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Role of single-dose intravenous iron therapy for the treatment of anemia after orthopaedic trauma: protocol for a pilot randomised controlled trial.

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ABSTRACT

Introduction: Orthopaedic trauma and fracture care have a high prevalence of perioperative anemia, which is associated with functional iron deficiency due to a systemic inflammatory state. Historically the surgical community has been unsuccessful in adequately treating or advancing therapies to manage anemia in the post-operative period. Evaluation and treatment of perioperative anemia is critical to improving outcomes in orthopaedic surgery. The primary objective of this study is to determine feasibility of a pilot study design aimed at evaluating intravenous iron therapy (IVIT) as a means to improve patient well-being following orthopaedic injury.

Methods and analysis: This is a single center, pilot, double-blind randomised controlled trial (RCT) investigating the use of IVIT for acute blood loss anemia in traumatically injured orthopaedic patients. Patients are randomised to receive either a single dose infusion of low molecular weight iron dextran (1000mg) or placebo (normal saline) during their hospital stay for trauma management. Eligible subjects include adult patients admitted for lower extremity or pelvis operative fracture care with a hemoglobin of 7-11g/dL post-operatively during their hospital stay. Exclusion criteria include history of intolerance to IV iron supplementation, active hemorrhage requiring ongoing resuscitation with blood products, planned staged procedures, pre-existing hematologic disorders or chronic inflammatory states, iron overload on screening, or vulnerable populations. Patients with clinically normal ferritin are included as these iron stores may not be readily available for use in inflamed states such as trauma or surgery. We follow patients for three months to measure the effect of iron supplementation on clinical outcomes (resolution of anemia and functional iron deficiency), patient reported outcomes (fatigue, physical function, depression), and immune cell function.

Ethics and dissemination: This study has ethics approval (Oregon Health & Science University Institutional Review Board, STUDY00022441). We will disseminate the findings through peer-reviewed publications and conference presentations.

Registration details: ClinicalTrials.gov (NCT05292001)

Strengths and limitations of this study

- Our study is a single center, pilot, double-blind randomised controlled trial investigating the use of IVIT for acute blood loss anemia in injured orthopaedic patients
- Use of a single high dose infusion of low molecular weight iron dextran is a safe method of body iron store repletion that optimizes study design logistics, patient capture, cost and efficacy of iron delivery.
- We aim to assess the feasibility for a future large scale randomised controlled trial evaluating IVIT as a means to improve time to anemia and functional iron deficiency resolution, as well as standardized patient reported quality of life indices (PROMIS fatigue, physical function, depression).

INTRODUCTION

Background and rationale: Clinical

Acute blood loss in orthopaedic trauma and operative fracture care contributes substantially to perioperative anemia and functional iron deficiency. The prevalence of preoperative anemia has been observed as up to 50% in patients undergoing surgical stabilization for hip fractures[1,2] and, unsurprisingly, up to 87% following hip fracture fixation.[2] Perioperative anemia is associated with increased hospital length of stay (LOS), need for blood transfusion, risk of surgical site infection (SSI), genitourinary and cardiovascular complications, and death.[1,2] Additionally, anemia has clinical implications in quality of life (QOL) measures and is associated with fatigue, impaired physical performance, decreased exercise capacity, and mood disturbances.[3–5] The broad impact of anemia is often underestimated by clinicians; treatment may have profound benefits to patients' well-being.[4] Therefore, evaluation and treatment of perioperative anemia is critical to improving outcomes in orthopaedic surgery.

The standard of care for perioperative anemia in orthopaedic trauma is pRBC transfusion; however, this has been associated with increased mortality, nosocomial infection, postoperative venous thromboembolism, multi-system organ dysfunction, and acute respiratory distress syndrome.[1,6,7] Current recommendations for the treatment of anemia in orthopaedic trauma center upon a restrictive strategy for management (i.e., hemoglobin <7g/dL for pRBC transfusion initiation) to minimize transfusion reactions while ignoring the long term recovery effects of anemia. A safer alternative to pRBCs is desirable because a critical number of patients do not meet this restrictive transfusion threshold and may suffer negative effects from anemia during recovery from the acute insult.

Currently, there is no standard practice of iron supplementation for treatment of anemia in the acute trauma setting. Previous studies have shown promising results for the use of intravenous iron therapy (IVIT) in orthopaedic patients. A recent meta-analysis by Shin et al. concluded that use of IVIT perioperatively in orthopaedic surgery significantly decreased the proportion of patients receiving packed red blood cell (pRBC) transfusions by 31%, shortened hospital stay by 1.6 days, and reduced post-operative infection rate by 33%.[6] Serrano-Trenas et al. demonstrated IV iron sucrose therapy reduced transfusion requirements in a subset of geriatric hip fracture patients but no difference was found between groups for morbidity, mortality, or LOS.[8] Ten percent of the patients enrolled in the studied died prior to their post-hospitalization check-up. Additionally, only 16% of patients in the investigational arm received the three full doses of IVIT sufficient to replete body iron stores. Shortcomings in study design and restrictive study population limit the utility of the findings in this study. Non-orthopaedic studies suggest improved patient-reported outcomes with IVIT after hemorrhagic events.[3,9] Unfortunately, there are a lack of high-quality randomised controlled trials (RCT) in the orthopaedic literature investigating the routine use of IVIT. Further, no studies within orthopaedics have looked at the effect of IVIT on patient reported quality of life outcomes.

Background and rationale: Translational

The consequence of functional iron deficiency, in which insufficient iron is available for erythropoiesis despite normal iron stores in bone marrow macrophages[10,11] has not been fully investigated in the trauma and orthopedic settings. Functional iron deficiency results from two

main pathways – conditions that incite a systemic inflammatory response (i.e. surgery, trauma)[6,12] and situations of increased erythropoiesis mediated by endogenous or exogenous erythropoietin stimulation.[12] In the former, there is a hepcidin mediated down-regulation of intestinal iron absorption and impaired mobilization of body iron stores.[13] In the latter, there is a mismatch between iron demand and supply as in the setting of acute blood loss.[12] Both of these clinical scenarios play a role in the setting of orthopaedic trauma requiring operative surgical stabilization.

We aim to evaluate the consequence of IVIT on immune cell physiology. The general effect of these cells on bone regeneration in the setting of fracture are three-fold; they promote migration and proliferation of osteogenic cells, increase blood vessel formation, and induce inflammatory reactions.[14,15] During fracture repair, multiple immune cell types work in harmony to modulate healing, including those of myeloid origin (neutrophils, macrophages, osteoclasts) and lymphoid origin (T- and B-lymphocytes, natural killer cells).[15] Further work must be done to understand the biological significance of immune cells and their regulatory factors in bone regeneration, as well as potential areas for modulation.

Platelets are of particular interest to investigate as they not only affect wound healing, but also play a critical role in surgical hemostasis. Platelet production is known to be intimately linked with iron stores, as iron deficiency often causes increased platelet counts, however the role of iron in platelet function remains unclear. While platelet numbers increase in anemia, platelet response to inflammation, trauma and conditions with excessive bleeding are more complex – where platelet activities cause increased clotting as well as exacerbate bleeding.[16] Low but persistent levels of platelet stimulation in inflammation and trauma can cause a dulling of platelet activity (i.e. platelet exhaustion).[16,17] Specific mechanisms of platelet dysfunction under these conditions remain largely unspecified.[18–20]

Recent work by our multidisciplinary team has identified several clinically relevant physiologic changes of platelets in iron deficient premenopausal women which are reversed with IVIT. Preliminary findings demonstrated (1) IV iron repletion decreases platelet count in iron deficiency, (2) iron repletion significantly increases platelet integrin activation and alpha-granule secretion in response to ADP and collagen related peptide, and (3) platelet adhesion to type-1 collagen is enhanced after IVIT.[21] This suggests that iron is vital for optimal platelet function and hemostasis. We seek to understand the alterations IVIT has on similar platelet profiles in injured patients, which has not previously been studied.

Objectives and study hypothesis

The primary objective of this study is to determine feasibility of study design, recruitment, randomisation, intervention implementation, blinded procedures, and follow-up. The central hypothesis motivating the research is that acute blood loss anemia may be one modifiable risk factor which can be addressed with IVIT to improve patient well-being following traumatic orthopaedic injury. The secondary objectives of the study include:

(I) measure the time to return to normal hemoglobin as a marker for resolution of anemia and normalization of body iron stores as a marker for resolution of functional iron deficiency following orthopaedic fracture care

(II) evaluate the effect of IVIT on patient reported quality of life measures of fatigue, physical function, and depression following traumatic orthopaedic injury through standardized PROMIS questionnaires

(III) determine the role of IVIT on immune cell physiology in the setting of acute blood loss anemia and inflammation from orthopaedic trauma

METHODS AND ANALYSIS

Overview of study design

This is a single center, double-blind randomised controlled trial (RCT) investigating the use of IVIT (N=75) compared to placebo (N=75) for acute blood loss anemia in traumatically injured orthopaedic patients. The intervention consists of a single dose infusion of low molecular weight iron dextran (1000mg LMW ID) during the patient's hospital stay for initial trauma management. Patients in the placebo arm are given an equal volume normal saline infusion (Figure 1). Both the investigator and participants are blinded to the study treatment administered.

Eligibility criteria

Inclusion criteria

1. Patients age 18-89 admitted for operative fracture care of a lower extremity or pelvis fractures
2. Acute blood loss anemia as defined by hemoglobin concentration between 7.0 – 11.0g/dL post-operatively during the hospital admission

Exclusion criteria

Patients who meet any one or more of the following will be excluded from the study:

1. History of intolerance or hypersensitivity to IV iron supplementation
2. Active hemorrhage requiring greater than two units (whole blood or pRBCs) transfused perioperatively
3. Planned staged orthopaedic procedures
4. Pre-existing hematologic or coagulation disorder (e.g., thalassemia, sickle cell disease, hemophilia, von Willibrand's disease, or myeloproliferative disease)
5. Diagnosis of chronic kidney disease and/or chronic liver disease
6. Known infection, inflammatory condition (e.g., systemic lupus erythematosus, rheumatoid arthritis, and ankylosing spondylitis), or malignancy
7. Pregnancy
8. Iron overload (defined as serum ferritin concentration ≥ 1,000ng/mL, serum iron concentration > 160µg/ dL, or serum transferrin saturation ≥ 50%) or any condition associated with iron overload (e.g., hemochromatosis and aceruloplasminemia)
9. Patients that are tenets of the Jehovah's Witness faith
10. Vulnerable populations including pediatric patients, geriatric populations 90 or older, incarcerated individuals, those unable to provide informed consent
11. Inability to refrain from oral iron supplementation during study period
12. Current or recent (within 30 days) use of immunosuppressive agents
13. Use of any recombinant human erythropoietin formulation within the previous 30 days

Study intervention and blinding

LMW ID was chosen over IV iron alternatives to optimize logistics, patient capture, cost and efficacy of iron delivery. LMW ID has a stable formulation for safe administration of sufficient iron for repletion of body iron stores in a single high dose infusion over one hour, as compared to alternative regimens that require several small dose administrations over the course of days to weeks.[22,23] Use of this iron formulation improves upon prior RCTs with incomplete adherence when utilizing multiple infusions of alternative low-dose regimens. Importantly, the Food and Drug Administration (FDA) has recognized the incidence of life-threatening adverse effects and deaths associated with IVIT (2.2 per million doses and 0.4 per million doses, respectively) is significantly lower than that associated with blood transfusion (10 per million units and 4 per million units, respectively).[13] Oral iron supplementation is an alternative to IVIT, but is associated with increased risk of adverse reactions,[22] poor medication adherence,[12,22] lower efficacy,[12] and limited use in settings where rapid iron repletion is required.[12,13] IVIT is preferred when rapid, significant correction is necessary as it has higher efficiency and shorter time to improvement.[13,24,25]

Research Pharmacy Services (RPS) at our institution is responsible for all study drug related tasks including randomisation and blinding of the study drug and placebo. RPS follows a published protocol for drug shipment/receipt, packaging, storage, preparation, dispensing and accountability, and administration. Consent will be required from the patient or a legally authorized representative. Unblinding will be considered in emergency situations (i.e. severe infusion reaction). Verbal permission from the principle investigator or co-investigator will suffice in order to unblind, followed by subsequent written documentation after the unblinding has occurred. Drug destruction will be performed by RPS at the study drug expiration date or the completion of the study. Given the single dose design of the study drug, medication compliance assessment will not be required; however, it will be documented if the treatment had to be discontinued prior to completion of infusion due to adverse reaction.

Recruitment

Our institution is a level 1, tertiary care center with high volume fracture care and over 4000 trauma activations yearly. Our recruitment pool consists of all patients admitted with orthopaedic trauma during the enrollment period. Screening includes review of laboratory studies, injuries, and comorbidities to assess for inclusion.

