Health England (Public Health England 2014), Myocardial infarction (heart attack), transient ischaemic attack or cerebrovascular accident (stroke), acute kidney injury (AKI) within 30-days of surgery, In-patient Deep vein thrombosis (DVT) or in-patient pulmonary embolism (PE) within 30-days of surgery, Mortality (30- and 90-day)

6 Data

6.1 SOPs

This statistical analysis plan should be used in conjunction with the following documents

Retrospective analysis SOP version 1.0

Non-Anaemic Iron Deficiency and Lower Limb Arthroplasty: A Retrospective Data Analysis statistics plan Version 1.0

6.2 Management of Datasets and Data verification

The principal investigator will perform audit and quality control of the data to ensure data accuracy. The database is stored on an NHS password protected Server. Electronic files created to analyse the data will be kept on a password protected NHS server.

6.3 External Datasets

Data anonymised will be shared with YTU PhD supervisors for assistance and guidance during the statistical analysis process.

7 Analysis

Analyses will be conducted in STATA statistical analysis package, with the version stated in the final report. Significance tests will be two-sided at the 5% significance levels unless otherwise stated. Parameter estimates will be presented with associated 95% confidence intervals and p-values as appropriate.

7.1 Baseline data

Baseline data will be summarised using descriptive statistics overall and as analysed in the primary analysis model (see Appendix for draft table). No formal comparisons will be made between the groups.

7.2 Missing Data

There is no plan to adjust the statistical comparison based on missing data.

7.3 Primary analysis

The primary analysis will compare the Haemoglobin postoperatively using a linear regression model adjusting for baseline Haemoglobin, age, gender and type of arthroplasty (hip or knee).

7.4 Secondary analyses

Length of hospital stay (in days) will be analysed using a poisson regression.

Continuous outcomes will be analysed using a linear regression

- **Eq5d5l**

Binary outcomes will be analysed using a logistic regression

- Transfusion Rate up to 30 days
- 30 day readmission rate.
- mortality
- Readmission within 30 days of surgery
- Inpatient DVT within 30 days of surgery
- Inpatient PE within 30 days of surgery
- Pneumonia
- Cerebrovascular incident
- Myocardial infarction

7.5 Sensitivity analyses

It is possible that some patients will require blood transfusion. The expected time point for requiring transfusion is usually soon after surgery, expected to be <5 days. Clinical intervention for transfusions occurs if the patient has Hb<8g/dl. The number of patients this affects by group, will be reported and a brief description will be given of when this occurred by patient.

Therefore, for these patients Hb levels would be influenced by transfusion. While it is important to investigate how this might have influenced the primary analysis, we expect <1% of patients to be affected (Pujol-Nicolas, et al 2017). We will therefore, undertake a sensitivity analysis excluding these patients.

Co-morbidities were a potential confounding factor, therefore analyse were planned to assess for the effect on the primary outcome, adjusting for the number of co-morbidities in the primary analysis model.

8 Baseline Characteristics

Table 1. Baseline characteristics of randomised patient and analysed patient data

Patient Characteristics	**NAID**	**Control**	**Total**
Gender Male, n(%)			
Age (years)			

Mean (sd) Median (min, max)			
Ethnicity White(black)asian(chinese(other)			
Smoking status Current Ex-smoker Never smoked			
Diabetes 1			
Diabetes 2			
IHD			
Hypertension			
TIA/CVA			
COPD			
Asthma			
Thyroid Disease			
AF			
Liver Disease			
Renal Disease			
Hb (g/dl) Mean (sd) Median (min, max)			
Ferritin Mean (sd) Median (min, max)			
CRP Mean (sd) Median (min, max)			
Type of surgery Knee			

Hip			

9. SAP amendment log

Please note all changes that are made to the Statistical Analysis Plan following initial sign-off in the box below. Include details of the changes made, any notes/justification for these changes, the new version number if applicable, who the changes were made by, and the date.

Amendment/addition to SAP and reason for change	New version number, name and date
SAP completed and signed-off	

10 Signatures of approval

Sign-off of the final approved version of the Statistical Analysis Plan by the principle investigator and trial statistician(s) (can also include Trial Manager/Co-ordinator)

Name	Trial Role	Signature	Date
Mike Reed	Chief Investigator		
John Randall	Principal investigator/ PhD Student		
Catherine Hewitt	Statistician YTU/ PhD Supervisor		

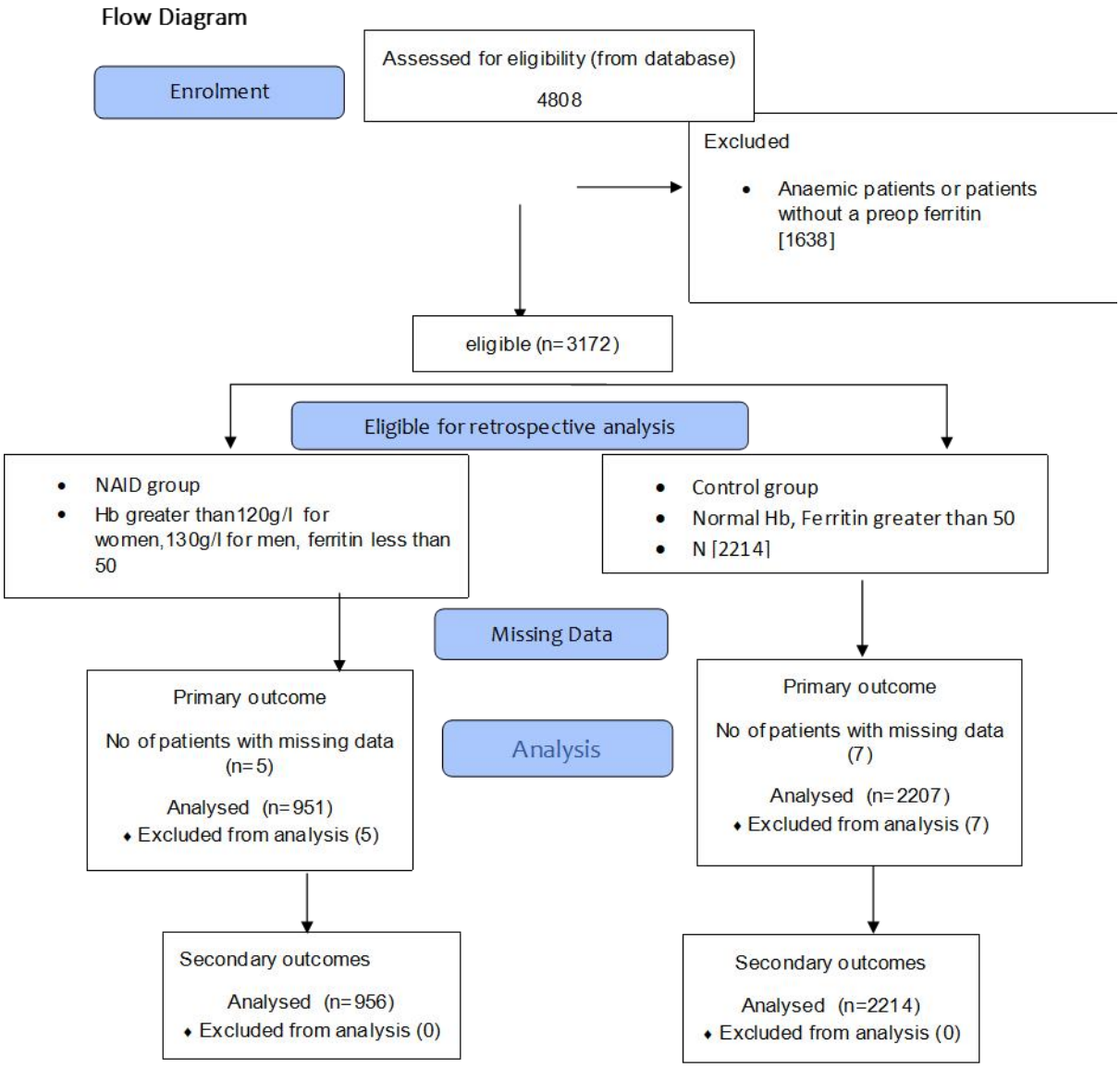
11 References

Public Health England (2014) Surgical site infection surveillance service (SSISS) https://www.gov.uk/guidance/surgical-site-infection-surveillance-service-ssiss

Pujol-Nicolas, A, Morrison, R, Casson, C, Khan, S, Marriott, A, Tiplady, C, Kotze, A, Gray, W, Reed, M, [2017] Preoperative screening and intervention for mild anemia with low iron stores in elective hip and knee arthroplast. Transfusion volume 57 pg3049-3057

World Health Organisation. (2011) Haemoglobin concentrations for the diagnosis of anaemia and assessment of severity. Vitamin and Mineral Nutrition Information System.

World Health Organization, Geneva. (http://www.who.int/vmnis/indicators/haemoglobinpdf, accessed [April 2018]).



Appendix 4: Regression Model assumptions

Determining appropriate model assumptions to be used was decided through analysing the literature from Bland (2015) and Bobbitt (2020)

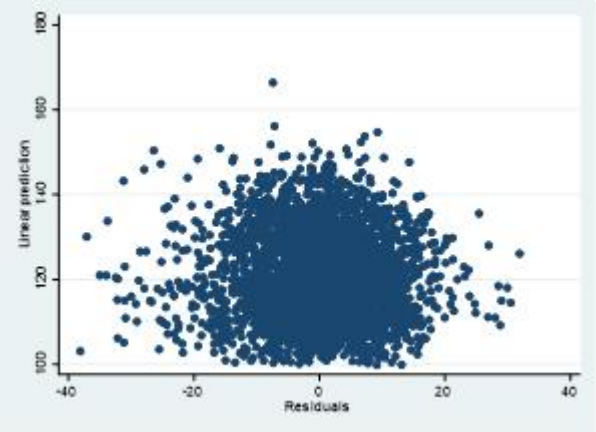
Linear regression model assumptions: for the primary outcome post-operative haemoglobin

Assumption 1. Independence: observations are independent from each other.

By design, the data was separated into two groups hence observations are independent. It was collected from single NHS trust so not clustered by hospital. It could not be clustered by family. There are not repeated measurements from the same individual in this analysis so there should not be clustering by individual.

Assumption 2. Homoscedasticity: The residuals have constant variance at every level of x.

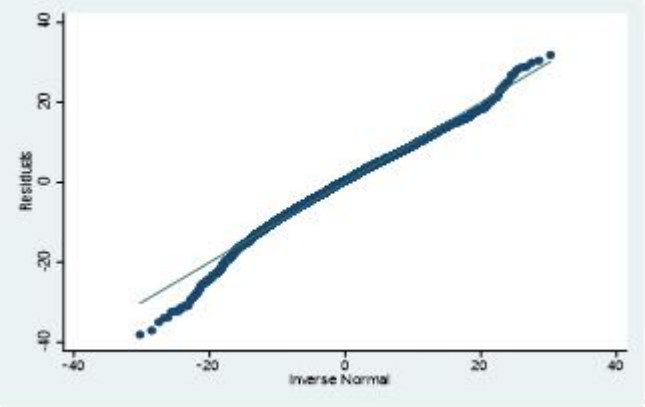
To detect homoscedasticity a fitted value vs. residual plot was created. Demonstrating the model assumption was appropriate.

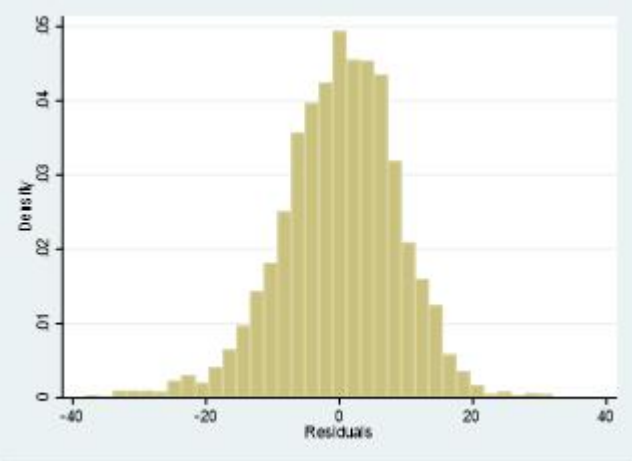


Assumption 3. Normality: The residuals of the model are normally distributed.

The assumption was checked visually using a Q-Q plot [see below].

A Q-Q plot was used to determine if the data were normally distributed.





Assumption 4: There is No Multicollinearity Among Explanatory Variables

Linear regression assumes that there is no severe multicollinearity among the explanatory variables. The VIF was less than 5 therefore, multicollinearity was not present.

Mean VIF 1.19 [range 1.02 -.52]

Logistic regression model assumptions: for all secondary binary outcomes

Assumption 1: The Response Variable is Binary

Logistic regression assumes only two possible outcomes. The response variables are whether someone had the condition under study or not so by design meets this assumption.

Assumption 2: The Observations are Independent

Logistic regression assumes that the observations in the dataset are independent of each other. By design, the two data streams analysed are separated into non-anaemic iron deficient and a control group.

Assumption 3: There is No Multicollinearity Among Explanatory Variables

Logistic regression assumes that there is no severe multicollinearity, this occurs when variables are correlated to each other, which effects their independence within the regression model. A higher correlation can cause problems when interpreting the model.

Mean VIF was performed in STATA using the collin command, results for each logistic regression. Mean VIF was 1.16, with a range from 1-1.52 suggesting there was no severe multicollinearity. VIF could not be performed on TIA, deaths as there were no incidences.

Assumption 4: The sample size is Sufficient

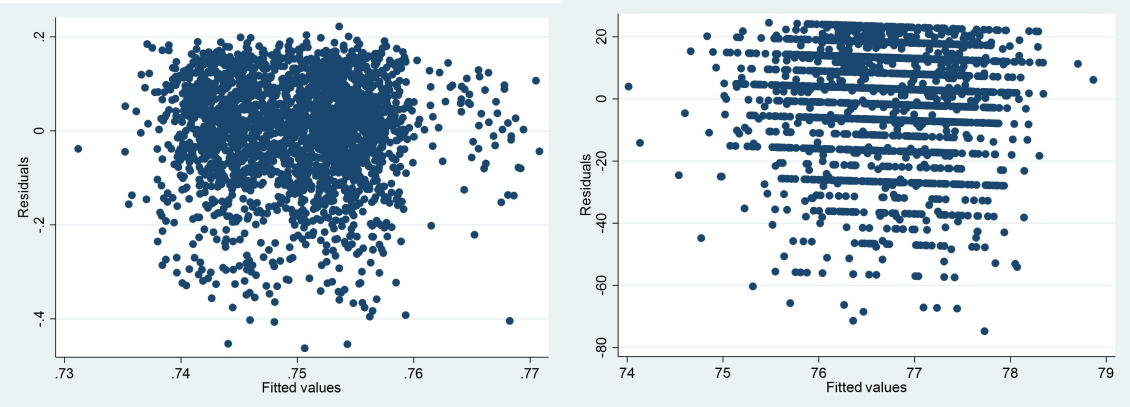
The sample size is suggested to be greater than 10 incidences per variable. There were 127 patients who were readmitted within 30 days of surgery: 42 (4.4%) NAID and 85 (3.8%) control. There were 3 transfusions 1 NAID, 1 control: There was one myocardial infarction in each group and 8 DVTs; 2 NAID; 6 control. Overall, there were 5 cases of pneumonia with 1 in the NAID group and 4 in the control, 2 cerebrovascular incidents in the control group but none in the NAID group and 19. pulmonary embolisms 8 NAID, 11. There were no deaths or TIAs attacks. Therefore, this assumption [10 per variable] was met in the readmission and PE was only.

Linear regression model assumptions: for EQ-5D-5L index score and VAS SCORE Assumption 1. Independence: observations are independent from each other.

By design, the data was separated into two groups hence observations are independent. It was collected from single NHS trust so not clustered by hospital. It could not be clustered by family. There are not repeated measurements from the same individual in this analysis so there should not be clustering by individual.

Assumption 2. Homoscedasticity: The residuals have constant variance at every level of x.

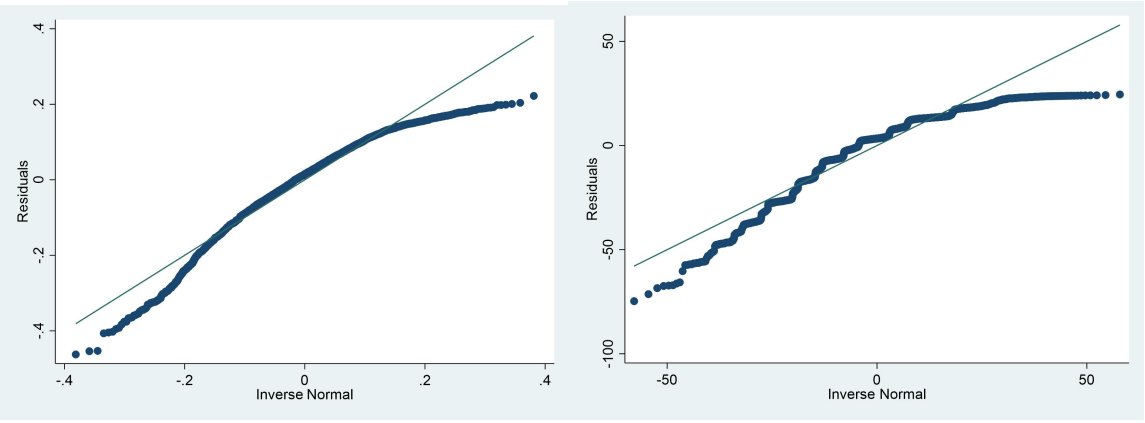
To detect homoscedasticity a fitted value vs. residual plot was created, demonstrating variance.

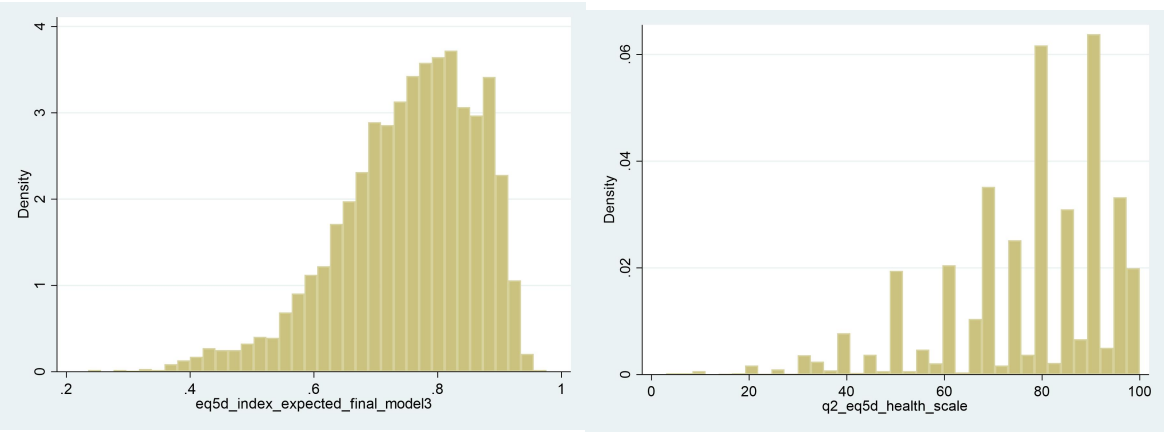


Assumption 3. Normality: The residuals of the model are normally distributed.

The assumption was checked visually using a Q-Q plot [see below].

A Q-Q plot was used to determine if the data was skewed , outliers were present at either end of the qqplot, however this was unlikely to effect the overall data assumptions.





Assumption 4: There is No Multicollinearity Among Explanatory Variables

Linear regression assumes that there is no severe multicollinearity among the explanatory variables. The VIF was less than 5 therefore, multicollinearity was not present.

Mean VIF 1.19

Poisson regression model assumptions

Assumption 1: The number of events can be counted.

By design, the number of nights stay was easily identifiable in the data sets.

Assumption 2: The occurrence of events are independent.

By design the data was separated into non-anaemic iron deficiency versus the control group, therefore data sets were independent.

Assumption 3: The average rate at which events occur can be calculated.

By design, the average rate of length of stay was measurable.

Assumption 4: The values are non-negative

There values were non-negative

Assumption 5: Events cannot occur at exactly the same instant time.

Due to design, two events could not occur at the same time, data pools were separate, and the patients couldn't be simultaneously in and out of hospital

ASSUMPTION 6: Is the variance larger than mean

Yes it was, a negative binomial was performed, which demonstrated overdispersion [see below]. Therefore, the model assumption to use poisons was not met and the negative binomial was more appropriate , as it accounts for overdispersion..

xi: poisson I i.NAID AB G i.F i.AA i.H, irr

Poisson regression Number of obs = 3,170

LR chi2(6) = 497.01

Prob > chi2 = 0.0000

Log likelihood = -6062.3782 Pseudo R2 = 0.0394

I	IRR	Std. err.	z	P>|z|	[95% conf. interval]	
_INAID_1	1.092077	.02618	3.67	0.000	1.041952	1.144613
AB	.9974132	.0012465	-2.07	0.038	.9949732	.9998593
G	1.026258	.0013213	20.13	0.000	1.023672	1.028851

```
      _IF_2 |   1.079296   .0288625     2.85   0.004     1.024183    1.137374
     _IAA_2 |    .861117   .0422548    -3.05   0.002     .7821569    .9480482
      _IH_2 |   1.022612   .0225372     1.01   0.310     .9793802    1.067752
      _cons |   .6596947   .1458808    -1.88   0.060     .4276747    1.017589
------------------------------------------------------------------------------


xi: nbreg I i.NAID AB G i.F i.AA i.H, irr
Negative binomial regression                    Number of obs =  3,170
                                                LR chi2(6)    = 354.72
Dispersion: mean                                Prob > chi2   = 0.0000
Log likelihood = -5925.9144                     Pseudo R2     = 0.0291
------------------------------------------------------------------------------
           I |        IRR   Std. err.      z    P>|z|     [95% conf. interval]
-------------+----------------------------------------------------------------
  _INAID_1 |1.088709   .0300626     3.08   0.002     1.031354    1.149255
          AB |   .9973824   .0014209    -1.84   0.066     .9946014    1.000171
           G |   1.025312   .0014818    17.30   0.000     1.022412     1.02822
      _IF_2 |   1.078142   .0329893     2.46   0.014     1.015385    1.144778
     _IAA_2 |   .8593039   .0484169    -2.69   0.007     .7694606    .9596375
      _IH_2 |   1.026948   .0259486     1.05   0.293     .9773281    1.079086
      _cons |   .7077555   .1778735    -1.38   0.169     .4324728    1.158265
-------------+----------------------------------------------------------------
   /lnalpha |  -2.193067   .0843874                     -2.358463   -2.027671
-------------+----------------------------------------------------------------
```

alpha | .111574 .0094154 .0945655 .1316418

LR test of alpha=0: chibar2(01) = 272.93 Prob >= chibar2 = 0.000

BLAND, M. 2015. *An Introduction to Medical Statistics,* Oxford, Oxford University Press.

BOBBITT, Z. 2020. *The Four Assumptions of Linear Regression* [Online]. https://www.statology.org/linear-regression-assumptions/. [Accessed 06/03/2022 2022].

Appendix 5: PROSPERO registered Systematic Review protocol

Title: Non-anaemic iron deficiency and surgery: A systematic review.

Identification

1a) This document is a protocol designed to perform a systematic review of non-anaemic iron deficiency (NAID) and Surgery.

Update

1b) This is a primary systematic review; it is not an update of a previously performed systematic review.

Registration

2) This systematic review protocol has been registered with PROSPERO

PROSPERO Identification Number: CRD42020164485

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3a) **Contributions**

3b) John Randall will act as the first reviewer of the literature and has designed protocol for the systematic review. Dr William Fishley will act as second reviewer.

Professor David Torgerson, Ms Catherine Arundel and Professor Catherine Hewitt are PhD supervisors at the University of York for John Randall, they will provide advice and guidance and assist in the development and review of the protocol.

Professor Mike Reed is a Consultant Orthopaedic surgeon, who will provide speciality expertise and assist in reviewing the protocol design, he will act in the role of third assessor, if required to resolve disputes between the two primary reviewers.

Amendments

4) This is an original version of the systematic review protocol. Amendments will be documented, if they arise. PROSPERO will be updated with any changes to the systematic review protocol.

Support:

Sources

5a) This systematic review is being performed by a PhD student primarily for academic purposes. No funding has been sought. SALUS HAUS are funding PhD fees for John Randall, Northumbria Healthcare NHS Foundation Trust are paying the salary for John Randall. However, neither organisation is directly contributing financially to this systematic review.

Sponsor

5b) University of York

Role of sponsor or funder

5c) University of York will provide the assistance expected to support a PhD student to undertake their studies successfully. This will be performed by Professor Torgerson, Ms Arundel and Professor Hewitt in their role as supervisors for John Randall PhD Student.

SALUS HAUS and Northumbria Healthcare NHS Foundation Trust have had no role or direct involvement in producing or funding this systematic review protocol.

INTRODUCTION

Rationale

6) An international consensus statement suggesting the need to treat preoperative anaemia was published in 2016 (1). In this statement anaemia and non-anaemic iron deficiency were identified as patient groups that would benefit from the introduction of preoperative anaemia screening, assessment and treatment (1). They suggest patients with low iron levels, with or without anaemia, should be given supplementation to enable them to recover from surgery. Benefits of monitoring and intervention have been demonstrated in the anaemic (2) and non-anaemic iron deficient (3) lower limb arthroplasty population. However, there is limited published research in the non-anaemic iron deficient population. The anaemic patient screening and treatment element of the consensus statement is currently being applied successfully within our surgical population. This systematic review aims to evaluate the known evidence surrounding screening and treatment in the non-anaemic iron deficient population as suggested in the consensus statement.

