The differential effect of intravenous iron in patients with non-dialysis chronic kidney disease in terms of fibroblast growth factor 23, phosphate, bone metabolism, functional status and quality of life and cardiovascular variables

"Iron and Phosphaturia in CKD"

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Abstract

Background: Modern intravenous iron preparations (e.g. ferric derisomaltose (FDI), ferric carboxymaltose (FCM)) are commonly used in non-dialysis-dependent chronic kidney disease (CKD) patients. Despite similar efficacy and safety in terms of previously described side-effect profile, a differential effect in hypophosphatemia has been noted with FCM. This appears to be related to fibroblast growth factor 23 (FGF23). Fibroblast growth factor 23 is an important phosphatonin with intertwined effects to phosphate metabolism, relevant to vitamin D and parathyroid hormone, leading to enhanced phosphaturia. In addition such alterations may lead to changes in bone turnover, and may be responsible for subtle differences in clinical and patient reported outcome measures. No previous randomised study evaluated this phenomenon in patients with non-dialysis dependent CKD. Moreover, no prior study adopting such a methodology evaluated any differential effect in terms of clinical and patient reported outcome measures, alongside potential cardiovascular implications. The primary hypothesis under study was whether any differential effect could arise secondary to the use of various modern intravenous iron compounds. This study primarily examined the comparative effects of FDI and FCM on FGF23, phosphate and other bone metabolism markers. In addition, it secondarily evaluated the impact of intravenous iron on functional status and cardiovascular variables, and assessed for any difference between the two compounds.

Methods: This single-center double-blind randomised controlled trial primarily assessed the effects of FCM and FDI on intact FGF23 and phosphate, whilst also studying the impact on vitamin D, parathyroid hormone and phosphaturia. Bone turnover markers (alkaline phosphatase, bone-specific alkaline phosphatase, cross-linked C-telopeptide of type I collagen, N-terminal propeptides of Type I collagen), functional status (fatigue severity scale, 36-Item Short Form Health Survey, Duke Activity Status Index and 1-minute-sit-to-stand

test) and cardiovascular variables (NT-pro-BNP, troponin T and pulse wave velocity) were monitored. Non-dialysis-dependent CKD patients with iron deficiency with/without anemia (serum ferritin <200µg/L or transferrin saturation ≤20% and serum ferritin 200-299µg/L) were randomised to receive FDI or FCM (1:1). At baseline 1000mg of intravenous iron was administered followed by 500-1000mg at one month. Measurements were performed at baseline, 1-2 days following iron administration, 14 days, 1 month, 1-2 days following second administration, 2 months and 3 months following initial infusion. Safety was assessed throughout the study.

Results: Twenty-six patients were randomized to FDI (n=14) and FCM (n=12). Intact FGF23 increased following iron administration, which was significantly higher with FCM compared to FDI (Baseline to 1-2 days following 1st administration: FDI: 3.0 (IQR: - 15.1 - 13.8) % vs. FCM: 146.1 (IQR: 108.1-203.1) %; $p < 0.001$ and Baseline to 1-2 days following 2nd administration: FDI: 3.2 (IQR: - 3.5 - 25.4) % vs. FCM: 235.1 (138.5-434.6) %; $p = 0.001$). Phosphate levels decreased in the FCM group, causing a significant difference versus FDI at 14 days (FDI: 1.26 (IQR: 1.05–1.66) mmol/L vs. FCM: 1.09 (IQR: 0.94–1.23) mmol/L; $p = 0.049$). No clinically significant hypophosphataemia was detected ad either group. A significantly greater decrease in 1,25 $(OH)_2$ Vitamin D was noted with FCM. A trend for increased phosphaturia was noted with FDI, albeit not statistically significant. Several bone turnover markers significantly changed following FCM administration but not FDI, both in terms of resorption and formation. Functional status improved in the total cohort and more specifically FDI in certain domains. This was particularly evident in those pertinent to fatigue and physical function as indicated both by the 36-Item Short Form Health Survey questionnaire and the Fatigue Severity Scale. No cardiovascular detriment was identified. Clinical efficacy and safety were similar between the two groups.

Conclusions: The study suggests a differential effect in FGF23 following FCM administration in non-dialysis-dependent CKD patients. This may lead to changes consistent with hypovitaminosis D and alterations in bone turnover with potential clinical consequences. A common complication of non-dialysis-dependent CKD is mineral bone disease, characterised by impact on quality of life, strength and succeptibility to fractures. This is fueled by changes in the metabolism of both vitamin D and parathyroid hormone. It is hence possible that any further detriment to the already fragile kidney-bone axis secondary to such differential effect can have a significant clinical impact on patients with reduced kidney function. This becomes increasingly important, as the cumulative effect of repeated intravenous iron infusions is yet to be established. The positive effect of iron on patient reported outcome measures and functional status, alongside the similar efficacy and safety displayed, complement evidence relevant to intravenous iron, and can increase the confidence of clinicians in the use of such compounds. Nonetheless, as this study was small in nature with certain inherent limitations, further definitive studies are required to understand these differences and provide further insights, both clinical and mechanistic, into any arising differences.

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Author's declaration

I confirm that this work is original and that if any passage(s) or diagram(s) have been copied from academic papers, books, the internet or any other sources these are clearly identified by the use of quotation marks and the reference(s) is fully cited.

I certify that, other than where indicated, this is my own work and does not breach the regulations of HYMS, the University of Hull or the University of York regarding plagiarism or academic conduct in examinations.

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1: Introduction

Chronic Kidney Disease (CKD) is a progressive disorder, an umbrella term representing a disease state characterised by either functional or structural damage of the kidneys persisting for more than three months (1). It is defined as an estimated glomerular filtration rate (eGFR) of < 60 ml/min/1.73m^2, by the presence of persistent abnormalities on urinalysis such as haematuria or proteinuria or by structural changes even in the presence of a normal eGFR (2). Chronic kidney disease can be further divided into non-dialysis (ND-CKD) not requiring renal replacement therapy, and dialysis dependent CKD, which can be provided through peritoneal means (peritoneal dialysis) or haemodialysis (HD-CKD). Approximately 10% of the global population suffers from CKD, and this is associated with 1.2 million deaths and 28.0 million years of life lost annually (3). Despite improvements in prevention of kidney function deterioration, CKD often progresses to a point of no cure, causing significant impact on both patients and healthcare economics (4,5).

The kidneys are intricately involved in a number of metabolic pathways and can be considered as master regulators in a number of physiological processes (figure 1). As such, derangements in normal kidney function can have a number of consequences, and figure 1 highlights these complications. These potential complications necessitate treatment and if left untreated they can affect this patient group further (6).

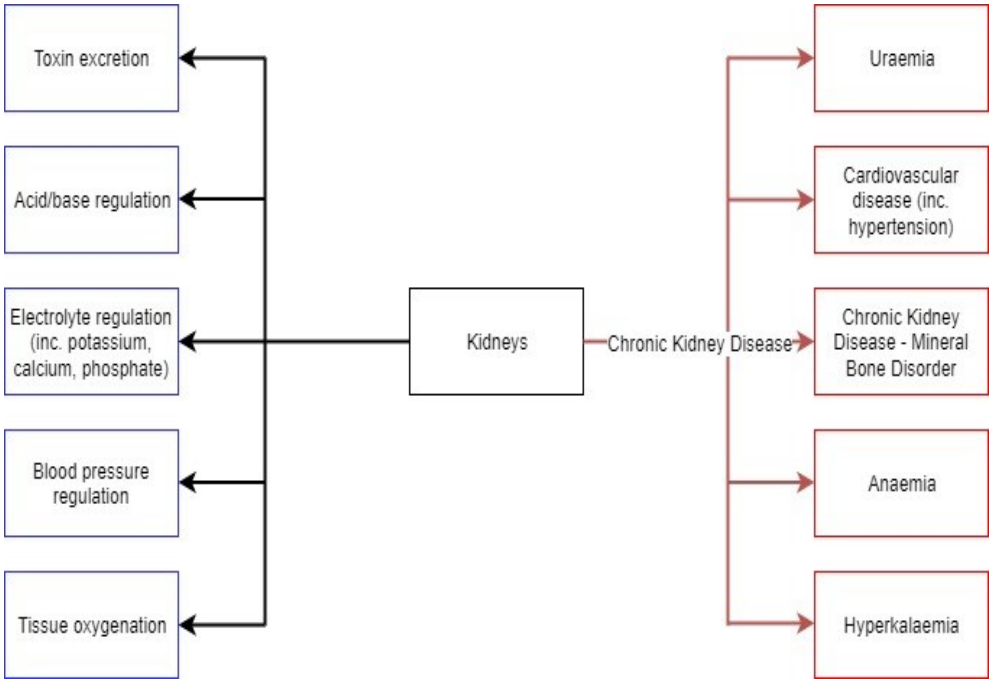

Figure 1: Physiological processes of the kidney and complications of CKD (in red).

Management of complications of CKD, including anaemia and iron deficiency are paramount in limiting the impact of disease. As further understanding of pathophysiological processes is gained, and newer interventions are created in the field, it is important to assess their differences, especially in cases where a differential effect has been seen. Anaemia can be treated using erythropoiesis stimulating agents (ESA), blood transfusion, iron supplementation and hypoxia-inducible factor–prolyl hydroxylase inhibitors (7). Iron supplementation, and more specifically intravenous iron is commonly used in the treatment of iron deficiency anaemia in ND-CKD population, with most recent studies suggest high efficacy and a good side-effect profile (8). The advent of newer intravenous iron compounds has led to the discovery of hypophosphataemia as a distinct side effect of certain preparations; whilst this has been explored in numerous conditions, it has not yet been fully elicited in patients with CKD. An analysis of the potential differential effect and any subsequent consequences is needed. In

order, nonetheless, to appreciate how such effects may arise, it is important to consider:

- the pathophysiology of anaemia and iron deficiency,
- the different treatment modalities focusing on intravenous iron,
- the concerns surrounding intravenous iron and finally,
- how modern intravenous iron products may exert such a differential effect

Emphasis must be placed to the pathophysiological mechanism behind this hypophosphataemic effect that is possibly related to fibroblast growth factor 23 (FGF23), with a review of the potential downstream consequences that this differential effect may exert.

1.1: Anaemia and iron deficiency of CKD

Anaemia of CKD was first identified and described by Sir Richard Bright in the 19th century (9). Anaemia is defined by the World Health Organisation as a haemoglobin concentration below 130 g/L for adult males and 120 g/L for non-pregnant women. In CKD however, other parameters can be used such as those delineated by the National Institute of Health and Care Excellence (NICE) and Kidney Disease: Improving Global Outcomes (KDIGO); NICE guidelines suggest that a haemoglobin concentration 100-120 g/L can be described as adequate (2). KDIGO guidelines suggest that target haemoglobin concentration in this patient group should be < 115 g/L in those treated with ESA (10).

1.1.1 Prevalence of renal anaemia and its implications

Data from the National Health and Nutrition Examination Survey in the United States of America has suggested a prevalence of renal anaemia of 15.4% (11). A more recent multicentre cross sectional study of 1,066 patients with ND-CKD noted that 55.9% of the patient group had anaemia (haemoglobin <120 g/L) and 14.9% had severe anaemia (haemoglobin <100 g/L) (12). An international cross-sectional analysis using data from the Chronic Kidney Disease Outcomes and Practice Patterns Study (CKDOPPS) ND-CKD patient (n=6,766) identified an anaemia prevalence range of 28 to 52%, with increasing prevalence as eGFR decreases (13).

Anaemia of CKD as a disease process has a number of implications to care. It represents a significant burden to the patient with CKD, with repercussions on mortality/morbidity, quality of life and healthcare economics. Appendix table 1 presents evidence from the CKDOPPS and indeed reflects on these repercussions (14–16).

Beyond mortality, patients with anaemia of CKD have a greater risk for cardiovascular disease, hospitalisations and cognitive impairment, and report worse health related quality of life outcomes (17,18). Observational studies have also linked the presence of anaemia, alongside untreated anaemia as a risk factor for worsening renal function but also as predictive factor for the development and progression of CKD (19–21).

Symptoms of anaemia can be debilitating and include weakness, low energy, fatigue and shortness of breath; they have a detrimental effect on the quality of life of patients with CKD (22). Despite heterogeneity

in studies assessing quality of life and functional status due to variability in instruments used, links exist suggesting poorer health related outcomes (23). A prospective study of 1,186 CKD patients identified a significant difference between the physical and mental component scores of patients with anaemia compared to those without ($p<0.001$ and $p=0.004$, respectively) (24). Data from the CKDOPPS registry, suggests that anaemia irrespective of severity is associated with worsening health related quality of life outcomes (as assessed by KDQOL-36) and are less physically active than non-anaemic individuals (25).

Similarly, iron deficiency in CKD is common and is an important co-factor towards the presence of renal anaemia. A more detailed review of the haematinic and iron profile of patients with CKD (n=6,766) noted that 8-18% of patients had absolute iron deficiency and 15-34% had contrasting iron parameters potentially associated with functional iron deficiency (13). A different observation was noted through analysis of the US Veterans Administration on 933,463 CKD patients – 30% of the patients with anaemia had absolute iron deficiency and 19% had functional iron deficiency anaemia (26).

Iron is involved in a number of haematopoietic and non-haematopoietic functions, and as such its deficiency is associated with a number of significant implications (Appendix table 2) (27).

Evidence from two large cohort studies (n = 1,012,014) focusing on cardiovascular associated hospitalisation in CKD-associated iron deficiency indicates a relationship between mortality and hospitalisation especially where functional iron deficiency is present (26,28). The impact of iron deficiency alone, is underestimated - a recent review of the CKDOPPS registry (n=5,145) highlighted this, as

patients with lower transferrin saturation (TSAT) had a higher risk of all-cause mortality or major adverse cardiovascular events, irrespective of anaemia status or ferritin, especially in cases where transferrin saturation <15% (29).

In addition, iron deficiency is characterised by debilitating symptoms including dyspnoea, fatigue, headache, angina, dry and damaged skin and hair and loss of concentration (Lopez et al., 2016). More specific to CKD, one of its most incapacitating symptoms is restless leg syndrome with studies linking its pathophysiology to imbalances in brain iron metabolism, and higher risk of its presentation in patients undergoing dialysis (30,31). This has been recently demonstrated aptly by the CKDOPPS registry (n=2,513, ND-dependent CKD). Looking primarily at the impact of absolute/functional iron deficiency (TSAT <20% and ferritin >300 or <50 ng/ml) on the mental and physical component of KDQOL-36, they noted that absolute iron deficiency was associated with worse physical component scores, with a similar effect in patients with functional iron deficiency. This scoring disadvantage was maintained irrespective of confounders and was most strongly related to transferrin saturation values. This was also translated into lower probability of self-reported intense physical activity in those with a TSAT <20%, compared to the other groups. Nonetheless, the results were neutral in terms of the mental component score (32). Upon reviewing the results of CKDOPPS registry associated studies it is important to remember its observational nature, which therefore is open to confounders.

1.1.2: Pathophysiology of anaemia of CKD and iron deficiency:

There are several putative causes of anaemia of CKD (Appendix table 3) some of which are related directly to erythropoiesis and other

associated with risk factors in the environment, therapy and as a result of the development of further CKD complications associated with progression of disease (17). Anaemia of CKD is partly a product of dysregulation of erythropoiesis secondary to reduction in erythropoietin production and decreased iron (33). Defective erythropoiesis associated anaemia in the form of reduction of endogenous erythropoietin production has classically been considered to play an essential role in the pathogenesis of anaemia. It is therefore fundamental for an understanding of normal erythropoiesis to be set prior to embarking on attempts to classify the causes of renal anaemia.

Erythropoietin is a 165 amino acid glycoprotein containing four oligosaccharide chains (34). Erythropoietin is mainly produced by the kidneys, with liver being the other organ with erythropoietin-producing capabilities albeit in a lower concentration. Theories suggest that fibroblast like-interstitial cells surrounding the cortical and juxtamedullary tubules (peritubular cells), which are the areas of most oxygen consumption in the kidney, are responsible for erythropoietin secretion (34). Additionally epithelial cells, able to sense tissue hypoxia, could potentially variably secrete erythropoietin (34).

Erythropoietin is secreted in response to hypoxia-inducible factors (HIF) 1α and 2, which are released secondary to renal tissue hypoxia (34,35). Hypoxia-inducible factor binds to the erythropoietin gene, inducing transcription of mRNA and thereby the synthesis of erythropoietin; in essence HIF is the major transcription factor for the erythropoietin gene. Additionally, HIFs are associated with the genetic upregulation of erythropoietin receptors, transferrin, transferrin reception and the downregulation of hepcidin, potentially aiding in the mobilisation of iron and the reduction of inflammation that has additive effects on the development of anaemia (36). Hypoxia-inducible factors

are only active in the presence of hypoxia; they are otherwise hydroxylated through the action of prolyl hydroxylase domain proteins 1-3 (PHD 1-3) which specifically eliminate the α subunit (34,35). However, PHDs require oxygen and iron to operate, therefore in conditions of hypoxia, no degradation of HIF takes place leading to induction of erythropoietin production (35). Iron chelation has previously shown induction of HIFα protein stability highlighting a physiological cross talk exists between iron metabolism and erythropoietin (37,38). Other factors associated with the production of erythropoietin include norepinephrine and epinephrine, androgens and various prostaglandins (34).

Erythropoietin plays an essential role in the production of proerythroblasts, committed stem cells in the haematopoietic centres that are the pre-genitors of red blood cells. Erythropoietin binds to the specialised erythropoietin receptor on the erythroid progenitor cells and is a preeminent stimulus for the production, survival, proliferation, and differentiation of red cells (39). In addition, erythropoietin can accelerate erythropoiesis in the bone marrow (34). This continues either until hypoxia is resolved or until enough red blood cells exist to maintain oxygen delivery despite hypoxia. It is important to note that maturation of red blood cells, alongside oxygen carrying capacity of the newly formed red blood cells is not only controlled by erythropoietin but vitamin B12, folic acid and iron are also crucial in the formation and performance of red blood cells (34).

Erythropoietin concentration reduces relatively early in the course of CKD, however the deficiency becomes more evident as disease progresses at CKD stage 4 (eGFR < 30 ml/min/1.73m^2) (40). This reduction is brought about by a combination of reduced erythropoietin production and errors in erythropoietin sensing. As CKD progresses blood flow to the kidneys is reduced, and whilst originally this causes

an increase in HIF activity, eventually kidney tissue adapts in consuming less oxygen maintaining normal tissue oxygen gradient causing increased activity of PHD enzymes (41). In addition, CKD represents a state of inflammation, and experimental evidence suggests that inflammatory cytokines inhibit the hypoxia-induced erythropoietin production pathway (42,43). Inflammation is also important in the development of erythropoietin resistance through effects on erythroid progenitor cells, the downregulation of erythropoietin receptors expression on their surfaces, and erythropoietin antagonism (44,45). Additionally, CKD associated inflammation leads to increased hepcidin, causing downstream effects on iron metabolism, erythroid proliferation and erythropoietin resistance (46).

Iron plays a key role in metabolism affecting a number of reactions intracellularly and extracellularly (47). Iron is involved in the Krebs cycle leading to adenosine triphosphate formation, cellular respiration as part of the mitochondrial machinery, oxygen transport in haemoglobin and myoglobin and DNA synthesis (47). An iron recycling system exists within the body: most of the iron exists within haemoglobin (≈70%) or stored in the reticuloendothelial system and liver cells in the form of ferritin (15-30%). The remaining iron exists either as myoglobin, part of various haeme compounds or attached to transferrin and circulating in the blood plasma (33). However, as iron is an indispensable part of several reactions in the body, it has a number of deleterious effects as well (48). This is due to its ability to be an electron donor and acceptor, partaking in reactions that could trigger oxidative stress and free radical formation leading to cellular and tissue damage. Therefore, a tight regulatory process is necessary to maintain iron homeostasis.

Iron is obtained through the diet in the ferric form (Fe^{3+}) and is absorbed in the small intestine, mostly at the duodenum. The amount of iron obtained is enough to counterbalance the minimal iron loss occurring due to epithelial desquamation and/or bleeding (≈ 1-2 mg). Iron in the ferric form is difficult to be absorbed and therefore reduction takes place to the ferrous form (Fe^{2+}) at the apical aspect of the duodenal endothelium via the action of duodenal cytochrome B (33,49,50). The ferrous form is transported in the enterocyte through the divalent metal transporter 1. Haeme-bound iron can also be transported into the enterocyte via the haeme carrier protein 1. In the enterocyte, iron can be stored as ferritin or exported to bind with transferrin. The transfer of Fe^{2+} into the bloodstream takes place through the transporter ferroportin. Ferroportin is tightly regulated by the action of hepcidin – considered a “master-key” in iron homeostasis maintenance. Hepcidin is released by hepatocytes in response to raised iron stores in macrophages and inflammation, and at high levels promotes endocytosis of ferroportin, limiting the transfer of Fe^{2+} into the blood stream (46,50). Iron in ferrous form is thereby oxidised to ferric iron by the action of hephestin and binds to transferrin that is the main transport protein of iron in the body.

The flow of iron in the body is not limited to absorption through the duodenum. It includes iron recycling and mobilisation from senescent erythrocytes within macrophages and ferritin in macrophages and hepatocytes. The export of iron through these channels also requires the action of ferroportin, therefore regulated by hepcidin. Iron in the plasma is bound to transferrin to travel to the haematopoietic centres, peripheral tissues and the liver (46,50).

Fundamentally, iron is a cofactor for haeme formation and aids in oxygen transport. Haeme is in turn essential for haemoglobin

formation, the oxygen-carrying molecule of the body, as it is composed of four haeme chains around globin.

Once the cell cycle of red blood cells is complete, macrophages recover iron from senescent red blood cells through phagocytosis. The haeme aspect of haemoglobin is degraded via haeme oxygenase. The iron not used within the cells, will either be stored as ferritin or be exported back into circulation through ferroportin channels expressed on macrophage membrane, re-entering circulation as part of the recycling process explained above (50).

A distinct relationship exists between hypoxia, erythropoiesis and iron metabolism with crosstalk at various levels. The hepcidin-ferroportin axis and the hypoxia-inducible factor regulatory pathway are heavily dependent on iron concentrations (51). Increased iron stimulates the action of PHD enzymes, which catalyse HIFs and lead to decreased erythropoietin production. Simultaneously, hypoxia is a triggering factor in increased absorption and mobilisation of iron to haematopoietic centres through the action of HIFs enhancing duodenal absorption and action of ferroportin, and downregulating hepcidin production (52).

The discovery of erythroferrone has identified new links between erythropoieisis, hepcidin and iron homeostasis (53). Erythroferrone maximises iron acquisition, through suppression of hepcidin production. It is released by erythroid precursor cells, following erythropoietin stimulation (50). The presence of erythroferrone additionally complicates iron metabolism, as it appears that erythropoiesis, inflammation, hypoxia and iron status all regulate iron-related pathways.

The pathophysiology of CKD can therefore interact with iron metabolism, leading to either absolute or functional iron deficiency (54). Iron metabolism in CKD is affected by increased hepcidin, potential enhanced losses and the increased use of ESAs (55). Absolute iron deficiency occurs where iron stores are inadequate to maintain erythropoiesis or absent, whilst functional (relative) iron deficiency is defined by normal total body iron stores that are not efficiently utilised due to a 'block' of the iron pathway (49).

As anaemia and iron deficiency play an integral role in disease progression, complications of comorbidities, development of complications and affects the quality of life of patients, it is important for them to be screened for, and managed accordingly in this patient group.

1.1.3: Investigations of CKD associated anaemia and iron deficiency

KDIGO defines anaemia following the same principles as the World Healthy Organisation (haemoglobin < 130 g/L in males, <120 g/L in females); NICE and the Renal Association in the United Kingdom suggest investigations and evaluation of anaemia if haemoglobin is <110 g/L irrespective of gender or if they have symptoms consistent with anaemia (2,10,56). The frequency of investigations is dictated by the eGFR. As part of investigations it is important to identify the true haematological picture (through full blood count, paying attention to red cell indices) and iron status (through a combination of tests that could include percentage of hypochromic cells, reticulocyte haemoglobin content or the most widely available options of TSAT and ferritin).

Both NICE and Renal Association guidelines identify iron deficiency in terms of absolute iron deficiency as those being serum ferritin < 100 µg/L and TSAT < 20% in ND-CKD (2,56). Functional iron deficiency can be defined as ferritin 100-500 µg/L and TSAT <20%. The treatment targets triggering supplementation are delineated in Appendix table 4 (54).

An essential step prior to the initiation of management of anaemia is the exclusion of any pathological process contributing to the anaemia alongside with the identification of anaemia type, as that can be key in non-response to treatment. This actually forms part of the Renal Association guidelines (1B) and the KDIGO guidelines (not graded). Other causes of anaemia, include vitamin B12 and folate deficiency, haemolysis, multiple myeloma, and occult blood loss secondary to malignancy (10,56).

1.1.4: Management of anaemia of CKD and iron deficiency

Contemporary management is based on targeting the haematopoietic deficiencies that arise due to CKD through ESA and iron (10). Red blood cell transfusions remain an option especially where correction of anaemia is urgently required, such as active bleeding or unstable ischaemic heart disease. Newer agents such as HIF-PH inhibitors have been developed as modulators, therapeutic agents that target the erythropoietin pathway and could lead to greater erythropoietin production without administration of exogenous erythropoietin/erythropoietin-stimulating agents (57).

Erythropoietin / erythropoiesis stimulating agents

The purification of human erythropoietin in 1977 revolutionised the management of anaemia of CKD enabling the production of ESA, which are based on recombinant human erythropoietin. Indeed, ESA have remained in the cornerstone of management of renal anaemia since the late 1980s (58).

There are three generations of ESA available; newer generations are benefited from a longer half-life and greater stability compared to the original generation enabling greater spacing between administrations (58,59). These act through stimulation of the erythropoietin receptor and can be administered intravenously or subcutaneously in different regimens (58,59).

Despite the benefits of treatment with ESA in a number of clinical aspects (such as avoidance of blood transfusions and improvement in symptomatology), there is no strict consensus between Associations on the use of specific targets for haemoglobin concentration (7). Intriguingly most guidelines exploring this notion avoid any “recommendations” and guide modern clinicians through the subject via “suggestions”. KDIGO suggests that in adults ESA should not be commenced with a haemoglobin > 100 g/L (10). The Renal Association and NICE do not specify an initiation point, however they both underline the importance of excluding absolute iron deficiency prior to the initiation of management with ESA, as iron deficiency plays an integral role in hyporesponsiveness towards these agents (2,56). All guidelines follow a cautious approach into the treatment of anaemia using ESA, with suggestions of review of their use once haemoglobin concentration reaches 120 g/L as per NICE and Renal Association and 115 g/L as per KDIGO (2,10,56). These suggestions are based on evidence from three large randomised controlled trials (RCT) which failed in identifying improved outcomes with higher haemoglobin targets, but noted the possibility of increasing side effect incidence

where higher haemoglobin targets were set (60–62). Concerns surrounding the use of ESA revolve around increased cardiovascular event risk (e.g., strokes) secondary to potentially greater blood viscosity (7). Additionally historic links of ESA with cancer (progression and recurrence), despite lack of in vivo evidence have casted doubt on their liberal use (63). Another side effect associated with the use of ESA includes worsening hypertension that could lead to hypertensive encephalopathy or seizures in extreme cases (59). An extremely serious and rare complication of ESA use is pure red cell aplasia secondary to antibodies against erythropoietin acting in the bone marrow (58,59). Erythropoiesis stimulating agents are also affected by hyporesponsiveness and loss of response. Hyporesponsiveness to ESA is the lack of response following one month following initiation of treatment, and this can be due to a number of causes (Appendix table 5). Loss of ESA response on the other hand, should be considered where despite subsequent increase in ESA doses, the concentration of haemoglobin is not maintained at a level previously achieved with a lower ESA dose (7,64).

Erythropoiesis stimulating agents, therefore, should be used proactively but with caution aiming to minimise the impact of anaemia on patients without full haemoglobin normalisation.

Red blood cells transfusion

Prior to the advent in use of ESA therapy, red blood cells transfusion was fundamental in the management of the extreme symptomatology of renal anaemia. A number of guidelines suggest the avoidance of red blood cell transfusion unless absolutely necessary, such as in cases there is intolerance of contraindication to any other treatment offered (iron, ESA) or have bone marrow failure (10,56). A number of

factors exist why red blood cell transfusion is not a preferred treatment in the era of ESA and other modes of management (Appendix table 6).

Hypoxia inducible factor prolyl-hydroxylase inhibitors (HIF-PHI) and future therapies

Hypoxia inducible factor prolyl-hydroxylase inhibitors are oral medications, which may be beneficial especially in ND-CKD and peritoneal dialysis dependent patients, with potential treatment benefits compared to ESA in terms of greater response closer to the physiological ranges (65).

The gathered interest in the use of HIF-PHIs has led to extensive studies taking place, indicating that HIF-PHIs led to increases in haemoglobin concentration, erythropoietin concentration and decreased hepcidin concentration, and potential signalling benefits through the up-regulation of the HIF-pathway both with regards to erythropoietin and iron metabolism (65–67). A global study (n=922) monitored both the efficacy and safety profile of roxadustat against placebo in ND-CKD. Over a total of 52 weeks, the haemoglobin mean change was significantly larger than roxadustat, with a greater proportion of patients achieving response by week 24 (roxadustat: 86% (95% confidence interval (CI): 83.0 – 88.7%) vs. placebo: 6.6% (95% CI: 4.1% - 9.9%); $p<0.001$). The incidence of treatment related adverse and serious adverse events was comparable (68). Similarly a pooled analysis of roxadustat use in patients on dialysis (n=1,530, 3 phase III studies) indicated a significantly greater mean change ($p=0.0013$) and lower risk of major adverse cardiovascular events with roxadustat compared to epoetin alfa (69). Daprodustat has been evaluated in both HD-CKD and ND-CKD as part of the ASCEND-D

and ASCEND-ND randomised open labelled controlled trials, comparing daprodustat with darbapoietin-alfa (total n=6,836) (70,71). In both studies, daprodustat was non-inferior to ESA for both haemoglobin increase and major adverse cardiovascular event; interestingly better iron utilisation was observed with daprodustat in patients with ND-CKD as that was suggested by a greater decrease in hepcidin and TSAT (70,71). A meta-analysis (n=2,804) indicated greater change from baseline in terms of haemoglobin concentration irrespective of renal replacement mode and significant heterogeneity across included trials (72). In addition, they noted significant reduction in hepcidin and reduced ferritin. The incidence of adverse events was similar between groups. Risk of bias analysis took place, with no risk identified in terms of publication; however significant heterogeneity was noted (72).

The use of such agents has potentially pleiotropic advantages. They can cause a physiological response leading to endogenous production of erythropoietin, avoiding the potential of overshoot while also addressing iron metabolism (73). Furthermore, they are orally administered, which is important in terms of compliance, convenience, and avoidance of any pain/discomfort of administration (74). In addition, their production and administration are cost-effective compared to the current costs relevant to ESA, while also causing reduced ESA needs, alongside with improved iron economy and potential limitation of the need of intravenous iron use (66,67,72–74). Given these potential benefits, the use of HIF-PHIs has been adopted in Asia as highlighted in the recent Asian Pacific Society of Nephrology guidelines on the management of renal anaemia (75). The guidelines recommend that HIF-PHIs are an appropriate alternative to ESA therapy both in non-dialysis and dialysis-dependent CKD patients, and that treatment should be initiated once absolute iron deficiency is resolved. A similar approach has been witnessed in the United Kingdom with the recent approval of the use of roxadustat in the

management patients with CKD stages 3-5 and anaemia without iron deficiency by NICE (76).

1.1.5: Iron deficiency anaemia – iron supplementation

Iron supplementation and alleviation of iron deficiency are helpful in minimising and limiting the need for blood transfusion and ESA use in CKD. Iron therapy is at the forefront of anaemia management with all guidelines suggesting treatment with iron prior to initiation of ESA where iron deficiency exists (2,10,56,77,78). In HD-CKD, intravenous iron plays a mainstay role, while in patients that are non-dialysis dependent, or receive dialysis through the peritoneal route, a trial with oral iron can be first attempted.

Oral iron

A trial with oral iron therapy appears reasonable in the ND-CKD population given its inexpensive nature, ease of administration, elimination of continuous hospital interaction and effectiveness (79). Nonetheless, oral iron in the form of the widespread ferrous salts is accompanied by poor response due impaired absorption and mobilisation, poor tolerability and increased interaction risk with other drugs that can affect the pH of the stomach (8). The absorption of oral iron in CKD is limited by the increased concentration of hepcidin, and a significant proportion of it remains in the gut lumen (93%) with potential negative connotations to the gut flora and endothelium (79,80). Gastro-intestinal side effects therefore are frequent and translate into poor adherence. A systematic review and meta-analysis of 43 RCTs (n=6,831) noted that ferrous sulphate (a ferrous salt supplementation) was associated with a significant increase of side

effects (including nausea, constipation, abdominal pain, dark stools etc.) irrespective of population or dose (81). Longer treatments of up to 6 months may be necessary to replete iron stores adequately (47). Novel oral iron preparations have been developed to address these problems such as ferric citrate, ferric maltol and sucrosomial iron with increasing popularity (82); alternative regimes have also been described to minimise the impact of the hepcidin/ferroportin axis on absorption (83).

In an effort to by-pass the effect of hepcidin on iron absorption through its effect on divalent metal transpore 1 receptors, heme iron polypeptides were developed. Despite small-scale early positive outcomes regarding haemoglobin concentration and ESA efficiency in a prospective study in HD-CKD against placebo (n=34), this was not translated in an open RCT in patients on peritoneal dialysis against alternative oral iron preparation (n=62) (84,85). A further review of the topic yielding three clinical studies indicated no benefit of heme iron polypeptide over traditional treatment could be identified, while an increasing cost was incurred through their use (86). The area of oral iron supplementation and its unmet needs are still under investigation. Further research is undergoing on utilisation of bioengineering and nanotechnology for the creation of novel oral iron formulations (47).

Intravenous iron preparations

Intravenous iron has been utilised in the management of iron deficiency for over a century. Original ferric hydroxide preparations were linked to unacceptable levels of toxicity due to their high levels of labile iron release (47). To counter this, carbohydrate shells have been used in the development of further iron compounds. An unacceptable level of severe hypersensitivity reactions and well-documented

dextran-induced anaphylaxes limited their use and led to extreme caution in the medical community (47). As such further developments took place with newer preparations developed in 1990s and late 2000s, with each generation characterised by stronger carbohydrate cores, leading to more controlled release of labile iron (79). Second generation intravenous iron preparations (iron sucrose, low-molecular-weight iron dextran) are limited to their dose, as they are associated with a relatively high amount of non-transferrin bound iron; this leads to a need for several infusions for replenishment to be successful. Third generation intravenous iron preparations (ferric carboxymaltose (FCM), ferric derisomaltose (FDI) and ferumoxytol) are characterised by an ability to slowly release iron, limiting non-transferrin bound iron concentration. Both second and third generation intravenous iron prepartions are currently licensed for use in the CKD population, with all displaying similar efficacy in terms of iron deficiency correction (8).

Chemistry and pharmacodynamics of intravenous iron

Intravenous iron preparations consist of an iron core (typically comprised by ferrous (Fe^{2+}) molecules), surrounded by a carbohydrate ligand within a colloidal suspension (47). These products essentially act as pro-drugs, retaining iron until metabolism of the carbohydrate ligand, therefore controlling iron release and limiting iron-associated injury. The administration of intravenous iron bypasses the intestine and therefore does not rely on the hepcidin/ferroportin axis for absorption. Instead, following infusion the iron complex is endocysed the macrophages within the reticuloendothelial system. Within the macrophage, endolysosomes form with an acidic pH causing iron release from the iron-carbohydrate structure. This is the point where the carbohydrate ligand is vital, as the type and concentration of the ligand is highly relevant to the reaction occurring and iron release following acidification. The precise mechanism of iron release is not

well defined, with theories also suggesting that the type of macrophage plays a part in the quantity of iron released. As iron is released from the complex it becomes part of the labile iron pool within the macrophage – there it is either stored as ferritin or exported to the plasma by ferroportin to be incorporated with transferrin and be transferred where it is necessary. Iron that enters circulation unbound to transferrin, exhibits the same pro-oxidant properties as labile iron, and is able to part-take in the Fenton and Haber and Weiss reactions leading to the creation of free radicals (87). The carbohydrate ligand and the size of the core are not only important in relation to the macrophage-associated iron release. A more tightly compact, heavier core limits the release of iron in the blood stream prior to macrophage uptake. As such, the generation of large amounts of labile plasma iron is restricted, minimising the possibility of immediate reactions to the administration of iron, and the generation of oxidative stress.

Modern intravenous iron products (FDI, FCM, ferumoxytol) utilise the properties discussed above, and are iron-carbohydrate complexes that are able to provide high doses of iron in a stable, non-toxic form. The differential attribute and unique feature of each preparation is indeed the carbohydrate ligand that it carries, which in turn affects stability, immunogenicity and modes of iron release (47). A striking difference between second and third generation compounds is the size of the carbohydrate ligand and overall molecule. This in turn improves stability, iron release and immunogenicity, rendering third generation preparations able to deliver large doses of iron on a single sitting (47). The biochemical particularities and properties of each compound differ, and these differences rely on the iron-carbohydrate structure, both in terms of architecture and carbohydrate ligand used. Ferric derisomaltose is characterised by a shorter, linear non-ionic structure, which is unlike the classical iron core-carbohydrate shell alignment. It is composed of matrices created through the arrangement of interchanging layers of linear derisomaltose and iron atoms (88).

Ferumoxytol is based on a different iron molecule compared to FDI and FCM. Ferumoxytol has an iron-oxyhydroxide molecule based on magnetite, whereas the other two have a structure similar to akaganeite (88). Unlike FDI, and ferumoxytol, the cores of FCM tend to cluster together, and have the propensity to release iron depending on pH of solution (88). These differences are subtle; however explain some phenomena akin to each preparation. In vitro evidence analyses have indicated that FDI has a 7-fold lower non-transferrin-bound iron compared to FCM, likely secondary to its matrix structure, which allows for a longer half-life (20.3 hours vs. 6.82 hours) (89). In addition, this may explain why ferritin levels appear to be higher with FCM compared to FDI imminently following administration without reflecting an increase in bioavailable iron (90). In vivo studies have also underlined the differences in half-life with FCM having the shortest (7-12 hours), followed by ferumoxytol (15 hours) and FDI (27 hours) and have highlighted that at least in vivo FDI appears to be the most stable compound out of the three (90).

Clinical efficacy and other benefits of intravenous iron

A number of studies have highlighted the potential superiority of intravenous iron when compared to oral iron in achieving an appropriate and early erythropoietic response in patients with CKD. Two large-scale systematic reviews and meta-analyses have highlighted the potential benefits of using intravenous iron relevant to superiority of treatment when compared to oral iron (91,92). A research group performed two systematic reviews and meta-analyses in 2008 and 2015; the updated review included only RCTs irrespective of language and set as a primary outcome the percentage of patients reaching an increase in haemoglobin > 10 g/L. The group also looked into other outcomes such as need for ESA, blood transfusion and iron profile, while also assessing safety. The study incorporated both ND-

CKD and HD-CKD patients (24 studies, n=3,187) and identified that patients treated with intravenous iron were more likely to reach the primary outcome (ND-CKD risk ratio (RR): 1.61 (95%CI: 1.39-1.87), HD-CKD RR: 2.14 (95%CI: 1.68-2.72)) (91). This trend in benefit remained irrespective of iron formulation. Similar rates of adverse events and mortality were noted, however intravenous iron was associated with a greater risk of hypotension but fewer gastrointestinal side effects compared to oral iron preparations. Significant heterogeneity was noted in the review, while the authors also commented on the short follow-up period nature of the studies. On comparison of the results of this meta-analysis to current practice, it is also important to acknowledge that third generation intravenous iron preparations were not represented heavily in the sample of trials reviewed (91). A later systematic review and meta-analysis conducted by the Cochrane network included 39 randomised controlled and quasi-randomised controlled studies (n=3,852) both of adults and children with CKD stages 3-5D (92). The review focused primarily on mortality and quality of life and secondarily on markers of response (haemoglobin, iron profile, ESA use) and other markers of safety. No difference was noted in any of the primary outcomes, indicating no superiority or inferiority of intravenous iron to oral iron, in terms of death, need to initiate dialysis or number needing blood transfusion. Five studies were also analysed in terms of quality of life, with four of them reporting no difference between treatment groups, but one favouring the use of intravenous iron. The group also identified a potential of greater number of patients achieving haemoglobin target or increased haemoglobin and decreased need to ESA. Nonetheless, the certainty of evidence in both cases as low. In this review, the authors highlighted the high degree of heterogeneity in the studies included, and the need for further research on the field, focusing on patient reported outcome measures (92).

From these systematic reviews and meta-analyses intravenous iron preparations represent a more efficacious treatment of iron deficiency in CKD, at least in terms of haematopoietic response. The rapid nature of repletion and potential decrease of ESA requirements may also have positive implications in relation to cost-effectiveness and treatment adherence (93). Evidence from cost-effectiveness analyses in HD-CKD and ND-CKD point towards that direction, with intravenous iron being more economical than oral iron in maintenance and achieving improvement of anaemia irrespective of the use of ESA (94,95). More specifically upon comparing ferumoxytol (a third generation intravenous iron, similar to FDI and FCM) with oral iron alongside ESA in ND-CKD, ferumoxytol was identified as more cost-effective than oral iron both when used as a monotherapy and when combined with ESA. This was more evident upon comparing incremental costs per g/dL increase in haemoglobin (95). Using a similar approach, a study within the dialysis setting highlighted that on comparing oral with intravenous iron, the possibility of achieving a greater haematopoietic response early with its accompanied improvement in quality adjusted life years, led to greater cost-effectiveness despite the costs incurred through administration (94). Therefore intravenous iron poses a sensible route-of-administration and this is reflected in the KDIGO guidelines (number 2.1.4) which state that in ND-CKD patients "*who require iron supplementation, one should select the route of iron administration on the basis of the severity of iron deficiency, availability of venous access, response to prior oral iron therapy, side effects with prior oral or IV iron therapy, patient compliance, and cost (not graded)*" (10).

The dose of intravenous iron prescribed, alongside the target aimed for have been the subject of controversy for a number of years. Evidence now indicates that iron repletion targeting higher iron values or through administration of higher doses may have beneficial results in terms of cardiovascular mortality, both in HD-CKD and ND-CKD

(96,97). The landmark randomised open-label Proactive IV Iron Therapy in Haemodialysis Patients (PIVOTAL) trial included more than 2,000 HD-CKD patients receiving intravenous iron. Patients were randomised to receive iron either proactively (high cumulative dose) or reactively (low cumulative dose) and were followed up of over a median of 2.1 years. A significantly reduced composite of death, myocardial infarction, cerebrovascular event or heart failure hospitalisation existed in those receiving higher cumulative doses (19.4 per 100 patient years vs. 24.6 per 100 patient-years) was witnessed (97). There was no significant difference in infection rate between the two groups (97). Heart failure events (both fatal and non-fatal) were reduced by 34% in patients receiving higher doses, which was also associated with a 44% reduction in heart failure hospitalisation. The incidence of first heart failure event was significantly lower in the higher dose compared to the lower dose group (98). In the same cohort 8.4% of patients had a myocardial infarction over 2 years, with a significant reduction in composite endpoint of non-fatal and fatal myocardial infarction and non-fatal myocardial infarction on comparing high dose iron regime to low dose iron replenishment (99). In addition, an open-label randomised comparative trial (n=1,538) comparing FDI (1 dose: 1000 mg) and iron sucrose (5 doses: 200 mg each) concluded that there was a reduced incidence of composite cardiovascular adverse events with FDI (4.1% vs. 6.9%; p=0.025) (96). The time to first composite cardiovascular adverse event was significantly longer with FDI, indicating that possibly faster iron repletion is associated with improved cardiovascular outcomes (96). Such results indicate the potential cardioprotective nature of iron repletion and anaemia resolution in patients with CKD, and tie well with evidence from an earlier RCT conducted by Toblli who studied forty patients with reduced creatinine clearance and heart failure with reduced ejection fraction, and randomised them to receive either weekly iron sucrose or placebo. By the end of the 6-month trial, patients receiving iron sucrose had a significantly lower NT-proBNP compared to their counterparts (117.5

+/- 87.4 pg/ml vs. 450.9 +/- 248.8 pg/ml; p < 0.01) with improvements in quality of life and functional status (100).

As indicated by the work of Shepsphelovich and colleagues and O'Lone and colleagues, despite evidence of improved haematopoietic response given intravenous iron in CKD, no strong evidence exists highlighting the impact of intravenous iron on the quality of life in this population (91,92). Therefore, in terms of quality of life, researchers rely on the experience of patients with heart failure following intravenous iron administration (27). The Ferinject Assessment in Patients with Iron Deficiency and Chronic Heart Failure (FAIR-HF) trial (n=459, heart failure with reduced ejection fraction) noted significant improvement in quality of life and 6-minute-walk-test within 24 weeks of iron administration irrespective of presence of anaemia (101,102). The results led to changes in the current guidelines of management of heart failure. The utility of iron administration in iron deficiency in heart failure, has also been recently demonstrated in patients with acute heart failure. In the randomised double-blinded placebo controlled AFFIRM-AHF (n=1,058) inpatient administration of iron following an acute episode of heart failure decompensation was associated with better health related quality of life outcomes as early as 4 weeks, and up to 24 weeks following hospitalisation (103). Similarly, intravenous iron has been associated with improvement in quality of life in a number of clinical conditions such as cancer and autoimmune pathologies, potentially through the alleviation of anaemia (104). Improvements in quality of life in patients with CKD have also been demonstrated irrespective of dose. The observational NIMO-CKD UK (n=258) noted a significant improvement in the FACIT-Fatigue score following administration of iron independent of dose (mean change from baseline: 7.1; p<0.0001) (105). Similarly, the RCT IRON-CKD, demonstrated an increase, but non-significant change in all domains of Short Form 36 questionnaire following administration of intravenous iron, irrespective of dose and preparation (87).The multi-centre RCT

Iron & Heart Study (n=54) aimed to primarily identify any potential improvement of functional status in iron deficient CKD patients. Upon comparing FDI with placebo there were no statistically significant difference between the two arms in 6-minute-walk test results (1 month: p=0.74; 3 months: 0.74). Nonetheless, a non-significant increase in the 6-minute-walk test distance was recorded within the FDI arm (106). Such changes may be attributed to the mechanistic benefits of intravenous iron and correction of iron deficiency on skeletal muscle function and mitochondrial energetics. Charles-Edwards and colleagues have previously displayed in a RCT composed of 40 patients with reduced ejection fraction and iron deficiency that FDI was associated with significantly improved half-life of both adenosine triphosphate and phosphocreatinine compared to placebo implying improved mitochondrial function (107). This was further associated with significant improvements in the New York Heart Association Class, resting respiratory rate and post-exercise Borg dyspnoea score (107).

Despite the progress made in the development of new, third generation, intravenous iron preparations (Appendix table 7), and their widespread use in CKD and other conditions, concerns still exist regarding the potential complications associated with its use (figure 2) (8,47).

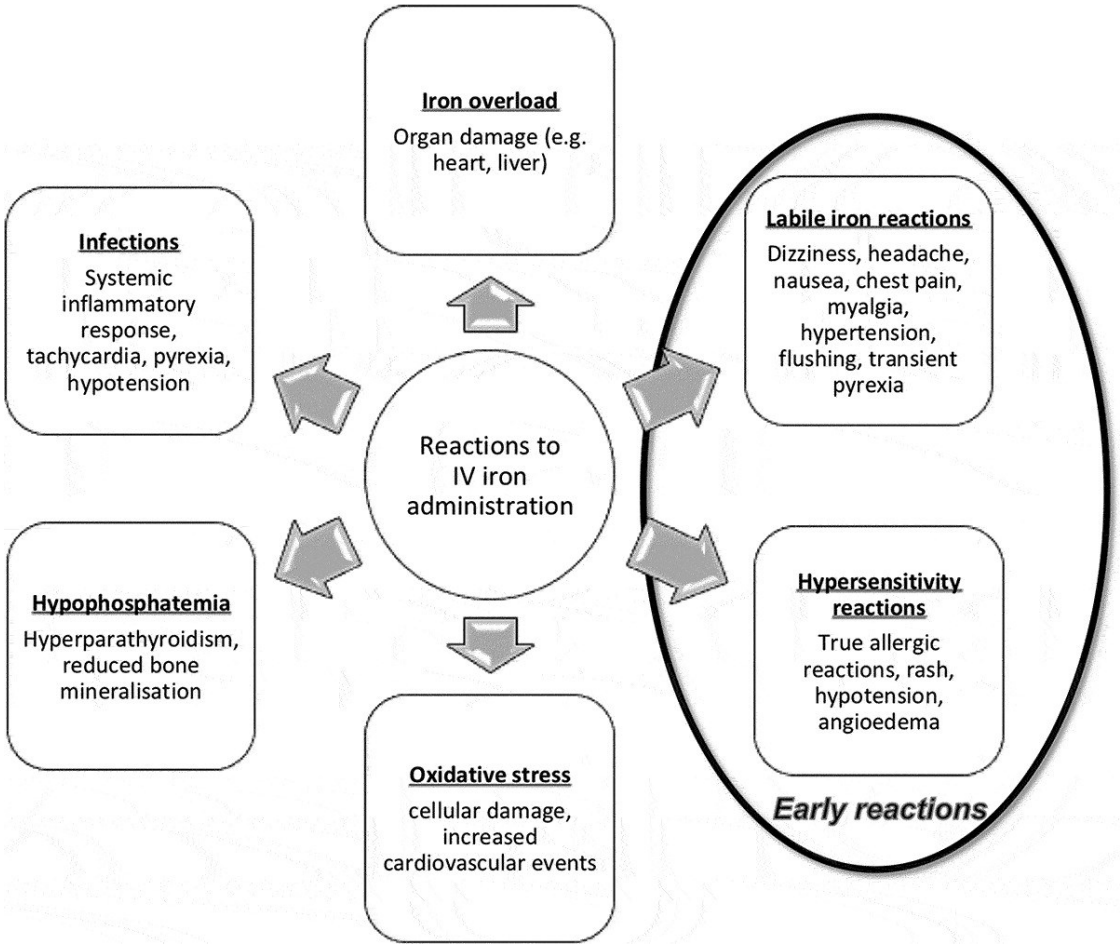

Figure 2: Concerns regarding intravenous iron use (8)

1.1.6: Complications of intravenous iron administration

On addressing and considering the safety of intravenous iron preparations, one could categorise complications into short-term and long-term side effects (8). It is also important to recognise that certain distinct side effects are either generation or preparation-specific, secondary to similarities and differences of their structure (8).

Early complications: hypersensitivity/anaphylactic reactions and labile iron reactions

Early complications are associated with the structure and stability of the iron-carbohydrate complex of each preparation (8). A great and rapid iron release in circulation can overwhelm the availability of transferrin leading to a greater amount of non-transferrin bound iron, yielding hypersensitivity and labile iron (8). Moreover, the carbohydrate ligand itself could represent an anaphylaxis and hypersensitivity trigger, with historical concerns associated with dextran-based compounds (8).

Wang and colleagues identified that the relative risk of anaphylaxis was that of one event in every 1,500-4,000 infusions, irrespective of preparation (108). A more recent pooled analysis of 5 large RCTs (n=5,247) identified no difference in the incidence of hypersensitivity reactions between the preparations with low incidence of moderate, severe or serious hypersensitivity reactions (0.2-1.7%) (109). Given the similar rate of incidence of such reactions in dextran-based (FDI, ferumoxytol), and non-dextran based (FCM, iron sucrose), the authors derived no associated between ligand and reaction (109). A similar incidence of hypersensitivity reactions was noted through real-world evidence in CKD patients receiving low-molecular weight iron dextran (110). A recent indirect comparison (21 prospective trials; n=8,599) between FCM, FDI and iron sucrose highlighted a low incidence was noted with all the involved preparations, especially with FDI upon comparison with FCM and iron sucrose (Odds ratio using Bayesian approach: 0.41 and 0.51 respectively) (111).

