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Published in:
BMJ Open

DOI:
[10.1136/bmjopen-2022-065423](https://doi.org/10.1136/bmjopen-2022-065423)

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
2023

[Link to publication in University of Groningen/UMCG research database](#)

Citation for published version (APA):

Vinke, J. S. J., Eisenga, M. F., Sanders, J. S. F., Berger, S. P., Spikman, J. M., Abdulahad, W. H., Bakker, S. J. L., Gaillard, C. A. J. M., Van Zuilen, A. D., van der Meer, P., & de Borst, M. H. (2023). Effect of Intravenous Ferric Carboxymaltose on Exercise Capacity After Kidney Transplantation (EFFECT-KTx): rationale and study protocol for a double-blind, randomised, placebo-controlled trial. *BMJ Open*, *13*(3), Article e065423. <https://doi.org/10.1136/bmjopen-2022-065423>

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BMJ Open Effect of Intravenous Ferric Carboxymaltose on Exercise Capacity After Kidney Transplantation (EFFECT-KTx): rationale and study protocol for a double-blind, randomised, placebo-controlled trial

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To cite: Vinke JSJ, Eisenga MF, Sanders J-SF, *et al.* Effect of Intravenous Ferric Carboxymaltose on Exercise Capacity After Kidney Transplantation (EFFECT-KTx): rationale and study protocol for a double-blind, randomised, placebo-controlled trial. *BMJ Open* 2023;**13**:e065423. doi:10.1136/bmjopen-2022-065423

► Prepublication history for this paper is available online. To view these files, please visit the journal online (<http://dx.doi.org/10.1136/bmjopen-2022-065423>).

Received 04 June 2022
Accepted 07 March 2023



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ABSTRACT

Introduction Iron deficiency (ID) is common and has been associated with an excess mortality risk in kidney transplant recipients (KTRs). In patients with chronic heart failure and ID, intravenous iron improves exercise capacity and quality of life. Whether these beneficial effects also occur in KTRs is unknown. The main objective of this trial is to address whether intravenous iron improves exercise tolerance in iron-deficient KTRs.

Methods and analysis The Effect of Ferric Carboxymaltose on Exercise Capacity after Kidney Transplantation study is a multicentre, double-blind, randomised, placebo-controlled clinical trial that will include 158 iron-deficient KTRs. ID is defined as plasma ferritin <100 µg/L or plasma ferritin 100–299 µg/L with transferrin saturation <20%. Patients are randomised to receive 10 mL of ferric carboxymaltose (50 mg Fe³⁺/mL, intravenously) or placebo (0.9% sodium chloride solution) every 6 weeks, four dosages in total. The primary endpoint is change in exercise capacity, as quantified by the 6 min walk test, between the first study visit and the end of follow-up, 24 weeks later. Secondary endpoints include changes in haemoglobin levels and iron status, quality of life, systolic and diastolic heart function, skeletal muscle strength, bone and mineral parameters, neurocognitive function and safety endpoints. Tertiary (explorative) outcomes are changes in gut microbiota and lymphocyte proliferation and function.

Ethics and dissemination The protocol of this study has been approved by the medical ethical committee of the University Medical Centre Groningen (METc 2018/482;) and is being conducted in accordance with the principles of the Declaration of Helsinki, the Standard Protocol Items: Recommendations for Interventional Trials checklist and the Good Clinical Practice guidelines provided by the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use. Study results will be disseminated through publications in peer-reviewed journals and conference presentations.

STRENGTHS AND LIMITATIONS OF THIS STUDY

- ⇒ This double-blind, randomised, placebo-controlled trial will be the first intervention study to provide information on the effect of intravenous iron supplementation on exercise capacity in kidney transplant recipients with iron deficiency.
- ⇒ Several clinically relevant additional endpoints are assessed, including changes in quality of life, cardiac function, skeletal muscle strength, neurocognitive function and safety endpoints including effects of phosphate homeostasis.
- ⇒ A limitation of this study is that it is insufficiently powered to detect differences on clinical endpoints such as cardiovascular events or mortality.

Trial registration number NCT03769441.

INTRODUCTION

After kidney transplantation, exercise capacity and skeletal muscle strength improve compared with the dialysis setting, but this improvement is often incomplete.^{1,2} Reduced physical activity and muscle strength have been associated with an excess risk of mortality and worse graft outcomes in kidney transplant recipients (KTRs).^{3,4} Although patient and graft survival have substantially improved over the past decades, few therapeutic strategies are available to ameliorate physical fitness and well-being. Therefore, it is key to identify new modifiable risk factors related to impaired exercise tolerance.