Objectives

7) The main objective of this systematic review is to compile and review the evidence surrounding non-anaemic iron deficiency and surgery. The review will address evidence with reference to participants, interventions, comparators, and outcomes (PICOS). The aim of the review is to assess the prevalence and effectiveness of treatment of the non-anaemic iron deficient surgical patient.

METHODS

Eligibility criteria

8a)

Criteria for including studies in the review If the PICOS format does not fit the research question of interest, please split up the question into separate concepts and put one under each heading	
Population, or participants and conditions of interest	People with Non-Anaemic Iron Deficiency undergoing surgery; any age, any gender and any severity of NAID and any definition of NAID, and any type of surgery No restrictions on study location but must be published in English.
Interventions or exposures	Treatment or monitoring of non-anaemic iron deficiency in patients undergoing surgery
Comparisons or control groups	No treatment (usual care, no intervention, placebo)
Outcomes of interest	Primary Outcome: Effectiveness of treatment, using measures of haemoglobin, ferritin levels after surgery and transfusion rate up to 30 days. Secondary Outcomes: length of stay, infection, morbidity, mortality up to 30 days, Patient reported outcome measures (PROMs).
Setting	Patients undergoing surgery, including private patients
Study designs	Randomised controlled trials and non-randomised controlled studies

b) **Criteria for excluding studies not covered in inclusion criteria** Any specific populations excluded, date range, language, whether abstracts or full text available.

Paediatric patient populations, although unlikely, will be excluded

No further exclusions are planned

Information sources

9) Articles published in English text will be sought utilising the following databases:

Electronic databases- no date restrictions to be applied

CENTRAL (Cochrane Library)

OVID (Embase/Medline)

PubMed

WHO International Clinical Trials Registry Platform (ICTRP) Search Portal

Clinicaltrials.gov

CINAHL

Web of Science

Search strategy

10a) The search strategy will be designed to access both unpublished and published materials and will incorporate two stages:

(1) Search terms and any synonyms used by respective databases, will be used in an extensive literature search, using the following search terms: 'iron deficiency', 'non-anaemic iron deficiency', 'low ferritin' and 'surgery' (See Appendix 1 for full search strategy).

(2) Bibliographies and reference lists of the articles collected in stage one above will be searched and further scrutinised to enhance literature capture.

Search term	Potential spelling or synonyms
Iron deficiency	Deficiency, deificiencies
Non-Anaemic Iron deficiency	Anaemic, Anemic, Anaemia, Anemia,

Low Ferritin	Ferritin, No synonyms
Surgery	Surgery, surgeries, surgical proceduce
Intervention	Treatment, intervention, Iron, iron compounds, ferric, ferrous
Clinical trial	Randomised controlled trial, clinical trial
Adults	Not children, not pregnant

Study records:

Data management

11a) Data will be stored securely on a password protected computer system. Data will be downloaded into reference management software and deduplicated.

Selection process

11b) For this systematic review, we will use two main reviewers (John Randall and William Fishley) and a third reviewer will be used to resolve any disagreements (Professor Mike Reed) through each phase of the review (that is, screening, eligibility and inclusion in meta-analysis). We will begin by screening titles and abstracts, full texts will be sought for eligible papers and included in the meta-analysis where data permits.

Data collection process

11c) This systematic review will develop and utilise a Data extraction form in google doc format (Appendix 1). The first (John Randall) and second reviewer (William Fishley) will independently screen titles and abstracts and extract data using the data extraction form, encompassing the elements identified below in section 12. A third reviewer (Professor Mike Reed) will review if there are any disparities between the two initial reviews after discussion. Efforts will be made to contact individual investigators to obtain or confirm relevant data.

Data items

12) The following data will be extracted from all studies - author identification, year of publication, language of publication, source of study funding, study design, study population, sample size (including main study inclusion and exclusion criteria), patient characteristics (age, sex, ethnicity, country of residence), intervention (drugs utilized, dose, route of administration) and its comparator, and results reported for the outcomes of interests

(including; length of stay, infection, transfusion rate, morbidity, mortality, Patient reported outcome measures (PROMs)).

Observational studies comparing NAID against a control group of patients will extract the following data - author identification, year of publication, language of publication, source of study funding, study design, study population (including main study inclusion and exclusion criteria), patient characteristics (age, sex, ethnicity, county of residence), Patients with NAID and its comparator (Control Group), and results reported for the outcomes of interest.

Risk of bias in individual studies

13) For this systematic review, the Cochrane risk of bias tool will be used to assess the risk of bias within each individual clinical trial and STROBE will be used for observational studies.
Data synthesis

14a) The number of participants in each group, means and standard deviations will be extracted for continuous outcomes and the number of participants and those experiencing the event of interest in each group will be extracted for categorical data. Data will be pooled using a random effects meta-analysis model, based on the assumption that clinical and methodological heterogeneity is likely to exist and to influence the results. Standardised mean differences (continuous data), odds ratio or relative risk ratio (categorical data) and their 95% confidence intervals will be presented. Analyses will be undertaken in Rev Man. Heterogeneity will be explored using standard chi-square and quantified using I^2 statistic. If statistical comparison is not possible, the systematic review findings will be presented narratively. Meta-analyses will be conducted if comparable outcome data from two or more studies are available.

14b) If there is sufficient data, then the following subgroup analyses are proposed. Gender (male vs. female), mode of iron therapy, Haemoglobin level, Ferritin level and measurement used for identifying fatigue

14c) If quantitative synthesis is not appropriate, then a narrative synthesis will be conducted. If statistical comparison is not possible, the systematic review findings will be presented narratively, describing the included studies and commenting on the methodological quality (risk of bias) of each study.

Assessing systematically and comprehensively the results of each study, highlighting important characteristics of the study, including changes to Haemaglobin, length of stay, infection, transfusion rate, morbidity and mortality. Synthesis of studies will be divided by study design into groups, for example, RCTs, quasi-RCTs, retrospective analysis and observational studies. We will explore any potential heterogeneity in the results that might be due to differences in study designs.

Bias

15) If there are more than 10 studies included in each meta-analysis, then a funnel plot will be used to assess for publication bias.

Confidence in cumulative evidence

16) Grade of evidence will not be formally assessed

References

1. Munoz M, Acheson A.G, Auerbach M, Besser M, Habler O, Kehlet H, Liumbruno G.M, Lasocki S, Meybohm P, Rao-Baikady R, Richards T, Shander A, So-Osman C, Spahn D.R, Klein A.A, (2017) International consensus statement on the peri-operative management of anaemia and iron deficiency. Anaesthesia, 72 233-247

2. Khan S, Jameson S, Fishley W, Tate R, Petheram T, Partington P.F, Reed M.R, (2012) The influence of pre-operative anaemia on outcomes after primary hip and knee arthroplasty under an Enhanced Recovery programme Podium presentation at the North East Surgical Society Meeting. Hexham.

3. Cuenca J, García-Erce J.A, Martínez F, Cardona R, Pérez-Serrano L, Muñoz M (2007) Preoperative haematinics and transfusion protocol reduce the need for transfusion after total knee replacement. *International Journal of Surgery*. Apr;5(2):89-94.

Appendix 1

Search Strategy

Cochrane Library		**Search Results**
#1	MeSH descriptor: [Iron] explode all trees	2502
#2	MeSH descriptor: [Iron Compounds] explode all trees	2358
#3	MeSH descriptor: [Ferrous Compounds] explode all trees	556
#4	MeSH descriptor: [Ferric Compounds] explode all trees	1286
#5	#1 or #2 or #3 or #4	4202
#6	low ferritin	1097
#7	iron deficiency	4019
#8	non-an?emic	164
#9	#5 and #6 or #7 or #8	4131
#10	surgery	235144

#11	#9 and #10	275

275 titles/abstracts reviewed

6 trials not completed

2 meet criteria and full texts reviewed

1.

Perioperative Iron With Erythropoietin in Bilateral Total Knee Replacement Arthroplasty (TKRA) https://clinicaltrials.gov/show/NCT01012063, 2009 | added to CENTRAL: 31 May 2018 | 2018 Issue 5

2.

Impact of Preoperative Treatment of Anemia and Iron Deficiency in Cardiac Surgery on Outcome https://clinicaltrials.gov/show/NCT02031289, 2014 | added to CENTRAL: 31 January 2020 | 2020 Issue 01

OVID Embase and Medline	**Search results**
1 iron	515898
2 iron compounds	4118
3 ferrous compounds	9912
4 ferric compounds	20005
5 1 or 2 or 3 or 4	527536
6 iron defi*	68702
7 low ferritin	1050
8 5 and (6 or 7)	69171
9 surg*	7070056
10 8 and 9	6476
11 preoperative	712334
12 before surg*	94905

13 10 and (11 or 12)	932
14 randomised controlled trial	53457
15 randomized controlled trial	1306604
16 clinical trial	2227073
17 Observatio*	1997738
18 Retrospective	2162740
19 13 and (14 or 15 or 16 or 17 or 18)	380
20 remove duplicates from 19	296

296 titles/abstracts reviewed

9 meet criteria full texts to be reviewed

1. Effect of ultra-short-term treatment of patients with iron deficiency or anaemia undergoing cardiac surgery: a prospective randomised trial.

Spahn D.R., Schoenrath F., Spahn G.H., Seifert B., Stein P., Theusinger O.M., Kaserer A., Hegemann I., Hofmann A.,mMaisano F., Falk V.

Embase The Lancet. 393 (10187) (pp 2201-2212), 2019. Date of Publication: 1 - 7 June 2019.

2. Postoperative outcomes following cardiac surgery in non-anaemic iron-replete and iron-deficient patients – an exploratory study.

Miles L.F., Kunz S.A., Na L.H., Braat S., Burbury K., Story D.A.

Embase Anaesthesia. 73 (4) (pp 450-458), 2018. Date of Publication: April 2018.

3. A cost-effective implementation of preoperative protocol with Sucrosomial iron supplementation.

Scardino M.

Embase Expert Review of Hematology. Conference: 5th International Multidisciplinary Course on Iron Anemia. Italy. 10

(Supplement 1) (pp 4), 2017. Date of Publication: 2017.

[Conference Abstract]

4. Pre-operative iron deficiency in bariatric surgery: Diagnosis and treatment.

Omelanczuk P., Sanchez M., Pampillon N., Ojeda A., Abaurre M., Penuto C., Berducci M., Lasagni V., Palma R., Omelanczuk S.

Embase Obesity Surgery. Conference: 20th International Federation for the Surgery of Obesity and Metabolic Disorders

World Congress, IFSO 2015. Vienna Austria. Conference Publication: (var.pagings). 25 (1 SUPPL. 1) (pp S119-S120), 2015.

Date of Publication: August 2015.

[Conference Abstract]

5. Preoperative iron deficiency increases transfusion requirements and fatigue in cardiac surgery patients: A prospective

observational study.

Piednoir P., Allou N., Driss F., Longrois D., Philip I., Beaumont C., Montravers P., Lasocki S.

Embase European Journal of Anaesthesiology. 28 (11) (pp 793-801), 2011. Date of Publication: November 2011.

[Article]

6. Iron deficiency is associated with higher mortality in patients undergoing cardiac surgery: a prospective study.

Rossler J., Schoenrath F., Seifert B., Kaserer A., Spahn G.H., Falk V., Spahn D.R.

Embase British Journal of Anaesthesia. 124 (1) (pp 25-34), 2020. Date of Publication: January 2020. è

[Article]

7. The role of intravenous iron carboxymaltose supplementation in non-anaemic patients undergoing elective hip or knee

arthroplasty.

D'Amato T., Fenocchio G., Martorelli F., Scardino M., Simili V., Gurgone A.

Embase Transfusion Medicine. Conference: 18th Annual NATA Symposium on Patient Blood Management, Haemostasis and

Thrombosis. Italy. 27 (Supplement 1) (pp 54-55), 2017. Date of Publication: April 2017.

[Conference Abstract]

Maybe view full text

8. Iron deficiency in preoperative period of bariatric surgery.

Omelanczuk P., Pampillon N., Sanchez M., Lasagni V., Penutto C., Omelanczuk S., Abaurre M. Embase Obesity Surgery. Conference: 18th World Congress of the International Federation for the Surgery of Obesity and Metabolic Disorders, IFSO 2013. Istanbul Turkey. Conference Publication: (var.pagings). 23 (8) (pp 1077), 2013. Date of

Publication: August 2013.

[Conference Abstract]

9. Iron pre-load for major joint replacement.

Andrews C.M., Lane D.W., Bradley J.G.

Embase Transfusion Medicine. 7 (4) (pp 281-286), 1997. Date of Publication: 1997 Dec.

[Article] AN: 28051962

PubMed

((((((((((((((((((((comparative studies[Publication Type]) OR randomized controlled trial[Publication Type]) OR controlled clinical trial[Publication Type]) OR observational study[Publication Type]) OR randomised[Title/Abstract]) OR randomised[Title/Abstract]) OR placebo[Title/Abstract]) OR randomly[Title/Abstract]) OR group[Title/Abstract]) OR groups[Title/Abstract]) AND preoperative) OR before operation) OR period, preoperative[MeSH Terms]) AND surgery[MeSH Terms]) AND non-anaemic iron deficiency[Title/Abstract]) OR non-anemic iron deficiency NOT Children)))) NOT child)) NOT pregnancy:

123 titles/abstracts reviewed

2 full texts reviewed

0 meet criteria

WHO International Clinical Trials Registry Platform (ICTRP) Search Portal

Title: Surgery

AND

Condition: iron deficiency

AND

Recruitment status: ALL

And

Intervention: Iron

21 reviewed

0 meet Criteria

Clinicaltrials.gov

(Surgery) AND "All" [STUDY-TYPES] AND iron deficiency [DISEASE] or low ferritin NOT anemia NOT anaemia

1 title/abstract reviewed

0 meet criteria

Cinahl
TX non anaemic iron deficiency OR TX non anemic iron deficiency AND TX low ferritin AND TX Surgery

42 titles and abstracts reviewed

0 meet criteria

Web of Science Index Expanded (SCI-EXPANDED) & Conference Proceedings Citation Index-Science (CPCI-S)

#13 #7 and #12

#12 #5 and #9 and #11

#11TS=(surgery or surgical or operation)

#10 #5 and #6 and #7 and #9

#9TS=(non-anemic iron deficiency or non-anaemic iron deficiency or low ferritin)

#8 #5 and #6 and #7

#7TS=(preoperative or pre-operative or before surgery)

#6TS=(Iron deficiency AND low ferritin)

#5 #1 OR #2 OR #3 OR #4

#4TS=(human)

#3TS=(observational OR Retrospective)

#2TS=(controlled clinical trial OR controlled trial OR clinical trial OR placebo)

#1TS=(randomised OR randomized OR randomly OR random order OR random sequence OR random allocation OR randomly allocated OR at random OR randomized controlled trial)

41 reviewed

6 meet criteria

1. PT J, AU Hubert, Marine Gaudriot, Baptiste Biedermann, Sebastien Gouezec, Herve Sylvestre, Emmanuelle Bouzille, Guillaume Verhoye, Jean-Philippe Flecher, Erwan Ecoffey, Claude

TI Impact of Preoperative Iron Deficiency on Blood Transfusion in Elective

Cardiac Surgery

SO JOURNAL OF CARDIOTHORACIC AND VASCULAR ANESTHESIA

VL 33 IS 8 BP 2141EP 2150 DI 10.1053/j.jvca.2019.02.006 PD AUG 2019 PY 2019 TC 2 ZR 0 ZB 1 Z8 0 ZS 0 Z9 2 SN 1053-0770 EI 1532-8422 UT WOS:000478706100006 PM 30857851 ER

2. PT J

AU Miles, Lachlan F. Sandhu, Ravinder N. S. Grobler, Anneke C. Heritier, Stephane Burgess, Adele Burbury, Kate L. Story, David A.

TI Associations between non-anaemic iron deficiency and outcomes following surgery for colorectal cancer: An exploratory study of outcomes relevant to prospective observational studies

SO ANAESTHESIA AND INTENSIVE CARE

VL 47 IS 2 BP 152 EP 159 DI 10.1177/0310057X19838899 PD MAR 2019 PY 2019

OI Grobler, Anneke/0000-0002-7809-7688 ZS 0 ZR 0 TC 1 ZB 0 Z8 Z9 1 SN 0310-057X EI 1448-0271 UT WOS:000489308400008 PM 31090438 ER

3.PT J

AU Scardino, Marco Di Matteo, Berardo Martorelli, Federica Tanzi, Dario Kon, Elizaveta D'Amato, Tiziana

TI Improved patient blood management and cost saving in hip replacement surgery through the implementation of pre-operative Sucrosomial (R) iron supplementation: a quality improvement assessment study

SO INTERNATIONAL ORTHOPAEDICS

VL 43 IS 1 SI SI BP 39 EP 46 DI 10.1007/s00264-018-4149-7 PD JAN 2019 PY 2019 TC 3 ZS 0 ZB 0 ZR 0 Z8 0 Z9 3 SN 0341-2695 EI 1432-5195 UT WOS:000455631800006 PM 30232527 ER

4. PT J

AU Miles, L. F. Kunz, S. A. Na, L. H. Braat, S. Burbury, K. Story, D. A.

TI Postoperative outcomes following cardiac surgery in non-anaemic iron-replete and iron-deficient patients - an exploratory study

SO ANAESTHESIA

VL 73 IS 4 BP 450 EP 458 DI 10.1111/anae.14115 PD APR 2018 PY 2018

OI Story, David/0000-0002-6479-1310; Kunz, Stephen/0000-0001-7424-9472 ZS 0 TC 9 ZB 3 ZR 0 Z8 0 Z9 9 SN 0003-2409 EI 1365-2044 UT WOS:000427417200008 PM 29197079 ER

5. PT J

AU Piednoir, Pascale Allou, Nicolas Driss, Fathi Longrois, Dan Philip, Ivan Beaumont, Carole Montravers, Philippe Lasocki, Sigismond

TI Preoperative iron deficiency increases transfusion requirements and fatigue in cardiac surgery patients: a prospective observational study

SO EUROPEAN JOURNAL OF ANAESTHESIOLOGY

VL 28 IS 11 BP 796 EP 801 DI 10.1097/EJA.0b013e32834ad97b PD NOV 2011 PY 2011

RI lasocki, sigismond/G-9443-2016 ZR 1 TC 32 Z8 0 ZB 9 ZS 1 Z9 33 SN 0265-0215 EI 1365-2346

UT WOS:000295865300008 PM 21885979 ER

6. Peri-operative correction of non-anaemic iron deficiency. A reply

By: Munoz, M.Group Author(s): Panel Int Consensus Statement Peri

ANAESTHESIA Volume: 72 Issue: 7 Pages: 911-912 Published: JUL 2017

1 further information required

Effects of intravenous iron combined with low-dose recombinant human erythropoietin on transfusion requirements in iron-deficient patients undergoing bilateral total knee replacement arthroplasty (CME)

By: Na, Hyo-Seok; Shin, Soon-Young; Hwang, Jin-Young; et al.

TRANSFUSION Volume: 51 Issue: 1 Pages: 118-124 Published: JAN 2011

WHO International Clinical Trials Registry Platform (ICTRP) Search Portal

0 meet criteria

Appendix 2

Articles to be included after viewing full text

1.
Effect of ultra-short-term treatment of patients with iron deficiency or anaemia undergoing cardiac surgery: a
prospective randomised trial.
Spahn D.R., Schoenrath F., Spahn G.H., Seifert B., Stein P., Theusinger O.M., Kaserer A., Hegemann I., Hofmann A.,
Maisano F., Falk V.
m m nnn Embase The Lancet. 393 (10187) (pp 2201-2212), 2019. Date of Publication: 1 - 7 June 2019.
[Article]
AN: 2001871184
PMID
31036337 [http://www.ncbi.nlm.nih.gov/pubmed/?term=31036337]
Embase

2.

Pre-operative iron deficiency in bariatric surgery: Diagnosis and treatment.
Sanchez M., Pampillon N., Ojeda A., Abaurre M., Penuto C., Berducci M., Lasagni V., Palma R., Omelanczuk
S.
Embase Obesity Surgery. Conference: 20th International Federation for the Surgery of Obesity and Metabolic Disorders
World Congress, IFSO 2015. Vienna Austria. Conference Publication: (var.pagings). 25 (1 SUPPL. 1) (pp S119-S120), 2015.

Date of Publication: August 2015.
[Conference Abstract]
AN: 72002909

3.
The role of intravenous iron carboxymaltose supplementation in non-anaemic patients undergoing elective hip or knee
arthroplasty.
D'Amato T., Fenocchio G., Martorelli F., Scardino M., Simili V., Gurgone A.
Embase Transfusion Medicine. Conference: 18th Annual NATA Symposium on Patient Blood Management, Haemostasis and
Thrombosis. Italy. 27 (Supplement 1) (pp 54-55), 2017. Date of Publication: April 2017.
[Conference Abstract]
AN: 615441255

4.
Na, Hyo-Seok; Shin, Soon-Young; Hwang, Jin-Young; et al. (2011) Effects of intravenous iron combined with low-dose recombinant human erythropoietin on transfusion requirements in iron-deficient patients undergoing bilateral total knee replacement arthroplasty (CME)

TRANSFUSION Volume: 51 Issue: 1 Pages: 118-124

5.

AU Scardino, Marco Di Matteo, Berardo Martorelli, Federica Tanzi, Dario Kon, Elizaveta D'Amato, Tiziana

TI Improved patient blood management and cost saving in hip replacement surgery through the implementation of pre-operative Sucrosomial (R) iron supplementation: a quality improvement assessment study

SO INTERNATIONAL ORTHOPAEDICS

VL 43 IS 1 SI SI BP 39 EP 46 DI 10.1007/s00264-018-4149-7 PD JAN 2019 PY 2019 TC 3 ZS 0 ZB 0 ZR 0 Z8 0 Z9 3 SN 0341-2695 EI 1432-5195 UT WOS:000455631800006 PM 30232527 ER

6.
Iron deficiency in preoperative period of bariatric surgery.
Omelanczuk P., Pampillon N., Sanchez M., Lasagni V., Penutto C., Omelanczuk S., Abaurre M.
Embase Obesity Surgery. Conference: 18th World Congress of the International Federation for the Surgery of Obesity and
Metabolic Disorders, IFSO 2013. Istanbul Turkey. Conference Publication: (var.pagings). 23 (8) (pp 1077), 2013. Date of

Publication: August 2013.
[Conference Abstract]
AN: 71128314

7.

Scardino M.
Embase Expert Review of Hematology. Conference: 5th International Multidisciplinary Course on Iron Anemia. Italy. 10
(Supplement 1) (pp 4), 2017. Date of Publication: 2017.
[Conference Abstract]
AN: 619950847

EXCLUDED DUE TO LACK OF CLARIFICATION IN THE DATA,AUTHORS CONTACTED WITHOUT REPLY.

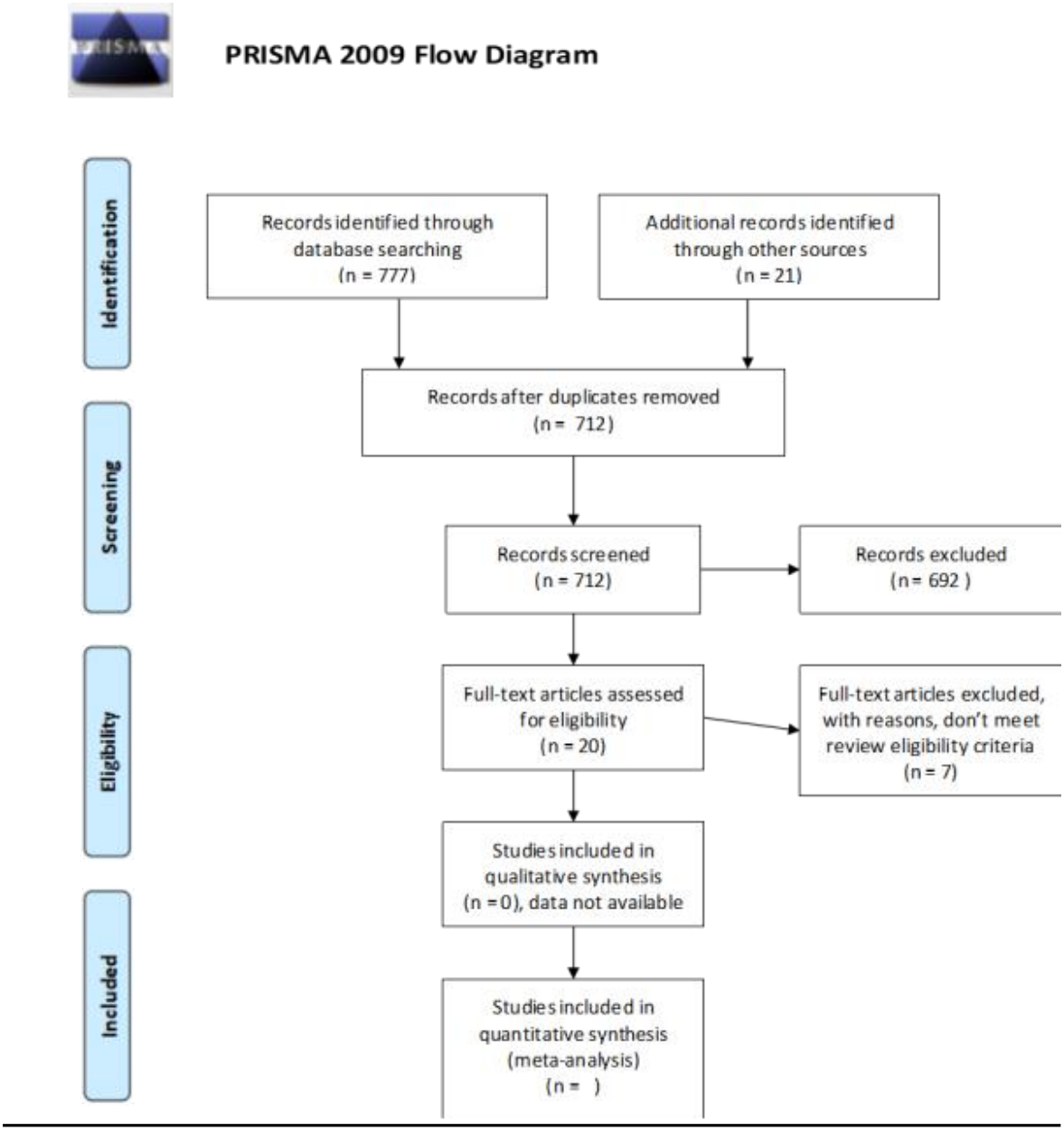
Peri-operative correction of non-anaemic iron deficiency. A reply

By: Munoz, M.Group Author(s): Panel Int Consensus Statement Peri

ANAESTHESIA Volume: 72 Issue: 7 Pages: 911-912 Published: JUL 2A cost-effective implementation of preoperative protocol with Sucrosomial iron supplementation.