Allocation of patients to study groups

Following informed consent, patients are randomised one-to-one into a treatment arm by RPS and receive the allocated therapy via a computer-generated random number schema. RPS is responsible for all blinding procedures per their documented protocol. Medication related study documents are stored in an electronic pharmacy binder on Vestigo only accessible by unblinded personnel. The study medication is prepared, delivered, labeled, and covered with blinding bags and tubing covers by the unblinded pharmacy personnel to ensure that both the investigators and subjects are blinded to the treatment received.

Outcome measures

Appraisal of feasibility will primarily be qualitative in nature, including documentation of blinding failures, review of challenges in recruitment and retention, and assessment of data

management and survey administration. We will evaluate quantitatively with recruitment rate of all eligible patients, as well as calculation of screening failure and retention rates.

Outcome measures to fulfill the secondary objectives will be collected to assess the feasibility of their collection and relevance of timing in anticipation of a future large-scale RCT.

Laboratory data

- *Complete blood count.* Concentration of hemoglobin (oxygen carrying protein) in whole blood and percentage of blood volume (hematocrit) occupied by RBCs are of primary interest. These are markers of anemia (defined as hemoglobin <12g/dL in females and <13.5g/dL in males) measured for inclusion assessment and to monitor for resolution of anemia at all study follow-up visits.
- *Ferritin.* Evaluated at enrollment to assess for iron overload (Ferritin ≥ 1,000ng/mL) and tracked throughout the study to measure participants' body stores of iron.
- *Additional iron studies (Serum iron, transferrin, total iron binding capacity).* Additional indicators of body iron stores and iron carrying capacity within blood. Utilized to further define patients' anemia and iron available for functional use.

Quality of life measures

- *PROMIS Fatigue Questionnaire.* Computer adaptive survey administered via REDCap to evaluate feelings of tiredness likely to decrease one's ability to execute daily activities and function normally in family or social roles.
- *PROMIS Physical Function Questionnaire.* Computer adaptive survey administered via REDCap to measure self-reported capability to perform physical activities including activities of daily living.
- *PROMIS Depression Questionnaire.* Computer adaptive survey administered via REDCap to assess negative mood, views of self, social cognition, and decreased social engagement.

Immune cell functional testing will be performed through a variety of novel laboratory techniques, including but not limited to the following

- Flow cytometry and Fluorescence-Activated Cell Sorting (FACS) to quantify and evaluate platelets, cytokines and other immune cells.[26]
- Assessment of platelet aggregation under venous shear in chambers coated with type I collagen.
- Use of proteomics tools to systematically measure the molecular composition of immune cells as well as the activation of signaling systems in response to relevant agonists.[27]
- Analysis of immune cells, biomarkers, and relevant circulating factors using Luminex technology and ELISA.

Participant timeline

Table 1 delineates the schedule for enrollment, interventions, laboratory studies and patient reported outcome surveys.

Table 1. Schedule of enrolment, study drug allocation, quality of life and laboratory assessments.

	Enrollment	Allocation	Follow-up			
Time point	POD1 through hospital discharge		2 weeks	4 weeks	6 weeks	3 months
Screening/Enrollment						
Eligibility screen	x					
Informed consent	x					
Randomisation		x				
Allocation of study drug vs placebo		x				
Assessments						
PROMIS Fatigue	x		x	x	x	x
PROMIS Physical Function	x		x	x	x	x
PROMIS Depression	x		x	x	x	x
Laboratory studies						
CBC	x		x	x	x	x
Ferritin	x		x	x	x	x
Iron, TIBC, transferrin, %sat	x					x
Immune cell studies	x		x	x	x	x

POD1 = post-operative day one; TIBC = total iron binding capacity; %Sat = transferrin saturation

Safety considerations

Adverse events are documented in a secure REDCap database, including description of the symptoms, management provided, and outcome. Adverse events are categorized as mild, moderate and severe in relation to the infusion itself as described hereafter. Patients are additionally monitored for other complications in their care (not necessarily related to study drug administration) including surgical site infection, non-union, and need for post-infusion pRBC transfusion per clinical threshold criteria.

Serious adverse events including severe infusion reactions (e.g., cardiac arrest, cyanosis, loss of consciousness, periorbital edema, wheezing, stridor) will be reported as required by the IRB. Management of such events will include stopping the infusion, activating the rapid response team, oxygen supplementation, epinephrine, IV steroids, and initiating ACLS (if necessary).

Other infusion related reactions are documented and managed as described by DeLoughery and Rampton et al. (Figure 2).[22,28] Hypersensitivity medications are ordered with the study medication per our standard institution order set, including diphenhydramine, famotidine, hydrocortisone sodium succinate injection, epinephrine IM, and normal saline bolus.

Procedures for completion

Completion occurs at the last follow-up visit when all patient reported outcome measures and laboratory data have been collected. In an effort to optimize retention, PROMIS surveys will be emailed to study participants via REDCap (which has pre-built computer adaptive testing for the chosen instruments) at the appropriate follow-up timepoints. These may be completed upon email receipt or during scheduled study visits. Therefore, patient report outcomes may still be completed virtually in the event patients are otherwise unable to complete in person follow-up visits. Patients may freely withdraw their informed consent at any time during the clinical trial. Further, the investigator may terminate a subject's participation in the research study if they are found to have any of the exclusion criteria during the study period (including use of oral iron supplements, new malignancy, or newly diagnosed inflammatory disease) with the exception of

post-operative infection. Subjects are considered lost to follow-up if they do not attend scheduled study visits or complete study surveys.

Sample size consideration

The primary objective of this study is to pilot for feasibility; therefore, preliminary sample size calculations were only informative in nature. Preliminary sample size estimates have been calculated based on similar studies; however, ultimately the results of this pilot study will inform sample size requirements in a larger scale RCT.

Sample size calculations were performed in R, using α=0.05 and β=0.20. Based on prior studies, a significant increase from baseline hemoglobin of 1.2 g/dL ± 1.4 was observed within a median follow-up time of three weeks after administration of LMW ID.[29,30] Therefore, when using hemoglobin as a marker of anemia improvement, the minimum number of subjects required to detect a difference in hemoglobin was estimated to be 23 subjects per group. The minimum number of subjects required to detect a clinically meaningful change in PROMIS instrument score defined as 5 points with a standard deviation of 10 (minimally important change has been defined for several PROMIS measures as 3-6 points[31]), was estimated to be 64 subjects per group. Based on the aforementioned calculations, we elected to target a sample size of 150 subjects, which accounts for a 15% lost to follow-up (LTFU) rate.

Data analysis plan

We will use hemoglobin as a marker for resolution of anemia, as defined as >12g/dL in females and >13.5g/dL in males. Based on previous studies, administration of IVIT improves hemoglobin levels within the first week, and normalization is typically achieved within 3-4 weeks.[1] We anticipate that this will hold true in our IVIT cohort, with resolution of anemia occurring around 3 months for the placebo cohort. We will evaluate for statistical difference of change in hemoglobin at all study visit timepoints with t tests.

We will use PROMIS fatigue, physical function, and depression scores as indicators of important quality of life metrics that relate to recovery from traumatic injury and fracture healing. The aggregated change in PROMIS score will be calculated as a percent change from baseline at all time-points for both measures. An analysis of covariance model (ANCOVA) will be used to access for clinical significance, with treatment (IVIT vs placebo) as factors and baseline score as covariate.

Significance level set to 95% for all statistical measures and we will perform a multivariate analysis to examine layered contributions of critical variables such as age, sex and BMI known to contribute to anemia, fatigue, and depression. Analysis will be performed according to a modified intention-to-treat paradigm in which all patients, except those who are deemed ineligible after randomisation, will be analyzed according to the treatment group to which they are randomly assigned. We will use multiple-imputation to handle missing data

Potential impact of study

Successful completion of this project has the potential to provide relevant clinical information for the development of a large-scale, multicenter randomised trial. Ultimately, a better understanding of the effects of IVIT both clinically and at a biological level may alter our

treatment approach of anemia in patients who sustain orthopaedic injuries, thereby leading to decreased risks and improved recovery. If IVIT is proven to be effective in improving quality of life after traumatic lower extremity fracture, clinical relevance to other fracture types and more broadly in orthopaedic surgery will follow. We plan to further evaluate the efficacy of IVIT for reduction of blood transfusion and as an adjunct therapy to blood transfusion in the future.

ETHICS AND DISSEMINATION

Research ethics approval: This study has ethics approval from the Oregon Health & Science University Institutional Review Board (STUDY00022441). Protocol modifications and annual continuing review will be submitted as necessary for IRB approval prior to implementation and continuation of the study, respectively.

Consent: Informed consent is performed using IRB approved forms with a trained study provider. Patients may freely withdraw their informed consent at any time during the clinical trial.

Confidentiality: All data from this work is maintained in security and confidentiality at our institution. A secure REDCap database (encrypted and password protected) is used for data collection, administration of PROMIS surveys, organization of data reports for statistical analysis, and documentation of adverse events. Research medication management, randomisation, blinding, and related record keeping is performed by RPS per their published protocol.

Dissemination policy: The findings of this study will be disseminated through peer-reviewed publications and conference presentations. This protocol has been reported following the Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) statement.[32] Results will be published following the Consolidated Standards of Reporting Trials (CONSORT) guidelines for pilot and feasibility trials.[33,34] In addition, appropriate publication requirements will be upheld for the use of PROMIS instruments.

Registration details: ClinicalTrials.gov (NCT05292001)

Authors' contributions: DFP and ZMW are co-principle investigators responsible for conceptualization and funding of the study. DFP further designed and drafted the protocol for the study. DMF, NW, JJS, and JEA contributed to study design as pertinent to their respective specialties, providing specific content and edits to the manuscript. MAS acted as an advisor to the project and edited the manuscript. KL oversees the clinical aspects of the study and CJY oversees the translational laboratory component. All authors have reviewed and approved the manuscript.

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Competing interests: None

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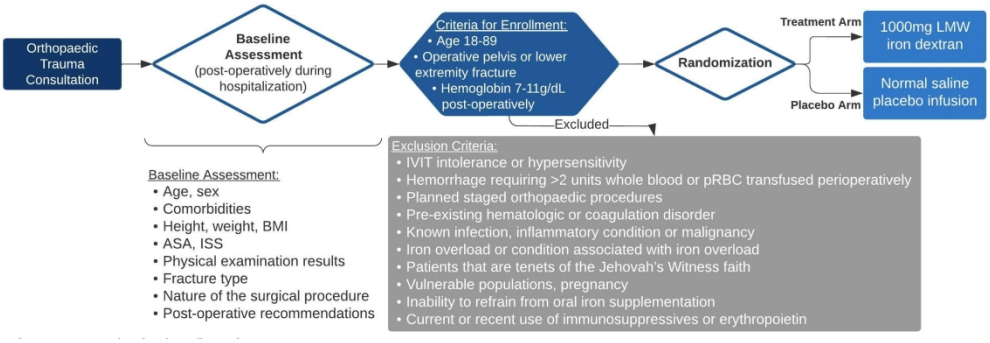

Figure 1. Study design flowchart.

214x72mm (300 x 300 DPI)

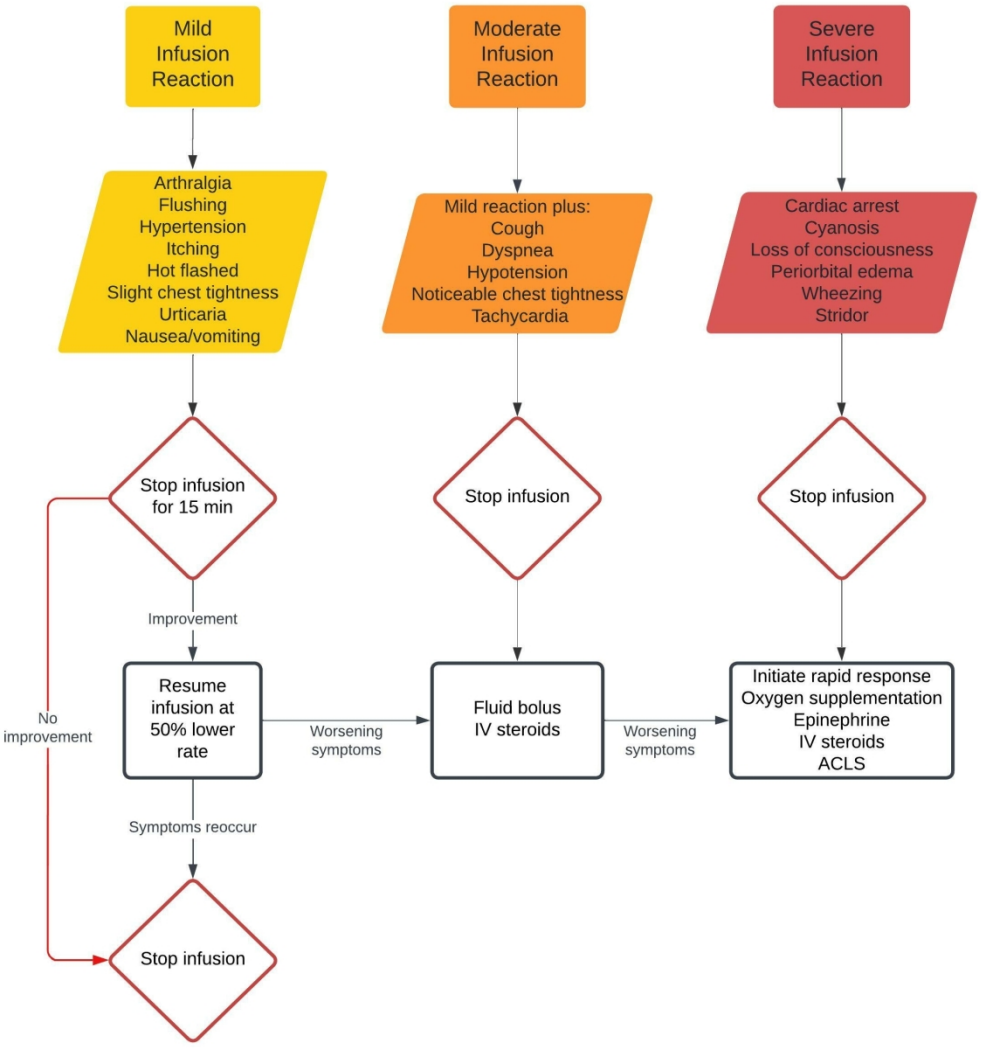

Figure 2. Infusion reaction treatment algorithm

185x195mm (300 x 300 DPI)

Reporting checklist for protocol of a clinical trial.