The sudden surge of non-transferrin bound iron can also be associated with labile iron reactions, also known as ‘Fishbane’ phenomena (8,90). These reactions are classically transient events that present as chest pain, erythema and myalgia, and without the characteristic hypotension associated with true anaphylactic responses. Different theories have been postulated relevant to the

pathophysiology of these reactions and involve complement activation-related pseudo-allergy processes, mast cell activation and labile iron (8). As they can occur on first exposure, it is unlikely that they are mediated by immunoglobin-E (8). Such events tend to be short-lived and transient and resolve through decreasing the rate of infusion (90).

Despite the encouraging evidence surrounding the use of intravenous iron, concerns regarding the possibility of anaphylaxis remain. International guidelines advocate that intravenous iron should be given in an environment with resuscitative facilities available and appropriately trained personnel in the proximity (10). Given the interest surrounding the area, useful algorithms have been created outlining the management strategy of acute hypersensitivity reactions (109,112).

Late complications

Late complications can be secondary to specific iron compounds or due to long-standing iron accumulation in the body. Iron can be deleterious, secondary to its pro-oxidant nature, the potential for direct cell injury, and its involvement in inflammation. The potential for iron overload also exists, due to iron deposition to various organs of the body. Additionally, concerns exist, regarding the potential of iron to act as catalyst in bacterial growth, yielding higher infection incidence. Finally, iron preparations exert compound-specific side effects, unrelated to the iron within the iron carbohydrate complex, as in the phenomenon of drug-induced hypophosphataemia (8).

Labile plasma iron can result from the administration of intravenous iron. Earlier compounds may ‘leak’ iron prior to endocytosis by macrophages; in addition, as transferrin becomes saturated (>65%) following release of ferric iron from the reticuloendothelial macrophages, a greater proportion of it remains non-transferrin bound (47). This iron is available to part-take in certain reactions (Fenton and Haber-Weiss reactions) yielding free radicals and radical oxygen and nitrogen species. These, in turn, generate oxidative stress affecting various processes through atherosclerosis including cardiac, vascular, renal and cognitive function (47). Beyond the notion of increased oxidative stress, free iron can be directly cytotoxic, especially to the kidneys (48,113). More specifically considering the kidneys, oxidative stress could be harmful to the renal parenchyma, renal vasculature and cells directly. Iron is implicated in regulated cell death, through ferroptosis, necroptosis and pyroptosis (114). The fact that proximal tubule cells are rich in ferritin, may therefore suggest that increased iron within, can lead to such detrimental effects to renal function, acute kidney injury and CKD progression (114).

Transient elevations of biomarkers of oxidative stress have been previously observed in vitro and in vivo studies, with potential links to cardiac and renal toxicity (113,115–117). Agarwal and colleagues previously demonstrated transient proteinuria secondary to iron sucrose administration, as that was exhibited 15 minutes following administration in ND-CKD (118). In a further randomised trial (n=136, ND-CKD) comparing intravenous iron sucrose with oral iron, that was primarily focused on renal dysfunction, the same group identified an increased relative risk of serious cardiovascular events in patients treated with intravenous iron (119). Further analysis from the CKDOPPS consortium (n=32,435) monitoring the association between iron and mortality in patients on haemodialysis, identified a greater hazard ratio of mortality (including cardiovascular) and hospitalisation with increasing intravenous iron dose (120). This

analysis however was observational in nature, with inherent limitations and bias. Nonetheless, such results stimulated further research in order to identify whether pathogenic effects could be exerted by intravenous iron use. A large number of RCTs and observational studies specific to CKD have produced evidence superseding these results (8). The large-scale RCTs in ND-CKD, FERWON-Nephro and FIND-CKD (combined n=2,164), indicated improved cardiovascular results. FIND-CKD compared oral iron with FCM (two doses: high and low) and identified that over the course of the study (1-year) the incidence of adverse events and cardiac disorders was similar between the groups formed (121). Similarly, FERWON-Nephro compared FDI with iron sucrose (1000 mg x 1 dose vs. 200 mg x 5 doses) and identified significantly smaller rate of cardiovascular events in the high dose group (4.1%) vs. the iron sucrose group (6.5%) (96). Taking in consideration the potential nephrotoxic nature of intravenous iron, FIND-CKD also monitored renal function. Over the course of 52 weeks, eGFR was noted to be 35.6, 32.1 and 33.4 ml/min/1.73m^2 in the high ferritin FCM, low ferritin FCM and oral iron, from 32.8, 31.5 and 32.3 ml/min/1.73m^2 at baseline respectively (121). An analysis of the FAIR-HF RCT, which was specifically designed in patients with heart failure with reduced ejection fraction and iron deficiency, identified that the use of intravenous iron (FCM) was associated with improved eGFR compared to placebo throughout the study (2.93 +/- 1.44 ml/min/1.73m^2 (p=0.0039)) (122). Mechanistic evidence from Kassianides and colleagues indicate that intravenous iron is not associated with prolonged oxidative stress or renal impact (87). In a RCT of 40 ND-CKD patients comparing different intravenous iron preparations and doses (iron sucrose, low-molecular weight iron dextran, 200 mg FDI and 1000 mg FDI), no resultant differences between markers of oxidative stress (Thiobarbituric acid reactive substances) were noted over the course of three months (87). In addition, the Iron and Heart multicentre RCT (n=58) noted in a secondary analysis a non-significant decrease in markers of oxidative stress (F2-isoprostanes, thiobarbituric acid reactive substances)

following administration of high dose FDI in patients with ND-CKD and iron deficiency (123). No detriment to renal function or nephrotoxicity was identified at either of these studies in agreement with the results of FIND-CKD – more specifically looking at renal function markers through creatinine and cystatin C and markers of renal injury through proteinuria and serum neutrophil gelatinase-associated lipocalin (124). The results of these trials confirm the safety profile of new intravenous iron products. The observations made could be secondary to the iron-carbohydrate molecule representative of the newer compounds that limits labile iron release. Additionally, the alleviation of iron deficiency with/without anaemia may itself be anti-oxidant, a notion previously supported in murine experiments (125).

Narrative reviews have provided valuable insights from in vitro studies on the mechanisms in which iron may play a key role in the infectivity of pathogens (126,127). Observational studies in patients with haemochromatosis suggest that they are more susceptible to bacterial infections (128). The KDIGO 2012 guidelines, have suggested avoiding administration of intravenous iron where active systemic infection exists (64). A systematic review and meta-analysis of RCTs of patients of varying comorbidities (72 trials, n=10,605) suggested an association between relative risk of infection and intravenous iron administration when compared to oral or no iron provided (RR: 1.33 (95% CI: 1.10-1.64) (129). A large observational study in haemodialysis (n=117,050) identified higher risks of hospitalisation for infection or death with higher doses (hazard ratio (HR): 1.05; 95% CI: 1.02-1.08) (130). However, there are conflicting studies relevant to the topic, with further research on the topic providing re-assuring answers on the safety of intravenous iron (8). Evidence from CKD indicates safety. The FIND-CKD multicentre RCT comparing FCM and oral iron identified no such links, similar to results published earlier in ND-CKD by Qunibi and colleagues also comparing FCM to oral iron (121,131). An observational study in 22,820 HD-CKD adults that were

hospitalised due to bacterial infection highlighted that inpatient administration of intravenous iron did not affect hospital outcomes, in terms of mortality or length of stay (132). In addition, two large observational studies which included over 55,000 patients on dialysis failed to identify any association between dose and infection related hospital admission or mortality (120,133). Finally, a comprehensive review and meta-analysis investigating the safety of intravenous iron (103 RCT, n=10,390) concluded that there was no increased risk in infection rates following intravenous iron (134). Similar to oxidative stress, the relationship between infections and intravenous iron may be preparation dependent, as experimental studies have shown potential for modulation of monocyte differentiation to macrophages and mature dentritic cells through iron sucrose and no other preparations (FCM, FDI low-molecular weight iron dextran and ferumoxytol) (135,136). This in vitro evidence was replicated in the Randomised Trial to Evaluate IV and Oral Iron in CKD (REVOKE) trial where in the secondary analysis a significantly greater incidence of infections in patients receiving intravenous iron (iron sucrose) compared to oral iron was noticed (37 episodes in 19 patients vs. 27 episodes in 11 patients; $p<0.006$) (119). In HD-CKD, a comparative study between ferric gluconate and iron sucrose (n=66,207) identified increased risk for infection-related death and infection-related hospitalisation with iron sucrose (137). It is essential to bear in mind the bi-directional nature of the relationship between iron and susceptibility to infection. Iron is important for cell growth and therefore represents a part of innate immunity and host response (138). Furthermore, iron deficiency anaemia can trigger impaired immunity leading to a greater vulnerability to infections (139–141).

As part of iron metabolism and homeostasis, a balance exists between tissue distribution, loss and absorption. Chronic kidney disease and the associated anaemia represent states of “negative iron balance” (142). The potential of “positive iron balance” nonetheless exists,

secondary to repetitive administration of intravenous iron that overcomes the losses aforementioned. The clinical relevance of these changes in iron balance, and whether they lead to further complications and toxicity remains to be elicited (8). The consequences of overcorrection of anaemia and repetitive non-prudent iron prescribing would be those of iron toxicity and overload. These terms are frequently interchangeable, and are phenomena observed in conditions where frequent and repeated blood transfusions are required (such as thalassaemia, myelodysplasia or haemodialysis) (143,144). Iron overload is defined as a "*condition of increased total body iron content that is possibly associated with a time-dependent risk of organ dysfunction*", which is considered pathological once an established disease process takes place (142). Reports of end-organ damage due to iron overload remain rare in the population with CKD, however it is important to highlight that no universal blood marker exists to identify early iron overload prior to the disease process becoming evident (142). Magnetic Resonance Imaging (MRI) studies on HD-CKD patients have highlighted the potential of iron deposition in the liver (143,145,146). Nonetheless, the duration and pathological connotations of this deposition have not been elicited, as MRI technology is unable to differentiate between parenchymal and reticuloendothelial distribution of iron in the liver. It is also important to highlight that deposition was mostly displayed in organs associated with the reticuloendothelial system (spleen and liver) and no cardiac deposition was noted (142,147). No similar studies have been performed or elicited similar results in patients not receiving renal replacement therapy. Interestingly the size of the carbohydrate ligand appears to play a key role in distribution of iron. Positron emission tomography has also been employed to identify the pathway of radiolabelled iron in terms of organ deposition and bone marrow utilisation in anaemia. Both iron sucrose and iron polymaltose indicated a constant uptake/flux into the bone marrow, however this release was much more controlled in the case of iron polymaltose, with less iron deposited in the liver/spleen which in the case of iron sucrose

acted as a “buffer” (148,149). This may indicate that stronger, more compact cores that are a feature of newer products may release iron in such a controlled manner that minimises deposition in other organs and becomes more readily available for erythropoiesis. Additionally, the strict guidelines advising upon iron replenishment have minimised the presentation of iatrogenic iron overload in CKD, as observed through the lack of case reports on the topic (8).

Hypophosphataemia

Hypophosphataemia, while originally perceived as potentially a side-effect of intravenous iron administration irrespective of preparation, now appears to be dependent on compound used (150). This association became more evident through numerous case reports from the early 2010s that appeared to describe this phenomenon with FCM specifically (151–154).

Hypophosphataemia is usually graded according to laboratory values and phosphate concentration with values of 0.6–0.8 mmol/L consistent with mild hypophosphataemia, 0.3–0.59 mmol/L moderate, and <0.3 mmol/L severe hypophosphataemia. Interestingly, symptomatology does not only depend on the actual phosphate concentration, but also the duration and onset of hypophosphataemia (150). Symptoms consistent with hypophosphatemia (Appendix table 8) can be systemic and mimic that of iron deficiency anaemia, raising the need for high index of suspicion in the treating physician.

A detailed narrative review on the phenomenon of drug induced hypophosphataemia looking at third generation intravenous iron compounds including studies from 2003 to 2020 (FCM, FDI and

ferumoxytol) studied 55 RCTs and observational studies, explored mechanistic links and highlighted the association between low phosphate and FCM (150). An updated literature search was performed through PubMed up to December 2022 using “ferric carboxymaltose”, “iron isomaltoside”, “ferric derisomaltose” and “feruoxytol” as keywords in title and abstract Information was obtained through PubMed using “ferric carboxymaltose”, “iron isomaltoside”, “ferric derisomaltose” and “ferumoxytol” as keywords in the title/abstract. This was combined with a search relevant to hypophosphataemia in the terms of “phosph*” or “phosphorus” or “phosphate” or “hypophosphateamia”. Only RCTs and observational trials were reviewed and 76 studies were considered relevant and are presented on Appendix table 9 (96,103,106,110,155–226).

This association was explored by a systematic review focusing on products licenced in the United States of America until 2019 (227). Forty studies (n=10,444; 19 RCTs (n=8,863), 10 observational studies (n=1,570), 11 case reports (n=11)) were included studying the effect caused by FCM, iron sucrose, ferumoxytol and low-molecular weight iron dextran. A greater incidence of hypophosphataemia was noted with FCM (0.0-92.1%) compared to iron sucrose (0.0-40.0%), ferumoxytol (0.4%) and low-molecular-weight iron dextran (0.0%). Studies incorporating FDI were not included in the review, as at the time of publication this preparation was not licensed for use in the USA. The reported hypophosphataemia was transient, and case reports (exclusively involving FCM) reported symptoms dependable on the nature and onset of hypophosphataemia (acute/chronic) (227).

A further systematic review and Bayesian network meta-analysis identified 8 RCTs (n=5,989) where FCM, FDI, ferumoxytol, iron sucrose, and low-molecular-weight iron dextran were compared (228). Hypophosphataemia incidence was higher following administration of

FCM (45.0%) when compared to FDI (RR: 7.90 (95% CI: 2.10 - 28.0) and ferumoxytol and all other preparations (RR: 24.0 (95% CI: 2.50 - 220.0)) (228). No significant differences in incidence were identified in-between all other compounds included in the study. They also noted that severe hypophosphataemia (defined as < 0.40 mmol/L) was greater with FCM (10.0-11.0%) and had greater duration(228). Schaefer and colleagues compiled a systematic review and meta-analysis focusing particularly on FDI and FCM, incorporating 11,700 patients (42 trials, 36 RCT, 6 observational studies) (229). They concluded that a significantly higher incidence of hypophosphataemia existed following FCM administration compared to FDI (47.0% (95% CI: 36.0-58.0) vs. 4.0% (95% CI: 2.0-5.0)), alongside a greater mean decrease in phosphate (FCM: 0.40 mmol/L; FDI: 0.06 mmol/L). Hypophosphataemia persisted in up to 45.0% of the patients receiving FCM for up to 3 months (229). Investigating the potential for hypophosphataemia to be severely restricted in cases of reduced kidney function, the authors also attempted to review studies pertaining to CKD patients only. Hypophosphataemia remained more prevalent in the FCM group when compared to FDI (27.0% (95% CI: 1.0-54.0%) vs. 2% (95% CI: 1.0 - 3.0)) (229). The meta-analysis also concluded a greater percentage decrease in phosphate concentration after FCM use compared to FDI in patients with CKD (229). Rosano and colleagues performed a pooled analysis of serum phosphate measurements from trials on FCM (45 studies, n= 15,080, receiving FCM= 6,879) (230). Mild hypopshosphataemia was noted in 41.4% of participants receiving FCM, and 49 (0.7%) exhibited severe hypophosphataemia (serum phosphate < 0.3 mmol/L). The hypophosphataemia reported was transient as 89% of study participants had a normal serum phosphate by week 8 (230). These results reinforce the theory of hypophosphataemia being a molecule drug-specific side effect in terms of FCM, as it is not consistently observed at the same incidence with other intravenous iron preparations. Nonetheless, it is important to stress that hypophosphataemia was not universally defined in a number of

studies involving intravenous iron, and the side-effect profile in terms of symptomatology and duration were not followed up (150). This was further confounded by the similarity in clinical features between iron deficiency and hypophosphataemia. Additionally, it is important to note that iron dosing and follow up varied considerably between different studies (150). All the systematic reviews and attempted meta-analyses concluded with the need for conducting further comparative RCTs, especially incorporating modern intravenous iron compounds, in various clinical conditions.

Head-to-head comparisons in RCTs incorporating third generation intravenous iron preparations have also explored this association (150). Five RCTs have been conducted up to the end of 2021 comparing third generation intravenous iron compounds (n=2,365). Comparisons have taken place between FCM and FDI (4 trials) and FCM and Ferumoxytol (1 trial), with four focusing in patients with iron deficiency anaemia, greatly associated with gynaecological comorbidities and one on patients with inflammatory bowel disease (155,157,162,163). Evidence specific to other disease processes (such as heart failure, CKD etc.) arise through review of studies comparing third generation intravenous iron products either with earlier preparations, oral iron or placebo, or through observational studies (150).

The findings discussed were reciprocated in the large FIRM RCT assessing the comparative safety of ferumoxytol and FCM in 1,997 patients with iron deficiency anaemia. Despite a comparable incidence of hypersensitivity reactions, anaphylaxes, and cardiovascular events, confirming non-inferiority of ferumoxytol, hypophosphataemia was witnessed in 50.8% of patients receiving FCM, compared to 0.9% of those receiving ferumoxytol ($p<0.001$) (155,168). Extreme hypophosphataemia (defined as <0.40 mmol/L) was exclusively

witnessed in patients that were administered FCM (155). The duration of hypophosphataemia was demonstrated again to persist until day 35 in some of the patients treated with FCM; whilst no participant randomised to receive ferumoxytol had persistent hypophosphataemia (155). These results led to the PHOSPHARE-IDA RCTs, primarily investigating the differential effect of intravenous iron preparations on phosphate in patients with iron deficiency anaemia. These large-scale RCTs (n=245) concluded that on comparison of FCM to FDI in patients with iron deficiency anaemia the incidence of hypophosphataemia at any point following administration of iron was significantly higher with FCM (FCM: 74.4%; FDI: 8.0%; $p<0.001$) (162). In addition, a serum phosphate of < 0.32 mmol/L (defined in clinical terms as severe hypophosphataemia by the authoring group) was only recorded in patients randomised to FCM (11.3%) (162). Where hypophosphataemia was noted (n=90; FCM: 82; FDI: 8), this completely recovered in the patients receiving FDI, whilst it persisted in 47 patients receiving FCM (229). The team led by Wolf and colleagues further completed a secondary analysis which highlighted changes in bone metabolism with FCM but not FDI, in particular PTH, 1,25 $(OH)_2$ vitamin D and FGF23 (229). All three studies were limited by their non-uniform comparative dosing (150). The recently completed HOMburg evaluations on application of Ferrum study (HOMe aFers) (n=26) comparing FCM and FDI at the same dose (1000 mg) produced results in concordance with the studies described. Similar efficacy in treatment of iron deficiency anaemia was noted, however FCM caused significantly greater hypophosphataemia when compared with FDI (75.0% vs. 7.7% respectively; $p=0.001$) (163). These comparative studies were composed greatly by female participants with a background of gynaecological comorbidities, therefore one could argue the potential bias and confounding in establishing a drug-specific association (150). An inflammatory bowel disease specific RCT (PHOSPHARE-IBD) has recently been completed (157). A total of 97 patients with iron deficiency anaemia and inflammatory bowel disease were randomised to receive either

FCM or FDI (FCM: 48, FDI: 49), 1500 mg or 2000 mg of either comparator depending on weight over two sittings a month apart (157). Hypophosphataemia incidence (serum phosphate <0.65 mmol/L at any time after first dose to day 35 (prior to second administration) was 51.0% in the FCM group and 8.3% in the FDI group. Peak incidence of hypophosphataemia with either agent was seen 2 weeks following administration. Incidence of hypophosphataemia throughout the study remained significantly higher with FCM when compared to FDI ($p<0.0001$) (157). The study was powered to detect incidence based on randomisation of 49 vs. 49 with a goal to recruit 120 patients, however recruitment had to stop early due to the COVID-19 pandemic, hence limiting generalisability of results. A key observation is the absence of inclusion of patients with limited kidney function, with most studies excluding patients with CKD; as such, these results cannot be extrapolated to patients with altered kidney function.

A number of observational studies especially in patients with inflammatory bowel disease have compared third-generation intravenous iron preparations (216,220,223). Such retrospective reviews have highlighted the period in which the development of this phenomenon is expected (2 weeks), alongside the potential duration that it may last. Single dose FCM (n=52) was compared with FDI (n=54) and found a significantly higher incidence of hypophosphataemia at 2 and 6 weeks with FCM ($p<0.001$ and $p=0.0013$, respectively) (216). A review of data from 231 patients that were treated with FCM and/or FDI over a 3-year period concluded that there was a significantly greater incidence of hypophosphataemia with FCM, and more cases of hypophosphataemia compared to FDI at week 2 and 5 ($p<0.001$) (220). Prolongation of hospitalisation and necessity of treatment has been associated with FCM-induced hypophosphataemia in a real-world observational study including 162 patients with iron deficiency anaemia (199). Predictive factors for the development of hypophosphataemia were low baseline phosphate

and intravenous iron preparation selection (223). More risk factors have been associated with the development of this phenomenon (Appendix table 10).

As explored later on, hypophosphataemia may arise due to inadequate phosphate intake, increased phosphate excretion and movement of extracellular phosphate to the intracellular space. Initial theories attributed the development of hypophosphataemia to increased cellular uptake of phosphate secondary to increased rate of erythropoiesis, however, intravenous iron induced hypophosphataemia appears to be associated with an interplay of factors relevant to bone metabolism, such as parathyroid hormone (PTH), vitamin D and FGF23 (150). Useful hypothesis generating evidence, potentially identifying FGF23 as a key player in this side effect, yielded from an earlier RCT by Wolf and colleagues (187). Fifty-five women with iron deficiency anaemia secondary to heavy uterine bleeding were randomised to either FCM or low-molecular-weight iron dextran, with evidence suggesting significant increase in FGF23 associated with the administration of FCM but not low-molecular-weight iron dextran, potentially playing a key role in the manifestation of the significant difference in hypophosphataemia incidence (187). Therefore, to understand this process further, it is important to consider phosphate metabolism.

1.2: Phosphate metabolism

Phosphorus (as phosphate - PO_4^{3-}) is essential for a number of functions and plays an integral role in energy storage and liberation, skeletal structure and function, cellular metabolism and signalling, and smooth muscle contraction (231). A complex system involving diet, multi-organ crosstalk, hormones, and other factors coordinates

regulation of phosphate, preserving serum levels within a range of 0.8-1.2 mmol/L for adults; any dysregulation can lead to serious clinical complications such as fatigue and neurological symptoms (including seizures), arrhythmias and respiratory difficulties due to paralysis of diaphragm (232).

The majority of phosphorus exists as phosphate in the skeleton (85% - as hydroxyapatite); the remaining phosphate exists in skeletal muscle, with <1% present in extracellular fluid (231). Key organs regulating the concentration of phosphate include the kidneys, bone, alimentary canal and parathyroid glands. Additionally, given the extensive involvement of vitamin D in the regulation of phosphate, the liver and skin also have important roles in phosphate metabolism.

Dietary phosphate absorption takes place in the small intestine through passive paracellular diffusion and active cell-mediated transport of phosphate. The sodium-phosphate (NaPi)-2b co-transporter located on the luminal side of the enterocyte (also known as brush border) is integral in this process. Dietary phosphate intake and 1,25 $(OH)_2$ Vitamin D regulate NaPi-2b, alongside increasing evidence surrounding FGF23 (150,232). It is important to note that not all phosphate within the small intestine gets absorbed, with around 490 mg of phosphate excreted gastrointestinally per day (233). Absorbed phosphate recycles between extracellular fluid and skeletal pools, and surpasses the normal requirements. Therefore, for maintenance of phosphate concentration within expected limits, phosphate is also excreted (and partially reabsorbed) at the kidneys. Phosphate is freely filtered through the glomerulus allowing for a decrease in concentration. Reabsorption takes place at the proximal tubules, through the renal NaPi type 2 co-transporters, NaPi-2a and NaPi-2c, and (232). These co-transporters are expressed on the apical aspect of the epithelial cells of the proximal tubules and allow for 75-85% of

filtered phosphate to be reabsorbed (232). The rate of reabsorption depends on the abundance of these co-transporters on the apical surface; their transcription is in turn regulated (akin to gut absorption) by concentrations of phosphate, PTH, FGF23 and other factors (Appendix table 11).

Key in the metabolism of both these minerals, especially absorption and excretion, is FGF23; it is therefore important to discuss its actions, and how it may be related to iron deficiency, CKD and indeed iron therapy.

1.2.1: Fibroblast growth factor 23

Fibroblast growth factor 23 is a 251 amino acid glycoprotein that was first identified in 2003, in association with a number of rare hereditary and acquired syndromes such as vitamin D-resistant hypophosphataemic rickets and tumour-induced osteomalacia (234). Fibroblast growth factor 23 belongs to the family of phospatonin, has plethora of functions and is associated with a number of disease processes (235,236). It is an endocrine growth factor, applying its effect alongside the co-receptors α- and β-Klotho mainly on phosphate and calcium homeostasis, and vitamin D metabolism (236). It displays a diurnal variation (similar to PTH), but its concentration is not affected by fasting, and has a half-life of 45-60 minutes, which is longer than other animals (237,238).

The main source of FGF23 is the bone, with osteocytes, osteoblasts and bone marrow able to synthesise and secrete it (239). No

quantitative studies have confirmed ectopic production of FGF23 with the exception of tumour-induced osteomalacia (239,240).

Fibroblast growth factor 23 acts in cooperation with PTH and 1,25 $(OH)_2$ Vitamin D regulating phosphate and calcium homeostasis through the kidneys. Activation of the FGF23-Klotho complex at the kidneys causes a decrease in the reabsorption of phosphate by the proximal tubule, secondary to down-regulation and internalisation of the NaPi-2a and NaPi-2c co-transporters, via the fibroblast growth factor receptor 1 (241). This leads to a greater degree of phosphaturia in a manner similar to the action of PTH at the proximal tubule (242). Additionally, FGF23 has been shown to increase the transient receptor potential vanilloid type 5 receptors at the distal convoluted tubules in a Klotho-dependent way, therefore, it is implicated in calcium reabsorption, and can be seen as a calcium-conserving hormone (243,244)

Outside the direct action on phosphate and calcium reabsorption, FGF23 acts as regulator for both PTH and active vitamin D affecting mineral balance further. Fibroblast growth factor 23 limits the hydroxylation of vitamin D through its inhibitory action on 1-α-hydroxylase stimulation of 24-hydroxylase (245). Fibroblast growth factor 23 also affects vitamin D metabolism further, as it accelerates the inactivation of 1,25 $(OH)_2$ Vitamin D through the stimulation of 24-hydoxyvitamin D-24-hydroxylase (236). In addition, FGF23 exerts inhibitory effects on PTH secretion by the parathyroid gland, likely through both Klotho-dependent (via fibroblast growth factor receptor 1) and Klotho-independent mechanisms (235). As a result, the concentration of biologically active vitamin D decreases, leading to decreased calcium reabsorption and by proxy a reduction in intestinal absorption of phosphate. In order for the effects of FGF23 to be restored, and limit the FGF23 driven suppression of serum calcium

and vitamin D, PTH production increases, causing greater phosphaturia and leading to secondary hyperparathyroidism (246). Therefore, in states of FGF23 excess (such as hereditary hypophosphataemic rickets and tumour induced osteomalacia) but normal renal function, the biochemical phenotype often witnessed is that of hypophosphataemia (due to increased renal phosphate excretion), inappropriately low 1,25 $(OH)_2$ Vitamin D concentration, normal/low serum calcium and secondary hyperparathyroidism (239).

1.2.2: Fibroblast growth factor 23 production and cleavage

Transcription of FGF23 is promoted via different stimuli, both classical and non-classical. Once transcribed FGF23 undergoes post-translational modification through a series of reactions including glycosylation and phosphorylation producing a biologically active phosphatonin. O-glycosylation produces a full length FGF23 that is responsible for the effects that FGF23 has in relation to the bone metabolism. Phosphorylation marks FGF23 for proteolytic cleavage therefore rending it inert. Any FGF23 that does not undergo glycosylation can be cleaved, and its fragments can enter circulation (247,248). Cleavage of the full length (also known as intact) FGF23 leads to the formation of two fragments the N-terminal fragment (the end of iFGF23 responsible for binding to fibroblast growth factor receptors) and the C-terminal fragment (which is the area that binds to Klotho). The bioactivity of these fragments is not yet recognised, and indeed they are currently considered mostly inert in relation to the phosphaturic effects of FGF23 (239). Hence, post-translation modification and cleavage represent an important gateway in the regulation of FGF23 activity, limiting the net amount of FGF23 versus fragmentised FGF23.

Bone sampling is the gold standard method in the detection, analysis and quantification of FGF23; this technique involves western blotting and can differentiate between intact FGF23 and fragments (239). Bone sampling however is restricted in research as it is an expensive, impractical and invasive, and can induce pain and anxiety to the participants (249). This led to the development of enzyme-linked immunosurbent assays (ELISAs), which can detect either full-length FGF23 (intact FGF23, iFGF23) or both full-length and its fragments (C-terminal assay, cFGF23, detecting C-terminal fragments and full-length). ELISAs have revolutionised the field of FGF23 research as they have allowed 'liquid bone biopsy' and enabled non-invasive large-scale longitudinal studies. The ratio between iFGF23 and cFGF23 (iFGF23:cFGF23) can provide us with an assessment of the rate of cleavage, which is important in discriminating between different FGF23-mediated syndromes (239).

A number of classical stimuli of FGF23 production have been identified and these are noted on Appendix table 12, with the main drivers being dietary phosphate content, PTH, high calcium concentration and 1,25 $(OH)_2$ Vitamin D (242). Interestingly, FGF23 part-takes in a number of negative feedback loops affecting the concentrations of its stimulants. In particular it is important to consider the loop between 1,25 $(OH)_2$ Vitamin D and FGF23 – activated vitamin D appears to stimulate FGF23 description, whereas aforementioned FGF23 limits both the enzymatic conversion of vitamin D and also leads to increased degradation. Similarly FGF23 inhibits the release of PTH, whereas PTH directly and indirectly (via vitamin D activation) leads to an increase in FGF23 (242).

The exact nature of the stimulation of FGF23 production has not yet been fully elicited, however, Ratsma and colleagues have recently performed a literature review the regulation of FGF23 (250). Local

regulation within the osteocytes exists involving the dentin matrix protein 1 and the phosphate regulating endopeptidase homolog X-linked, which both act as inhibitory mechanisms to the transcription of FGF23, and their mutations are associated with disease states of raised FGF23 such as autosomal recessive hypophosphataemic rickets and X-linked hypophosphataemia (250). Interestingly, PTH and calcium can affect the dentin matrix protein 1 process and stimulate FGF23 transcription via cessation of its inhibitory actions. Parathyroid hormone also has directly stimulatory actions relevant to FGF23 transcription. 1,25 $(OH)_2$ Vitamin D can promote directly FGF23 transcription intracellularly. Phosphate on the other hand, is related to protection from cleavage, leading to higher levels of circulating iFGF23 (250).

Focusing further on the non-classical inducers of FGF23 transcription, the common pathway appears to be that of stabilisation and increase of HIF1α (239,250). Remarkably, inflammation is associated with raised PTH and 1,25 $(OH)_2$ Vitamin D, potentially acting as a link both the classical and non-classical mediators of FGF23 production. Hypoxia, inflammation and iron deficiency lead to greater HIF1α stabilisation, which in turn stimulate FGF23 transcription (250). Hypoxia-inducible factor 1α is also associated with increase in erythropoietin, which has been shown to be directly associated with FGF23 transcription, however may also lead to greater FGF23 cleavage as well (250). This indicates that non-classical regulators of FGF23 may have an impact on both transcription and cleavage of FGF23, and appear to be coupled, potentially not affecting the iFGF23:cFGF23 ratio, whilst classical regulators act mainly to support transcription (239,251). Finally, other less recognised potential regulators of FGF23 metabolism include leptin, adiponectin, insulin and aldosterone (252).

In individuals with normal kidney function, iFGF23 demonstrates fluctuations similar to that of plasma PTH and phosphate concentration (238). A lag exists between phosphate loading and iFGF23 peak (8-12 hours), with diurnal variation; cFGF23 did not exhibit similar tendencies (238). Under physiological conditions, levels of both iFGF23 and cFGF23 are low and matched, resulting in a mid-range iFGF23: cFGF23 ratio (238). This equilibrium is frequently distorted through a number of diseased states, such as CKD and iron deficiency, while it is also affected by inflammation, intravenous iron and other inherited conditions.

Liquid biopsy has enabled research to gain a firmer understanding of the physiological implications of FGF23. Nonetheless, it carries a number of limitations, such as the lack of standardised FGF23 assays with poor analytical agreement between assays in terms of cFGF23 and iFGF23 particularly in healthy individuals (253). In addition interpretation of liquid biopsy and analysis of dynamics and ratios necessitates a semi-quantitative approach as there are different units of report between cFGF23 and iFGF23 (239). Liquid bone biopsy also assumes that all FGF23 analysed is sourced from the bone and cleaved at bone tissue, therefore making interpretation of results very difficult in cases of ectopic FGF23 production or cleavage of FGF23 at another tissue (239).

1.2.3: Fibroblast growth factor 23 – beyond vitamin D and PTH:

Fibroblast growth factor 23, beyond its role calcium and phosphate metabolism, and its effects on vitamin D and PTH, has physiological actions on the bone, the main source of its production. Bone mineralisation (calcification) is an essential lifelong process, whereas minerals are deposited on the bone matrix for the formation of bone.

Evidence from murine studies suggests that FGF23 expresses an inhibitory effect to bone mineralisation, via the suppression of tissue non-specific alkaline phosphatase (ALP) mRNA transcription, in a Klotho-independent fashion through the fibroblast growth factor receptor 3 (254). Other elements of bone metabolism that appear to be suppressed by FGF23 in both in vitro and murine models of CKD include osteoblast differentiation, matrix mineralisation and bone formation (255,256). Osteoclastic regulation has also been explored in murine models (257,258). In addition, through its inhibitory action on 1,25 (OH) Vitamin D and PTH it can affect both bone formation and bone resorption (259,260). As such, changes in FGF23 metabolism may be associated with inadequate bone microarchitecture, formation and strength. Nonetheless, the exact nature of osteoblastic/osteoclastic activity has not been fully elicited, and this may be concentration and disease-state dependent (261).

Fibroblast growth factor 23 has been linked to direct cardiovascular effects according to murine studies. Injection of recombinant FGF23 protein in mice myocardium induced cardiac hypertrophy irrespective of presence of Klotho, alongside the identification of the FGF receptor 4 as the one responsible for the hypertrophic effects of FGF23 (262,263). Autopsy results in 24 patients with end-stage-kidney disease have revealed (when compared to age- and sex-matched controls) a strong association between left ventricular hypertrophy and levels of FGF23, FGF receptor 4, calcineurin and nuclear factor of activated T cells (264). Indeed, attenuation of cardiac FGF23/FGF receptor 4 signalling through the use of 1,25 $(OH)_2$ vitamin D in uraemic rats blocked FGF23 driven ventricular hypertrophy (265). Interestingly, excess iFGF23 of potential cardiac origin did not induce left ventricular hypertrophy in rats with normal renal function, with its downward cardiac stream attenuated by the action of soluble Klotho. This may support an absence of direct cardiotoxicity by iFGF23, with its cardiovascular effect secondary to Klotho deficiency (266).

Overexpression of FGF23 in experimental rat models has also been associated with greater degree of myocardial fibrosis and diastolic failure following myocardial infarction (267). In addition, cultured human atrial fibroblasts with and without FGF23 have been used in in vitro experiments, confirming increased proliferative and migratory abilities of human atrial fibroblasts following recombinant FGF23 administration (268). Such results further support the potential of FGF23 involvement in cardiac fibrosis – at least in vitro. Furthermore, FGF23 has been associated with reduced myocardial contractility and arrhythmogenic properties secondary to effects leading to intracellular calcium iron mishandling in murine experiments both in vitro and in vivo (269). Nonetheless, real-life evidence do not support these theories as FGF23-excess associated genetic syndromes do not exhibit a particular phenotype conferring added cardiovascular risk (270–272).

Fibroblast growth factor 23 is also involved in renal sodium handling and blood pressure regulation (273). Murine models have indicated that FGF23 is involved in the upregulation of the sodium/chloride co-transporter in the distal convoluted tubule, leading to greater sodium reabsorption and volume expansion. This hypertensive effect of FGF23 may be directly implicated to cardiac hypertrophy (273). Additionally, the downregulation of 1,25 $(OH)_2$ Vitamin D by FGF23 can lead to increased renin production, and further activation of the renin angiotensin aldosterone system (RAAS) (274). Rat models with CKD further alluded to a synergistic effect between FGF23 and the RAAS in the promotion of cardiac hypertrophy and fibrosis (275). Additionally angiotensin II has been shown to induce FGF23 expression and transcription in angiotensin II treated wild-type rodents (276). Autopsy evidence from patients with CKD also confirmed an intertwined relationship between the two systems (277).

Oxidative stress and endovascular dysfunction have also been associated with FGF23, through increased production of reactive oxygen species and decreased production of nitric oxide (278). Evidence coming from murine studies and in vitro human coronary artery endothelium cell analysis have highlighted the protective role of Klotho, and the independent action of FGF23 in states of Klotho deficiency leading to enhanced reactive oxygen species formation and reduced bioavailability of nitric oxide (279,280). These vasoconstricting effects of FGF23 were further demonstrated on mouse aortic rings, human umbilical vein endothelial cells and human with Klotho attenuating the effect of FGF23 due to promotion of nitric oxide production (281). In addition, human umbilical vein endothelial cells cultured in FGF23 had an increased expression of both e-selectin and vascular cell adhesion indicating vascular endothelium activation, a feature common in essential hypertension (282). Such results have been replicated in small number studies in patients with ND-CKD (283).

1.2.4: Distortions in the metabolism of FGF23

Upon considering the disease states associated with distorted FGF23 metabolism, one could review the examples of autosomal dominant hypophosphataemic rickets, autosomal recessive hypophosphataemic rickets and tumoural calcinosis (239,284). In the case of autosomal dominant hypophosphataemic rickets, the genetic prototype displaying derangements in FGF23 metabolism and resultant variation in the ratio of iFGF23:cFGF23, mutations affect the cleavage recognition site, leading to a high iFGF23:cFGF23 ratio, as most circulating FGF23 is full length (284). Similarly, a mutation at the FAM20C site renders FGF23 cleavage impaired, again leading to raised iFGF23:cFGF23 ratio. Such mutations can lead to phosphaturia and therefore hypophosphataemia, given the right circumstances

(239). On the contrary, tumoural calcinosis is characterised by mutation in the GalNT3, which leads to lesser FGF23 glycosylation and as a result greater cleavage of iFGF23 (239,284). As a result, a moderate increase in phosphate can be seen, given the absence of action by iFGF23. Reflecting upon these pathological entities, can aid in understanding the changes witnessed in CKD, iron deficiency and the association of particular intravenous iron preparations with hypophosphataemia.

1.2.5: Fibroblast growth factor 23 metabolism in CKD

Chronic kidney disease represents a state of raised FGF23, potentially as an attempt for maintenance of phosphorus within normal range in a state of decreased phosphate output (242). This has been exhibited within large cohort studies – using data from the Chronic Renal Insufficiency Cohort Study (CRIC) (CKD stages 2-4, n=3,879), an early rise in FGF23 was detected following a decrease in eGFR with eGFR representing the strongest univariate correlate of FGF23 ($r = -0.52$, $p<0.0001$) (285,286). This increase in FGF23 predated any changes in PTH and co-existed with enhanced urinary phosphate excretion (286). Evidence from murine studies provide mechanistic basis as to the potential of a physiological “trade-off” mechanism giving rise to increased FGF23 in CKD in order to minimise the effect of hyperphosphataemia (287). Given these findings, it is possible that this rise in FGF23 leads a pathological series of events. Indeed, FGF23 may act as one of the main culprits leading to the development of CKD-MBD.

The precise driver behind the rise in FGF23 with decreasing eGFR has yet to be identified, and theories include primary Klotho deficiency (the main transmembrane protein aiding in FGF23 renal actions) and

primary FGF23 excess (242). Klotho and FGF23 have an interdependent relationship as both ligand and co-receptor, with Klotho promoting the phosphaturic effect of FGF23 (243). The FGF23-Klotho complex acts on the proximal renal convoluted tubule, through activation of the extracellular signal-regulated kinase-1/2 and of serum/glucocorticoid-regulated-kinase-1 that leads in the degradation of the NaPi2a and NaPi2c co-transporters (243). A number of studies have consistently demonstrated that CKD represents a state of Klotho deficiency, with reduced Klotho concentration as kidney function decreases (288–290). A review and meta-analysis (n=1,457) confirmed this, as a significant positive correlation was witnessed between soluble α-Klotho and reduced eGFR, however the studies included had a high degree of heterogeneity (291). The absence in Klotho could therefore lead to tubular resistance to FGF23, potentially causing a compensatory increase in FGF23. Nonetheless, the clinical applicability of this theory is questionable, as no derangements in terms of phosphate, calcium and 1,25 $(OH)_2$ Vitamin D appear to exist early in CKD that would be consistent with Klotho deficiency (242). A hint towards that has also been pointed out in the aforementioned meta-analysis where no correlation between PTH, phosphate and calcium was seen in these patient group and Klotho (291). On the contrary, evidence from the work of Isakova and colleagues indicated that in early CKD the clinical picture is that of increased PTH and reduction in serum calcium, phosphate and 1,25 $(OH)_2$ Vitamin D, with an increase in FGF23 predating these (286). Therefore, the most likely scenario appears to be that of FGF23 excess which as a result drives a compensatory negative feedback decrease in Klotho; however, the key trigger causing remains unknown.

Upon reviewing studies surrounding FGF23 dynamics in CKD, evidence suggests alterations in iFGF23: cFGF23 ratio, with uncoupling between iFGF23 transcription and a reduction in cleavage rate which appears to be eGFR dependent (238). In patients on

peritoneal dialysis (n=34) iFGF23 was expressed in large quantities, while fragments of FGF23 were rarely identified (292). These results indicate that an increased iFGF23: cFGF23 ratio approaches one in ESRD. Nonetheless, evidence also indicates that a degree of the total FGF23 rise may also be related to decreased cFGF23 clearance, similar to PTH metabolism, secondary to decreasing renal function. In a cohort study of 3,246 elderly individuals an increase in the log10(c/iFGF23 ratio) was noted with decreasing eGFR, which could reflect decreased degradation/excretion of cFGF23, accompanying a reduction in iFGF23 cleavage (293). Given these findings, CKD potentially represents a state of increased FGF23 transcription, with unknown, but potentially inhibitory effects on post-translational modification and cleavage, leading to a greater quantity of biologically active FGF23.

As previously discussed, iron deficiency and inflammation are associated with an increased transcription and cleavage FGF23. Where iron deficiency arises in CKD, a mechanistic profile similar to autosomal dominant hypophosphataemic rickets may exist. In murine models, induction of inflammation and iron sequestration were associated with a significant increase in both cFGF23 and iFGF23 indices, which was however far greater in terms of cFGF23, suggesting an increased production of FGF23 accompanied by satisfactory cleavage compensatory response (294). Induction of inflammation and functional iron deficiency however in CKD murine models caused a significant rise in both iFGF23 and cFGF23; nonetheless the rise in iFGF23 was such that it was significantly greater than all models used, suggesting a preferential rise of iFGF23, linked with increased transcription and impaired cleavage (294). These results have been reflected by a systematic review and meta-analysis of RCTs (9 RCTs; n= 906) investigating the effect of iron supplementation (oral and intravenous) on levels of iFGF23 and cFGF23 in CKD patients (295). Iron repletion was associated with a

significant reduction in iFGF23 (weighted mean differences: − 60.56 pg/ml (95% CI: − 92.17 - − 28.95); I^2 = 96%) but not cFGF23 (a significant reduction in cFGF23 took place in the oral iron group). These results indicate that iron repletion may be associated with improved FGF23 metabolism and decreased transcription, which can be dependent however on the mode of replacement. It is important to acknowledge that this systematic review was limited by the fact that the studies included pertaining to intravenous iron were only related to one form of intravenous iron (saccharated ferric oxide), which potentially can alter FGF23 metabolism (295). Therefore, given the results discussed, derangements exist in CKD that could be partly caused by increased transcription and reduced cleavage that is amplified through iron deficiency.

As previously noted, different intravenous iron preparations have a differential impact in terms of phosphate metabolism likely associated with FGF23. It is therefore important to elucidate this impact in research in order to identify the optimal management of this patient group, especially considering the potential negative implication of raised FGF23.

1.3: Intravenous iron and hypophosphataemia – the FGF23 association

Intravenous iron induced hypophosphataemia appears to be related to certain iron preparations such as iron polymaltose, sacharated ferric oxidate and FCM, potentially due to the moiety of the carbohydrate ligand present in each compound (150). The presence of case reports in the literature linking certain iron compounds with hypophosphataemia, prompted Schouten and colleagues to explore mechanistically the link between hypophosphataemia, FGF23 and iron

treatment. Eight female patients with iron deficiency anaemia with no prior history of CKD, hypophosphataemia and/or parathyroid-related comorbidity received intravenous iron polymaltose (296). There was a significant fall in phosphate and 1,25 $(OH)_2$ Vitamin D, with a significant increase on iFGF23 (43.5 pg/ml to 177 pg/ml; $p<0.001$) within one week of administration (296). Likewise in a cohort study in HD-CKD (n=27), a significant increase in iFGF23 was noted following intravenous saccharated ferric oxide (a compound similar to FCM) within 3 weeks of treatment (3,453 pg/ml to 4,701 pg/ml; p=0.002). (3,453 pg/ml to 4,701 pg/ml; p=0.002) (297). The results of these studies alluded to the potential of disruption in FGF23 metabolism with changes in either transcription (increased) or cleavage (reduced) as the pathophysiological link between intravenous iron and hypophosphataemia, however as they did not include a “liquid biopsy”, they could not verify the potential causative mechanism behind this. These results led to the work by Wolf and colleagues that enhanced our understanding of the link between FGF23 and intravenous iron (187). Iron deficiency was associated with elevated cFGF23 levels, but normal iFGF23 levels. Alleviation of iron deficiency was associated with a reduction in cFGF23 within 24 hours in both groups; a significant increase in urinary phosphate excretion and decrease in phosphate concentration was only noted with FCM. Preceding the decrease in phosphate concentration that was exhibited by FCM, there was a significant increase in iFGF23 within day 1 that remained elevated throughout until day 35 – this change correlated significantly with the magnitude of decrease in serum phosphate. A significant reduction in 1,25 $(OH)_2$ Vitamin D was seen within 1 day in the FCM cohort reaching its nadir on day 7, and normalising by the end of the study, while PTH concentration increased albeit not significantly (187). These findings aided in the formation of a theory whereby iron deficiency represents a state of increased FGF23 transcription with satisfactory compensatory mechanism, which improves on alleviation. As such both iFGF23 and cFGF23 decrease. Nonetheless, this mechanism appears to be affected by FCM – this could be secondary to inhibition

of cleavage (centrally or peripherally) or an ectopic production of iFGF23 leading to changes in serum phosphate concentration, urinary phosphate excretion, PTH and 1,25 $(OH)_2$ Vitamin D metabolism. Upon reviewing the differences between iron molecules, the striking difference relies on their carbohydrate core, whereby the "phosphaturic" preparations have similar ligand molecules. This phenomenon exhibited by these compounds led Heinz Zoller and colleagues to coin the term of 6Hs to describe the effects expressed: High iFGF23, Hypophosphataemia, Hyperphosphaturia, Hypovitaminosis D, Hypocalcaemia and Hyperparathyroidism) (298) (figure 3). Indeed, iatrogenic high iFGF23 secondary to FCM and its effects may represent the result of a "two-hit" phenomenon similar to autosomal dominant hypophosphataemic rickets. As discussed, iron deficiency acts as a strong promoter of FGF23 production, with a satisfactory cleavage mechanism. In cases of defective cleavage (such as autosomal dominant hypophosphataemic rickets) however, hypophosphataemia ensues. A similar sequale appears to exist with certain carbohydrate moieties, similar to FCM, leading to an increase in iFGF23 given the raised background transcription of FGF23 that is unopposed (239) (figure 4). Other possible explanations for the rise in FGF23 may be that of ectopic production of FGF23 or increased FGF23 transcription (284). Nevertheless, the matched upregulation of cleavage to transcription occurring in iron deficiency in murine models, and the absence of hypophosphataemic effect in iron replete animals following administration of FCM, indicate the higher possibility for the explanation behind this rise in iFGF23 to be secondary to ineffective cleavage due to certain carbohydrate moieties (299,300). Evidence supporting the notion of compound-specific FGF23 metabolism alterations leading to the 6Hs syndrome can be derived from further RCTs and observational studies (Appendix table 13).

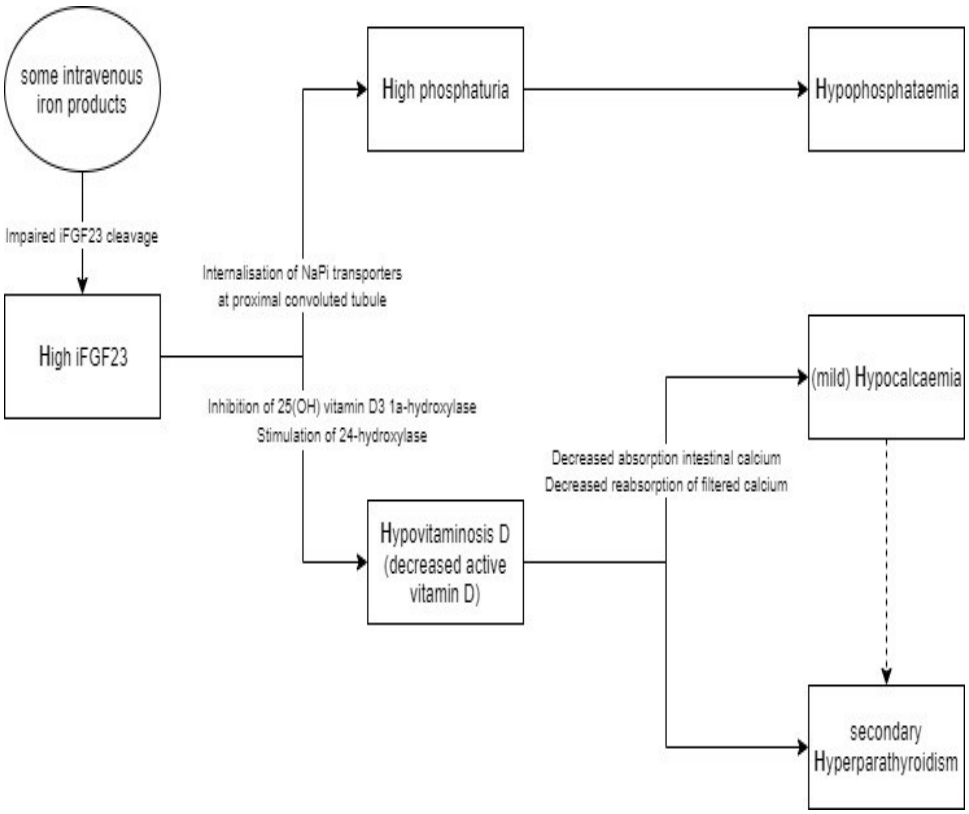

Figure 3: The 6H syndrome

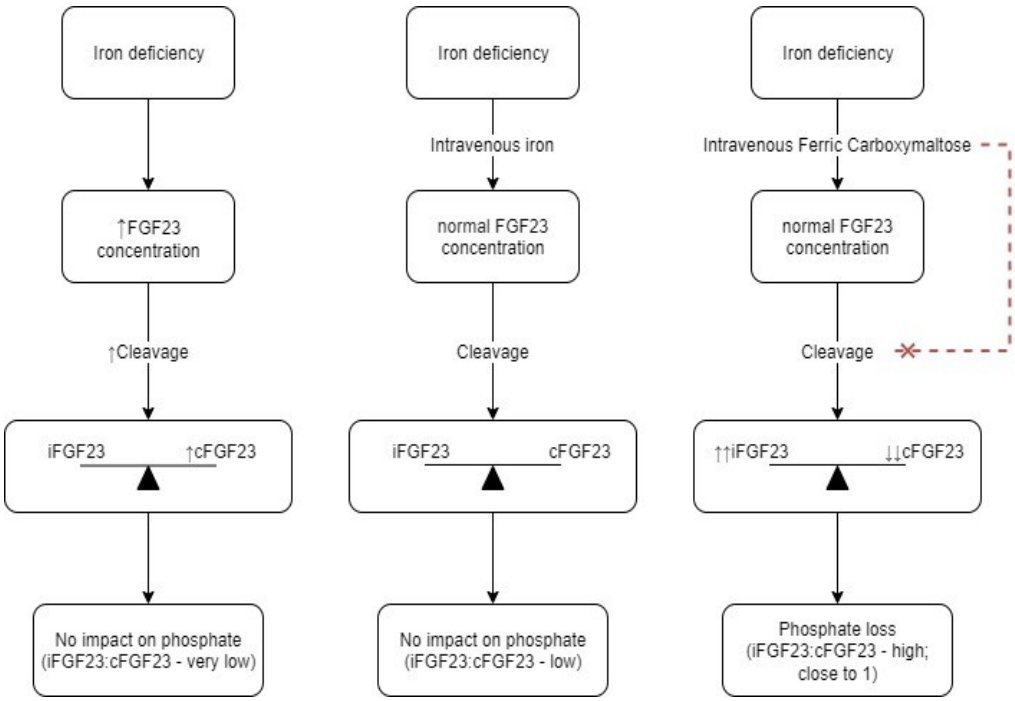

Figure 4: FGF23 metabolism and alterations due to iron deficiency and intravenous iron use

As Appendix table 13 demonstrates, there are conflicting results in terms of patients with CKD. In HD-CKD iFGF23, cFGF23 and iFGF23:cFGF23 ratio analyses have not demonstrated a similar trend as above (155,157,162,163,178,187,204,211–213,217,218,222,226,296,297,301–305). These findings may suggest differential metabolism in patients receiving haemodialysis. Indeed, the authors of these studies argued that these results could reflect the participants' iron status, anuria, low cFGF23 as baseline given ESRD and the low dose of iron administered. Observations however in ND-CKD have identified similar changes in terms of FGF23 metabolism as those in patients with normal kidney function. Certain elements of the 6H syndrome (such as hypovitaminosis D) were also demonstrated, alongside a propensity for phosphate decrease (218). The absence however of consensus and direct comparative studies in ND-CKD, make the need for further research imperative, in order to isolate trends and quantify the extent of potential complications.