Iron deficiency (ID) is common after kidney transplantation, with reported prevalences ranging between 11% and 47%, depending on the definition and time

after transplantation.^{5–9} ID has been associated with an increased risk of mortality in KTRs, independent of coexisting anaemia.⁵ Iron is involved in oxygen transport as a component of haemoglobin. Moreover, ID has implications beyond impaired erythropoiesis as iron is crucial to myriad physiological processes. Iron is a cofactor of many enzymes involved in different cellular functions: as a constituent of aconitase and succinate dehydrogenase, both enzymes involved in the Krebs cycle, and as a constituent of different complexes involved in the mitochondrial respiratory chain, iron is pivotal to cellular energy metabolism. Additionally, iron is involved in nucleotide synthesis for replication and production of DNA and RNA.¹⁰ Therefore, ID may affect growth and function of many cell types, in particular those with high energy consumption or mitogenic activity.

Skeletal muscle cells and cardiomyocytes are among the cells with the highest energy demand, and ID compromises their aerobic metabolism and function.^{11–16} In patients with chronic heart failure (CHF), ID is associated with impaired exercise tolerance, skeletal muscle strength and quality of life, and worse prognosis.^{17–23} Intravenous correction of ID improves exercise capacity, quality of life,^{24–27} left^{28 29} and right^{29 30} ventricular function and skeletal muscle energy metabolism³¹ in CHF patients and reduces the incidence of heart failure hospitalisations.³² In chronic obstructive pulmonary disease patients, intravenous correction of ID also improves exercise capacity and quality of life.³³ In patients with chronic kidney disease (CKD), ID has been associated with fatigue,³⁴ lower physical quality of life³⁵ and higher risk of cardiovascular events and premature mortality.^{36–40} In a relatively small trial in patients with non-anaemic CKD, there was a non-significant trend towards an improvement of exercise capacity and quality of life after intravenous iron supplementation.⁴¹ Furthermore, a high-dose intravenous iron regime in patients undergoing haemodialysis was associated with a better prognosis, compared with a low-dose regime in the Proactive IV Iron Therapy in Haemodialysis Patients (PIVOTAL) trial.⁴² The impact of ID and its correction on muscle strength and physical function in KTRs is yet unknown. The Effect of Ferric Carboxymaltose on Exercise Capacity after Kidney Transplantation (EFFECT-KTx) trial has been designed to test the hypothesis that iron supplementation improves exercise capacity, skeletal muscle strength, cardiac function and quality of life in iron-deficient KTRs.

Additionally, the study provides an opportunity to assess the effect of iron supplementation on several other organ systems and physical functions. First, correction of ID might improve neurocognitive functions such as memory, mental speed and executive functioning, which are compromised after kidney transplantation.^{43 44} In addition to its role in energy metabolism, iron is involved in neurotransmitter synthesis and uptake and neuron myelination.⁴⁵ ID is associated with impaired cognitive function,^{46–51} which can be improved by iron supplementation.^{47 52 53} Second, ID correction after kidney

transplantation might ameliorate lymphocyte proliferation and function. Lymphocytes are among the cells with the highest mitogenic activity, and therefore it would be relevant to address whether ID correction changes lymphocyte proliferation and function, and potentially rates of infection and rejection, after kidney transplantation.^{54 55} While it is unknown whether iron supplementation improves resistance against pathogens in KTRs, prior studies dispute whether iron sufficiency and iron supplementation might increase rejection risk.^{56 57} Third, ID correction might alter the synthesis of fibroblast growth factor (FGF) 23, which is deregulated during ID.^{58 59} FGF23, a phosphaturic hormone secreted by osteocytes, is increased in CKD and often remains elevated after kidney transplantation.^{60 61} By promoting excessive phosphaturia and by lowering levels of 1,25-dihydroxycholecalciferol,⁶⁰ FGF23 might compromise bone mineral density^{62 63} and increase fracture risk.⁶⁴ In addition, FGF23 may induce left ventricular hypertrophy through off-target effects on cardiomyocytes,⁶⁵ and C-terminal FGF23 has been shown to (partially) mediate the association between ID and outcomes in KTRs and other populations.^{38 66–68} In KTRs, a higher plasma FGF23 level is associated with worse patient and graft survival.^{69 70} Interestingly, prior studies have shown that in the short-term, ferric carboxymaltose induces an increase of FGF23, potentially triggered by its carboxymaltose shell.^{58 71} While the long-term effect of ferric carboxymaltose on FGF23 levels in KTRs is currently unknown, this short-term upregulation might induce hypophosphatemia and may be a relevant potential safety signal that will be evaluated in the EFFECT-KTx trial.