Appendix 4: PRISMA Flow

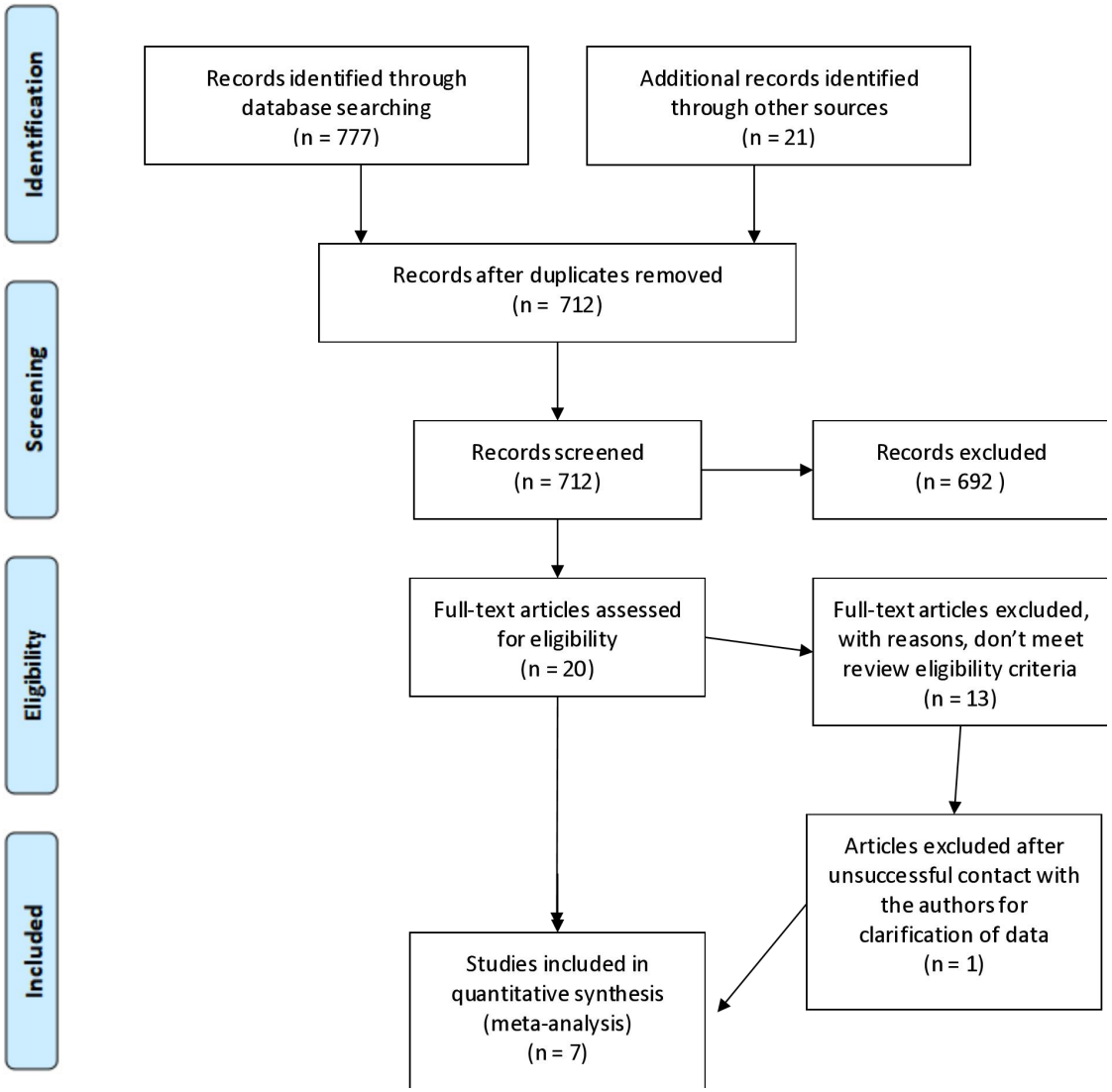
Diagram



Appendix 6: PRISMA Checklist
NAID

PRISMA Flow Diagram

(PRISMA flow diagram for systematic review, template taken from Moher, Liberati, Tetzlaff, Altman and The PRISMA Group (2009)



Appendix 7: Google forms data extraction form

NAID systematic review data extraction form

NAID Systematic review data extraction form

*Required

1. Email *

NAID systematic review, data extraction form v.2

2. author identification *

author names

3. Publication *

name source of publication

4. Language of publication *

5. Study funding *

details of how the study is funded

6. Study design *

7. detail study design (for example.. RCT, observational study)

8. Study population *

add the study participants

9. Inclusion criteria *

10. Exclusion criteria *

11. age *

12. sex *

Mark only one oval.

male femaleOther:

13. ethnicit y *

14. country of residence *

15. sample size overall *

16. sample size intervention arm

17. Intervention (drug) *

describe the drug utilised in the study

18. Intervention (dose) *

19. intervention (route of administration) *

Mark only one oval. oral Intravenous subcuticularOther:

20. haemaglobin preop [intervention arm] *

21. haemaglobin preop [intervention arm] *

standard deviation

22. haemaglobin postop [intervention arm] *

mean

23. haemaglobin postop [intervention arm] *

standard deviation

24. haemaglobin change [intervention arm] *

percentage

25. haemaglobin change [intervention arm] *

confidence intervals

26. ferritin pre-op [intervention arm] *

mean

27. ferritin pre-op [intervention arm] *

standard deviation

28. ferritin pre-op [intervention arm] *

standard deviation

29. ferritin change [intervention arm] *

percentage

30. ferritin change [intervention arm] *

confidence intervals

31. comparators (length of stay) Intervention arm *

in number of nights stay

32. comparators (infection rate) intervention arm *

extract mean

33. comparators (infection rate) intervention arm *

standard deviation

mean

34. comparators [transfusion rate] intervention arm

mean

35. comparators (transfusion rate) Intervention arm *

extract standard deviation

36. comparators (morbidity) intervention arm *

binary percentage

37. comparators [mortality] intervention arm *

38. proms score [intervention arm] *

include type of proms

39. proms score [intervention arm] *

mean score

40. proms score [intervention arm] *

standard deviation

41. control group *

identify type of control group

42. type of treatment

43. Intervention (dose) Control group *

write N/A if control group

44. intervention (route of administration) If applicable *

Mark only one oval.

C) oral

C) Intravenous

C) subcuticular

C) N/A control group

C) Other:

45. comparators (length of stay) control group *

in number of nights stay

46. haemaglobin preop [control group] *

mean

47. haemaglobin preop [control group] *

standard deviation

48. haemaglobin postop [control arm] *

standard deviation

49. haemaglobin postop [control arm] *

standard deviation

50. haemaglobin change [control arm] *

percentage

51. haemaglobin change [control arm] *

confidence interventional

52. ferritin preop [control arm] *

mean

53. ferritin preop [control arm] *

standard deviation

54. ferritin postop [control arm] *

mean

55. ferritin postop [control arm

Standard deviation

56. ferritin change [control arm] *

percentage

57. ferritin change [control arm] *

confidence intervals

58. comparators (infection rate) control group *

extract mean

59. comparators (infection rate) control group *

extract standard deviation

60. comparators (transfusion rate) control group *

extract mean

61. comparators (transfusion rate) control group *

extract standard deviation

62. comparators (mortality) control group *

extract binary percentage

63. proms score [control group] *

include type of proms

64. proms score [control group] *

include mean

65. proms score [control group] *

include standard deviation

Appendix 8: Systematic Review: tabulation

Study characteristics

author identification	Spahn et al	Sanchez et al	D'Amato et al	Na et al	Scardino et al	omelanczuk et al	M Scardino
Publication	Lancet	Nutricion Hospitalaria	Transfusion Medicine	Transfusion	International orthopaedics	Obesity surgery	Conference Abstract
year of publication	2019	2015	2017	2011	2018	2017	2017
Language of publication	English	Spanish (Abstract in English)	English	English	English	English	English
Study funding	Multiple sources	not reported	not reported	not reported	Pharmanutra	not reported	not reported
Study design	Prospective Randomised Controlled Trial	Prospective Observational study	observational cohort study	Randomised Controlled Trial	Observational retrospective study	Observational prospective study	Retrospective Observational study

Study population	Patients with iron deficiency or anaemia undergoing cardiac surgery	Morbidly obese patients undergoing bariatric surgery	iron deficient patients undergoing knee/hip arthroplasty	Iron deficient patients scheduled for primary knee arthroplasty	patients admitted for elective hip surgery	Morbidly obese patients undergoing bariatric surgery	Prosthetic Hip Replacement
Inclusion criteria	Patients undergoing elective coronary artery bypass surgery Patients undergoing elective cardiac valve surgery Patients undergoing elective combined valve-CABG surgery Patients who have signed the informed consent after explanation of the study	not reported	Hb between 12-14 Ferritin less than 100	undergoing knee arthroplasty hb greater than 100 Ferritiin less than 100 or ferritin 100-300 with a TSAT less than 20%	patients who underwent elective prosthetic hip surgery in 2016	not reported	Hip Arthroplasty, low ferritin < 100

Exclusion criteria	Patients in need of urgent surgery the day of admission Participation in another clinical trial during the last 4 weeks prior to patient screening Impairments, diseases or language problems which do not allow the patient to fully understand the consequences of study participation Age <18 years Pregnant and/or breastfeeding women Jehovah's Witnesses Patients suffering from endocarditis Known allergy against iron-carboxymaltose or mannitol	not reported	Patients with kidney failure, liver failure, intravenous iron allergy and sepsis	Haematological disease, Thromboembolic disease, Hepatic disease, Renal disease, Coagulation disorders, Infection, Malignancy, anticoagulant therapy, blood transfusion within the las month, Sensitivity to iron	Infection, diabetes, heart disease, coronary disease, kidney or liver disease, haematological disease, anticoagulant or anti-platelet therapy, malignancy	not reported	not reported

	need for intraoperative extra-corporeal membrane oxygenation Untractable surgical bleeding with massive transfusion (>10 Red blood cell (RBC) transfusions per 24h						
Age (Overall)	not reported	not reported	not reported	not reported	not reported	not reported	not reported
Age (Overall)	not reported	not reported	not reported	not reported	not reported	not reported	not reported

age (Intervention arm)	69	not reported	not reported	69.4	68.8	not reported	not reported
age (intervention Arm)	11	not reported	not reported	4.1	9.4	not reported	not reported
age (control arm)	67	not reported	not reported	67.9	68.4	not reported	not reported
age (control Arm)	12	not reported	not reported	5.2	9.5	not reported	not reported
sex (Overall)	n/a	not reported	not reported	100	not reported	not reported	not reported
sex (Overall)	n/a	not reported	not reported	0	not reported	not reported	not reported
sex (Intervention arm)	40	not reported	94	100	56	not reported	not reported
sex (Intervention Arm)	60	not reported	6	0	44	not reported	not reported
sex (Control arm)	43	not reported	84	100	57	not reported	not reported
sex (Control arm)	57	not reported	16	0	43	not reported	not reported
Ethnicity	not reported	not reported	not reported	not reported	not reported	not reported	not reported

Intervention and control group allocation

author identification	Spahn et al	Sanchez et al	D'Amato et al	Na et al	Scardino et al	omelanczuk et al	M Scardino
Intervention (drug)	ferric carboxymaltose	Ferric Carboxymaltose	Ferric Carboxymaltose	Iron Succrose	Succrosomial Iron	Ferric Carboxymaltose	Succrosomial iron
Intervention (dose)	20mg/kg	500	1g	200mg	30mg	500mg	1 capsule
intervention (route of administration)	Intravenous	Intravenous	Intravenous	Intravenous	oral Daily for for weeks pre-op	Intravenous	Oral administered daily
Intervention (drug)	Erythropoetin	n/a	n/a	Erythropoetin	n/a	n/a	n/a
Intervention (dose)	1mg	n/a	n/a	3000	n/a	n/a	n/a
intervention (route of administration)	Subcuticular	n/a	n/a	subcuticular	n/a	n/a	n/a
Intervention (dose) Control group	Placebo	no intervention	n/a	n/a	n/a	n/a	n/a
intervention (route of administration) If applicable	Intravenous	N/A control group	N/A control group	N/A control group	N/A control group	N/A control group	N/A control group

Haemoglobin data table

author identification	Spahn et al	Sanchez et al	D'Amato et al	Na et al	Scardino et al	omelanczuk et al	M Scardino
Haemagloin preop Intervention arm Mean	140	12.8	125	121	134.5	12.46	not reported
haemoglobin preop [intervention arm] Standard Deviation	10	12	not reported	13	26	12.7	not reported
haemoglobin preop [intervention arm] IQR	not reported	not reported	not reported	not reported	not reported	not reported	not reported
haemoglobin postop 1 [intervention arm] Mean	102	13.5	122	not reported	97	11.92	not reported
haemoglobin postop 1 [intervention arm] No of days reported	1	30	30	not reported	1	not reported	not reported
haemoglobin postop 1 [intervention arm] Standard Deviation	not reported	0.7	not reported	not reported	12.4	1.24	not reported
haemoglobin postop 1 [intervention arm] IQR	89-114	not reported	not reported	not reported	not reported	not reported	not reported
haemoglobin change 1 [intervention arm] Percentage	not reported	not reported	not reported	not reported	not reported	not reported	not reported

haemoglobin change 1 [intervention arm] Confidence Intervals	not reported	not reported	not reported	not reported	not reported	not reported	not reported
haemoglobin postop 2 [intervention arm] Mean	89	not reported	not reported	not reported	112	not reported	not reported
haemoglobin postop 2 [intervention arm] No of days reported	3	not reported	not reported	not reported	discharge	not reported	not reported
haemoglobin post op 2 [intervention arm] Standard Deviation	not reported	not reported	not reported	not reported	13.7	not reported	not reported
haemoglobin post op 2 [intervention arm] IQR	79-104	not reported	not reported	not reported	not reported	not reported	not reported
haemoglobin change 2 [intervention arm] Percentage	not reported	not reported	not reported	not reported	not reported	not reported	not reported
haemoglobin change 2 [intervention arm] Confidence Intervals	not reported	not reported	not reported	not reported	not reported	not reported	not reported
haemoglobin postop 3 [intervention arm] Mean	96	not reported	not reported	not reported	133	not reported	not reported
haemoglobin postop 3 [intervention arm] No of days	5	not reported	not reported	not reported	30	not reported	not reported
haemoglobin post op 3 [intervention arm] Standard Deviation	not reported	not reported	not reported	not reported	15.4	not reported	not reported

haemoglobin post op 3 [intervention arm] Standard Deviation	86-110	not reported	not reported	not reported	not reported	not reported	not reported
haemoglobin change 3 [intervention arm] Percentage	not reported	not reported	not reported	not reported	not reported	not reported	not reported
haemoglobin change 3 [intervention arm] Confidence Intervals	not reported	not reported	not reported	not reported	not reported	not reported	not reported
Haemoglobin postop 4 [intervention arm] Mean	96	not reported	not reported	not reported	not reported	not reported	not reported
Haemoglobin postop 4 [intervention arm] Mean	7	not reported	not reported	not reported	not reported	not reported	not reported
haemoglobin post op 4 [intervention arm] Standard Deviation	not reported	not reported	not reported	not reported	not reported	not reported	not reported
haemoglobin post op 4 [intervention arm] IQR	84-110	not reported	not reported	not reported	not reported	not reported	not reported
haemoglobin change 4 [intervention arm] Percentage	not reported	not reported	not reported	not reported	not reported	not reported	not reported
haemoglobin change 4 [intervention arm] Confidence Intervals	not reported	not reported	not reported	not reported	not reported	not reported	not reported
haemoglobin preop [control group] Mean	140	13.7	131	121	135	not reported	not reported

haemoglobin preop [control group] Standard Deviation	10	0.9	not reported	12	21	not reported	not reported
haemoglobin preop [control group] IQR	not reported	not reported	not reported	not reported	not reported	not reported	not reported
haemoglobin postop 1 [control group] Mean	94	12.3	112	not reported	84	117	not reported
haemoglobin postop 1 [control group] No of days reported	1	not reported	30	not reported	1	not reported	not reported
haemoglobin postop 1 [control arm] Standard Deviation	not reported	not reported	not reported	not reported	8.2	not reported	not reported
haemoglobin postop 1 [control arm] IQR	85-107	not reported	not reported	not reported	not reported	not reported	not reported
haemoglobin change 1 [control arm] Percentage	not reported	not reported	not reported	not reported	not reported	not reported	not reported
haemoglobin change 1 [control arm] Confidence Intervals	not reported	not reported	not reported	not reported	not reported	not reported	not reported
haemoglobin postop 2 [control group] Mean	86	not reported	not reported	not reported	96	not reported	not reported

haemoglobin postop 2 [control group] no of days reported	3	not reported	not reported	not reported	discharge	not reported	not reported
haemoglobin postop 2 [control arm] Standard deviation	not reported	not reported	not reported	not reported	11.6	not reported	not reported
haemoglobin postop 2 [control arm] IQR	78-98	not reported	not reported	not reported	not reported	not reported	not reported
haemoglobin change 2 [control arm] Percentage	not reported	not reported	not reported	not reported	not reported	not reported	not reported
haemoglobin change 2 [control arm] Confidence Intervals	not reported	not reported	not reported	not reported	not reported	not reported	not reported
haemoglobin postop 3 [control group] Mean	89	not reported	not reported	not reported	102	not reported	not reported
haemoglobin postop 3 [control group] No of days	5	not reported	not reported	not reported	30	not reported	not reported
haemoglobin postop 3 [control arm] Standard Deviation	not reported	not reported	not reported	not reported	11.9	not reported	not reported
haemoglobin postop 3 [control arm] IQR	82-101	not reported	not reported	not reported	not reported	not reported	not reported
haemoglobin change 3 [control arm] Percentage	not reported	not reported	not reported	not reported	not reported	not reported	not reported

haemoglobin change 3 [control arm] Confidence Intervals	not reported	not reported	not reported	not reported	not reported	not reported	not reported
haemoglobin postop 4 [control group] Mean	85	not reported	not reported	not reported	not reported	not reported	not reported
haemoglobin postop 4 [control group] No of days	7	not reported	not reported	not reported	not reported	not reported	not reported
haemoglobin postop 4 [control arm] Standard Deviation	not reported	not reported	not reported	not reported	not reported	not reported	not reported
haemoglobin postop 4 [control arm] IQR	80-95	not reported	not reported	not reported	not reported	not reported	not reported
haemoglobin change 4 [control arm] Percentage	not reported	not reported	not reported	not reported	not reported	not reported	not reported
haemoglobin change 4 [control arm] Confidence Intervals	not reported	not reported	not reported	not reported	not reported	not reported	not reported

Ferritin Data Table

author identification	Spahn et al	Sanchez et al	D'Amato et al	Na et al	Scardino et al	omelanczuk et al	M Scardino
ferritin pre-op [intervention arm] Mean	61	not reported	not reported	80.2	65.4	70.64	not reported
ferritin pre-op [intervention arm] Standard Deviation	25	not reported	not reported	40	12.37	84.79	not reported
ferritin post-op [intervention arm] Mean	not reported	not reported	not reported	195	not reported	136	not reported
ferritin post-op [intervention arm] no of days reported	not reported	not reported	not reported	1	not reported	not reported	not reported

ferritin post-op [intervention arm] Standard deviation	not reported	not reported	not reported	57.4	not reported	157.96	not reported
ferritin change [intervention arm] Percentage	not reported	not reported	not reported	not reported	not reported	not reported	not reported
ferritin change [intervention arm] Confidence Intervals	not reported	not reported	not reported	not reported	not reported	not reported	not reported
ferritin post-op 2 [intervention arm] Mean	not reported	not reported	not reported	not reported	not reported	not reported	not reported
ferritin post-op 2 [intervention arm] no of days reported	not reported	not reported	not reported	not reported	not reported	not reported	not reported

ferritin post-op 2 [intervention arm] Standard Deviation	not reported	not reported	not reported	not reported	not reported	not reported	not reported
ferritin change 2 [intervention arm] Percentage	not reported	not reported	not reported	not reported	not reported	not reported	not reported
ferritin change 2 [intervention arm] Confidence intervals	not reported	not reported	not reported	not reported	not reported	not reported	not reported
ferritin preop [control arm] Mean	65	not reported	not reported	68.7	66	not reported	not reported
ferritin preop [control arm] Standard deviation	23	not reported	not reported	31.9	10.25	not reported	not reported

ferritin postop 1 [control arm] Mean	not reported	not reported	not reported	39.5	not reported	not reported	not reported
ferritin postop 1 [control arm] no of days	not reported	not reported	not reported	1	not reported	not reported	not reported
ferritin postop 1 [control arm] Standard Deviation	not reported	not reported	not reported	32.2	not reported	not reported	not reported
ferritin change 1 [control arm] Percentage	not reported	not reported	not reported	not reported	not reported	not reported	not reported
ferritin change 1 [control arm] Confidence Intervals	not reported	not reported	not reported	not reported	not reported	not reported	not reported
ferritin postop 2 [control arm] Mean	not reported	not reported	not reported	not reported	not reported	not reported	not reported

ferritin postop 2 [control arm] no of days	not reported	not reported	not reported	not reported	not reported	not reported	not reported
ferritin postop 2 [control arm] Standard Deviation	not reported	not reported	not reported	not reported	not reported	not reported	not reported
ferritin change 2 [control arm] Percentage	not reported	not reported	not reported	not reported	not reported	not reported	not reported
ferritin change 2 [control arm] confidence Intervals	not reported	not reported	not reported	not reported	not reported	not reported	not reported
ferritin pre-op [intervention arm] Mean	65	not reported	not reported	80.2	65.4	70.64	not reported
ferritin pre-op [intervention arm] Standard Deviation	23	not reported	not reported	40	12.37	84.79	not reported

ferritin post-op [intervention arm] Mean	not reported	not reported	not reported	195	not reported	136	not reported
ferritin post-op [intervention arm] no of days reported	not reported	not reported	not reported	1	not reported	not reported	not reported
ferritin post-op [intervention arm] Standard deviation	not reported	not reported	not reported	57.4	not reported	157.96	not reported
ferritin change [intervention arm] Percentage	not reported	not reported	not reported	not reported	not reported	not reported	not reported
ferritin change [intervention arm] Confidence Intervals	not reported	not reported	not reported	not reported	not reported	not reported	not reported

ferritin post-op 2 [intervention arm] Mean	not reported	not reported	not reported	not reported	not reported	not reported	not reported
ferritin post-op 2 [intervention arm] no of days reported	not reported	not reported	not reported	not reported	not reported	not reported	not reported
ferritin post-op 2 [intervention arm] Standard Deviation	not reported	not reported	not reported	not reported	not reported	not reported	not reported
ferritin change 2 [intervention arm] Percentage	not reported	not reported	not reported	not reported	not reported	not reported	not reported
ferritin change 2 [intervention arm] Confidence intervals	not reported	not reported	not reported	not reported	not reported	not reported	not reported

Length of Stay Data Table

author identification	Spahn et al	Sanchez et al	D'Amato et al	Na et al	Scardino et al	omelanczuk et al	M Scardino
comparators (length of stay) control group (Mean)	12.3	not reported	not reported	not reported	6.5	not reported	15
comparators (length of stay) control group (Standard Deviation)	12.2	not reported	not reported	not reported	not reported	not reported	not reported
Length of Stay (Intervention) Mean no of Days	10.5	not reported	not reported	not reported	4	not reported	10

Length of Stay (Intervention) Standard Deviation	8	not reported	not reported	not reported	not reported	not reported	not reported