1.4: The pathological and patient-related implications of FGF23

As a differential effect appears to exist in terms of FGF23 and phosphate secondary to different intravenous iron compounds, it is important to consider what the consequences of excess FGF23 could be in terms of disease processes and quality of life. The symptoms of hypophosphataemia has been previously discussed (section 1.1.6). Fibroblast growth factor 23 exerts effects on numerous organs and interacts in a number of metabolic pathways. Given its endocrine, paracrine and autocrine actions, FGF23 has the potential of being involved in a number of pathological processes, potentially amplifying the effect of disease and conferring a worse prognosis.

1.4.1: Bone and mineral disorders

Disorders in FGF23 metabolism can lead to both direct and indirect effects, secondary to its actions on phosphate and calcium metabolism. Pathologies characterised by high circulating FGF23 levels display hypophopshataemia, decreased 1,25 $(OH)_2$ Vitamin D production and osteomalacia/rickets, whereby where low FGF23 levels exist, hyperphosphataemia, increased 1,25 $(OH)_2$ Vitamin D concentration and soft tissue calcification and hyperostosis result (306). Mutations of the FGF23 gene lineage are associated with a number of hereditary disorders and malignant processes (Appendix table 14) (307). Iatrogenic osteomalacia has been reported in a number of cases following intravenous iron administration, and has led to research discussed in section 1.3. These resultant derangements in calcium/phosphate metabolism secondary to FGF23 lead to pain, fatigue and fractures of progressive nature. Additionally, neurological symptoms such as fatigue and asthenia have been described (308).

Conventionally, hypophosphataemic disorders associated with raised FGF23 were treated with oral phosphate and vitamin D analogue supplementation (309). Monoclonal antibody technology has been developed, showing promise both in vitro and in vivo. Burosumab is a human anti-FGF23 monoclonal antibody that has been approved for use in children with X-linked hypohosphataemic rickets (310). Burosumab has been demonstrated to improve both the metabolic (phosphate, 1,25 $(OH)_2$ Vitamin D concentrations) and clinical picture (reduced stiffness, improved healing) of patients (310–313). Burosumab has also been utilised in the treatment of tumour-induced osteomalacia, indicating in a phase 2 study an acceptable safety profile with improvements in phosphate metabolism and osteomalacia (314). Successful use of burosumab has also been reported in the treatment of iatrogenic osteomalacia due to intravenous iron (315).

One patient with extensive inflammatory bowel disease displayed several insufficiency fractures, pseudofractures, and severe hypophosphataemia following chronic FCM use, intractable to vitamin D and phosphate supplementation (intravenous). A significant resolution of symptoms, and improvement in biochemical picture and fractures, as displayed by MRI was noted (315).

Beyond the disorders that are traditionally associated with distorted FGF23 metabolism, high FGF23 concentrations (using c-terminal immunoassays) have been associated with decreased bone mineral density in patients with normal and reduced renal function in Mendelian randomisation and cohort studies (316–318). Other study groups highlighted a negative correlation between FGF23 (both cFGF23 and iFGF23) with variables of mineralisation such as osteoid thickness and osteoid maturation time in children on peritoneal dialysis and bone mineral density at the femoral neck and lumbar spine (as measured by dual-energy x-ray absorptiometry (DEXA) scan) (319,320). Nonetheless, other cohort studies (combined n=294; CKD stages 2-5D) failed to show to show similar results in terms of the T-score of the lumbar spine and femoral neck and bone mineral density measurements through DEXA scan and computed tomography (321–323). Additionally a large-scale cohort study investigating the relationship between bone mineral density (n=2,234) and fracture risk (2,786) in elderly individuals failed to identify a relationship between fracture risk and iFGF23 following both crude and adjusted models. A temporal association between iFGF23 and bone mineral density was recognised, however this was attenuated when adjustment for eGFR and PTH took place (324). These studies however were all affected by their observational nature, and given the previously discussed potential implications of FGF23 in bone metabolism, it is possible that FGF23 exerts effects on the bone both in an autocrine fashion but also secondary to its effects on PTH and Vitamin D.

1.4.2: Mortality, multimorbidity and FGF23

The implications of FGF23 on the metabolism of PTH and vitamin D may affect mortality. Secondary hyperparathyroidism has been linked both with increased mortality and greater incidence of cardiovascular events as indicated by a 1.3-fold increased risk of death and a greater hazard ratio for cardiovascular events in two independent observational studies enlisting a total of 5,001 patients with CKD of all stages (325,326). No information on FGF23 was collected. Earlier evidence from the Evaluation of Cinacalcet Hydrochloride Therapy to Lower Cardiovascular Events (EVOLVE) RCT and real-world observational data suggested that treatment of secondary hyperparathyroidism through the use of cinacalcet was associated with reduced cardiovascular events and mortality especially in older individuals but not in younger patients (327–329). No specific analysis of FGF23 was taken in consideration, however a post-hoc analysis of the EVOLVE study noted that in participants where a ≥ 30% decrease in FGF23 was achieved a significant reduction in cardiovascular events took place (330). Despite this, a systematic review and meta-analysis on the topic of cinacalcet use (10 RCTs, 4 observational studies; n=38.219) failed to identify a decrease in overall mortality despite an association with significant reduction in FGF23 (331).

Active Vitamin D, which decreases in response to FGF23, is indispensable for skeletal health and considered protective in a number of other processes such cardiovascular health, immunity and malignancy. Meta-analyses incorporating patients from a multitude of pathologies have already displayed an association between low levels of 25 $(OH)_2$ Vitamin D and risk of death due to cardiovascular disease and cancer, with improvement in patients with CKD following vitamin D supplementation (332–334). Evidence pertaining to 1,25 $(OH)_2$

Vitamin D from the Homocysteinemia in Kidney and End Stage Kidney Disease Study suggest that patients with lower concentrations of 1,25 $(OH)_2$ Vitamin D had an increased risk of death and initiation of dialysis (335). Interestingly, these relationships were attenuated by FGF23 levels (335).

As indicated above, the results on the exact effect of FGF23 on mortality and morbidity are inconclusive. A correlation between raised FGF23 concentration and mortality has been noted in other studies that extends beyond secondary hyperparathyroidism and vitamin D. In a nested case control study based on the Accelerated Mortality on Renal Replacement study on 400 patients commencing haemodialysis, FGF23 measurements were taken prior to initiation of treatment. Increasing FGF23 levels were associated with risk of death both through odds ratio and quartile analysis, with confounder analysis and adjustment amplifying the relationship between the two (336). In addition, two large-scale registries of combined 4,978 patients (ND-CKD) concluded that patients on higher FGF23 quartiles had a nearly two-fold risk of mortality compared to those with low baseline FGF23, even after adjustment for classical cardiovascular risk factors and traditional markers of CKD-MBD (337,338). This association is enhanced through the results of a systematic review and meta-analysis (studies: 34, n>22,000; normal renal function, ND-CKD and HD-CKD) highlighting an increased risk of cardiovascular and non-cardiovascular mortality in patients with higher FGF23 concentration (339)

Most studies conferring an association between FGF23 and mortality are based on single measurements of FGF23; nonetheless a subcohort study (n=1,135) from CRIC monitored longitudinal FGF23 and mortality trajectories. In patients with rising levels of FGF23 a higher risk of death was noted (slow-rising FGF23 trajectory: 4.5-fold

risk of death (95% CI: 3.17-6.35); rapidly-rising FGF23 trajectory: 15.23-fold higher risk of death (95%CI: 8.24-28.14)) (340). Raised FGF23 concentrations have been independently associated with tachyarrhythmias, infection-related hospitalisations and death in critical illness as well (341–344). Nonetheless, data arising from observational studies does not confirm causation, but exhibits associations that could be attributed to exogenous factors and do not portray an exposure-response relationship.

1.4.3: Cardiovascular effects of FGF23

Given the mechanistic involvement of FGF23 in cardiovascular disease (section 1.2.3) and the association between FGF23 and mortality, a number of studies have attempted to use FGF23 (both intact and c-terminal) with prognostication in cardiology, with mixed results. Echocardiographic analyses indicated an association between higher cFGF23 and iFGF23 concentrations and markers of left ventricular hypertrophy (345,346). Cardiac MRI employed in a multi-centre cohort study (n=2,276), identified an association between baseline iFGF23 and left ventricular and atrial mechanical function at a 10-year follow up development of atrial fibrillation, thereby linking mechanistic in vitro evidence with changes in cardiac architecture that may lead to heart failure with preserved ejection fraction and atrial fibrillation. These associations were irrespective of race and eGFR (347). Nevertheless, the Coronary Artery Risk Development In young Adults study (n > 3,000; no prior cardiac disease) failed to note an association between incidence of left ventricular hypertrophy and worsening left ventricular mass index and baseline FGF23 concentration, despite greater odds of left ventricular hypertrophy at higher quartiles of FGF23 (348). These results signify that FGF23 can be a risk marker for incident left ventricular hypertrophy, but not necessarily linked with the development of pathological changes

(348). The full involvement of FGF23 in cardiac disease is still unknown, with no evidence categorising it into a bystander or a mediator (272).

The association between FGF23, mortality and morbidity and disease progression may extend beyond the potential cardiac assault and be relevant to vascular effects. As discussed in section 1.2.3 FGF23 has an intertwined relationship with the RAAS system, alongside potential vasoconstricting effects secondary to oxidative stress and impact on nitric oxide (283). Summarising the mechanistic effect of FGF23 on the endothelium, FGF23 is associated with increased vasoconstriction and associated decreased vasorelaxation, an increased radical oxygen speciees production and a decrease in nitric oxide production (278). Fibroblast growth factor 23 may indeed be related to vascular calcification, but results are conflicting (Appendix table 15) (323,349–359).

Randomised placebo controlled interventional studies and single arm studies using phosphate binders (such as lanthanum and sevelamer) have failed to identify any significant difference between intervention and placebo in terms of pulse wave velocity or abdominal aortic calcification and iFGF23 or cFGF23 (360,361). Such results cast a shadow on the potential causal relationship between FGF23 and arterial stiffness and a clinically meaningful association between FGF23 and vascular dysfunction.

1.4.4: Functional status/Quality of Life and FGF23

Patients with FGF23-excess clinical syndromes associated with hypophosphataemia are often characterised by an impact on physical,

emotional and social aspects of life (362,363). A cohort study (n>2,900) using data from the Cardiovascular Health Study aimed to assess frailty scores in elderly individuals living in the community, and their potential link with cFGF23 (364). An association was noted between cFGF23 and frailty, with increasing odds ratio accompanying doubling of cFGF23 concentration, which remained significant following multiple adjustments. Symptoms reported as associated with FGF23 were exhaustion, slowness and reduced physical activity (364). The authors proposed that this phenomenon may be secondary to the independent association between inflammation and FGF23, or due to FGF23 representing a marker of chronic disease states. This study had several strengths such as the number of patients involved, and the multiple models of analysis employed to limit covariate impact, however as it is observational causality and reverse causality cannot be fully examined. In patients with heart failure with preserved ejection fraction both cFGF23 and iFGF23 levels were associated with markers consistent with decreased exercise capacity at baseline, namely 6 minute walking distance and peak oxygen uptake (VO_2) (365). These relationships were maintained using linear regression, and following adjustment for demographics and cardiovascular risk factors. This study was however non-powered and failed to identify relevant associations between FGF23 and change in exercise capacity (365). Ghuman and colleagues also highlighted the possibility of FGF23 representing a constant state of low-grade inflammation therefore leading to the exercise capacity results displayed.

Additionally, the association between quality of life and FGF23 has been demonstrated through open label, phase 1/2 studies (total n=45) focusing on the FGF23 lowering treatment burosumab. In both studies, treatment with burosumab significantly improved all of the scores used, especially in terms of physical function (314,366). These changes coincided with FGF23 decrease; no correlation analysis took place. The changes observed may be relevant to improvement in

disease status, therefore FGF23 causality cannot be explored whilst phosphate concentrations were not taken in consideration (314,366). The PHOSPHARE-IBD monitored fatigue in response to treatment using the Functional Assessment of Chronic Illness Therapy (FACIT) Fatigue Scale. Fatigue improved in both groups, however a significantly greater increase was witnessed following FDI compared to FCM (coinciding with the differential effect on iFGF23), which also showed an inverse association with the magnitude of phosphate decrease (157). As such, the differential effect could be secondary to the phosphate decrease, rather than the increase in FGF23 per se.

1.5: The "Iron and Phospaturia – ExplorIRON-CKD" trial

Iron deficiency anaemia poses a common and debilitating complication of CKD. Treatment using newer intravenous iron preparations appears a reasonable option given the ability to deliver large volumes of iron in a single sitting, achieving faster repletion, safely. However, a differential effect on phosphate and FGF23 metabolism has been observed with FCM and this may have effects on bone turnover and other aspects relevant to the care of the CKD patient. No prior comparative trials have examined this in the CKD population, whereby intravenous iron administration is common. Given the additional concerns on the use of high dose intravenous iron including potential cardiovascular effects, the "Iron and Phospaturia – ExplorIRON-CKD" trial was set up.

The aim of this study was to explore

1. The differential effect of modern intravenous iron products on FGF23 and phosphate in patients with CKD
2. The effect of intravenous iron on markers of the 6H syndrome within ND-CKD

3. The differential effect on patient reported outcome measures and function
4. The impact on markers of cardiovascular function and injury

Moreover, clinical efficacy and safety were monitored.

In order to examine these areas of uncertainty aid in the identification of future research questions and hypothesis generation, the ExplorIRON-CKD Trial was designed to explore primarily the effect of two different modern intravenous iron products (FCM and FDI) on FGF23 and phosphate. The extended effects of iFGF23 on 6H syndrome, bone metabolism and cardiovascular health were taken in consideration as part of the protocol design of the study. As Kidney Research UK has identified health related quality of life as an important research target (367), and taking in consideration the potential effects of iron deficiency, hypophosphataemia and increase in FGF23, quality of life and functional status monitoring were investigated as secondary outcomes.

To ascertain the aims above certain outcomes were set up, as part of the study protocol (Appendix table 16). A plan for post-hoc analysis on markers of phosphaturia and bone turnover was implemented as per the agreement with health research regulatory authorities.

2: Methods

2.1: Design and approval and governance

This was an investigator led single centre randomised double-blind pilot clinical trial aiming to recruit a total of 30 participants with stage 3a-5 (non-dialysis) stable CKD. Stability was <20% variation in eGFR in the preceding 3 months). The participants were randomised in a 1:1 ratio into two groups to receive either intravenous Ferric Derisomaltose (FDI) (Monofer® - Pharmacosmos UK, Reading, UK) or intravenous Ferric Carboxymaltose (FCM) (Ferinject® - Vifor Pharma UK, Staines-upon-Thames, UK) with dosage based on summary product characteristics (SmPC) (368,369).

The number of recruits was not based on a statistical calculation. This was a pilot study looking for proof of concept to be used as hypothesis generating and further stimulate research in the topic of iron biochemistry, bone disease and CKD.

2.1.1: Approvals and governance

The study was performed subject the favourable opinion of Health Research Authority (HRA) and the Research Ethics Committee Leeds West (20/YH/0005), Medicines and Healthcare Products Regulatory Agency (MHRA) Clinical Trial Authorisation and Hull University Teaching Hospitals (HUTH) NHS Trust permissions (Research and Development Department reference number: R2451). The study was registered with and processed via the Integrated Research Application System (IRAS Project ID: 272279) and the European Union Drug

Regulating Authorities Clinical Trials Database (EudraCT number: 2019-004370-26). Further amendments to the study protocol and design received the favourable opinion of the aforementioned organisations. The study was adopted in the portfolio of the National Institute for Health Research Clinical Research Network.

This study was conducted in accordance with The Medicines for Human Use (Clinical Trials) Regulations 2004 (370), the International Conference for Harmonisation of Good Clinical Practice (ICH GCP) guidelines (371) and the UK Policy Framework for Health and Social Care Research (372). All study members completed the Good Clinical Practice as delineated by the UK Policy Framework for Health and Social Care Research for clinical trials of investigational medicinal products.

The overall responsibility of the trial relied with the sponsor of the study, the Research and Development Department of the HUTH NHS Trust. The Principal Investigator (PI) managed the study proceedings concerning recruitment, follow up, investigational assessments and review of adverse events. Study monitoring was performed by the HUTH NHS Trust Research and Development Department Clinical Trials Monitor according to the standard operating procedures of the trust, ensuring quality assurance.

2.1.2: Setting

The trial was a single centre trial led by the Academic Renal Research Department at HUTH NHS Trust, United Kingdom. The hosting department had already displayed a considerable track record in trials related to iron deficiency and the provision of IV iron in patients with

CKD, having led the IRON-CKD (EudraCT number: 2010-020452-64) and Iron & Heart studies previously (EudraCT number: 2014-004133-16).

2.1.3: Population and patient identification

Patients referred to the renal anaemia services aged 18 years or over with stable CKD stage 3a-5 (not on any form of dialysis) from Hull, East Riding of Yorkshire, North and North East Lincolnshire were considered as potential participants. Patients were referred to the service either via primary care (if patient belonged to the renal registry) or through secondary care as part of their routine outpatient clinic appointments.

The past medical history and blood results of these individuals were compared against the inclusion and exclusion criteria. Patients that clearly met those criteria, alongside borderline cases were contacted via telephone in order to gauge interest. During the telephone conversation, the study was described in lay terms following verbal consent. An explanation of the reasons behind the study, the benefits and drawbacks of partaking and the safety netting in-place were presented. The right to decline alongside the fact that this was not to affect any of their healthcare rights and normal routine care provision were discussed. Patients that declined were asked, where possible, to provide the reason behind this. Patients that expressed interest in pursuing the study further were provided with further written information including the participant information sheet (Appendix 3 – section 9.2).

Once those patients had read the patient information summary, they were re-contacted in order to arrange a screening interview, at least following 24-hours from receiving the written information. During the screening interview, all questions related to the study alongside an explanation of the purpose of the study and the study schedule were addressed comprehensively. Capacity was assessed in order to ensure informed consent. Once this was completed, a consent form was signed (Appendix 3 – section 9.1). At the screening visit, all relevant patient information was compared against the inclusion/exclusion criteria to ensure study eligibility.

All patients that were reviewed were recorded in a password protected screening log. The reasons of decline or reasons for not fulfilling the eligibility criteria were also recorded. No further information was collected relevant to ineligible individuals or those declining enrolment.

2.1.4: Inclusion and exclusion criteria

The inclusion criteria were designed to identify patients benefited by intravenous iron administration whilst the exclusion criteria were designed to identify individuals where a repeat infusion in a relatively short period of time (4 weeks) would carry any detrimental effects (e.g. iron overload). Any confounders and contraindications to intravenous iron administration were considered exclusion factors. The criteria in terms of haemoglobin and haematinics were composed based on both national and local guidelines (56,64,373,374). Dosage, weight adjustments and limitations were derived based on the SmPC of each preparation. Phosphate concentration as an excluding factor was proposed given the incidence of hypophosphataemia previously recorded in studies in order to allow full completion of study participation (150). Adhering to the rules and regulations of the MHRA

pregnant and/or lactating patients were excluded from the trial, while childbearing potential was examined and its implications with regards to contraception were discussed between the study personnel and the participants. The inclusion/exclusion criteria are displayed in Appendix table 17.

2.2: Screening and evaluation

Patients identified as being potential participants underwent a screening interview. Baseline data were collected from all participants who have consented to take part in the study. Data collected included:

1. Age
2. Gender
3. Ethnicity
4. Aetiology of CKD
5. Weight in kilograms
6. Height in metres
7. Smoking status (current, previous, never)
8. Alcohol Intake in units
9. Concurrent Medication Check
10. Relevant medical history

Blood investigation results were accepted as screening bloods up to four weeks prior to screening.

2.2.1: Concurrent medication check

A complete listing of all concomitant medication received during the treatment phase was recorded in the case report form at baseline and at the end of the study. The sources accepted for concurrent

medication check were provided either by the patient (i.e. a copy of their repeat prescription) or through access at the online summary care records following consent from the patient. All routine concomitant medication were continued during the trial with the exception of iron supplementation. Any medication changes were recorded.

2.2.2: Informed consent

Informed consent (Appendix 3 – section 9.1) was obtained for any procedures that were specifically for the purposes of the trial and out-with standard routine care. Full explanation of the reasons behind any investigation performed, alongside the course of action in case that the investigations yielded abnormal results was provided. Consent was provided by patients deemed as having mental capacity as per the principles underlining mental capacity (375). The consent process was only completed by an authorised and trained study doctor.

Once study information was communicated and the study doctor was assured that each potential participant understood the implications of participating in the study, the consent forms were signed and dated by both parties. The original consent forms were retained and filed according to Good Clinical Practice guidelines in an allocated investigator site file, while copies were stored in their clinical notes, with the patient and at the hospital secure Y:drive.

The consent form governed aspects of voluntary participation and right of withdrawal use of samples, data access, protection and transfer, communication between healthcare providers and handling and transfer of samples.

2.3: Randomisation and blinding/unblinding

Randomisation took place using a web-based randomisation system (www.sealedenvelope.com) (376), which allocated the treatment arm using permuted blocks in a 1:1 ratio.

As this was a double-blind trial, the principal investigator and study doctors were not aware of the group allocation and the treatment delivered to each patient. Information regarding randomisation was provided only to one 'unblinded' nurse, who was also responsible for administration of the allocated investigational medicinal product. Unblinding would only take place in cases of medical emergency (e.g. anaphylactic episodes).

The participants were randomised to receive either:

FDI - 100ml of normal saline 0.9% with the addition of FDI infused intravenously over 15-30 minutes at Visit 2 and Visit 5 for total dose repletion.

Or

FCM - 100ml of normal saline 0.9% with the addition of FCM infused intravenously over 15-30 minutes at Visit 2 and Visit 5 for total dose repletion.

2.4: Trial investigational medical products

Patients entering the trial would receive 1500 or 2000 mg of FDI or FCM as part of the replenishment dose offered based on their weight and haemoglobin. Participants received the same dose during the first administration (1000 mg) irrespective of preparation.

The iron dose for total repletion was estimated based on Appendix table 18, which was created using the SmPC of each product. The dosing table was reviewed and accepted by the MHRA as part of the authorisation process.

Both products are third generation intravenous iron formulations able to provide near-full replenishment of iron need on a single sitting. They are indicated for the treatment of iron deficiency when oral iron preparations are ineffective or cannot be used, where there is intolerance to oral iron and where there is a clinical need to deliver iron rapidly. They are both contra-indicated in cases of known hypersensitivity to the named product or other iron products, evidence or known iron overload disturbances and non-iron deficiency anaemia. Additionally FDI is contraindicated in patients with decompensated liver disease. Both products are approved for use by the MHRA since 2009 (FDI) and 2007 (FCM).

The SmPC of each preparation was regularly reviewed in order to ensure the safety of patients and that procedures and safety measures in-place are consistent with up-to-date practice.

The Pharmacy Clinical Trials Unit of the HUTH NHS Trust was responsible for the reception and validation of the supplied medicines. The medicinal products were stored in a locked cupboard on the Medical Day Unit of the Hull Royal Infirmary according to their storage instructions. Expiry date check, stock control and administration checks were the responsibility of the unblinded nurse involved in the trial. As part of drug accountability, the unblinded nurse maintained a drug accountability log where the randomisation treatment that each participant received was documented. The randomised treatment was prescribed on the participant's drug chart. The drug chart stated that the participant had been prescribed either FDI or FCM and the dose of each in order for the blind to be maintained.

2.5. Planned interventions and investigational assessments

2.5.1: Outcomes (pre-specified and post-hoc)

This pilot study aimed to explore the potential differential impact of different iron compounds on iFGF23 and phosphate in patients with established CKD. As per protocol outcomes of interest revolved around iFGF23, phosphate, 6H syndrome. Given the impact that iFGF23 can have on phosphaturia, we post-hoc explored markers of urinary phosphate excretion (fractional excretion of phosphate (FEPi)), alongside markers of bone metabolism. In addition, haematinic response was explored, alongside any implications on kidney function. Markers of inflammation and cardiovascular variables were monitored. The differential response to iron preparations in terms of functional status and quality of life was assessed. A safety assessment was performed as part of pharmacovigilance (see section 7.0).

2.5.2: Investigational assessments

Demographics and co-morbidities

At screening, details relevant to demographics and co-morbidities were collected. Co-morbidities recorded for analysis included stage and aetiology of CKD and presence of cardiovascular pathologies, diabetes mellitus or malignancy.

Physical Examination

Physical examination was also performed at baseline, in order to establish an understanding of the participant's well-being and background status prior to inclusion in the trial. This included examination of the following systems:

1. Cardiovascular
2. Respiratory
3. Abdominal
4. Neurological
5. Skin

Bone metabolism screen

Bloods to assess iFGF23, phosphate, calcium, PTH, 1,25 $(OH)_2$ Vitamin D, 25(OH) Vitamin D, 24(R),25$(OH)_2$, were measured at every visit following screening (excluding visit 8). Bone turnover markers were monitored including ALP, bone-specific ALP (BALP), carboxy-terminal collagen cross-linked telopeptide of type I collagen (CTx) and

N-terminal propeptide of type I procollagen (P1NP). These can used to investigate the impact that iron and iFGF23 could have on bone turnover, as they are related both with formation and resorption processes (377). Bone-specific ALP is involved in bone matrix calcification, and is synthesised by osteoblasts (378). It is considered a marker of bone formation, similar to P1NP (379). Procollagen type 1 N-terminal propeptide is released during the synthesis of type I collagen, which is the most copious bone protein and important in bone formation (380). In the meantime CTx is a collagen I degradation product, therefore signifying bone destruction and resorption (380). The participants were asked to provide the team with 24-hour urinary collection samples for assessment of 24-hour urinary phosphate excretion and calculation of FEPi. Fractional excretion of phosphate was calculated using the following equation as described by Walton and Bijvoet (381):

FEPi = (24-h urine phosphate × serum creatinine) × 100/(serum phosphate x 24-h urine creatinine)

Efficacy of treatment and markers of inflammation and kidney function

Blood investigations to assess haematinic response, kidney function and injury and inflammation. Haematinic response was assessed through measurements of haemoglobin, serum ferritin and TSAT. Kidney function and injury were quantified through serum creatinine, eGFR (CKD-EPI 2009) and calculation of urinary protein:creatinine ratio.

Cardiovascular screen

Bloods to assess cardiac variables N-terminal (NT)-pro hormone BNP (NT-proBNP) and Troponin T were measured as markers of prognostication and cardiac dysfunction. Troponins are proteins that play a fundamental role in the sarcomere action of the cardiac muscle, and are released in response to stress or necrosis of cardiac cells (382). They are widely accepted as a marker of ischaemia and myocyte damage, and have an important role in the diagnosis of acute myocardial events (383). However, as modern highly-sensitive assays have evolved, troponins can remain elevated for a longer period of time, especially in chronic disease, representing continuing myocardial insult. Therefore, troponin assays can be used to detect the potential pathogenic consequence to cardiac muscle resulting from the administration of a medication. B-type natriuretic peptide (BNP) and its N-terminal fragment (NT-proBNP) are natriuretic proteins released by the ventricular myocardium at periods of increased myocardial wall stress (384). They are important prognosticators in heart failure and have been implicated in research on multiple occasions (384). Electrocardiography to monitor for the presence of arrhythmias and intervals suggestive of electrical misconductance (PR interval, QRS interval, QTc interval). Trained cardiology department technicians using the same ECG machine throughout the study performed electrocardiography.

Pulse wave velocity is the speed of arterial pressure waves travelling through the aorta and large arteries; the greater the speed indicates a greater pressure and a greater degree of atherosclerosis and loss of elasticity (385). The American Heart Association advocates the use of pulse wave velocity as a marker of arterial stiffness as a *Class IIa; Level of Evidence A*, with pulse wave velocity carotid-femoral (PWV(cf)) being the variable measured to determine non-invasively

arterial stiffness (Class Ia; Level of Evidence A) in previous guidelines on standardisation of arterial stiffness measurements (385). Carotid-femoral pulse wave velocity measurements and Augmentation Index in the central aorta (AIx (ao)) were recorded as indicators of arterial stiffness. This was obtained by a single clinician using the Enverdis ® Vascular Explorer (Enverdis GmbH Medical Solutions, Jena, Germany). The device works via an oscillometric method, which produces automatic measurement and determination of central and central-peripheral pulse wave velocities. Measurements were taken with the patient in a semi-recumbent position (45º angle), in order to standardise the process of recording. The limb preferred was dictated by the presence of an arteriovenous fistula, or the expectation for the creation of one. The limb used in the first measurement, was thereafter used for the rest of the study. In order for the measurement to be complete, an accurate weight, height and distance between the suprasternal notch and pubic bone.

Functional status and patient reported outcome measures

Patient reported outcomes were monitored via the use of the Fatigue Severity Scale (FSS) and the Short Form (36) Health Survey (SF-36). Functional status was monitored through the Duke Activity Status Index (DASI) and the 1-minute-sit-to-stand-test.

1. The SF-36 (Appendix 3 – Section 9.5): A thirty-six question based health-related quality of life patient-reported survey assessing eight variables including vitality, physical functioning, bodily pain, physical role functioning, social functioning, emotional functioning, mental health and general health (386). It has been extensively used in research with good reliability and validity extending to CKD and anaemia (387–390). The SF-36 v1.0 was used following relevant authorisation from the

RAND Corporation. Scoring was performed according to the manual provided.

2. The FSS (Appendix 3 – Section 9.4)**:** A nine-item unidimensional questionnaire assessing fatigue severity (391). This is scored on a seven-point Likert scale where the participant scores elements of their fatigue such as motivation, impact of exercise, ease of fatigue, fatigue interference, reflection of fatigue in normal function, impact of fatigue on activities, fatigue interference, fatigue frequency and fatigue interference with social life; the higher the score, the greater the fatigue. It has been previously used in multiple chronic diseases including fibromyalgia, multiple sclerosis, chronic hepatitis, and Parkinson's disease (391–395). It also incorporates a visual analogue scale. The FSS has been showing to have good test-retest reliability and a high internal consistency (395).
3. The DASI score (Apppendix 3 – Section 9.3): This is a 12-part questionnaire assessing functional capacity of patients; it can be used to calculate the maximal oxygen consumption of an individual (VO_2), a surrogate marker of a participant's maximal aerobic capacity without invasive and lengthy procedures (396). It has been validated and advocated in cardiac patients whilst validity has been established in patients with low clearance CKD (eGFR < 30 ml/kg/1.73 m^2 (397,398). Additionally thinkkidneys.nhs.uk an active campaign launched by the Renal Association UK advocates the use of DASI score as "*a measurement tool that the Measurement and Understanding workstream have identified as relevant for use in quality improvement projects within the renal setting*" (399). The maximum DASI score can be that of 58.2 points. Any measurements were converted to VO_2 peak (mL/kg) and metabolic equivalents based on the following equations:
    1. VO_2 peak (mL/kg)= 0.43 x DASI + 9.6
    2. METs (metabolic equivalents) = VO_2 peak / 3.5

4. The 1-minute-sit-to-stand-test: Each participant was asked to sit on a standard height chair (46 cm) without armrests from the ground and stand up as many times as possible within one minute with arms crossed across chest (400). The chair was placed against the wall for safety purposes. Full motion (complete sitting at 90º flexion of knees and hip and complete upright) was required for a complete action to be counted. The same chair was used throughout the study and was isolated in the room allocated to study visits. Timing of 1 minute took place using a professional timer stopwatch. No encouragement was given during the 1 minute but participants were advised to inform the investigator if any chest pain, dizziness or shortness of breath arose during the test. All participants were informed that they could stop as necessary prior to the 1-minute mark. The 1-minute-sit-to-stand carries good validity and reliability in assessing and quantifying lower extremity function, exercise capacity and fatiguability (400–402).

Safety Assessment

Safety assessment took place throughout the trial, and any changes in health status were monitored for incidence of adverse events. The participants were asked about any potential side effects of the use of iron and their general health at every visit. Additionally their online medical records were accessed to ensure no urgent hospitalisations or presentations to hospital occurred between appointments. The participants were asked to contact the PI or study nurse if they had any concerns about safety at any time in between follow-up visits for the trial. Any blood investigations deemed abnormal were noted and acted upon at the time of trial and the necessary referrals were made and documented. Any changes in health were monitored throughout the study and until their resolution. Adverse event were recorded as

part of pharmacovigilance duties towards the sponsor based on the standard of practice “Hull University Teaching Hospitals NHS Trust R&D GCP SOP 07 – Safety Reporting”. Relationship to the investigational medicinal product administered was assessed based on section 4.8 of the SmPC for each individual product. Severity status was adjudicated based on criteria delineated on the reporting process. Additionally as part of collection of data relevant to ‘changes in health’ questions relevant to COVID-19 were asked from every individual patient on every visit in order to ensure no symptomatology developing associated with COVID-19 (new onset fever, cough, change in sense of smell or taste, shortness of breath). Anyone having such symptoms was advised to follow national guidelines and request testing as applicable.

The participants were monitored during iron administration, which took place at the Medical Day Unit of Hull Royal Infirmary, where appropriate resuscitative equipment was present. Any allergic, hypersensitivity, and Fishbane reactions were recorded, alongside any irritation at the site of infusion. The participants were followed up for a total of 15 minutes following intravenous iron administration as per hospital policy.

In order to minimise the risk of iron overload and increase in labile iron content a TSAT target of 25-60% was set. Ferritin was closely monitored; the dosing regime was reviewed if the ferritin appeared to rise above >500 micrograms/ml as per the NICE guidelines on the management of CKD associated anaemia. Additionally in order to ensure that no iron was administered to patients developing hypophosphataemia, a clinical visit between visit 4 and 5 (18-30 days) was arranged in order for phosphate measurements to be repeated. If the measured phosphate level was <0.65 mmol/L prior to second planned dose of iron, the participant in the ExplorIRON-CKD Trial was

ineligible for a second dose of iron but would continue follow-up in the trial as detailed in the schedule of visits. The rate of failed completed treatments due to the development of hypophosphataemia was recorded.

2.5.3: Planned trial procedures and follow-up

The trial ran over 12 weeks, with eight distinct visits. The study points enabled examination of early bone related outcomes, cardiovascular marker changes, efficacy and safety. In addition, enough time for positive or negative signals to develop in terms of patient reported outcome measures existed. Appendix table 19 indicates the schedule of trial events.

Given the nature of the evolving COVID-19 pandemic and the impact that this had on healthcare provision and research alongside with restrictions on participants' travelling and their wishes to minimise time away from home, the team counselled with participants of the trial in order to seek and implement appropriate changes that would safeguard both participation and minimise the degree of loss-to-follow-up data. This was as part of our continuous consultation with the participants, as the aim of the team was to have a solid interaction with all participants regarding the status of the trial from the design of the trial until the end. As such, the team redesigned an aspect of the planned trial procedures in order to maximise data gathering without limiting study integrity about the outcomes relevant to phosphate and iFGF23. Visit 8 was redeveloped to happen in an "online/virtual/remote" setting in order to accommodate the patients that may not wish to visit the hospital due to isolation/shielding but were already enrolled in the study and wished to remain part of it during the months of peak pandemic incidence. Where applicable

(such as in patients with on-going care needs requiring frequent blood investigations) NT-pro-BNP was added to the routine bloods taken.

Upon completion of involvement to the trial, participants' care continued under their renal physician or general practitioner.

2.6: Handling of samples and analysis

2.6.1: Blood and urine handling and storage

Venesection was performed by trained HUTH NHS Trust staff. Venesection was performed using butterfly needles (BD Vacutainer Safety-Lok). Blood samples were collected in EDTA and serum bottles (BD Vacutainer K3E 7.2 mg and BD Vacutainer SST II Advance blood collection tubes respectively; Becton Diskinson, Franklin Lakes, New Jersey, United States). Expiry date was reviewed prior to use. Urine for protein-creatinine ratio calculation was collected using a standardised white-top specimen container, while 24-hour urine collection for analysis of phosphate excretion was performed using 2L containers enriched with 20 mL of hydrochloric acid (50%). Strict advice on safety measures when using this container was provided verbally and in written format. The participants were explained when urinary collection should be performed and that no urine transfer between containers should take place.

The samples collected were transferred to the pathology laboratory within one hour. The transfer time between study site to the pathology laboratory ranged between 5 and 10 minutes. On arrival to pathology certain samples were immediately processed according to the HUTH

NHS Trust pathology laboratory standard operating procedures, while others required freezing for transfer to University of East Anglia following centrifugation for 10 minutes at 3000 rpm (Heraeus Multifuge 1S - Thermo Fisher Scientific, Waltham, Massachusetts, USA). Once centrifugation was completed, the separated plasma was pipetted to appropriate aliquots (Sarstedt 2m microtubule; Sarstedt AG and Co, Nümbrecht, Germany). The aliquots were labelled according to destination and sample type. The aliquots were stored at a secure -80^{o}C freezer at the pathology laboratory. Transfer between sides was performed using accredited courier service over dry ice, under the umbrella of Category B biological samples, with next day delivery. On receipt to external site, the samples were stored in -70^{o}C freezers located in The Norwich Research Park Biorepository. All blood handling, storage, transfer and analysis was as per the Human Tissue Act 2004 (403).

2.6.2: Sample analysis

Analysis of samples occured either in HUTH NHS Trust or in University of East Anglia, depending on variable monitored. The analysis was as follows:

HUTH NHS Trust

All sample analysis took place in real-time (with the exception of Troponin T) and therefore did not require freezing and storage.

1. Markers of haematological response and efficacy: The SYSMEX XN-9100 (Sysmex Corporation, Kobe, Japan) was used for the measurement of haemoglobin. Ferritin was

analysed using the UniCel DxIAccess Immunoassay System (Beckman Coulter, Nyon, Switzerland), utilising the Access Ferritin assay, which is a two-site immunoenzymatic ("sandwich") assay based on the incubation of ferritin with goat anti-ferritin-alkaline phosphatase conjugate and mouse anti-ferritin complexes and the subsequent detection of chemiluminesccence. Beckman Coulter technology was used to analyse transferrin and TSAT through the AU5800 analyser (Beckman Coulter, Nyon, Switzerland). The method of analysis includes an immune-turbidimetric technique for the quantitative determination of transferrin. The technique is based on the formation of insoluble aggregates of transferrin following mixing of the blood sample with a buffer and an antiserum solution containing anti-human transferrin antibodies. The absorbance of these aggregates is proportional to the transferrin concentration in the sample.

2. Kidney function and kidney injury: Kidney function was measured using creatinine. Creatinine (both serum and urinary) was measured an automated enzymatic assay through the AU5800 analyser (Beckman Coulter, Nyon, Switzerland). A series of chemical reactions lead to the catalysis of creatinine to hydrogen peroxide. Hydrogen peroxide then undergoes quantitative oxidative condensation yielding a blue pigment, which is used to estimate the creatinine concentration through absorbance quantification. eGFR was automatically calculated at source using the CKD-EPI Creatinine equation:

$eGFR = 141 \times \min(S_{Cr}/\kappa, 1)^{\alpha} \times \max(S_{Cr}/\kappa, 1^{)-1.209} \times 0.993^{Age}$ x 1.018 [if female] x 1.159 [if Black] (where κ =0.7 (females) or 0.9 (males) ; α = -0.329 (females) or -0.411 (males); S_{Cr} = standardised serum creatinine).

Kidney injury was quantified through proteinuria, a marker of glomerular damage (124). Urinary protein quantification was an

automated procedure using the AU5800, based on photometric colour testing. Once urinary protein was quantified, urinary protein:creatinine ratio was calculated.

3. Inflammatory markers: C-reactive protein was measured using the AU5800 analyser. The sample was combined with an antiserum solution containing anti-human CRP antibodies. As such insoluble aggregates were formed which were absorbed and measured, yielding a result proportional to the CRP concentration of the sample.
4. Bone metabolism markers: Calcium, phosphate and ALP were analysed using an AU5800 automated analyser. Total calcium was measured using a photometric colour test, based on the formation of an intense purple colour on the reaction between calcium and the compound Arsenazo III. The intensity of the colour is proportional to the concentration of calcium. Adjusted calcium was calculated through an automated process excluding albumin. Both serum and urinary phosphate were quantified using a photometric ultraviolet method based on the reaction between inorganic phosphorous and molybdate. The absorbance displayed at a certain wavelength is proportional to the concentration of inorganic phosphorus in the sample. Alkaline phosphatase was quantified through a kinetic colour test involving enzymatic action of ALP on p-nitro-phenylphosphate to p-nitrophenol. The rate of absorbance of the newly formed p-nitrophenol is directly proportional to the ALP activity, and by-proxy to the ALP concentration. Parathyroid hormone was analysed using the UniCel DxIAccess Immunoassay System (Beckman Coulter, Nyon, Switzerland), which, similar to ferritin, made use of a two-site immunoenzymatic (“sandwich”) assay (Access Intact PTH). Light generated and detected through a luminometer following the incubation of PTH with monoclonal anti-PTH antibody and goat polyclonal anti-PTH antibody, was proportional to the concentration of PTH in the sample. Total P1NP was analysed

through the Roche cobas e411 analyser (Roche Diagnostics, Risch-Rotkreuz, Switzerland) using the Elecsys total P1NP assay, which operates based on electrochemiluminescence. The sample is incubated with monoclonal P1NP-specific antibodies and other substances and then aspirated into a measuring cell. The microparticles formed are magnetically captured on an electrode.The results are plotted on a calibration curve, and based on those the concentration of P1NP is calculated.

5. Cardiac biomarkers: The Roche cobas e411 analyser was used in the analysis of both cardiac biomarkers monitored in this study (NT-pro-BNP and Troponin T). This analyser utilises patented electrochemiluminescence technology in the analysis of immunoassays. In order to quantify NT-pro-BNP the Elecsys proBNP II immunoassay was utilised. This method employs the sandwich principle with incubation of a sample with biotinylated monoclonal NT-proBNP-specific antibody, monoclonal NT-proBNP-specific antibody labelled with a ruthenium complex and other substances in order for a solid product to be formed. The solid mixture is aspirated and magnetically applied onto an electrode. In a technique similar to P1NP immunoassay analysis, application of voltage through the electrode causes chemiluminescence, with light captured and measured through a photomultiplier. A calibration curve is then plotted and the concentration of NT-proBNP is hence calculated. Troponin T was initially centrifuged, frozen and stored as aforementioned. It was then brought to room temperature over one hour, centrifuged at 3000 rpm for 5 minutes immediately prior to dilution. Troponin T was analysed and quantified using the Elecsys Troponin T hs electrochemiluminescence immunoassay making use of the sandwich principle and electrochemiluminescence as explained in the immunoassays associated with NT-proBNP and P1NP. The chemiluminescent

emission generated is proportional to the troponin T concentration as plotted on a calibration curve.

All automated procedures took place in accordance to the standard of operating procedures of the HUTH NHS Trust and following the manufacturers' instructions for each individual test performed.

University of East Anglia

Prior to analysis samples were defrosted and brought to room temperature over one hour, and then centrifuged at 4000 rpm for 5 minutes immediately prior to dilution. On the same day, the defrosted samples were used for analysis.

1. Bone metabolism markers: Serum iFGF23, BALP, $1,25(OH)_2$ Vitamin D, $25(OH)_2$ Vitamin D, $24(R),25(OH)_2$ Vitamin D, and CTX , were analysed at the University of East Anglia. Plasma iFGF23 was analysed through a quantitative 3-step chemiluminescence immunoassay where two FGF23 monoclonal antibodies to FGF23 are used to capture and detect the intact protein (Liaison XL, DiaSorin S.p.A., Saluggia, Italy). Plasma CTX was measured on the COBAS 6000 (Roche Diagnostics, Switzerland) utilizing an electrochemiluminescence sandwich immunoassay. Serum CTx was quantified through biotinylated monoclonal and ruthenium labelled antibodies against CTx. Serum BALP was analysed using an Enzyme-Linked Immunosorbent Assays purchased from Quidel (Quidel, San Diego, California, USA) following manufacturer's instructions. $1,25(OH)_2$ Vitamin D and iFGF23 were analysed using the Liaison XL analyser (Liaison XL, DiaSorin S.p.A., Saluggia, Italy) that can utilise fully

automated assays following manufacturer's instructions. A chemiluminescent immumnoassay was used to quantify $1,25(OH)_2$ Vitamin D through a recombinant fusion protein for capture of the $1,25(OH)_2$ Vitamin D molecule and a murine monoclonal antibody which specifically recognizes the complex formed by the recombinant fusion protein with the $1,25(OH)_2$ Vitamin D molecule. Manual analysis of $25(OH)_2$ Vitamin D and $24(R),25(OH)_2$ Vitamin D, took place and this was measured by Liquid Chromatography Tandem Mass Spectrometry as previously described by Tang and colleagues (404).

All automated procedures took place in accordance to the standard of operating procedures of the University of East Anglia and following the manufacturers' instructions for each individual test performed.

Appendix table 20 summarises the assays used in terms of cardiovascular and bone metabolism markers.

2.7: Pharmacovigilance

2.7.1: Definitions

In order to ensure pharmacovigilance throughout the duration of the trial the following set of definitions was used in accordance to the standard of operating procedures regarding safety reporting (Safety Reporting SOP – R&D GCP SOP 07) of HUTH NHS Trust, the study sponsor (Appendix table 21).

2.7.2: Adverse events and reporting

Reporting Period

The adverse event (AE) reporting period for this trial began as soon as participants were consented to the trial and ended after the patient's final study visit (Visit 8).

Reporting Process

All reportable adverse events (serious and non-serious) were recorded on the adverse event form at the back of the case report form. The study team followed up all events until resolution or decision of no further follow-up. All serious adverse events (SAE), serious adverse reactions (SARs) and suspected unexpected serious adverse reactions (SUSARs) were reported to the sponsor using the study specific SAE report form, within 24 hours of the research staff becoming aware of the event.

The PI was responsible for the assessment of severity, seriousness and causality using the Appendix table 22. As part of the duty to report and the safety assessment-taking place during the study, the study team completed follow-ups of every SAE, providing a concluding statement and re-assessing causality and severity appropriately as indicated through the SAE duration. The sponsor and an independent board was responsible with review and reassessment of all SAEs.

All SAEs with the exception of elective surgery or planned admission, or prolongation of existing hospitalisation for pre-existing conditions

required expedited reporting. As part of clinical governance and the regulations of MHRA and the relevant research ethics committee, an annual Development Safety Update Report was produced and submitted to the relevant parties.

2.8: Withdrawal criteria

Any participant involved in the trial had the right to withdraw from the trial at any time without any detrimental effect on their on-going care delivered by the HUTH NHS Trust and the Renal Department. Participation was discontinued in cases where:

1. The participant withdrew consent; the reasons for withdrawal would be sought and recorded, respecting the individual patient's right to give no reason
2. The participant was withdrawn from the trial by the research team: such option applied in patients that declined or where unable to attend follow-up (loss to follow-up) or in those that had undergone an intervention altering results according to the discretion of the research team.
3. The trial was stopped due to safety reasons.

Any individuals withdrawing consent had the right to decline use of their data and samples up to the point of withdrawal.

2.9: Data Management

All data management (use, control, storage) was in accordance to the General Data Protection Regulation 2018 (405). The database generated was password protected and stored on a drive belonging to

the HUTH NHS Trust. Access was granted to study staff that already abide to the HUTH CP134 – Confidentiality and Information Security Policy. A list of staff authorised to access and make data changes was maintained. Direct access to data was granted to authorised representatives from the sponsor and the regulatory authorities to permit trial-related monitoring, audits and inspections and the research staff involved in the execution of the trial.

The security of stored data was guaranteed by the HUTH NHS Trust IT Services Department, which had a backup procedure approved by auditors for disaster recovery of files held on the Y: drive servers.

2.9.1: Source data

Online case note sheets were prepared mirroring the case report form and standard outpatient practice. A 'web-alert' within the shared clinical records software of the trust was created as soon as the patient enrolled in the study. The Trust's online system (Lorenzo) and the online Summary Care Records were also considered as source. Any information collected on the case report forms was reviewed to ensure that it matched the source data. Additionally, evidence of results of investigations performed (PWV reports, functional status tests scores) were considered as source.

2.9.2 Case report forms

Originally, a paper-format case report form was created for the recording of study-relevant findings, however due to the on-going COVID-19 pandemic this was redesigned to an electronic format. The case report form was anonymised with no links present between the

results and the individual patient except by the study research team, monitoring team and regulatory authorities. The protocol number, subject initials, and subject number were included in all pages. The case report form was used to record all relevant data to the study as including investigation results, adverse events, changes to concomitant medication and health status.

2.10: Statistical analysis plan

The flow of individual participants through each stage of the trial is reported in accordance with the CONSORT 2010 statement extension to pilot and feasibly trials; this includes the number of persons evaluated for potential enrolment, randomly assigned to each group, who received treatment as allocated, in an intention to treat method of analysis.