As exploratory endpoints, the effects of intravenous iron supplementation on gut microbiome composition and the presence of gastrointestinal symptoms will be assessed. Oral iron supplements are known to change the gut microbiome in favour of pathogenic bacteria, which are iron dependent,⁷² and it has been suggested that intravenous iron supplementation also affects the gut microbiome⁷³ and metabolome.⁷⁴ Furthermore, since ID has been linked to restless legs syndrome^{75 76} and ID correction has been shown to alleviate restless legs symptoms,^{77–79} intravenous iron might reduce these complaints in KTRs.⁸⁰

The EFFECT-KTx trial addresses the effects of iron treatment using an intravenous solution. Apart from the potential adverse effects of oral iron supplements on the gut microbiome and its gastrointestinal side effects, which may impair quality of life as well as treatment adherence,⁸¹ intravenous iron supplementation may also have a better treatment effect in KTRs. In patients with CHF⁸² or CKD,⁸³ it has been shown that intravenous iron supplementation is a more effective way to correct iron parameters, compared with oral iron treatment. In a patient with CHF, several trials show a positive effect of intravenous iron supplementation on exercise capacity,^{25–27 84} but do not show the same effect of oral iron treatment.⁸⁵ Mechanistically, patients with CHF or CKD may suffer from

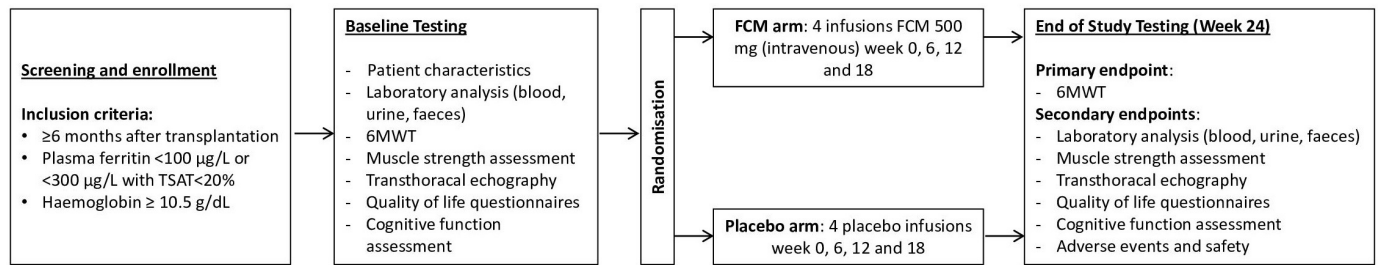


Figure 1 Design of the Effect of Ferric Carboxymaltose on Exercise Capacity after Kidney Transplantation study. 6MWT, 6 min walk test; FCM, ferric carboxymaltose; TSAT, transferrin saturation.

chronic inflammation, leading to higher hepcidin levels, which hypothetically could hamper the intestinal absorption of iron. This may also apply to KTRs.

Together, the EFFECT-KTx trial will provide important information on health effects of ID correction by intravenous ferric carboxymaltose in KTRs, with a primary focus on exercise capacity.

METHODS AND ANALYSIS

Overall design

The EFFECT-KTx study is designed as an investigator-initiated, multicentre, randomised placebo-controlled clinical superiority trial with two parallel treatment arms. The study design is shown in [figure 1](#). Study medication (ferric carboxymaltose or placebo, allocation ratio 1:1) is administered at a baseline visit and at subsequent study visits 6, 12 and 18 weeks later. Study participants are screened and recruited at the University Medical Centre Groningen (UMCG) and the University Medical Centre Utrecht, both in the Netherlands. KTRs with ID, defined as plasma ferritin <100 µg/L or plasma ferritin 100–299 µg/L with transferrin saturation (TSAT) <20%, and a haemoglobin level of ≥10.5 g/dL, who are at least 6 months after transplantation, may be eligible for participation. Inclusion and exclusion criteria are listed in [box 1](#).

Patient and public involvement

During the feasibility stage, a concept version of the project plan was presented to the Dutch Kidney Patient Association (NVN) and was reviewed by an NVN employee and a kidney patient. Their advice to minimise the effort patients are required to make while participating in the trial was taken into consideration during the writing of the study design. They approved the choice of outcome parameters. In accordance with the NVN recommendation, an informative event will be organised and a study newsletter will be composed to inform the participants and other KTRs about the results of the study after they have been published.

Sample size calculation

Based on an average 6 min walking distance of 495±92 m, as previously reported in stable KTRs,¹ an anticipated improvement in walking distance of at least 10%,

a desired power of 0.90 with a significance level of 0.05, we calculated that a sample size of 72 participants per arm is needed to establish a clinically meaningful effect of intravenous iron on the primary endpoint, using a two-sided independent samples t-test. To account for possible dropout of up to 10% of enrolled participants, we aim to include 79 participants per arm (158 participants in total).