Based on the SPIRIT guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

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		Reporting Item	Page Number
Administrative information			
Title	#1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	#2a	Trial identifier and registry name. If not yet registered, name of intended registry	2 & 10
Trial registration: data set	#2b	All items from the World Health Organization Trial Registration Data Set	
Protocol version	#3	Date and version identifier	N/A
Funding	#4	Sources and types of financial, material, and other support	10
Roles and responsibilities: contributorship	#5a	Names, affiliations, and roles of protocol contributors	10

Roles and responsibilities: sponsor contact information	#5b	Name and contact information for the trial sponsor	N/A
Roles and responsibilities: sponsor and funder	#5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	N/A
Roles and responsibilities: committees	#5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	N/A
Introduction			
Background and rationale	#6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	3-4
Background and rationale: choice of comparators	#6b	Explanation for choice of comparators	6
Objectives	#7	Specific objectives or hypotheses	4-5
Trial design	#8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, non-inferiority, exploratory)	4-5
Methods: Participants, interventions, and outcomes			
Study setting	#9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	6
Eligibility criteria	#10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will	5

		perform the interventions (eg, surgeons, psychotherapists)	
Interventions: description	#11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	5
Interventions: modifications	#11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving / worsening disease)	8
Interventions: adherance	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)	6
Interventions: concomitant care	#11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	5
Outcomes	#12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	6-7
Participant timeline	#13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	8
Sample size	#14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	9
Recruitment	#15	Strategies for achieving adequate participant enrolment to reach target sample size	6
Methods: Assignment of interventions (for controlled trials)			
Allocation: sequence generation	#16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	6

Allocation concealment mechanism	#16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	6
Allocation: implementation	#16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	6
Blinding (masking)	#17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	6
Blinding (masking): emergency unblinding	#17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	6
Methods: Data collection, management, and analysis			
Data collection plan	#18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	7 & 10
Data collection plan: retention	#18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	8
Data management	#19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	10
Statistics: outcomes	#20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	9
Statistics: additional analyses	#20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	9

Statistics: analysis population and missing data	#20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	9
Methods: Monitoring			
Data monitoring: formal committee	#21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	6
Data monitoring: interim analysis	#21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	N/A
Harms	#22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	8
Auditing	#23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	N/A
Ethics and dissemination			
Research ethics approval	#24	Plans for seeking research ethics committee / institutional review board (REC / IRB) approval	10
Protocol amendments	#25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC / IRBs, trial participants, trial registries, journals, regulators)	10
Consent or assent	#26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	10
Consent or assent: ancillary studies	#26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	N/A
Confidentiality	#27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to	10

		protect confidentiality before, during, and after the trial	
Declaration of interests	#28	Financial and other competing interests for principal investigators for the overall trial and each study site	10
Data access	#29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	10
Ancillary and post trial care	#30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	N/A
Dissemination policy: trial results	#31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	10
Dissemination policy: authorship	#31b	Authorship eligibility guidelines and any intended use of professional writers	10
Dissemination policy: reproducible research	#31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	N/A
Appendices			
Informed consent materials	#32	Model consent form and other related documentation given to participants and authorised surrogates	Uploaded
Biological specimens	#33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	N/A

BMJ Open

Role of single-dose intravenous iron therapy for the treatment of anemia after orthopaedic trauma: protocol for a pilot randomised controlled trial.

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Role of single-dose intravenous iron therapy for the treatment of anemia after orthopaedic trauma: protocol for a pilot randomised controlled trial.

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ABSTRACT

Introduction: Orthopaedic trauma and fracture care commonly cause perioperative anemia and associated functional iron deficiency due to a systemic inflammatory state. Modern, strict transfusion thresholds leave many patients anemic; managing this perioperative anemia is an opportunity to impact outcomes in orthopaedic trauma surgery. The primary outcome of this pilot study is feasibility for a large RCT to evaluate intravenous iron therapy (IVIT) to improve patient well-being following orthopaedic injury. Measurements will include rate of participant enrollment, screening failure, follow up, missing data, adverse events, and protocol deviation.

Methods and analysis: This single center, pilot, double-blind randomised controlled trial (RCT) investigates the use of IVIT for acute blood loss anemia in traumatically injured orthopaedic patients. Patients are randomised to receive either a single dose infusion of low molecular weight iron dextran (1000mg) or placebo (normal saline) post-operatively during their hospital stay for trauma management. Eligible subjects include adult patients admitted for lower extremity or pelvis operative fracture care with a hemoglobin of 7-11g/dL within seven days post-operatively during inpatient care. Exclusion criteria include history of intolerance to IV iron supplementation, active hemorrhage requiring ongoing blood product resuscitation, multiple planned procedures, pre-existing hematologic disorders or chronic inflammatory states, iron overload on screening, or vulnerable populations. Patients with clinically normal ferritin are included; iron stores may not be readily available for use in inflamed states such as trauma or surgery. We follow patients for three months to measure the effect of iron supplementation on clinical outcomes (resolution of anemia and functional iron deficiency), patient reported outcomes (fatigue, physical function, depression, and quality of life), and translational measures of immune cell function.

Ethics and dissemination: This study has ethics approval (Oregon Health & Science University Institutional Review Board, STUDY00022441). We will disseminate the findings through peer-reviewed publications and conference presentations.

Registration details: ClinicalTrials.gov (NCT05292001)

Strengths and limitations of this study

- Our study is a single center, pilot, double-blind randomised controlled trial investigating the use of IVIT for acute blood loss anemia in injured orthopaedic patients
- Use of a single high dose infusion of low molecular weight iron dextran is a safe method of body iron store repletion that optimizes study design logistics, patient capture, cost and efficacy of iron delivery.
- We aim to assess the feasibility for a future large scale randomised controlled trial evaluating IVIT as a means to improve time to anemia and functional iron deficiency resolution, as well as standardized patient reported quality of life indices (PROMIS fatigue, physical function, depression).

INTRODUCTION

Background and rationale: Clinical

Acute blood loss in orthopaedic trauma and operative fracture care contributes substantially to perioperative anemia and functional iron deficiency. The prevalence of preoperative anemia has been observed as up to 50% in patients undergoing surgical stabilization for hip fractures[1,2] and, unsurprisingly, up to 87% following hip fracture fixation.[2] Perioperative anemia is associated with increased hospital length of stay (LOS), need for blood transfusion, risk of surgical site infection (SSI), genitourinary and cardiovascular complications, and death.[1,2] Additionally, anemia has clinical implications in quality of life (QOL) measures and is associated with fatigue, impaired physical performance, decreased exercise capacity, and mood disturbances.[3–5] The broad impact of anemia is often underestimated by clinicians; treatment may have profound benefits to patients' well-being.[4] Therefore, evaluation and treatment of perioperative anemia is critical to improving outcomes in orthopaedic surgery.

The standard of care for perioperative anemia in orthopaedic trauma is packed red blood cell (pRBC) transfusion; however, this has been associated with increased mortality, nosocomial infection, postoperative venous thromboembolism, multi-system organ dysfunction, and acute respiratory distress syndrome.[1,6,7] Current recommendations for the treatment of anemia in orthopaedic trauma center upon a restrictive strategy for management (i.e., hemoglobin <7g/dL for pRBC transfusion initiation) to minimize transfusion reactions while ignoring the long term recovery effects of anemia. A safer alternative to pRBCs is desirable because a critical number of patients do not meet this restrictive transfusion threshold and may be negatively impacted by anemia during recovery.

Currently, there is no standard practice of iron supplementation for treatment of anemia in the acute trauma setting. Previous studies have shown promising results for the use of intravenous iron therapy (IVIT) in orthopaedic patients. A recent meta-analysis by Shin et al. concluded that use of IVIT perioperatively in orthopaedic surgery significantly decreased the proportion of patients receiving packed red blood cell (pRBC) transfusions by 31%, shortened hospital stay by 1.6 days, and reduced post-operative infection rate by 33%.[6] Serrano-Trenas et al. demonstrated IV iron sucrose therapy reduced transfusion requirements in a subset of geriatric hip fracture patients without differences between groups for morbidity, mortality, or LOS.[8] Ten percent of the patients enrolled in the studied died prior to their post-hospitalization check-up. Additionally, only 16% of patients in the investigational arm received the three full doses of IVIT sufficient to replete body iron stores. Shortcomings in study design and restrictive study population limit the utility of the findings in this study. Non-orthopaedic studies suggest improved patient-reported outcomes with IVIT after hemorrhagic events.[3,9] Unfortunately, there are a lack of high-quality randomised controlled trials (RCT) in the orthopaedic literature investigating the routine use of IVIT. Further, no studies within orthopaedics have looked at the effect of IVIT on patient reported quality of life outcomes.

Background and rationale: Translational

Our preliminary analysis of iron store derangements following a traumatic event have prompted us to simultaneously investigate the underlying pathophysiology of anemia during the inflammatory conditions of trauma and surgery. We have found that less than 5% of patients

demonstrated normal serum iron, total iron binding capacity, transferrin saturation and transferrin values following orthopaedic fracture care, with abnormally low values in some or all of these assessments being seen in the vast majority of patients. Despite this, ferritin level is normal in approximately two-thirds of patients overall and normal or high in nearly all patients with all other iron studies low.[10] This phenomenon may indicate that iron becomes sequestered and unavailable for use in replenishing blood cell volume in the setting of orthopaedic trauma, leading to a state of functional iron deficiency.

The consequence of functional iron deficiency, in which insufficient iron is available for erythropoiesis despite normal iron stores in bone marrow macrophages[11,12] has not been fully investigated in the trauma and orthopedic settings. Functional iron deficiency results from two main pathways – conditions that incite a systemic inflammatory response (i.e. surgery, trauma)[6,13] and situations of increased erythropoiesis mediated by endogenous or exogenous erythropoietin stimulation.[13] In the former, there is a hepcidin mediated down-regulation of intestinal iron absorption and impaired mobilization of body iron stores.[14] In the latter, there is a mismatch between iron demand and supply as in the setting of acute blood loss.[13] Both of these clinical scenarios play a role in the setting of orthopaedic trauma requiring operative surgical stabilization.

We aim to evaluate the consequence of IVIT on immune cell physiology given the connection of functional iron deficiency to pro-inflammatory states. The general effect of these cells on bone regeneration in the setting of fracture are three-fold; they promote migration and proliferation of osteogenic cells, increase blood vessel formation, and induce inflammatory reactions.[15,16] During fracture repair, multiple immune cell types work in harmony to modulate healing, including those of myeloid origin (neutrophils, macrophages, osteoclasts) and lymphoid origin (T- and B-lymphocytes, natural killer cells).[16] Further work must be done to understand the biological significance of immune cells and their regulatory factors in bone regeneration, as well as potential areas for modulation.