A table showing baseline demographic and characteristics for the whole group of participants and each group is presented to indicate any differences between groups. Patient characteristics were summarised using appropriate statistics. Mean (standard deviation (SD) is used for normally distributed data, median (interquartile range (IQR) for non-normally distributed data and raw count (number, %) for nominal data. Percentage change regarding the two outcomes of particular interest (iFGF23 and phosphate) was calculated alongside the associated factors of urinary phosphate excretion and fractional excretion from baseline to individual time points. Normality of distribution was assessed using the Shapiro-Wilk test. Between groups analysis (i.e. FDI vs. FCM) was conducted using independent T-test and Mann-Whitney U test depending on distribution. Differences between the two groups in terms of categorical data at baseline were investigated using the Fisher's exact test. Spearmann's correlation

coefficient was used to assess the relationship between different variables at baseline, in order to identify potential confounders. Spearmann's correlation coefficient was also used to assess the relationship between percentage change (%change) in phosphate and iFGF23 from baseline to every different point between variables of interest, i.e. the ones relevant to the 6H syndrome (PTH, FEPi, 1,25 $(OH)_2$ Vitamin D, Calcium)

The combined effect of iron supplementation as composite of change was examined using %change of iFGF23 from baseline and %change in serum phosphate from baseline to day 2 and week 2. Based on previous work by Huang and colleagues (maximum %change iFGF23 noted on day 2: 248% (significant – $p<0.0001$), maximum %change in phosphate noted on day 7: -23% (significant – $p<0.0001$) and Stohr and colleagues (maximum %change iFGF23 noted on day 1: 80% (non-significant), maximum % change in phosphate noted on day 14: -20% (non-significant)) on patients with ND-CKD administered FCM, and the exploratory nature of the study, we included in the analysis of this outcome values of either >200% change in iFGF23 and/or > -20% in phosphate concentration (217,218).

A post-hoc analysis took place to identify any within-group trends (FDI group, FCM group, whole cohort); due to the nature of the sample (small, with loss of data), the Skillings-Mack test was performed which is a variation of the Friedman test taking in consideration any block design with randomly present missing data (406).

A statistical software package was used to aid in analysis (IBM SPSS Statistics Version 26, IBM Corp. 2019). Statistical significance was inferred from a p-value <0.05.

3: Results

3.1: Screening process

The study ran between March 2020 and July 2021. In total 168 patients were referred to the renal specialist anaemia service and were pre-screened. Ninety-nine patients were contacted as they fulfilled on first principle the inclusion/exclusion criteria of the study. The common reasons for not being contacted were “clear-cut” cases where patients were ineligible. These included ferritin concentration outside the eligibility criteria (i.e. a serum ferritin >200), weight restrictions (i.e. a weight less than 70 kg where the haemoglobin was > 100) and active malignancy (26.0%, 18.8% and 13.0% respectively). The consort diagram (figure 5) delineates the process of pre-screening, screening and study proceedings.

Following pre-screening 99 patients were invited for screening. The reasons behind not joining the trial were explored in the 64 individuals that declined participation, however not all of them provided a reason. The most frequent reasons for not being interested in the study where they were provided (30 out of the 64 individuals that declined) were the COVID-19 pandemic (13 individuals – 43.3%) and inability/difficulty to frequently travel to hospital (11 individuals – 36.7%).

Thirty-five patients of those contacted (35.4%) agreed to attend a screening visit. The mean age of the screened group was 64.9 years old ((SD): 15.4), with 22 males and 13 females. Nine patients were ineligible for the study following screening. One was later re-screened

successfully. One participant completed screening successfully, however withdrew prior to randomisation and provision of medication.

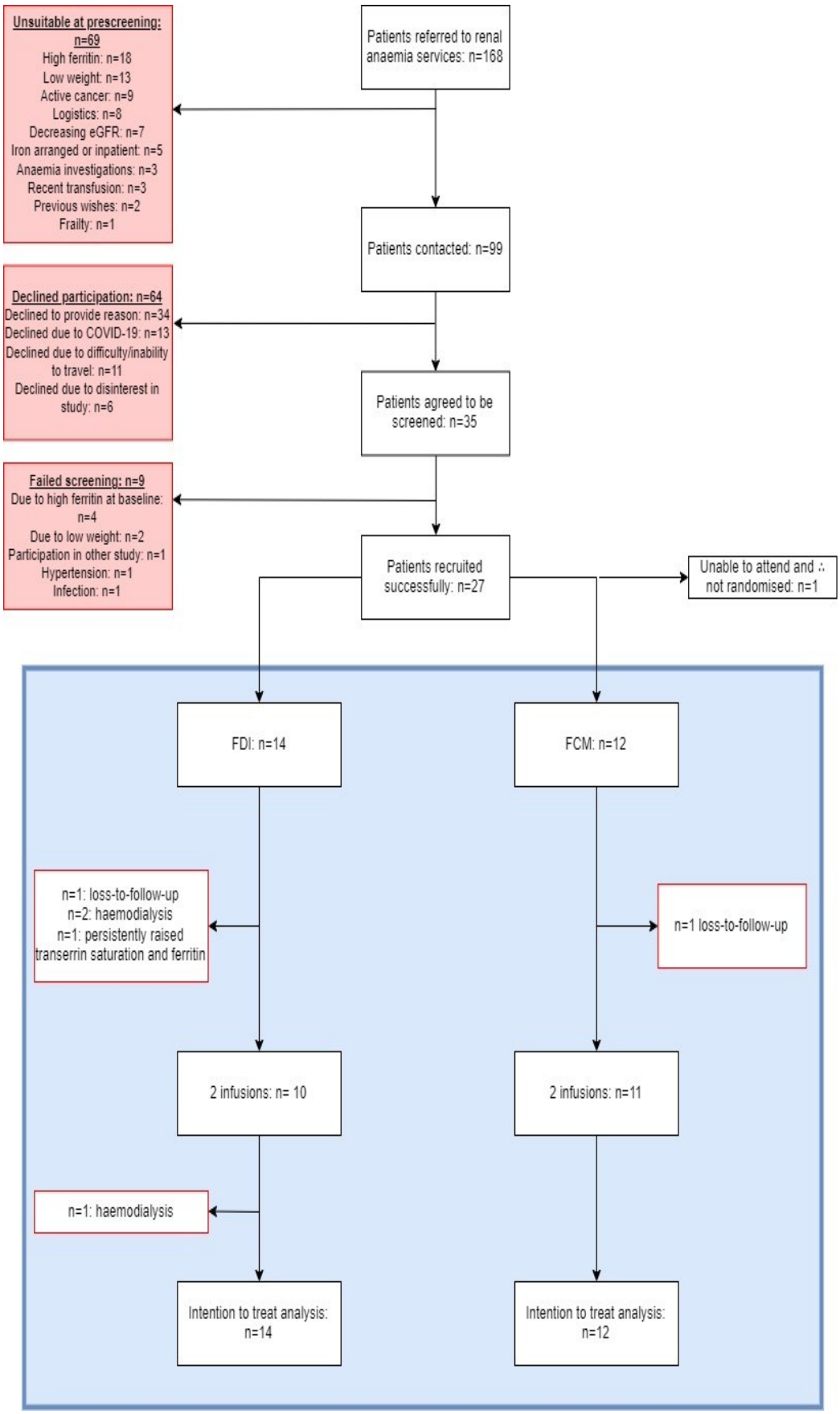

Figure 5: Consort diagram

3.2: Baseline data

3.2.1: Total cohort

Twenty-six participants were randomised and received at least one dose of the medication. The distribution of variables is delineated in table 1. The mean age of the randomised population was 68 years old (67.9 years, SD: 12.4) and 17 (65%) were male. Twenty-three (88.5%) participants had CKD stage 4 or worse. The median eGFR was 18.0 (IQR: 11.3) ml/min/1.73 m^2. Mean creatinine concentrations was 269.5 (SD: 88.3) µmol/L (table 2). Thirteen different causes of renal disease were identified in the group (table 3). Six patients (23.1%) had more than one causative process and they were adjudicated as multifactorial. The most common intrinsic causative pathology was membranous nephropathy (3 participants (11.5%)).

As primary outcome measures the cumulative population median iFGF-23 was 212.1 pg/ml (IQR: 166.4) and median phosphate concentration was 1.28 (IQR: 0.31) mmol/L at baseline. In terms of haematinics, the baseline concentrations were consistent with absolute iron deficiency anaemia (mean haemoglobin: 100.3 g/L (SD: 13.5); median serum ferritin and TSAT: 76.5 (IQR: 118.8) µg/L and 15.0 (IQR: 6.8) % respectively) Baseline data of continuous variables are displayed in table 3.

In terms of background medical history, 21 (80.8%) of the participants had hypertension and 13 (50.0%) had an underlying diagnosis of diabetes. Other important comorbidities are on display on table 3. Nineteen (72.9%) of the participants were either current or former

smokers. On medication review (table 3), 18 (69.2%) participants were not receiving ESA at the time of inclusion to the trial.

Table 1: Distribution of data and reported values

Normally distributed data (reported as means and (SD))	Not normally distributed data (reported as medians and interquartile range (IQR))	
• Age • Haemoglobin • Creatinine • Calcium • 1,25 $(OH)_2$ Vitamin D • Pulse wave velocity • Augmentation Index • 1-minute-sit-stand • Energy score of SF-36	• Serum ferritin • TSAT • eGFR • CRP • Urine PCR • Urinary phosphate excretion • FEPi • Phosphate • PTH • 25 $(OH)_2$ Vitamin D • 24(R),25 $(OH)_2$ Vitamin D • ALP • BALP • CTX • P1NP	• iFGF23 • BNP • Troponin T • ECG parameters • DASI • FSS total • FSS visual analogue scale • All components of SF-36 except energy

3.2.2: Randomised groups

Fourteen patients were allocated to receive FDI, and 12 FCM. Out of the 26 that were enrolled, 21 patients received both doses of iron supplementation and they attended visit 6 of follow up. All patients that received at least one dose of iron are included in the analysis, as intention-to-treat.

Table 2 displays the two groups formed and their baseline characteristics in terms of all continuous data variables that were investigated as part of the study. No statistical difference existed between the two groups with the exception of age, 24-hour urinary phosphate excretion, fatigue severity scale, and QRS interval. The mean age for the FDI group was 63.4 (SD: 12.2) years old and for the FCM group 73.2 (SD: 10.8) years old (p=0.04). The median 24-hour urinary phosphate excretion at baseline was 21.0 (IQR: 8.5) mmol/24hr and 12.5 (IQR: 7.5) mmol/24hr for FDI and FCM respectively (p=0.023). In terms of fatigue severity scale, the median score reported in those receiving FDI was 54.5/63 (IQR: 13.0) and in those receiving FCM it was reported as 42.0/63 (IQR: 30.0) (p=0.035). The median QRS interval duration at baseline in the FDI group was 92.0 (IQR: 20.0) ms and in the FCM group the median QRS interval duration was 136.0 (IQR: 66.0) ms (p=0.018)

In terms of primary outcome measures (iFGF23 and phosphate) baseline iFGF23 median concentration was 257.3 (IQR: 448.4) pg/ml and 186.5 (IQR: 83.0) pg/ml for FDI and FCM groups respectively. Phosphate concentration was 1.30 (0.43) mmol/L and 1.20 IQR: 0.31) mmol/L in the FDI and FCM groups respectively. No statistically significant difference existed.

Table 3 displays the two groups formed and their baseline characteristics in terms of categorical data, including co-morbidities and medications. A significant difference between the two groups existed in terms of heart failure incidence; six (50.0%) participants in the FCM group had a diagnosis of heart failure (p=0.026). There was no significant difference between the two groups in terms of other comorbidities and medications prescribed at baseline.

Table 2: Baseline values of continuous variables

Variable	Iron group	Value	p-value	Variable	Iron group	Value	p-value
Age* / years	Total	67.9 (12.4)		1,25 (OH)2 Vitamin D* / pmol/L	Total	45.6 (22.2)	
	FDI	63.4 (12.2)	0.043		FDI	41.3 (20.8)	0.290
	FCM	73.2 (10.8)			FCM	50.7 (23.5)	
BMI / kg/m^2	Total	27.8 (8.4)		25 (OH)2 Vitamin D / nmol/L	Total	57.4 (63.6)	
	FDI	28.8 (10.1)			FDI	44.2 (64.8)	
	FCM	26.8 (9.7)	0.279		FCM	67.5 (67.3)	0.252
iFGF23 / pg/ml	Total	212.1 (116.4)		24(R),25 (OH)2 Vitamin D / nmol/L	Total	2.2 (2.8)	
	FDI	257.3 (448.4)	0.212		FDI	1.2 (4.0)	0.631
	FCM	186.5 (83.0)			FCM	2.9 (2.3)	
Phosphate / mmol/L	Total	1.28 (0.31)		Calcium* / mmol/L	Total	2.35 (0.08)	
	FDI	1.30 (0.43)			FDI	2.35 (0.08)	0.813
	FCM	1.20 (0.31)	0.193		FCM	2.34 (0.09)	
Hemoglobin* / g/L	Total	100.3 (13.5)		PTH / pmol/L	Total	17.4 (11.3)	
	FDI	99.2 (12.2)	0.664		FDI	18.9 (15.9)	
	FCM	101.6 (15.3)			FCM	16.3 (13.0)	0.145

Serum Ferritin / µg/L	Total	76.5 (118.8)		24hr urinary phosphate / mmol	Total	17.5 (11.3)	
	FDI	76.5 (158.5)			FDI	21.0 (8.5)	
	FCM	72.7 (104.6)	0.899		FCM	12.5 (7.5)	0.023
TSAT / %	Total	15.0 (6.8)		FEPi / %	Total	43.2 (22.7)	
	FDI	15.0 (10.0)	0.781		FDI	49.7 (26.1)	
	FCM	14.5 (5.8)			FCM	36.4 (21.6)	0.374
Creatinine* / µmol/L	Total	269.5 (88.2)		ALP / [iU]/L	Total	97.0 (65.3)	
	FDI	277.6 (98.8)			FDI	96.0 (74.0)	
	FCM	260.2 (77.3)	0.626		FCM	107.0 (52.0)	0.667
eGFR / ml/min/1.73m^2	Total	18.0 (11.3)		BALP / [U]/L	Total	19.5 (11.4)	
	FDI	18.0 (11.3)	1.000		FDI	21.3 (10.2)	0.462
	FCM	18.0 (11.3)			FCM	18.7 (13.4)	
CRP / mg/L	Total	7.4 (14.0)		CTx / µg/ml	Total	0.89 (0.55)	
	FDI	8.0 (17.6)			FDI	0.84 (0.45)	
	FCM	4.3 (9.9)	0.462		FCM	0.98 (0.69)	0.560
urinary PCR / mg/mmol	Total	87.5 (311.3)		P1NP / µg/L	Total	103.0 (111.3)	
	FDI	155.0 (550.0)	0.082		FDI	112.0 (108.5)	0.820
	FCM	30.0 (290.0)			FCM	103.0 (103.3)	

Table 3: Baseline values of categorical variables									
Variable	**Total**	**FDI**	**FCM**	**P-value**	**Variable**	**Total**	**FDI**	**FCM**	**P-value**
Gender					Medications				
· Male	17 (65.3)	8 (57.1)	9 (75.0)		· Erythropoiesis stimulating agents	8 (30.8)	5 (35.7)	3 (25.0)	0.683
· Female	9 (34.6)	6 (42.8)	3 (25.0)	0.429	· Renin-angiotensin-aldosterone system associated medications	16 (61.5)	9 (64.3)	7 (58.3)	1.000
Smoking status					· Vitamin D supplementation	6 (23.1)	4 (28.6)	2 (16.7)	0.652
· Smoker	5 (19.2)	5 (35.7)	2 (16.7)		· Diuretics	14 (53.8)	7 (50.0)	7 (58.3)	0.713
· Ex-smoker	14 (53.8)	5 (35.7)	9 (75.0)		· β-blockers	18 (69.2)	9 (64.3)	9 (75.0)	0.683
· Non-smoker	7 (27.0)	4 (28.6)	1 (8.3)	N/A	Cause				
CKD stage					· Autosomal Dominant Polycystic Kidney Disease	1 (3.8)	1 (7.1)	0 (0.0)	N/A
· 3b	3 (11.5)	1 (7.1)	2 (16.7)		· Multifactorial	6 (23.1)	2 (14.3)	4 (33.3)	N/A
· 4	16 (61.5)	9 (64.3)	7 (58.3)		· Primary renovascular	2 (7.7)	1 (7.1)	1 (8.3)	N/A
· 5	7 (26.9)	4 (28.6)	3 (25.0)	N/A	· Glomerulosclerosis	1 (3.8)	0 (0.0)	1 (8.3)	N/A

Ethnicity					· Unknown	1 (3.8)	1 (7.1)	0 (0.0)	N/A
· White	26 (100.0)	14 (100.0)	12 (100.)		· Diabetic nephropathy	3 (11.5)	2 (14.3)	1 (8.3)	N/A
· Black	0 (0.0)	0 (0.0)	0 (0.0)		· IgA nephropathy	2 (7.7)	1 (7.1)	1 (8.3)	N/A
· Other	0 (0.0)	0 (0.0)	0 (0.0)	N/A	· Nephrectomy	1 (3.8)	0 (0.0)	1 (8.3)	N/A
Hypertension	21 (80.8)	13 (92.9)	8 (66.7)	0.148	· Kidney aplasia and obstructive uropathy	1 (3.8)	1 (7.1)	0 (0.0)	N/A
Type I Diabetes Mellitus	2 (7.7)	2 (14.3)	0 (0.0)	0.483	· Chronic pyelonephritis	1 (3.8)	1 (7.1)	0 (0.0)	N/A
Type II Diabetes Mellitus	11 (42.3)	6 (42.8)	5 (41.7)	1.000	· Membranous nephropathy	3 (11.5)	3 (21.4)	0 (0.0)	N/A
Heart failure	7 (26.9)	1 (7.1)	6 (50.0)	0.003	· Cardiorenal syndrome	2 (7.7)	0 (0.0)	2 (16.7)	N/A
Ischemic Heart disease	10 (38.5)	4 (28.6)	6 (50.0)	0.422	· Hypertension	1 (3.8)	1 (7.1)	0 (0.0)	N/A
Previous cancer	5 (19.2)	2 (14.3)	3 (25.0)	0.635	· Systemic Lupus Erythematosus	1 (3.8)	0 (0.0)	1 (8.3)	N/A

3.2.3: Baseline cohort correlations

Baseline cohort correlations are displayed in table 4. Intact FGF23 and eGFR had a negative moderately strong correlation at baseline (Spearman's rho: -0.63; p<0.001). A moderately strong negative correlation also existed between haemoglobin and iFGF23 value (Spearman's rho correlation coefficient of -0.68, p<0.001). A moderately strong positive correlation existed between iFGF23 and phosphate concentration (Spearman's rho: 0.70, p<0.001), with a weak negative correlation between eGFR and phosphate (Spearman's rho: -0.39, p=0.047). A moderately strong negative correlation between 1,25 $(OH)_2$ Vitamin D and iFGF23 (Spearman's rho: -0.59, p=0.002) was noted. A moderately strong positive correlation was observed between FEPi and iFGF23 (Spearman's rho: 0.62; p<0.001). No correlation existed between iFGF23 and calcium and PTH. No correlation existed between FEPi, 1,25 $(OH)_2$ Vitamin D, PTH or calcium and phosphate at baseline.

Table 4: Baseline correlations with outcomes of interest (iFGF23 and phosphate)

Variable of interest	Correlation coefficient	Significance	Variable of interest	Correlation coefficient	Significance
Phosphate with:			***iFGF23 with***		
eGFR	**-0.39**	**0.047**	**eGFR**	**-0.63**	**< 0.001**
Haemoglobin	**-0.63**	**< 0.001**	**Haemoglobin**	**-0.68**	**< 0.001**
Serum ferritin	-0.07	0.736	Serum ferritin	-0.29	0.15
TSAT	-0.25	0.219	TSAT	-0.22	0.283
CRP	0.25	0.225	CRP	0.08	0.694
Calcium	0.02	0.939	Calcium	0.18	0.383
PTH	0.29	0.146	PTH	0.25	0.223
1,25 $(OH)_2$ Vitamin D	-0.38	0.057	**1,25 $(OH)_2$ Vitamin D**	**-0.59**	**0.002**
FEPi	0.15	0.443	**FEPi**	**0.62**	**< 0.001**

Bold letter-type indicates significant correlation

3.2.4: Intravenous iron administered

Intravenous iron was administered at baseline (visit 2) and at 1 month (visit 5) (table 5). A total of 38000 mg of intravenous iron was administered. Participants received 1000 mg (n=5 – 19.2%), 1500 mg (n=18 – 69.2%) and 2000 mg (n=3 – 11.5%) during the study. The mean dose administered was 1461.5 mg (SD: 280.1) in the whole of the population. The mean dose administered in the FDI group was 1428.6 mg (SD: 331.5) while the mean dose administered in the FCM group was of 1500 mg (SD: 213.2) (p=0.53). More specifically, in the FDI group, 4 participants were administered 1000 mg, 8 were administered 1500 mg and 2 were administered 2000 mg. In the FCM group, 1 participant was administered 1000 mg, 10 were administered 1500 mg and 1 was administered 2000 mg.

Table 5: Visit allocation / time period (corresponding)

Visit 1 →	Screening
Visit 2 →	Baseline (1st intravenous iron administration)
Visit 3 →	1-2 days following 1st infusion
Visit 4 →	2 weeks
Visit 5 →	1 month (2nd intravenous iron administration)
Visit 6 →	1-2 days following 2nd infusion
Visit 7 →	2 months
Visit 8 →	3 months

3.3: Intact FGF23, phosphate and markers of phosphaturia

3.3.1: Comparison between groups in terms of iFGF23 and phosphate

Table 6: Markers of 6H syndrome									
Visit	**Iron group (n)**	**Mean/Median (SD/IQR)**	**p-value**	**p- value (within group analysis)**	**Visit**	**Iron group (n)**	**Mean/Median (SD/IQR)**	**p-value**	**p- value (within group analysis)**
iFGF23 / pg/ml					**1,25 (OH)2 Vitamin D * / pmol/L**				
Baseline	FDI (14)	257.3 (448.4)	0.212		Baseline	FDI (14)	41.3 (20.8)	0.290	
	FCM (12)	186.5 (83.0)				FCM (12)	50.7 (23.5)		
Visit 3	FDI (14)	251.0 (426.4) l	0.066		Visit 3	FDI (14)	41.0 (25.6)	0.702	
	FCM (11)	467.1 (321.1)				FCM (11)	44.8 (21.9)		
Visit 4	FDI (13)	233.6 (256.0)	0.410		Visit 4	FDI (13)	37.5 (18.6)	0.841	
	FCM (10)	199.7 (184.7)				FCM (10)	39.0 (15.1)		
Visit 5	FDI (12)	226.5 (291.2)	0.487		Visit 5	FDI (12)	40.0 (20.5)	0.398	
	FCM (11)	212.1 (137.7)				FCM (11)	47.5 (20.9)		
Visit 6	FDI (9)	262.4 (339.0)	0.035		Visit 6	FDI (9)	45.8 (42.2)	0.283	
	FCM (10)	662.7 (1257.9)				FCM (10)	36.2 (12.6)		
Visit 7	FDI (12)	301.2 (389.5)	0.497	Within FDI: 0.058	Visit 7	FDI (12)	41.9 (22.2)	0.491	Within FDI: 0.264
	FCM (10)	227.8 (120.0)		Within FCM		FCM (10)	49.0 (25.3)		Within FCM: 0.026

				0.001					
Phosphate / mmol/L					**25(OH)$_2$ Vitamin D / nmol/L**				
Baseline	FDI (14)	1.30 (0.43)	0.193		Baseline	FDI (14)	44.2 (64.8)	0.252	
	FCM (12)	1.20 (0.31)				FCM (12)	67.5 (67.3)		
Visit 3	FDI (14)	1.37 (0.46)	0.647		Visit 3	FDI (14)	44.9 (65.0)	0.467	
	FCM (11)	1.23 (0.31)				FCM (11)	54.5 (65.0)		
Visit 4	FDI (13)	1.26 (0.72)	0.049		Visit 4	FDI (13)	45.2 (73.5)	0.832	
	FCM (10)	1.09 (0.29)				FCM (10)	69.0 (67.6)		
Visit 5	FDI (12)	1.18 (0.50)	0.449		Visit 5	FDI (12)	35.7 (67.8)	0.235	
	FCM (11)	1.14 (0.36)				FCM (11)	68.1 (75.8)		
Visit 6	FDI (9)	1.23 (0.51)	0.065		Visit 6	FDI (9)	57.8 (67.8)	0.604	
	FCM (10)	1.11 (0.45)				FCM (10)	63.3 (71.5)		
Visit 7	FDI (13)	1.33 (0.54)	0.057	Within FDI: 0.278	Visit 7	FDI (12)	47.4 (74.0)	0.346	Within FDI: 0.945
	FCM (10)	1.13 (0.22)		Within FCM: 0.129		FCM (10)	70.2 (83.4)		Within FCM: 0.977
Calcium * / mmol/L					**24(R),25 (OH)2 Vitamin D / nmol/L**				
Baseline	FDI (14)	2.35 (0.08)	0.813		Baseline	FDI (14)	1.2 (4.0)	0.631	
	FCM (12)	2.34 (0.09)				FCM (12)	2.9 (2.3)		

Visit 3	FDI (14)	2.39 (0.11)	0.286		Visit 3	FDI (14)	1.2 (3.7)	0.727	
	FCM (11)	2.34 (0.10)				FCM (11)	2.5 (2.5)		
Visit 4	FDI (13)	2.36 (0.11)	0.123		Visit 4	FDI (13)	1.2 (3.7)	0.693	
	FCM (10)	2.29 (0.06)				FCM (10)	3.1 (3.2)		
Visit 5	FDI (12)	2.35 (0.09)	0.698		Visit 5	FDI (12)	1.0 (3.1)	0.347	
	FCM (11)	2.32 (0.08)				FCM (11)	2.6 (3.1)		
Visit 6	FDI (9)	2.38 (0.10)	0.063		Visit 6	FDI (9)	1.7 (3.4)	1.000	
	FCM (10)	2.31 (0.06)				FCM (10)	2.9 (2.7)		
Visit 7	FDI (13)	2.35 (0.11)	0.807	Within FDI: 0.473	Visit 7	FDI (12)	1.3 (4.3)	0.539	Within FDI: 0.902
	FCM (10)	2.36 (0.07)		Within FCM: 0.544		FCM (10)	2.8 (2.6)		Within FCM: 0.406
FEPi / %					**PTH / pmol/L**				
Baseline	FDI (14)	49.7 (26.1)	0.374		Baseline	FDI (14)	18.9 (15.9)	0.145	
	FCM (12)	36.4 (21.6)				FCM (12)	16.3 (13.0)		
Visit 3	FDI (11)	41.8 (20.1)	0.918		Visit 3	FDI (14)	16.2 (17.1)	0.344	
	FCM (10)	41.3 (15.6)				FCM (11)	13.2 (11.6)		
Visit 4	FDI (12)	41.9 (17.6)	0.722		Visit 4	FDI (13)	19.6 (26.2)	0.564	
	FCM (10)	40.8 (18.9)				FCM (10)	17.2 (9.6)		

Visit 5	FDI (9)	40.3 (22.9)	0.968		Visit 5	FDI (12)	18.2 (21.2)	0.651	
	FCM (10)	42.6 (24.8)				FCM (11)	17.5 (13.0)		
Visit 6	FDI (8)	40.1 (23.0)	0.897		Visit 6	FDI (9)	14.4 (18.4)	0.604	
	FCM (10)	40.3 (22.4)				FCM (10)	13.4 (9.9)		
Visit 7	FDI (10)	48.3 (26.3)	0.631	Within FDI: 0.927	Visit 7	FDI (12)	20.2 (13.2)	0.283	Within FDI: 0.299
	FCM (10)	42.2 (16.4)		Within FCM: 0.412		FCM (10)	12.8 (12.0)		Within FCM: 0.081
24-hour urinary phosphate / mmol/24hr									
Baseline	FDI (14)	21.0 (8.5)	0.023						
	FCM (12)	12.5 (7.5)							
Visit 3	FDI (12)	22.0 (8.5)	0.381						
	FCM (10)	14.5 (13.5)							
Visit 4	FDI (12)	20.0 (11.8)	0.228						
	FCM (10)	15.0 (9.3)							
Visit 5	FDI (9)	19.0 (11.5)	0.017						
	FCM (10)	13.0 (9.5)							
Visit 6	FDI (9)	24.0 (14.5)	0.043						
	FCM (10)	12.0 (9.8)							

Visit 7	FDI (10)	22.0 (13.5)	0.015	Within FDI: 0.371				
	FCM (10)	12.5 (7.3)		Within FCM: 0.198				
* variables characterised by asterisk are described as mean (SD); the remaining variables are described as median (IQR) based on distribution								
	FCM (11)	0.3 (3.8)	0.916					
Baseline to Visit 6	FDI (9)	1.5 (2.2)						
	FCM (10)	-1.0 (2.4)	0.035					
Baseline to Visit 7	FDI (12)	0.5 (4.7)						
	FCM (10)	1.5 (3.2)	0.566					
* variables characterised by asterisk are described as mean (SD); the remaining variables are described as median (IQR) based on distribution								

Table 7: %change relevant to markers of the 6H syndrome

Variable	**Iron group (n)**	**Mean/Median (SD/IQR)**	**p-value**	**Variable**	**Iron group (n)**	**Mean/Median (SD/IQR)**	**p-value**
iFGF23				**1,25 $(OH)_2$ Vitamin D ***			
Baseline to Visit 3	FDI (14)	3.0 (28.9)		Baseline to Visit 3	FDI (14)	-2.8 (14.2)	
	FCM (11)	146.1 (94.9)	< 0.001		FCM (11)	-15.6 (12.8)	0.027
Baseline to Visit 4	FDI (13)	11.9 (40.6)		Baseline to Visit 4	FDI (13)	-14.7 (14.6)	

	FCM (10)	24.3 (52.7)	0.284		FCM (10)	-18.1 (14.9)	0.580
Baseline to Visit 5	FDI (12)	6.5 (30.3)		Baseline to Visit 5	FDI (12)	-9.8 (16.5)	
	FCM (11)	17.1 (32.3)	0.566		FCM (11)	-5.4 (27.2)	0.642
Baseline to Visit 6	FDI (9)	3.2 (28.9)		Baseline to Visit 6	FDI (9)	-4.7 (13.0)	
	FCM (10)	235.1 (296.1)	0.001		FCM (10)	-24.9 (22.5)	0.031
Baseline to Visit 7	FDI (12)	15.7 (104.7)		Baseline to Visit 7	FDI (12)	-5.4 (30.5)	
	FCM (10)	8.1 (62.1)	0.497		FCM (10)	-5.3 (29.1)	0.933
Phosphate				**25 $(OH)_2$ Vitamin D**			
Baseline to Visit 3	FDI (14)	-6.5 (15.1)		Baseline to Visit 3	FDI (14)	1.6 (14.0)	
	FCM (11)	-3.3 (18.2)	0.893		FCM (11)	-3.5 (17.5)	0.267
Baseline to Visit 4	FDI (13)	-1.6 (20.6)		Baseline to Visit 4	FDI (13)	5.8 (17.7)	
	FCM (10)	-11.0 (17.8)	0.077		FCM (10)	-0.3 (10.6)	0.483
Baseline to Visit 5	FDI (12)	-7.5 (25.1)		Baseline to Visit 5	FDI (12)	-11.0 (28.8)	
	FCM (11)	-6.1 (14.3)	1.000		FCM (11)	-7.1 (26.0)	0.880
Baseline to Visit 6	FDI (9)	1.8 (30.3)		Baseline to Visit 6	FDI (9)	0.8 (20.0)	
	FCM (10)	-14.9 (14.7)	0.013		FCM (10)	-8.5 (21.7)	0.549
Baseline to Visit 7	FDI (13)	9.2 (25.1)		Baseline to Visit 7	FDI (12)	-1.0 (48.8)	
	FCM (10)	-13.2 (18.5)	0.131		FCM (10)	-3.0 (25.7)	0.923
24 hour urinary phosphate				**24(R), 25 $(OH)_2$ Vitamin D**			

Baseline to Visit 3	FDI (12)	5.2 (16.8)		Baseline to Visit 3	FDI (14)	0.0 (7.7)	
	FCM (10)	38.6 (80.6)	0.093		FCM (11)	-8.3 (14.3)	0.085
Baseline to Visit 4	FDI (12)	0.0 (36.7)		Baseline to Visit 4	FDI (13)	0.0 (7.3)	
	FCM (10)	32.0 (71.5)	0.107		FCM (10)	12.0 (21.1)	0.067
Baseline to Visit 5	FDI (9)	12.0 (30.5)		Baseline to Visit 5	FDI (12)	-4.2 (29.3)	
	FCM (10)	29.5 (111.1)	0.720		FCM (11)	13.5 (37.5)	0.695
Baseline to Visit 6	FDI (9)	0.0 (27.3)		Baseline to Visit 6	FDI (9)	-3.0 (21.6)	
	FCM (10)	13.5 (40.6)	0.400		FCM (10)	6.3 (27.8)	0.842
Baseline to Visit 7	FDI (10)	9.3 (32.4)		Baseline to Visit 7	FDI (12)	0.0 (48.4)	
	FCM (10)	11.7 (94.4)	0.684		FCM (10)	-4.1 (26.8)	0.923
FEPi				**PTH ***			
Baseline to Visit 3	FDI (11)	-7.1 (26.2)		Baseline to Visit 3	FDI (14)	-7.8 (22.0)	
	FCM (10)	6.4 (27.9)	0.314		FCM (11)	-6.9 (25.4)	0.927
Baseline to Visit 4	FDI (12)	-3.7 (18.1)		Baseline to Visit 4	FDI (13)	2.9 (30.8)	
	FCM (10)	-5.0 (28.9)	0.872		FCM (10)	19.6 (32.9)	0.226
Baseline to Visit 5	FDI (9)	-2.7 (13.0)		Baseline to Visit 5	FDI (12)	-12.1 (32.0)	
	FCM (10)	3.6 (33.9)	0.661		FCM (11)	13.6 (27.7)	0.054
Baseline to Visit 6	FDI (9)	-8.2 (31.6)		Baseline to Visit 6	FDI (9)	-3.7 (29.5)	
	FCM (10)	13.0 (48.4)	0.182		FCM (10)	-5.0 (24.3)	0.913

Baseline to Visit 7	FDI (10)	5.0 (24.8)		Baseline to Visit 7	FDI (12)	1.8 (36.7)	
	FCM (10)	0.3 (28.0)	1.000		FCM (10)	-1.6 (28.1)	0.810
Calcium *							
Baseline to Visit 3	FDI (14)	1.5 (2.7)					
	FCM (11)	0.4 (2.5)	0.297				
Baseline to Visit 4	FDI (13)	0.4 (3.4)					
	FCM (10)	-1.8 (3.1)	0.131				
Baseline to Visit 5	FDI (12)	0.5 (2.9)					

Intact FGF23

The median concentrations of iFGF23 in either group at each visit are displayed in table 6 and as clustered boxplot in figure 6. In the FDI group, the maximum median concentration of iFGF23 was recorded at visit 7 at 301.2 (IQR: 389.5) pg/ml. The minimum median concentration was noted at visit 5 (226.5 (IQR 291.2) pg/ml). In the FCM group the maximum median concentration was recorded at visit 6 at 662.7 (IQR: 1257.9) pg/ml, and the minimum at baseline at 186.5 (IQR: 83.0) pg/ml. No statistical difference existed between the two groups with the exception of visit 6 (1-2 days following second IV iron infusion). In particular median concentration in the FDI group was 262.4 (IQR: 339.0) pg/ml and in the FCM group was 662.7 (IQR: 1257.9) pg/ml (p=0.035). Both groups had outliers throughout the study as noted in figure 6.

There was a significant difference between the concentrations within the FCM group (p=0.001) in terms of iFGF23 but no significant difference between the concentrations within the FDI group.

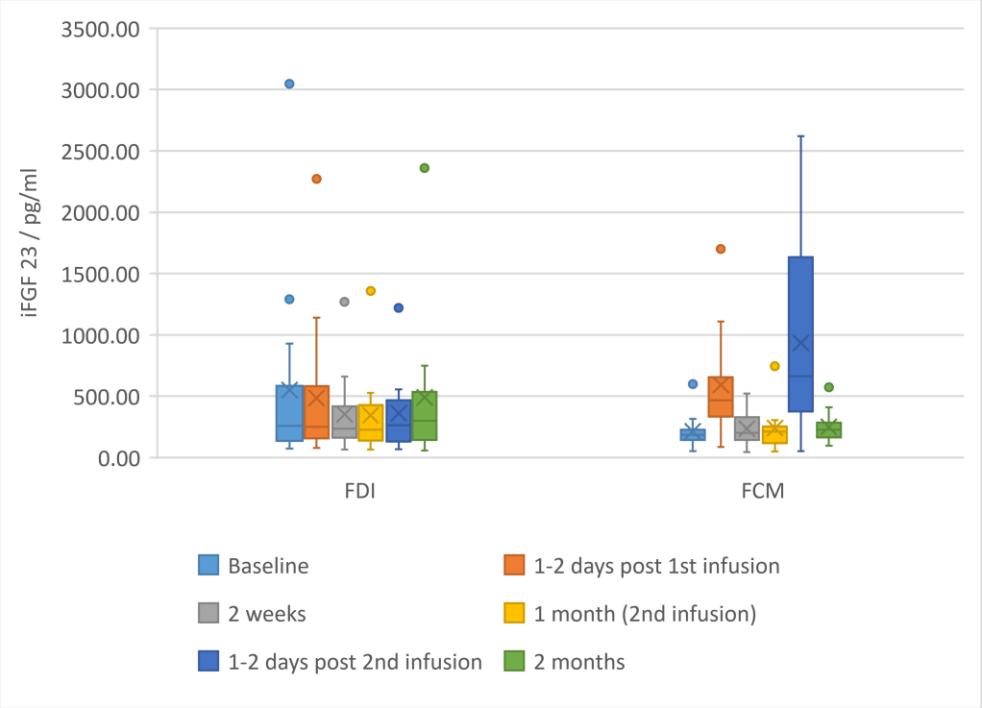

Figure 6: iFGF23 concentrations

The median %change of iFGF23 in either group throughout the study is displayed in table 7 and as clustered boxplot in figure 7. A statistically significant difference in the distribution of percentage difference caused by each product was noted between baseline and visit 3 (FDI: 3.0 (IQR: 28.9)% vs. FCM: 146.1 (IQR: 94.9) %; p<0.001) and baseline and visit 6 (FDI: 3.2 (IQR: 28.9)% vs. FCM: 235.1 (296.1) %; p=0.001).

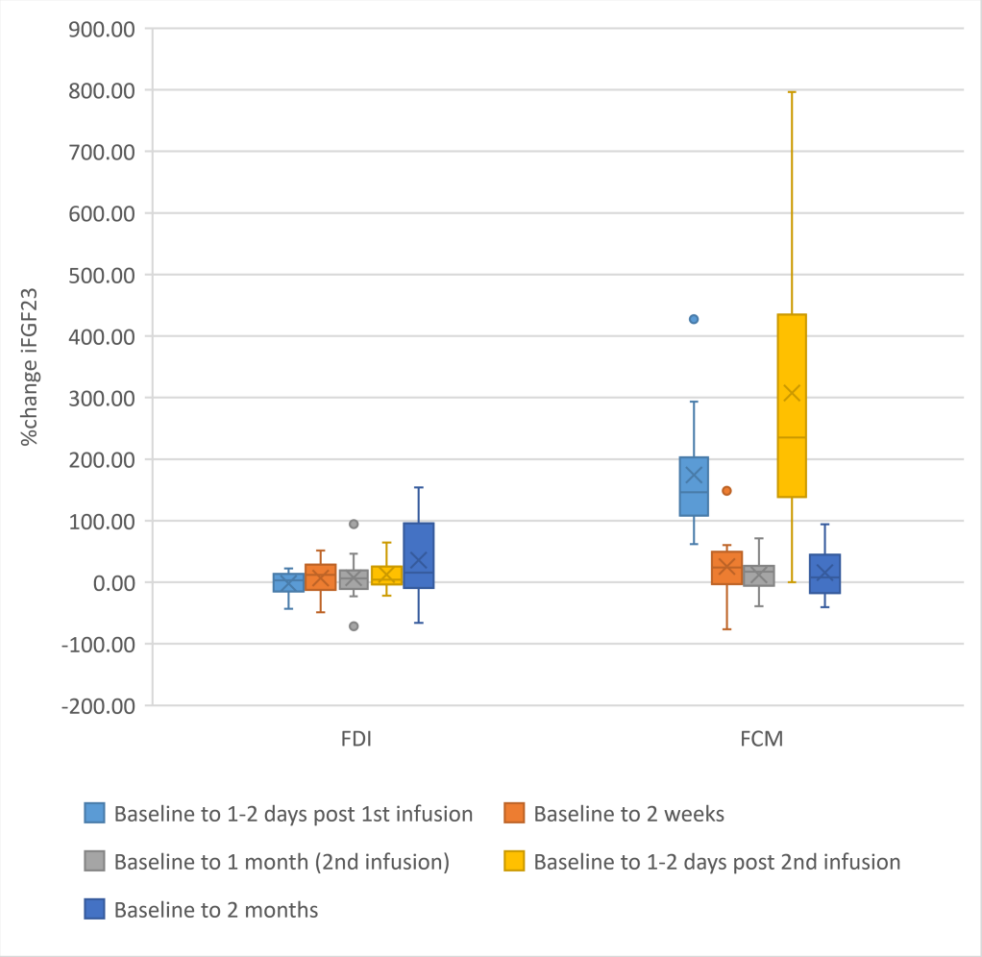

Figure 7: Percentage change of iFGF23

Phosphate

The median concentrations of serum phosphate in either group at each visit are displayed in table 6 and as clustered boxplot in figure 8. Median phosphate concentration reached its maximum in the FDI group at visit 3 (1.37 (IQR: 0.46) mmol/L) and its minimum at visit 5 (1.18 (IQR: 0.50) mmol/L). Median phosphate concentration reached its maximum in the FCM group at visit 3 (1.23 (IQR: 0.31) mmol/L) and its minimum at visit 4 (1.09 (IQR: 0.29) mmol/L). A significant difference between the two preparations existed at visit 4 (FDI: 1.26 (IQR: 0.72) mmol/L vs. FCM: 1.09 (IQR: 0.29) mmol/L; p=0.049). One

outlier existed in the FDI group at baseline and visit 3, alongside one outlier in the FCM group at visit 7.

No statistically significant difference within the FDI group nor within the FCM group was noted.

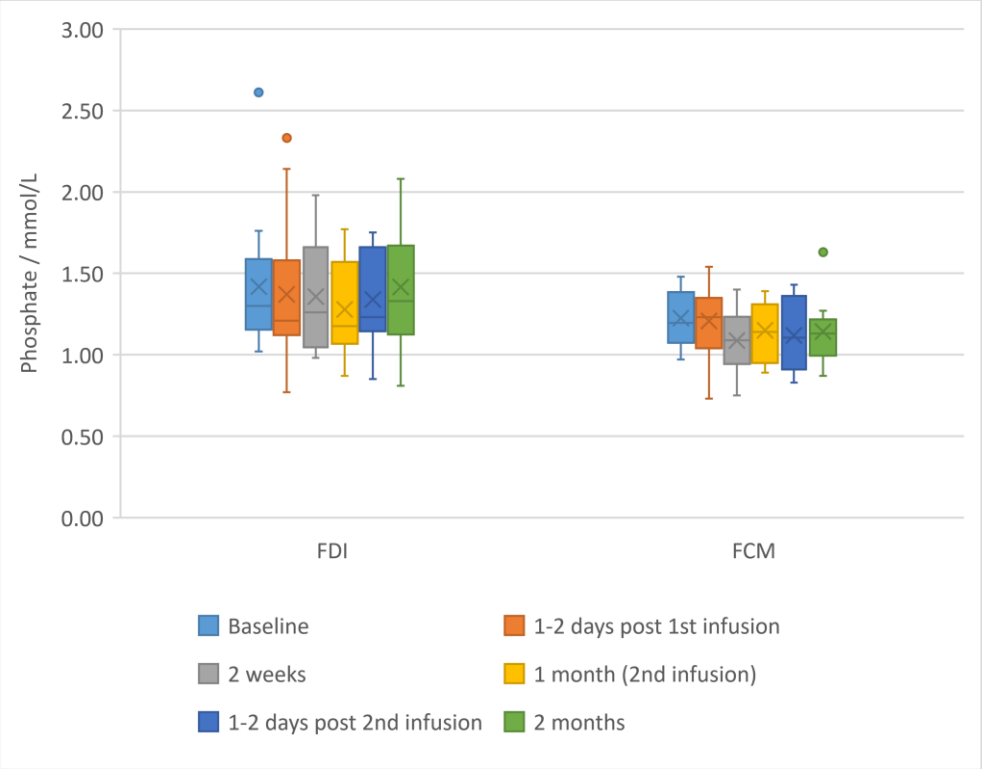

Figure 8: Serum phosphate concentrations

The median %change of serum phosphate in either group throughout the study is displayed in table 7 and as clustered boxplot in figure 9. A statistically significant difference between the distribution of %change caused by the products was observed between baseline and visit 6 (FDI: 1.8 (IQR: 30.3) % vs. FCM: -14.9 (IQR: 14.7); p=0.013).

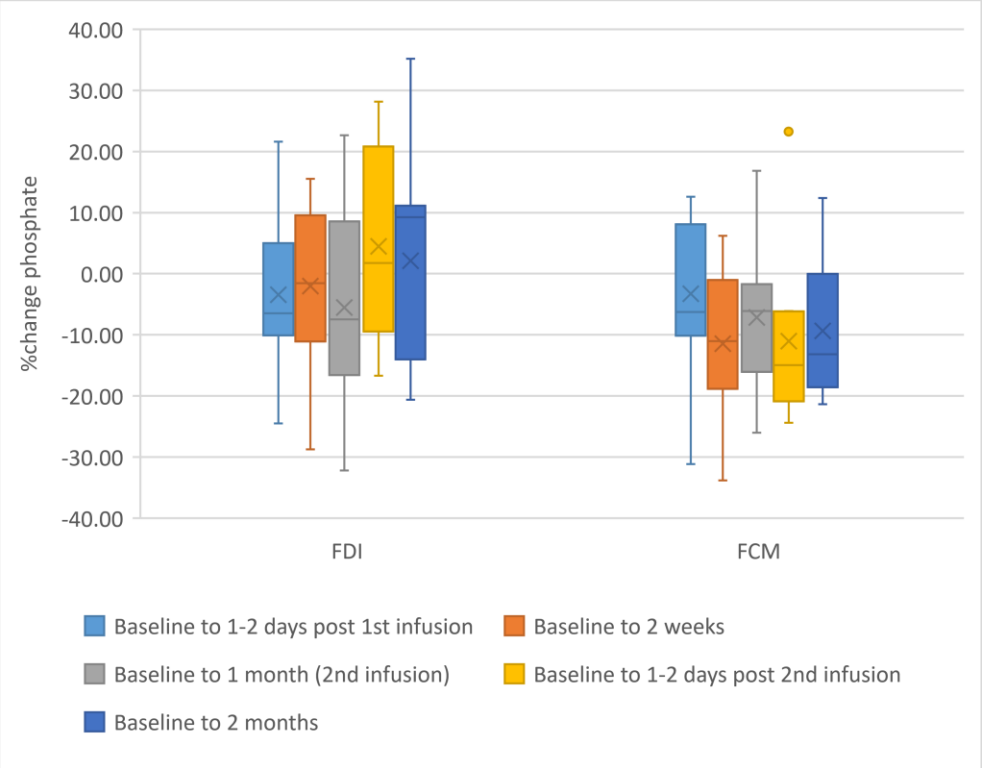

Figure 9: Percentage change of serum phosphate

3.3.2: Combined effect of intravenous iron administration on iFGF23 and phosphate concentration in terms of %change

At day 2 and week 2

One (7.1%) participant developed either a decrease in phosphate of > 20% or an increase in iFGF23 > 200% within 2 days from administration of FDI (in particular they had a phosphate decrease > 20%). By week 2, two further (14.2%) patients developed a phosphate decrease of > 20%. In total three patients (21.4%) in the FDI group developed either a decrease in phosphate of > 20% or an increase in iFGF23 > 200% within 2 weeks from administration of intravenous iron. No participant developed an increase in iFGF23 >200% and a decrease in phosphate of > -20% during this period.

Four (33.3%) participants developed either a decrease in phosphate of > 20% or an increase in iFGF23 > 200% within 2 days from administration of FCM (in particular 3 patients had an iFGF23 increase of >200%, and 1 had a phosphate decrease > 20%). By week 2, two (16.7%) further patients developed a phosphate decrease >-20%. In total five patients (41.7%) in the FCM group developed either a decrease in phosphate of > 20% or an increase in iFGF23 > 200% within 2 weeks from administration of intravenous iron. One patient (8.3%) was noted to have an increase in iFGF23 > 200% at day 2 and a decrease in phosphate concentration of > 20% at week 2, following FCM administration.

Post-hoc whole duration of the study

Three (21.4%) participants in the FDI group a serum phosphate decrease > 20%, throughout the study. No participant in the FDI group had a %change in iFGF23 >200%.

Ten (83.3%) participants in the FCM group developed either a decrease of phosphate > -2 0% or an increase in iFGF23 >200% at any point in the study. Eight (66.7%) participants in the FCM group developed an increase in iFGF23 >200%, and five (41.7%) participants developed a decrease in serum phosphate > - 20%. Three (25.0%) participants developed both a decrease in serum phosphate > 20% and an increase in iFGF23 > 200% following FCM administration FCM.

3.3.3: Post-hoc analysis of markers of phosphaturia: 24-hour urinary phosphate excretion and FEPi

24-hour urinary phosphate excretion:

The median 24-hour urinary phosphate excretion in either group throughout the study are displayed in table 6 and as clustered boxplot in figure 10. Median urinary phosphate excretion (24-hour) reached its maximum in the FDI group at visit 6 (24.0 (IQR: 14.3) mmol/24hr) and its minimum at visit 5 (19.0 (IQR: 11.5) mmol/24hr). Median 24-hour urinary phosphate excretion reached its maximum in the FCM group at visit 4 (15.0 (IQR: 9.3) mmol/24hr) and its minimum at visit 6 (12.0 (IQR: 9.8) mmol/24hr). A significant difference between the two preparations existed at baseline (FDI: 21.0 (IQR: 8.5) mmol/24hr vs. FCM: 12.5 (IQR: 7.5) mmol/24hr; p=0.023), visit 5 (FDI: 19.0 (IQR: 11.5) mmol/24hr vs. FCM: 13.0 (9.5) mmol/24hr; p=0.017), visit 6 (FDI: 24.0 (IQR: 14.5) mmol/24hr vs. FCM: 12.0 (IQR: 9.8) mmol/24hr; p=0.043) and visit 7 (FDI: 22.0 (13.5) mmol/24hr vs. FCM: 12.5 (7.3) mmol/24hr; p=0.015). One outlier existed in the FDI group at visit 3.

No statistically significant difference within the FDI group nor within the FCM group was noted.

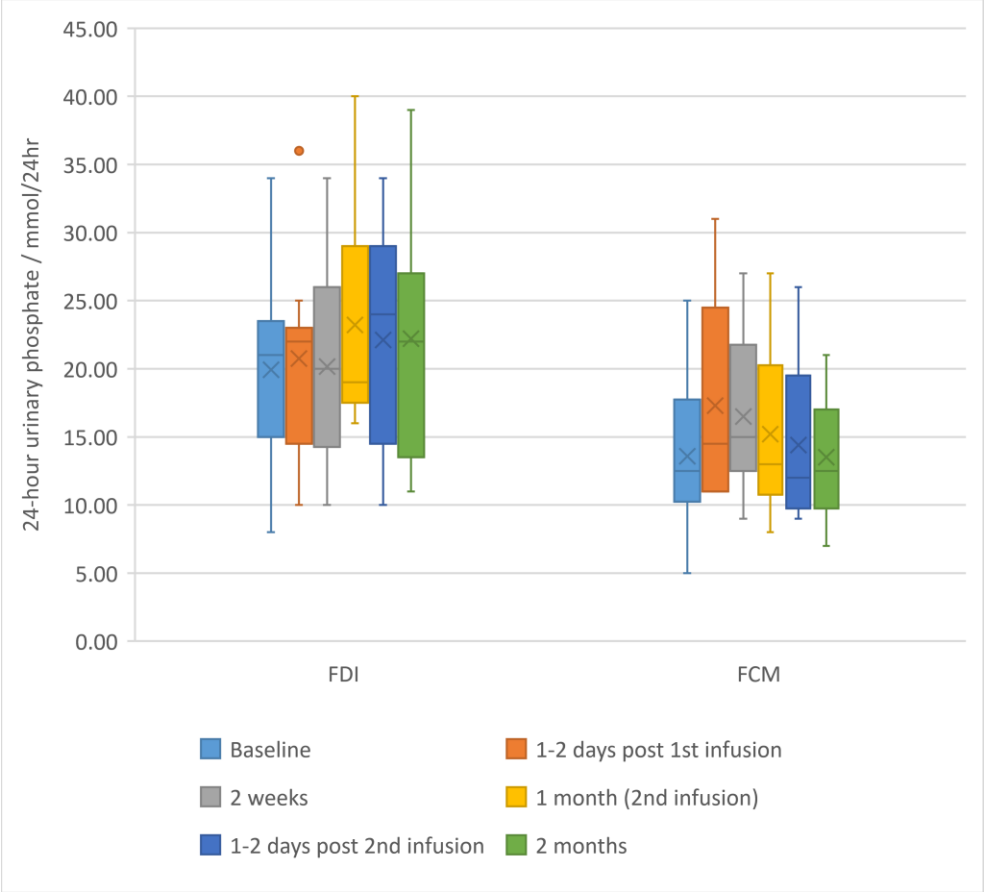

Figure 10: Urinary phosphate excretion (24-hour) concentration

The median 24-hour %change in urinary phosphate excretion in either group throughout the study is displayed in table 7 and as clustered boxplot in figure 11. No statistical difference was noted between the distributions of %change caused by either preparation at any point in the study.