Randomisation

Participants are randomised by the UMCG hospital pharmacy using a precomposed list of usable participant numbers, which are assigned to a study arm. The

Box 1 Inclusion and exclusion criteria for the EFFECT-KTx trial

Inclusion criteria

- ⇒ Kidney transplant recipient
- ⇒ Iron deficiency at two consecutive measurements with an interval of at least a week, defined as plasma ferritin <100 µg/L or plasma ferritin 100–299 µg/L with transferrin saturation <20%
- ⇒ ≥6 months after kidney transplantation
- ⇒ ≥18 years of age
- ⇒ Ability to comply with the study protocol
- ⇒ Informed consent

Exclusion criteria

- ⇒ Known intolerance to intravenous iron products
- ⇒ Severe (Hb <10.5 g/dL) or progressive anaemia (Hb decline of ≥3.2 g/dL in 2 months) or a reduced mean corpuscular erythrocyte volume (<80 fL)
- ⇒ Recent history of gastrointestinal or urogenital blood loss or a positive faecal occult blood test
- ⇒ Erythrocyte transfusion ≤6 weeks prior to inclusion
- ⇒ Polycythaemia (Hb >15.3 g/dL) or a known history of haemochromatosis
- ⇒ Estimated glomerular filtration rate of ≤30 mL/min per 1.73 m²
- ⇒ Symptomatic coronary artery disease or myocardial infarction ≤4 weeks prior to inclusion
- ⇒ Disability to walk
- ⇒ Severe hypophosphatemia (plasma phosphate <0.35 mmol/L) ≤4 weeks prior to inclusion
- ⇒ For women at childbearing age: pregnancy or disagreement to take adequate contraceptive precautions
- ⇒ Signs of an active systemic infection
- ⇒ Participation in another intervention study

participant numbers on the list are grouped in blocks, with an equal distribution and a random order of treatment arm allocations within each block. To stratify for treatment centre, all study numbers within a certain block are assigned to participants in the same centre, in the order of the list. Other than to the pharmacist, the list is not accessible to any of the investigators or to others. In case of a medical emergency possibly related to the study treatment, treatment allocation may be revealed by the pharmacist.

Study drug and blinding

Patients in the active treatment arm receive ferric carboxymaltose (Ferinject, provided by Vifor Pharma, Glattbrugg, Switzerland), administered intravenously as 10 mL doses, each containing 500 mg of elemental iron, dissolved in 240 mL 0.9% sodium chloride. Patients in the placebo arm receive 250 mL 0.9% sodium chloride, administered intravenously as well. All treatments are followed by a 30 min observation period. Treatments are performed by an unblinded study nurse, who is not involved in any other study activities. Since ferric carboxymaltose is a dark brown liquid, participant blinding is maintained by using non-transparent infusion lines (Codan, Lensahn, Germany) or by opaque infusion line protection covers (Medipak, Winchester, Virginia, USA). Additionally, infusion lines and bags are covered with aluminium foil. All patients receive four doses of study medication, with 6 week intervals, at the baseline visit and after 6, 12 and 18 weeks. Only in case of a systemic infection or hypophosphatemia, defined as a phosphate level of ≤ 0.50 mmol/L, on the day of the scheduled treatment, a dose is skipped. In case of imminent iron overload, defined as a plasma ferritin level of ≥ 800 $\mu\text{g/L}$ or a plasma ferritin level of 500 to 799 $\mu\text{g/L}$, combined with a TSAT of $\geq 45\%$ on the day of the scheduled treatment, patients in the intervention arm receive placebo instead of ferric carboxymaltose. This is decided by the unblinded study nurse according to a standard operating procedure that was designed for this purpose, without interference of the blinded study personnel. For optimal adherence, participants are encouraged to attend all study visits. To evaluate adherence to the protocol, drug accountability is performed by the pharmacy. In case the allocated treatment is not administered, the medication is returned to the pharmacy with an explanation from the study doctor or study nurse. Other healthcare providers such as the general practitioner and the nephrologist of the participant are discouraged to assess iron status and/or start oral iron treatment during trial participation, unless there is a strict medical indication.

Data collection and study endpoints

At the baseline visit, a full medical history is taken, including any current medication use. A physical examination, including assessment of weight, height and blood pressure, is performed as well. Blood pressure is measured three times, with at least 1 min between assessments,

using a Dinamap pressure metre. The mean of three measurements is calculated and recorded in the study file. Furthermore, iron intake is estimated using a food diary, which is completed by the participants on three representative days during the week before the baseline visit and after 24 weeks.