Platelets are of particular interest to investigate as they not only affect wound healing, but also play a critical role in surgical hemostasis. Platelet production is known to be intimately linked with iron stores, as iron deficiency often causes increased platelet counts, however the role of iron in platelet function remains unclear. While platelet numbers increase in anemia, platelet response to inflammation, trauma and conditions with excessive bleeding are more complex – where platelet activities cause increased clotting as well as exacerbate bleeding.[17] Low but persistent levels of platelet stimulation in inflammation and trauma can cause a dulling of platelet activity (i.e. platelet exhaustion).[17,18] Specific mechanisms of platelet dysfunction under these conditions remain largely unspecified.[19–21]

Recent work by our multidisciplinary team has identified several clinically relevant physiologic changes of platelets in iron deficient premenopausal women which are reversed with IVIT. Preliminary findings demonstrated (1) IV iron repletion decreases platelet count in iron deficiency, (2) iron repletion significantly increases platelet integrin activation and alpha-granule secretion in response to ADP and collagen related peptide, and (3) platelet adhesion to type-1 collagen is enhanced after IVIT.[22] This suggests that iron is vital for optimal platelet function

and hemostasis. We seek to understand the alterations IVIT has on similar platelet profiles in injured patients, which has not previously been studied.

Objectives and study hypothesis

The primary objective of this study is to determine feasibility of study design, recruitment, randomisation, intervention implementation, blinded procedures, and follow-up. Feasibility outcomes will be quantified as rate of participant enrollment (60 patients randomised in 2 years), proportion of participants completing each follow up visit, proportion of missing data, rate of transfusion reactions, and rate of protocol adherence. The primary clinical outcome is patient reported quality of life measures of fatigue on PROMIS questionnaire. The central hypothesis motivating the research is that acute blood loss anemia may be one modifiable risk factor which can be addressed with IVIT to improve patient well-being following traumatic orthopaedic injury. The secondary objectives of the study include:

(I) measure the time to return to normal hemoglobin as a marker for resolution of anemia and normalization of body iron stores as a marker for resolution of functional iron deficiency following orthopaedic fracture care

(II) evaluate the effect of IVIT on patient reported quality of life measures of physical function and depression following traumatic orthopaedic injury through standardized PROMIS questionnaires

(III) appraise cost effectiveness of IVIT with a cost-utility analysis using quality-adjusted life-years (QALYs)

(IV) determine the role of IVIT on immune cell physiology in the setting of acute blood loss anemia and inflammation from orthopaedic trauma

METHODS AND ANALYSIS

Overview of study design

This is a single center, double-blind parallel design randomised controlled trial (RCT) investigating the use of IVIT (N=30) compared to placebo (N=30) for acute blood loss anemia in traumatically injured orthopaedic patients. The intervention consists of a single dose infusion of low molecular weight iron dextran (1000mg LMW ID) post-operatively during the patient's hospital stay for initial trauma management. Patients in the placebo arm are given an equal volume normal saline infusion (Figure 1). Both the investigator and participants are blinded to the study treatment administered.

Eligibility criteria

Inclusion criteria

1. Patients age 18-89 admitted with a lower extremity or pelvis fracture requiring surgical stabilization
2. Acute blood loss anemia as defined by hemoglobin concentration between 7.0 – 11.0g/dL within seven days post-operatively from definitive fracture stabilization during the hospital admission

Exclusion criteria

Patients who meet any one or more of the following will be excluded from the study:

1. History of intolerance or hypersensitivity to IV iron supplementation

2. Active hemorrhage requiring greater than two units (whole blood or pRBCs) transfused perioperatively
3. Multiple planned operative procedures during the trauma admission, excluding orthopaedic staged procedures for the fracture meeting inclusion criterion one (such as temporizing external fixator application and washout for open fracture) in which subjects otherwise meet qualifications for enrollment after definitive stabilization
4. Pre-existing hematologic or coagulation disorder (e.g., thalassemia, sickle cell disease, hemophilia, von Willibrand's disease, or myeloproliferative disease)
5. Diagnosis of chronic kidney disease and/or chronic liver disease
6. Known infection, inflammatory condition (e.g., systemic lupus erythematosus, rheumatoid arthritis, and ankylosing spondylitis), or malignancy
7. Pregnancy
8. Iron overload (defined as serum ferritin concentration ≥ 1,000ng/mL, serum iron concentration > 160μg/ dL, or serum transferrin saturation ≥ 50%) or any condition associated with iron overload (e.g., hemochromatosis and aceruloplasminemia)
9. Patients that are tenets of the Jehovah's Witness faith
10. Vulnerable populations including pediatric patients, geriatric populations 90 or older, incarcerated individuals, those unable to provide informed consent
11. Inability to refrain from oral iron supplementation during study period
12. Current or recent (within 30 days) use of immunosuppressive agents
13. Use of any intravenous iron therapy or recombinant human erythropoietin formulation within the previous 30 days

Study intervention and blinding

The study intervention consists of a single dose infusion of low molecular weight iron dextran (1000mg LMW ID). Patients in the placebo arm are given an equal volume normal saline infusion (250mL NS). Given the dark colour of the LMW ID compared to the clear, translucent normal saline, opaque bags and tube covers will be utilized in order to blind all study staff and patients to the allocated treatment group. The blinding covers will be applied by an unblinded pharmacist who prepares the infusion and remain in place during transport, infusion, and disposal of the infusion packaging.

LMW ID was chosen over IV iron alternatives to optimize logistics, patient capture, cost and efficacy of iron delivery. LMW ID has a stable formulation for safe administration of sufficient iron for repletion of body iron stores in a single high dose infusion over one hour, as compared to alternative regimens that require several small dose administrations over the course of days to weeks.[23,24] Use of this iron formulation improves upon prior RCTs with incomplete adherence when utilizing multiple infusions of alternative low-dose regimens. Importantly, the Food and Drug Administration (FDA) has recognized the incidence of life-threatening adverse effects and deaths associated with IVIT (2.2 per million doses and 0.4 per million doses, respectively) is significantly lower than that associated with blood transfusion (10 per million units and 4 per million units, respectively).[14] Oral iron supplementation is an alternative to IVIT, but is associated with increased risk of adverse reactions,[23] poor medication adherence,[13,23] lower efficacy,[13] and limited use in settings where rapid iron repletion is required.[13,14] IVIT is preferred when rapid, significant correction is necessary as it has higher efficiency and shorter time to improvement.[14,25,26]

Research Pharmacy Services (RPS) at our institution is responsible for all study drug related tasks including randomisation and blinding of the study drug and placebo. RPS follows a published protocol for drug shipment/receipt, packaging, storage, preparation, dispensing and accountability, and administration. Consent will be required from the patient or a legally authorized representative. Unblinding will be considered in emergency situations (i.e. severe infusion reaction). Verbal permission from the principle investigator or co-investigator will suffice in order to unblind, followed by subsequent written documentation after the unblinding has occurred. Drug destruction will be performed by RPS at the study drug expiration date or the completion of the study. Given the single dose design of the study drug, medication compliance assessment will not be required; however, it will be documented if the treatment had to be discontinued prior to completion of infusion due to adverse reaction.

Recruitment

Our institution is a level one, tertiary care center with high volume fracture care and over 4000 trauma activations yearly. Our recruitment pool consists of all patients admitted with orthopaedic trauma during the enrollment period, planned June 2022 through May 2024. Patients are eligible for enrollment if they meet the aforementioned criteria within seven days post-operatively from definitive surgical stabilization of their fracture. Screening includes review of laboratory studies, injuries, and comorbidities to assess for inclusion.

Allocation of patients to study groups

Following informed consent, patients are randomised one-to-one into a treatment arm by RPS and receive the allocated therapy via a computer-generated random number schema from randomization.com. RPS is responsible for all blinding procedures. Medication related study documents are stored in an electronic pharmacy binder on Vestigo only accessible by unblinded personnel. The study medication is stored with restricted access in the hospital inpatient pharmacy and prepared, delivered, labeled, and covered with blinding bags and tubing covers by the unblinded pharmacy personnel upon subject enrollment to ensure that both the investigators and subjects are blinded to the treatment received.

Outcome measures

Feasibility outcome measures

Appraisal of feasibility will be based on rate of participant enrollment per year, rate of screening failures, proportion of participants completing each follow up visit, proportion of missing data, rate of transfusion reactions, and rate of protocol adherence. Other feasibility concerns will be qualitative in nature, including documentation of blinding failures, review of challenges in recruitment and retention, and assessment of data management and survey administration.

Primary clinical outcome

The primary clinical outcome of this pilot study will be Health Related Quality of Life (HRQoL) over the 3 months postoperatively. HRQoL will be assessed using the PROMIS Fatigue Questionnaire, a computer adaptive survey (Table 1). This will measure feelings of tiredness likely to decrease one's ability to execute daily activities and function normally in family or social roles.

Secondary clinical outcomes

Outcome measures to fulfill the secondary objectives will be collected to assess the feasibility of their collection and relevance of timing in anticipation of a future large-scale RCT.

Laboratory data

- *Complete blood count.* Concentration of hemoglobin (oxygen carrying protein) in whole blood and percentage of blood volume (hematocrit) occupied by RBCs are of primary interest. These are markers of anemia (defined as hemoglobin <12g/dL in females and <13.5g/dL in males) measured for inclusion assessment and to monitor for resolution of anemia at all study follow-up visits.
- *Ferritin.* Evaluated at enrollment to assess for iron overload (patients with a ferritin level ≥ 1,000ng/mL will be excluded) and tracked throughout the study to measure participants' body stores of iron. Importantly, patients will not be required to have a ferritin level consistent with iron deficiency as we have observed that the majority of patients have normal to high post-traumatic ferritin levels
- *Additional iron studies (Serum iron, transferrin, total iron binding capacity).* Additional indicators of body iron stores and iron carrying capacity within blood. Utilized to further define patients' anemia and iron available for functional use. Similar to ferritin level, only patients with iron values consistent with overload on post-operative laboratory work will be excluded (as defined by exclusion criterion 8).

Quality of life measures

- *PROMIS Fatigue Questionnaire.* Computer adaptive survey administered via REDCap to evaluate feelings of tiredness likely to decrease one's ability to execute daily activities and function normally in family or social roles.
- *PROMIS Physical Function Questionnaire.* Computer adaptive survey administered via REDCap to measure self-reported capability to perform physical activities including activities of daily living.
- *PROMIS Depression Questionnaire.* Computer adaptive survey administered via REDCap to assess negative mood, views of self, social cognition, and decreased social engagement.
- *EQ-5D-5L Quality of Life Questionnaire.* Instrument assesses HRQoL with five dimensions of health, each with five levels ranging from no problem (Level 1) to extreme problem (Level 5). Answers correspond to 3,125 possible health states that can be converted into a single 'utility' score. This will be utilized for the assessment of quality-adjusted life years (QALYs) and cost effectiveness of IVIT for the treatment of acute blood loss anemia following surgical fracture stabilization.

Immune cell functional testing will be performed through a variety of novel laboratory techniques, including but not limited to the following

- Flow cytometry and Fluorescence-Activated Cell Sorting (FACS) to quantify and evaluate platelets, cytokines and other immune cells.[27]
- Assessment of platelet aggregation under venous shear in chambers coated with type I collagen.

- Use of proteomics tools to systematically measure the molecular composition of immune cells as well as the activation of signaling systems in response to relevant agonists.[28]
- Analysis of immune cells, biomarkers, and relevant circulating factors using Luminex technology and ELISA.

Participant timeline

Table 1 delineates the schedule for enrollment, interventions, laboratory studies and patient reported outcome surveys.

Table 1. Schedule of enrolment, study drug allocation, quality of life and laboratory assessments.

	Enrollment	Allocation	Follow-up			
Time point	POD1 through POD7 during hospitalization		2 weeks	4 weeks	6 weeks	3 months
Screening/Enrollment						
Eligibility screen	x					
Informed consent	x					
Randomisation		x				
Allocation of study drug vs placebo		x				
Assessments						
PROMIS Fatigue	x		x	x	x	x
PROMIS Physical Function	x		x	x	x	x
PROMIS Depression	x		x	x	x	x
EQ-5D-5L	x		x	x	x	x
Laboratory studies						
CBC	x		x	x	x	x
Ferritin	x		x	x	x	x
Iron, TIBC, transferrin, %sat	x					x
Immune cell studies	x		x	x	x	x

POD1 = post-operative day one; TIBC = total iron binding capacity; %Sat = transferrin saturation

Safety considerations

Adverse events are documented in a secure REDCap database, including description of the symptoms, management provided, and outcome. Adverse events are categorized as mild, moderate and severe in relation to the infusion itself as described hereafter. Patients are additionally monitored for other complications in their care (not necessarily related to study drug administration) including surgical site infection, non-union, and need for post-infusion pRBC transfusion per clinical threshold criteria.

Serious adverse events including severe infusion reactions (e.g., cardiac arrest, cyanosis, loss of consciousness, periorbital edema, wheezing, stridor) will be reported as required by the IRB. Management of such events will include stopping the infusion, activating the rapid response team, oxygen supplementation, epinephrine, IV steroids, and initiating ACLS (if necessary).

Other infusion related reactions are documented and managed as described by DeLoughery and Rampton et al. (Figure 2).[23,29] Hypersensitivity medications are ordered with the study medication per our standard institution order set, including diphenhydramine, famotidine, hydrocortisone sodium succinate injection, epinephrine IM, and normal saline bolus.