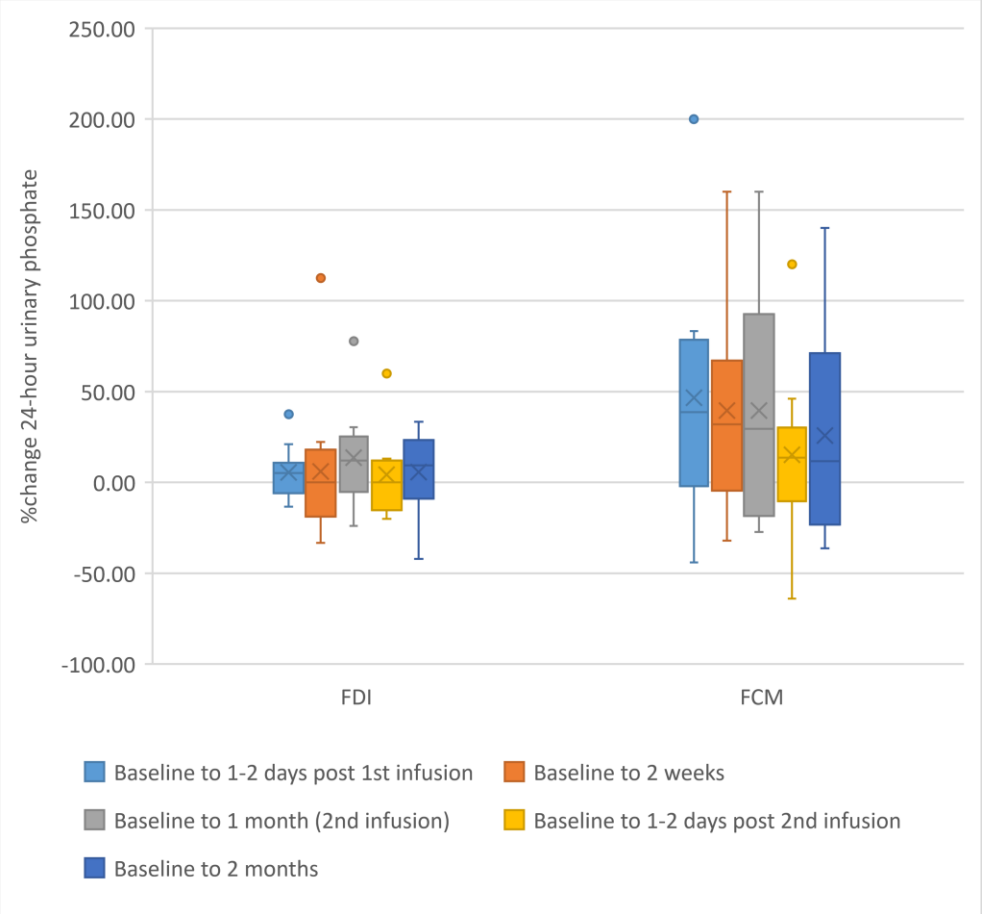

Figure 11: Percentage change of urinary phosphate excretion (24-hour)

Fractional excretion of phosphate

The median FEPi in either group throughout the study are displayed in table 6 and as clustered boxplot in figure 12. Median FEPi was at its maximum in the FDI group at baseline (49.7 (IQR: 26.1) %) and its minimum at visit 6 (40.1 (IQR: 23.0) %). Median FEPi reached its maximum in the FCM group at visit 7 (42.2 (IQR: 16.4) %) and was at its minimum at baseline (36.4 (IQR: 21.6) %). No statistically significant difference between the distributions of FEPi in either group was noted

throughout the study. One outlier existed in the FDI group at visit 4. One outlier existed in the FCM group at visit 7.

No statistically significant difference within the FDI group nor within the FCM group was noted.

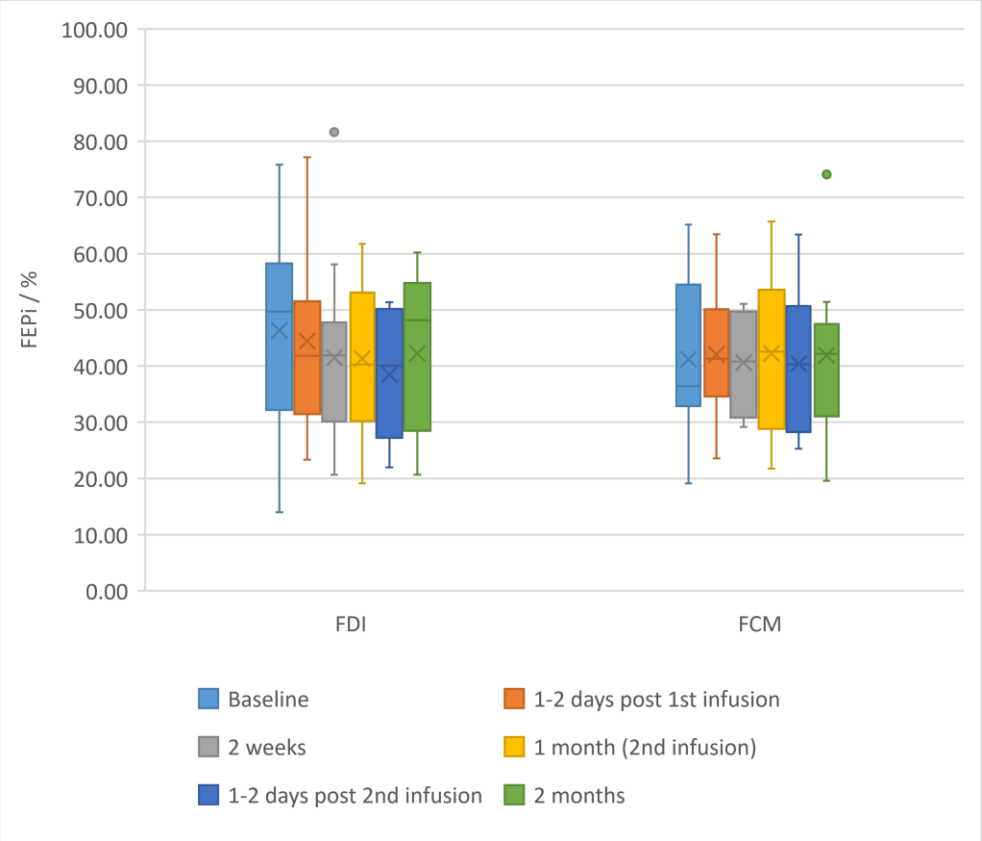

Figure 12: FEPi

The median %change in FEPi in either group throughout the study is displayed in table 7 and as clustered boxplot in figure 13. No statistical difference was noted between the distribution of %change caused by either preparation at any point in the study.

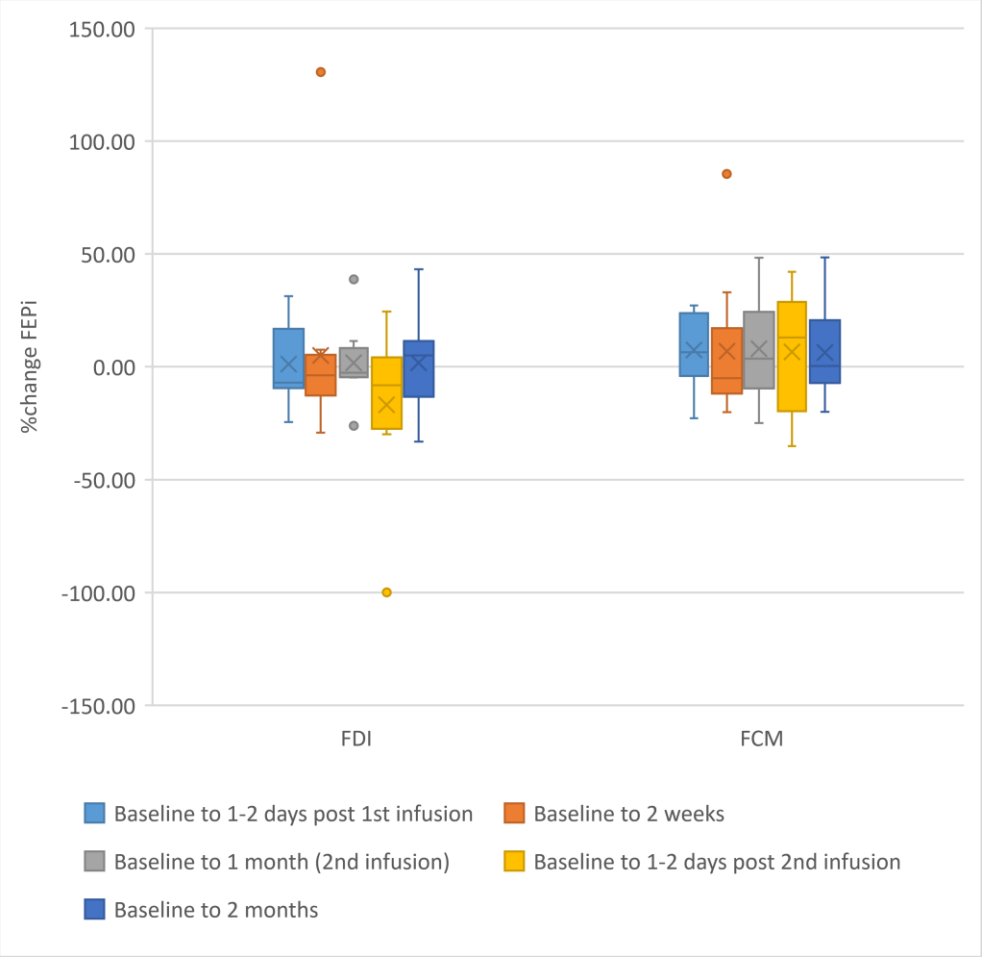

Figure 13: Percentage change FEPi

3.3.4: Correlation between %change in iFGF23 and %change in phosphate with markers of the 6H syndrome

Table 8 indicates the Spearman's rho correlation coefficients and their significance in terms of contemporaneous changes in serum phosphate and iFGF23 and changes in other parameters associated with 6H syndrome.

There was a statistically significant moderately positive correlation between %change in serum phosphate and %change in iFGF23 at visit 5 (Spearman's rho =0.55; p=0.006). There was no other

statistically significant correlation between contemporaneous changes throughout the study. A significant strong negative correlation existed between %change in serum phosphate and % change in FEPi at visit 3 (Spearman's rho =-0.67; p=0.002), and a significant moderately negative correlation between the changes in the two variables at visit 6 (Spearman's rho =-0.47; p=0.040). There was no other statistically significant correlation between concomitant changes in the study in terms of serum phosphate and other variables.

A statistically significant moderately negative correlation between %change in iFGF23 and %change in 1,25 (OH)2 Vitamin D was noted between baseline and visit 3 (Spearman's rho =-0.45; p=0.024). There was a statistically significant moderately negative correlation between the concomitant changes in iFGF23 and 1,25 (OH)2 Vitamin D at visit 6 (Spearman's rho =-0.49; p=0.034). A statistically significant moderately positive correlation between the change in iFGF23 and the change in FEPi was noted at visit 4 (Spearman's rho =0.56; p=0.007). No other statistically significant correlation between concomitant changes in iFGF23 concentration and other variables was noted.

Table 8: Contemporaneous %change in variables relevant to the 6H syndrome

Visits	**Correlation coefficient**	**p-value**	**Visits**	**Correlation coefficient**	**p-value**
iFGF23 with Phosphate			***iFGF23 with PTH***		
Baseline to Visit 3	0.18	0.398	Baseline to Visit 3	-0.01	0.953
Baseline to Visit 4	0.26	0.229	Baseline to Visit 4	0.13	0.562
Baseline to Visit 5	**0.55**	**0.006**	Baseline to Visit 5	-0.28	0.198
Baseline to Visit 6	-0.11	0.647	Baseline to Visit 6	-0.21	0.399
Baseline to Visit 7	0.36	0.097	Baseline to Visit 7	-0.20	0.363
Phosphate with PTH			***iFGF23 with 1,25 (OH)2 Vitamin D***		
Baseline to Visit 3	0.13	0.553	**Baseline to Visit 3**	**-0.45**	**0.024**
Baseline to Visit 4	-0.07	0.761	Baseline to Visit 4	0.05	0.825
Baseline to Visit 5	-0.20	0.352	Baseline to Visit 5	-0.22	0.324

Baseline to Visit 6	0.03	0.898	**Baseline to Visit 6**	**-0.49**	**0.034**
Baseline to Visit 7	-0.15	0.506	Baseline to Visit 7	-0.07	0.770
Phosphate with 1,25 (OH)2 Vitamin D			***iFGF23 with FEPi***		
Baseline to Visit 3	-0.13	0.535	Baseline to Visit 3	0.21	0.354
Baseline to Visit 4	0.29	0.175	**Baseline to Visit 4**	**0.56**	**0.007**
Baseline to Visit 5	0.07	0.764	Baseline to Visit 5	0.30	0.209
Baseline to Visit 6	0.38	0.111	Baseline to Visit 6	0.18	0.459
Baseline to Visit 7	-0.28	0.206	Baseline to Visit 7	0.32	0.169
Phosphate with FEPi			***iFGF23 with Calcium***		
Baseline to Visit 3	**-0.67**	**0.001**	Baseline to Visit 3	-0.39	0.057
Baseline to Visit 4	-0.24	0.282	Baseline to Visit 4	-0.26	0.226
Baseline to Visit 5	0.16	0.509	Baseline to Visit 5	0.11	0.615
Baseline to Visit 6	**-0.47**	**0.040**	Baseline to Visit 6	-0.36	0.128
Baseline to Visit 7	-0.19	0.427	Baseline to Visit 7	-0.13	0.553
Phosphate with Calcium					
Baseline to Visit 3	-0.29	0.161			
Baseline to Visit 4	0.07	0.769			
Baseline to Visit 5	-0.03	0.89			
Baseline to Visit 6	0.44	0.058			
Baseline to Visit 7	-0.01	0.968			
Bold letter-type indicates significant correlation					

3.3.5: Incidence of hypophosphataemia and failed repeat infusions due to hypophosphataemia

No incidents of hypophosphataemia as defined by the protocol (<0.65mmol/L) were noted at any time point. No severe hypophosphataemia (<0.3 mmol/L) occurred. Accepting a higher cut-off for hypophosphataemia as defined in other studies (<0.8 mmol/L), there were two episodes of mild hypophasphataemia, one at each group on visit 3 (0.77 mmol/L and 0.73 mmol/L; FCM and FDI respectively). These recovered by visit 4. The incidence of mild hypophosphataemia was 1.64% in the FDI group (1 out of 61 total

measurements) and 1.92% in the FCM group (1 out of 52 total measurements). There were no failed repeat infusions due to hypophosphataemia.

3.4: Bone metabolism markers (including bone turnover)

3.4.1: Markers of bone metabolism

Vitamin D

The mean concentrations of 1,25 $(OH)_2$ Vitamin D in either group throughout the study are displayed in table 6 and as line graph in figure 14. In the FDI group, the maximum mean concentration of 1,25 (OH)2 Vitamin D was recorded at visit 6 (45.8 (SD: 42.2) pmol/L). The minimum mean concentration was noted at visit 4 (37.5 (SD: 18.6) pmol/L). In the FCM group the maximum mean concentration was recorded at baseline (50.7 (SD: 23.5) pmol/L), and the minimum mean concentration at visit 6 (36.2 (SD: 12.6) pmol/L).No significant statistical difference existed between the two groups.

There was a significant difference between the concentrations within the FCM group (p=0.026) in terms of 1,25 $(OH)_2$ Vitamin D but no significant difference between the concentrations within the FDI group.

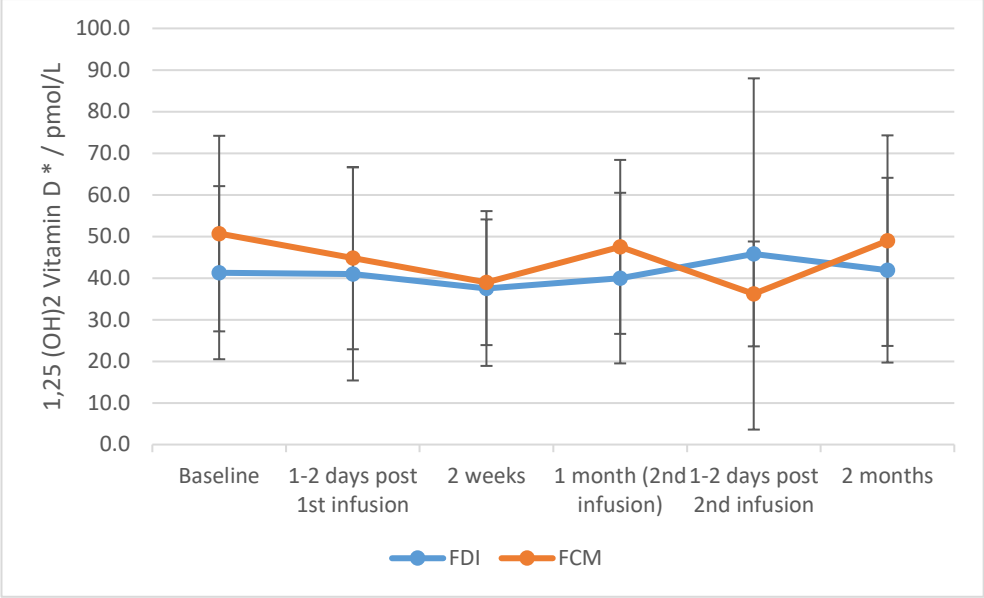

Figure 14: 1,25 $(OH)_2$ Vitamin D concentrations

The mean %change in 1,25 $(OH)_2$ Vitamin D concentration in either group throughout the study is displayed in table 7 and as boxplot in figure 15. In the FDI group mean %change was negative throughout the study. A statistically significant difference between the %change caused by each intravenous iron was noted between baseline and visit 3 (FDI: -2.8 (SD: 14.2)% vs. FCM: -15.6 (SD: 12.8)%; p=0.027), baseline and visit 6 (FDI: -4.7 (SD: 13.0) % vs. FCM: -24.9 (SD: 22.5)% ; p=0.031).

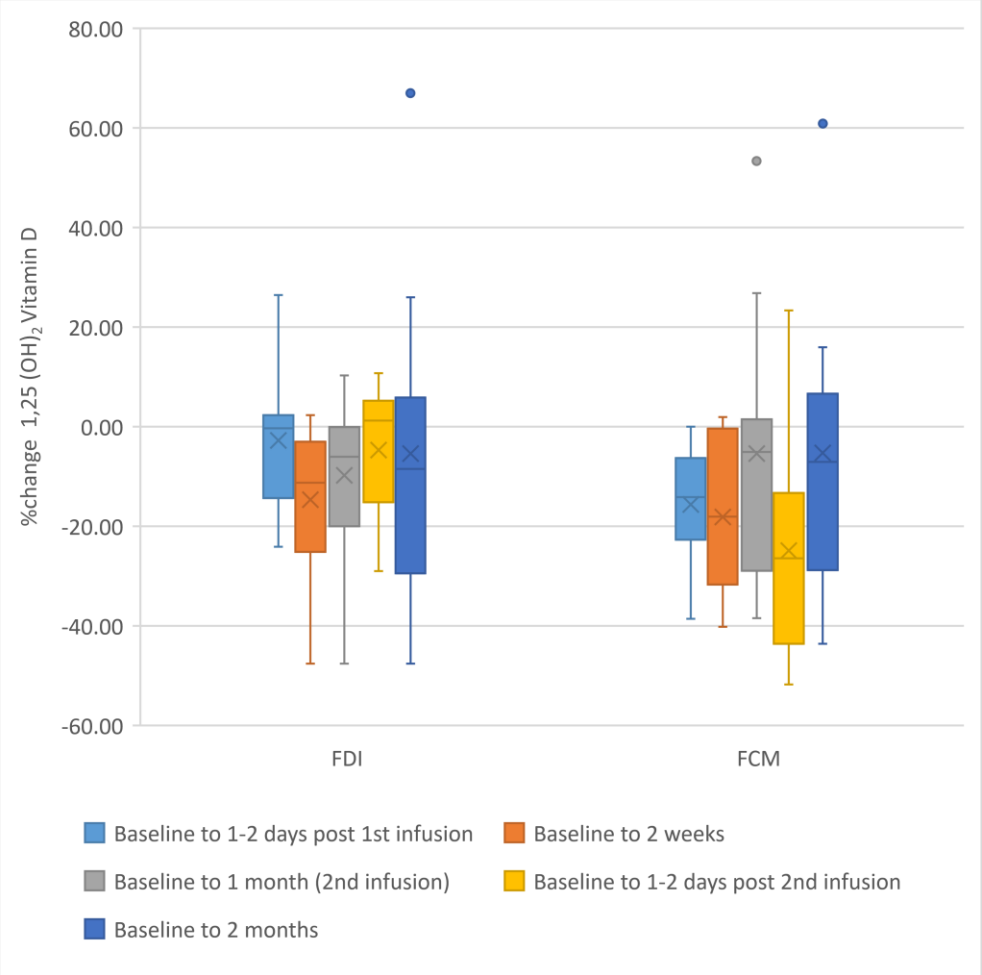

Figure 15: Percentage change 1,25 $(OH)_2$ Vitamin D

The median concentrations of 25 $(OH)_2$ Vitamin D in either group throughout the study are displayed in table 6 and as clustered boxplot in figure 16. In the FDI group, the maximum median concentration of 25 $(OH)_2$ Vitamin D was recorded at visit 6 (57.8 (IQR: 67.8) nmol/L). The minimum median concentration was noted at visit 5 (35.7 (IQR: 67.8) nmol/L). In the FCM group the maximum median concentration was recorded at visit 7 (70.2 (IQR: 83.4) nmol/L), and the minimum median concentration at visit 3 (54.5 (IQR: 65.0) nmol/L). No significant statistical difference existed between the two groups at any time point in the study. No outliers existed at any group at any time point of the study as indicated in figure 16.

No statistically significant difference within the FDI group nor within the FCM group was noted.

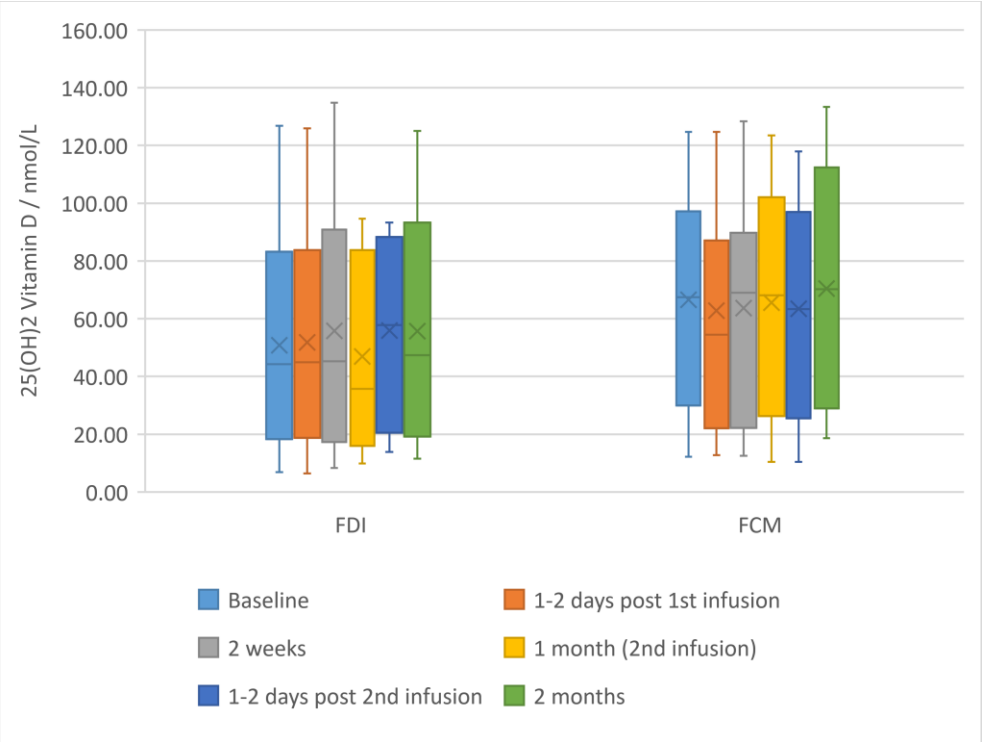

Figure 16: 25 $(OH)_2$ Vitamin D concentrations

The median %change in 25 $(OH)_2$ Vitamin D concentration in either group throughout the study is displayed in table 7 and as clustered boxplot in figure 17. No statistically significant difference existed between the mean %change caused by each IV preparation at any time point in the study. One outlier existed between baseline and visit 7 for the FDI group.

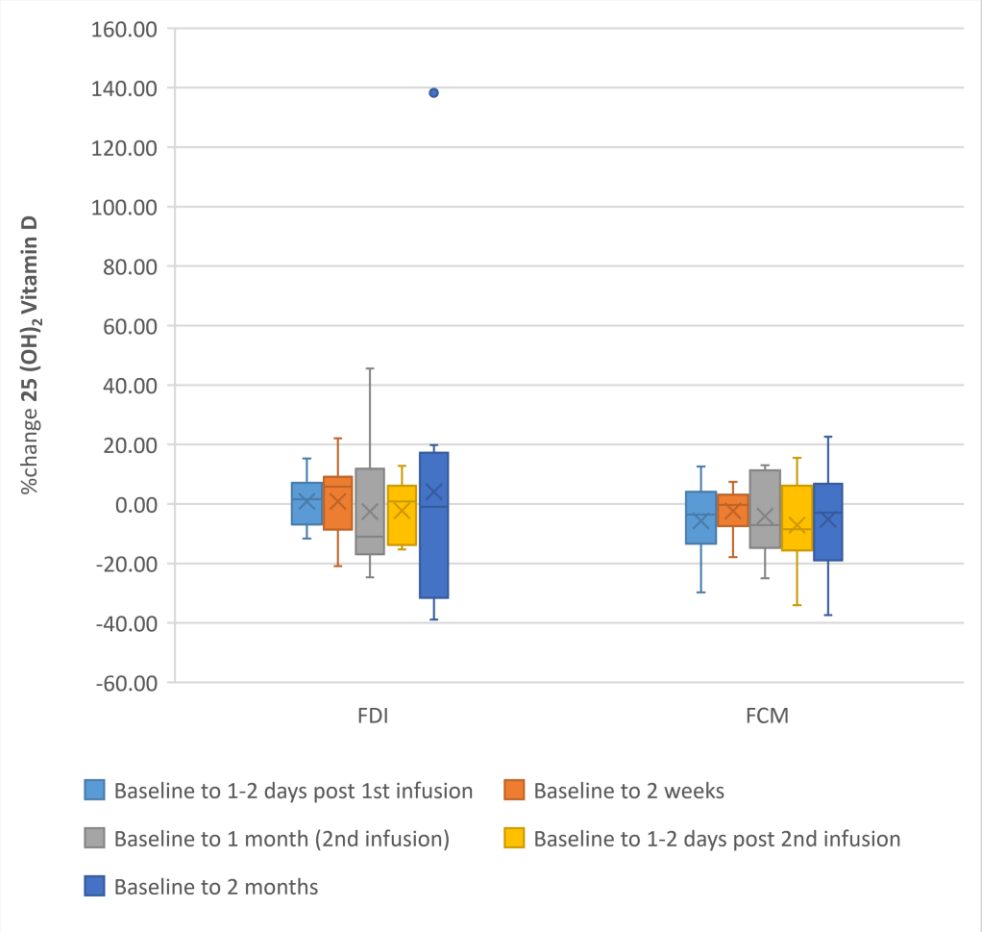

Figure 17: Percentage change 25 $(OH)_2$ Vitamin D

The median concentrations of 24(R),25 $(OH)_2$ Vitamin D in either group throughout the study are displayed in table 6 and as clustered boxplot in figure 18. In the FDI group, the maximum median concentration of 24(R),25 $(OH)_2$ Vitamin D was recorded at visit 6 (1.7 (IQR: 3.4) nmol/L). The minimum median concentration was noted at visit 5 (1.0 (IQR: 3.1) nmol/L). In the FCM group the maximum median concentration was recorded at visit 4 (3.1 (IQR: 3.2) nmol/L), and the minimum median concentration at visit 3 (2.5 (IQR: 2.5) nmol/L). No significant statistical difference existed between the two groups at any time point in the study. No outliers existed at any group at any time point of the study as indicated in figure 18.

No statistically significant difference within the FDI group nor within the FCM group was noted.

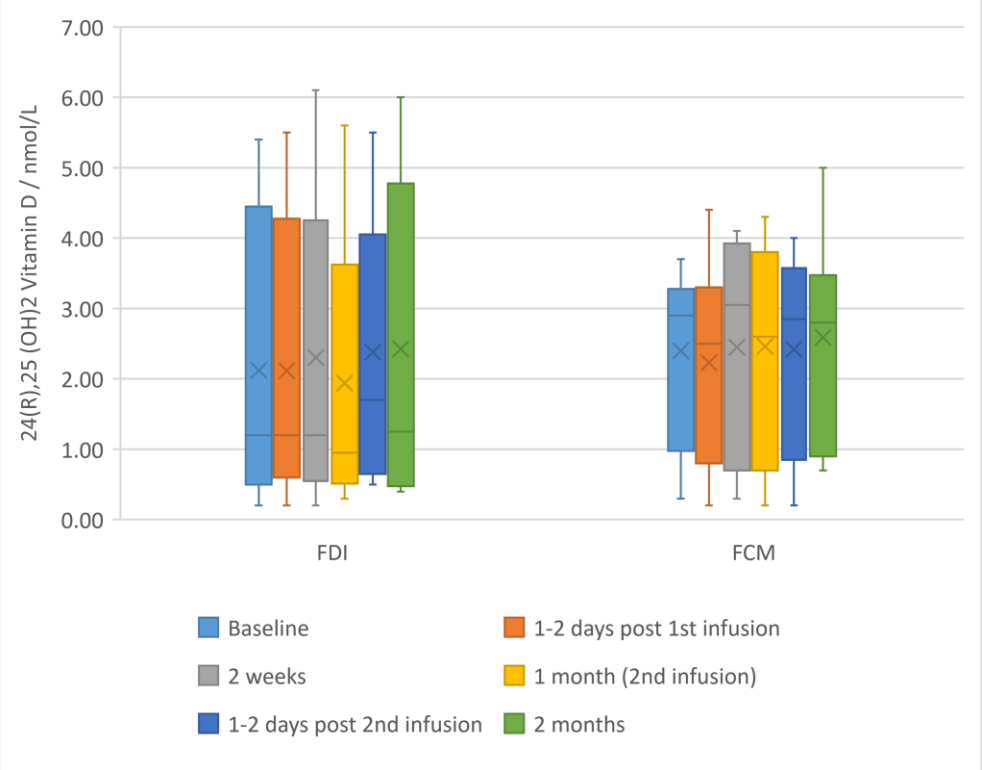

Figure 18: 24(R),25 $(OH)_2$ Vitamin D concentrations

The median %change in 24(R), 25 $(OH)_2$ Vitamin D concentration in either group throughout the study is displayed in table 7 and as clustered boxplot in figure 19. No statistically significant difference existed between the mean %change caused by each IV preparation at any time point in the study.

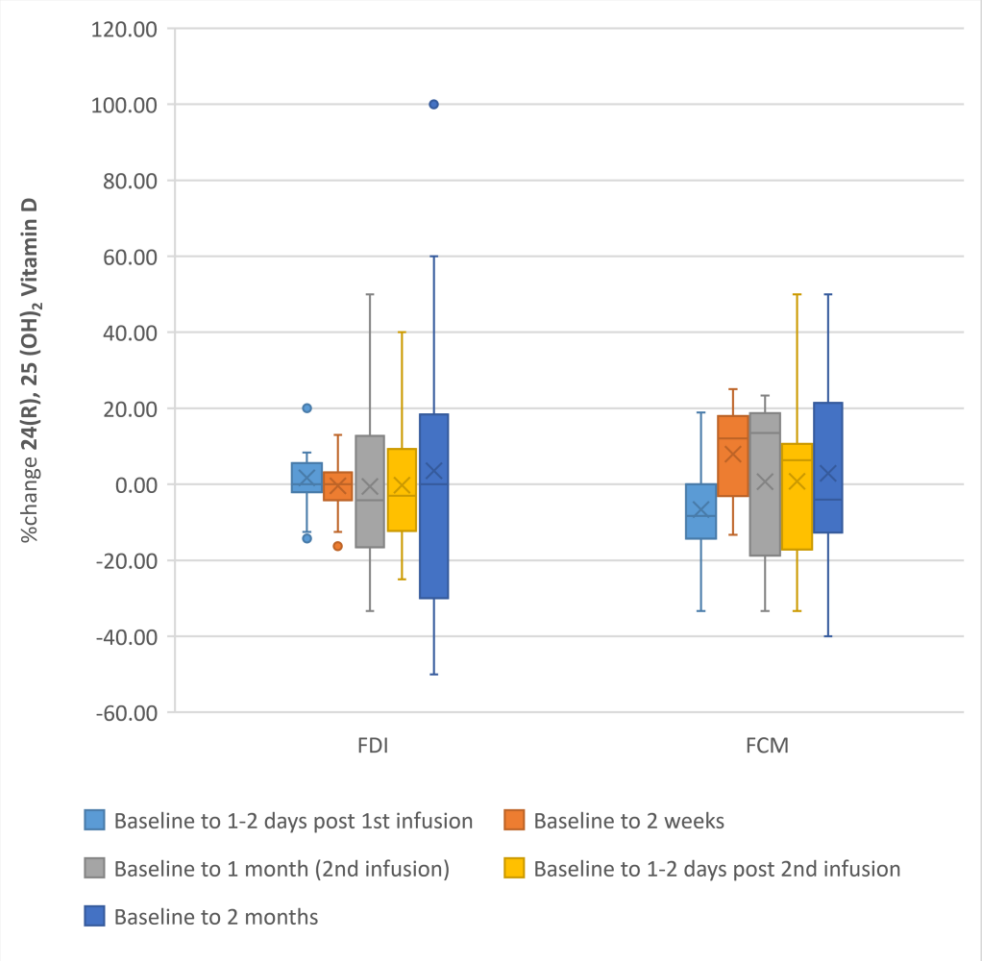

Figure 19: Percentage change 24(R),25 $(OH)_2$ Vitamin D

Calcium

The mean concentrations of serum calcium in either group throughout the study are displayed in table 6 and as line graphs in figure 20. In the FDI group, the maximum mean concentration of serum calcium was recorded at visit 3 at 2.39 (SD: 0.11) mmol/L. The minimum mean concentration was noted at baseline, visit 5 and visit 7 (2.35 (SD: 0.08) mmol/L, 2.35 (SD: 0.09) mmol/L and 2.35 (SD: 0.11) mmol/L respectively). In the FCM group the maximum mean concentration was recorded at visit 7 at 2.36 (IQR: 0.07) mmol/L, and the minimum mean concentration at visit 4 at 2.29 (SD: 0.06) mmol/L. No significant

statistical difference existed between the two groups at any time point of the study.

No statistically significant difference within the FDI group nor within the FCM group was noted.

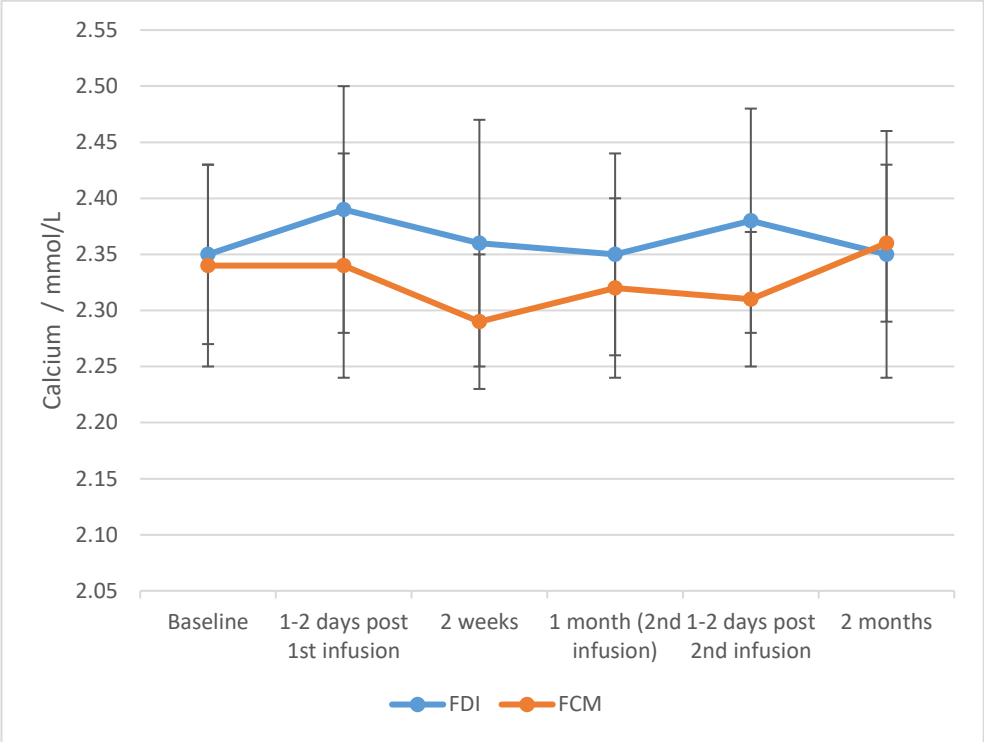

Figure 20: Serum calcium concentrations

The mean %change in calcium concentration in either group throughout the study is displayed in table 7 and as clustered boxplot in figure 21. A statistically significant difference between the %change caused by each intravenous iron was noted between baseline and visit 6 (FDI: 1.5 (SD: 2.2)% vs. FCM: -1.0 (SD: 2.4); p=0.035)

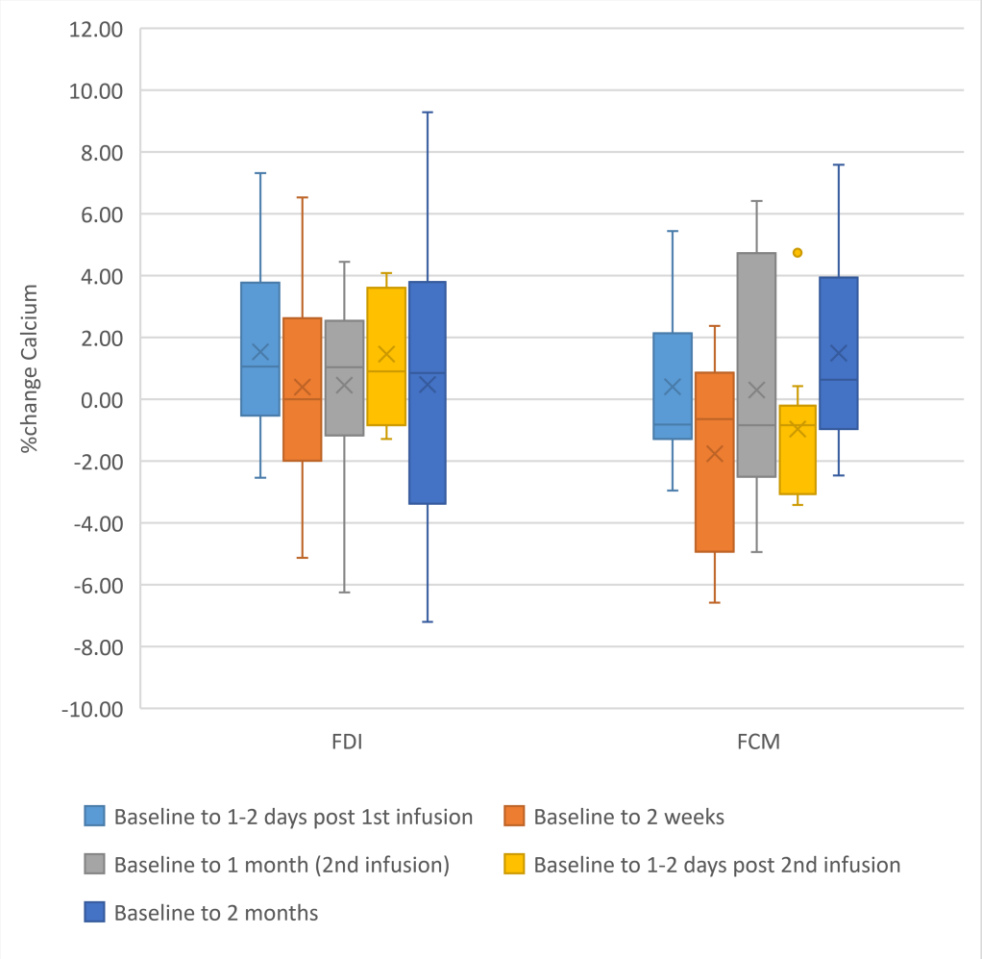

Figure 21: Percentage change serum calcium

Parathyroid Hormone

The median concentrations of PTH in either group throughout the study are displayed in table 6 and as clustered boxplot in figure 22. In the FDI group, the maximum median concentration of PTH was recorded at visit 7 (20.2 (IQR: 13.2) pmol/L). The minimum median concentration was noted at visit 6 (14.4 (IQR: 18.4) pmol/L). In the FCM group the maximum median concentration was recorded at visit 5 (17.5 (IQR: 13.0) pmol/L), and the minimum at visit 7 (12.8 (IQR: 12.0) pmol/L). No significant statistical difference existed between the two groups at any time point of the study. One outlier existed in the

FDI group at baseline, visits 3, 4, 5 and 7. No outliers existed in the FCM group as noted in figure 22.

No statistically significant difference within the FDI group nor within the FCM group was noted.

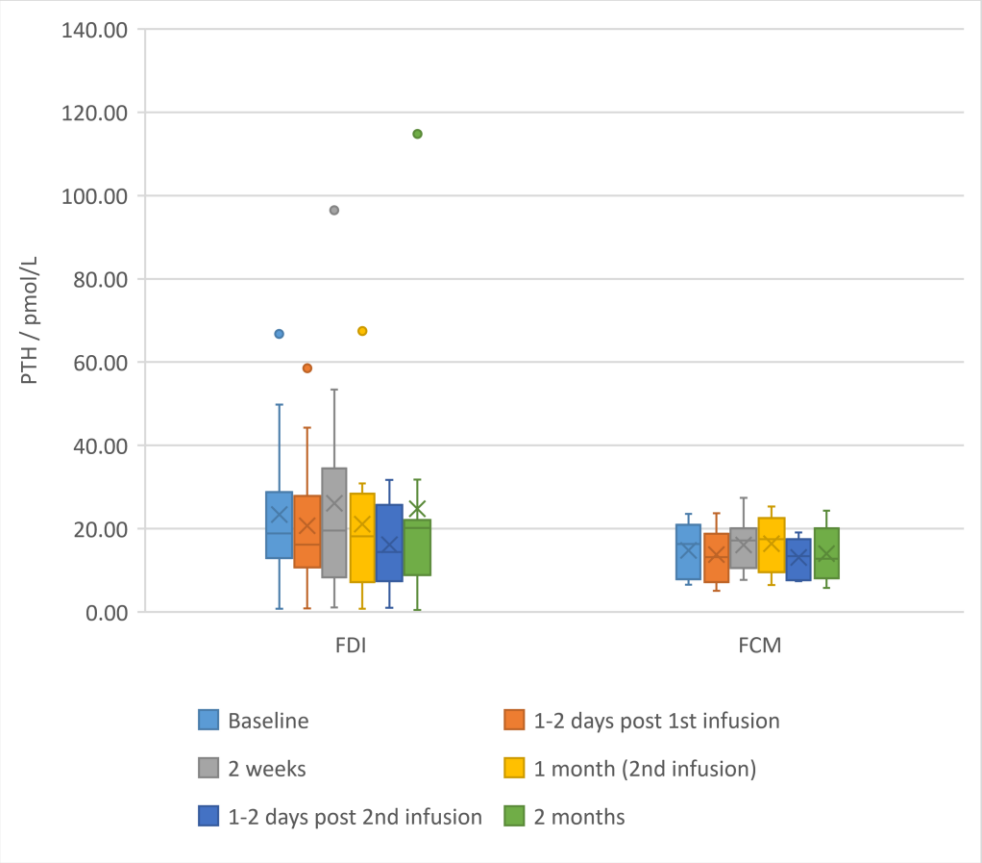

Figure 22: PTH concentrations

The mean %change in PTH concentration in either group throughout the study is displayed in table 7 and as clustered boxplot in figure 23. No statistically significant difference existed between the mean %change caused by each IV preparation at any time point in the study.

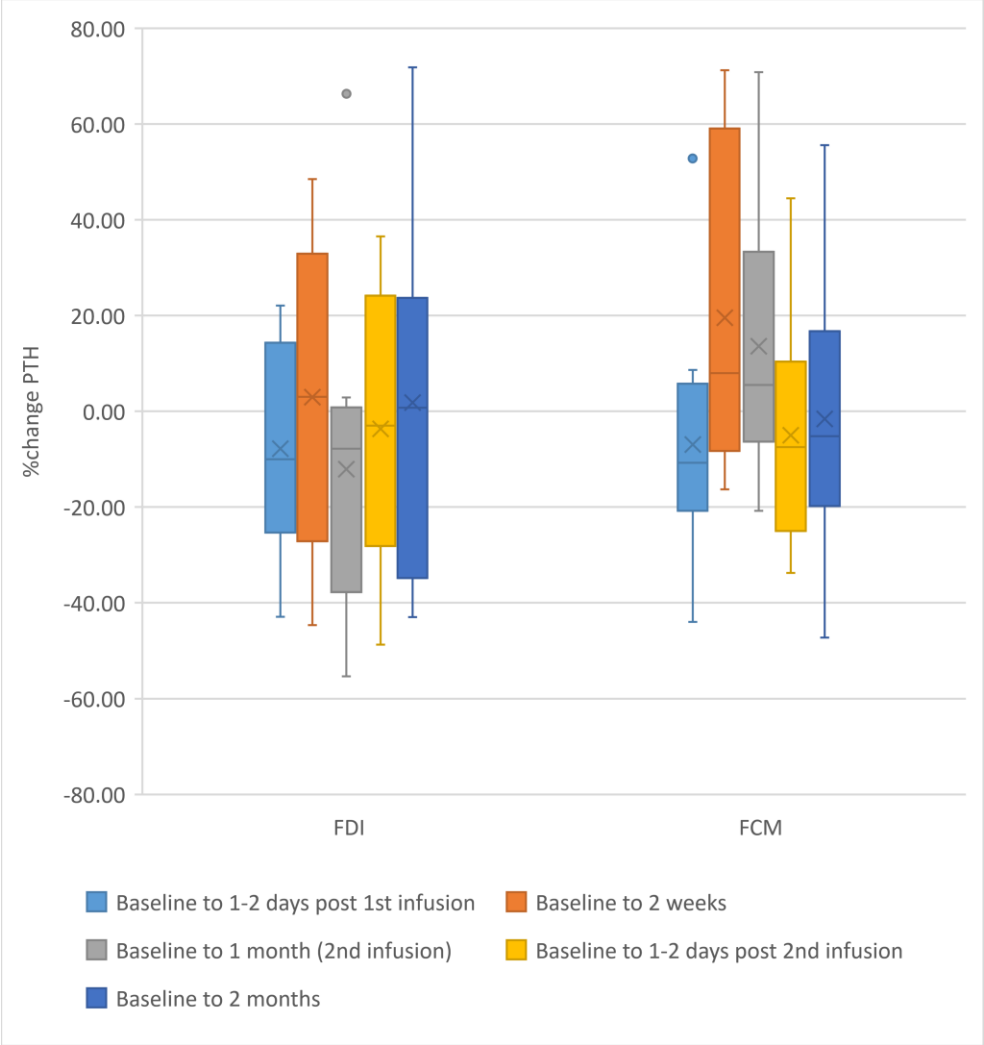

Figure 23: Percentage change PTH

3.4.2: Markers of bone turnover

Table 9: Markers of bone turnover									
Visit	**Iron group (n)**	**Median (IQR)**	**p-value**	**p- value (within group analysis)**	**Visit**	**Iron group (n)**	**Median (IQR)**	**p-value**	**p- value (within group analysis)**
ALP / [iU]/L					**CTx / µg/ml**				
Baseline	FDI (14)	96.0 (74.0)	0.667		Baseline	FDI (14)	0.84 (0.45)	0.560	
	FCM (12)	107.0 (52.0)				FCM (12)	0.98 (0.69)		
Visit 3	FDI (14)	104.0 (77.0)	0.893		Visit 3	FDI (14)	0.81 (0.46)	0.767	
	FCM (11)	110.0 (68.0)				FCM (11)	0.73 (0.75)		
Visit 4	FDI (13)	110.0 (62.5)	0.832		Visit 4	FDI (13)	0.77 (0.32)	0.927	
	FCM (10)	112.5 (48.5)				FCM (10)	0.69 (0.54)		
Visit 5	FDI (12)	99.5 (53.3)	0.525		Visit 5	FDI (12)	0.74 (0.56)	0.316	
	FCM (11)	112.0 (50.0)				FCM (11)	0.99 (0.74)		
Visit 6	FDI (9)	110.0 (58.0)	0.604		Visit 6	FDI (9)	0.71 (0.38)	0.211	
	FCM (10)	123.5 (65.3)				FCM (10)	0.88 (0.67)		
Visit 7	FDI (13)	124.0 (55.0)	0.879	Within FDI: 0.427	Visit 7	FDI (12)	0.84 (0.63)	0.582	Within FDI: 0.905

	FCM (10)	118.0 (77.3)		Within FCM: 0.016		FCM (10)	0.94 (0.88)		Within FCM: 0.006
BALP / [U]/L					**P1NP / µg/ml**				
Baseline	FDI (14)	21.3 (10.2)	0.462		Baseline	FDI (14)	112.0 (108.5)	0.820	
	FCM (12)	18.7 (13.4)				FCM (12)	103.0 (103.3)		
Visit 3	FDI (14)	18.6 (11.0)	0.767		Visit 3	FDI (14)	108.0 (93.3)	0.767	
	FCM (11)	17.0 (19.2)				FCM (11)	89.0 (109.0)		
Visit 4	FDI (13)	20.4 (7.3)	0.738		Visit 4	FDI (13)	107.0 (62.0)	0.784	
	FCM (10)	17.9 (7.4)				FCM (10)	77.5 (102.5)		
Visit 5	FDI (12)	20.6 (9.2)	0.740		Visit 5	FDI (12)	98.0 (55.0)	0.748	
	FCM (11)	18.5 (18.3)				FCM (11)	85.0 (110.0)		
Visit 6	FDI (9)	20.9 (12.1)	0.905		Visit 6	FDI (9)	80.0 (65.5)	0.780	
	FCM (10)	19.9 (18.4)				FCM (10)	71.0 (117.8)		
Visit 7	FDI (12)	19.8 (7.4)	0.203	Within FDI: 0.883	Visit 7	FDI (12)	97.0 (105.8)	0.722	Within FDI: 0.439
	FCM (10)	22.9 (8.9)		Within FCM<0.001		FCM (10)	104.5 (126.5)		Within FCM: 0.459

Alkaline Phosphatase

The median concentrations of ALP in either group throughout the study are displayed in table 9 and as clustered boxplot in figure 24 In the FDI group, the maximum median concentration of ALP was recorded at visit 7 (124.0 (IQR: 55.0) [iU]/L). The minimum median concentration was noted at baseline (96.0 (IQR: 74.0) [iU]/L). In the FCM group the maximum median concentration was recorded at visit 6 (123.5 (IQR: 65.3) [iU]/L), and the minimum median concentration at baseline (107.0 (IQR: 52.0) [iU]/L). No significant statistical difference existed between the two groups at any time point of the study. One outlier existed in the FDI group at baseline and visit 3 as noted in figure 24.