At all study visits, blood samples are taken for direct assessments (table 1) or storage. Furthermore, participants are asked to bring a 24-hour urine sample. Briefly, participants are instructed to discard their first morning urine on the day before the visit and collect urine throughout the following 24 hours, including the first morning urine of the day of the study visit. Participants are also asked to collect a faeces sample in a specific tube during the week before the baseline study visit and after 24 weeks, and to bring the frozen sample to the study centre, where it is stored for later analysis.

Once per year, a study monitor visits both study sites to inspect the accuracy of data collection and overall implementation of the study protocol. In addition, trial conduct will be audited at least once, independent from the investigators and sponsors. Since the safety risks of participation are considered low, no data monitoring board has been installed.

Primary endpoint

The primary endpoint of the study is change in exercise capacity, which is measured using the 6 min walk test (6MWT) at baseline and 24 weeks later.⁸⁶ The 6MWT, a validated measure of exercise capacity,⁸⁷ requires a quiet walking course with a flat, hard surface. Two traffic cones are placed at the beginning and at the end of a 15 m straight course and the participant is asked to walk around the two cones as fast as possible during 6 min, without running. If needed, participants may use a cane. During the test, the participant is encouraged verbally by the researcher. Before and immediately after the test, the heart rate is measured.

Secondary endpoints

Haematinic indices and iron parameters

Blood haemoglobin and haematocrit are measured at each study visit. Levels of plasma iron, ferritin and transferrin are assessed but are only available to the unblinded study nurse until after completion of the trial to avoid disclosure of treatment allocation to the blinded study team. TSAT is calculated as $100 \times \text{plasma iron } (\mu\text{mol/L}) \div (\text{plasma transferrin } (\text{g/L}) \times 25)$. Blood is stored for measurement of additional iron parameters, including hepcidin and soluble transferrin receptor.

Muscle mass and strength and functional mobility

At each study visit, 24-hour urinary creatinine excretion rate is measured to estimate muscle mass.⁸⁸ Skeletal muscle strength and functional mobility are assessed at the baseline visit and after 24 weeks using the Five Times Sit-to-Stand (FTSTS) Test, the Timed Up-and-Go (TUG) Test and a handgrip dynamometry. The FTSTS Test is

Table 1 Primary and secondary endpoints and exploratory outcomes

Primary endpoint	Measurements and tests	Study visits				
		Baseline	Week 6	Week 12	Week 18	Week 24
Exercise capacity	6 min walk test	x				x
Secondary endpoints						
Haematinic indices and iron status	Plasma haemoglobin	x	x	x	X	x
	Plasma iron, ferritin, transferrin saturation	x	x*	x*	x*	x*
	Soluble transferrin receptor	x		x		x
Muscle mass and strength and functional mobility	24-hour urinary creatinine excretion rate	x	x	x	x	x
	Five Times Sit-to-Stand Test	xx				x
	Timed Up-and-Go Test	x				x
	Handgrip dynamometry					x
Cardiac structure, function and strain	Resting transthoracic echocardiography	x				x
	Troponin-T	x		x		x
	N-terminal pro-B-type natriuretic peptide	x		x		x
Quality of life	Short Form-36	x				x
	Dutch 'Checklist Individuele Spankrant'	x				x
	Dutch Multifactor Fatigue Scale	x				x
	EuroQol-5D-5L	x				x
Bone and mineral metabolism	Plasma phosphate and 24-hour urinary phosphate excretion rate	x	x	x	x	x
	Plasma calcium and 24-hour urinary calcium excretion rate	x	x	x	x	x
	Parathyroid hormone	x	x	x	x	x
	C-terminal and total fibroblast growth factor 23	x	x	x	x	x
	25-hydroxycholecalciferol	x		x		x
	1,25-dihydroxycholecalciferol	x		x		x
Cognitive function	Cognitive Screening Test	x				
	Clock Drawing Test	x				
	Nederlandse Leestest voor Volwassenen	x				
	Digit Span Forward	x				x
	15 Words Test	x				x
	Word Fluency	x				x
	Symbol Digit Modalities Test	x				x
	Trail Making Test part A	x				x
	Trail Making Test part B	x				x
	Controlled Oral Word Association Test	x				x
	Digit Span Backward	x				x
Adverse effects and safety	Gastrointestinal Symptom Rating Scale	x	x	x	x	x
	Incidence of adverse symptoms and new clinical events	x	x	x	x	x
	Plasma creatinine					x
	Plasma aspartate transaminase	x	x	x	x	x
	Plasma alanine transaminase	x	x	x	x	x
Exploratory outcomes						
Incidence of restless leg syndrome (RLS)	Report of RLS-related symptoms	x	x	x	x	x
Kidney graft rejection and injury	Urine markers (kidney injury marker 1, neutrophil gelatinase-associated lipocalin)	x	x	x	x	x