Procedures for completion

Completion occurs at the last follow-up visit when all patient reported outcome measures and laboratory data have been collected. In an effort to optimize retention, PROMIS surveys will be emailed to study participants via REDCap (which has pre-built computer adaptive testing for the chosen instruments) at the appropriate follow-up timepoints. These may be completed upon email receipt or during scheduled study visits. Therefore, patient report outcomes may still be completed virtually in the event patients are otherwise unable to complete in person follow-up visits. Patients may freely withdraw their informed consent at any time during the clinical trial. Further, the investigator may terminate a subject's participation in the research study if they are found to have any of the exclusion criteria during the study period (including use of oral iron supplements, new malignancy, or newly diagnosed inflammatory disease) with the exception of post-operative infection. Subjects are considered lost to follow-up if they do not attend scheduled study visits or complete study surveys.

Sample size consideration

The primary objective of this study is to pilot for feasibility; therefore, traditional quantitative sample size calculations are not well suited for this study. Given the exploratory nature of pilot studies, we plan to enroll a sample of 60 patients to assess the feasibility of a definitive large RCT.

Preliminary sample size calculations were only informative in nature. Preliminary sample size estimates have been calculated based on similar studies; however, ultimately the results of this pilot study will inform sample size requirements in a larger scale RCT.

Sample size calculations were performed in R, using α=0.05 and β=0.20. Based on prior studies, a significant increase from baseline hemoglobin of 1.2 g/dL ± 1.4 was observed within a median follow-up time of three weeks after administration of LMW ID.[30,31] Therefore, when using hemoglobin as a marker of anemia improvement, the minimum number of subjects required to detect a difference in hemoglobin was estimated to be 23 subjects per group. The minimum number of subjects required to detect a clinically meaningful change in PROMIS instrument score defined as 5 points with a standard deviation of 10 (minimally important change has been defined for several PROMIS measures as 3-6 points[32]), was estimated to be 64 subjects per group. Based on the aforementioned calculations, we elected to target a sample size of 150 subjects, which accounts for a 15% lost to follow-up (LTFU) rate.

Data analysis plan

Analysis of feasibility outcomes

Rate of participant enrollment per year, percentages of screening failures, and proportions of completed follow up visits and missing data will be summarized as counts with percentages or means with standard deviations.

Analysis of clinical outcomes

The intervention arm (IVIT) will be compared to the placebo for all prespecified analyses. We will use hemoglobin as a marker for resolution of anemia, as defined as >12g/dL in females and >13.5g/dL in males. Based on previous studies, administration of IVIT improves hemoglobin levels within the first week, and normalization is typically achieved within 3-4 weeks.[1] We anticipate that this will hold true in our IVIT cohort, with resolution of anemia occurring around

3 months for the placebo cohort. We will evaluate for statistical difference of change in hemoglobin at all study visit timepoints with t tests.

We will use PROMIS fatigue, physical function, and depression scores as indicators of important quality of life metrics that relate to recovery from traumatic injury and fracture healing. The aggregated change in PROMIS score will be calculated as a percent change from baseline at all time-points for both measures. An analysis of covariance model (ANCOVA) will be used to access for clinical significance, with treatment (IVIT vs placebo) as factors and baseline score as covariate.

We will perform a multivariate analysis adjusting for the potential confounders of age, sex, BMI, and transfusion status (transfusion versus no transfusion) known to contribute to anemia, fatigue, and depression. Both unadjusted and adjusted results will be reported. Analysis will be performed according to a modified intention-to-treat paradigm in which all patients, except those who are deemed ineligible after randomisation, will be analyzed according to the treatment group to which they are randomly assigned.

Given the investigative nature of pilot studies, we plan to additionally conduct several exploratory analyses to inform the definitive trial analysis. Participants will be placed in subgroups based on transfusion requirements. We will compare patients who received perioperative blood transfusions (whole blood or pRBCs), not exceeding 2 units, to those who do not. These subgroups will be assessed for change in hemoglobin with t tests and analysis of variance (ANOVA). Bivariate linear regression analyses will assess the relationship between patient factors, injury characteristics, as well as recovery parameters (age, sex, post-operative weightbearing status, fracture type, fixation type, length of hospital stay, degree of iron panel derangements, degree of post-operative anemia, and transfusion requirements) and fatigue, physical function, and depression questionnaires PROMIS scores.

We will use multiple-imputation to handle missing data. Pilot studies are exploratory in nature and will be underpowered for clinical outcomes due to sample size, and multiple testing will not be adjusted for. Therefore, all clinical pilot study data should be interpreted as exploratory. Significance level set to 95% for all statistical measures. Statisticians blinded to treatment arms will conduct all analyses in the most updated version of R (R Core Team).

Potential impact of study

Successful completion of this project has the potential to provide relevant clinical information for the development of a large-scale, multicenter randomised trial. Ultimately, a better understanding of the effects of IVIT both clinically and at a biological level may alter our treatment approach of anemia in patients who sustain orthopaedic injuries, thereby leading to decreased risks and improved recovery. If IVIT is proven to be effective in improving quality of life after traumatic lower extremity fracture, clinical relevance to other fracture types and more broadly in orthopaedic surgery will follow. We plan to further evaluate the efficacy of IVIT for reduction of blood transfusion and as an adjunct therapy to blood transfusion in the future.

Patient and Public Involvement

Patients and the public will not be involved in the design, conduct, reporting, or dissemination plans of this research.

ETHICS AND DISSEMINATION

Research ethics approval: This study has ethics approval from the Oregon Health & Science University Institutional Review Board (STUDY00022441). Protocol modifications and annual continuing review will be submitted as necessary for IRB approval prior to implementation and continuation of the study, respectively.

Consent: Informed consent is performed using IRB approved forms with a trained study provider. Patients may freely withdraw their informed consent at any time during the clinical trial.

Confidentiality: All data from this work is maintained in security and confidentiality at our institution. A secure REDCap database (encrypted and password protected) is used for data collection, administration of PROMIS surveys, organization of data reports for statistical analysis, and documentation of adverse events. Research medication management, randomisation, blinding, and related record keeping is performed by RPS per their published protocol.

Dissemination policy: The findings of this study will be disseminated through peer-reviewed publications and conference presentations. This protocol has been reported following the Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) statement.[33] Results will be published following the Consolidated Standards of Reporting Trials (CONSORT) guidelines for pilot and feasibility trials.[34,35] In addition, appropriate publication requirements will be upheld for the use of PROMIS instruments.

Registration details: ClinicalTrials.gov (NCT05292001)

Authors' contributions: DFP and ZMW are co-principle investigators responsible for conceptualization and funding of the study. DFP further designed and drafted the protocol for the study. DMF, GJD, NW, JJS, and JEA contributed to study design as pertinent to their respective specialties, providing specific content and edits to the manuscript. MAS acted as an advisor to the project and edited the manuscript. NSM, CEH, and KL oversee the clinical aspects of the study and CJY oversees the translational laboratory component. NSM additionally provided guidance for planned statistical analysis. All authors have reviewed and approved the manuscript.

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Competing interests: None

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FIGURE LEGEND/CAPTION

Figure 1. Study design flowchart.

Figure 2. Infusion reaction treatment algorithm

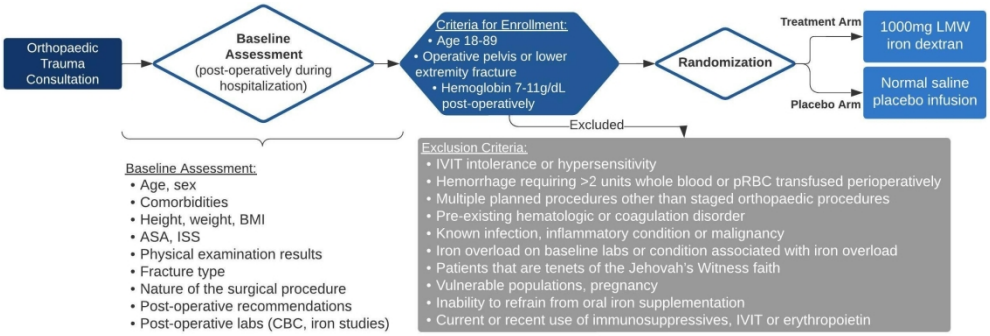

Figure 1. Study design flowchart.

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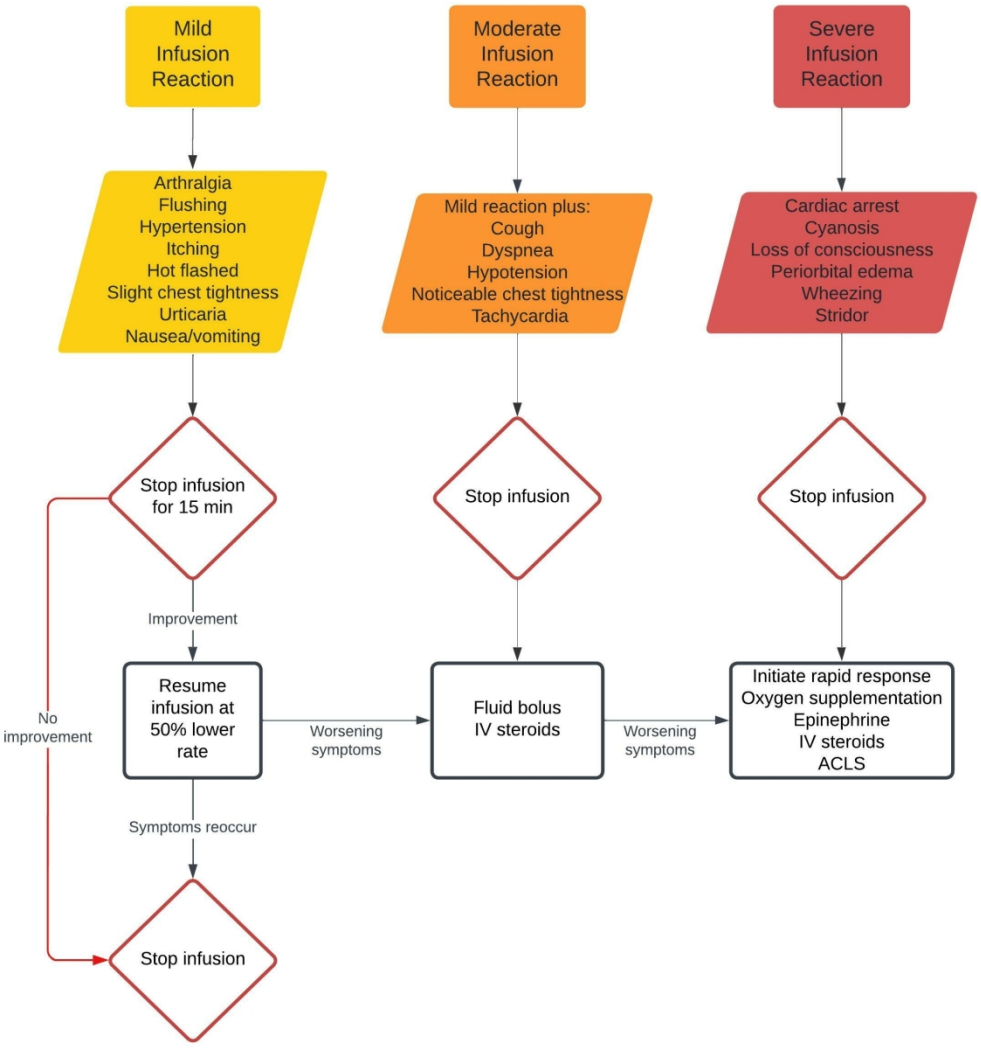

Figure 2. Infusion reaction treatment algorithm

185x195mm (300 x 300 DPI)

Reporting checklist for protocol of a clinical trial.

Based on the SPIRIT guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

Upload your completed checklist as an extra file when you submit to a journal.