There was a significant difference between the concentrations within the FCM group (p=0.016) in terms of ALP but no significant difference between the concentrations within the FDI group.

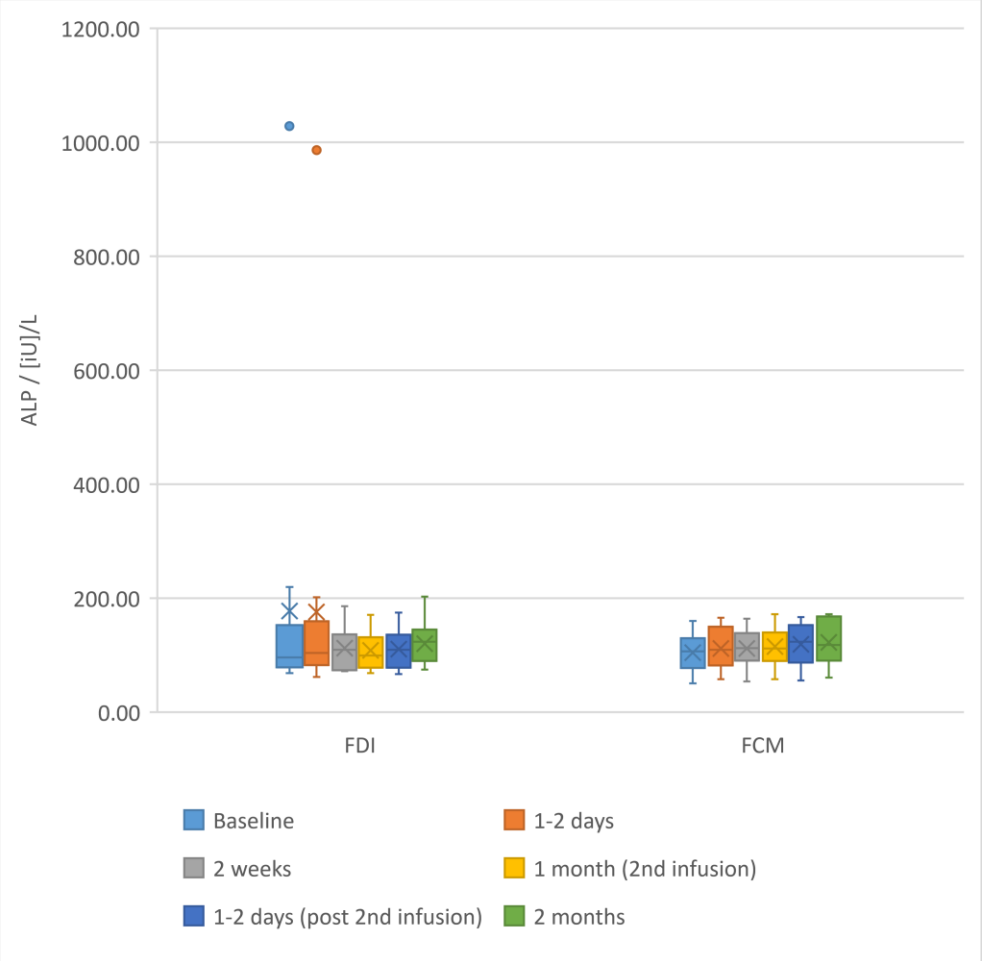

Figure 24: ALP concentrations

Bone specific ALP

The median concentrations of BALP in either group throughout the study are displayed in table 9 and as clustered boxplot in figure 25. In the FDI group, the maximum median concentration of bone specific ALP was recorded at baseline (21.3 (IQR: 10.2) [iU]/L). The minimum median concentration was noted at visit 3 (18.6 (IQR: 11.0) [iU]/L). In the FCM group the maximum median concentration was recorded at visit 7 (22.9 (IQR: 8.9) [iU]/L), and the minimum median concentration at visit 3 (17.0 (IQR: 19.2) [iU]/L). No significant statistical difference

existed between the two groups at any point of the study. There were outliers in both groups throughout the study. One outlier was noted in the FDI group at baseline and visit 3; one outlier was noted in the FCM group at visit 4 and visit 7 as noted in figure 25.

There was a significant difference between the concentrations within the FCM group ($p<0.001$) in terms of BALP but no significant difference between the concentrations within the FDI group.

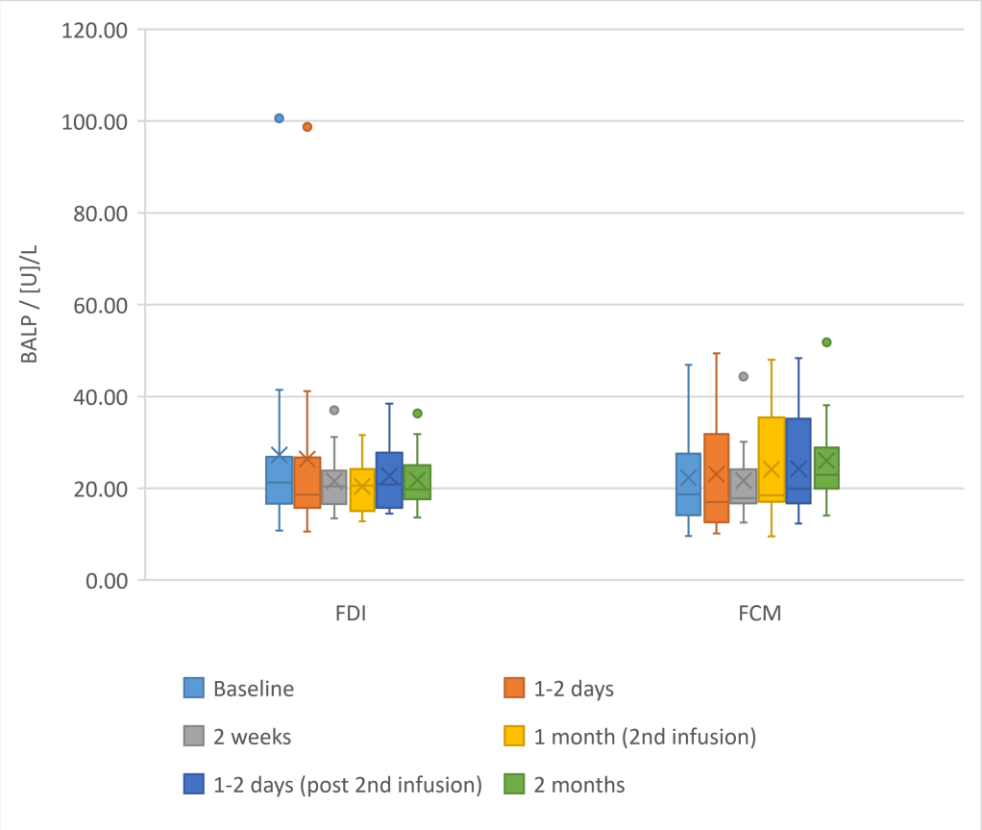

Figure 25: BALP concentrations

Carboxy-terminal collagen cross-linked telopeptide

The median concentrations of CTx in either group throughout the study are displayed in table 9 and as clustered boxplot in figure 26. In the

FDI group, the maximum median concentrations of CTx were recorded at baseline and visit 7 (0.84 (IQR: 0.45) µg/ml and 0.84 (IQR: 0.63) µg/ml respectively). The minimum median concentration was noted at visit 6 (0.71 (IQR: 0.38) µg/ml). In the FCM group the maximum median concentration was recorded at visit 5 (0.99 (IQR: 0.74), and the minimum median concentration at visit 4 (0.69 (IQR: 0.54) µg/ml). No significant statistical difference existed between the two groups at any point of the study. There was one outlier in the FDI group on baseline, visit 3 and visit 4 as noted in figure 26. No outliers existed in the FCM group.

There was a significant difference between the concentrations within the FCM group (p=0.006) in terms of CTx but no significant difference between the concentrations within the FDI group.

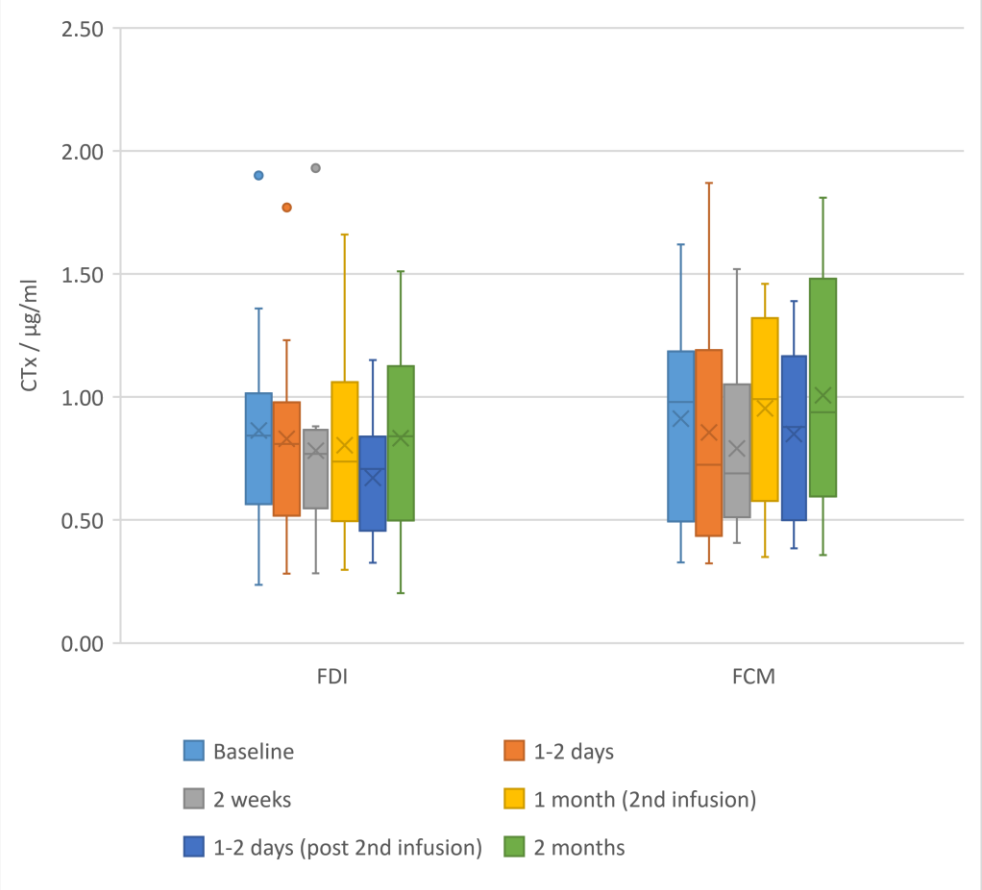

Figure 26: CTx concentrations

Procollagen 1 Intact N-Terminal Propeptide

The median concentrations of P1NP in either group throughout the study are displayed in table 9 and as clustered boxplot in figure 27. In the FDI group, the maximum median concentration of P1NP was recorded at baseline (112.0 (IQR: 108.5) µg/L). The minimum median concentration was noted at visit 6 (80.0 (IQR: 65.5) µg/L). In the FCM group the maximum median concentration was recorded at visit 7 (104.5 (IQR: 126.5) µg/L), and the minimum median concentration at visit 6 (71.0 (IQR: 117.8) µg/L). No significant statistical difference existed between the two groups at any point of the study. There was one outlier in the FDI group at baseline and visits 4, 5 and 6. Two outliers in the FDI group were noted at visit 3 No outliers existed in the FCM group as noted in figure 27

No statistically significant difference within the FDI group nor within the FCM group was noted.

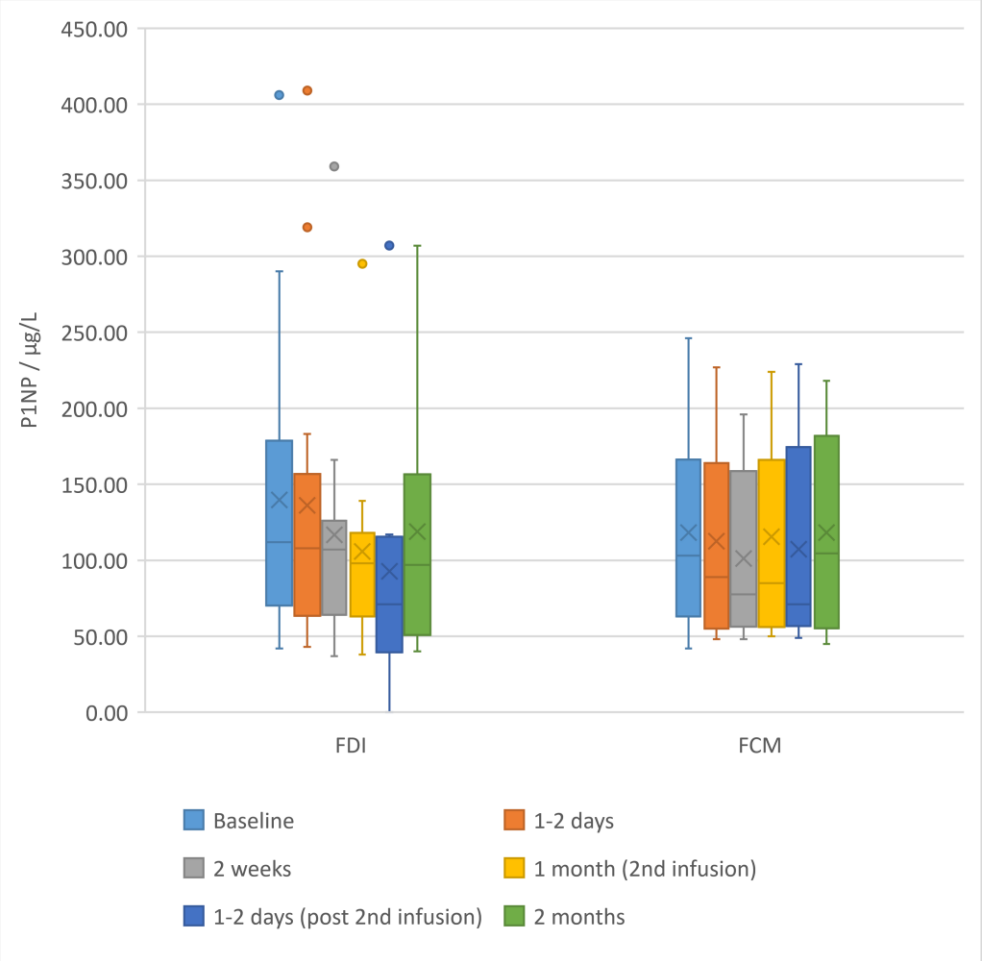

Figure 27: P1NP concentrations

3.5: Functional status and patient reported outcome measures – cumulative effect of iron and between group comparisons

3.5.1: Functional status

Table 10: Functional status markers

Visit	Iron group (n)	Mean (SD)	p-value	p- value (within group analysis)	Visit	Iron group (n)	Median (IQR)	p-value	p- value (within group analysis)
1-minute-sit-to-stand					**DASI / METs**				
Baseline	Total (21)	16.0 (8.0)			Baseline	Total (26)	4.9 (1.7)		
	FDI (11)	16.5 (5.9)	0.731			FDI (14)	5.1 (1.1)	0.145	
	FCM (10)	15.3 (10.2)				FCM (12)	4.1 (1.9)		
Visit 5	Total (20)	20.9 (9.8)			Visit 5	Total (23)	4.6(1.4)		
	FDI (11)	20.4 (5.4)	0.796			FDI (12)	4.8 (3.0)	0.211	
	FCM (9)	21.6 (13.9)				FCM (11)	4.4 (1.5)		
Visit 7	Total (17)	22.0 (9.8)			Visit 7	Total (22)	5.1 (2.1)		
	FDI (8)	21.0 (4.5)	0.704			FDI (12)	5.1 (2.3)	0.228	
	FCM (9)	22.9 (13.1)				FCM (10)	4.7 (2.3)		
Visit 8	Total (17)	25.6 (8.7)		Total cohort <0.001	Visit 8	Total (22)	5.1 (2.0)		Total cohort: 0.294
	FDI (10)	24.3 (4.7)	0.465	FDI < 0.001		FDI (12)	5.1 (2.4)	0.346	FDI: 0.376
	FCM (7)	27.6 (12.7)		FCM < 0.001		FCM (10)	4.8 (1.4)		FCM: 0.419

Duke Activity Status Index

The median cumulative scores for the study are displayed in table 10 and as clustered boxplot in figure 32. The maximum median score was noted on visits 7 and 8 (5.1 (IQR: 2.1) METs and 5.1 (IQR: 2.0) METs, respectively). The minimum median score was noted at visit 5 (4.6 (IQR: 1.4) METs). Outliers were noted throughout the study as indicated at figure 28.

In the FDI group, the maximum median DASI score was recorded on baseline, visit 7 and visit 8 (5.1 (IQR: 1.1) METs, 5.1 (IQR: 2.3) METs, 5.1 (IQR: 2.4) METs respectively). The minimum median DASI score in the FDI group was recorded at visit 5 (4.8 (IQR: 4.4) METs). In the FCM group the maximum DASI score was recorded at visit 8 (4.8 (IQR: 1.4) METs, and the minimum median DASI score was recorded at baseline (4.1 (IQR: 1.9) METs).The median scores for the DASI for each group at each visit are displayed in table 10 and as clustered boxplot in figure 28. There was no statistically significant difference between the distribution of DASI scores displayed at each group at any time point of the trial. There were no outliers in the FDI group. Two outliers at visit 5 and one outlier at visit 8 existed for FCM as noted in figure 28.

There was no significant difference within the scores of the total cohort and the individual groups.

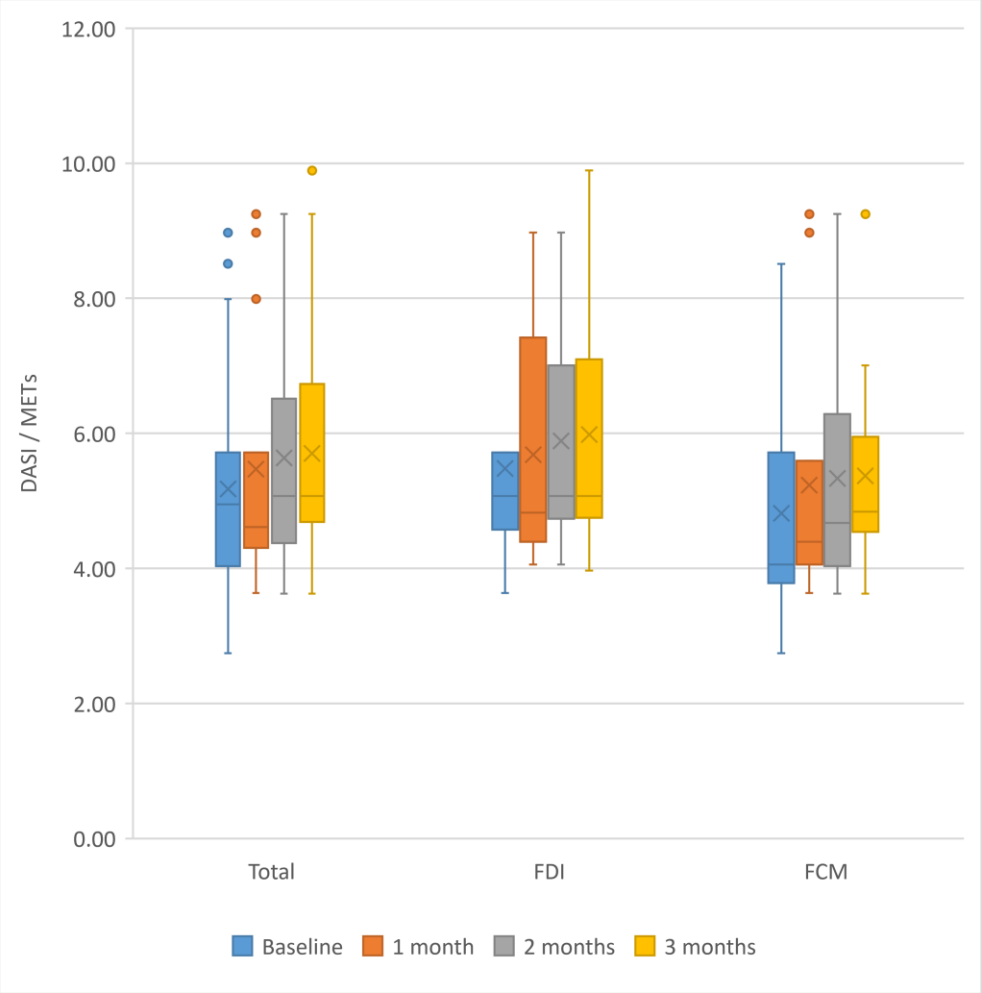

Figure 28: DASI scores

1-minute-sit-to-stand test

The 1-minute-sit-to-stand test was only performed where the participant was able to undertake the demands of the test (n=21). Five participants were unable to do the test due to limb prosthesis (n=1), permanent foot deformities (n=1) or severe arthritis documented (n=3). The mean cumulative scores for the test are displayed in table 10 and as a line graph in figure 29. The maximum mean score was noted on visit 8 (25.6 (SD: 8.7) /min) and the minimum mean score on baseline (16.0 (SD: 8.0) /min).

In the FDI group, the maximum mean 1-minute-sit-to-stand performance was recorded on visit 8 (24.3 (SD: 4.7)/min). The minimum mean 1-minute-sit-to-stand performance was recorded at baseline (16.5 (SD: 5.9) /min). In the FCM group the maximum mean 1-minute-sit-to-stand performance was recorded at visit 8 (27.6 (SD: 12.7) /min), and the minimum mean 1-minute-sit-to-stand performance was recoded at baseline (15.3 (SD: 10.2) /min). There was no statistically significant difference between the performances recorded at each group at any given time point of the study. The 1-minute-sit-to-stand mean performances for each group at each visit are displayed in table 10 and as a line graph in figure 29.

There was a significant difference between performances of 1-minute-sit-to-stand recorded at various points within the total cohort, the FDI group and the FCM group ($p<0.001$ respectively).

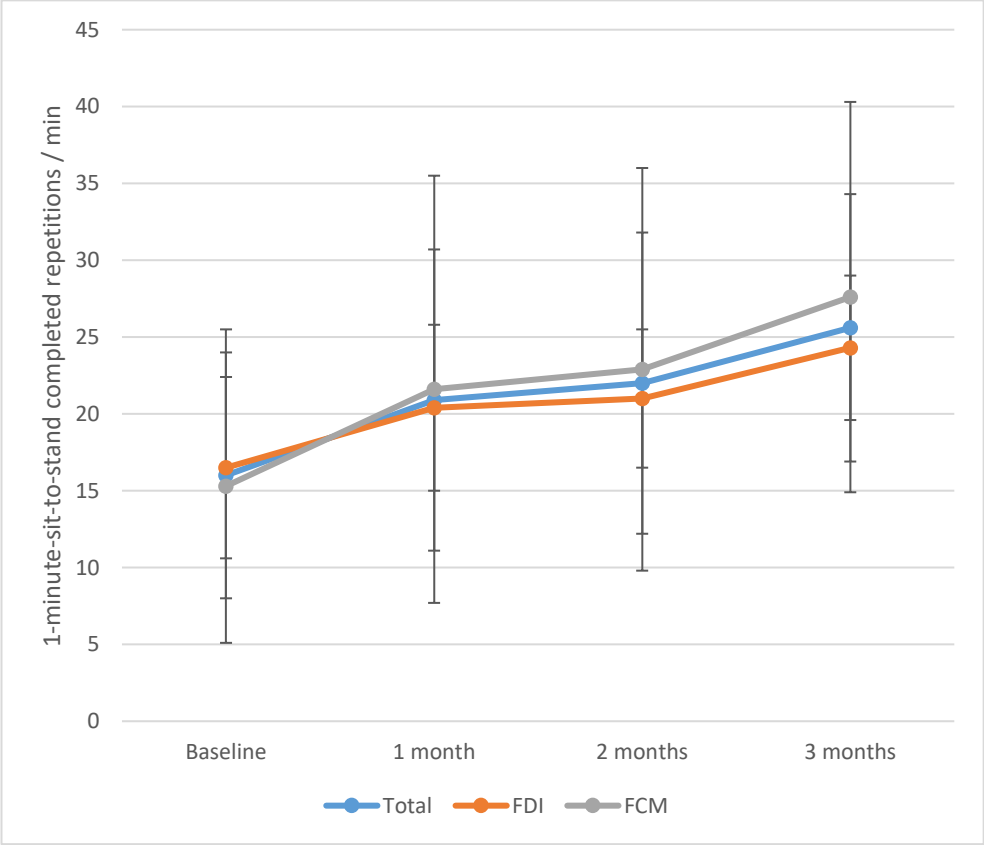

Figure: 29: 1-minute-sit-to-stand test scores

3.5.2: Patient reported outcome measures

Fatigue Severity Scale

Table 11: Fatigue Severity Scale scores

Visit	Iron group (n)	Median (IQR)	p-value	p- value (within group analysis)	Visit	Iron group (n)	Median (IQR)	p-value	p- value (within group analysis)
FSS total score					**FSS Visual analogue scale**				
Baseline	Total (26)	50.0 (24.3)			Baseline	Total (26)	3.5 (4.5)		
	FDI (14)	54.5 (12.5)	0.036			FDI (14)	3.0 (6.0)	0.403	
	FCM (12)	42.0 (30.0)				FCM (12)	4.5 (2.0)		
Visit 5	Total (23)	48.0 (21.0)			Visit 5	Total (23)	5.0 (4.0)		
	FDI (12)	52.5 (25.5)	0.424			FDI (12)	4.5 (3.0)	0.413	
	FCM (11)	44.0 (19.0)				FCM (11)	6.0 (4.0)		
Visit 7	Total (23)	49.0 (18.0)			Visit 7	Total (23)	5.0 (3.0)		
	FDI (13)	55.0 (19.0)	0.284			FDI (13)	5.0 (4.5)	0.605	
	FCM (10)	48.0 (18.0)				FCM (10)	5.0 (2.5)		
Visit 8	Total (22)	47.5 (21.8)		Total cohort : 0.884	Visit 8	Total (22)	6.0 (3.3)		Total cohort: 0.026
	FDI (12)	49.5 (25.0)	0.771	FDI: 0.653		FDI (12)	7.0 (4.0)	0.159	FDI: 0.048
	FCM (10)	42.5 (20.0)		FCM: 0.249		FCM (10)	5.0 (2.5)		FCM: 0.152

The median cumulative total scores for the FSS are displayed in table 11 In terms of FSS total score the maximum median score in the whole-group analysis was noted at baseline (50.0 (IQR: 24.3) / 60) and the minimum median score was noted at visit 8 (47.5 (IQR: 21.8) /60). In terms of the FSS visual analogue scale the maximum median score was noted at visit 8 (6.0 (IQR: 3.3) /10) and the minimum median score was noted at baseline (3.5 (IQR: 4.5) / 10).

In terms of the FSS total score, in the FDI group the maximum median was recorded at visit 7 (55.0 (IQR: 19.0) /63) and the minimum median FSS total score was recorded at visit 8 (49.5 (IQR: 25.0) / 63). In the FCM group the maximum median FSS total score was recorded at visit 7 (48.0 (IQR: 18.0)/ 63) and the minimum median FSS total score was recorded at baseline (42.0 (IQR: 30.0) / 63). There was a statistically significant difference in the distribution of FSS total scores at baseline (FDI: 54.5 (IQR: 12.5) / 63) vs. FCM: 44.0 (IQR: 19.0) /63; p=0.036). The median FSS total scores for each group at each visit are displayed in table 11 and as a clustered boxplot in figure 30. Two outliers were noted at baseline at the FDI group.

No significant difference was noted in terms of FSS total scores recorded at various points in the study, within the total cohort, the FDI group nor the FCM group.

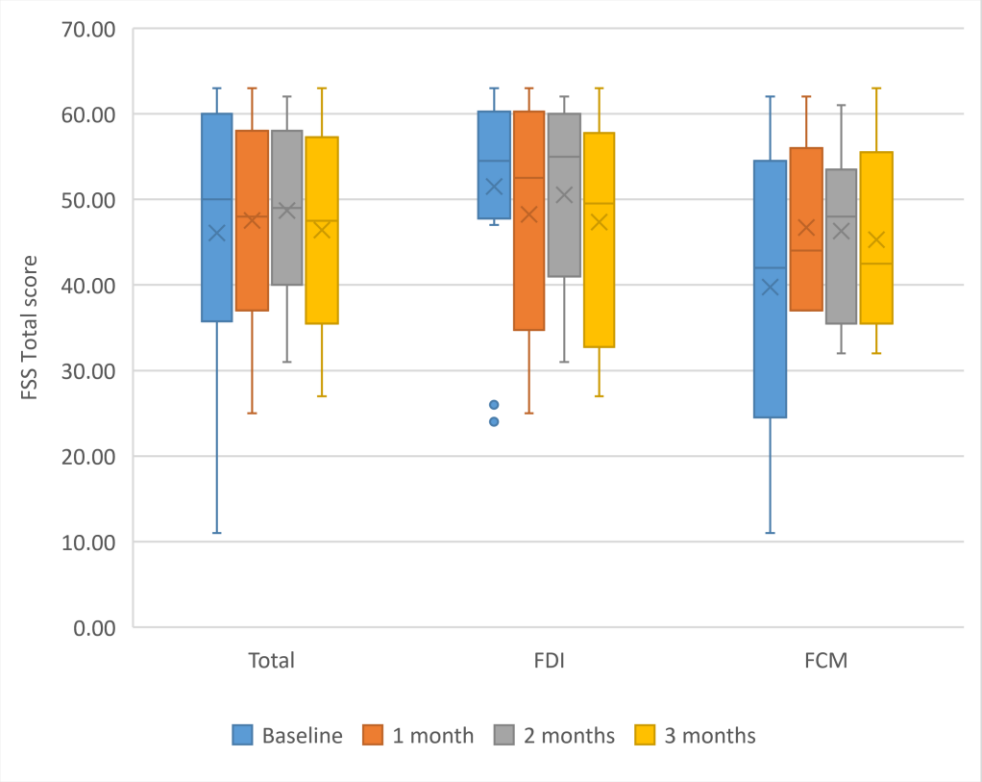

Figure 30: FSS Total scores

In terms of FSS visual analogue score, in the FDI group the maximum median score was recorded at visit 8 (7.0 (IQR: 4.0)/ 10) and the minimum median FSS visual analogue scale score was recorded at baseline (3.0 (IQR: 6.0) / 10). In the FCM group the maximum median FSS visual analogue scale score was recorded at visit 5 (6.0 (IQR: 4.0)/ 10) and the minimum median FSS visual analogue scale score was recorded at baseline (4.5 (IQR: 2.0)/ 10). There was no statistically significant difference between the distribution of FSS visual analogue scale scores displayed at each group at any time point of the trial. The median FSS visual analogue scale scores for each group at each visit are displayed in table 11 and as a clustered boxplot in figure 31. No outliers existed.

There was a significant difference in terms of FSS visual analogue scores within the total cohort and the FDI group (p=0.026 and p=0.048

respectively). No significant difference between the scores within the FCM group was noted.

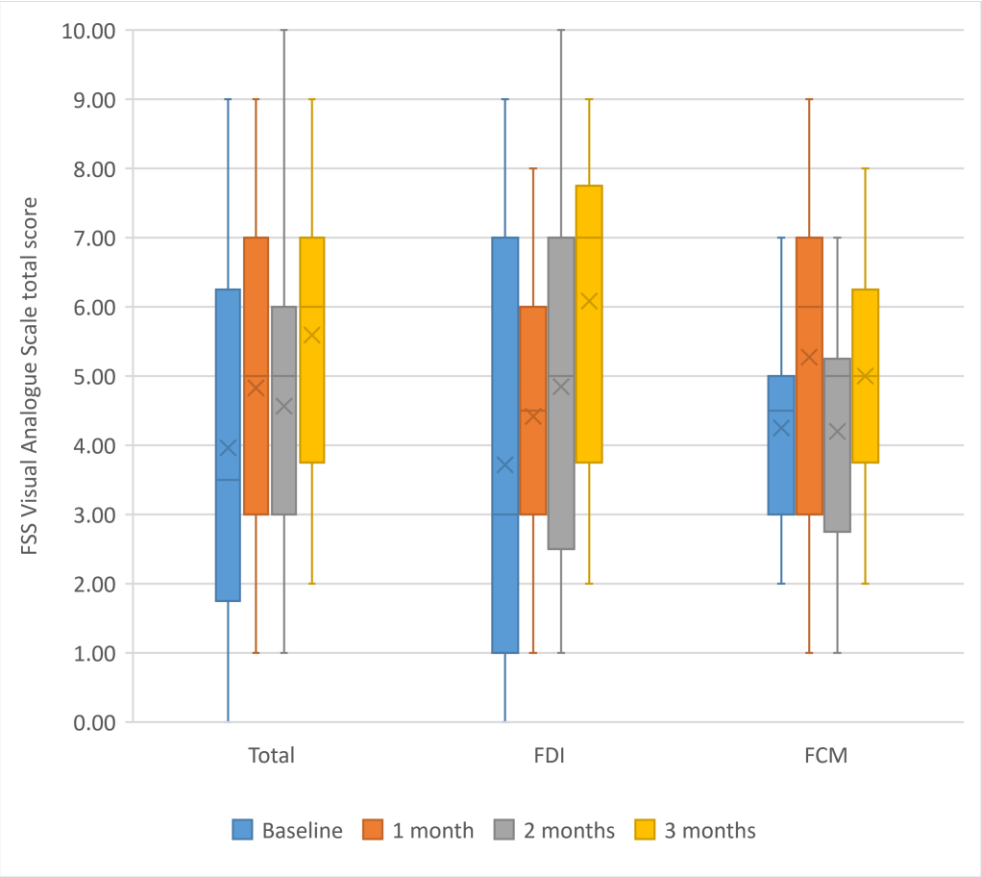

Figure 31: FSS Visual analogue scores

Short form (36) questionnaire

Table 12: SF-36 questionnaire results									
Visit	**Iron group (n)**	**Mean/Median (SD/IQR)**	**p-value**	**p- value (within group analysis)**	**Visit**	**Iron group (n)**	**Mean/Median (SD/IQR)**	**p-value**	**p- value (within group analysis)**
Physical Function					**Role limitation (physical)**				
Baseline	Total (26)	25.0 (27.5)			Baseline	Total (26)	0.00 (25.0)		
	FDI (14)	22.5 (23.8)	0.494			FDI (14)	0.00 (31.3)	0.297	
	FCM (12)	34.4 (27.5)				FCM (12)	0.00 (0.00)		
Visit 5	Total (25)	35.0 (50.0)			Visit 5	Total (25)	25.0 (75.0)		
	FDI (14)	35.0 (46.3)	0.651			FDI (14)	25.0 (56.3)	0.608	
	FCM (11)	35.0 (55.0)				FCM (11)	0.00 (100.0)		
Visit 7	Total (23)	30.0 (50.0)			Visit 7	Total (23)	0.00 (50.0)		
	FDI (13)	30.0 (45.0)	0.410			FDI (13)	25.0 (50.0)	0.832	
	FCM (10)	25.0 (61.3)				FCM (10)	0.00 (62.5)		
Visit 8	Total (22)	37.5 (55.0)		Total cohort: 0.015	Visit 8	Total (22)	25.0 (56.3)		Total cohort: 0.149
	FDI (12)	35.0 (45.0)		FDI: 0.042		FDI (12)	37.5 (43.8)	0.722	FDI: 0.323
	FCM (10)	42.5 (65.0)	0.582	FCM: 0.116		FCM (10)	12.5 (100.0)		FCM: 0.373
Role limitation (emotional)					**Energy ***				

Baseline	Total (26)	33.3 (100.0)			Baseline	Total (26)	25.6 (20.2)		
	FDI (14)	33.3 (75.0)	0.231			FDI (14)	26.4 (24.8)	0.815	
	FCM (12)	0.00 (91.7)				FCM (12)	24.6 (14.2)		
Visit 5	Total (25)	66.7 (100.0)			Visit 5	Total (25)	40.0 (21.3)		
	FDI (14)	83.4 (58.4)	0.413			FDI (14)	42.5 (19.2)	0.569	
	FCM (11)	66.7 (100.0)				FCM (11)	37.3 (24.0)		
Visit 7	Total (23)	66.7 (66.7)			Visit 7	Total (23)	38.9 (21.3)		
	FDI (13)	66.7 (83.4)	0.784			FDI (13)	38.1 (21.9)	0.836	
	FCM (10)	83.4 (66.7)				FCM (10)	40.0 (28.8)		
Visit 8	Total (22)	100.0 (41.7)		Total cohort: 0.053	Visit 8	Total (22)	39.5 (18.8)		Total cohort: 0.002
	FDI (12)	100.0 (91.7)	0.872	FDI: 0.364		FDI (12)	39.2 (19.8)	0.920	FDI: 0.015
	FCM (10)	83.4 (33.3)		FCM: 0.043		FCM (10)	40.0 (18.6)		FCM: 0.079
Emotional well-being					**Social functioning**				
Baseline	Total (26)	68.0 (21.0)			Baseline	Total (26)	43.8 (53.1)		
	FDI (14)	66.0 (31.0)	0.595			FDI (14)	43.8 (65.6)	0.560	
	FCM (12)	68.0 (15.0)				FCM (12)	43.8 (34.3)		
Visit 5	Total (25)	72.0 (16.0)			Visit 5	Total (25)	62.5 (62.5)		
	FDI (14)	68.0 (17.0)	0.316			FDI (14)	62.5 (71.9)	0.976	
	FCM (11)	80.0 (16.0)				FCM (11)	50.0 (50.0)		

Visit 7	Total (23)	72.0 (16.0)			Visit 7	Total (23)	50.0 (50.0)		
	FDI (13)	72.0 (18.0)	0.208			FDI (13)	50.0 (56.3)	0.784	
	FCM (10)	80.0 (21.0)				FCM (10)	62.5 (53.1)		
Visit 8	Total (22)	78.0 (32.0)		Total cohort: 0.780	Visit 8	Total (22)	75.0 (65.63)		Total cohort: 0.621
	FDI (12)	72.0 (18.0)	0.821	FDI: 0.725		FDI (12)	75.0 (62.5)	0.771	FDI: 0.990
	FCM (10)	80.0 (21.0)		FCM: 0.764		FCM (10)	68.8 (65.6)		FCM: 0.248
Pain					**General health**				
Baseline	Total (26)	45.0 (37.5)			Baseline	Total (26)	37.5 (21.3)		
	FDI (14)	45.0 (47.5)	0.899			FDI (14)	37.5 (28.8)	0.940	
	FCM (12)	45.0 (31.9)				FCM (12)	37.5 (15.0)		
Visit 5	Total (25)	55.0 (55.0)			Visit 5	Total (25)	35.0 (30.0)		
	FDI (14)	56.3 (63.1)	0.786			FDI (14)	30.0 (38.8)	0.260	
	FCM (11)	45.0 (67.5)				FCM (11)	35.0 (35.0)		
Visit 7	Total (23)	55.0 (45.0)			Visit 7	Total (23)	30.0 (25.0)		
	FDI (13)	55.0 (61.3)	0.784			FDI (13)	30.0 (32.5)	0.784	
	FCM (10)	51.3 (47.5)				FCM (10)	37.0 (26.3)		
Visit 8	Total (22)	45.0 (31.9)		Total cohort: 0.038	Visit 8	Total (22)	27.5 (17.5)		Total cohort: 0.245
	FDI (12)	56.3 (43.8)	0.782	FDI: 0.079		FDI (12)	22.5 (31.3)	0.346	FDI: 0.439
	FCM (10)	45.0 (55.0)		FCM: 0.230		FCM (10)	30.0 (17.5)		FCM: 0.301

* variables characterised by asterisk are described as mean (SD); the remaining variables are described as median (IQR) based on distribution

Physical function domain

Physical function median scores are displayed in table 12 and as clustered boxplot at figure 32 for the entire population. The maximum physical function median score for the entire population was recorded at visit 8 (37.5 (IQR: 55.0) and the minimum physical function median score was noted at baseline (25.0 (IQR: 27.5)).

In the FDI group the maximum median score was recorded at visits 5 and 8 (35.0 (IQR: 46.3) and 35.0 (IQR: 45.0) respectively) and the minimum median physical function score was recorded at baseline (22.5 (IQR: 23.8)). In the FCM group the maximum median physical function was recorded at visit 8 (42.5 (IQR: 65.0) and the minimum median physical function score was recorded at visit 7 (25.0 (IQR: 61.3)). There was no statistically significant difference between the distribution of physical function scores displayed at each group at any time point of the trial. The median physical function scores for each group at each visit are displayed in table 12 and as clustered boxplot in figure 32.

There was a significant difference in terms of physical function scores visual analogue scores within the total cohort ($p=0.015$) and the FDI group ($p=0.042$). No significant difference between the scores within the FCM group was noted.

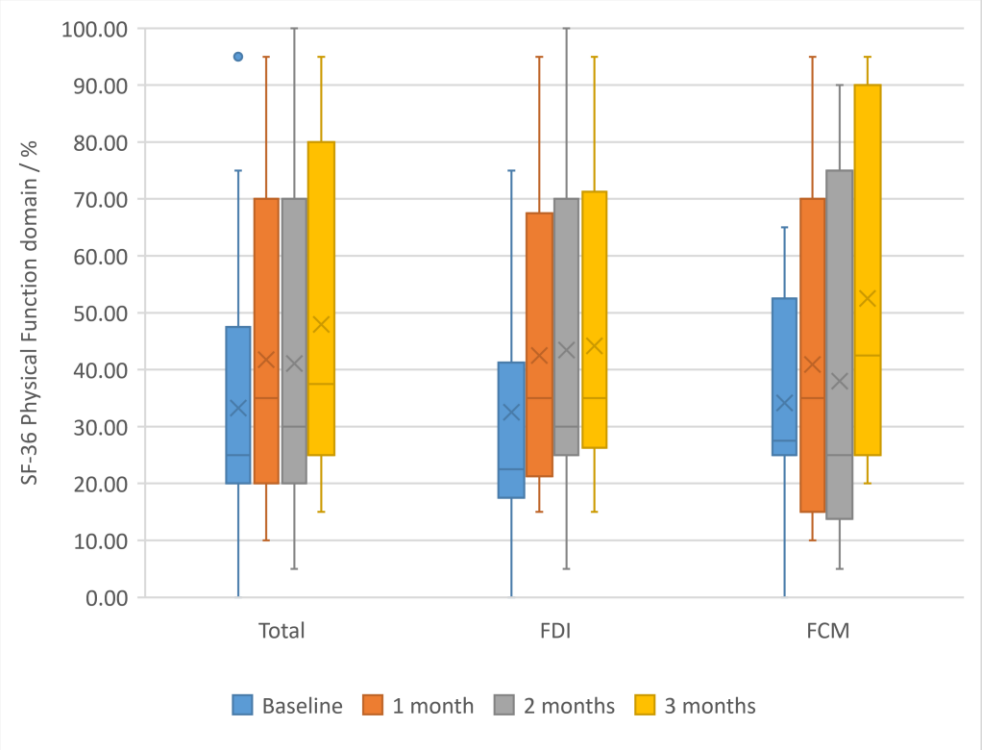

Figure 32: SF-36 Physical function domain

Role limitation due to physical restrictions / Role limitation due to emotional restrictions domains

Median scores relevant to role limitation due to physical restrictions are displayed in table 12 and as clustered boxplot at figure 33 for the entire population. The maximum median score for the entire population was recorded at visits 5 and 8 (25.0 (IQR: 75.0) and 25.0 (IQR: 56.3)) respectively. The minimum median score was recorded at baseline and visit 7 (0.0 (IQR: 25.0) and 0.0 (IQR: 50.0) respectively).

In the FDI group the maximum median score was recorded at visit 8 (37.5 (IQR: 43.8) and the minimum median score was recorded at baseline (0.0 (IQR: 31.3)). In the FCM group the maximum median score was recorded at visit 8 (12.5 (IQR: 100.0)). There was no statistically significant difference between the distribution of scores

displayed at each group at any time point of the trial. The median role limitation due to physical restriction scores for each group at each visit are displayed in table 12 and as clustered boxplot in figure 33.

No significant difference in terms of role limitation due to physical restriction was noted within the total cohort, the FDI group nor the FCM group.

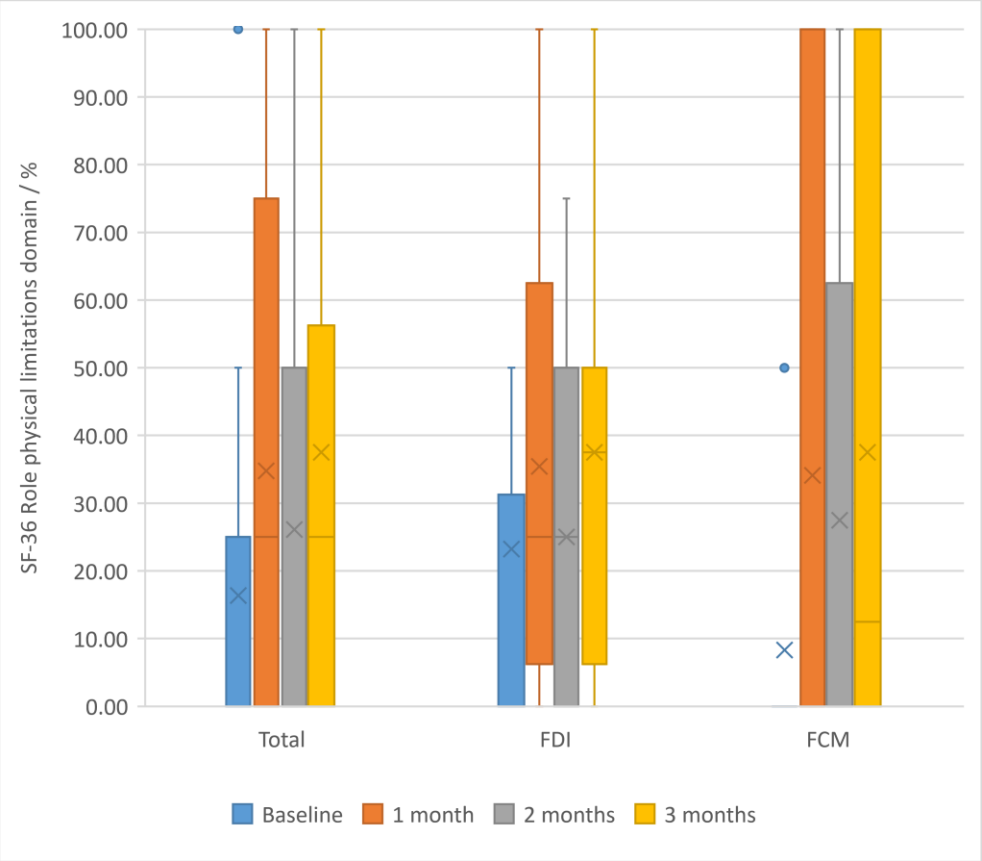

Figure 33: SF-36: Role limitations physical domain

Median scores relevant to role limitation due to emotional restrictions are displayed in table 12 and as clustered boxplot at figure 34 for the entire population. The maximum median score for the entire population was recorded at visit 8 (100.0 (IQR: 41.7)). The minimum median score was recorded at baseline (33.3 (IQR: 100.0)).

In the FDI group the maximum median score was recorded at visit 8 (100.0 (IQR: 91.7)) and the minimum median score was recorded at baseline (33.3 (IQR: 75.0)). In the FCM group the maximum median score was recorded at visits 7 and 8 (83.4 (IQR: 66.7) and 83.4 (IQR: 33.3) respectively) and the minimum median score was recorded at baseline (0.0 (IQR 91.7)). There was no statistically significant difference between the distribution of scores displayed at each group at any time point of the trial. The median role limitations due to emotional restriction scores for each group at each visit are displayed in table 12 and as a clustered boxplot in figure 34.

There was a significant difference in terms of role limitation due to emotional restriction scores within the FCM group (p=0.043). No significant difference between the scores within the FDI group nor the total cohort was noted.

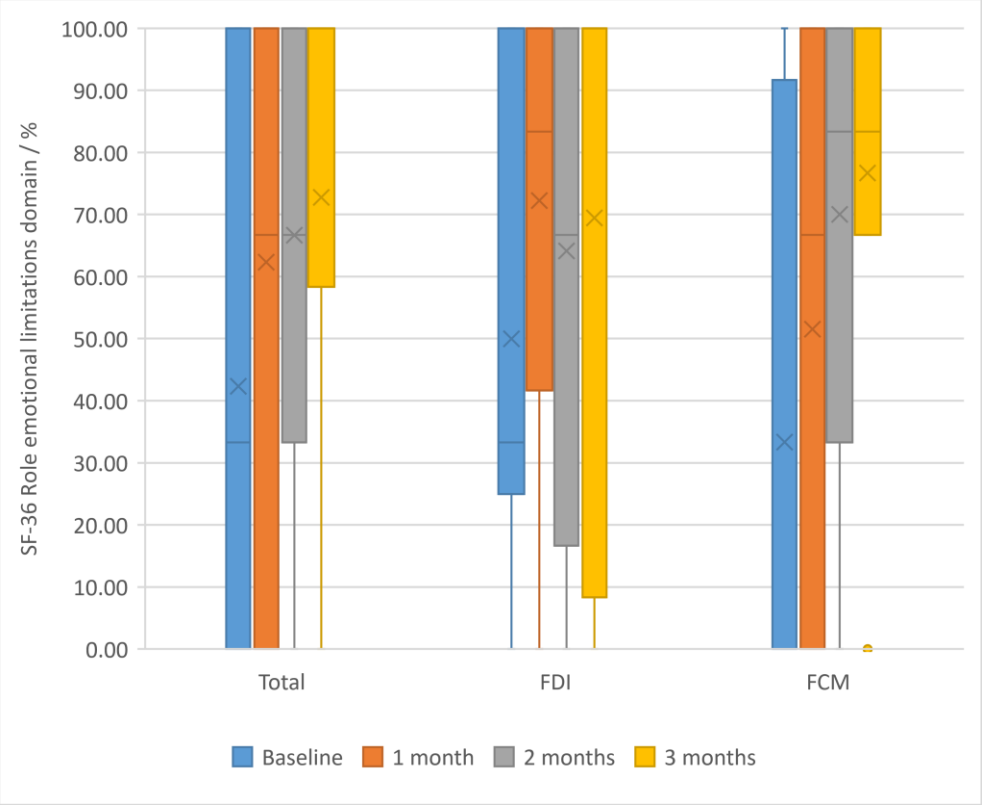

Figure 34: SF-36 Role limitations emotional domain

Energy domain

The mean cumulative scores for the test are displayed in table 12 and as a line graph in figure 35. The maximum mean score was noted on visit 5 as 40.0 (SD: 21.3) and the minimum was noted at baseline (25.6 (SD: 20.2).