Continued

**Table 1** Continued

Primary endpoint	Measurements and tests	Study visits				
		Baseline	Week 6	Week 12	Week 18	Week 24
Function of the immune system	Analysis of peripheral blood mononuclear cells (growth, differentiation, immunoglobulin production, cytokine production)	x				x
	IgA, IgG, IgM	x				x
	Complement	x	x	x	x	x
	Cytokine levels	x				x
Gut microbiota	DNA sequencing of faecal samples	x				x

*Concealed to blinded study personnel.

used to measure leg strength by using the participant's body weight as resistance. Participants are asked to stand up from a chair five times successively as fast as possible, without support of their arms. The time required to do this is recorded. The task is repeated three times, and the mean of the attempts is calculated. The TUG Test is an instrument to assess leg strength, coordination and balance.^{89 90} A traffic cone is placed 3 m before a chair and participants are instructed to stand up from the chair, again without support of their arms, walk around the cone as fast as possible and take place on the chair again, while the time is recorded. The TUG Test is also performed three times, and the mean of the attempts is calculated. Handgrip strength is measured with the Jamar Hydraulic Hand Dynamometer (Jamar 5030J1, Patterson Medical, Warrenville, Canada). Participants are asked to clench the device with maximum force to perform an isometric contraction, first with the right hand and then with the left hand, while sitting on a chair. Participants are not allowed to rest their elbow on a table. Handgrip strength is tested three times with an interval of 30 s between each attempt, and the mean is calculated. The second handle position of the hand dynamometer is used because it has been shown to be the most accurate position.⁹¹

Cardiac structure, function and strain

Cardiac structure, volumes, systolic and diastolic function and global longitudinal strain are analysed with a resting transthoracic echocardiography at the baseline study visit and after 24 weeks. A Vivid E90 cardiovascular ultrasound system (GE Healthcare, Chicago, USA) is used. Imaging and analysis are performed by a blinded sonographer under supervision of a blinded cardiologist. Ventricular wall thickness and atrial and ventricular volumes are measured using two-dimensional echocardiography. Left ventricular ejection fraction as the main parameter of cardiac function is calculated from these measurements using the Simpson biplane method if image quality is sufficient, or is alternatively assessed by eyeballing. Ventricular mass is also calculated. Tricuspid annular plane systolic excursion is measured as a parameter of right ventricular function. Doppler imaging is used to assess diastolic function and blood flow velocity distal to the valves, from which pressure gradients are calculated.

Global longitudinal strain is evaluated as an early measure of impaired myocardial contractility, using cardiac performance analysis. Furthermore, troponin T and N-terminal pro-B-type natriuretic peptide as markers of myocardial injury and dysfunction are measured at baseline and after 12 and 24 weeks.

Quality of life

Participants are being requested to complete four questionnaires at baseline and after 24 weeks, to assess quality of life and level of fatigue. The Short Form-36 Questionnaire is used to measure health-related quality of life.⁹² Subjective fatigue, is assessed using the Dutch 'Checklist Individuele Spankrant' and the Dutch Multifactor Fatigue Scale.⁹³ Overall quality of life is measured with the EuroQol-5D-5L Questionnaire and Visual Analogue Scale.⁹⁴

Neurocognitive function

Neurocognitive performance of study participants is assessed by neuropsychology students at baseline and after 24 weeks, using a set of neuropsychological tests. Raw scores and norm scores, specific for age, sex and educational level, will be used for analysis.

At baseline, three tests are used to screen for signs of neurocognitive disorders and intellectual disability. The Cognitive Screening Test (CST) is a Dutch orientation questionnaire to screen for neurodegenerative disorders such as dementia.⁹⁵ The Clock Drawing Test (CDT) is a second CST for neurodegenerative disorders.⁹⁶ Participants are asked to draw a clock and set the time to 'a quarter to two'. The Nederlandse Leestest voor Volwassenen (NLV), the Dutch version of the National Adult Reading Test, provides an estimation of premorbid intelligence.⁹⁷ The participant has to read out 50 irregularly spelled words. Participants with signs of a neurodegenerative disorder based on the CST or the CDT or with an intellectual disability based on the NLV (estimated IQ<80) will be excluded from further cognitive analyses.