In your methods section, say that you used the SPIRITreporting guidelines, and cite them as:

Chan A-W, Tetzlaff JM, Gøtzsche PC, Altman DG, Mann H, Berlin J, Dickersin K, Hróbjartsson A, Schulz KF, Parulekar WR, Krleža-Jerić K, Laupacis A, Moher D. SPIRIT 2013 Explanation and Elaboration: Guidance for protocols of clinical trials. BMJ. 2013;346:e7586

		Reporting Item	Page Number
Administrative information			
Title	#1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	#2a	Trial identifier and registry name. If not yet registered, name of intended registry	2 & 10
Trial registration: data set	#2b	All items from the World Health Organization Trial Registration Data Set	
Protocol version	#3	Date and version identifier	N/A
Funding	#4	Sources and types of financial, material, and other support	10
Roles and responsibilities: contributorship	#5a	Names, affiliations, and roles of protocol contributors	10

Roles and responsibilities: sponsor contact information	#5b	Name and contact information for the trial sponsor	N/A
Roles and responsibilities: sponsor and funder	#5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	N/A
Roles and responsibilities: committees	#5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	N/A
Introduction			
Background and rationale	#6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	3-4
Background and rationale: choice of comparators	#6b	Explanation for choice of comparators	6
Objectives	#7	Specific objectives or hypotheses	4-5
Trial design	#8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, non-inferiority, exploratory)	4-5
Methods: Participants, interventions, and outcomes			
Study setting	#9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	6
Eligibility criteria	#10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will	5

		perform the interventions (eg, surgeons, psychotherapists)	
Interventions: description	#11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	5
Interventions: modifications	#11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving / worsening disease)	8
Interventions: adherance	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)	6
Interventions: concomitant care	#11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	5
Outcomes	#12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	6-7
Participant timeline	#13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	8
Sample size	#14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	9
Recruitment	#15	Strategies for achieving adequate participant enrolment to reach target sample size	6
Methods: Assignment of interventions (for controlled trials)			
Allocation: sequence generation	#16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	6

Allocation concealment mechanism	#16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	6
Allocation: implementation	#16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	6
Blinding (masking)	#17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	6
Blinding (masking): emergency unblinding	#17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	6
Methods: Data collection, management, and analysis			
Data collection plan	#18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	7 & 10
Data collection plan: retention	#18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	8
Data management	#19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	10
Statistics: outcomes	#20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	9
Statistics: additional analyses	#20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	9

Statistics: analysis population and missing data	#20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	9
Methods: Monitoring			
Data monitoring: formal committee	#21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	6
Data monitoring: interim analysis	#21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	N/A
Harms	#22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	8
Auditing	#23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	N/A
Ethics and dissemination			
Research ethics approval	#24	Plans for seeking research ethics committee / institutional review board (REC / IRB) approval	10
Protocol amendments	#25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC / IRBs, trial participants, trial registries, journals, regulators)	10
Consent or assent	#26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	10
Consent or assent: ancillary studies	#26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	N/A
Confidentiality	#27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to	10

		protect confidentiality before, during, and after the trial	
Declaration of interests	#28	Financial and other competing interests for principal investigators for the overall trial and each study site	10
Data access	#29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	10
Ancillary and post trial care	#30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	N/A
Dissemination policy: trial results	#31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	10
Dissemination policy: authorship	#31b	Authorship eligibility guidelines and any intended use of professional writers	10
Dissemination policy: reproducible research	#31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	N/A
Appendices			
Informed consent materials	#32	Model consent form and other related documentation given to participants and authorised surrogates	Uploaded
Biological specimens	#33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	N/A

BMJ Open

Role of single-dose intravenous iron therapy for the treatment of anemia after orthopaedic trauma: protocol for a pilot randomised controlled trial.

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2022-069070.R2
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Role of single-dose intravenous iron therapy for the treatment of anemia after orthopaedic trauma: protocol for a pilot randomised controlled trial.

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ABSTRACT

Introduction: Orthopaedic trauma and fracture care commonly cause perioperative anemia and associated functional iron deficiency due to a systemic inflammatory state. Modern, strict transfusion thresholds leave many patients anemic; managing this perioperative anemia is an opportunity to impact outcomes in orthopaedic trauma surgery. The primary outcome of this pilot study is feasibility for a large RCT to evaluate intravenous iron therapy (IVIT) to improve patient well-being following orthopaedic injury. Measurements will include rate of participant enrollment, screening failure, follow up, missing data, adverse events, and protocol deviation.

Methods and analysis: This single center, pilot, double-blind randomised controlled trial (RCT) investigates the use of IVIT for acute blood loss anemia in traumatically injured orthopaedic patients. Patients are randomised to receive either a single dose infusion of low molecular weight iron dextran (1000mg) or placebo (normal saline) post-operatively during their hospital stay for trauma management. Eligible subjects include adult patients admitted for lower extremity or pelvis operative fracture care with a hemoglobin of 7-11g/dL within seven days post-operatively during inpatient care. Exclusion criteria include history of intolerance to IV iron supplementation, active hemorrhage requiring ongoing blood product resuscitation, multiple planned procedures, pre-existing hematologic disorders or chronic inflammatory states, iron overload on screening, or vulnerable populations. We follow patients for three months to measure the effect of iron supplementation on clinical outcomes (resolution of anemia and functional iron deficiency), patient reported outcomes (fatigue, physical function, depression, and quality of life), and translational measures of immune cell function.

Ethics and dissemination: This study has ethics approval (Oregon Health & Science University Institutional Review Board, STUDY00022441). We will disseminate the findings through peer-reviewed publications and conference presentations.

Registration details: ClinicalTrials.gov (NCT05292001)

Strengths and limitations of this study

- Our study is a single center, pilot, double-blind randomised controlled trial investigating the use of IVIT for acute blood loss anemia in injured orthopaedic patients
- Use of a single high dose infusion of low molecular weight iron dextran is a safe method of body iron store repletion that optimizes study design logistics, patient capture, cost and efficacy of iron delivery.
- We aim to assess the feasibility for a future large scale randomised controlled trial evaluating IVIT as a means to improve time to anemia and functional iron deficiency resolution, as well as standardized patient reported quality of life indices (PROMIS fatigue, physical function, depression).

INTRODUCTION

Background and rationale: Clinical

Acute blood loss in orthopaedic trauma and operative fracture care contributes substantially to perioperative anemia and functional iron deficiency. The prevalence of preoperative anemia has been observed as up to 50% in patients undergoing surgical stabilization for hip fractures[1,2] and, unsurprisingly, up to 87% following hip fracture fixation.[2] Perioperative anemia is associated with increased hospital length of stay (LOS), need for blood transfusion, risk of surgical site infection (SSI), genitourinary and cardiovascular complications, and death.[1,2] Additionally, anemia has clinical implications in quality of life (QOL) measures and is associated with fatigue, impaired physical performance, decreased exercise capacity, and mood disturbances.[3–5] The broad impact of anemia is often underestimated by clinicians; treatment may have profound benefits to patients' well-being.[4] Therefore, evaluation and treatment of perioperative anemia is critical to improving outcomes in orthopaedic surgery.

The standard of care for perioperative anemia in orthopaedic trauma is packed red blood cell (pRBC) transfusion; however, this has been associated with increased mortality, nosocomial infection, postoperative venous thromboembolism, multi-system organ dysfunction, and acute respiratory distress syndrome.[1,6,7] Current recommendations for the treatment of anemia in orthopaedic trauma center upon a restrictive strategy for management (i.e., hemoglobin <7g/dL for pRBC transfusion initiation) to minimize transfusion reactions while ignoring the long term recovery effects of anemia. A safer alternative to pRBCs is desirable because a critical number of patients do not meet this restrictive transfusion threshold and may be negatively impacted by anemia during recovery.

Currently, there is no standard practice of iron supplementation for treatment of anemia in the acute trauma setting. Previous studies have shown promising results for the use of intravenous iron therapy (IVIT) in orthopaedic patients. A recent meta-analysis by Shin et al. concluded that use of IVIT perioperatively in orthopaedic surgery significantly decreased the proportion of patients receiving packed red blood cell (pRBC) transfusions by 31%, shortened hospital stay by 1.6 days, and reduced post-operative infection rate by 33%.[6] Serrano-Trenas et al. demonstrated IV iron sucrose therapy reduced transfusion requirements in a subset of geriatric hip fracture patients without differences between groups for morbidity, mortality, or LOS.[8] Ten percent of the patients enrolled in the studied died prior to their post-hospitalization check-up. Additionally, only 16% of patients in the investigational arm received the three full doses of IVIT sufficient to replete body iron stores. Shortcomings in study design and restrictive study population limit the utility of the findings in this study. Non-orthopaedic studies suggest improved patient-reported outcomes with IVIT after hemorrhagic events.[3,9] Unfortunately, there are a lack of high-quality randomised controlled trials (RCT) in the orthopaedic literature investigating the routine use of IVIT. Further, no studies within orthopaedics have looked at the effect of IVIT on patient reported quality of life outcomes.

Background and rationale: Translational

Our preliminary analysis of iron store derangements following a traumatic event have prompted us to simultaneously investigate the underlying pathophysiology of anemia during the inflammatory conditions of trauma and surgery. We have found that less than 5% of patients

demonstrated normal serum iron, total iron binding capacity, transferrin saturation and transferrin values following orthopaedic fracture care, with abnormally low values in some or all of these assessments being seen in the vast majority of patients. Despite this, ferritin level is normal in approximately two-thirds of patients overall and normal or high in nearly all patients with all other iron studies low.[10] This phenomenon may indicate that iron becomes sequestered and unavailable for use in replenishing blood cell volume in the setting of orthopaedic trauma, leading to a state of functional iron deficiency.

The consequence of functional iron deficiency, in which insufficient iron is available for erythropoiesis despite normal iron stores in bone marrow macrophages[11,12] has not been fully investigated in the trauma and orthopedic settings. Functional iron deficiency results from two main pathways – conditions that incite a systemic inflammatory response (i.e. surgery, trauma)[6,13] and situations of increased erythropoiesis mediated by endogenous or exogenous erythropoietin stimulation.[13] In the former, there is a hepcidin mediated down-regulation of intestinal iron absorption and impaired mobilization of body iron stores.[14] In the latter, there is a mismatch between iron demand and supply as in the setting of acute blood loss.[13] Both of these clinical scenarios play a role in the setting of orthopaedic trauma requiring operative surgical stabilization.

We aim to evaluate the consequence of IVIT on immune cell physiology given the connection of functional iron deficiency to pro-inflammatory states. The general effect of these cells on bone regeneration in the setting of fracture are three-fold; they promote migration and proliferation of osteogenic cells, increase blood vessel formation, and induce inflammatory reactions.[15,16] During fracture repair, multiple immune cell types work in harmony to modulate healing, including those of myeloid origin (neutrophils, macrophages, osteoclasts) and lymphoid origin (T- and B-lymphocytes, natural killer cells).[16] Further work must be done to understand the biological significance of immune cells and their regulatory factors in bone regeneration, as well as potential areas for modulation.

Platelets are of particular interest to investigate as they not only affect wound healing, but also play a critical role in surgical hemostasis. Platelet production is known to be intimately linked with iron stores, as iron deficiency often causes increased platelet counts, however the role of iron in platelet function remains unclear. While platelet numbers increase in anemia, platelet response to inflammation, trauma and conditions with excessive bleeding are more complex – where platelet activities cause increased clotting as well as exacerbate bleeding.[17] Low but persistent levels of platelet stimulation in inflammation and trauma can cause a dulling of platelet activity (i.e. platelet exhaustion).[17,18] Specific mechanisms of platelet dysfunction under these conditions remain largely unspecified.[19–21]

Recent work by our multidisciplinary team has identified several clinically relevant physiologic changes of platelets in iron deficient premenopausal women which are reversed with IVIT. Preliminary findings demonstrated (1) IV iron repletion decreases platelet count in iron deficiency, (2) iron repletion significantly increases platelet integrin activation and alpha-granule secretion in response to ADP and collagen related peptide, and (3) platelet adhesion to type-1 collagen is enhanced after IVIT.[22] This suggests that iron is vital for optimal platelet function

and hemostasis. We seek to understand the alterations IVIT has on similar platelet profiles in injured patients, which has not previously been studied.

Objectives and study hypothesis

The primary objective of this study is to determine feasibility of study design, recruitment, randomisation, intervention implementation, blinded procedures, and follow-up. Feasibility outcomes will be quantified as rate of participant enrollment (60 patients randomised in 2 years), proportion of participants completing each follow up visit, proportion of missing data, rate of transfusion reactions, and rate of protocol adherence. The primary clinical outcome is patient reported quality of life measures of fatigue on PROMIS questionnaire. The central hypothesis motivating the research is that acute blood loss anemia may be one modifiable risk factor which can be addressed with IVIT to improve patient well-being following traumatic orthopaedic injury. The secondary objectives of the study include:

(I) measure the time to return to normal hemoglobin as a marker for resolution of anemia and normalization of body iron stores as a marker for resolution of functional iron deficiency following orthopaedic fracture care

(II) evaluate the effect of IVIT on patient reported quality of life measures of physical function and depression following traumatic orthopaedic injury through standardized PROMIS questionnaires

(III) appraise cost effectiveness of IVIT with a cost-utility analysis using quality-adjusted life-years (QALYs)

(IV) determine the role of IVIT on immune cell physiology in the setting of acute blood loss anemia and inflammation from orthopaedic trauma

METHODS AND ANALYSIS

Overview of study design

This is a single center, double-blind parallel design randomised controlled trial (RCT) investigating the use of IVIT (N=30) compared to placebo (N=30) for acute blood loss anemia in traumatically injured orthopaedic patients. The intervention consists of a single dose infusion of low molecular weight iron dextran (1000mg LMW ID) post-operatively during the patient's hospital stay for initial trauma management. Patients in the placebo arm are given an equal volume normal saline infusion (Figure 1). Both the investigator and participants are blinded to the study treatment administered.