Comparators Data Table

author identification	Spahn et al	Sanchez et al	D'Amato et al	Na et al	Scardino et al	omelanczuk et al	M Scardino
comparators (infection rate) intervention arm (Mean)	not reported	not reported	not reported	not reported	not reported	not reported	not reported
comparators (infection rate) intervention arm (Standard Deviation)	not reported	not reported	not reported	not reported	not reported	not reported	not reported

comparators (infection rate) intervention arm (No of Days)	90	not reported	not reported	not reported	not reported	not reported	not reported
comparators (infection rate) intervention arm (percentage)	31	not reported	not reported	not reported	not reported	not reported	0
comparators (transfusion rate 1) intervention arm (Mean)	1	not reported	not reported	0.2	0	not reported	not reported

comparators (transfusion rate 1) intervention arm (Standard Deviation)	2.2	not reported	not reported	0.5	not reported	not reported	not reported
comparators (transfusion rate 1) intervention arm (No of days)	7	not reported	not reported	1	not reported	not reported	not reported
comparators (transfusion rate 1) intervention arm (percentage)	32	not reported	not reported	20.4	0	not reported	not reported

comparators (transfusion rate 1) Intervention arm (Confidence Intervals)	not reported	not reported	not reported	not reported	not reported	not reported	not reported
comparators (transfusion rate 2) intervention arm (Mean)	1.1	not reported	not reported	not reported	not reported	not reported	not reported

comparators (transfusion rate 2) intervention arm (standard Deviation)	2.5	not reported	not reported	not reported	not reported	not reported	not reported
comparators (transfusion rate 2) intervention arm (No of Days)	90	not reported	not reported	not reported	not reported	not reported	not reported
comparators (transfusion rate 2) intervention arm (Percentage)	not reported	not reported	not reported	not reported	not reported	not reported	not reported

comparators (transfusion rate 2) Intervention arm (Confidence Intervals)	not reported	not reported	not reported	not reported	not reported	not reported	not reported
comparators [mortality] intervention arm	3	not reported	not reported	not reported	not reported	not reported	not reported
comparators (morbidity) intervention arm (MI)	0	not reported	not reported	not reported	not reported	not reported	not reported

comparators (morbidity) intervention arm (stroke)	2	not reported	not reported	not reported	not reported	not reported	not reported
comparators (morbidity) intervention arm (acute kidney injury)	5	not reported	not reported	not reported	not reported	not reported	not reported
comparators (morbidity) intervention arm (bleeding)	3	not reported	not reported	not reported	not reported	not reported	not reported

comparators (morbidity) intervention arm (blood clots/thromboembolic events)	0	not reported	not reported	not reported	not reported	not reported	not reported
comparators (morbidity) intervention arm (serious adverse events)	22	not reported	not reported	not reported	not reported	not reported	not reported
comparators (infection rate) control group (Mean)	not reported	not reported	not reported	not reported	not reported	not reported	not reported

comparators (infection rate) control group (Standard Deviation)	not reported	not reported	not reported	not reported	not reported	not reported	not reported
comparators (infection rate) control group (No of Days Reported)	90	not reported	not reported	not reported	not reported	not reported	not reported
comparators (infection rate) control group (Percentage)	26	not reported	not reported	not reported	not reported	not reported	not reported

comparators (transfusion rate 1) control group (Mean)	1.3	not reported	not reported	0.8	not reported	not reported	not reported
comparators (transfusion rate 1) control group (Standard Deviation)	2.8	not reported	not reported	0.8	not reported	not reported	not reported

comparators (transfusion rate 1) control group (Number of Days reported)	7	not reported	not reported	1	not reported	not reported	not reported
comparators (transfusion rate 1) control group (Percentage)	37	not reported	not reported	53.7	not reported	not reported	10

comparators (transfusion rate 1) control group (Confidence Intervals)	not reported	not reported	not reported	not reported	not reported	not reported	not reported
comparators (transfusion rate 2) control group (Mean)	1.7	not reported	not reported	not reported	not reported	not reported	not reported

comparators (transfusion rate 2) control group (Standard deviation)	3.1	not reported	not reported	not reported	not reported	not reported	not reported
comparators (transfusion rate 2) control group (No of Days reported)	90	not reported	not reported	not reported	not reported	not reported	not reported

comparators (transfusion rate 2) control group (percentage)	not reported	not reported	not reported	not reported	not reported	not reported	not reported
comparators (transfusion rate 2) control group (Confidence Intervals)	not reported	not reported	not reported	not reported	not reported	not reported	not reported
comparators (mortality) control group (Percentage)	5	not reported	not reported	not reported	not reported	not reported	not reported

comparators (morbidity) control group (MI)	3	not reported	not reported	not reported	not reported	not reported	not reported
comparators (morbidity) control group (stroke)	3	not reported	not reported	not reported	not reported	not reported	not reported
comparators (morbidity) control group (acute kidney injury)	6	not reported	not reported	not reported	not reported	not reported	not reported

comparators (morbidity) control group (bleeding)	1	not reported	not reported	not reported	not reported	not reported	not reported
comparators (morbidity) control group (blood clots/thromboembolic events)	2	not reported	not reported	not reported	not reported	not reported	not reported
comparators (morbidity) control group (serious adverse events)	35	not reported	not reported	not reported	not reported	not reported	not reported

PROMs Data Table

author identification	Spahn et al	Sanchez et al	D'Amato et al	Na et al	Scardino et al	omelanczuk et al	M Scardino
proms score preop [intervention arm]	not reported	not reported	not reported	not reported	not reported	not reported	not reported
proms score preop [intervention arm]	not reported	not reported	not reported	not reported	not reported	not reported	not reported
proms score preop [intervention arm]	not reported	not reported	not reported	not reported	not reported	not reported	not reported
proms score postop [intervention arm]	not reported	not reported	not reported	not reported	not reported	not reported	not reported
proms score postop [intervention arm]	not reported	not reported	not reported	not reported	not reported	not reported	not reported
proms score [control group]	not reported	not reported	not reported	not reported	not reported	not reported	not reported
proms score pre-op[control group]	not reported	not reported	not reported	not reported	not reported	not reported	not reported
proms score pre-op [control group]	not reported	not reported	not reported	not reported	not reported	not reported	not reported
proms score post-op[control group]	not reported	not reported	not reported	not reported	not reported	not reported	not reported
proms score post-op [control group]	not reported	not reported	not reported	not reported	not reported	not reported	not reported

Appendix 9: Cochrane and STROBE risk assessments

Revised Cochrane risk-of-bias tool for randomized trials (RoB 2)
TEMPLATE FOR COMPLETION

Edited by Julian PT Higgins, Jelena Savović, Matthew J Page, Jonathan AC Sterne
on behalf of the RoB2 Development Group

Version of 22 August 2019

The development of the RoB 2 tool was supported by the MRC Network of Hubs for Trials Methodology Research (MR/L004933/2- N61), with the support of the host MRC ConDuCT-II Hub (Collaboration and innovation for Difficult and Complex randomised controlled Trials In Invasive procedures - MR/K025643/1), by MRC research grant MR/M025209/1, and by a grant from The Cochrane Collaboration.

Risk bias tool 1 for

Effect of ultra-short-term treatment of patients with iron deficiency or anaemia undergoing cardiac surgery: a prospective randomised trial.

Spahn D.R., Schoenrath F., Spahn G.H., Seifert B., Stein P., Theusinger O.M., Kaserer A., Hegemann I., Hofmann A.,mMaisano F., Falk V.

Embase The Lancet. 393 (10187) (pp 2201-2212), 2019. Date of Publication: 1 - 7 June 2019.

Study details

Reference	Spahn D.R., Schoenrath F., Spahn G.H., Seifert B., Stein P., Theusinger O.M., Kaserer A., Hegemann I., Hofmann A.,mMaisano F., Falk V. Embase The Lancet. 393 (10187) (pp 2201-2212), 2019. Date of Publication: 1 - 7 June 2019.

Study design

X Individually-randomized parallel-group trial

☐ Cluster-randomized parallel-group trial

☐ Individually randomized cross-over (or other matched) trial

For the purposes of this assessment, the interventions being compared are defined as

Experimental:	ferric carboxymaltose 20mg/kg Intravenous Erythropoetin 1mg Subcuticular	Comparator:	Control group

Specify which outcome is being assessed for risk of bias	Funding bias, attrition bias, reporting bias, selection bias, allocation bias
Specify the numerical result being assessed. In case of multiple alternative analyses being presented, specify the numeric result (e.g. RR = 1.52 (95% CI 0.83 to 2.77) and/or a reference (e.g. to a table, figure or paragraph) that uniquely defines the result being assessed.	No significant reduction of RBC transfusions by combination treatment (0 units [IQR 0–1]) versus placebo (0 units [0–2]) could be shown in the non-anaemic population. Univariable ordinal regression yielded an OR of 0·76 (95% CI 0·45–1·29) for each threshold of number of red blood cell transfusions

Is the review team's aim for this result…?

- ☑ to assess the effect of *assignment to intervention* (the 'intention-to-treat' effect)
- ☐ to assess the effect of *adhering to intervention* (the 'per-protocol' effect)

If the aim is to assess the effect of *adhering to intervention*, select the deviations from intended intervention that should be addressed (at least one must be checked):

- ☐ occurrence of non-protocol interventions
- ☐ failures in implementing the intervention that could have affected the outcome
- ☐ non-adherence to their assigned intervention by trial participants

Which of the following sources were <u>obtained</u> to help inform the risk-of-bias assessment? (tick as many as apply)

☑ Journal article(s) with results of the trial

☐ Trial protocol

☐ Statistical analysis plan (SAP)

☑ Non-commercial trial registry record (e.g. ClinicalTrials.gov record)

☐ Company-owned trial registry record (e.g. GSK Clinical Study Register record)

☐ "Grey literature" (e.g. unpublished thesis)

☐ Conference abstract(s) about the trial

☐ Regulatory document (e.g. Clinical Study Report, Drug Approval Package)

☐ Research ethics application

☐ Grant database summary (e.g. NIH RePORTER or Research Councils UK Gateway to Research)

☐ Personal communication with trialist

☐ Personal communication with the sponsor

Risk of bias assessment

Responses underlined in green are potential markers for low risk of bias, and responses in red are potential markers for a risk of bias. Where questions relate only to sign posts to other questions, no formatting is used.

Domain 1: Risk of bias arising from the randomization process

Signalling questions	Comments	Response options
1.1 Was the allocation sequence random?	Yes	Y / PY / PN / N / NI
1.2 Was the allocation sequence concealed until participants were enrolled and assigned to interventions?	Yes	Y / PY / PN / N / NI
1.3 Did baseline differences between intervention groups suggest a problem with the randomization process?	Yes	Y / PY / PN / N / NI
Risk-of-bias judgement	Low	Low / High / Some concerns
Optional: What is the predicted direction of bias arising from the randomization process?	NA	NA / Favours experimental / Favours comparator / Towards null /Away from null / Unpredictable

Signalling questions	Comments	Response options
2.1. Were participants aware of their assigned intervention during the trial?	Yes	Y / PY / PN / N / NI
2.2. Were carers and people delivering the interventions aware of participants' assigned intervention during the trial?	No	Y / PY / PN / N / NI
2.3. If Y/PY/NI to 2.1 or 2.2: Were there deviations from the intended intervention that arose because of the trial context?	No	NA / Y / PY / PN / N / NI
2.4 If Y/PY to 2.3: Were these deviations likely to have affected the outcome?	Na	NA / Y / PY / PN / N / NI
2.5. If Y/PY/NI to 2.4: Were these deviations from intended intervention balanced between groups?	Na	NA / Y / PY / PN / N / NI
2.6 Was an appropriate analysis used to estimate the effect of assignment to intervention?	Yes	Y / PY / PN / N / NI
2.7 If N/PN/NI to 2.6: Was there potential for a substantial impact (on the result) of the failure to analyse participants in the group to which they were randomized?	Yes	NA / Y / PY / PN / N / NI
Risk-of-bias judgement	Low	Low / High / Some concerns
Optional: What is the predicted direction of bias due to deviations from intended interventions?	Na	NA / Favours experimental / Favours comparator / Towards null /Away from null / Unpredictable

Domain 2: Risk of bias due to deviations from the intended interventions (*effect of assignment to intervention*)

Signalling questions	Comments	Response options
2.1. Were participants aware of their assigned intervention during the trial?	n	Y / PY / PN / N / NI
2.2. Were carers and people delivering the interventions aware of participants' assigned intervention during the trial?	n	Y / PY / PN / N / NI
2.3. [If applicable:] If Y/PY/NI to 2.1 or 2.2: Were important non-protocol interventions balanced across intervention groups?	y	NA / Y / PY / PN / N / NI
2.4. [If applicable:] Were there failures in implementing the intervention that could have affected the outcome?	n	NA / Y / PY / PN / N / NI
2.5. [If applicable:] Was there non-adherence to the assigned intervention regimen that could have affected participants' outcomes?	na	NA / Y / PY / PN / N / NI
2.6. If N/PN/NI to 2.3, or Y/PY/NI to 2.4 or 2.5: Was an appropriate analysis used to estimate the effect of adhering to the intervention?	na	NA / Y / PY / PN / N / NI
Risk-of-bias judgement	low	Low / High / Some concerns
Optional: What is the predicted direction of bias due to deviations from intended interventions?	na	NA / Favours experimental / Favours comparator / Towards null /Away from null / Unpredictable

Domain 2: Risk of bias due to deviations from the intended interventions (*effect of adhering to intervention*)

Domain 3: Missing outcome data

Signalling questions	Comments	Response options
3.1 Were data for this outcome available for all, or nearly all, participants randomized?	y	Y / PY / PN / N / NI
3.2 If N/PN/NI to 3.1: Is there evidence that the result was not biased by missing outcome data?	na	NA / Y / PY / PN / N
3.3 If N/PN to 3.2: Could missingness in the outcome depend on its true value?	Na	NA / Y / PY / PN / N / NI
3.4 If Y/PY/NI to 3.3: Is it likely that missingness in the outcome depended on its true value?	na	NA / Y / PY / PN / N / NI
Risk-of-bias judgement	low	Low / High / Some concerns
Optional: What is the predicted direction of bias due to missing outcome data?		NA / Favours experimental / Favours comparator / Towards null /Away from null / Unpredictable

Domain 4: Risk of bias in measurement of the outcome

Signalling questions	Comments	Response options
4.1 Was the method of measuring the outcome inappropriate?	n	Y / PY / PN / N / NI
4.2 Could measurement or ascertainment of the outcome have differed between intervention groups?	n	Y / PY / PN / N / NI
4.3 If N/PN/NI to 4.1 and 4.2: Were outcome assessors aware of the intervention received by study participants?	n	NA / Y / PY / PN / N / NI
4.4 If Y/PY/NI to 4.3: Could assessment of the outcome have been influenced by knowledge of intervention received?	Na	NA / Y / PY / PN / N / NI
4.5 If Y/PY/NI to 4.4: Is it likely that assessment of the outcome was influenced by knowledge of intervention received?	na	NA / Y / PY / PN / N / NI
Risk-of-bias judgement	low	Low / High / Some concerns
Optional: What is the predicted direction of bias in measurement of the outcome?		NA / Favours experimental / Favours comparator / Towards null /Away from null / Unpredictable

Domain 5: Risk of bias in selection of the reported result

Signalling questions	Comments	Response options
5.1 Were the data that produced this result analysed in accordance with a pre-specified analysis plan that was finalized before unblinded outcome data were available for analysis?	y	Y / PY / PN / N / NI
Is the numerical result being assessed likely to have been selected, on the basis of the results, from...		
5.2. ... multiple eligible outcome measurements (e.g. scales, definitions, time points) within the outcome domain?	n	Y / PY / PN / N / NI
5.3 ... multiple eligible analyses of the data?	n	Y / PY / PN / N / NI
Risk-of-bias judgement	low	Low / High / Some concerns
Optional: What is the predicted direction of bias due to selection of the reported result?		NA / Favours experimental / Favours comparator / Towards null /Away from null / Unpredictable

Overall risk of bias

Risk-of-bias judgement	Low	Low / High / Some concerns
Optional: What is the overall predicted direction of bias for this outcome?		NA / Favours experimental / Favours comparator / Towards null /Away from null / Unpredictable

Revised Cochrane risk-of-bias tool for randomized trials (RoB 2)
TEMPLATE FOR COMPLETION

Edited by Julian PT Higgins, Jelena Savović, Matthew J Page, Jonathan AC Sterne
on behalf of the RoB2 Development Group

Version of 22 August 2019

The development of the RoB 2 tool was supported by the MRC Network of Hubs for Trials Methodology Research (MR/L004933/2- N61), with the support of the host MRC ConDuCT-II Hub (Collaboration and innovation for Difficult and Complex randomised controlled Trials In Invasive procedures - MR/K025643/1), by MRC research grant MR/M025209/1, and by a grant from The Cochrane Collaboration.

Risk of Bias tool 2

By: Na, Hyo-Seok; Shin, Soon-Young; Hwang, Jin-Young; et al. (2011)Effects of intravenous iron combined with low-dose recombinant human erythropoietin on transfusion requirements in iron-deficient patients undergoing bilateral total knee replacement arthroplasty (CME)

TRANSFUSION Volume: 51 Issue: 1 Pages: 118-124 Published:

Study details

Reference	Effects of intravenous iron combined with low-dose recombinant human erythropoietin on transfusion requirements in iron-deficient patients undergoing bilateral total knee replacement arthroplasty (CME) By: Na, Hyo-Seok; Shin, Soon-Young; Hwang, Jin-Young; et al. TRANSFUSION Volume: 51 Issue: 1 Pages: 118-124 Published: JAN 2011

Study design

X Individually-randomized parallel-group trial

☐ Cluster-randomized parallel-group trial

☐ Individually randomized cross-over (or other matched) trial

For the purposes of this assessment, the interventions being compared are defined as

Experimental:	Iron Succrose 200mg Intravenous Erythropoetin 3000 subcuticular	Comparator:	Control group

Specify which outcome is being assessed for risk of bias	Funding bias, attrition bias, reporting bias, selection bias, allocation bias

Specify the numerical result being assessed. In case of multiple alternative analyses being presented, specify the numeric result (e.g. RR = 1.52 (95% CI 0.83 to 2.77) and/or a reference (e.g. to a table, figure or paragraph) that uniquely defines the result being assessed.	Not documented in enough detail for Non-anaemic iron deficiency, the outcome of interest to this review

Is the review team's aim for this result…?

☑ to assess the effect of *assignment to intervention* (the 'intention-to-treat' effect)

☐ to assess the effect of *adhering to intervention* (the 'per-protocol' effect)

If the aim is to assess the effect of *adhering to intervention*, select the deviations from intended intervention that should be addressed (at least one must be checked):

☑ occurrence of non-protocol interventions

☐ failures in implementing the intervention that could have affected the outcome

☐ non-adherence to their assigned intervention by trial participants

Which of the following sources were <u>obtained</u> to help inform the risk-of-bias assessment? (tick as many as apply)

☑ Journal article(s) with results of the trial

☐ Trial protocol

☐	Statistical analysis plan (SAP)
☐	Non-commercial trial registry record (e.g. ClinicalTrials.gov record)
☐	Company-owned trial registry record (e.g. GSK Clinical Study Register record)
☐	“Grey literature” (e.g. unpublished thesis)
☐	Conference abstract(s) about the trial
☐	Regulatory document (e.g. Clinical Study Report, Drug Approval Package)
☐	Research ethics application
☐	Grant database summary (e.g. NIH RePORTER or Research Councils UK Gateway to Research)
☑	Personal communication with trialist
☐	Personal communication with the sponsor

Risk of bias assessment

Responses underlined in green are potential markers for low risk of bias, and responses in red are potential markers for a risk of bias. Where questions relate only to sign posts to other questions, no formatting is used.

Domain 1: Risk of bias arising from the randomization process

Signalling questions	Comments	Response options
1.1 Was the allocation sequence random?	Yes	Y / PY / PN / N / NI
1.2 Was the allocation sequence concealed until participants were enrolled and assigned to interventions?	Yes	Y / PY / PN / N / NI
1.3 Did baseline differences between intervention groups suggest a problem with the randomization process?	No	Y / PY / PN / N / NI
Risk-of-bias judgement	Low	Low / High / Some concerns
Optional: What is the predicted direction of bias arising from the randomization process?		NA / Favours experimental / Favours comparator / Towards null /Away from null / Unpredictable

Domain 2: Risk of bias due to deviations from the intended interventions (*effect of assignment to intervention*)

2.1. Were participants aware of their assigned intervention during the trial?	Yes	Y / PY / PN / N / NI
2.2. Were carers and people delivering the interventions aware of participants' assigned intervention during the trial?	Yes	Y / PY / PN / N / NI
2.3. If Y/PY/NI to 2.1 or 2.2: Were there deviations from the intended intervention that arose because of the trial context?	Ni	NA / Y / PY / PN / N / NI
2.4 If Y/PY to 2.3: Were these deviations likely to have affected the outcome?	Ni	NA / Y / PY / PN / N / NI
2.5. If Y/PY/NI to 2.4: Were these deviations from intended intervention balanced between groups?	Ni	NA / Y / PY / PN / N / NI
2.6 Was an appropriate analysis used to estimate the effect of assignment to intervention?	Yes	Y / PY / PN / N / NI
2.7 If N/PN/NI to 2.6: Was there potential for a substantial impact (on the result) of the failure to analyse participants in the group to which they were randomized?	Na	NA / Y / PY / PN / N / NI
Risk-of-bias judgement	Some concerns, although randomised, patients and staff aware of allocation, could introduce bias	Low / High / Some concerns
Optional: What is the predicted direction of bias due to deviations from intended interventions?		NA / Favours experimental / Favours comparator / Towards null /Away from null / Unpredictable

Domain 2: Risk of bias due to deviations from the intended interventions (*effect of adhering to intervention*)

2.1. Were participants aware of their assigned intervention during the trial?	Yes	Y / PY / PN / N / NI
2.2. Were carers and people delivering the interventions aware of participants' assigned intervention during the trial?	Yes	Y / PY / PN / N / NI
2.3. [If applicable:] If Y/PY/NI to 2.1 or 2.2: Were important non-protocol interventions balanced across intervention groups?	Ni	NA / Y / PY / PN / N / NI
2.4. [If applicable:] Were there failures in implementing the intervention that could have affected the outcome?	Ni	NA / Y / PY / PN / N / NI
2.5. [If applicable:] Was there non-adherence to the assigned intervention regimen that could have affected participants' outcomes?	Ni	NA / Y / PY / PN / N / NI
2.6. If N/PN/NI to 2.3, or Y/PY/NI to 2.4 or 2.5: Was an appropriate analysis used to estimate the effect of adhering to the intervention?	Ni	NA / Y / PY / PN / N / NI
Risk-of-bias judgement	some concerns, not enough information available to assess the impact of adherence	Low / High / Some concerns
Optional: What is the predicted direction of bias due to deviations from intended interventions?		NA / Favours experimental / Favours comparator / Towards null /Away from null / Unpredictable

Domain 3: Missing outcome data

Signalling questions	Comments	Response options
3.1 Were data for this outcome available for all, or nearly all, participants randomized?	Py Yes for trial, but not enough separation for NAID, the purpose of this review	Y / PY / PN / N / NI
3.2 If N/PN/NI to 3.1: Is there evidence that the result was not biased by missing outcome data?	Py, see above	NA / Y / PY / PN / N
3.3 If N/PN to 3.2: Could missingness in the outcome depend on its true value?	Ni	NA / Y / PY / PN / N / NI
3.4 If Y/PY/NI to 3.3: Is it likely that missingness in the outcome depended on its true value?	ni	NA / Y / PY / PN / N / NI
Risk-of-bias judgement	Some concerns, for the outcome data im looking at some data is not reported	Low / High / Some concerns
Optional: What is the predicted direction of bias due to missing outcome data?		NA / Favours experimental / Favours comparator / Towards null /Away from null / Unpredictable

Domain 4: Risk of bias in measurement of the outcome

Signalling questions	Comments	Response options
4.1 Was the method of measuring the outcome inappropriate?	No	Y / PY / PN / N / NI
4.2 Could measurement or ascertainment of the outcome have differed between intervention groups?	No	Y / PY / PN / N / NI
4.3 If N/PN/NI to 4.1 and 4.2: Were outcome assessors aware of the intervention received by study participants?	Ni	NA / Y / PY / PN / N / NI
4.4 If Y/PY/NI to 4.3: Could assessment of the outcome have been influenced by knowledge of intervention received?	Ni	NA / Y / PY / PN / N / NI
4.5 If Y/PY/NI to 4.4: Is it likely that assessment of the outcome was influenced by knowledge of intervention received?	Ni	NA / Y / PY / PN / N / NI
Risk-of-bias judgement	Some concerns, not enough information on whether assessors were aware of allocation	Low / High / Some concerns
Optional: What is the predicted direction of bias in measurement of the outcome?		NA / Favours experimental / Favours comparator / Towards null /Away from null / Unpredictable

Domain 5: Risk of bias in selection of the reported result

Signalling questions	Comments	Response options
5.1 Were the data that produced this result analysed in accordance with a pre-specified analysis plan that was finalized before unblinded outcome data were available for analysis?	Yes	Y / PY / PN / N / NI
Is the numerical result being assessed likely to have been selected, on the basis of the results, from...		
5.2. ... multiple eligible outcome measurements (e.g. scales, definitions, time points) within the outcome domain?	No	Y / PY / PN / N / NI
5.3 ... multiple eligible analyses of the data?	No	Y / PY / PN / N / NI
Risk-of-bias judgement	Low concerns	Low / High / Some concerns
Optional: What is the predicted direction of bias due to selection of the reported result?		NA / Favours experimental / Favours comparator / Towards null /Away from null / Unpredictable

Overall risk of bias

Risk-of-bias judgement	Some concerns due to study design, lack of double blinding	Low / High / Some concerns
Optional: What is the overall predicted direction of bias for this outcome?		NA / Favours experimental / Favours comparator / Towards null /Away from null / Unpredictable

STROBE Statement—checklist of items that should be included in reports of observational studies

Risk of Bias 3

AU Scardino, Marco Di Matteo, Berardo Martorelli, Federica Tanzi, Dario Kon, Elizaveta D'Amato, Tiziana

TI Improved patient blood management and cost saving in hip replacement surgery through the implementation of pre-operative Sucrosomial (R) iron supplementation: a quality improvement assessment study

SO INTERNATIONAL ORTHOPAEDICS

VL 43 IS 1 SI SI BP 39 EP 46 DI 10.1007/s00264-018-4149-7 PD JAN 2019 PY 2019 TC 3 ZS 0 ZB 0 ZR 0 Z8 0 Z9 3 SN 0341-2695 EI 1432-5195 UT WOS:000455631800006 PM 30232527 ER