Three tests are performed to assess memory. The Digit Span Forward (Digit Span FW; subtest of the Wechsler Adult Intelligence Scale IV) measures memory span.⁹⁸ In this task, the participant is asked to repeat a series of numbers in the same order as the examiner did. The 15

Words Test (15WT; Dutch version of the Rey Auditory Verbal Learning Test) measures verbal memory, specifically immediate verbal memory (15WT Immediate Recall (IR)) and delayed verbal memory (15WT Delayed Recall (DR)).⁹⁹ In this task, 15 unrelated words are presented to the participant in 5 trials, who is asked to recall as many as possible after each trial (15WT IR). IR is the total score over five trials. After 20 min, participants are again asked to recall as many of the 15 words as possible (15WT DR). Word Fluency (subtest of the Groninger Intelligentie Test 2) is a verbal task measuring semantic memory.¹⁰⁰ Participants are asked to produce as many words within two semantic categories (animals and professions) within 1 min each.

Attention and mental speed are assessed using two tests. The Symbol Digit Modalities Test measures information processing speed.¹⁰¹ Participants are asked to combine as many symbols as possible with matching numbers within 90 s. The Trail Making Test part A (TMT-A) measures visuomotor and mental speed.¹⁰² In this paper and pencil task, participants are challenged to connect a series of randomly distributed numbers from 1 to 25 in an ascending order. The score is time in seconds to complete the task.

To assess executive functioning, three tests are performed. The Trail Making Test part B measures cognitive flexibility.¹⁰² This task is similar to the TMT-A, but numbers as well as letters have to be connected in an alternating ascending order (1-A-2-B- etc). Again, the score is time in seconds to complete the task. The Controlled Oral Word Association Test is used to measure executive control.¹⁰³ Participants are asked to produce as many words as possible that start with a specific letter (D-A-T) within 1 min. The three scores are added up. The Digit Span Backward (subtest of the Wechsler Adult Intelligence Test) measures working memory, which is considered an aspect of executive functioning.⁹⁸ The test is similar to the Digit Span FW, but this time, the participant is asked to repeat a series of numbers in the opposite order as the examiner did.

Adverse effects and safety

The occurrence of gastrointestinal side effects is examined using the Gastrointestinal Symptom Rating Scale Questionnaire, which is completed by the participant at baseline and after 24 weeks.¹⁰⁴ At all study visits, participants are being asked about adverse symptoms and new clinical events, particularly infections, hospitalisations, cardiovascular events and bone fractures. Plasma creatinine and hepatic transaminases are measured to screen for kidney or liver injury after treatment. Additionally, episodes of biopsy-proven rejection will be documented until the last visit of the last participant. Hypersensitivity reactions, cases of hypophosphatemia and other adverse events are registered systematically. To investigate the effect of ferric carboxymaltose on bone and mineral metabolism, blood levels of phosphate, calcium and parathyroid hormone are measured at all study visits. Phosphate and calcium

excretion are assessed using 24-hour urine. C-terminal and intact FGF23 levels, 25-hydroxycholecalciferol and 1.25-dihydroxycholecalciferol will be measured from stored blood samples after trial completion. Severe adverse events are reported to the medical ethical committee.

Exploratory outcomes

To investigate whether intravenous iron decreases the occurrence of restless leg syndrome, participants are asked whether in the past 2 weeks, they had experienced 'an often unpleasant or uncomfortable urge to move the legs that occurs during periods of inactivity, particularly in the evenings, and is transiently relieved by movement' at all study visits. Furthermore, to assess the potential effect of intravenous iron on kidney function, estimated glomerular filtration rate will be calculated using the 2021 CKD Epidemiology Collaboration (CKD-EPI) formula,¹⁰⁵ and 24-hour urine will be used to assess creatinine clearance and kidney damage markers including neutrophil gelatinase-associated lipocalin and kidney injury molecule-1.

At baseline and after 24 weeks, peripheral blood mononuclear cells (PBMCs) are isolated from whole blood. Stored PBMCs can be used to assess the effect of iron supplementation on lymphocytes. We will assess subset distribution of PBMCs, proliferation rate of in vitro stimulated T-lymphocytes and B-lymphocytes and plasma cell formation and cytokine expression of B-lymphocytes, using flow cytometry. Furthermore, B-lymphocyte immunoglobulin production is measured using ELISA. Blood samples are stored for the measurement of complement factors and cytokines as well.

Finally, participants are requested to bring a frozen faeces sample at baseline and after 24 weeks, which are stored for analysis. The effects of intravenous iron supplementation on gut microbiota will be assessed using 16S ribosomal RNA sequencing to identify different bacteria and liquid chromatography-mass spectrometry to analyse metabolomics.