In the FDI group, the maximum mean energy score was recorded on visit 5 (42.5 (SD: 19.2)). The minimum mean energy score was recorded at baseline (26.4 (SD: 24.8)). In the FCM group the maximum mean score was recorded at visits 7 and 8 (40.0 (SD: 28.8) and 40.0 (SD: 18.6) respectively) and the minimum mean energy score was recoded at baseline (24.6 (SD: 14.2)). There was no statistically significant difference between the energy scores recorded at each group at any given time point of the study. The mean energy scores for each group at each visit are displayed in table 12 and as a line graph in figure 35.

There was a significant difference in terms of energy scores within the total cohort (p=0.002) and the FDI group (p=0.015). No significant difference between the scores within the FCM group was noted.

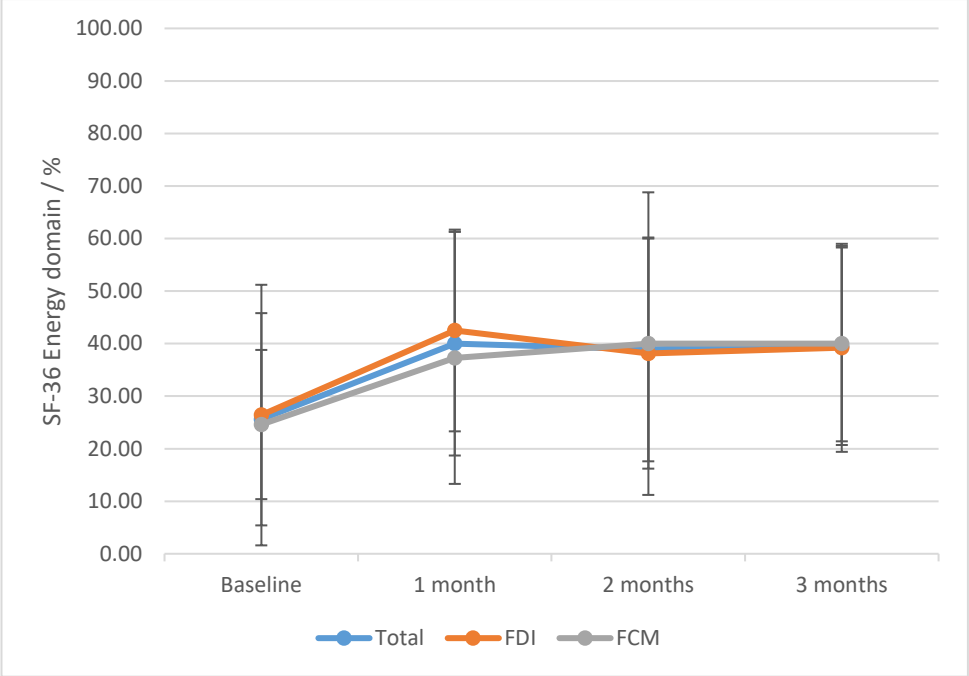

Figure 35: SF-36 Energy domain

Emotional well-being domain

Median scores relevant to emotional well-being are displayed in table 12 and as clustered boxplot at figure 36 for the entire population. The maximum median score for the entire population was recorded at visit 8 (78.0 (IQR: 32.0)). The minimum median score was recorded at baseline (68.0 (21.0)).

In the FDI group the maximum median score was recorded at visit 8 (78.0 (IQR: 31.0)) and the minimum median emotional well-being score was recorded at baseline (66.0 (IQR: 31.0)). In the FCM group the maximum median emotional well-being was recorded at visits 5, 7 and 8 (80.0 (IQR: 16.0), 80.0 (IQR: 21.0), 80 (IQR: 21.0) respectively) and the minimum median score was recorded at baseline (68.0 (IQR: 15.0). There was no statistically significant difference between the distribution of scores displayed at each group at any time point of the

trial. The median emotional well-being scores for each group at each visit are displayed in table 12 and as a clustered boxplot in figure 36. One outlier existed at visit 5 in the FCM group.

No significant difference in terms of emotional well-being scores was noted within the total cohort, the FDI group nor the FCM group.

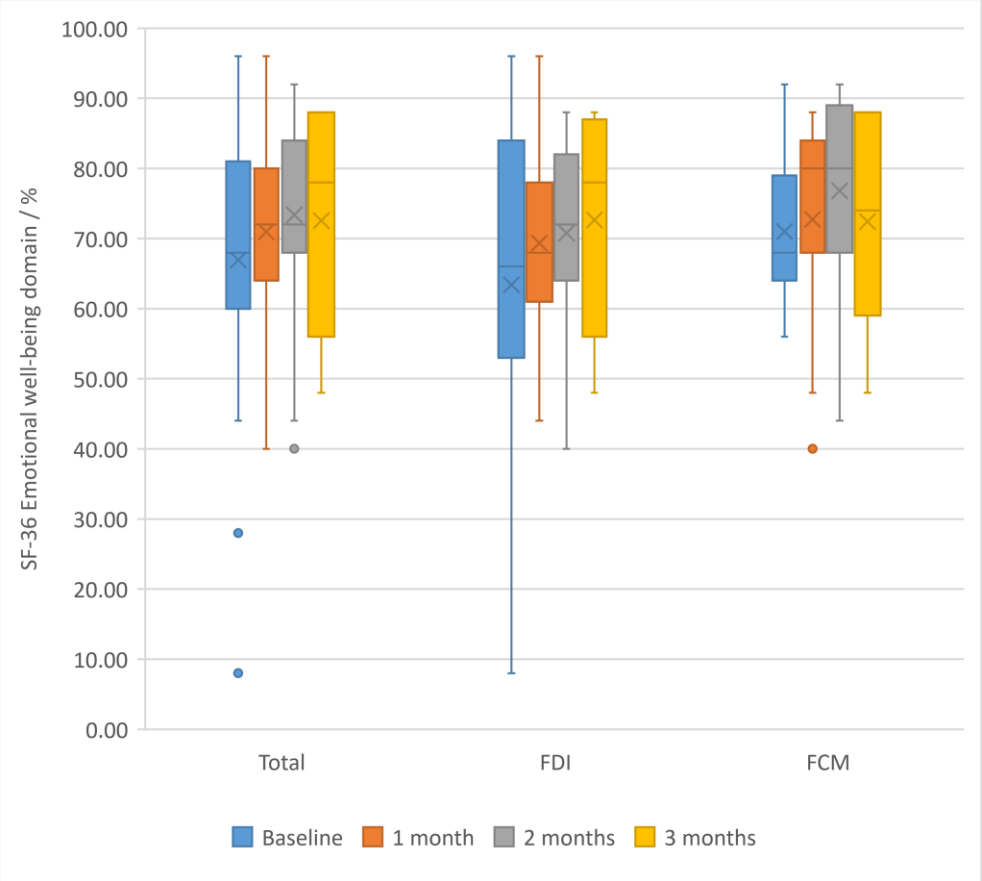

Figure 36: SF-36 Emotional well-being domain

Social functioning domain

Median scores relevant to social functioning are displayed in table 12 and as clustered boxplot at figure 37 for the entire population. The maximum median score for the entire population was recorded at visit

8 (75.0 (IQR: 65.6)). The minimum median score was recorded at baseline (43.8 (IQR: 53.1)).

In the FDI group the maximum median score was recorded at visit 8 (75.0 (IQR: 62.5)) and the minimum median social functioning score was recorded at baseline (43.8 (IQR: 65.6)). In the FCM group the maximum median social functioning score was recorded at visit 8 (68.8 (IQR: 65.6)) and the minimum median score was recorded at baseline (43.8 (IQR: 34.3)). There was no statistically significant difference between the distribution of scores displayed at each group at any time point of the trial. The median social functioning scores for each group at each visit are displayed in table 12 and as a clustered boxplot in figure 37.

No significant difference in terms of social functioning scores was noted within the total cohort, the FDI group nor the FCM group.

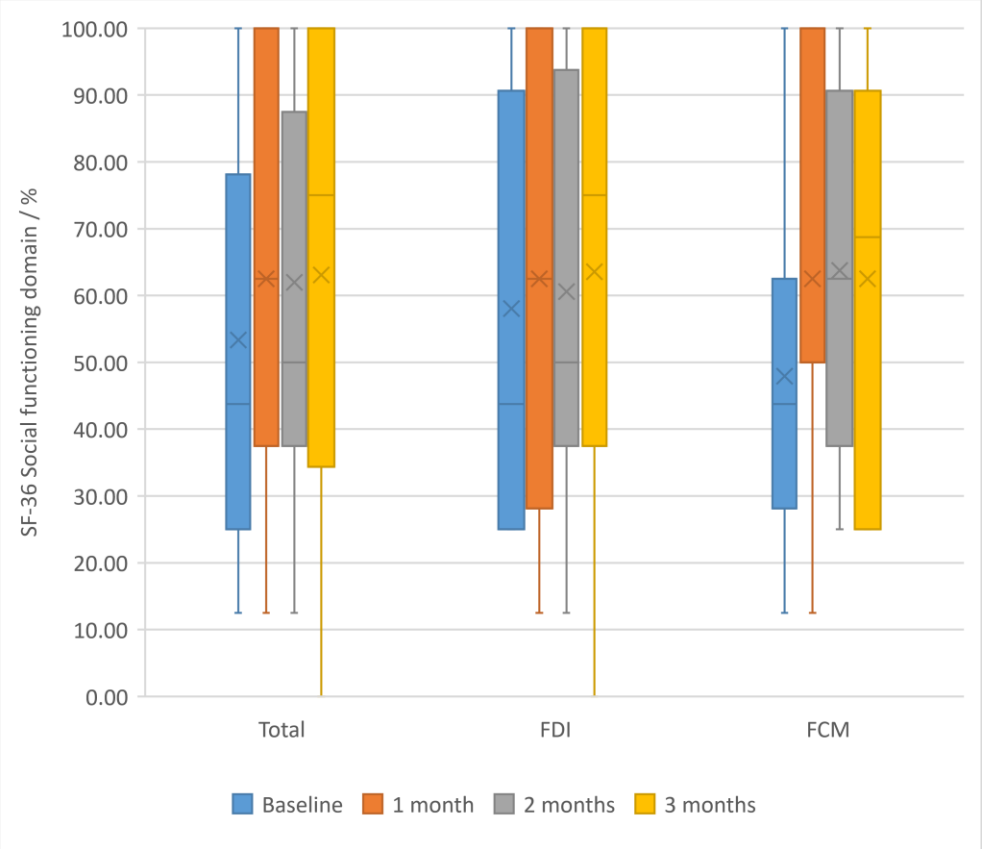

Figure 37: SF-36 Social functioning domain

Pain domain

Median scores relevant to pain are displayed in table 12 and as clustered boxplot at figure 38 for the entire population. The maximum median scores for the entire population was recorded at visits 5 and 7 (55.0 (IQR: 55.0) and 55.0 (IQR: 45.0). The minimum median scores was recorded at baseline and visit 8 (45.0 (IQR: 37.5) and 45.0 (IQR: 31.9)).

In the FDI group the maximum median score was recorded at visits 5 and 8 (56.3 (IQR: 63.1) and 56.3 (IQR: 43.8) respectively) and the minimum median pain score was recorded at baseline (45.0 (IQR: 47.5)). In the FCM group the maximum median pain score was

recorded at visit 7 (55.0 (IQR: 61.3)) and the minimum was at baseline and visit 8 (45.0 (IQR: 31.9) and 45.0 (IQR: 55.0) respectively).There was no statistically significant difference between the distribution of scores displayed at each group at any time point of the trial. The median pain scores for each group at each visit are displayed in table 12 and as a clustered boxplot in figure 38.

There was a significant difference in terms of pain scores within the total cohort (p=0.038). No significant difference between the scores within the FDI group nor the FCM group was noted.

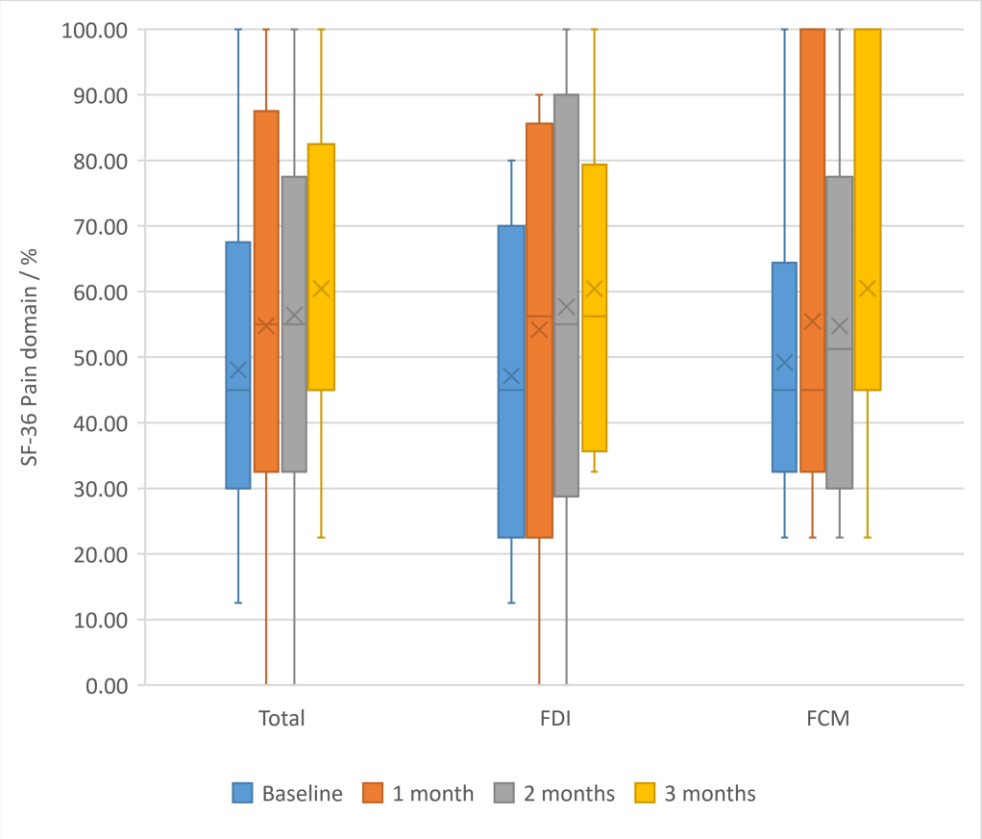

Figure 38: SF-36 Pain domain

General health domain

Median scores relevant to general health are displayed in table 12 and as clustered boxplot at figure 39 for the entire population. The maximum median score for the entire population was recorded at baseline (37.5 (IQR: 21.3)). The minimum median score was recorded at visit 8 (27.5 (17.5)).

In the FDI group the maximum median score was recorded at baseline (37.5 (IQR: 28.8)) and the minimum median general health score was recorded at visit 8 (22.5 (IQR: 31.3)). In the FCM group the maximum median general health score was recorded at baseline (37.5 (IQR: 15.0)) and the minimum median score was recorded at visit 8 (30.0 (IQR: 17.5)). There was no statistically significant difference between the distribution of scores displayed at each group at any time point of the trial. The median general health scores for each group at each visit are displayed in table 12 and as a clustered boxplot in figure 39. One outlier existed at baseline at the FCM group.

No significant difference in terms of general health domain scores was noted within the total cohort, the FDI group nor the FCM group.

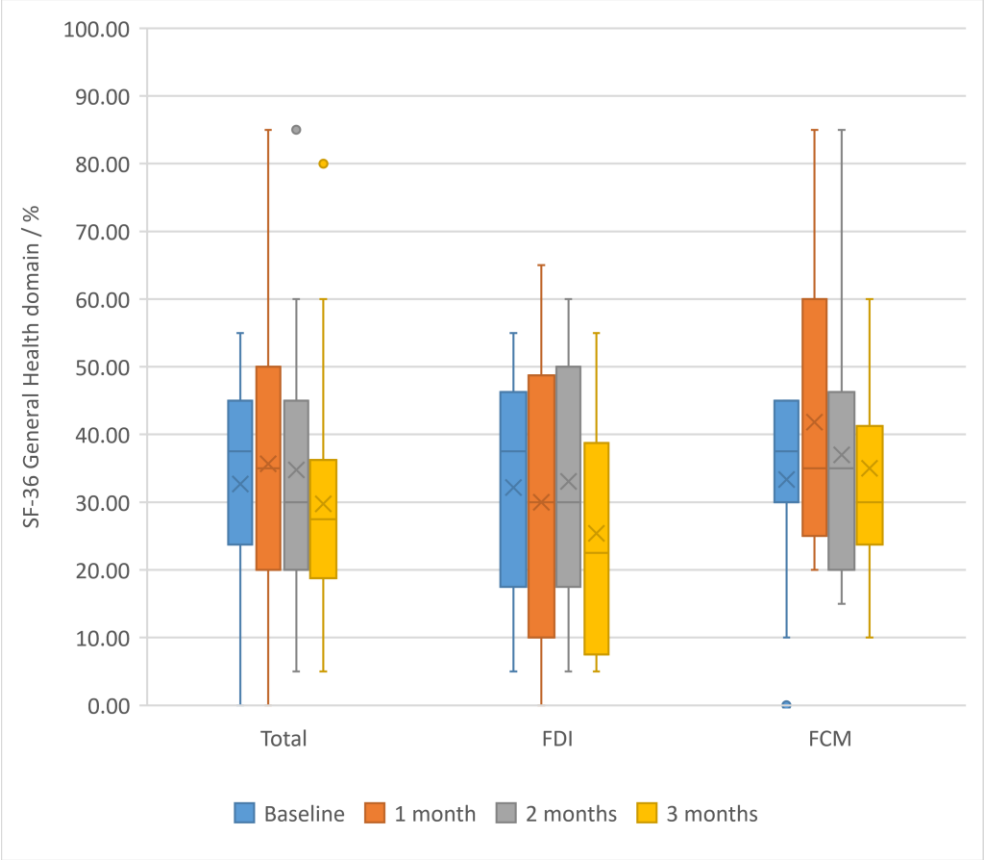

Figure 39: SF-36 General health domain

3.6: Clinical measures – comparison between groups

Table 13: Haematinic response and markers of kidney function/injury and inflammation									
Visit	**Iron group (n)**	**Mean/Median (SD/IQR)**	**p-value**	**p-value (within group)**	**Visit**	**Iron group (n)**	**Mean/Median (SD/IQR)**	**p-value**	**p-value (within group)**
Haemoglobin * / g/L					**eGFR / ml/min/1.73m^2**				
Baseline	FDI (14)	99.2 (12.2)			Baseline	FDI (14)	18.0 (11.3)		
	FCM (12)	101.6 (15.3)	0.664			FCM (12)	18.0 (11.3)	1.000	
Visit 4	FDI (13)	105.2 (9.5)			Visit 4	FDI (13)	21.0 (12.5)		
	FCM (10)	102.7 (16.3)	0.645			FCM (10)	18.5 (11.8)	0.738	
Visit 5	FDI (12)	103.8 (13.0)			Visit 5	FDI (12)	19.5 (10.5)		
	FCM (11)	106.0 (14.3)	0.707			FCM (11)	19.0 (14.0)	0.880	
Visit 7	FDI (13)	106.0 (13.3)		Within FDI: 0.041	Visit 7	FDI (13)	19.0 (14.0)		Within FDI: 0.811
	FCM (10)	109.1 (8.3)	0.526	Within FCM: 0.002		FCM (10)	16.5 (8.5)	1.000	Within FCM: 0.726
Creatinine * / µmol/L					**urinary PCR / mg/mmol**				
Baseline	FDI (14)	277.6 (98.8)			Baseline	FDI (13)	155.0 (550.0)		
	FCM (12)	260.2 (77.3)	0.626			FCM (11)	30.0 (290.0)	0.082	
Visit 4	FDI(13)	278.2 (106.0)			Visit 4	FDI (11)	85.0 (265.0)		
	FCM (10)	256.4 (61.0)	0.570			FCM (9)	20.0 (92.5)	0.056	
Visit 5	FDI (12)	275.3 (95.1)	0.526		Visit 5	FDI (10)	130.0 (457.5)	0.085	

	FCM (11)	252.4 (72.4)				FDI (11)	30.0 (130.0)		
Visit 7	FDI (13)	288.6 (100.9)		Within FDI: 0.527	Visit 7	FDI (10)	157.5 (531.3)		Within FDI: 0.663
	FCM (10)	271.0 (65.9)	0.638	Within FCM: 0.421		FCM (10)	35.0 (173.8)	0.089	Within FCM: 0.793
Serum ferritin / µg/L					**TSAT / %**				
Baseline	FDI (14)	76.5 (158.5)			Baseline	FDI (14)	15.0 (10.0)		
	FCM (12)	72.7 (104.6)	0.899			FCM (12)	14.5 (5.8)	0.781	
Visit 3	FDI (14)	190.5 (90.3)			Visit 3	FDI (14)	100.0 (9.0)		
	FCM (11)	231.0 (208.0)	0.107			FCM (11)	100.0 (12.0)	1.000	
Visit 4	FDI (13)	344.0 (222.5)			Visit 4	FDI (13)	22.0 (16.5)		
	FCM (10)	483.0 (398.0)	0.115			FCM (10)	25.5 (14.0)	0.605	
Visit 5	FDI (12)	299.5 (188.8)			Visit 5	FDI (12)	26.0 (13.8)		
	FCM (11)	318.0 (239.0)	0.740			FCM (11)	22.0 (13.0)	0.740	
Visit 6	FDI (9)	331.0 (96.5)			Visit 6	FDI (9)	89.0 (38.0)		
	FCM (10)	409.5 (282.3)	0.156			FCM (9)	67.0 (20.5)	0.161	
Visit 7	FDI (13)	406.0 (122.5)		Within FDI <0.001	Visit 7	FDI (13)	29.0 (9.5)		Within FDI <0.001
	FCM (10)	415.5 (253.5)	0.563	Within FCM <0.001		FCM (10)	26.0 (17.5)	0.927	Within FCM <0.001
CRP / mg/L									
Baseline	FDI (14)	8.0 (17.6)							
	FCM (12)	4.3 (9.9)	0.462						

Visit 4	FDI (13)	7.7 (20.6)			
	FCM (10)	6.4 (18.1)	0.738		
Visit 5	FDI (12)	8.0 (15.1)			
	FCM (11)	4.9 (18.5)	0.651		
Visit 7	FDI (13)	8.6 (27.9)		Within FDI: 0.456	
	FCM (10)	6.2 (12.0)	0.832	Within FCM: 0.114	
* variables characterised by asterisk are described as mean (SD); the remaining variables are described as median (IQR) based on distribution					

3.6.1: Haemoglobin and markers of iron metabolism

Haemoglobin

At baseline, one patient from each group had a haemoglobin > 120 g/L (FDI: 7.1% vs. FCM: 8.3%). The mean haemoglobin concentration in either group throughout the study is displayed in table 13 and as a line graph in figure 40. In the FDI group, the maximum mean haemoglobin concentration was recorded at visit 7 (106.0 (SD: 13.3) g/L). The minimum mean haemoglobin concentration was noted at baseline (99.2 (SD: 12.2) g/L). In the FCM group the maximum mean concentration was recorded at visit 7 (109.1 (SD: 8.3) g/L) and the minimum mean concentration at baseline (101.6 (SD: 15.3) g/L). No statistically significant difference existed between the two groups at any time point of the study.

There was a statistically significant difference in terms of haemoglobin concentrations within both the FDI and the FCM groups over time ($p=0.041$ and $p=0.002$ respectively).

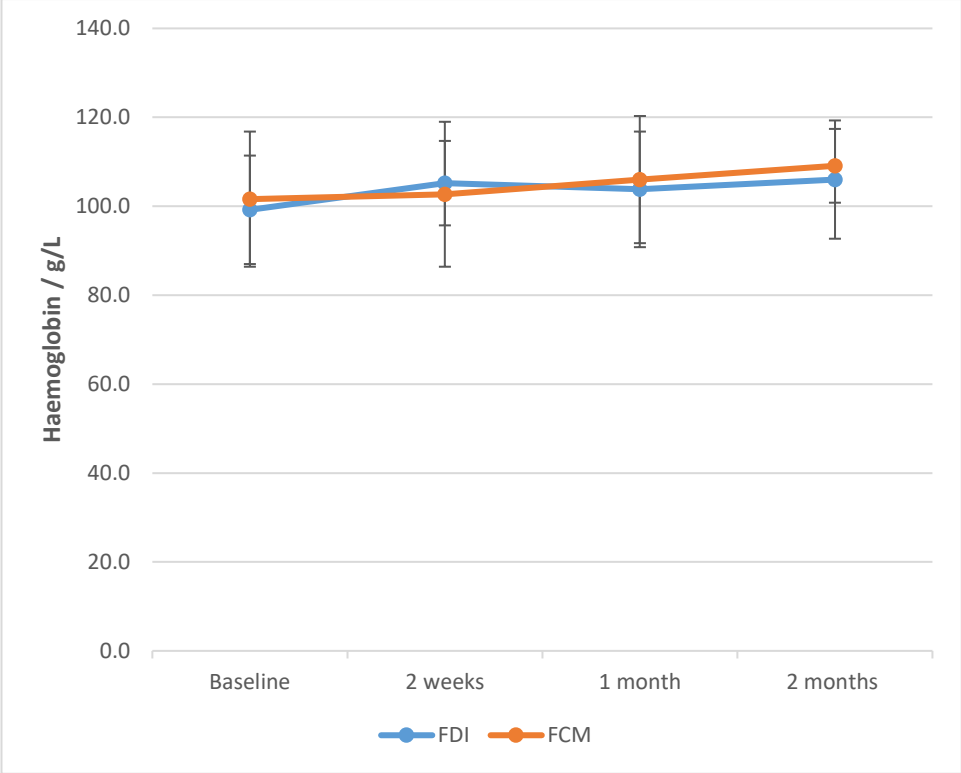

Figure 40: Haemoglobin concentrations

Serum ferritin and TSAT

Median measurements of serum ferritin and TSAT are displayed for each group in table 13 and as clustered boxplot in figures 41 and 42 respectively.

In terms of serum ferritin, in the FDI group the maximum median concentration of serum ferritin was noted at visit 7 (406.0 (IQR: 122.5) µg/L) and the minimum median concentration at baseline (76.5 (IQR: 158.5) µg/L). In the FCM group, the maximum median concentration of serum ferritin was noted at visit 4 (483.0 (IQR: 398.0) µg/L) and the minimum median concentration at baseline (72.7 (IQR: 104.6) µg/L). No statistically significant difference existed between the distribution of serum ferritin concentration due to either preparation at any time

point in the study. Two outliers were noted on visits 6 and 7 in the FDI group as indicated in figure 41.

There was a statistically significant difference in terms of serum ferritin concentrations within both the FDI and the FCM groups over time ($p<0.001$ respectively).

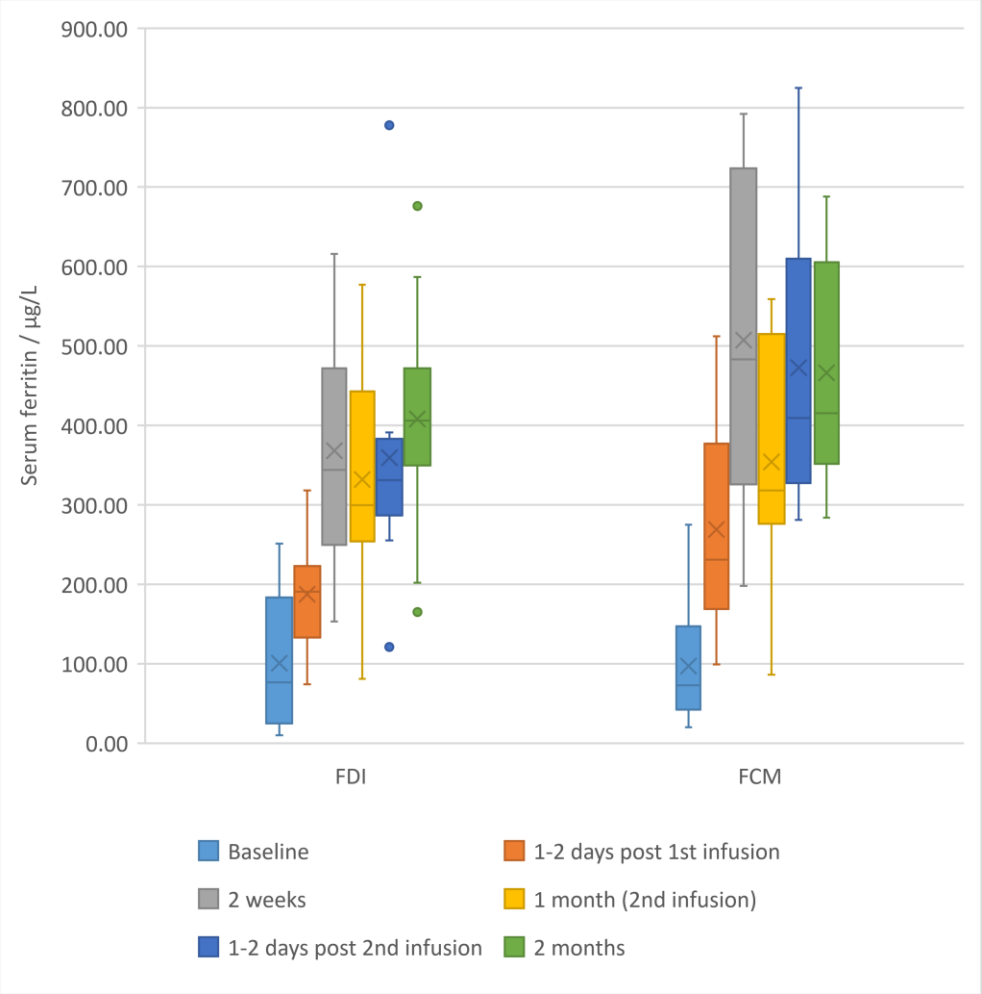

Figure 41: Serum ferritin concentrations

In terms of TSAT, in the FDI group the maximum median transferrin saturation was noted at visit 3 (100.0 (IQR: 9.0) %) and the minimum median transferrin saturation at baseline (15.0 (IQR: 10.0) %). In the

FCM group, the maximum median transferrin saturation was noted at visit 3 (100.0 (IQR: 12.0) %) and the minimum median concentration at baseline (14.5 (5.8) %). No statistically significant difference existed between the distribution of TSAT due to either preparation at any time point in the study. Two outliers were noted on visit 3, and one outlier at visits 5 and 7 in the FDI group as indicated in figure 42. One outlier existed at visit 6 in the FCM group.

There was a statistically significant difference in terms of TSAT within both the FDI and the FCM groups over time ($p<0.001$ respectively).

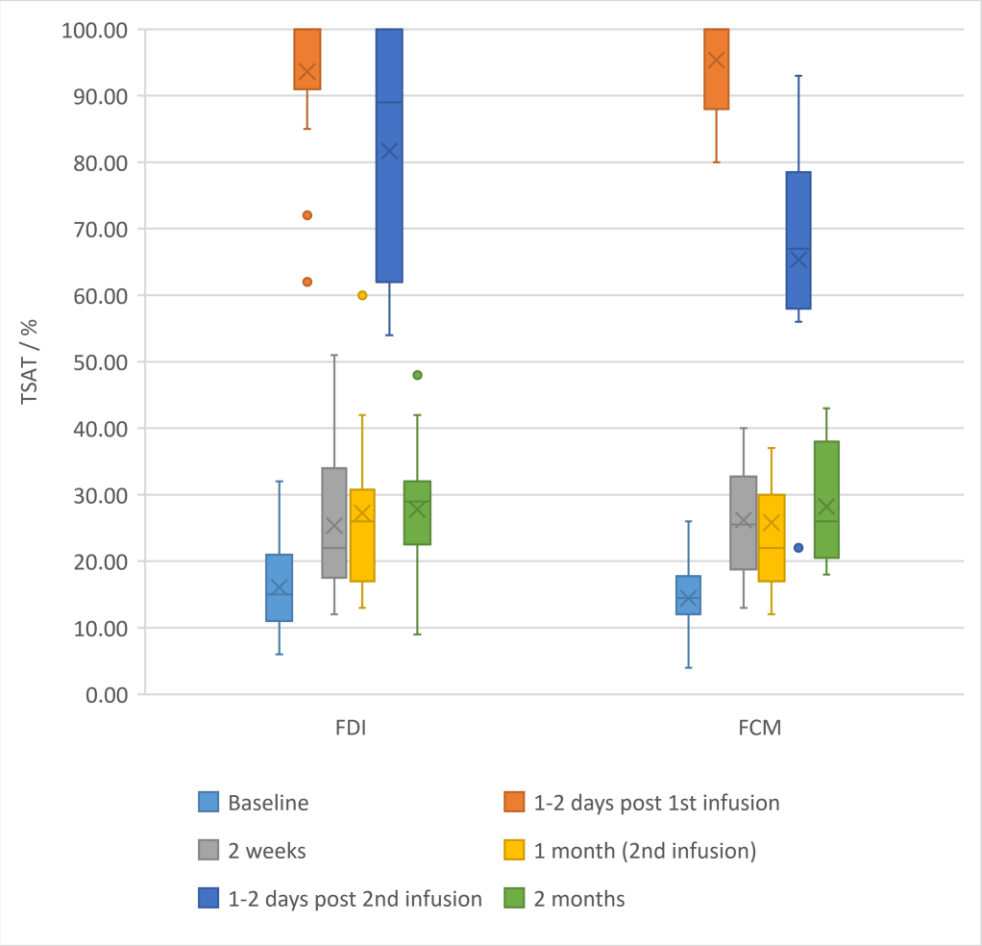

Figure 42: TSATs

3.6.2: Kidney function and injury

Serum creatinine, eGFR and urinary protein:creatinine ratio

The mean serum creatinine concentration in either group throughout the study is displayed in table 13 and as a line graph in figure 43. Median calculations of eGFR are displayed for each group in table 13 and as clustered boxplot in figure 44.

In terms of serum creatinine, in the FDI group, the maximum mean creatinine concentration was recorded at visit 7 (288.6 (SD: 100.9) µmol/L). The minimum mean creatinine concentration was noted at visit 5 (275.3 (SD: 95.1) µmol/L). In the FCM group the maximum mean creatinine concentration was recorded at visit 7 (271.0 (SD: 65.9) µmol/L) and the minimum mean concentration at visit 5 (252.4 (SD: 72.4) µmol/L). No statistically significant difference existed between the two groups at any time point of the study.

No statistically significant difference within the FDI group nor within the FCM group was noted.

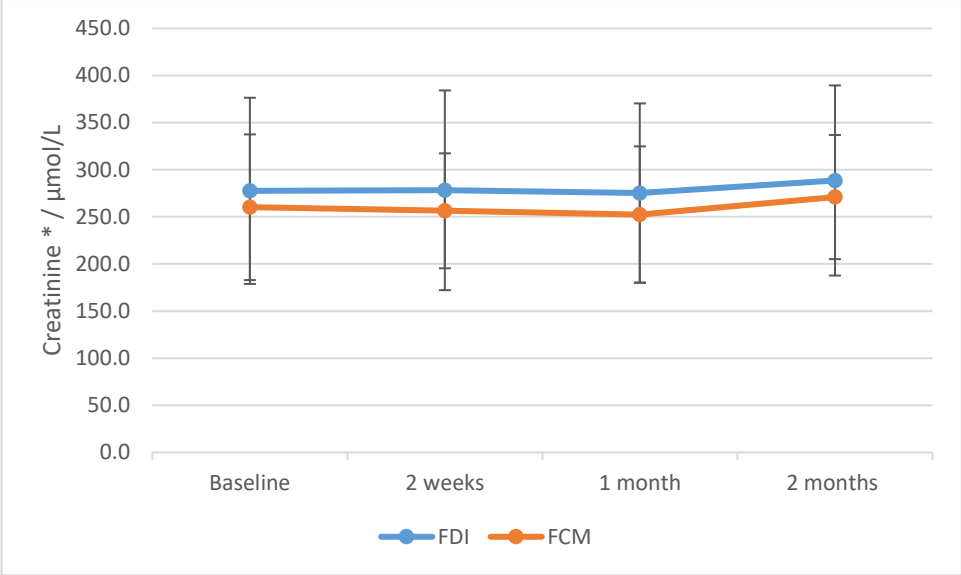

Figure 43: Serum creatinine concentrations

In terms of eGFR, in the FDI group the maximum median eGFR was recorded at visit 4 (21.0 (IQR: 12.5) ml/min/1.73 m^2). The minimum median eGFR calculated was noted at baseline (18.0 (IQR: 11.3) ml/min/1.73 m^2). In the FCM group the maximum median eGFR calculated was recorded at visit 5 (19.0 (IQR: 14.0) ml/min/1.73 m^2) and the minimum median calculation at visit 7 (16.5 (IQR: 8.5) ml/min/1.73 m^2). No statistically significant difference existed between the two groups at any time point of the study. One outlier existed in the FDI group on visit 5, while one outlier existed in the FCM group at baseline and visit 7.

No statistically significant difference between eGFR values were noted at any point of the study within each group.

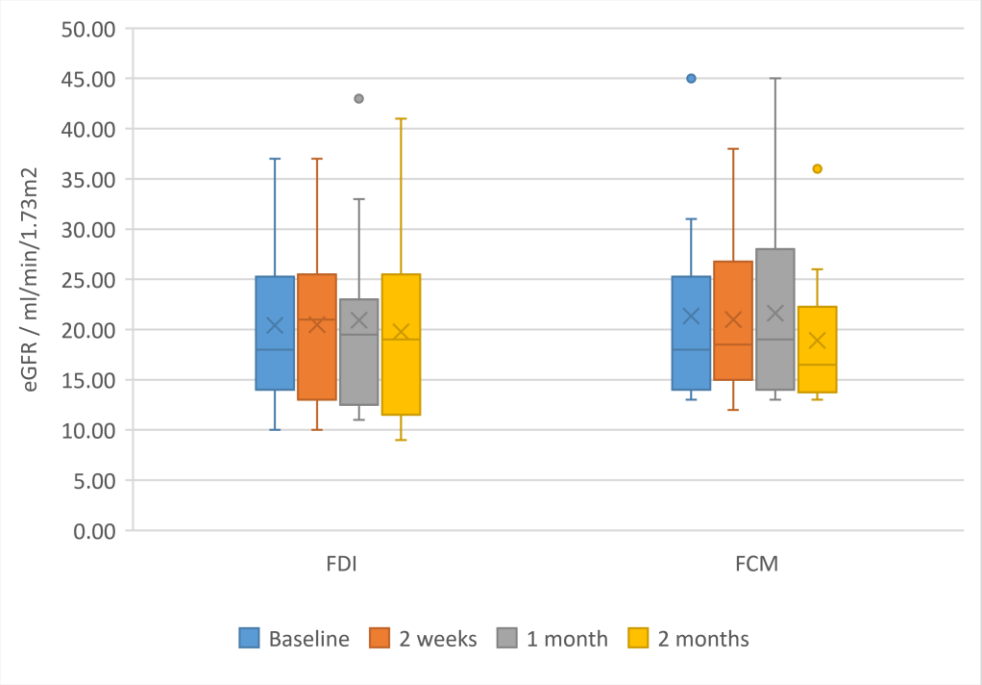

Figure 44: eGFR

Median urinary PCR are displayed for each group in table 13 and as clustered boxplot in figure 45 In the FDI group, maximum median urinary PCR was noted at visit 7 (157.5 (IQR: 531.3) mg/mmol), and the minimum was noted at visit 4 (85.0 (IQR: 265.0) mg/mmol). In the FCM group the maximum median urinary PCR was noted at visit 7 (35.0 (IQR: 173.8) mg/mmol) and the minimum was noted at visit 4 (20.0 (IQR: 92.5) mg/mmol).No statistically significant difference existed between the distribution of urinary PCR between the two groups at any time point on the study. One outlier existed in the FDI group at visits 4 and 7, whereas one outlier existed in the FCM group at visit 5, as indicated in figure 45.

No statistically significant difference within the FDI group nor within the FCM group was noted.

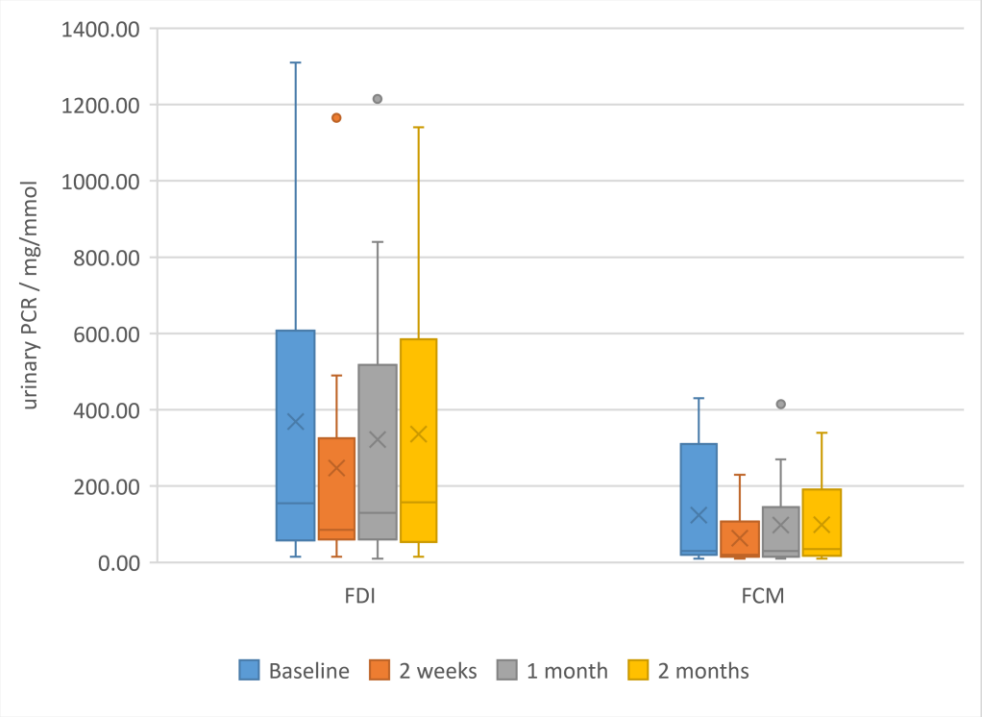

Figure 45: Urinary PCR

3.6.3: Inflammation

CRP

Median CRP concentrations are displayed for each group in table 13 and as clustered boxplot in figure 46. In the FDI group, maximum median CRP concentration was noted at visit 7 (8.6 (IQR: 27.9) mg/L), and the minimum was noted at visit 4 (7.7 (IQR: 20.6) mg/L). In the FCM group the maximum median CRP concentration was noted at visit 4 (6.4 (IQR: 18.1) mg/L) and the minimum was noted at baseline (4.3 (IQR: 9.9) mg/L). No statistically significant difference existed between the distribution of CRP concentration between the two groups at any time point on the study. One outlier existed in the FDI group throughout the study, whereas one outlier existed in the FCM group at visits 4, 5 and 6 as indicated in figure 46.

No statistically significant difference within the FDI group nor within the FCM group was noted.

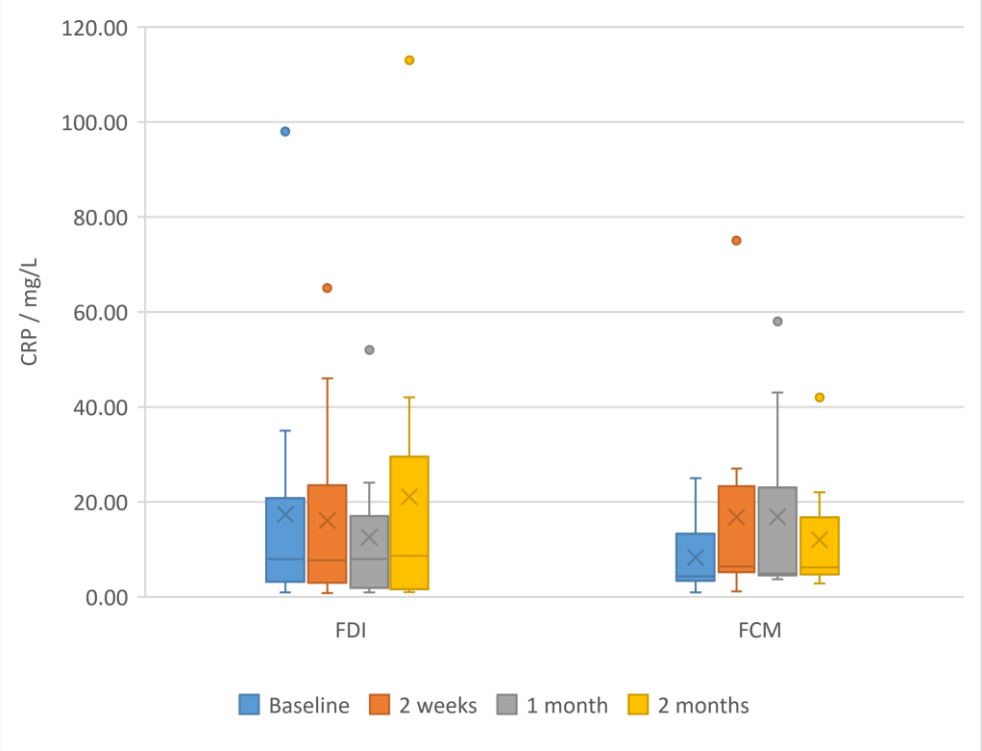

Figure 46: CRP concentrations

3.7: Mechanistic aspect of trial: cardiac markers, electrocardiography and pulse wave velocity - cumulative effect of iron and between group comparisons

3.7.1: Cardiac markers

NT-proBNP

As a whole-group analysis the median NT-proBNP concentration was at maximum on visit 7 (1386.0 (IQR: 4360.0) ng/L and at minimum at baseline (875.0 (IQR: 3033.0) ng/L).The median concentrations of NT-proBNP for the whole population are displayed in table 14 and as clustered boxplot at figure 47.

In the FDI group, the maximum median concentration of NT-proBNP was recorded at visit 7 (1204.0 (IQR: 2342.0) ng/L) and the minimum at visit 5 (611.0 (1853.0) ng/L). In the FCM group, the maximum median concentration of NT-proBNP was recorded at visit 7 (2548.0 (IQR: 8197.0) ng/L) and the minimum at visit 8 (893.0 (IQR: 4607.0) ng/L).The median concentrations of NT-proBNP for each group at each time point are displayed in table 14 and as clustered boxplot at figure 47. There was no statistical difference in the distribution of NT-proBNP concentrations at each group at any given time point in the study. Outliers existed in both groups throughout the study as indicated in figure 47.

No statistically significant difference was noted between concentration of NT-proBNP at various points within each group, including total cohort, FDI group and FCM group.

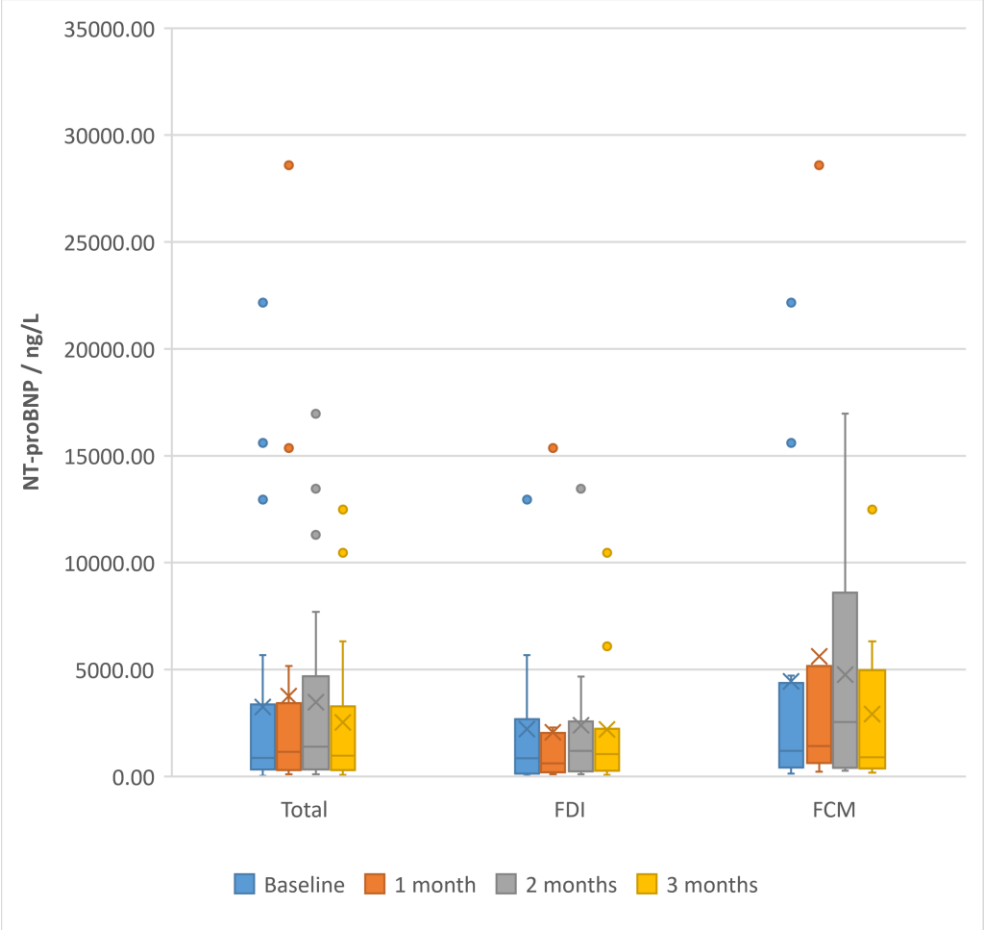

Figure 47: NT-proBNP concentrations

Table 14: NT-proBNP and electrocardiography

Visit	Iron group (n)	Median (IQR)	p-value	p- value (within group analysis)	Visit	Iron group (n)	Median (IQR)	p-value	p- value (within group analysis)
PR interval / ms					**QTc / ms**				
Baseline	Total (20)	172.0 (42.0)			Baseline	Total (25)	442.0 (36.0)		
	FDI (14)	172.0 (43.0)	0.547			FDI (14)	440.0 (25.0)	0.291	
	FCM (6)	173.0 (50.0)				FCM (11)	460.0 (84.0)		
Visit 3	Total (15)	166.0 (48.0)			Visit 3	Total (21)	442.0 (68.0)		
	FDI (11)	168.0 (48.0)	0.571			FDI (12)	439.5 (33.0)	0.219	
	FCM (4)	160.0 (57.0)				FCM (9)	483.0 (85.0)		
Visit 5	Total (15)	172.0 (26.0)			Visit 5	Total (20)	430.0 (53.0)		
	FDI (10)	173.0 (31.0)	0.679			FDI (10)	429.5 (15.0)	0.579	
	FCM (5)	164.0 (48.0)				FCM (10)	455.0 (77.0)		
Visit 7	Total (14)	171.0 (26.0)			Visit 7	Total (19)	426.0 (47.0)		
	FDI (9)	176.0 (24.0)	0.606			FDI (10)	423.0 (15.0)	0.156	
	FCM (5)	170.0 (38.0)				FCM (9)	465.0 (86.0)		
Visit 8	Total (13)	168.0 (28.0)		Total cohort: 0.026	Visit 8	Total (17)	438.0 (41.0)		Total cohort: 0.861

	FDI (8)	171.0 (25.0)	0.354	FDI: 0.116		FDI (8)	428.0 (36.0)	0.606	FDI: 0.458
	FCM (5)	158.0 (30.0)		FCM: 0.079		FCM (9)	444.0 (87.0)		FCM: 0.113
QRS / ms					**NT-proBNP / ng/L**				
Baseline	Total (25)	98.0 (37.0)			Baseline	Total (26)	875.5 (3033.0)		
	FDI (14)	92.0 (20.0)	0.0.18			FDI (14)	856.0 (2555.8)	0.347	
	FCM (11)	136.0 (66.0)				FCM (12)	1192.5 (3953.5)		
Visit 3	Total (21)	94.0 (39.0)			Visit 5	Total (23)	1156.0 (3132.0)		
	FDI (12)	92.0 (19.0)	0.041			FDI (12)	611.0 (1853.0)	0.091	
	FCM (9)	128.0 (71.0)				FCM (11)	1420.0 (4542.0)		
Visit 5	Total (20)	97.0 (40.0)			Visit 7	Total (22)	1386.0 (4360.0)		
	FDI (10)	93.0 (20.0)	0.063			FDI (12)	1204.0 (2342.0)	0.254	
	FCM (10)	115.0 (69.0)				FCM (10)	2548.0 (8197.0)		
Visit 7	Total (19)	98.0 (52.0)			Visit 8	Total (20)	967.0 (2976.0)		Total cohort: 0.626
	FDI (10)	90.0 (15.0)	0.156			FDI (11)	1041.0 (1962.0)	0.710	FDI: 0.921
	FCM (9)	138.0 (73.0)				FCM (9)	893.0 (4607.0)		FCM: 0.449
Visit 8	Total (17)	96.0 (53.0)		Total cohort: 0.422					
	FDI (8)	90.0 (15.0)	0.059	FDI: 0.351					
	FCM (9)	130.0 (89.0)		FCM: 0.400					

Troponin T

As a whole-group analysis the median Troponin T concentration was at maximum on visit 7 (40.5 (IQR: 40.3) ng/L) and at minimum at visit 5 following infusion of iron (31.5 (34.3) ng/L). The median concentrations of Troponin T for the whole population are displayed in table 15 and as clustered boxplot at figure 48.