	Item No	Recommendation
Title and abstract	1	*(a)* Indicate the study's design with a commonly used term in the title or the abstract Yes
		(b) Provide in the abstract an informative and balanced summary of what was done and what was found Yes
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported Yes
Objectives	3	State specific objectives, including any prespecified hypotheses Yes
Methods		
Study design	4	Present key elements of study design early in the paper

		Yes
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants Yes
		(c) Cohort study—For matched studies, give matching criteria and number of exposed and unexposed *yes* *Case-control study*—For matched studies, give matching criteria and the number of controls per case
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable Yes
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group Yes
Bias	9	Describe any efforts to address potential sources of bias Not documented
Study size	10	Explain how the study size was arrived at Yes
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why

		Completed
Statistical methods	12	*(a)* Describe all statistical methods, including those used to control for confounding Described
		(b) Describe any methods used to examine subgroups and interactions Described
		(c) Explain how missing data were addressed Described
		(d) *Cohort study*—If applicable, explain how loss to follow-up was addressed described *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy
		(*e*) Describe any sensitivity analyses described

Results

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed Described
		(b) Give reasons for non-participation at each stage
		(c) Consider use of a flow diagram
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders
		(b) Indicate number of participants with missing data for each variable of interest
		(b) *Cohort study*—Summarise follow-up time (eg, average and total amount)

		All described
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time
		Case-control study—Report numbers in each exposure category, or summary measures of exposure
		Cross-sectional study—Report numbers of outcome events or summary measures Fully described
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included
		(*b*) Report category boundaries when continuous variables were categorized
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses Fully completed
Discussion		
Key results	18	Summarise key results with reference to study objectives Completed
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias Addressed
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence Completed
Generalisability	21	Discuss the generalisability (external validity) of the study results

		Completed
Other information		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based

Not documented

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

All elements completed appropriately with a low risk of bias, except the study design, comparing a quality improvement with retrospective data, leading to a judgement of some risk of concern, which is observational in nature and not a blinded study

Risk of Bias 4

Iron deficiency in preoperative period of bariatric surgery. Omelanczuk P., Pampillon N., Sanchez M., Lasagni V., Penutto C., Omelanczuk S., Abaurre M. Embase Obesity Surgery. Conference: 18th World Congress of the International Federation for the Surgery of Obesity and Metabolic Disorders, IFSO 2013. Istanbul Turkey. Conference Publication: (var.pagings). 23 (8) (pp 1077), 2013. Date of Publication: August 2013. [Conference Abstract] AN: 71128314

STROBE Statement—Items to be included when reporting observational studies in a conference abstract

Item	Recommendation
Title	Indicate the study's design with a commonly used term in the title (e.g cohort, case-control, cross sectional) Described
Authors	Contact details for the corresponding author Completed
Study design	Description of the study design (e.g cohort, case-control, cross sectional) Completed
Objective	Specific objectives or hypothesis Completed
Methods	
Setting	Description of setting, follow-up dates or dates at which the outcome events occurred or at which the outcomes were present, as well as any points or ranges on other time scales for the outcomes (e.g., prevalence at age 18, 1998-2007). Completed
Participants	*Cohort study*—Give the most important eligibility criteria, and the most important sources and methods of selection of participants. Describe briefly the methods of follow-up *Completed* *Case-control study*—Give the major eligibility criteria, and the major sources and methods of case ascertainment and control selection *Cross-sectional study*—Give the eligibility criteria, and the major sources and methods of

	selection of participants
	Cohort study—For matched studies, give matching and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case
Variables	Clearly define primary outcome for this report. Completed
Statistical methods	Describe statistical methods, including those used to control for confounding, Partially, means and standard deviations presented with p values, but doesn't state the actual test used
Results	
Participants	Report Number of participants at the beginning and end of the study Completed
Main results	Report estimates of associations. If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period percentage decrease identified Report appropriate measures of variability and uncertainty (e.g., odds ratios with confidence intervals
Conclusions	General interpretation of study results completed

Most elements completed in-line with STROBE for conference extracts, prospective and observational in nature, randomised, although method not identified. Limitations on assessing risk of bias due to the lack of reported evidence and the lack of blinding in the study design lead to the risk of bias being judged as high.

Risk of Bias 5

The role of intravenous iron carboxymaltose supplementation in non-anaemic patients undergoing elective hip or knee arthroplasty.
D'Amato T., Fenocchio G., Martorelli F., Scardino M., Simili V., Gurgone A.
Embase Transfusion Medicine. Conference: 18th Annual NATA Symposium on Patient Blood Management, Haemostasis and Thrombosis. Italy. 27 (Supplement 1) (pp 54-55), 2017. Date of Publication: April 2017. [Conference Abstract] AN: 615441255

STROBE Statement—Items to be included when reporting observational studies in a conference abstract

Item	Recommendation
Title	Indicate the study's design with a commonly used term in the title (e.g cohort, case-control, cross sectional) completed
Authors	Contact details for the corresponding author partially completed with name and hospital but no address
Study design	Description of the study design (e.g cohort, case-control, cross sectional) described
Objective	Specific objectives or hypothesis described
Methods	
Setting	Description of setting, follow-up dates or dates at which the outcome events occurred or at which the outcomes were present, as well as any points or ranges on other time scales for the outcomes (e.g., prevalence at age 18, 1998-2007). described
Participants	*Cohort study*—Give the most important eligibility criteria, and the most important sources and methods of selection of participants. Describe briefly the methods of follow-up described *Case-control study*—Give the major eligibility criteria, and the major sources and methods of case ascertainment and control selection *Cross-sectional study*—Give the eligibility criteria, and the major sources and methods of

	selection of participants
	Cohort study—For matched studies, give matching and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case
Variables	Clearly define primary outcome for this report. described
Statistical methods	Describe statistical methods, including those used to control for confounding partially completed, means and standard deviations reported, but method of measurement not described
Results	
Participants	Report Number of participants at the beginning and end of the study reported
Main results	Report estimates of associations. If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period Report appropriate measures of variability and uncertainty (e.g., odds ratios with confidence intervals p-values documented, but not CI intervals
Conclusions	General interpretation of study results described appropriately

Most elements completed in-line with STROBE for conference extracts, prospective and observational in nature, randomised, although method not identified. Limitations on assessing risk of bias due to the lack of reported evidence and the lack of blinding in the study design lead to the risk of bias being judged as high.

Risk of Bias 6

Pre-operative iron deficiency in bariatric surgery: Diagnosis and treatment. Sanchez M., Pampillon N., Ojeda A., Abaurre M., Penuto C., Berducci M., Lasagni V., Palma R., Omelanczuk S.

Embase Obesity Surgery. Conference: 20th International Federation for the Surgery of Obesity and Metabolic Disorders World Congress, IFSO 2015. Vienna Austria. Conference Publication: (var.pagings). 25 (1 SUPPL. 1) (pp S119-S120), 2015. Date of Publication: August 2015. [Conference Abstract] AN: 72002909

STROBE Statement—Items to be included when reporting observational studies in a conference abstract

Item	**Recommendation**
Title	Indicate the study's design with a commonly used term in the title (e.g cohort, case-control, cross sectional) described
Authors	Contact details for the corresponding author partially described,
Study design	Description of the study design (e.g cohort, case-control, cross sectional) described
Objective	Specific objectives or hypothesis described
Methods	
Setting	Description of setting, follow-up dates or dates at which the outcome events occurred or at which the outcomes were present, as well as any points or ranges on other time scales for the outcomes (e.g., prevalence at age 18, 1998-2007). described

Participants	*Cohort study*—Give the most important eligibility criteria, and the most important sources and methods of selection of participants. Describe briefly the methods of follow-up described
	Case-control study—Give the major eligibility criteria, and the major sources and methods of case ascertainment and control selection
	Cross-sectional study—Give the eligibility criteria, and the major sources and methods of selection of participants
	Cohort study—For matched studies, give matching and number of exposed and unexposed
	Case-control study—For matched studies, give matching criteria and the number of controls per case
Variables	Clearly define primary outcome for this report. described
Statistical methods	Describe statistical methods, including those used to control for confounding partially described, means and standard deviations and p-values given, methods not specified
Results	
Participants	Report Number of participants at the beginning and end of the study reported
Main results	Report estimates of associations. If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period not described
	Report appropriate measures of variability and uncertainty (e.g., odds ratios with confidence intervals p-values yes, no CI intervals documented
Conclusions	General interpretation of study results

Downloaded from https://www.strobe-statement.org/checklists/ accessed (April 2021)

Most elements completed in-line with STROBE for conference extracts, prospective and observational in nature, randomised, although method not identified. Limitations on assessing risk of bias due to the lack of reported evidence and the lack of blinding in the study design lead to the risk of bias being judged as high.

Risk of Bias 7

Scardino M. Embase Expert Review of Hematology. Conference: 5th International Multidisciplinary Course on Iron Anemia. Italy. 10 (Supplement 1) (pp 4), 2017. Date of Publication: 2017. [Conference Abstract] AN: 619950847

STROBE Statement—Items to be included when reporting observational studies in a conference abstract

Item	Recommendation
Title	Indicate the study's design with a commonly used term in the title (e.g cohort, case-control, cross sectional) described
Authors	Contact details for the corresponding author described
Study design	Description of the study design (e.g cohort, case-control, cross sectional) described
Objective	Specific objectives or hypothesis described
Methods	
Setting	Description of setting, follow-up dates or dates at which the outcome events occurred or at which the outcomes were present, as well as any points or ranges on other time scales for the outcomes (e.g., prevalence at age 18, 1998-2007). described

Participants	*Cohort study*—Give the most important eligibility criteria, and the most important sources and methods of selection of participants. Describe briefly the methods of follow-up described
	Case-control study—Give the major eligibility criteria, and the major sources and methods of case ascertainment and control selection
	Cross-sectional study—Give the eligibility criteria, and the major sources and methods of selection of participants
	Cohort study—For matched studies, give matching and number of exposed and unexposed
	Case-control study—For matched studies, give matching criteria and the number of controls per case
Variables	Clearly define primary outcome for this report. Not described
Statistical methods	Describe statistical methods, including those used to control for confounding partially described, means, standard deviations and p-values described, methods not stated
Results	
Participants	Report Number of participants at the beginning and end of the study reported
Main results	Report estimates of associations. If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period
	Report appropriate measures of variability and uncertainty (e.g., odds ratios with confidence intervals not reported
Conclusions	General interpretation of study results completed

Downloaded from https://www.strobe-statement.org/checklists/ accessed (April 2021)

Most elements completed in-line with STROBE for conference extracts, retrospective versus prospective and observational in nature, comparing a quality improvement with retrospective data. Limitations on assessing risk of bias due to the lack of reported evidence and the study design led to the risk of bias being judged as high.

APPENDIX 10: CONSORT Checklist

CONSORT 2010 checklist of information to include when reporting a randomised trial*

Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a randomised trial in the title	
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale	
	2b	Specific objectives or hypotheses	
Methods			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	
Participants	4a	Eligibility criteria for participants	
	4b	Settings and locations where the data were collected	
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	
	6b	Any changes to trial outcomes after the trial commenced, with reasons	
Sample size	7a	How sample size was determined	
	7b	When applicable, explanation of any interim analyses and stopping guidelines	

Randomisation:			
Sequence generation	8a	Method used to generate the random allocation sequence	
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those assessing outcomes) and how	
	11b	If relevant, description of the similarity of interventions	
Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	
Results			
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome	
	13b	For each group, losses and exclusions after randomisation, together with reasons	
Recruitment	14a	Dates defining the periods of recruitment and follow-up	
	14b	Why the trial ended or was stopped	
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	
Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	

Discussion			
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	
Generalisability	21	Generalisability (external validity, applicability) of the trial findings	
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	
Other information			
Registration	23	Registration number and name of trial registry	
Protocol	24	Where the full trial protocol can be accessed, if available	
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	

*We strongly recommend reading this statement in conjunction with the CONSORT 2010 Explanation and Elaboration for important clarifications on all the items. If relevant, we also recommend reading CONSORT extensions for cluster randomised trials, non-inferiority and equivalence trials, non-pharmacological treatments, herbal interventions, and pragmatic trials. Additional extensions are forthcoming: for those and for up to date references relevant to this checklist, see www.consort-statement.org.

Appendix 11: Randomised Controlled trial protocol .

STUDY PROTOCOL

PROTOCOL TITLE:

Effectiveness of iron supplementation in the non-anaemic iron deficient patient population undergoing lower limb arthroplasty,

A Randomised controlled trial.

SHORT TITLE:

Iron supplementation for iron deficiency before arthroplasty (ISIDA)

PROTOCOL VERSION: 2.0

PROTOCOL DATE: 12th March 2019

PROTOCOL NUMBER: NA

ISRCTN NUMBER: 48118194

SWAT REPOSITORY: SWAT 83

SPONSOR: Northumbria Healthcare NHS Foundation Trust

CORRESPONDING CHIEF INVESTIGATOR:

Professor Mike Reed

Consultant Orthopaedic Surgeon

Northumbria Healthcare NHS Foundation Trust

Authorised by:

Name:	Professor Mike Reed	Name:	Peta Heslop
Role:	Chief Investigator	Role:	Sponsor Representative
Signature:	Date:	Signature:	Date:

1 TRIAL CONTACTS

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Professor Mike Reed Consultant Orthopaedic Surgeon, Northumbria Healthcare NHS Foundation Trust

Email: mike.reed@nhs.net

2 Abbreviations and Glossary

AE	Adverse event
AR	Adverse reaction
CF	Consent form
CI	Chief Investigator
CRF	Case Report Form
DCF	Data Clarification Form
DMEC	Data Monitoring Ethics Committee
ERC	Endpoint Review Committee
GCP	Good Clinical Practice
HES	Hospital episode statistic
HRA	Health Research Authority
IB	Investigator's Brochure
ICH	International Conference on Harmonisation

IDMC Independent Data Monitoring Committee

ISRCTN International Standard Randomised Controlled Trial Number

NHS National Health Service

PAS Patient Administration System

PI Principal Investigator

PIS Patient information Sheet

QoL Quality of life

REC Research Ethics Commitee

SAE Serious Adverse Event

SAR Serious Adverse Reaction

SOP Standard Operating Procedures

SSA Site Specific Assessment

SUSAR Suspected Unexpected Serious Adverse Reaction

TMG Trial Management Group

THUG Total Hip Users Group

TSC Trial Steering Committee

UAR Unexpected adverse reaction

3 SUMMARY OF STUDY

3.1 Summary Table

Acronym	**ISIDA** (**I**ron **S**upplementation for **I**ron **D**eficiency in **A**rthroplasty)
Long title	Effectiveness of iron supplementation in the non-anaemic iron deficient patient population undergoing lower limb arthroplasty, A Randomised controlled trial.
Type of trial	Non-CTIMP
Study design	Two-arm, open, parallel group, randomised controlled trial
Type of participants	Non-anaemic Iron deficient patients, undergoing lower limb

to be studied	arthroplasty
Setting	Northumbria Healthcare NHS Foundation Trust
Treatment	All patients will have a Full Blood count (FBC), CRP and ferritin checked prior to surgery. Patients identified as non-anaemic (Hb > 12 for Women and 13 for Men) but iron deficient, (Ferritin <50) undergoing lower limb hip and knee arthroplasty, will be randomised to treatment with oral iron supplementation or no intervention. Patients with normal Ferritin will not be eligible for the study. Patients inadvertently found to have a high ferritin will be referred to haematology for further investigation as a safety mechanism and will not be eligible for the study.
Primary Objective	To assess the effectiveness of oral iron supplementation to improve outcomes for patients undergoing lower limb arthroplasty.
Primary Outcome	Haemoglobin drop/recovery from pre-operative to 3 weeks postoperative
Secondary Outcomes	• Length of hospital stay. • Transfusion Rate and number of units. • Readmission rate at up to 30 Days of surgery for any reason • Morbidity and Mortality at 30 and 90 days, defined in table 3.3 • Hb, ferritin and CRP every four weeks whilst on supplementation Patients further randomised to receive follow-up questionnaire via post or telephone, SWAT analysis • Patient symptom questionnaire (FACIT Fatigue) preoperative and at 4 weeks postoperative. • Patient outcome measures EQ5D 5L preoperative and at 3 months postoperative.
Estimated recruitment period	April 2019 – January 2020
Duration per patient	6 months
Estimated total trial duration	36 months including write up, 12 months planning and funding, 12 months data collection, 12 months write-up
Planned trial sites	Wansbeck General Hospital, North Tyneside General hospital,

	Hexham General hospital, Northumbria Specialist Emergency Care Centre
Number of patients	188
Sponsor	Northumbria Healthcare NHS Foundation Trust
Funder	SALUS Haus
Chief Investigator	Professor Mike Reed

3.2 TRIAL SUMMARY

The aim of this trial is to analyse the effect of iron supplementation in non-anaemic iron deficient patients undergoing lower limb arthroplasty. Patients with a normal haemoglobin (greater than 12 in women and 13 in men), who are deemed to be iron deficient (Ferritin <50) will be recruited to this randomised controlled trial. 188 patients will be randomised 1 to 1 to receive oral iron supplementation or no intervention for six months duration, covering preoperative and postoperative phases of care. All patients will have blood tests 4 weekly throughout the trial and a sample 3 weeks postoperative. To ease patient burden, blood tests will be arranged with the patient in home or hospital clinic. All participants must receive at least 4 weeks of supplementation prior to surgery to be eligible for the trial. The hypothesis being that patients with reduced overall iron stores will benefit from iron supplementation to enable their haemoglobin to recover after surgery.

Primary Outcome: Hb at 3 weeks postoperative

Secondary Outcomes

- Length of hospital stay (midnights in hospital).
- Transfusion Rate and number of units transfused up to 30 days
- Adverse events (including all cause morbidity and mortality at 30 and 90 days)
- FBC, CRP and Ferritin every 4 weeks throughout the six month trial.
- Readmission within 30 days of surgery
- Inpatient DVT/PE within 30 days of surgery
- Pneumonia
- Cerebrovascular incident
- Myocardial infarction

3.3 STUDY WITHIN A TRIAL SUMMARY

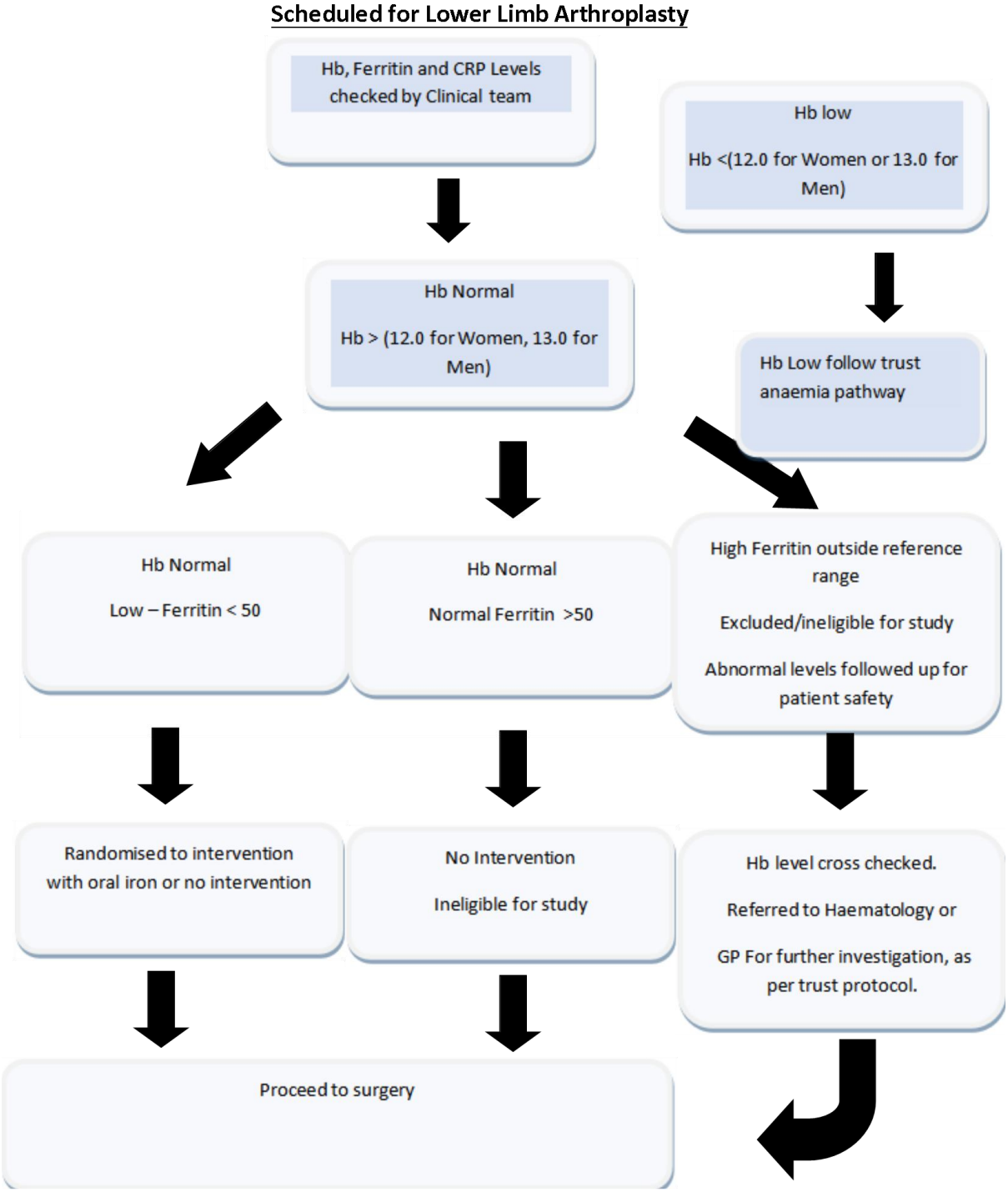
The aim of this study within a trial is to analyse response rates and quality using two different methods to administer post operative quality of life questionnaires.

All trial participants will be eligible for the SWAT follow-up study.

Patients will be randomised to postal and telephone follow-up initially, with further contact if necessary to improve response or completion rate.

Patients randomised to postal follow-up, will receive an anaemia screening questionnaire (FACIT Fatigue) at 4 weeks and QOL (eq 5d 5l) at 3 months to be completed and returned. Patients randomised to telephone follow-up, will receive telephone calls to complete the same questionnaires.

3.4 Study Flow Chart



3.5 Study Assessment Schedule

Assessment (D=day, W=week, M=month)	**Routine practice**	**PRE-CLINIC SCREENING**	**D0**	**WK3**	**WK4**	**30 and 90 days postop**	**4 weekly blood tests**
Allowed variation in days							
Eligibility screen		X	X				
Blood Test	X						X
Screen for anaemia	X						
Consent			X				
Randomise			X				
Commence oral supplement		X					
Repeat blood screen				X			
Symptom Questionnaire and Eq 5d 5l pre-op and 3 months post-op		X X			X	 X	
Routine data use including transfusion requirement up to 30 days post-operative, and mortality at 30 and 90 days	X					X	
Morbidity 30 days including: Infection rate Myocardial Infarction (heart attack), Transient ischaemic attack (TIA) Cerebrovascular accident (stroke/ CVA) Acute Kidney Injury (AKI) Deep vein thrombosis (DVT) or pulmonary embolism (PE)							

within 60-days of surgery							

4 BACKGROUND AND RATIONALE

4.1 General Introduction

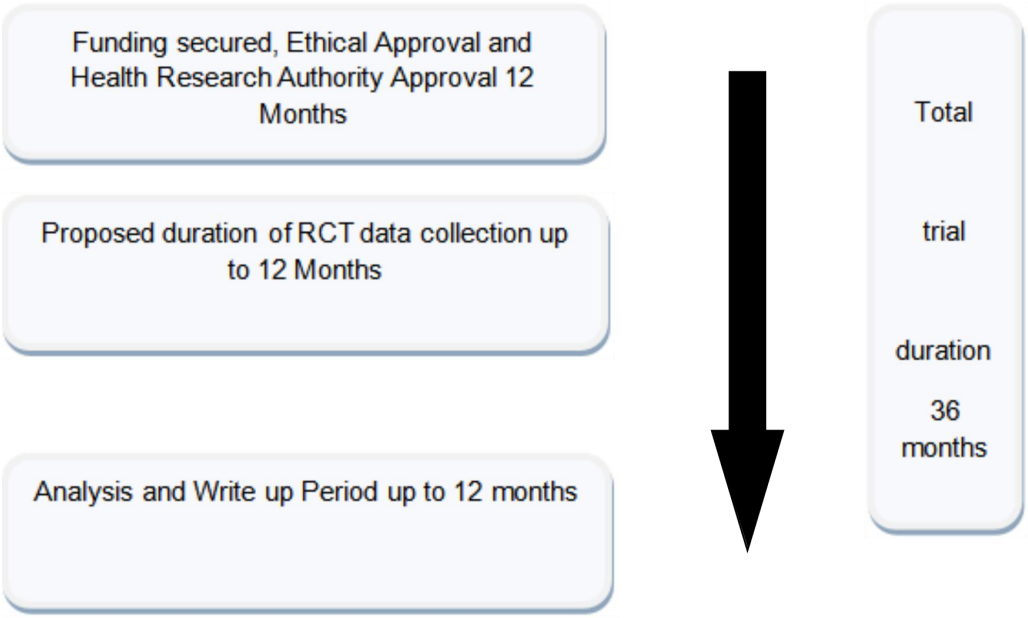
The prevalence of joint replacement within the UK is continually increasing due to an ageing population. For the year 2016/17, 242,629 cases in the UK, were recorded National Joint Registry (1) The risk of blood transfusion within the normal patient population following lower limb arthroplasty has been estimated between 21 and 70% (2). Currently within our organisation, retrospective analysis has shown 20% of anaemic patients receive blood transfusions, with an overall transfusion rate of approximately 5%. Joint replacement surgery within the UK is estimated to utilise approximately 10% of blood available (3).