Data analysis

Data analysis will be performed primarily according to the intention-to-treat principle for the allocated treatment and will include all participants who received at least one dose of study medication. An additional sensitivity analysis will be performed, in which only participants who received at least one treatment according to the allocated arm and who were still (patients in the placebo arm) or no longer (patients in the intervention arm) iron deficient at the last study visit will be included. Continuous data will be presented as mean with standard deviation (normally distributed data), median with interquartile range (data with a skewed distribution) or as number with percentages (categorical data). To evaluate changes in the continuous variables from baseline to week 6, 12, 18 and 24, paired t-tests or Wilcoxon signed-rank tests will be used. The



primary endpoint and secondary endpoints with data at baseline and the end-of-study visit will be analysed using mixed model analysis, thereby adjusting for baseline values. A p value of <0.05 will be considered significant. We do not expect to encounter a high number of missing data, but in the case of >10% incompleteness, missing values will be replaced by estimates. Statistical analyses will be performed with SPSS (IBM, Armonk, New York, United States of America, version 28.0) software.

Data management

Study data will be recorded digitally using the secured REDCap electronic data capture tool (REDCap, Nashville, USA)¹⁰⁶ hosted at the UMCG. Data and biological materials will be stored for 15 years. Data analysis will take place with validated and pseudoanonymised data. To protect participant privacy, public access to the dataset will not be provided. After the last visit of the last participant and before disclosure of treatment arm allocation, data will be extracted from REDCap and exported to SPSS for analysis. No interim analyses will be performed.

Ethics and dissemination

The study protocol has been approved by the local ethics committee (Medisch Ethische Toetsingscommissie UMCG, METc 2018/482, Protocol version: V.6.2, May 2022). Protocol modifications are reviewed by the ethics committee and are communicated to the full study team, current participants and trial registries. All study participants must provide written informed consent to the study doctor before enrolment. Study funders are not involved in the composition of the study design and data collection and will not be involved in data analysis or the decision to publish the results. Study results will be presented in publications in peer-reviewed scientific journals and at relevant international conferences. Authors will be selected according to eligibility guidelines and no professional writers will be involved.

Trial status

Recruitment started in October 2019 and is currently ongoing. Between March and June 2020, recruitment was temporarily halted because of the COVID-19 pandemic. Between March and May 2020, no in-hospital study visits were allowed. Given the uncertainty about the duration of the pandemic, it was decided that for patients who missed two or more study treatments, of which at least one because of the pandemic restrictions, participation would be terminated. In that case, the participant would not be included in the enrolment number and a new participant would be recruited instead. Participants who were excluded because of missed study drug administrations during the COVID-19 epidemic were invited for a rescreening under a new study number, providing they were still iron deficient. End-of-study visits were either postponed, with a maximum of 2 weeks, or were

performed at the participant's home if possible. This procedure has been approved by the medical ethical committee of the UMCG. We expect to include the last participant in early 2023 and to complete the trial in the second half of 2023.

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Contributors JSJV primarily wrote the study design and coordinated the trial. MFE and CAJMG contributed to the study design with expertise in iron metabolism. JMS cooperated in the setup of the design as an expert in cognitive assessment. J-SS and WHA contributed to the study design with expertise as a transplant immunology specialists. SJLB and SPB contributed to the study design with expertise in kidney transplantation research. PvdM supervised the writing of the design of the cardiological assessments. ADVz coordinated the local trial execution at the University Medical Centre Utrecht. MdB is the principal investigator of the study and supervised composition of the study design.

Funding This work was supported by the Dutch Kidney Foundation (grant no 170KG18) and by Vifor Fresenius Medical Care Pharma.

Competing interests JSJV has received consultancy fees from Vifor Pharma (to employer). MFE has declared receiving consultant fees from Vifor Pharma; serving on the Advisory Board for Cablon Medical; and receiving speakers bureaus from Vifor Pharma. CAJMG takes part in the advisory board of Vifor Pharma, GlaxoSmithKline and Pharmacosmos. PvdM received consultancy fees and/or grants from Novartis, Pharmacosmos, Vifor Pharma, AstraZeneca, Pfizer, Abbott, Pharma Nord, Novo Nordisk and Ionis. MdB has consultancy agreements with Amgen, Astellas, AstraZeneca, Bayer, Kyowa Kirin, Vifor Fresenius Medical Care Renal Pharma and Sanofi Genzyme and received grant support from Sanofi Genzyme and Vifor Pharma (all to employer).

Patient and public involvement Patients and/or the public were involved in the design, or conduct, or reporting, or dissemination plans of this research. Refer to the Methods and analysis section for further details.

Patient consent for publication Not applicable.

Provenance and peer review Not commissioned; externally peer reviewed.

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