Eligibility criteria

Inclusion criteria

1. Patients age 18-89 admitted with a lower extremity or pelvis fracture requiring surgical stabilization
2. Acute blood loss anemia as defined by hemoglobin concentration between 7.0 – 11.0g/dL within seven days post-operatively from definitive fracture stabilization during the hospital admission

Exclusion criteria

Patients who meet any one or more of the following will be excluded from the study:

1. History of intolerance or hypersensitivity to IV iron supplementation

2. Active hemorrhage requiring greater than two units (whole blood or pRBCs) transfused perioperatively
3. Multiple planned operative procedures during the trauma admission, excluding orthopaedic staged procedures for the fracture meeting inclusion criterion one (such as temporizing external fixator application and washout for open fracture) in which subjects otherwise meet qualifications for enrollment after definitive stabilization
4. Pre-existing hematologic or coagulation disorder (e.g., thalassemia, sickle cell disease, hemophilia, von Willibrand's disease, or myeloproliferative disease)
5. Diagnosis of chronic kidney disease and/or chronic liver disease
6. Known infection, inflammatory condition (e.g., systemic lupus erythematosus, rheumatoid arthritis, and ankylosing spondylitis), or malignancy
7. Pregnancy
8. Iron overload (defined as serum ferritin concentration ≥ 1,000ng/mL, serum iron concentration > 160μg/ dL, or serum transferrin saturation ≥ 50%) or any condition associated with iron overload (e.g., hemochromatosis and aceruloplasminemia)
9. Patients that are tenets of the Jehovah's Witness faith
10. Vulnerable populations including pediatric patients, geriatric populations 90 or older, incarcerated individuals, those unable to provide informed consent
11. Inability to refrain from oral iron supplementation during study period
12. Current or recent (within 30 days) use of immunosuppressive agents
13. Use of any intravenous iron therapy or recombinant human erythropoietin formulation within the previous 30 days

Study intervention and blinding

The study intervention consists of a single dose infusion of low molecular weight iron dextran (1000mg LMW ID). Patients in the placebo arm are given an equal volume normal saline infusion (250mL NS). Given the dark colour of the LMW ID compared to the clear, translucent normal saline, opaque bags and tube covers will be utilized in order to blind all study staff and patients to the allocated treatment group. The blinding covers will be applied by an unblinded pharmacist who prepares the infusion and remain in place during transport, infusion, and disposal of the infusion packaging.

LMW ID was chosen over IV iron alternatives to optimize logistics, patient capture, cost and efficacy of iron delivery. LMW ID has a stable formulation for safe administration of sufficient iron for repletion of body iron stores in a single high dose infusion over one hour, as compared to alternative regimens that require several small dose administrations over the course of days to weeks.[23,24] Use of this iron formulation improves upon prior RCTs with incomplete adherence when utilizing multiple infusions of alternative low-dose regimens. Importantly, the Food and Drug Administration (FDA) has recognized the incidence of life-threatening adverse effects and deaths associated with IVIT (2.2 per million doses and 0.4 per million doses, respectively) is significantly lower than that associated with blood transfusion (10 per million units and 4 per million units, respectively).[14] Oral iron supplementation is an alternative to IVIT, but is associated with increased risk of adverse reactions,[23] poor medication adherence,[13,23] lower efficacy,[13] and limited use in settings where rapid iron repletion is required.[13,14] IVIT is preferred when rapid, significant correction is necessary as it has higher efficiency and shorter time to improvement.[14,25,26]

Research Pharmacy Services (RPS) at our institution is responsible for all study drug related tasks including randomisation and blinding of the study drug and placebo. RPS follows a published protocol for drug shipment/receipt, packaging, storage, preparation, dispensing and accountability, and administration. Consent will be required from the patient or a legally authorized representative. Unblinding will be considered in emergency situations (i.e. severe infusion reaction). Verbal permission from the principle investigator or co-investigator will suffice in order to unblind, followed by subsequent written documentation after the unblinding has occurred. Drug destruction will be performed by RPS at the study drug expiration date or the completion of the study. Given the single dose design of the study drug, medication compliance assessment will not be required; however, it will be documented if the treatment had to be discontinued prior to completion of infusion due to adverse reaction.

Recruitment

Our institution is a level one, tertiary care center with high volume fracture care and over 4000 trauma activations yearly. Our recruitment pool consists of all patients admitted with orthopaedic trauma during the enrollment period, planned June 2022 through May 2024. Patients are eligible for enrollment if they meet the aforementioned criteria within seven days post-operatively from definitive surgical stabilization of their fracture. Screening includes review of laboratory studies, injuries, and comorbidities to assess for inclusion.

Allocation of patients to study groups

Following informed consent, patients are randomised one-to-one into a treatment arm by RPS and receive the allocated therapy via a computer-generated random number schema from randomization.com. RPS is responsible for all blinding procedures. Medication related study documents are stored in an electronic pharmacy binder on Vestigo only accessible by unblinded personnel. The study medication is stored with restricted access in the hospital inpatient pharmacy and prepared, delivered, labeled, and covered with blinding bags and tubing covers by the unblinded pharmacy personnel upon subject enrollment to ensure that both the investigators and subjects are blinded to the treatment received.

Outcome measures

Feasibility outcome measures

Appraisal of feasibility will be based on rate of participant enrollment per year, rate of screening failures, proportion of participants completing each follow up visit, proportion of missing data, rate of transfusion reactions, and rate of protocol adherence. Other feasibility concerns will be qualitative in nature, including documentation of blinding failures, review of challenges in recruitment and retention, and assessment of data management and survey administration.

Primary clinical outcome

The primary clinical outcome of this pilot study will be Health Related Quality of Life (HRQoL) over the 3 months postoperatively. HRQoL will be assessed using the PROMIS Fatigue Questionnaire, a computer adaptive survey (Table 1). This will measure feelings of tiredness likely to decrease one's ability to execute daily activities and function normally in family or social roles.

Secondary clinical outcomes

Outcome measures to fulfill the secondary objectives will be collected to assess the feasibility of their collection and relevance of timing in anticipation of a future large-scale RCT.

Laboratory data

- *Complete blood count.* Concentration of hemoglobin (oxygen carrying protein) in whole blood and percentage of blood volume (hematocrit) occupied by RBCs are of primary interest. These are markers of anemia (defined as hemoglobin <12g/dL in females and <13.5g/dL in males) measured for inclusion assessment and to monitor for resolution of anemia at all study follow-up visits.
- *Ferritin.* Evaluated at enrollment to assess for iron overload (patients with a ferritin level ≥ 1,000ng/mL will be excluded) and tracked throughout the study to measure participants' body stores of iron. Importantly, patients will not be required to have a ferritin level consistent with iron deficiency (≤ 50 ng/mL) as we have observed that the majority of patients have normal (51-200 ng/mL) to high (>200 ng/mL) post-traumatic ferritin levels
- *Additional iron studies (Serum iron, transferrin, total iron binding capacity).* Additional indicators of body iron stores and iron carrying capacity within blood. Utilized to further define patients' anemia and iron available for functional use. Similar to ferritin level, only patients with iron values consistent with overload on post-operative laboratory work will be excluded (as defined by exclusion criterion 8).

Quality of life measures

- *PROMIS Fatigue Questionnaire.* Computer adaptive survey administered via REDCap to evaluate feelings of tiredness likely to decrease one's ability to execute daily activities and function normally in family or social roles.
- *PROMIS Physical Function Questionnaire.* Computer adaptive survey administered via REDCap to measure self-reported capability to perform physical activities including activities of daily living.
- *PROMIS Depression Questionnaire.* Computer adaptive survey administered via REDCap to assess negative mood, views of self, social cognition, and decreased social engagement.
- *EQ-5D-5L Quality of Life Questionnaire.* Instrument assesses HRQoL with five dimensions of health, each with five levels ranging from no problem (Level 1) to extreme problem (Level 5). Answers correspond to 3,125 possible health states that can be converted into a single 'utility' score. This will be utilized for the assessment of quality-adjusted life years (QALYs) and cost effectiveness of IVIT for the treatment of acute blood loss anemia following surgical fracture stabilization.

Immune cell functional testing will be performed through a variety of novel laboratory techniques, including but not limited to the following

- Flow cytometry and Fluorescence-Activated Cell Sorting (FACS) to quantify and evaluate platelets, cytokines and other immune cells.[27]
- Assessment of platelet aggregation under venous shear in chambers coated with type I collagen.

- Use of proteomics tools to systematically measure the molecular composition of immune cells as well as the activation of signaling systems in response to relevant agonists.[28]
- Analysis of immune cells, biomarkers, and relevant circulating factors using Luminex technology and ELISA.

Participant timeline

Table 1 delineates the schedule for enrollment, interventions, laboratory studies and patient reported outcome surveys.

Table 1. Schedule of enrolment, study drug allocation, quality of life and laboratory assessments.

	Enrollment	**Allocation**	**Follow-up**			
Time point	POD1 through POD7 during hospitalization		2 weeks	4 weeks	6 weeks	3 months
Screening/Enrollment						
Eligibility screen	x					
Informed consent	x					
Randomisation		x				
Allocation of study drug vs placebo		x				
Assessments						
PROMIS Fatigue	x		x	x	x	x
PROMIS Physical Function	x		x	x	x	x
PROMIS Depression	x		x	x	x	x
EQ-5D-5L	x		x	x	x	x
Laboratory studies						
CBC	x		x	x	x	x
Ferritin	x		x	x	x	x
Iron, TIBC, transferrin, %sat	x					x
Immune cell studies	x		x	x	x	x

POD1 = post-operative day one; TIBC = total iron binding capacity; %Sat = transferrin saturation

Safety considerations

Adverse events are documented in a secure REDCap database, including description of the symptoms, management provided, and outcome. Adverse events are categorized as mild, moderate and severe in relation to the infusion itself as described hereafter. Patients are additionally monitored at all follow up visits for other complications in their care (not necessarily related to study drug administration) including fracture related infection (FRI), non-union, and need for post-infusion pRBC transfusion per clinical threshold criteria. Infections will be determined using the criteria for fracture-related infection (FRI) as validated by Metsemakers et al.[29]

Serious adverse events including severe infusion reactions (e.g., cardiac arrest, cyanosis, loss of consciousness, periorbital edema, wheezing, stridor) will be reported as required by the IRB. Management of such events will include stopping the infusion, activating the rapid response team, oxygen supplementation, epinephrine, IV steroids, and initiating ACLS (if necessary).

Other infusion related reactions are documented and managed as described by DeLoughery and Rampton et al. (Figure 2).[23,30] Hypersensitivity medications are ordered with the study

medication per our standard institution order set, including diphenhydramine, famotidine, hydrocortisone sodium succinate injection, epinephrine IM, and normal saline bolus.

Procedures for completion

Completion occurs at the last follow-up visit when all patient reported outcome measures and laboratory data have been collected. In an effort to optimize retention, PROMIS surveys will be emailed to study participants via REDCap (which has pre-built computer adaptive testing for the chosen instruments) at the appropriate follow-up timepoints. These may be completed upon email receipt or during scheduled study visits. Therefore, patient report outcomes may still be completed virtually in the event patients are otherwise unable to complete in person follow-up visits. Patients may freely withdraw their informed consent at any time during the clinical trial. Further, the investigator may terminate a subject's participation in the research study if they are found to have any of the exclusion criteria during the study period (including use of oral iron supplements, new malignancy, or newly diagnosed inflammatory disease) with the exception of post-operative infection. Subjects are considered lost to follow-up if they do not attend scheduled study visits or complete study surveys.

Sample size consideration

The primary objective of this study is to pilot for feasibility; therefore, traditional quantitative sample size calculations are not well suited for this study. Given the exploratory nature of pilot studies, we plan to enroll a sample of 60 patients to assess the feasibility of a definitive large RCT.

Based on prior studies, a significant increase from baseline hemoglobin of 1.2 g/dL ± 1.4 was observed within a median follow-up time of three weeks after administration of LMW ID.[31,32] The minimum number of subjects required to detect a clinically meaningful change in PROMIS instrument score defined as 5 points with a standard deviation of 10 (minimally important change has been defined for several PROMIS measures as 3-6 points[33]). However, the results of this pilot study will ultimately inform sample size requirements in a larger scale RCT.

Data analysis plan

Analysis of feasibility outcomes

Rate of participant enrollment per year, percentages of screening failures, and proportions of completed follow up visits and missing data will be summarized as counts with percentages or means with standard deviations.

Analysis of clinical outcomes

The intervention arm (IVIT) will be compared to the placebo for all prespecified analyses. We will use hemoglobin as a marker for resolution of anemia, as defined as >12g/dL in females and >13.5g/dL in males. Based on previous studies, administration of IVIT improves hemoglobin levels within the first week, and normalization is typically achieved within 3-4 weeks.[1] We anticipate that this will hold true in our IVIT cohort, with resolution of anemia occurring around 3 months for the placebo cohort. We will evaluate for statistical difference of change in hemoglobin at all study visit timepoints with t tests.