Pre-operative anaemia in patients undergoing elective hip and knee replacement is associated with increased post-operative morbidity and mortality as well as increased red blood cell transfusion rates, hospital readmission and length of stay (4-11).

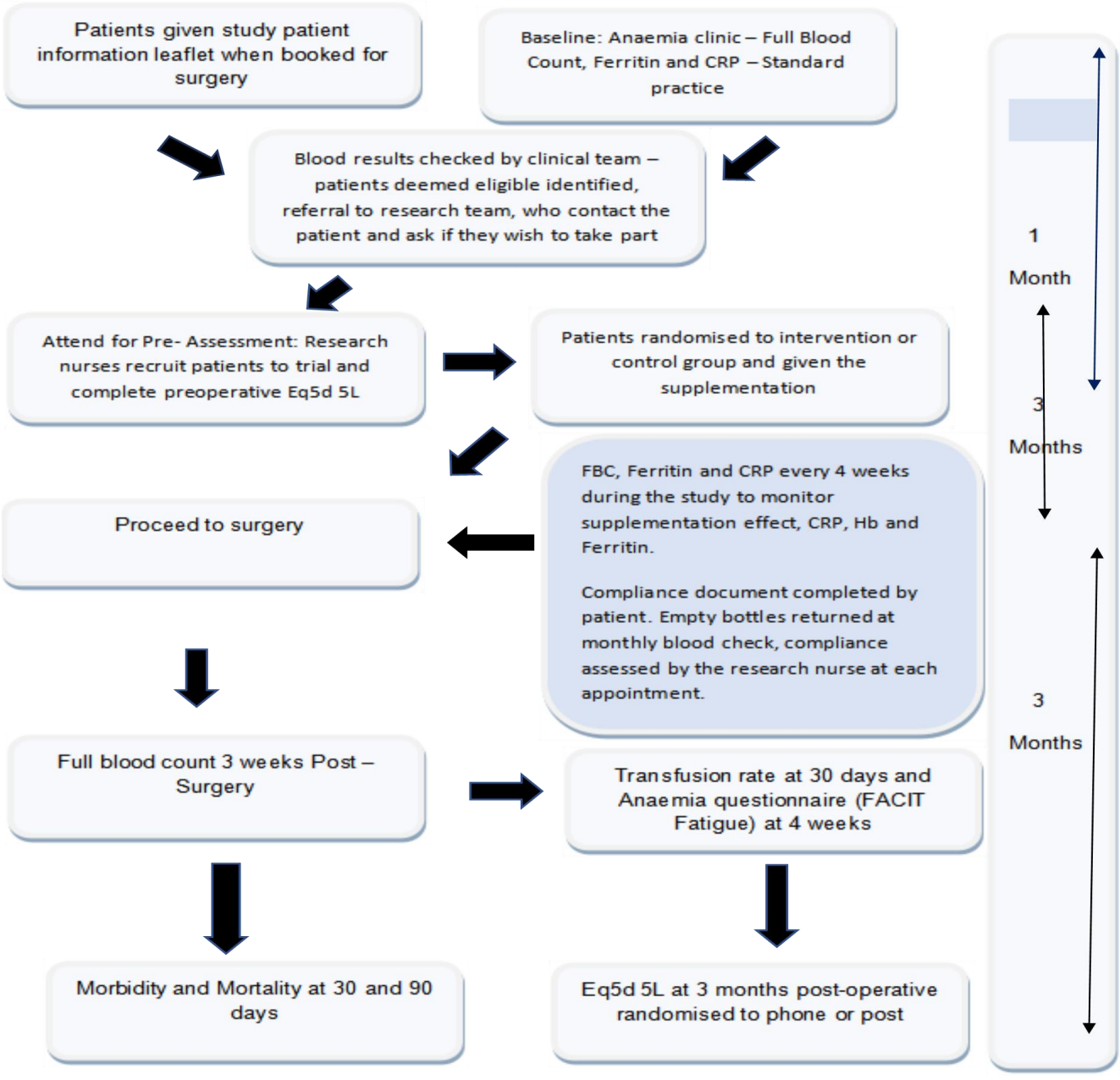
4.1.1 Population

The trial will be conducted utilising patients identified is non-anaemic but iron deficient undergoing lower limb arthroplasty.

Study Timeline Figure 1.2



Participant Timeline (figure 1.3) - 6 months from consent/randomisation to completion.



4.1.2 Intervention(s)

Patients randomised to the intervention group, will be contacted and offered oral iron supplementation

Floradix with iron (also known as Floradix mit Eisen)

DOSAGE: Iron 36.8 mg

DURATION: 6 months from diagnosis of non-anaemic iron deficiency, this aims to encompass both the pre-operative and post-operative phases until the final outcome measures at 90 days.

Patients randomised to the Control/No intervention group will have the same outcome data measures collected but without intervention.

Patients will be further randomised to receive initally postal or telephone follow up following the procedure, they may be reminded via text.

4.1.3 Risks and benefits

Screening for anaemia or iron deficiency is not associated with any greater risk it is standard practice, and is part of patient's routine bloods through the arthroplasty clinic. All patients undergoing arthroplasty have FBC, CRP and Ferritin samples taken prior to surgery as standard practice. However, there will be additional blood tests required for research purposes, outwith standard practice, which expose the patient to additional blood testing and associated risks. Common risks include, discomfort, bleeding and bruising have little overall lasting impact on the the patient. Infection as an extremely rare complication of phlebotomy, all samples will be obtained using aseptic technique.

Oral iron treatment are usually well tolerated but have well documented gastrointestinal symptoms including abdominal pain, constipation and diarrhoea, nausea, and dark stools. There are few significant risks with oral iron supplements. Any side effects or adverse events will be documented and supplementation discontinued if appropriate. Patient FBC, CRP and Ferritin will be monitored every 4 weeks during the trial to monitor the effect of the supplementation.

Although considered best practice, correction of non-anaemic iron deficiency is not routinely performed in most hospitals prior to major surgery so any correction would provide better care than most UK hospitals. The 2015 National Comparative Audit of Blood Transfusion was performed in 190 hospitals and concluded that hospitals should have a preoperative management protocol (12). They concluded there is a need to increase the investigation and management of preoperative anaemia and iron deficiency in the UK. They stated improvement in practice to help ensure appropriate use of transfusion and alternatives would benefit patients and reduce healthcare costs.

For those who have anaemia or iron deficiency with parameters outside the realms of the trial, will be treated as per our existing protocol. In summary these patients are referred to GP for further investigation. Patients in the intervention and non-intervention groups will be monitored for adverse events. With appropriate intervention to maintain patient safety.

Patients who have a reaction or do not tolerate the intervention may have the treatment discontinued.

For the follow-up trial, there are no inherent risks to patients whether randomised to telephone or postal follow up initially. This is simply a way of analysing which method is most effective for the patient population.

4.2 Rationale and justification for the study

An international consensus statement suggesting the need to treat preoperative anaemia was published in 2016 (13). In this statement anaemia and non-anaemic iron deficiency were identified as patient groups that would benefit from the introduction of preoperative anaemia screening, assessment and treatment (13). They suggest patients with low iron levels, with or without anaemia, should be given supplementation to enable them to recover from surgery. Benefits of monitoring and intervention have been demonstrated in the anaemic (8) and non-anaemic iron deficient (14), lower limb arthroplasty population population. However, there is limited published research in the Non-anaemic iron deficient population. The anaemic patient screening and treatment element of the consensus statement is currently being applied within successfully our surgical population. This research aims to implement screening and treatment in the non-anaemic iron deficient population as suggested in the consensus statement, questioning if iron supplementation can demonstrate similar improvements in defined patient outcomes, in non-anaemic iron deficient patients, undergoing lower limb arthroplasty. This will be performed by screening lower limb arthroplasty patients for non-anaemic iron deficiency (defined as a haemoglobin >12 in women and 13 in Men (15) with a ferritin below 50), randomising patients to intervention or no-intervention. Patients identified as having a high ferritin will not be eligible for the trial but will be referred for further investigation by haematology, as a safety mechanism, as per trust protocol (Availiable if required at trust level) (See flow chart 1). Recent publications have suggested lower dose iron may be more effective and have less side effects than traditional treatment (16,17,18), whilst achieving the same benefits. The iron supplementation chosen for this study adheres to this ethos and is a lower dose than traditional therapy at 36.8mg of elemental iron. Previous research has shown patients Hb continues to drop following arthroplasty until day five, before naturally improving as the patient recovers from surgery(19). The drop between day 0 and 5 is quite pronounced (19), with improvement beginning at day five. The impact of oral iron on this sudden surgical insult may be difficult to detect at day five, as the body as still recovering. Therefore, Hb at three weeks postoperative will be measured to analyse improvements in Hb recovery. Supplementation with iron has shown to improve Hb recovery in patients with low ferritin(20). Patients with a low ferritin below 50, but not anaemic have been shown to benefit from supplementation(20) in Quality of Life studies, with improvement of symptoms. This is the level below which we have chosen to treat.

5. HYPOTHESIS AND OBJECTIVES

5.1 Hypothesis

Compared to no intervention, supplementation with iron in the non-anaemic iron deficient population improves patients haemoglobin recovery post surgery, reduces symptoms of anaemia (FACIT fatigue) and improves patient outcomes (EQ5D 5L). It may also reduce length of stay, readmission rate at 30 days, and transfusion rate post surgery.

5.2 Objective

The primary objective is to determine the effectiveness of iron supplementation in the non-anaemic iron deficient lower limb arthroplasty population.

The secondary objective is to analyse postal versus telephone follow-up

6 STUDY DESIGN

This project is 36-months in length (12 months funding, trial design and ethical approval, 12 month clinical trial duration (including 3 month follow up) and 12 months for analysis and write-up). It is designed as a parallel group, randomised controlled trial comparing arm 1 (intervention) to arm 2 (control):

Arm 1: Intervention with oral iron supplementation

Arm 2: No intervention

Patients will be randomly allocated to intervention or control arm using simple randomisation.

All patients will be further randomised to receive initially anaemia symptoms questionnaire (FACIT Fatigue) and health related Quality of Life (QOL) (Eq 5d 5l) via post or telephone after surgery using simple randomisation and may receive text reminders.

6.1 Trial Registration

The trial has been registered for an ISRCTN Number www.isrctn.com. The Study within a Trial (SWAT) analysis has been registered with the SWAT repository www.qub.ac.uk/TheNorthernIrelandNetworkforTrialsMethodologyResearch/SWATSWARInformation/ApplicationForms/

6.2 Study Setting

This study will collect data across four hospital sites within a single NHS Foundation Trust based in England. The research sponsor Northumbria Healthcare NHS Foundation Trust.

6.3 Site inclusion criteria

Wansbeck General Hospital, North Tyneside General Hospital, Hexham General Hospital, Northumbria Special Emergency Care Centre.

6.4 Selection of patients

The flow of patients through this trial is illustrated at the beginning of the protocol (particpant timeline figure 1.3). A total of 188 non-anaemic iron deficient patients undergoing primary lower limb arthroplasty will be enrolled in the study over a period of up to 28 months. Patients will be identified by anaemia/iron deficiency screening, contacted and consented for the study and randomised. Research nurses will be responsible for collating data, obtaining written consent at preassessment for use of data, participation in the trial and adherence to the intervention.

6.4.1 Eligibility assessment

Any questions raised about eligibility should be addressed prior to entering the patient into the study.

The date of surgery can sometimes be delayed or cancelled, with preassesment occasionally close to the date of surgery. In order to adequately assess the treatment impact, patients in the intervention group must have received at least four weeks of treatment prior to surgery to be eligible to participate in this trial. Patients surgical date can sometimes brought forward, if this occurs, participation in this trial will not alter this process. Patients will be given surgery at the most optimal date.

The eligibility criteria have been carefully considered and are standards used to ensure both the safety of the participants and that the trial results can be appropriately used to make future treatment decisions for other people with similar disease or medical condition. It is therefore vital that exceptions are not made to the following detailed selection criteria. Deviations from the eligibility criteria are considered to be protocol violations.

6.5 Participant inclusion criteria

- Patients must be undergoing primary Knee or Hip replacement surgery.
- Patients aged over 18 years

- Non anaemic iron deficiency must be identified criterion; Hb>12 for women 13 for men, ferritin < 50.
- Patients must not already be taking regular oral iron
- Patients must provide informed consent.

6.6 Participant exclusion criteria

- Patients who lack capacity to consent to inclusion in the trial.
- Patients with a known allergy/intolerance to floradix
- Pregnancy (Unlikely due to expected age range of patients)
- Patients listed for surgery within four weeks of commencing iron supplementation.
- Patients with a history of Haemochromatosis
- Patients with a history of Thalassemia
- Patients with a ferritin less than 15, who have not had it investigated

6.7 Screening procedures and pre-randomisation investigations

All patients who are listed for hip and knee joint replacement surgery in clinic have their blood checked for anaemia and iron stores and inflamation as standard practice prior to surgery. A GCP trained "gatekeeper", who currently manages the anaemia screening and optimisation programme and is part of the clinical team, will screen laboratory results to identify potential participants. Those who fit the inclusion criteria outlined above will be referred to the research team. Patient information along with an accompanying letter will be given to potential participants in clinic. There will be no additional pre-randomisation investigations. If patients agree to participate they will be randomised into one of two groups – oral iron supplementation or no intervention. They will receive their oral iron supplements at preassessment and asked to follow the intervention.

Informed consent

A detailed Patient Information Sheet (PIS see section 16), which clearly explains the risks and benefits of trial participation will be produced and will be given to all patients in clinic. Patients that are found to fit the inclusion criteria will be asked if they wish to participate in the trial when they attend for preassessment. The GCP trained research team may contact the patient via telephone or post to ensure information has been received and understood. The participants will be given a contact phone number so that they can have the opportunity to ask questions to clinical staff and to discuss the trial with friends/family prior to agreement to take part. This will give them the opportunity to discuss the trial from receiving initial information to reach a decision on whether to take part. Written informed consent will be obtained by the research team at the preassessment clinic, who will randomize and distribute the supplementation as required.

In the unlikely event that new information arises during the trial that may affect participants' willingness to take part, this will be reviewed by the TSC for addition to the patient information sheet. A revised consent form will also be completed if necessary.

All consent forms will be stored in accordance with local requirements. A copy of the consent form (see section 16) will be given to the patient and the original signed copy kept in the trial site file.

A screening log will be used to capture numbers of ineligible or non-consenting patients at each participating site.

Randomisation & Enrolment procedure

Those patients that are found to be non-anaemic but iron deficient will be recruited to the study and will be randomised by a Research Nurse or recruiting clinician.

6.8 Randomisation practicalities

Randomisation would be managed centrally by the lead site R&D team. We propose to use simple randomisation. A telephone call by the recruiting research nurse or clinician would be made to the R&D administrator. The patient will be identified by their trial number and T-Number Using the website www.randomization.com, the administrator will allocate the patient to either intervention or no intervention arm. They will verbally relay this treatment option to the referring clinician, and record the allocation in a password-protected database, stored on a Trust computer. A confirmation email using NHS.net email will be used to provide written confirmation of the treatment allocation and follow-up method.

6.9 Randomisation codes and unblinding

Patients will be informed of their allocations as will the clinician managing each patient. Therefore, procedures for breaking codes/un-blinding are not necessary. Laboratory staff sampling bloods will be blinded to the intervention.

6.10 Study Treatment

Patients included in the study will be split into two groups: Oral supplementation with iron (Floradix mit Eisen, DOSAGE: Iron 36.8mg daily as Ferrous Gluconate) or no intervention. Blood results will be reviewed postoperatively at 3 weeks to assess haemoglobin drop/recovery. The research nursing team will provide the supplementation for patients.

6.10.1 Control Group

Non-anaemic iron deficient patients undergoing lower limb arthroplasty randomised to no intervention. Blood results will be reviewed postoperatively at 3 weeks to assess haemoglobin drop/recovery. Patients will have blood tests every 4 weeks (Hb, CRP, Ferritin) throughout the six month trial duration, as a contol group to measure against the supplementation group. These bloods are trial specific bloods and not part of routine clinical practice, results will be stored until the end of the trial and analysed when patients have completed their participation.

6.10.2 Intervention Group

Non-anaemic iron deficient patients undergoing lower limb arthroplasty randomised to Oral supplementation with iron (Floradix mit eisen DOSAGE: Iron 36.8 mg in three doses daily as Ferrous Gluconate) Blood results will be reviewed postoperatively at 3 weeks to assess haemoglobin drop/recovery. Patients will have blood tests every 4 weeks (Hb, CRP, Ferritin) throughout the six month trial duration, to monitor effects of the supplementation. These bloods are trial specific bloods and not part of routine clinical practice, results will be stored until the end of the trial and analysed when patients have completed their participation.

6.11 Rehabilitation

For both the intervention and control groups, patients will receive standard physiotherapy and rehabilitation, inline with current trust protocols.

7 ASSESSMENTS AND FOLLOW-UP

The assessment schedule is tabled at the beginning of the protocol and assessment details are given below.

At clinic.

Patients who are eligible for joint replacement surgery will have routine bloods taken as is standard practice (baseline). A patient information sheet explaining the trial will be given to all patients.

Baseline:

Bloods; full blood count, serum ferritin, CRP, urea and electrolytes, liver function and eGFR will be checked (as per standard practice).

The results from the clinic will be screened and those that meet the inclusion criteria will be recruited by the research nursing team at preassessment or alternative clinic.

For patients that consent, they will be randomised into one of two groups before receiving the oral iron supplement regimen with clear instructions.

Recruited patients

Proceed to surgery after intervention or no intervention:

Each patient will have repeat haemoglobin taken postoperatively at three weeks. The mean levels of haemoglobin will be compared between the groups adjusting for baseline values.

Each patient will be given a compliance questionairre exploring common gastrointestinal symptoms linked to oral iron supplement intake

- Diarrhoea, constipation, abdominal pain, compliance

All patients will have blood tests every 4 weeks (Hb, CRP, Ferritin) throughout the six month trial duration, to monitor effects of the supplementation. Tests will be performed in hospital or the patients home as arranged by the research nursing team. As patients may have extra trial specific blood samples taken prior to surgery which they would not normally have, we have chosen to blind the research and clinical team to these trial specific results until the patient has completed their trial participation. This is to reduce the risk of biasing the study or altering the patients normal pathway.

Each patient will complete follow up questionnaires at three weeks (Facit-Fatigue) and three months post surgery (EQ-5D-5L).

Compliance

Adherence to the treatment arm will be assessed by the research nursing team, who will confirm with the patient, that they have adhered to the study regimen at each of the regular blood tests. Patients in the no intervention arm will be asked if they have taken and additional iron supplements without the trial teams direction.

To further enhance monitoring of patient compliance, we have designed a patient compliance document, whereby patients will complete a compliance chart to indicate that they have taken the required three daily doses of Floradix mit eisen as instructed. Patients will return the empty bottles to the research team when they have their four weekly blood test. The research team will perform a bottle count and complete compliance documentation, including a percentage compliance measurement. Patients will be given a trial ID card and appointment reminder card, to better inform clinicians to feedback to the research team and to optimise the repeated four weekly blood testing.

Following surgery:

Complications during and after surgery are recorded as standard. Patients included in the study will be followed up for a further 30 days after their surgery to check if they required a blood transfusion.

A report generated from the Trust information based on PAS data from the episode of care. Data will be supplied by the Trust at the end of recruitment.

1) Demographic Data to be collected will include:

- Age at surgery
- Gender
- Smoking status

2) Comorbidities:

- Hypertension
- Atrial Fibrillation
- Ischaemic Heart Disease
- Type I diabetes
- Type II diabetes
- Chronic Obstructive Pulmonary Disease.

3) Hospital episode statistics data:

- Length of stay
- Readmission within 30-days of surgery,

4) Complication data:

- Infection rate (superficial and deep) Public Health England(21)
- Myocardial infarction (heart attack), transient ischaemic attack or cerebrovascular accident (stroke), acute kidney injury (AKI) within 30-days of surgery
- In-patient Deep vein thrombosis (DVT) or in-patient pulmonary embolism (PE) within 60-days of surgery
- Mortality (30- and 90-day).

7.1 PRIMARY OUTCOME ASSESSMENT

We are investigating the efficacy of an oral iron supplementation in the non-anaemic iron deficient population undergoing lower limb arthroplasty. This is a randomised two arm study.

The question is does oral iron supplementation in the non-anaemic iron deficient population undergoing lower limb arthroplasty improve Haemoglobin recovery from pre-operative, to 3

weeks postoperative. Hb measured preoperatively will be compared against the 3 week postoperative sample, to analyse the difference in haemoglobin recovery, with the least drop considered the most positive outcome. Transfused patients will be accounted for in the analysis.

7.2 SECONDARY OUTCOME ASSESSMENT

The following outcomes will be assessed by recording each secondary outcome and comparing each outcome measure against the two arms of the trial.

These will include

- Length of hospital stay (midnights in hospital).
- Transfusion Rate and number of units transfused up to 30 days (no transfusion Haemoglobin threshold has been set, transfusion will be a purely clinical decision.
- 30 day readmission rate.
- Adverse events (including all cause morbidity and mortality at 30 and 90 days)
- FBC, CRP and Ferritin every 4 weeks throughout the six month trial.

Local Trust PAS data will be used to generate data on:

- Readmission within 30 days of surgery
- Inpatient DVT within 30 days of surgery
- Inpatient PE within 30 days of surgery
- Blood transfusion rate and number of units
- Pneumonia
- Cerebrovascular incident
- Myocardial infarction

FBC, CRP and Ferritin will be collected from Northumbria Healthcare NHS Foundation Trust ICE data, to assess change in haemoglobin. Blood transfusion data will also be collected.

7.3 RATIONALE FOR THE FOLLOW-UP TRIAL

The aim of the sub-study is to analyse whether different methods utilised for completion of follow-up questionnaires, postal versus telephone, provide similar qualitative and quantitative data in this patient population.

Previous research undertaken in stroke patients, has shown completion rates to be overall similar, although the postal route takes longer and the telephone route is more expensive(22). Telephone follow up has been shown to be effective in the initial phase postoperatively(23), and as a secondary measure to improve the rate of response when patients fail to respond to postal questionnaires(23,24). A Cochrane review of strategies to improve recruitment to randomised trials (25) suggested that clinical trials should consider

including a SWAT (Study Within A Trial), to effectively gather further evidence on methodologies within trial recruitment, thus improving recruitment and retention to clinical trials in the future.

This sub study designed as a parallel group, randomised controlled trial comparing arm 1 (telephone follow-up) to arm 2 (postal follow-up):

All participants in the trial will be eligible for the SWAT follow-up study.

Patients will be randomised to postal and telephone follow-up initially, with further contact if necessary to improve response or completion rate. Quantitative and qualitative analysis will then be undertaken to analyse the effectiveness of each method.

Patients randomised to postal follow-up, will receive an anaemia screening questionnaire (FACIT Fatigue) at 4 weeks and QOL (eq 5d 5l) at 3 months (see documents sections 16) to be completed and returned. Patients randomised to telephone follow-up, will receive telephone calls to complete the same questionnaires. The telephone questionairres will be delivered by a single research assistant read directly from the questionairres.

We will use simple randomisation without stratification or blocking to generate the follow-up allocation schedule, at a ratio of 1:1. Randomisation will be conducted in the same way for the clinical and follow-up trials as detailed below.

Eligibility – All participants in the trial will be eligible for the SWAT follow-up study.

PRIMARY OUTCOMES– Completion rate and number of questionnaires completed, compared between the two methods, postal and telephone follow-up.

SECONDARY OUTCOMES – Quality of data compared between the two methods, analysing if either method improves the completion of all elements of the questionnaires.

7.4 Adverse Events assessment

For details on adverse event management and reporting see Section 8 (Safety Reporting).

7.5 Loss to follow-up and patient withdrawl

Participants will be able to withdraw from treatment, from postal follow-up, from hospital follow-up or from the trial (both questionnaires and collection of hospital data) at any time without giving a reason. Patients will be consented to use any collected data prior to withdrawl from the trial.

For operational management, a patient will be classified as 'lost to follow-up' (i.e. no further efforts to trace the patient are being made) when the trial ends. During this time

period attempts may be made to contact the patient via phone, text or post, if possible. If patients are lost to follow up at defined points in the trial, their collected data may be included, for example patient demographic data, blood results, transfusion rates or questionnaires.

7.6 Provision for cancelled/delayed operation

The NHS can fluctuate greatly during times of pressure, operations can be cancelled or rearranged, leaving it difficult to accurately predict the day of surgery. For this reason we have decided to give all patients identified as non-anaemic iron deficient a six month course of treatment to either intervention or no intervention arm of the trial. This will enable us to negotiate the issue of cancellation or rearranged surgery, whilst continuing the proposed intervention until the last patient contact in the trial.

7.7 Trial closure

The end of the trial will be defined as the last patient last contact which will occur in approximately 30 months after the beginning of the recruitment period (24 month recruitment period, including, 4 week patient follow up period and 2 further month complication follow up and then 6 months to analyse data). An end of study declaration form will be submitted to the Research Ethics Committee (REC) and Trust R&D within 90 days of trial completion and within 15 days if the trial is discontinued prematurely. A summary of the trial report and/or publication will be submitted to the REC, Sponsor and Funders within 1 year of the end of the trial

An exit strategy will be created, patients who wish to continue the food supplement after the trial has ended. Will be advised to discuss with their GP and be given further information if requested.

7.8 Blinding

The patients will not be blinded to their treatment, nor will the treating clinicians. The laboratory technicians processing the blood samples will be blinded to the study. A member of the research and development team will store trial specific blood results, the clinical and greater research team will be blinded to these results.

8 SAFETY REPORTING

Safety reporting will incoporate serious adverse events, complications and adverse reaction related to the treatment proposed and applies to all trial participants.

A serious adverse event will be defined as the following:

- Death
- Life-threatening event (that is it places the participant, in the view of the Investigator, at immediate risk of death)
- Requires unplanned hospitalisation or prolongation of existing hospitalisation (Unplanned refers to emergency hospitalisations resulting in an inpatient stay. Prolonged hospitalisation is deemed to be where a patient's stay is longer than expected (e.g. patient is operated on as day case but remains in hospital overnight)
- Results in persistent or significant disability or incapacity (substantial disruption of one's ability to conduct normal life functions)
- Is another important medical condition

Adverse Events that are expected as part of surgical interventions and are deemed unlikely to be related, include: Wound infection, Venous thrombo-embolic phenomena, Pneumonia, Blood transfusion, Cerebrovascular accident, Myocardial infarction, Deep vein thrombosis, Readmission. These will be recorded as complications, and monitored as secondary outcomes.

An adverse reaction will be defined as the following: Untoward and unintended response in a subject which is caused by or related to a research treatment or procedure. Such as allergic reaction, gastrointestinal upset, abdominal pain, constipation and diarrhoea and nausea.

All participants experiencing SAEs will be followed-up as per protocol until the end of the trial.

In the context of this study, SAEs will be fully investigatedif they appear to be related to an aspect of taking part in the study and it is an unexpected occurrence.

Salus will be informed of all SAEs deemed related to the supplementation, so that they can be reported to MHRA.

8.1 Safety assessment

The Research Nurse will record all ARs related to the proposed intervention.

In addition, sites should follow their own local procedures for the reporting of any adverse events linked to clinical care (up to 90 day morbidity and mortality).

8.2 Collection, Recording and Reporting of Adverse Events

ARs whether expected or not, should be recorded in the patient's medical notes and recorded on the study AR Form by the RN and sent to Data Manager within an agreed timescale (usually five days). SAEs should be notified to the Chief Investigator, the Sponsor within 24 hours of the RN/clinical team becoming aware of the event.

At the time of reporting the investigator will be asked to record an assessment of causality (to trial treatment) selecting an option from the list below:

- Definitely related —there is clear evidence to suggest a causal relationship, and other possible contributing factors can be ruled out.
- Probably related —there is evidence to suggest a causal relationship, and the influence of other factors is unlikely.
- Possibly related —there is some evidence to suggest a causal relationship (e.g. the event occurred within a reasonable time after administration of the trial medication). However, the influence of other factors may have contributed to the event (i.e. the patient's clinical condition, other concomitant events).
- Unlikely to be related —there is little evidence to suggest there is a causal relationship (e.g. the event did not occur within a reasonable time after administration of the trial medication). There is another reasonable explanation for the event (e.g. the participant's clinical condition, or other concomitant treatments).
- Unrelated—there is no evidence of any causal relationship.

An event is defined as 'related' if the event was due to the administration of any research procedure. Whereas an 'unexpected event' is defined as a type of event not listed in the protocol as an expected occurrence. The relatedness of an event will be reviewed by the Chief Investigator and the Trial Steering Committee.

8.2.1 York Trials Unit responsibilities

YTU is undertaking the duties formally expected or a trials unit supervising a University of York PhD research student.

8.2.2 Annual progress reports

An Annual Progress Report will be submitted to the REC which gave the favourable ethics opinion 12 months after the date on which the favourable opinion was given and thereafter until the end of the study (if applicable).

8.2.3 Urgent safety measures

The CI may take appropriate urgent safety measures in order to protect research patients against any immediate hazard to their health or safety. These safety measures should be taken immediately and may be taken without prior authorization from the REC or local competent authorities.

9 DATA QUALITY ASSURANCE

9.1 Quality Assurance and Quality Control

Northumbria Healthcare NHS Foundation Trust has agreed to be the lead sponsor for this project and take overall responsibility for the quality of study conduct. This study will be fully compliant with the Research Governance Framework and MRC Good Clinical Practice Guidance. A trial specific data management plan agreed by the Chief Investigator, Sponsor, and other study investigators will be drafted to provide detailed instructions and guidance relevant to database set up, data entry, validation, review, query generation and resolution, quality control processes involving data access and transfer of data to the sponsor at the end of the study and archiving.

A rigorous programme of quality control will be undertaken. The day-to-day management of the trial will be the responsibility of the Trial Co-ordinator based at Northumbria Healthcare NHS Foundation Trust. Regular meetings with the Trial Management Group will be held and will monitor adherence to the trial protocols at the trial sites. The PI will regularly audit data collation and electronic input and will report to the TSC.

9.2 Direct access to source data/documents

A statement of permission to access source data by study staff and for regulatory and audit purposes will be included within the patient consent form with explicit explanation as part of the consent process and Participant Information Sheet.

In principle, anonymised data will be made available for meta-analysis and where requested by other authorised researchers and journals for publication purposes. Requests for access to data will be reviewed by the Chief Investigator and study Sponsor.

The Investigator(s)/Institutions will permit monitoring, audits, and REC review (as applicable) and provide direct access to source data and documents.

9.3 Data management

Study data will be recorded in a number of files for both the administration of the study and collection of patient data.

For the purposes of ongoing data management, once randomised, individual patients will only be identified by trial numbers. But will be identifiable to the research team for quality contorol of the data set during analysis. All data will be completely anonymised for any subsequent reports or publications.

Essential Trial documentation (i.e. the documents which individually and collectively permit evaluation of the conduct of a clinical trial and the quality of the data produced) will be kept with the Trial Master File and Investigator Site File. The Sponsor will ensure that this documentation will be retained for a minimum of five years after the conclusion of the trial to comply with standards of Good Clinical Practice.

The CRF data will be stored for a minimum of five years after the conclusion of the trial as paper records; and a minimum of 20 years in electronic format in accordance with guidelines on Good Research Practice (MRC Ethics Series, 2000, updated 2005). All paper records will be stored in a secure storage facility. All electronic records will be stored on a password protected server.

The PI will archive the trial essential documents generated at the site for the agreed archiving period in accordance with the signed Clinical Trial Site agreement.

The Investigator/institution will permit authorised representatives of the Sponsor and applicable regulatory agencies direct access to source data/documents to conduct trial-related monitoring, audits and regulatory inspection. Trial participants are informed of this during the informed consent discussion.

9.3.1 GDPR

Recent European Union regulations regarding data protection, GDPR(26), will be followed in all aspects of data management. Patients will be consented for there data to be stored and shared for the purpose of further research with the right to withdraw from the study at any time.

9.3.2 Data entry

All data will be stored and transferred following Health Insurance Portability and Accountability Act (HIPAA) protocol. The staff involved in the trial will receive training on data protection. The staff will be monitored to ensure compliance with privacy standards.

Data will be checked according to procedures detailed in the trial specific Data Management Plan.

9.3.3 Data storage

Data will be held according to the Data Protection Act 1998 and data will be collated in CRFs identified by a unique identification number (i.e. the Trial number) only. A Trial Enrolment Log at the site will list the ID numbers.

All essential documents, including source documents, will be retained for a minimum period of five years after study completion. The separate archival of electronic data will performed at the end of the trial, to safeguard the data indefinitely.

10 Statistical Considerations

10.1 Method of Randomisation

We will use simple randomisation without stratification or blocking to generate the treatment allocation schedule.

10.2 Determination of Sample Size

Previous research in patients undergoing lower limb arthroplasty has reported a mean drop in haemoglobin levels of -18.1 g/dl, with Standard Deviation (SD) of 6.6 in patients at 3 weeks (19). Our trial will recruit a similar population, investigating haemoglobin levels at 3 weeks. We therefore assume a similar variation in haemoglobin levels but in order to detect a more conservative effect size equivalent to a 0.5 SD, with 90% power for a comparison between the control and intervention group using 2-sided testing at 5% significance level, we estimate that a total of 188 patients are required (94 per group for a 1:1 randomisation). This includes 10% inflation of the sample size for attrition on primary outcome data. This is designed as a highly conservative estimate.

10.3 Statistical Analysis Plan

A statistical analysis plan detailing intended analyses will be drafted before the completion of data collection. This trial will be reported according to the CONSORT guidelines for clinical trials (Consolidated Standards Of Reporting Trials statement)(27).

Baseline data will be summarised using descriptive statistics by trial arm as randomised and as analysed in the primary analysis model. No formal comparisons will be made between the groups.

Our primary analysis will compare the treatment groups against predefined primary and secondary outcome data at 3 weeks, 4 weeks, 30 and 90 days, adjusting as fixed effects for age, gender baseline value, time point, treatment group, and a treatment group-by-time point interaction. The primary end point will be treatment effect at 3 weeks postoperative (recovery of Haemoglobin). Secondary endpoints are patient readmission rate at 30-days and transfusion rate and number of units transfused, morbidity and mortality at 30-90 days will serve as a secondary outcome. Patient functionality questionnaire (4 weeks) will be analysed similarly.

The proportion of participants who, within 30 days of surgery, are readmitted to hospital, or experience significant morbidity or mortality, will be compared between the two groups. Length of hospital stay in days will be analysed.

11 ETHICAL CONSIDERATIONS AND APPROVAL

11.1 Regulatory compliance

The trial will comply with the principles of the Declaration of Helsinki.

It will also be conducted in compliance with the approved protocol, the principles of GCP. The sites will comply with the principles of GCP and applicable national regulations. An agreement will be in place between the site PI and the Northumbria Healthcare, setting out respective roles and responsibilities.

All deviations from the protocol or GCP will be reported by PIs to the TMG. The Trial Coordinator will record deviations on the Protocol Deviation Form for the trial

For the purposes of this regulation, a 'serious breach' is one that is likely to affect to a significant degree:

- The safety or physical or mental integrity of the participants in the trial, or
- The scientific value of the trial

11.2 Ethical conduct of the trial

The study will be conducted to protect the human rights and dignity of the patient as reflected in the 1996 version of the Helsinki Declaration.

11.3 Ethical considerations

The key ethical issues relating to the patients participating in the trial will be dealt with as follows:

- Written consent will be obtained from all the patients who are eligible and after having received study information and sufficient time to consider, are willing to participate. The research teams will be trained to explain study information in simple terms. Recent European Union regulations regarding data protection, GDPR(26), will be followed, patients will be consented for their raw data to be stored and shared with the trial funder or for the purpose of further research.

- Patients will not be coerced to participate in the study. The study will also not interfere with any kind of treatment the patients are already receiving. No financial incentive will be offered to the patients urging them to participate, for those visits that are in addition to their routine care visits.
- Confidentiality will be maintained for all patients.

11.3.1 Patient confidentiality

The researchers and clinical care teams must ensure that patients' anonymity will be maintained and that their identities are protected from unauthorised parties. Patients will be assigned a Trial number and this will be used on CRFs; patients will not be identified by their name. Sites will keep securely and maintain the patient enrolment Log showing identification numbers names and date of birth of the patients. This unique Trial number will identify all CRFs and other records and no names will be used, in order to maintain confidentiality.

All records will be kept in locked locations. All consent forms will be secured safely in a separate compartment of a locked cabinet. Clinical information will not be released without written permission, except as necessary for monitoring by the trial monitors.

Any raw data shared for future studies or with the trial funder, will be anonymised.

At the end of the study, data will be securely archived for a minimum of five years.

11.3.2 Benefit sharing

An important goal of the study is to generate knowledge that improves health of patients after lower limb arthroplasty. Therefore, our target patients are the major beneficiaries of this research.

The aim is to publish this research and share new knowledge, anonymised data may be shared for further research or meta-analysis.

11.4 Ethics Approvals

Formal NHS Research Ethics approval will be sought via the Health Research Authority (HRA). Local R&D approvals will be obtained. Any further amendments will be submitted and approved by REC. York University ethics approval will also be obtained

11.5 Indemnity

This study will be sponsored by Northumbria Healthcare NHS Foundation Trust. If there is negligent harm during the trial, then the NHS Trust owes a duty of care to the person harmed, NHS Indemnity covers NHS staff and medical academic staff with honorary contracts only when the pilot trial has been approved by the R&D department. NHS indemnity does not offer no-fault compensation and is unable to agree in advance to pay compensation for non-negligent harm.

12 FINANCE AND CONTRACTS

12.1 Trial Funding

The financial arrangements for the study will be as contractually agreed between the funder SALUS Haus and the Sponsor (Northumbria Healthcare NHS Foundation Trust). Utilising agreed financing calculations (28).

12.2 Trial Contracts

A Contract will be prepared with SALUS Haus to fund the clinical trial. A contract will be agreed for postage of the food supplement, by an approved courier. Postal Contracts will be agreed to transport the supplementation and post-operative questionnaire.

12.3 Funders Responsibilities

The funder will provide the required funds to complete the research. The Funder will provide the food supplement. The funder will have no responsibilities, input or access to the clinical management of the trial

12.4 Funders Access to Data

Access to anonymised data raw data will be agreed between the trial sponsor Northumbria Healthcare NHS Foundation Trust and the funder SALUS Haus. Patient identifiable information will not be shared outside the research Team. Patients will be given full disclosure of any data sharing in the PIS and consent form.

13 TRIAL COMMITTEES

13.1 Project Management

John Randall (PI) in conjunction with guidance from PhD supervisors at the York University will be responsible for project management. Mike Reed as Chief Investigator is responsible for clinically leading the project.

13.2 Trial Management Group (TMG)

The TMG is the executive decision making body and is responsible for the day-to-day running and management of the trial. Led by the chief investigator, it will consist of members of project management group (principal, Co/PhD supervisors). The TMG will meet regularly, according to the needs of the study (via teleconference and face-to-face at least once a year).

13.3 Trial Steering Committee (TSC)

Due to the low risk nature of this study, approval will be sought from the funders to set up one Independent Steering and Monitoring Committee to undertake the roles traditionally undertaken separately by the TSC and the Data Monitoring Ethics Committee. This committee will comprise of an Independent Chair who will be a surgeon with expertise in lower limb arthroplasty surgery, a statistician, a member of the Patient Group, the Chief Investigator and Trial Principal investigator. Other study collaborators may also attend the meeting. The independent members of the committee will be allowed to see unblinded data. The role of this committee will include the review of all serious adverse events which are thought to be treatment related and unexpected. The committee will meet at least annually or more frequently if the committee requests.

If however, the funders do not agree to one committee being set up, then separate TSC and DMEC committees will be set up. The TSC will include an Independent Chair and at least two other independent members along with the Chief Investigator and the Trial Coordinator/Manager and other study collaborators. The DMEC will comprise of an Independent Chair, a statistician and surgeon. Both committees will meet annually. The role of the DMEC will be to immediately see all serious adverse events which are thought to be related to the intervention or being in the study and unexpected. The Principal investigator will inform the trial steering committee regarding audit a data quality management.

13.4 SOURCE DATA LIST

Type of Data	**Source Document**
Informed consent	Informed Consent Form
Relevant Medical History and Current Medical Conditions	Patient Medical Records
Fulfilment of eligibility criteria	Patient Medical Records
Demographics	Patient Medical Records /PAS
Transfusion data	Transfusion Database
Blood Results	ICE
Patient Questionnaire	Trial Data

14 PATIENT INFORMATION AND COMMUNICATION

We have specifically discussed research around the anaemia screening programme with Total Hip User Group, (THUG), Northumbria's Patient User Group for both hip and knee replacement (2016). They support this initiative and have agreed to support us in developing patient information. All patient information information have been submitted to THUG for approval, including consent forms and questionairres.

In addition Northumbria Healthcare has developed Patient Leaders as part of a separate Health Foundation funded grant improving care for patients with a hip fracture. Their roles are diverse and wide ranging. They help directly with patients and those that care for people with long term conditions and contribute directly to improving patient plans and experiences. These patient leaders offer a unique and valuable perspective and will be involved in the larger anaemia screening project across the NHS from 2018 to 2020. The results of this study have the potential to be disseminated rapidly to patient groups therefore directly improving the quality of care for our elderly patients.

15 PUBLICATION POLICY

The results will be disseminated in international, open-access peer-reviewed journals, through the local networks and at national and international meetings in surgical care within 12 months of trial completion in line with FDA rules. A dissemination and publication policy will be developed with an agreement between partners including ownership and exploitation of intellectual property, and publication rights. The publication policy and the agreement will ensure that any intellectual property generated during the project is protected and that the publication process is organised in a fair, balanced and transparent manner. The TMG will be responsible for overseeing these arrangements.

16 DOCUMENTATION

Health Questionnaire

English version for the UK

Under each heading, please tick the ONE box that best describes your health TODAY.

MOBILITY

I have no problems in walking about ❑

I have slight problems in walking about ❑

I have moderate problems in walking about ❑

I have severe problems in walking about ❑

I am unable to walk about ❑

SELF-CARE

I have no problems washing or dressing myself ❑

I have slight problems washing or dressing myself ❑

I have moderate problems washing or dressing myself ❑

I have severe problems washing or dressing myself ❑

I am unable to wash or dress myself ❑

USUAL ACTIVITIES *(e.g. work, study, housework, family or leisure activities)*

I have no problems doing my usual activities ❑

I have slight problems doing my usual activities ❑

I have moderate problems doing my usual activities ❑

I have severe problems doing my usual activities ❑

I am unable to do my usual activities ❑

PAIN / DISCOMFORT

I have no pain or discomfort ❑

I have slight pain or discomfort ❑

I have moderate pain or discomfort ❑

I have severe pain or discomfort ❑

I have extreme pain or discomfort ❑

ANXIETY / DEPRESSION

I am not anxious or depressed ❑

I am slightly anxious or depressed ❑

I am moderately anxious or depressed ❑

I am severely anxious or depressed ❑

I am extremely anxious or depressed ❑

We would like to know how good or bad your health is TODAY.

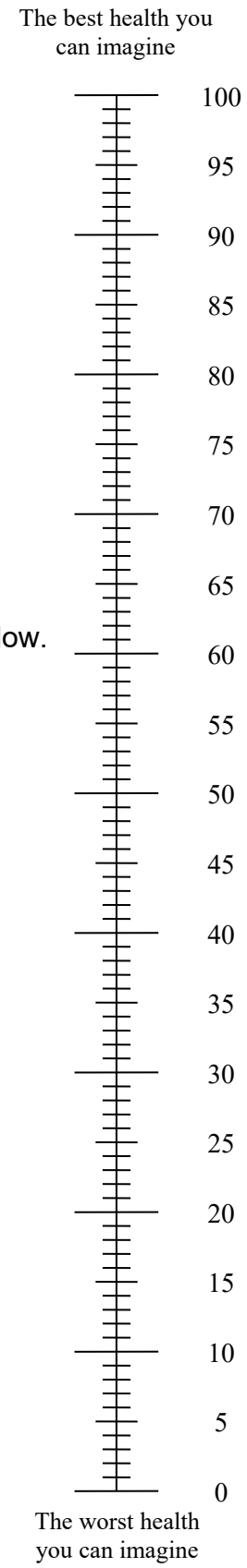
This scale is numbered from 0 to 100.

100 means the best health you can imagine.
0 means the worst health you can imagine.

Mark an X on the scale to indicate how your health is TODAY.

Now, please write the number you marked on the scale in the box below.

YOUR HEALTH TODAY = ☐



FACIT Fatigue Score

Below is a list of statements that other people with your illness have said are important**. Please circle or mark one number per line to indicate your response as it applies to the <u>past 7 days</u>.**

		Not at all	**A little bit**	**Some-what**	**Quite a bit**	**Very much**
HI7	I feel fatigued	0	1	2	3	4
HI12	I feel weak all over	0	1	2	3	4
An1	I feel listless (“washed out”)	0	1	2	3	4
An2	I feel tired	0	1	2	3	4
An3	I have trouble <u>starting</u> things because I am tired	0	1	2	3	4
An4	I have trouble <u>finishing</u> things because I am tired	0	1	2	3	4
An5	I have energy	0	1	2	3	4
An7	I am able to do my usual activities	0	1	2	3	4
An8	I need to sleep during the day	0	1	2	3	4
An12	I am too tired to eat	0	1	2	3	4
An14	I need help doing my usual activities	0	1	2	3	4
An15	I am frustrated by being too tired to do the things I want to do	0	1	2	3	4
An16	I have to limit my social activity because I am tired	0	1	2	3	4

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Appendix 12: Patient Information Sheet

Iron supplementation for iron deficiency before arthroplasty (ISIDA)

Chief Investigator: Professor Mike Reed

PROSPECTIVE PATIENT INFORMATION SHEET

We would like you to consider taking part in this research study investigating treating non-anaemic iron defficient patients having total knee and total hip replacements. Before you decide to participate in this study, please take some time to read through this information leaflet, so that you understand why the research is being done and what it would involve for you.

What is the purpose of this study?

Within our NHS trust, we have successfully introduced an anaemia screening and treatment programme. All patients undergoing knee and hip replacements are routinely screened for anaemia, (low red blood cells) and have another test for ferritin level, (low iron stores). Research shows that patients who are anaemic are more likely to have complications after their operation, may need to stay in hospital longer or need a blood transfusion. Currently anaemic patients are given iron supplements to increase their red blood cells prior to surgery.

This study will involve patients who are not anaemic, (their red blood cell level is normal), but their ferritin, (iron stores) are low. All patients lose blood during hip and knee replacements and after surgery the number of red blood cells drops initially, before returning to normal levels in the weeks after surgery. Patients with low ferritin (iron stores) may not have the required overall iron stores to return their red blood cells to normal levels quickly after surgery, which could prolong their recovery period.

The aim of this study is to see if iron supplementation in this group of patients reduces the drop in red blood cells and improves the return to normal levels. Patients will be randomally chosen to receive an iron food supplementation or no treatment and will be followed-up after surgery.

Who will be invited?

When patients like you are booked for their hip and knee replacement surgery, routine blood samples are taken including CRP (a measurement of inflammation), a Full Blood Count measuring anaemia (low red blood cells) and ferritin (low iron stores) screening tests are completed. For this study, we will check those blood test results and identify patients who have normal levels of red blood cells, but have low iron stores. If you are able to be in the study, when you attend for your anaesthetic preoperative assessment appointment, you will meet with a research nurse who will explain the study further and will ask if you would like to participate in this study.

We aim to recruit 188 patients across our organisation to be part of this study.

What will happen if I agree to be in the study?

Whether or not you agree to be part of this study, this will not alter or delay the timeframe for your surgery.

If you agree to be a part of this study, the research nursing team may contact you by phone and will ask for your written consent. At your pre-assessment appointment, you will be randomised to one of the below study arms.

Oral Iron supplements (Low dose Iron supplement)

If you are randomised to oral iron supplementation, you will be provided with an iron food supplement which contains a low amount of iron 36.8mg daily, taken in 3 doses, with instructions to take this medication for 6 months from that date. This will cover the time before your operation and your initial recovery period. You will be provided with a trial identity card.

No Iron Supplements

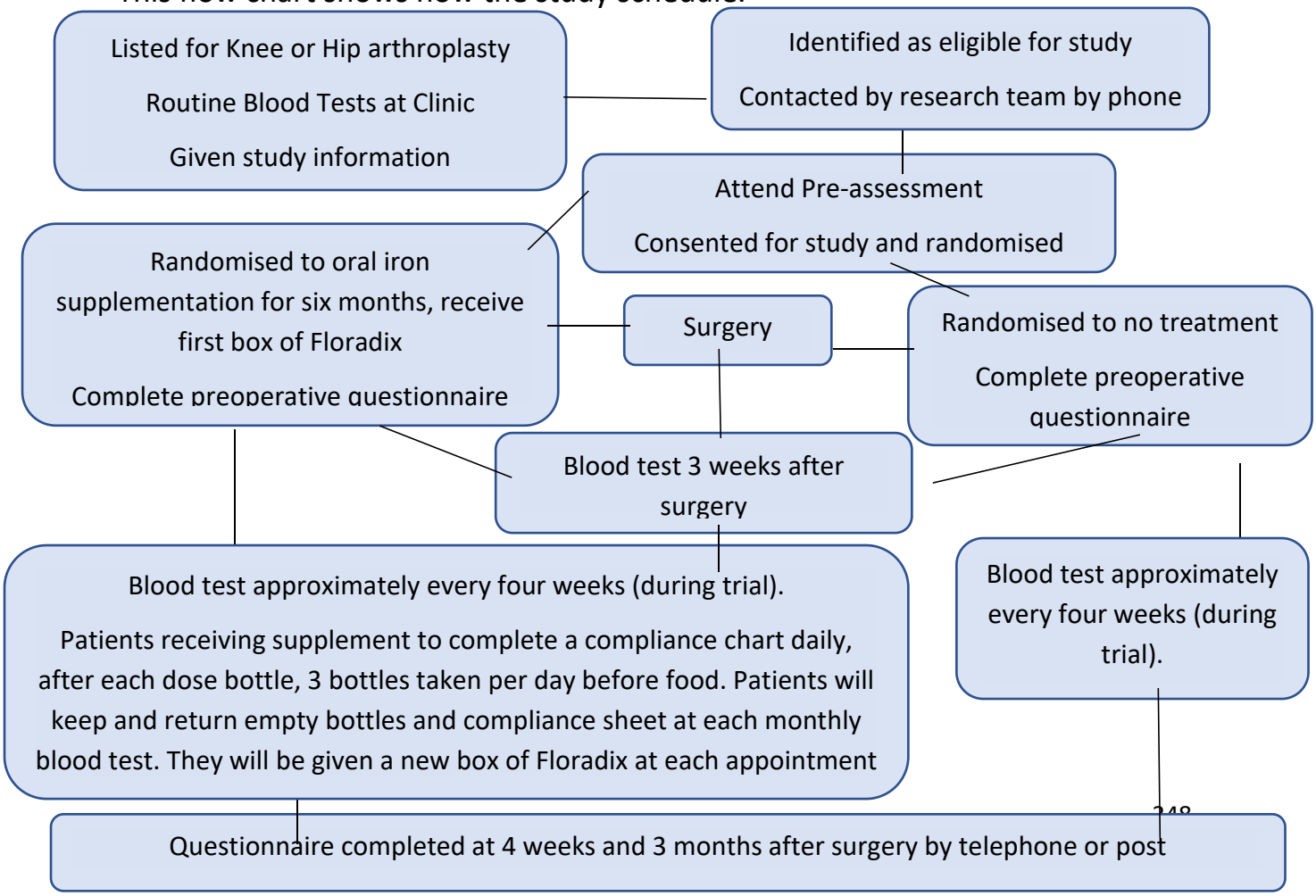
If you are randomised to no treatment, we request that you do not take iron supplementation without instruction, as this will make it more difficult to compare the 2 treatment arms. You will be provided with a trial identity card, if iron supplementation is advised by a medical professional throughout your participation in the trial, please inform the trial team.