We will use PROMIS fatigue, physical function, and depression scores as indicators of important quality of life metrics that relate to recovery from traumatic injury and fracture healing. The aggregated change in PROMIS score will be calculated as a percent change from baseline at all time-points for both measures. An analysis of covariance model (ANCOVA) will be used to access for clinical significance, with treatment (IVIT vs placebo) as factors and baseline score as covariate.

We will perform a multivariate analysis adjusting for the potential confounders of age, sex, BMI, and transfusion status (transfusion versus no transfusion) known to contribute to anemia, fatigue, and depression. Both unadjusted and adjusted results will be reported. Analysis will be performed according to a modified intention-to-treat paradigm in which all patients, except those who are deemed ineligible after randomisation, will be analyzed according to the treatment group to which they are randomly assigned.

Given the investigative nature of pilot studies, we plan to additionally conduct several exploratory analyses to inform the definitive trial analysis. Participants will be placed in subgroups based on transfusion requirements. We will compare patients who received perioperative blood transfusions (whole blood or pRBCs), not exceeding 2 units, to those who do not. These subgroups will be assessed for change in hemoglobin with t tests and analysis of variance (ANOVA). Bivariate linear regression analyses will assess the relationship between patient factors, injury characteristics, as well as recovery parameters (age, sex, post-operative weightbearing status, fracture type, fixation type, length of hospital stay, degree of iron panel derangements, degree of post-operative anemia, and transfusion requirements) and fatigue, physical function, and depression questionnaires PROMIS scores.

We will use multiple-imputation to handle missing data. Pilot studies are exploratory in nature and will be underpowered for clinical outcomes due to sample size, and multiple testing will not be adjusted for. Therefore, all clinical pilot study data should be interpreted as exploratory. Significance level set to 95% for all statistical measures. Statisticians blinded to treatment arms will conduct all analyses in the most updated version of R (R Core Team).

Potential impact of study

Successful completion of this project has the potential to provide relevant clinical information for the development of a large-scale, multicenter randomised trial. Ultimately, a better understanding of the effects of IVIT both clinically and at a biological level may alter our treatment approach of anemia in patients who sustain orthopaedic injuries, thereby leading to decreased risks and improved recovery. If IVIT is proven to be effective in improving quality of life after traumatic lower extremity fracture, clinical relevance to other fracture types and more broadly in orthopaedic surgery will follow. We plan to further evaluate the efficacy of IVIT for reduction of blood transfusion and as an adjunct therapy to blood transfusion in the future.

Patient and Public Involvement

Patients and the public will not be involved in the design, conduct, reporting, or dissemination plans of this pilot research protocol. Patient feedback from participation in this study will be considered upon finalizing a definitive large-scale study.

ETHICS AND DISSEMINATION

Research ethics approval: This study has ethics approval from the Oregon Health & Science University Institutional Review Board (STUDY00022441). Protocol modifications and annual continuing review will be submitted as necessary for IRB approval prior to implementation and continuation of the study, respectively.

Consent: Informed consent is performed using IRB approved forms with a trained study provider. Patients may freely withdraw their informed consent at any time during the clinical trial.

Confidentiality: All data from this work is maintained in security and confidentiality at our institution. A secure REDCap database (encrypted and password protected) is used for data collection, administration of PROMIS surveys, organization of data reports for statistical analysis, and documentation of adverse events. Research medication management, randomisation, blinding, and related record keeping is performed by RPS per their published protocol.

Dissemination policy: The findings of this study will be disseminated through peer-reviewed publications and conference presentations. This protocol has been reported following the Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) statement.[34] Results will be published following the Consolidated Standards of Reporting Trials (CONSORT) guidelines for pilot and feasibility trials.[35,36] In addition, appropriate publication requirements will be upheld for the use of PROMIS instruments.

Registration details: ClinicalTrials.gov (NCT05292001)

Authors' contributions: DFP and ZMW are co-principle investigators responsible for conceptualization and funding of the study. DFP further designed and drafted the protocol for the study. DMF, GJD, NW, JJS, and JEA contributed to study design as pertinent to their respective specialties, providing specific content and edits to the manuscript. MAS acted as an advisor to the project and edited the manuscript. NSM, CEH, and KL oversee the clinical aspects of the study and CJY oversees the translational laboratory component. NSM additionally provided guidance for planned statistical analysis. All authors have reviewed and approved the manuscript.

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FIGURE LEGEND/CAPTION

Figure 1. Study design flowchart.

Figure 2. Infusion reaction treatment algorithm

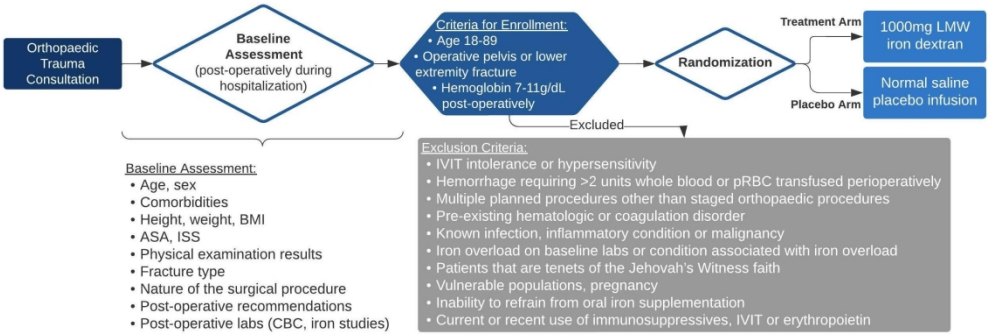

Figure 1. Study design flowchart.

214x72mm (300 x 300 DPI)

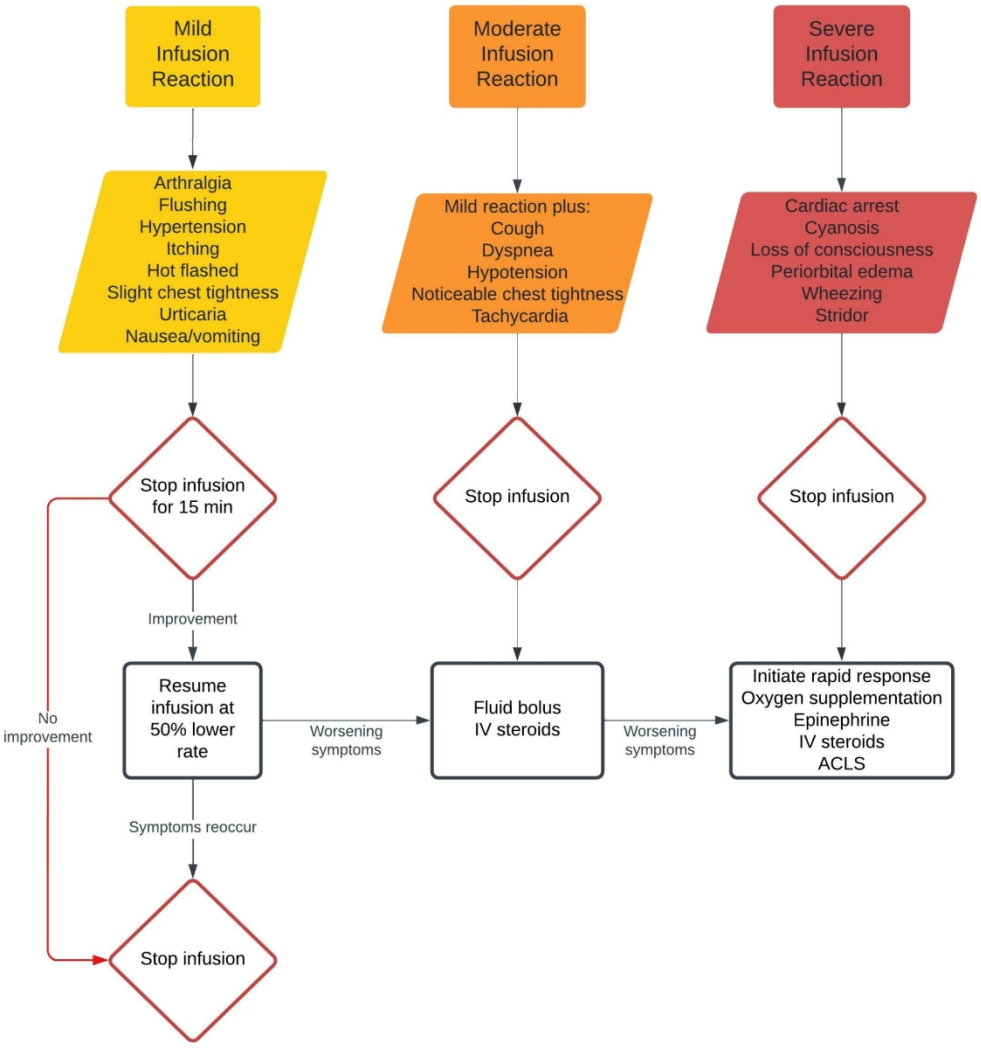

Figure 2. Infusion reaction treatment algorithm

185x195mm (300 x 300 DPI)

Reporting checklist for protocol of a clinical trial.

Based on the SPIRIT guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

Upload your completed checklist as an extra file when you submit to a journal.

In your methods section, say that you used the SPIRITreporting guidelines, and cite them as:

Chan A-W, Tetzlaff JM, Gøtzsche PC, Altman DG, Mann H, Berlin J, Dickersin K, Hróbjartsson A, Schulz KF, Parulekar WR, Krleža-Jerić K, Laupacis A, Moher D. SPIRIT 2013 Explanation and Elaboration: Guidance for protocols of clinical trials. BMJ. 2013;346:e7586

		Reporting Item	Page Number
Administrative information			
Title	#1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	#2a	Trial identifier and registry name. If not yet registered, name of intended registry	2 & 10
Trial registration: data set	#2b	All items from the World Health Organization Trial Registration Data Set	
Protocol version	#3	Date and version identifier	N/A
Funding	#4	Sources and types of financial, material, and other support	10
Roles and responsibilities: contributorship	#5a	Names, affiliations, and roles of protocol contributors	10

Roles and responsibilities: sponsor contact information	#5b	Name and contact information for the trial sponsor	N/A
Roles and responsibilities: sponsor and funder	#5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	N/A
Roles and responsibilities: committees	#5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	N/A
Introduction			
Background and rationale	#6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	3-4
Background and rationale: choice of comparators	#6b	Explanation for choice of comparators	6
Objectives	#7	Specific objectives or hypotheses	4-5
Trial design	#8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, non-inferiority, exploratory)	4-5
Methods: Participants, interventions, and outcomes			
Study setting	#9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	6
Eligibility criteria	#10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will	5

		perform the interventions (eg, surgeons, psychotherapists)	
Interventions: description	#11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	5
Interventions: modifications	#11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving / worsening disease)	8
Interventions: adherance	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)	6
Interventions: concomitant care	#11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	5
Outcomes	#12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	6-7
Participant timeline	#13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	8
Sample size	#14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	9
Recruitment	#15	Strategies for achieving adequate participant enrolment to reach target sample size	6
Methods: Assignment of interventions (for controlled trials)			
Allocation: sequence generation	#16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	6

Allocation concealment mechanism	#16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	6
Allocation: implementation	#16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	6
Blinding (masking)	#17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	6
Blinding (masking): emergency unblinding	#17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	6
Methods: Data collection, management, and analysis			
Data collection plan	#18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	7 & 10
Data collection plan: retention	#18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	8
Data management	#19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	10
Statistics: outcomes	#20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	9
Statistics: additional analyses	#20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	9

Statistics: analysis population and missing data	#20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	9
Methods: Monitoring			
Data monitoring: formal committee	#21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	6
Data monitoring: interim analysis	#21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	N/A
Harms	#22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	8
Auditing	#23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	N/A
Ethics and dissemination			
Research ethics approval	#24	Plans for seeking research ethics committee / institutional review board (REC / IRB) approval	10
Protocol amendments	#25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC / IRBs, trial participants, trial registries, journals, regulators)	10
Consent or assent	#26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	10
Consent or assent: ancillary studies	#26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	N/A
Confidentiality	#27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to	10

		protect confidentiality before, during, and after the trial	
Declaration of interests	#28	Financial and other competing interests for principal investigators for the overall trial and each study site	10
Data access	#29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	10
Ancillary and post trial care	#30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	N/A
Dissemination policy: trial results	#31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	10
Dissemination policy: authorship	#31b	Authorship eligibility guidelines and any intended use of professional writers	10
Dissemination policy: reproducible research	#31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	N/A
Appendices			
Informed consent materials	#32	Model consent form and other related documentation given to participants and authorised surrogates	Uploaded
Biological specimens	#33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	N/A