Both patient groups will be asked to complete a questionnaire at the pre-assessment appointment and will have an additional blood test three weeks after surgery and every four weeks for the duration of six month trial, either in clinic or in their home, by a member of the research team. These are trial specific bloods which you would not normally have taken, results will be stored by research and development and analysed at the end of trial. They will not be shared readily with the clinical team. This will stop the trial altering your normal surgery pathway and will reduce the risk of bias in the trial. Both groups will be further randomised to complete a postal or telephone questionnaire at 4 weeks and 3 months after their surgery.

Patients will be given a trial ID card and appropriate personal information will be collected, stored and used by the research team to contact participants in the study. Any information will be managed securely and confidentially.

Patients in the study may be contacted by post or phone for the follow up questionnaires and arranging the blood test three weeks after surgery.

This flow chart shows how the study schedule.



What blood tests will be performed 4 weekly?

The same blood tests taken routinely before surgery will be repeated approximately every 4 weeks, testing for anaemia, iron deficiency and inflammation levels throughout the trial. The research team will provide you an appointment reminder card for these appointments.

What are the possible disadvantages and risks of taking part?

Iron supplementation is associated with side effects and is not tolerated by all patients; side effects include bloating, constipation and dark stools. This study is categorised as low risk and we have specifically chosen a low dose oral iron food supplement to reduce the risk of any side effects from the treatment.

Patients will be required to complete questionnaires and have six approximately 4 weekly blood tests which you would not normally have throughout the trial duration. This will be performed in the patient's home, or the hospital, using standard aseptic precautions, to reduce the very small risk of infection, the blood test may lead to bruising.

What are the potential benefits of taking part?

Oral iron supplementation has shown to benefit some patient groups undergoing hip and knee replacements, the information we get from this study may help us to improve treatment for patients in the future having similar surgery.

What if new information becomes available?

Sometimes during a research project new information becomes available about the treatment being studied. If this happens, the research team will discuss its impact and inform patients and ask if they want to continue.

What happens if I decide to leave the study?

Your participation is voluntary and you can withdraw from the study at any time, this will not affect their treatment in any way.

What happens if something goes wrong?

In the highly unlikely event something goes wrong, Northumbria Healthcare NHS Trust, as sponsor, has appropriate safety measures in place to protect all trial participants. If any patient is unhappy with any aspect of their care in relation to this study, they can contact the research development team or the patient advice liaison service.

Will taking part in this study be kept confidential?

Patients participating in this trial will be consented to inform their GP that they are participating in a clinical trial, so that their GP is fully informed of the treatment All information collected during the study will be kept strictly confidential. Participant's anonymised data and personal information will be stored securely and separately in access restricted databases and filing systems and only viewed by authorised research staff. All data will be anonymised for publication and future relevant research; patients will not be identifiable.

Anonymised data may be shared with the trial funder SALUS HAUS.

When you agree to take part in a research study, the information about your health and care may be provided to researchers running other research studies in this organisation and in other organisations. These organisations may be universities, NHS organisations or companies involved in health and care research in this country or abroad. Your information will only be used by organisations and researchers to conduct research in accordance with the UK Policy Framework for Health and Social Care Research**.**

This information will not identify you and will not be combined with other information in a way that could identify you. The information will only be used for the purpose of health and care research, and cannot be used to contact you or to affect your care. It will not be used to make decisions about future services available to you, such as insurance.

What will happen the results of this study?

This study is expected to last approximately two years. We will publish the findings at the end of the study. If you would like to obtain a copy of the published results, please ask a member of our research team.

Who is organising and funding this study?

The study will be organised and managed by Northumbria Healthcare NHS Trust. York University will provide guidance and assistance with statistical analysis. The study will be funded by Northumbria Healthcare NHS trust in conjunction with SALUS HAUS.

How will we use your data?

Northumbria Healthcare NHS Trust will collect information from you and your medical records for this research study in accordance with our instructions.

Northumbria Healthcare NHS Trust will use your name, NHS number and contact details to contact you about the research study, and make sure that relevant information about the study is recorded for your care, and to oversee the quality of the study. Individuals from Northumbria Healthcare NHS Trust and regulatory organisations may look at your medical and research records to check the accuracy of the research study. Northumbria Healthcare NHS Trust will hold these details along with the information collected from you and your medical records. The only people in Northumbria Healthcare NHS Trust who will have access to information that identifies you will be people who need to contact you to complete follow-up questionnaires and blood tests or to audit the data collection process. The people who analyse the information will not be able to identify you and will not be able to find out your name, NHS number or contact details.

Northumbria Healthcare NHS Trust will keep identifiable information about you from this study for 5 years after the study has finished.

Ethical Approval

All research in the NHS is looked at by an independent group of people called a research ethics committee. North East-York Research Ethics Committee have reviewed and approved this study.

What if I don't want my bloods and data looked at for the purpose of this trial?

If you decide you don't want any contact from the research team or want any of your routine blood tests to accessed for the purpose of this research. You can contact the patient advice liaison service using the details below and they will inform the research team.

Contacts for further information

Research and Development

Northumbria Healthcare NHS Trust

Telephone: 0191 2934322 Email: researchanddevelopment@nhct.nhs.uk,

ISIDA trial contacts

Clinical Research Practitioners

Claire Walker

Telephone: 0191 2934096 Email: claire.walker1@nhct.nhs.uk

Maria Thompson

Telephone: 0191 2934096 Email: maria.thompson@nhct.nhs.uk

Patient Advice and Liaison Service (PALS)

Freephone: 0800 032 0202

Text: 01670 511098

Email: northoftynepals@nhct.nhs.uk

Appendix 13: Consent form

Informed Consent Form
ISIDA – Iron Supplementation before Arthroplasty
Chief Investigator: Professor Mike Reed

Patient ID: | | | | Please initial below:

1. I confirm I have read and understood the Patient Information Sheet version 1.0 dated 24/08/2018 for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily. ☐

2. I understand the purpose of this study, that my participation is voluntary and that I am free to withdraw at any time without giving any reason, without my medical care or legal rights being affected. ☐

3. I understand I have been found to have a low iron level during routine screening and I will be randomised to receive iron supplementation or not as a part of this clinical trial and further randomised to postal or telephone follow up at 4 weeks and 3 months post op. ☐

4. I understand that relevant sections of my medical notes and data collected from this study may be looked at by the research team and regulatory authorities, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records. ☐

5. I give permission that additional blood sample will be taken 4 weekly, and questionnaires will be completed to measure the impact of this study. ☐

6. I give permission for the anonymised raw data collected to be shared with the trial funder and for the purpose of future research, without further consent being taken. ☐

7. I understand that if I withdraw from the study, or I lose capacity to give further consent, any data that has already been collected will be used in analysing the results of the study, unless I specifically withdraw consent for this. I understand this data will be anonymous. ☐

8. I agree to my GP being informed of my participation in the study. ☐

9. I agree to take part in this study. ☐

Name _____ Signed _____ Date_____

Researcher _____ Signed _____ Date_____

Appendix 14: Statistical Analysis Plan

1. Trial Objectives

The aim of this trial is to analyse the effect of iron supplementation in non-anaemic iron deficient patients undergoing lower limb arthroplasty. Patients with a normal haemoglobin (greater than 12 in women and 13 in men) (World Health Organisation 2011), who are deemed to be iron deficient (Ferritin below 50) will be recruited to this randomised controlled trial. 188 patients will be randomised 1 to 1 to receive oral iron supplementation or no intervention for six months duration, covering preoperative and postoperative phases of care. All patients will have blood tests 4 weekly throughout the trial and a sample 3 weeks postoperative. To ease patient burden, blood tests will be arranged with the patient in home or hospital clinic. All participants must receive at least 4 weeks of supplementation prior to surgery to be eligible for the trial. The hypothesis being that patients with reduced overall iron stores will benefit from iron supplementation to enable their haemoglobin to recover after surgery.

Primary Outcome: Hb at 3 weeks postoperative

Secondary Outcomes

- Length of hospital stay (midnights in hospital).
- Transfusion Rate and number of units transfused up to 30 days
- Adverse events (including all cause morbidity and mortality at 30 and 90 days)
- Inpatient DVT/PE within 30 days of surgery
- Pneumonia
- Cerebrovascular incident
- Myocardial infarction
- Readmission within 30 days of surgery
- Repeated measures analysis FBC, CRP and Ferritin every 4 weeks throughout the six month trial.

Each patient allocated to intervention will be given a compliance questionnaire exploring common gastrointestinal symptoms linked to oral iron supplement intake

- Diarrhoea, constipation, abdominal pain, compliance

- Compliance to treatment

STUDY WITHIN A TRIAL SUMMARY

The aim of this study within a trial is to analyse response rates and quality of responses using two different methods to administer post operative quality of life questionnaires. All trial participants will be eligible for the SWAT follow-up study. Patients will be randomised to postal and telephone follow-up initially, with further contact if necessary to improve response or completion rate.

PRIMARY OUTCOMES– Overall response rate compared between the two methods, postal and telephone follow-up.

SECONDARY OUTCOMES – Quality of data compared between the two methods, analysing if either method improves the completion of all elements of the questionnaires, comparing time to return and number of phone calls required. EQ5D5L and FACIT Fatigue scores will be compared between postal and telephone follow up, to analyse for differences in scoring between the two methods.

Hypothesis

Compared to no intervention, supplementation with iron in the non-anaemic iron deficient population improves patients' haemoglobin recovery post-surgery, reduces symptoms of anaemia (FACIT fatigue) and improves patient outcomes (EQ5D 5L). It may also reduce length of stay, readmission rate at 30 days, transfusion rate, adverse events (including all cause morbidity and mortality at 30 and 90 days), inpatient DVT/PE within 30 days of surgery, pneumonia, cerebrovascular incident and myocardial infarction. Repeated measures analysis of FBC, CRP and Ferritin every 4 weeks throughout the six month trial, will provide added statistical power and analyse fluctuations between groups.

1.1 Primary objective

The primary objective is to determine the effectiveness of iron supplementation in the non-anaemic iron deficient lower limb arthroplasty population. This is a randomised controlled two arm study.

The question is does oral iron supplementation in the non-anaemic iron deficient population undergoing lower limb arthroplasty improve Haemoglobin recovery from pre-operative, to 3 weeks postoperative.

2. Design

The trial is designed as a parallel group, randomised controlled trial comparing arm 1 (intervention) to arm 2 (control):
Arm 1: Intervention with oral iron supplementation
Arm 2: No intervention
Patients will be randomly allocated to intervention or control arm using simple randomisation.

All patients will be further randomised to receive initially anaemia symptoms questionnaire (FACIT Fatigue) and health related Quality of Life (QOL) (EQ 5D 5L) via post or telephone after surgery using simple randomisation.

This study will collect data across four hospital sites within a single NHS Foundation Trust based in England. The study will take place within the surgical directorate utilising collaborative working between preoperative, perioperative, postopoerative care teams and the research and development team.

Refer to the trial protocol.

"Full details of the background and design of the trial are presented in the protocol (version 2.0)."

Any changes between the protocol and the analysis plan should be explained.

3. Sample Size

To calculate a sample size (taken form Torgerson and Torgerson 2008), previous research in patients undergoing lower limb arthroplasty has reported a mean difference in haemoglobin levels of -18.1 g/dl, with Standard Deviation (SD) of 6.6 in patients at 3 weeks (Zhou, Zhou, Wu, Wu, Qian, Zhao, Zhu, and Fu 2015). This trial will recruit a similar population, investigating haemoglobin levels at 3 weeks. The trial therefore assumes a similar variation in haemoglobin levels, in order to detect a more conservative effect size equivalent to a 0.5 SD (equating to a 3.3 mean difference), with 90% power for a comparison between the control and intervention group using 2-sided testing at 5% significance level

Approximate N = 42/d squared

N = 42/0.5squared

N = 42/0.25

N = 168

This calculation estimates a total of 168 patients are required including a 10% inflation of the sample size for attrition on primary outcome data requires 188 patients, (94 per group for a 1:1 randomisation).

4 Randomisation

The trial will utilise simple randomisation without stratification or blocking, to generate the participants allocation schedule, at a ratio of 1:1, utilising a computer based electronic process, to increase the reliability of the randomisation and reduce the risk of subversion (Jadad and Enkin 2007).

All patients undergoing hip or knee arthroplasty will meet the initial criteria to take part in this study and will be given a Patient Information Sheet. Normal preoperative investigations will be performed, with eligible patients meeting the inclusion criteria, being referred from the clinical team, to the research team for recruitment to the clinical trial. Patients will then be contacted by the research nursing team and if they agree to participate in the trial, will be brought to a research pre-assessment clinic and informed consent, pre-trial documentation and randomisation will be completed. Patients will then leave with the supplementation, if allocated to the treatment arm.

5. Definition of terms

Provide a definition of any terms which require explanation. If a more elaborate definition is required, include this in the Appendix.

6. Outcomes

6.1 Primary outcome(s)

The primary outcome of this trial, will be Hb level at three weeks postoperative

SWAT follow up trial

PRIMARY OUTCOME– Completion of the questionnaire in part or in full

6.2 Secondary outcome(s)

These will include

- Length of hospital stay (midnights in hospital).
- Transfusion Rate and number of units transfused up to 30 days
- 30 day readmission rate.
- Adverse events (including all cause morbidity and mortality at 30 and 90 days)
- FBC, CRP and Ferritin every 4 weeks throughout the six month trial.

Local Trust PAS data will be used to generate data on:

- Readmission within 30 days of surgery
- Inpatient DVT within 30 days of surgery
- Inpatient PE within 30 days of surgery
- Blood transfusion rate and number of units
- Pneumonia
- Cerebrovascular incident
- Myocardial infarction

Each patient allocated to intervention will be given a compliance questionnaire exploring both compliance and common gastrointestinal symptoms linked to oral iron supplement intake

- Compliance to treatment
- Diarrhoea, constipation, abdominal pain, compliance

SWAT follow up trial

SECONDARY OUTCOMES – Completion of all elements of the questionnaires, time to return and number of phone calls required. EQ5D5L and FACIT Fatigue scores will be compared between postal and telephone follow up, to analyse for differences in scoring between the two methods.

6.3 Follow-up

ASSESSMENTS AND FOLLOW-UP

At clinic.

Patients who are eligible for joint replacement surgery will have routine bloods taken as is standard practice (baseline). A patient information sheet explaining the trial will be given to all patients.

Baseline:

Bloods; full blood count, serum ferritin, CRP, urea and electrolytes, liver function and eGFR will be checked (as per standard practice).

The results from the clinic will be screened and those that meet the inclusion criteria will be recruited by the research nursing team at preassessment or alternative clinic.

For patients that consent, they will be randomised into one of two groups before receiving the oral iron supplement regimen with clear instructions.

Recruited patients

Proceed to surgery after intervention or no intervention:

Each patient will have repeat haemoglobin taken postoperatively at three weeks.

Each patient will be given a compliance questionnaire exploring common gastrointestinal symptoms linked to oral iron supplement intake

- Diarrhoea, constipation, abdominal pain, compliance

All patients will have blood tests every 4 weeks (Hb, CRP, Ferritin) throughout the six month trial duration, to monitor effects of the supplementation. Tests will be performed in hospital or the patients home as arranged by the research nursing team.

Each patient will complete follow up questionnaires at three weeks (Facit-Fatigue) and three months post surgery (EQ-5D-5L).

6.4 Other important information

Demographic Data to be collected will include:

Age at surgery, Gender, Smoking status, Comorbidities, Hypertension, Atrial Fibrillation, Ischaemic Heart Disease, Type I diabetes, Type II diabetes, Chronic Obstructive Pulmonary Disease.

Following surgery:

Complications during and after surgery are recorded as standard. Patients included in the study will be followed up for a further 30 days after their surgery to check if they required a blood transfusion.

Hospital episode statistics data:

Length of stay, Readmission within 30-days of surgery,

Complication data:

Infection rate (superficial and deep) Public Health England (Public Health England 2014), Myocardial infarction (heart attack), transient ischaemic attack or cerebrovascular accident (stroke), acute kidney injury (AKI) within 30-days of surgery, In-patient Deep vein thrombosis (DVT) or in-patient pulmonary embolism (PE) within 60-days of surgery, Mortality (30- and 90-day)

7. Data

7.1 SOPS and referral documents

This statistical analysis plan should be used in conjunction with the following documents

Trial Docs Pack V 1.0

ISIDA Clinical Trial Protocol V 2.0

Trial Specific Procedure Document V 1.0

7.2 CRFs

"A copy of the CRFs with the variable names from the database (known as 'specs') will be kept in the Trial Statistics folder."

7.3 Management of datasets and data verification

Trial data will be received on paper questionnaires and entered electronically into the clinical trial database by the research nursing team. The principal investigator will perform audit and quality control of the data to ensure data accuracy, checking 20% of the data with a 0% predefined error rate. If above that rate, then a further 20% sample will be taken and rechecked, it will be repeated if necessary. Paper research files will be kept in a locked cabinet. Electronic files will be kept on a password protected NHS server.

7.4 External datasets

Data anonymised will be shared with YTU PhD supervisors for assistance and guidance during the statistical analysis process.

8. Analysis

This trial will be reported according to the CONSORT guidelines for clinical trials (Consolidated Standards Of Reporting Trials statement - http://www.consort-statement.org/)). Analyses will be conducted following the principles of intention-to-treat with patient's outcomes analysed according to their original, randomised group irrespective of deviations based on non-compliance, unless otherwise specified. Analyses will be conducted in STATA statistical analysis package, with the version stated in the final report. Significance tests will be two-sided at the 5% significance levels unless otherwise stated. Parameter estimates will be presented with associated 95% confidence intervals and p-values as appropriate.

8.1 Screening and eligibility data

The number of patients screened, eligible and randomised will be reported. The flow of participants through the trial will be presented in a CONSORT diagram (see appendix 1 for draft diagram).

8.2 Withdrawal and loss to follow up

The following withdrawals will be recorded on a Withdrawal Form which distinguishes between types of withdrawal:

- The patient wishes to withdraw from treatment (patients agrees to provide outcome data)
- The patient has died (with date of death)
- The patient is withdrawing fully from the study i.e. no study treatment and no follow-up

Withdrawals will be summarised by treatment arm and type of withdrawal, with reasons and timings (in terms of length of follow-up completed) where possible.

Losses to follow up, for example, patients not returning questionnaires or not responding to telephone follow up will be logged and be described in the CONSORT diagram and text where appropriate.

8.3 Baseline data

Baseline data will be summarised using descriptive statistics overall and by trial arm as randomised and as analysed in the primary analysis model unless there is no loss to follow up (see Appendix for draft table). No formal comparisons will be made between the groups. Continuous measures will be reported using descriptive statistics (e.g., mean, standard deviation, median, minimum and maximum) while the categorical data will be reported as counts and percentages.

8.4 Primary analysis

The primary analysis will compare the Haemoglobin at 3 weeks post-surgery by trial arm using a linear regression model adjusting for baseline Haemoglobin, age, gender and type of arthroplasty (hip or knee).

Missing data: it is possible that some patients will not provide the post-op, or four weekly Hb because (a) they might not end up having the operation within the timeframe of study, (b) they die, (c) they decide to withdraw completely. statistically I dont plan to make any adjustments.

8.5 Sensitivity analysis

It is possible that some patients will require blood transfusion between pre-op and the 3 weeks post-surgery time points. The expected time point for requiring transfusion is usually soon after surgery, expected to be <5 days. Clinical intervention for transfusions occurs if the patient has Hb<8g/dl. The number of patients this affects by trial arm will be reported and a brief description will be given of when this occurred by patient.

Therefore, for these patients Hb levels at the 3 week post-op time point would be more related to transfusion levels than trial arm. While it is important to investigate how this might have influenced the primary analysis, we expect <1% of patients to be affected (ref). We will therefore, undertake a sensitivity analysis excluding these patients.

8.6 Hb over time repeated measure analysis

Hb levels will be plotted by trial arm for each of the assessment time points and a table will be presented to summarise descriptively the Hb levels at these time points (n, mean, sd, or median, IQR, minimum and maximum as appropriate to the distribution of the data) and including pre-operative assessment (see appendix for draft table).

Since time of surgery will vary among patients, we shall measure time from randomisation (the same date supplementation begins) to date of surgery and this will be reported descriptively by trial arm.

In a secondary analysis we will compare Hb between trial arm using a mixed model incorporating all time points where effects of interest and baseline covariates (Hb, age, gender and type of arthroplasty), time from randomisation to surgery are specified as fixed effects, and the correlation of observations within patients over time is modelled by a covariance structure.

8.7 SWAT analysis Follow-up

EQ-5D-5L and FACIT fatigue return rates and the proportion of questionnaires at least partially completed, will be assessed preoperatively and three months and four weeks post-operatively as per trial schedule.

Quality of data compared between the two methods will be conducted, analysing if either method improves the completion of all elements of the questionnaires or if there is a difference in responses when completing via post of telephone.

Completion rate is defined as fully completed questionnaires. Percentages will be presented descriptively by trial arm, and a logistic regression model, will be used to compare differences by trial arm to investigate optimal methods of data ascertainment (follow up).

In addition, it is of interest whether there are differences in patient reported outcomes by method of follow up. Therefore, the mean differences in ED5D and FACIT score will be compared overall by a using a logistic regression model with fully completed/partial. However, if there are chance imbalances in important baseline factors by randomised arm, then we shall use adjusted analyses such as linear regression adjusted for imbalanced factor.

8.8 Secondary analyses
These will be reported descriptively by trial arm

The number of days in hospital will be analysed using Poisson regression. Transfusion, readmission, inpatient deep vein thrombosis, inpatient pulmonary embolism, pneumonia, cerebrovascular incident, and myocardial infarction will all be analysed using logistic regression. The number of units transfused, EQ5D and FACIT scores will be analysed using

linear regression. All secondary outcome regression models will be adjusted for baseline haemoglobin, age, gender, and type of arthroplasty

8.9 Adverse events

All adverse events that occur during the trial will be documented on the trial adverse events document and will be fully investigated.

Adverse events will be summarised using descriptive statistics.

9 Baseline Characteristics

Table 1. Baseline characteristics of randomised patient and analysed patient data

Patient Characteristics	**Iron supplementation (n= 40)**	**Control (n=35)**	**Total (n=75)**
Gender Male, n(%)	14 (35)	4 (11)	18 (24)
Age (years) Mean (sd) Median (min, max)	 66.4 (7.2) 66.5 (50, 80)	 68.9 (10.4) 71 (38, 83)	 67.6 (8.9) 69 (38, 83)
Ethnicity White	 40 (100)	 35 (100)	 75 (100)
Smoking status Current Ex-smoker Never smoked	 3 (8) 16 (40) 21 (53)	 2 (6) 14 (40) 19 (54)	 5 (7) 30 (40) 40 (53)
Diabetes 1	1(3)	1 (3)	2 (3)
Diabetes 2	8 (20)	4 (11)	12 (16)
IHD	3 (8)	0 (0)	3 (4)
Hypertension	17 (43)	16 (46)	33 (44)
TIA/CVA	2 (5)	3 (9)	5 (7)
COPD	1 (3)	4 (11)	5 (7)
Asthma	5 (13)	8 (23)	13 (17)
Thyroid Disease	2 (5)	2 (6)	4 (5)
AF	2 (5)	2 (6)	4 (5)
Liver Disease	0 (0)	0 (0)	0 (0)
Renal Disease	0 (0)	0 (0)	0 (0)
Hb (g/dl)			

Mean (sd) Median (min, max)	137.7 (12.46) 138.5 (120, 165)	135.74 (10.17) 135 (122, 169)	136.79 (11.41) 136 (120, 169)
Ferritin Mean (sd) Median (min, max)	 33.88 (10.55) 35.5 (13, 49)	 32.03 (11.26) 33 (12, 49)	 33.01 (10.85) 35 (12, 49)
CRP Mean (sd) Median (min, max)	 5.03 (6.49) 3 (1, 39)	 3.74 (2.59) 3 (1, 11)	 4.43 (5.07) 3 (1, 39)
Type of surgery Knee Hip	 30 (75) 10 (25)	 25 (71) 10 (29)	 55 (73) 20 (27)

10. SAP amendment log

Please note all changes that are made to the Statistical Analysis Plan following initial sign-off in the box below. Include details of the changes made, any notes/justification for these changes, the new version number if applicable, who the changes were made by, and the date.

Amendment/addition to SAP and reason for change	New version number, name and date
SAP completed and signed-off	

11. Signatures of approval

Sign-off of the final approved version of the Statistical Analysis Plan by the principle investigator and trial statistician(s) (can also include Trial Manager/Co-ordinator)

Name	**Trial Role**	**Signature**	**Date**
Mike Reed	Chief Investigator		
John Randall	Principal investigator/ PhD Student		

Catherine Hewitt	Statistician YTU/ PhD Supervisor		