In the FDI group, the maximum median concentration of Troponin T was recorded at baseline (pre) and visit 3 (28.0 (IQR: 53.5) ng/L and 28.0 (IQR: 52.8) ng/L respectively) and the minimum at visit 4 (19.0 (IQR: 31.0) ng/L). In the FCM group, the maximum median concentration of Troponin T was recorded at visit 7 (45.5 (IQR: 26.0) ng/L) and the minimum at visits 3 and 5 (post) (37.0 (IQR: 37.0) ng/L and 37.0 (IQR: 24.5) ng/L). The median concentrations of Troponin T for each group at each time point are displayed in table 15 and as clustered boxplot at figure 48. There was no statistical difference in the distribution of Troponin T concentrations at each group at any given time point in the study. Outliers existed in both groups as indicated in figure 48.

No statistically significant difference was noted between concentration of Troponin T at various points within each group, including total cohort, FDI group and FCM group.

Table 15: Troponin T concentrations				
Variable	**Iron group (n)**	**Median (IQR)**	**p-value**	**p- value (within group analysis)**
Baseline (pre)	Total (24)	36.5 (40.0)		
	FDI (13)	28.0 (53.5)	0.134	
	FCM (11)	41.0 (21.0)		
Baseline (post)	Total (22)	34.5 (43.0)		
	FDI (12)	27.0 (51.0)	0.228	
	FCM (10)	38.0 (27.8)		
Visit 3	Total (25)	35.0 (41.5)		
	FDI (14)	28.0 (52.8)	0.467	
	FCM (11)	37.0 (37.0)		
Visit 4	Total (23)	32.0 (36.0)		
	FDI (13)	19.0 (31.00	0.067	
	FCM (10)	40.0 (27.8)		
Visit 5 (pre)	Total (22)	37.0 (41.3)		
	FDI (11)	20.0 (53.0)	0.088	
	FCM (11)	43.0 (21.0)		
Visit 5 (post)	Total (18)	31.5 (34.3)		
	FDI (9)	19.0(29.5)	0.094	
	FCM (9)	37.0 (24.5)		
Visit 6	Total (19)	39.0 (38.0)		
	FDI (9)	25.0 (28.5)	0.053	
	FCM (10)	41.5 (27.5)		
Visit 7	Total (22)	40.5 (40.3)		
	FDI (12)	27.5 (42.3)	0.159	
	FCM (10)	45.5 (26.0)		
Visit 8	Total (19)	34.0 (49.0)		Within group - total: 0.171
	FDI (10)	26.0 (57.3)	0.211	Within group - FDI: 0.128
	FCM (9)	42.0 (51.5)		Within group - FCM: 0.097

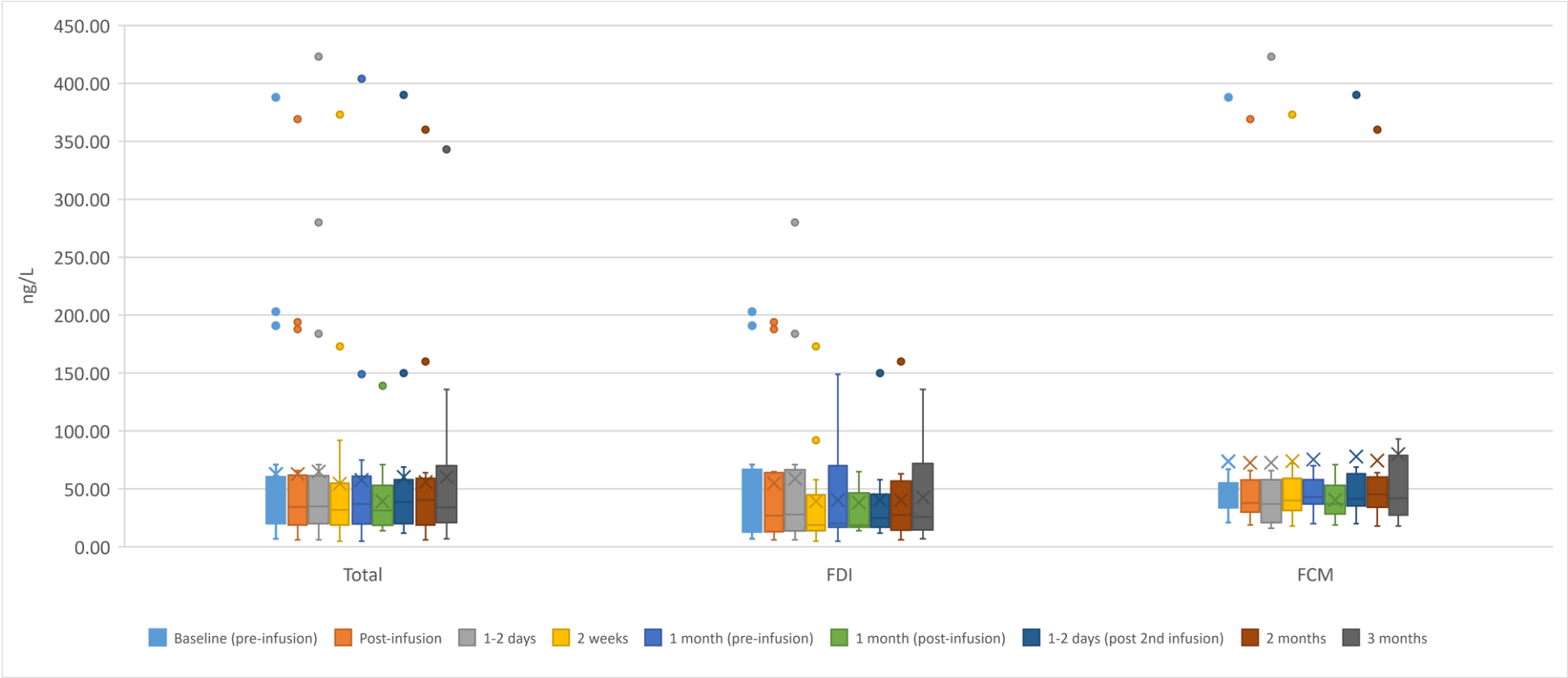

Figure 48: Troponin T concentration

3.7.2: Electrocardiography

At baseline 25 patients had ECGs, with 20 (80.0 %) participants in sinus rhythm, 3 (12.0%) with underlying paced rhythm, and 2 (8.0%) in atrial fibrillation. All patients in the FDI group were in sinus rhythm at baseline. Two patients with known paroxysmal atrial fibrillation that originally were in sinus rhythm at baseline, displayed atrial fibrillation at visit 3 and visits 3 and 5 respectively. Five patients in the FCM group were not in sinus rhythm at baseline; 3 patients had a paced rhythm due to permanent pacemaker device and 2 were in atrial fibrillation. Upon follow up one of the patients remained in atrial fibrillation, whilst the other reverted to sinus rhythm.

PR interval

The PR interval in the study could only be calculated in those in sinus rhythm. Table 14 displays the median values obtained in the whole-group analysis. The maximum median value obtained in the whole-group analysis was observed at baseline and visit 5 (172.0 (IQR: 42.0) ms and 172.0 (IQR: 26.0) ms respectively); the minimum median value obtained was observed at visit 3 (166.0 (IQR: 48.0) ms).

In terms of within group analysis and comparison between groups, the maximum median PR value in the FDI group was observed at visit 7 (176.0 (IQR: 24.0) ms) and the minimum at visit 3 (168.0 (IQR: 48.0) ms). The maximum median PR value in the FCM group was observed at baseline (173.0 (IQR: 50.0) ms) and the minimum at visit 8 ((158.0 (IQR: 30.0) m/s). No significant statistical difference between the distribution of results in the two populations was observed. Table 14

displays the median values obtained in the within and between group analysis.

A statistically significant difference was noted between PR intervals at various points of the study the FCM group (p=0.026). No statistically significant difference was noted within the FDI group nor the total cohort.

QRS interval

Table 14 displays the median values obtained in the whole-group analysis in terms of the QRS interval. The maximum median value obtained in the whole-group analysis was observed at baseline and visit 7 (98.0 (IQR: 37.0) ms and 98.0 (IQR: 52.0) ms respectively); the minimum median value obtained was observed at visit 3 (94.0 (IQR: 39.0) ms).

In terms of within group analysis and comparison between groups, the maximum median QRS value in the FDI group was observed at visit 5 (93.0 (IQR: 20.0) ms) and the minimum at visits 7 and 8 (90.0 (IQR: 15.0) ms and 90.0 (IQR: 15.0) ms). The maximum median QRS value in the FCM group was observed at visit 7 (138.0 (IQR: 73.0) ms) and the minimum at visit 5 (115.0 (IQR: 69.0) ms). A significant statistical difference between the distribution of values existed at baseline and visit 3 (Baseline: FDI: 92.0 (IQR: 20.0) ms vs. FCM: 136.0 (IQR: 66.0) ms; p= 0.018; Visit 3: FDI 92.0 (IQR: 19.0) ms vs. FCM: 128.0 (IQR: 71.0) ms; p=0.041). Table 14 displays the median values obtained in the within and between group analysis.

No statistically significant difference was noted between QRS intervals at various points within each group, including total cohort, FDI group and FCM group.

QTc interval

Table 14 displays the median values obtained in the whole-group analysis in terms of the QTc interval. The maximum median value obtained in the whole-group analysis was observed at baseline and visit 3 (442.0 (IQR: 36.0) ms and 442.0 (IQR: 68.0) ms); the minimum median value obtained was observed at visit 7 (426.0 (IQR: 47.0) ms).

In terms of within group analysis and comparison between groups, the maximum median QTc value in the FDI group was observed at baseline (440.0 (IQR: 25.0) ms) and the minimum at visits 7 (423.0 (IQR: 15.0) ms). The maximum median QTc value in the FCM group was observed at visit 3 (483.0 (85.0) ms) and the minimum at visit 8 (444.0 (IQR: 87.0) ms). No significant statistical difference between the distribution of results in the two populations was observed. Table 14 displays the median values obtained in the within and between group analysis.

No statistically significant difference was noted between QTc intervals at various points within each group, including total cohort, FDI group and FCM group.

3.7.3: Pulse wave velocity measurements

Table 16: Pulse wave velocity and Augmentation Index results

Visit	Iron group (n)	Median (IQR)	p-value	p- value (within group analysis)	Visit	Iron group (n)	Median (IQR)	p-value	p- value (within group analysis)
PWV (cf) / m/s					**AIx (ao) / %**				
Baseline	Total (23)	8.0 (2.6)			Baseline	Total (23)	26.0 (11.8)		
	FDI (12)	8.6 (2.6)	0.516			FDI (12)	23.4 (11.3)	0.283	
	FCM (11)	7.6 (2.7)				FCM (11)	28.8 (12.2)		
Visit 5	Total (19)	6.8 (2.5)			Visit 5	Total (19)	24.3 (11.8)		
	FDI (9)	7.1 (2.8)	0.624			FDI (9)	25.2 (13.3)	0.761	
	FCM (10)	6.5 (2.3)				FCM (10)	23.5 (11.0)		
Visit 7	Total (18)	6.2 (1.9)			Visit 7	Total (18)	20.4 (8.3)		
	FDI (8)	6.9 (1.6)	0.173			FDI (8)	19.8 (6.2)	0.781	
	FCM (10)	5.6 (1.8)				FCM (10)	20.9 (10.0)		
Visit 8	Total (17)	6.7 (1.9)		Total cohort : 0.303	Visit 8	Total (17)	22.2 (9.5)		Total cohort: 0.153
	FDI (9)	6.6 (1.9)	0.906	FDI: 0.491		FDI (9)	23.0 (9.1)	0.718	FDI: 0.878
	FCM (8)	6.7 (2.1)		FCM: 0.557		FCM (8)	21.3 (10.5)		FCM: 0.332

Two participants did not provide measurements of PWV throughout the study due to either false limb (n=1) or severe lymphoedema (n=1). Pulse wave velocity and AIx(ao) measurements exhibited normal distribution. Table 16 displays the mean measurements of pulse wave velocity and augmentation index as a whole-group analysis. Figures 49 and 50, respectively, display line graphs associated with whole-group analysis of PWV(cf) and AIx(ao) respectively. In the whole-group analysis mean PWV(cf) was maximum at baseline (8.0 (SD: 2.6) m/s) and minimum at visit 7 (6.2 (SD: 1.9) m/s). In the whole-group analysis mean AIx(ao) was maximum at baseline (26.0 (SD: 11.8) %) and minimum at visit 7 (20.4 (SD: 8.3) %).

In terms of within group analysis, maximum mean PWV(cf) measurement was noted at baseline (8.4 (SD: 2.6) m/s) for the FDI group, and minimum at visit 8 (6.6 (SD: 1.9) m/s). In the FCM group, the maximum mean PWV (cf) was recorded at baseline (7.6 (SD: 2.7) m/s) and the minimum at visit 7 (5.6 (SD: 1.8) m/s). In terms of AIx (ao), in the FDI group the maximum mean measurement was recorded at visit 5 (25.2 (SD: 13.3) %), whereas the minimum mean measurement was noted at visit 7 (19.8 (SD: 6.2) %). In the FCM group, the maximum mean measurement of AIx(ao) was recorded at baseline (28.8 (SD: 12.2) %), whereas the minimum mean measurement was noted at visit 7 (20.9 (SD: 10.0) %). There was no significant statistical difference at any point in the study between the measurements of PWV(cf) and AIx(ao) between the two groups. Table 16 displays the mean measurements of PWV(cf) and AIx(ao) for each group at any given point, whereas figures 49 and 50 display line graphs associated with the measurements.

No statistically significant difference was noted between PWV(cf) measurements intervals at various points within each group, including total cohort, FDI group and FCM group.

No statistically significant difference was noted between AIx(ao) measurements intervals at various points within each group, including total cohort, FDI group and FCM group.

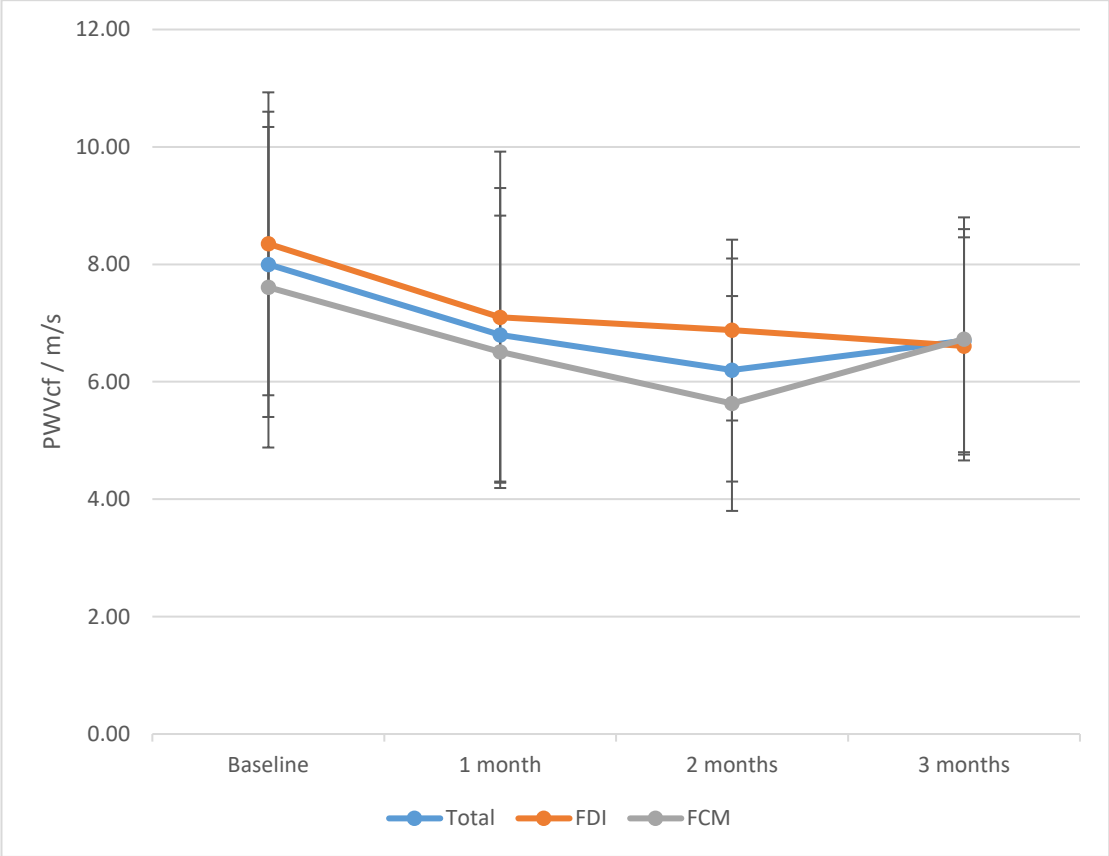

Figure 49: PWV (cf) measurements

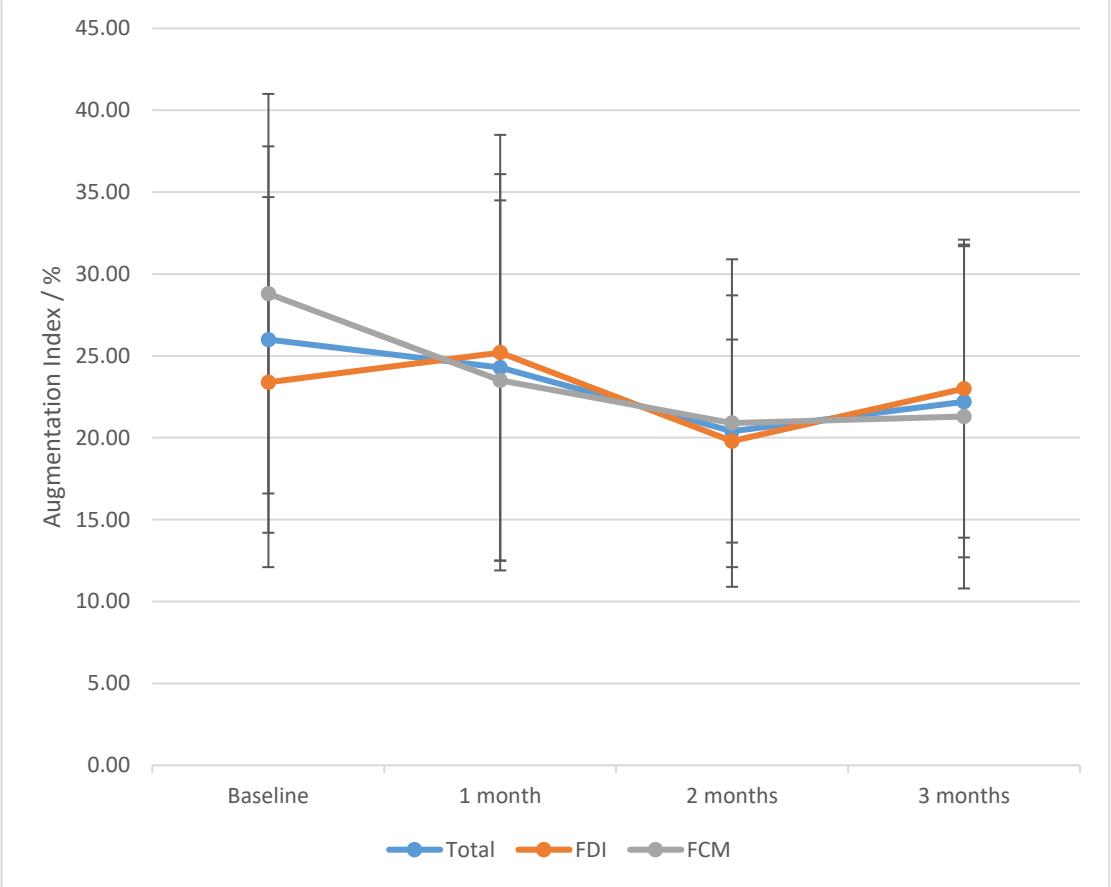

Figure 50: AIx (ao) measurements

3.8: Safety

There were SAEs in 6 (23.1%) patients (8 in total – 2 in FCM group, 6 in FDI group); these involved one death following intestinal perforation (table 17). All of the SAEs were adjudicated as not related to the investigational medicinal products medications review by the relevant bodies. There were 19 AEs recorded in the study (table 17). Serious adverse events and/or AEs took place in 20 patients (FDI: 12; FCM: 8). Most adverse events recorded were relevant to infection (FDI: 5; FCM: 5). There were 4 potential adverse reactions in the FDI group and 1 in the FCM group. There were no infusion-associated reactions throughout the study

During the study, 3 patients (11.5%) were initiated on dialysis all in the FDI group: two were unplanned and decision for renal replacement therapy was following hospitalisation. The remaining other one was due to long-term dialysis initiation plan.

There were 65 protocol deviations. Commonest protocol deviations included not attendance (n=16; 24.6%), no performance of 1-minute-sit-to-stand test (n=12; 18.5%) and no provision of urinary samples (n=14; 21.5%). Other causes for protocol deviations included non-performance of ECG, non-performance of pulse-wave-velocity measurement, no troponin collection and visit falling outside visit window.

Table 17: Safety profile

Adverse event	**Medication**	**SAE**
Infected liver cyst (leading to dialysis)	FDI	x
Atrial fibrillation	FDI	x
Vacant episode	FDI	x
Dialysis Initiation	FDI	x
Hyponatraemia/hypokalaemia	FDI	x
Infected rectal stump	FDI	x
Acute cholecystitis	FCM	x
Acute intestinal perforation	FCM	x
Nausea	FDI	
Diarrhoea	FDI	
Initiation of dialysis	FDI	
Urinary tract infection	FDI	
Lower limb cellulitis	FDI	
Gout	FDI	
Mild hypophosphatemia	FDI	
Urinary tract infection	FDI	
Asymptomatic rise in CRP	FDI	

Presyncope	FDI	
Neck stiffness/pain	FDI	
Pleurisy	FCM	
Urinary tract infection	FCM	
Otitis media	FCM	
Mild hypophosphataemia	FCM	
Urinary tract infection	FCM	
Urinary tract infection	FCM	
Cellulitis	FCM	
Gout	FCM	
Total SAEs: 8 in 6 patients (FDI: 4; FCM: 2) Total AEs: 27 in 18 patients (FDI: 12; FCM: 8) All SAEs required hospitalisation		

4: Discussion

The study “Iron and Phosphaturia – ExplorIRON-CKD” was an exploratory double-blind RCT that took place in a large tertiary centre in the United Kingdom. It was designed to primarily assess the differential effect of FCM and FDI in patients with ND-CKD on serum concentration of intact FGF23 and phosphate. The study also assessed the potential consequences on markers of the 6H syndrome (***h***igh iFGF23 leading to ***h***yperphosphaturic ***h***ypophosphatemia causing ***h***ypovitaminosis D, ***h***ypocalcemia and secondary ***h***yperparathyroidism) namely serum vitamin D and its metabolites, calcium, parathyroid hormone, and urinary phosphate. In addition, as secondary outcomes we monitored for signals associated with distortions of bone turnover and explored trends relevant to patient reported outcome measures and functional status, and variables of clinical relevance such as haematological and kidney response, and cardiovascular markers. The study was set up to aid signal detection, trend identification and hypothesis generation, including areas of research that have been identified as important by Kidney Research U.K and Kidney Disease U.K, KDIGO and Kidney Disease Outcomes Quality Initiative (367,407,408).

Prior to embarking on a further discussion of the results and their significance, it is important to focus on our baseline cohort. At baseline, the two groups were comparable in the majority of variables based on statistical analysis with the exception of age, urinary phosphate excreted (but not fractional excretion), fatigue severity scale total score and heart failure incidence. Patients receiving FDI were younger and had a higher urinary phosphate concentration than their counterparts, while also reporting significantly worse fatigue status. In terms of co-morbidities, the FCM group had a significantly greater incidence of heart failure when compared to FDI at baseline. It is imperative to

mention that in terms of primary outcome (iFGF23 and phosphate) there was no significant difference noted. Additionally in terms of other outcomes of concern (bone markers, cardiovascular markers) and other measurements that can affect the concentration of iFGF23 and phosphate, such as kidney function, inflammation and anaemia status, no significant difference existed at baseline.

4.1: Intact fibroblast growth factor 23 and phosphate concentration

4.1.1: Intact FGF23 concentration

At baseline, iFGF23 was above the normal reference range in both FDI and FCM groups (median (IQR): FDI: 257.3 (448.4) pg/ml, FCM: 186.5 (83.0) pg/ml; assay normal reference range: 28-121 pg/ml), with no statistical difference between them. As the study focused on patients with CKD, the results displayed are in accordance with previous evidence relevant to CKD, representing a condition of increased total FGF23 and iFGF23, potentially secondary to disorders in the transcription and post-translational modification (285,293,409). Indeed, prior work indicates that iFGF23 concentrations appear to increase exponentially with decreasing eGFR (238,292,293). This is further suggested in the present trial through the significant inverse correlation between eGFR and iFGF23 at baseline. Additionally, a significant correlation existed between iFGF23 and haemoglobin; previous studies have verified an inverse correlation between anaemia and iFGF23 however, it is not prudent to comment on a cause-effect given the intricate relationship between anaemia pathogenesis and iFGF23 (410). Additionally, despite conflicting results in terms of phosphate-iFGF23 correlation analyses in literature, a positive

correlation is mostly reported (285,411,412); in the present study a significant positive correlation was noted. An inverse correlation between 1,25 (OH)2 Vitamin D and iFGF23 was noted at baseline, which is in agreement with literature relevant to CKD. The correlations discussed are important as they verify that our sample, despite its small size, was behaving at baseline in accordance with previously published results (285).

Intact FGF23 concentrations remained close to baseline concentration in the FDI group throughout the trial; this finding was not present in the FCM group as a maximum increase was noted at visit 3 and visit 6 (both 1-2 days following administration of first and second intravenous iron dose respectively), causing a statistically significant greater %change when compared to FDI. In both cases, the concentration of iFGF23 following administration of FCM more than doubled. The primary outcome of the study, the %change from baseline to 1-2 days post-infusion was significantly greater in FCM group when compared to the FDI group, however the %change in iFGF23 from baseline to 2 weeks post-infusion was not significantly different. The concentration of iFGF23 in the FCM group remained higher than baseline in all visits following initial administration. This increase was statistically significant within the FCM group.

In comparison to previous RCTs exploring the differential effect of the use of modern intravenous iron products on phosphate (PHOSPHARE-IDA, HOMe aFers, PHOSPHARE-IBD and nested FIRM sub-study - FCM vs.FDI and FCM vs. ferumoxytol), baseline iFGF23 was higher (155,157,162,163). This may be representative of the lower median eGFR of the participants at baseline and underline a potential FGF23 metabolic discrepancy already present secondary to CKD. An important consideration is that different assays were used in each trial and inclusion criteria varied, as did treatment regimes,

therefore potentially introducing heterogeneity and variability in the results. More specifically in PHOSPHARE-IDA patients received either a single dose of 1000 mg of FDI or 2 doses of 750 mg FCM, a single dose of 1000 mg of either FCM or FDI in HOMe aFers and a single dose of either 510 mg of ferumoxytol or 750 mg of FCM in the FIRM-RCT (155,162,163). The comparative PHOSPHARE-IBD followed a treatment regime similar to that of the present trial (157).

Ferric carboxymaltose treated individuals in our cohort displayed an increasing trend in iFGF23 that was similar to that reported in the aforementioned studies (Section 1.3), with a large increase swiftly following administration of intravenous product, something not exhibited in the FDI group. The %change demonstrated was comparable to that previously reported in PHOSPHARE-IDA and FIRM-RCT within the first week of administration (ExplorIRON-CKD %change within 2 days: 146.1% (visit 3) and 235.1% (visit 6); PHOSPHARE-IDA within 2 days: 98.4% (following first administration), 282.2% (following second administration), FIRM-RCT within 1 week: 114.4% (121.5% in patients with defined CKD). Indeed, upon comparison of FCM to other modern intravenous iron preparations (either FDI or ferumoxytol), the %change was significantly higher following administration of FCM within one week (155,162,163). In addition, like the HOMe aFers trial, a significant trend of increased iFGF23 was demonstrated throughout the duration of the study (ExplorIRON-CKD – FCM within group analysis: $p<0.001$; HOMe aFers – FCM within group analysis: $p<0.001$). Moreover, participants in ExplorIRON-CKD displayed a similar pattern to those in PHOSPHARE-IDA following administration of the second dose of intravenous iron (ExplorIRON-CKD: 1-day post-administration: 235.1%; PHOSPHARE-IDA: 282.2%) which in both cases was more than double the original %change. This may suggest an additive effect within a primed system, and warrants further investigation, especially in patients receiving cumulative doses (162). However, it is important

to note that such an effect was not witnessed in PHOSPHARE-IBD, whereby a second administration of FCM was not associated with a greater increase compared to the one caused initially, although follow-up after administration was restricted to 7 days after second infusion (157).

In the participants receiving FDI there was no significant change in iFGF23 concentration throughout the study, akin to the results previously displayed by Emrich and colleagues, Wolf and colleagues and Zoller and colleagues (157,162,163). Similar to those studies, throughout a period of one month following intravenous FDI there was no increase in iFGF23, with the results being close to baseline. Nonetheless, in ExplorIRON-CKD a higher than baseline value was noted 2 months following initial administration – this may reflect the fact that 2 patients receiving FDI were on dialysis, which represents a state of increased iFGF23.

The results displayed by the FCM group are similar to other observational studies and RCTs including participants with various comorbidities ranging from patients with iron deficiency anaemia of unspecified cause, to bariatric patients and patients with heart failure (178,211,213,217,218). It is important however to compare our results with studies including or specific to patients with CKD. Such studies have been performed in patients with HD-CKD and ND-CKD. In an observational controlled trial which included 25 patients with CKD (median eGFR: 32 ml/min/1.73 m^2) that received 1000 mg of FCM, iFGF23 increased by 248% by day 2 to return to baseline levels by day 42, in a trend similar to that displayed in ExplorIRON-CKD (218). The exploratory IRON-Turtle trial in patients with heart failure and iron deficiency also found similar results in the population with ND-CKD (defined as eGFR <60ml/min/1.73 m^2) (n=12) following a single dose of 1000 mg of FCM (217). In HD-CKD, a different trend has been

noted; in 22 patients receiving haemodialysis a single 200 mg dose of FCM was administered (178). Median iFGF23 decreased from 843 (IQR: 313-1,922) pg/ml to 570 (IQR: 253-1,264) pg/ml by day 2 to return closer to (but lower than) baseline by day 42, therefore exhibiting an opposing trend to that indicated in ExplorIRON-CKD, other RCTs and observational studies (178). These results, however, do not necessarily contradict the status of FCM-induced FGF23 metabolic changes in the general population; as the authors report, their results may be secondary to the anuric status of the patients, haemodialysis, residual urinary phosphate output and iron status. Iron deficiency anaemia itself represents a state of increased transcription of iFGF23 matched by adequate cleavage to non-biologically active FGF23. As worsening kidney function can derange cleavage and excretion of FGF23, leading to a greater proportion of circulating iFGF23, it is possible that in anuric states resolution of iron deficiency leads to decreasing overall production of iFGF23, explaining the discrepancies shown in haemodialysis patients but not in ND-CKD individuals.

Given the evidence of increase in iFGF23 in the participants of this study correlating with previous results pertaining to the use of FCM and other similar iron products, it is important to discuss whether this change led to hypophosphataemic effects.

4.1.2: Serum phosphate concentration

At baseline serum phosphate concentration in either group was within the normal reference range (median (IQR): FDI: 1.30 (0.43) mmol/l, FCM: 1.20 (0.31) mmol/l; normal reference range: 0.80 – 1.50 mmol/l) despite advanced CKD. The prevalence of hyperphosphataemia increases with reduced kidney function, affecting up to 40% of patients

with an eGFR < 20 ml/min/1.73 m^2. However, incidence in this cohort of phosphate > 1.50 mmol/L was 11.5% (3 in the FDI group and 0 in the FCM group) (413). Baseline phosphate as predicted through literature review had an inverse correlation with eGFR (285).

A decrease in serum phosphate concentration in both groups following administration of iron has been recorded in most studies. In “Iron and Phosphaturia – ExplorIRON-CKD” %change was higher throughout the study in the FCM group compared to the FDI group, reaching significance at visit 6 (p=0.013). In addition, this was highlighted in the difference between the serum concentrations of phosphate at visit 4, with FCM having a significantly lower concentration than FDI. However, the decreasing trend exhibited in the FCM group did not reach statistical significance. The trends witnessed in the FDI group, did not reach statistical significance either.

As displayed in Appendix table 9, FCM has been linked with a greater hypophosphataemia incidence and a reduction trend which is larger than its comparators. Focusing specifically on RCTs comparing modern intravenous iron compounds (FCM vs. FDI and FCM vs. ferumoxytol), one could notice that nadir phosphate concentrations following FCM took place around 2-weeks following administration. The current ExplorIRON-CKD trial shares a similar trend in reduction following first administration with low concentration recorded at visit 4 (2 weeks following administration); nadir concentrations were recorded following the second administration, potentially highlighting an additive effect of FCM, which was not explored in PHOSPHARE-IDA, HOMe aFers or FIRM-RCT (162,163,168). The recently completed similarly designed PHOSPHARE-IBD on the other hand, identified a decreasing phosphate reaching nadir 1 week following administration irrespective of administration order (first or second) (157). In these four studies, an increase in iFGF23 predated the

decrease in phosphate concentration. However, the rate and significance of change were smaller in this population than what was previously witnessed in patients with higher kidney function. In our cohort the median nadir concentration of phosphate was 1.09 (0.29) mmol/L, which is comparable to the reported mean nadir concentration of phosphate reported in studies of FCM in patients with ND-CKD according to a meta-analysis by Schaefer and colleagues (1.11 mmol/L – 95% CI: 0.96-1.27) (414).

In order to appreciate the rate of change displayed in this study it is important to examine studies monitoring phosphate in patients with ND-CKD receiving FCM. Huang and colleagues reported that a 23.0% reduction was noted within one week of administration of 1000 mg of FCM (218). During the IRON-Turtle study a 20.0% decrease in phosphate was noted (217). Equally, a significant decrease in mean serum phosphate was witnessed in an observational study comprising of 47 patients with ND-CKD (mean eGFR: 26.1 ml/min/1.73m^2), with lower phosphate concentration noted by the end of study (week 12) (226). Moreover, in a RCT comparing FCM with oral iron in ND-CKD patients (FCM n=204), a mean decrease of 0.49 mmol/L was noted.The duration of symptoms, when these took place and the cohort kidney function alongside baseline phosphate concentration were not stated in the study (185). The REPAIR-IDA RCT comparing FCM and iron sucrose, also highlighted a greater decrease with FCM (n=1,276; mean eGFR: 32.5 ml/min/1.73 m^2) with a mean decrease in phosphate from baseline to nadir of - 0.41 mmol/L (-30.8%) and -0.16 mmol/L (-12.0%) from baseline to end of study (182). In the current study, we found a median decrease of -11.0% 2 weeks following administration of intravenous iron at the same dose, and the trend exhibited was not significant. These discrepancies in the rate of change and significance may be explained through a number of reasons. These include the difference in time of sample obtained (indicating a possibility for nadir phosphate concentration occurring

earlier than 2 weeks), a lower eGFR and worse kidney function at baseline, and a higher baseline fractional excretion of phosphate when compared to the above-mentioned trials (ExplorIRON-CKD: FEPi: median (IQR): 36.4 (21.6)%, Huang et al: FEPi: mean (SD): 26.9 (10.9)%, Stohr et al: mean FEPi (SD): 30.9 (11.8)%; not reported in other studies) (217,218). The impact of decreasing eGFR on phosphate change can be appreciated through reviewing the results of a meta-analysis on the impact of FCM on phosphate in 42 studies – mean change in serum phosphate in patients with ND-CKD was estimated at -0.38 mmol/L, whereas mean change in patients with normal kidney function was estimated at -0.42 mmol/L (414). As such, the reason behind the lower %change documented in the present trial may be indeed the lower kidney function of the participants.

Previous evidence pertaining to HD-CKD also demonstrated a decrease in phosphate following administration of FCM (178). Specifically, 200 mg of FCM were found to be associated with a decrease in phosphate by day 2 (median (IQR): 1.53 mmol/L (1.14-1.71) to 1.37 mmol/L (1.05-1.67); p=0.03), however the overall trend within the study lasting for 42 days was non-significant (p=0.64) (178). These results may imply that FCM affects phosphate concentrations irrespective of kidney function; nonetheless, the grade of kidney dysfunction may alter the rate, duration and significance demonstrated.

Median serum phosphate concentration in participants treated with FDI also decreased initially during the study, however this normalised by the end of follow-up (2 months following initial administration of FDI). In PHOSPHARE-IDA, HOMe aFers and PHOSPHARE-IBD trials, a similar trend was seen, with normalisation of phosphate concentrations closer to baseline by day 35 (157,162,163). A review of observational evidence pertaining to the use of FDI in patients with

gastrointestinal disorders reveals that nadir serum phosphate concentration was noted within 2 weeks of administration, similar to the results displayed here. It appears that any serum phosphate change taking place in patients treated with FDI as opposed to those treated with FCM, is smaller and more short-lived, in agreement with published research, however there are differences regarding duration and magnitude of decrease in phosphate. This may be explained by the fact that the analysis performed was intention-to-treat. It is important to clarify that the results relevant to later stages of phosphate concentration in patients receiving FDI may be affected by the use of haemodialysis in two patients in the FDI group, causing discrepancies between the populations of other studies and the one in the current study.

There were no episodes of hypophosphataemia as defined by the protocol (phosphate <0.65 mmol/l) throughout the trial, irrespective of the arm. Serum definition of hypophosphataemia, however, varies in literature, with studies defining hypophosphataemia either as a serum phosphate <0.80 mmol/L (i.e., mild) or serum phosphate <0.64 mmol/L (i.e., moderate) (150). Taking into consideration the alternative definitions, there were two episodes of mild hypophosphataemia during the trial (serum phosphate <0.8 mmol/L), one in each group, recorded on visit 3 (1-2 days post-administration of first intravenous iron dose) that recovered within 14 days following administration. No clinical symptoms were identified.

Upon considering the effects of hypophosphataemia, it is important to take into account the treatment of resultant hypophosphataemia and the potential for failed infusions secondary to its occurrence. As demonstrated by Fragkos and colleagues, hypophosphataemia secondary to FCM may necessitate an average of 4.4 infusions per person of intravenous phosphate and a longer hospital stay in order to

be corrected in patients with gastrointestinal pathologies (199). Moreover, hypophosphataemia may lead to cessation of administration of intravenous iron that could potentially have other cost implications (415). In “Iron and Phosphaturia – ExplorIRON-CKD”, there was no failed administration of iron due to hypophosphataemia, and no treatment requirement arose for the two episodes of transient hypophosphataemia.

The incidence of hypophosphataemia associated with FCM has been reported as up to 92%, with a higher likelihood of hypophosphataemia and a greater decrease in serum phosphate following administration of FCM compared to FDI irrespective of kidney function (47.0% (95% CI 36.0-58.0) vs. 4.0% (95% CI 2.0-5.0), and 0.40 mmol/L vs. 0.06 mmol/L, respectively) (227,414). In a pooled analysis of results of CKD-associated studies, the rates of incidence of hypophosphataemia between FCM and FDI were 27% and 2% respectively (414). In the present study, despite a trend for greater phosphate decrease with FCM, no difference existed in incidence of hypophosphataemia. This may be explained by the point raised in the aforementioned pooled analysis - mean nadir phosphate concentration after FCM was 1.11 mmol/L (95% CI: 0.96-1.27) and 1.57 mmol/L (95% CI: 1.54-1.60) following FDI in patients with CKD, which are values well above the cut-off for hypophosphataemia. Indeed, the median (IQR) nadir values in the current study were 1.09 (0.29) mmol/L in the FCM allocated group and 1.18 (0.50) mmol/L in the FDI allocated group, which are above the cut-off for hypophosphataemia. It is important to mention that the incidence of hypophosphataemia following FDI was 1.64% which falls within the rate to be anticipated following FDI administration in patients with ND-CKD (2.0% (95% CI: 1.0 – 3.0%)) (414). Such results indicate that the potential hypophosphataemic effect demonstrated following the administration of FCM is attenuated in patients with CKD, but not following FDI, therefore giving ground to theories of kidney function affecting the potential effects of FCM on

FGF23. In order to examine this further, it is important to relate and discuss the results of the co-primary outcome and the analysis on urinary phosphate excretion.

4.1.3: Combined effect of intravenous iron administration on %change of iFGF23 and phosphate concentration

The causative mechanism behind decreased phosphate following administration of certain intravenous iron compounds and not others has not been confirmed. The most prevalent theory is that of an iatrogenic halt in cleavage of iFGF23, limiting its breakdown by certain carbohydrate moieties associated with some intravenous iron compounds, such as FCM (239). This cessation in cleavage leads to an increase in iFGF23, leading to the previously discussed effects of the 6H syndrome (416). A key point in the development of hypophosphataemia is phosphaturia secondary to the inhibition of the renal NaPi type 2 cotransporters at the proximal convoluted tubule precipitated by increasing iFGF23 (150). Evidence suggestive of this sequence of events arises from studies performed by Wolf and colleagues whereby an initial increase of 128.0% in iFGF23 within one day of administration of FCM predated nadir mean phosphate taking place on day 14 of the study (187). In the nested study of the FIRM trial comparing ferumoxytol and FCM, a rise in iFGF23 by day 14 led to an increase in FEPi (representing phosphaturia) and therefore a decrease in phosphate concentration by day 14 (155). This trend was also exhibited in the PHOSPHARE-IDA trials (162). These trends have not been exhibited through the use of other modern intravenous iron compounds.

Nonetheless, phosphate output is affected greatly by kidney function, and CKD represents a hyperphosphataemic state caused by

decreased kidney filtration, bone metabolism disruption and alteration in the synthesis of vitamin D (417). Intriguingly, FGF23 and PTH increase in an effort to counter-act these changes, leading to an increase in NaPi co-transporters at the kidney proximal convoluted tubule, causing relative phosphaturia with an increased FEPi. However, a downward signal by decreased 1,25 $(OH)_2$ Vitamin D, alongside loss in PTH metabolism, and the action of phosphate itself on co-transporters in the proximal convoluted tubule affect the action of FGF23, despite its increased concentration, and potentially diminish effective excretion of phosphate (417). These alterations in homeostasis, may lead to changes in the ability of increased iFGF23 to induce hypophosphataemia, especially in a differential fashion, when examining two modern intravenous iron compounds. This outcome explored the relative change in both variables (iFGF23 and phosphate) at relevant time points. Previous evidence was used to design this based on study of patients with CKD including both iFGF23 and phosphate with a similar dosing regime (Section 2.10) (217,218).

In Iron and Phosphaturia – ExplorIRON-CKD no participant developed an increase in iFGF23 >200% and a decrease in phosphate of > -20% within the first two weeks of administration of FDI, whereas one patient had an increase in iFGF23 > 200% at day 2 and a decrease in phosphate concentration of > 20% at week 2, following administration of FCM. Throughout the study, no participant in the FDI group developed an increase in iFGF23 > 200%, but three developed a decrease in phosphate of > 20%; whereas five participants receiving FCM developed a decrease in serum phosphate > 20%, and eight developed an increase in iFGF23 >200%. These results suggest that an element of the decrease in phosphate following iron administration may be related to mechanisms outside FGF23, especially in the case of FDI, whereby no increase >200% was noted. Additionally, not all patients with an increase in iFGF23 >200% had a decrease of >20% in phosphate concentration, which raises the possibility that an

iatrogenic rise in iFGF23 secondary to a differential effect exerted by different compounds may lead to other complications outside hypophosphataemia/phosphaturia.

4.1.4: Kidney phosphate excretion (assessed through the 24-hour urinary phosphate concentration and FEPi)

As research suggests, the hypophosphataemic effect of certain intravenous iron compounds appears to be iFGF23-driven, leading to increased renal phosphate loss. Marked increase in urinary excretion of phosphate and impairment in tubular reabsorption of phosphate were noted with saccharate ferric oxide and iron polymaltose in case reports (418,419). The decrease in tubular reabsorption of phosphate in the case of iron polymaltose was also paired with an increase in FGF23, therefore suggesting an FGF23 induced phosphaturic effect, as physiologically expected (419). This was later confirmed by a small observational study with iron polymaltose, whereby a significant increase in FGF23 within one week was associated with a significant decrease in tubular reabsorption of phosphate, and therefore greater phosphaturia (296). A similar mechanism has been suggested with FCM, as evident in a RCT comparing low molecular weight iron dextran to FCM (187). At baseline, FEPi in both groups was comparable and within expected range, a significant increase in FEPi within 1 week accompanied the increase in iFGF23 seen with FCM one day following infusion of the product. This increase in FEPi led to a decrease in serum phosphate, reaching the nadir concentration (mean decrease: 0.23 mmol/L) by day 14 following administration. This did not take place with low molecular weight iron dextran. Further RCTs focusing on modern intravenous iron compounds including ferumoxytol, FCM and FDI displayed similar findings (155,157,162,163). These five RCTs quantified phosphaturia through FEPi based on spot urine samples.

At baseline and throughout most of the study urinary phosphate concentration was below the normal range in the patients with FCM (baseline: 12.5 (7.5) mmol/24hr – normal reference range: 16-48 mmol/24hr); this was not exhibited in the participants that received FDI. Despite a significantly greater degree of 24-hour urinary phosphate concentration in the FDI group at baseline, the present study reports an increase in 24-hour urinary phosphate concentration reaching maximum at visit 4 in the FCM group, at which point there was no statistically significant difference between the two comparators. Indeed, median percentage change between baseline and visit 4 (2 weeks following infusion) was 32.0% in the FCM group, compared to 0.0% in the FDI group (p=0.107), therefore signalling a non-significant trend for greater urinary phosphate excretion following FCM administration.

Fractional excretion of phosphate represents the reabsorption ability of the kidney; it is a calculation of the fraction of the filtered phosphate by the glomerulus that is excreted as urine (420). It is considered normal when below 20%, however increases as filtration rate falls due to nephron damage to compensate for the decrease in eGFR and maintain phosphate balance (420–422). As previously mentioned, urinary phosphate excretion and FEPi are tightly regulated by both FGF23 and PTH, and comparative studies have noted increased FEPi secondary to a rise in a FGF23 following administration of FCM in patients with intact kidney function. In “Iron and Phosphaturia – ExplorIRON-CKD”, FEPi was raised in both groups at baseline, reflecting a degree of kidney dysfunction; there was no statistically significant difference between the two groups. A distinct trend was however noted, with FEPi steadily increasing in the FCM group throughout the study, whereas no impact was identified in the patients that received FDI. Similarly, there was a positive %change almost

throughout the study for patients receiving FCM, whereas the opposite trend was seen in those given FDI. In addition, the second administration of FCM prompted a larger %change compared to the first one within only one day (visit 2-3: 6.4% vs. visit 2-6: 13.0%), potentially reflecting an amplified effect due to repeated administrations. This trend mirrored those seen in PHOSPHARE-IDA and PHOSPHARE-IBD trials (157,162). Nonetheless, unlike the comparative studies in patients with normal kidney function, these changes did not reach statistical significance. This may reflect the impact of low glomerular filtration rate on FEPi and the possibility of FGF23 resistance at the proximal convoluted tubule secondary to CKD progression.

Evidence regarding phosphaturia following intravenous iron administration in patients with CKD is scarce, particularly in relation to the use of modern intravenous iron compounds. Huang and colleagues, through their observational study comparing the effect of FCM in patients with normal kidney function, pregnant individuals and patients with CKD, identified a significant phosphaturic effect within 7 days of administration of 1000 mg FCM in all populations irrespective of kidney function. This was alleviated by day 21 in patients with CKD (baseline FEPi: 26.9 (10.9) %; day 7: 38.0 (12.0) %); $p<0.001$) (218). An analogous tendency was also reported in the observational IRON-Turtle study, whereby in patients with CKD (n=12; mean eGFR: 28.2 (9.1) ml/min/m^2) FEPi increased from baseline of 30.9 (11.8) % to its maximum (42.2 (8.8) %) by day 14, returning closer to baseline by day 28 (217). In this study, however, the displayed trend did not reach significance.

A probable explanation regarding the absence of significance may rely on the degree of CKD progression, especially when compared to the current study. As Bricker suggested in the “intact nephron hypothesis”,

as the number of functioning nephrons is reduced, the remaining nephrons must adapt and further contribute to total renal excretion (423). In doing so, certain complications of CKD-MBD arise, characterised by an increase in both PTH and FGF23 (424). These initially account for the increase in FEPi exhibited in patients with CKD, however, contribute eventually to the development of FGF23 resistance. Resistance to FGF23 can arise due to Klotho deficiency secondary to uraemic toxins or direct tubular damage associated with albuminuria, a phenomenon of worsening CKD (425). Downregulation of Klotho has been exhibited in tubular cells in in vitro studies secondary to albumin, whilst FGF23 serum measurements have correlated with proteinuria (425,426). In advanced CKD, the FEPi is already high, and as a result, the ability to enhance phosphaturia further can be limited (422). In the current study both creatinine and FEPi at baseline were higher than previous trials. Moreover, another potential reason behind the absence of significance is that there was no measurement within one week of intravenous iron administration, which was identified in certain studies (but not all) as the period in which peak phosphaturia occurred.

The possible presence of FGF23 resistance in patients with CKD underlines the need to explore whether any iatrogenic increase in FGF23 amplifies this phenomenon, which has been identified to be related to CKD progression, mortality and cardiovascular events (427,428). In addition, the absence of significant phosphaturia does not necessarily amount to absence of trend. In the present study, an increase in FEPi was recorded following administration of FCM, potentially related to an attenuated iFGF23 effect. It is hence important to consider the potential effects of raised iFGF23 on other markers of the 6H syndrome, namely hyperparathyroidism, hypovitaminosis D and hypocalcaemia, which are important markers of CKD complications.

4.2: Vitamin D, calcium and parathyroid hormone

As was described in previous sections, the administration of certain intravenous iron compounds leads to the 6H syndrome characterised by a sequence of events secondary to high iFGF23. Highlighted above, there was an absence of significant hypophosphataemia or phosphaturia in the participants of the study, however significant trends were seen in terms of a differential effect on both iFGF23 and phosphate concentration secondary to FCM when compared to FDI. This chapter has so far focused on the differential effect of the two products on iFGF23 and phosphate, but it is now necessary to explore any effects in terms of the other components of the 6H syndrome, namely vitamin D, calcium and PTH.

4.2.1: Vitamin D

Vitamin D metabolism has been altered secondary to iatrogenic increase in FGF23 in previous studies following intravenous iron. As explained previously, iFGF23 leads to inhibition of 25(OH) vitamin D3 1a-hydroxylase and stimulates 24-hydroxylase, therefore decreasing the hydroxylation of $25(OH)_2$ vitamin D to $1,25(OH)_2$ Vitamin D (active form) and increasing the degradation of both precursors and active form to $24(R),25(OH)_2$ Vitamin D products. In comparative RCTs of modern intravenous iron compounds (FCM vs. FDI and FCM vs. ferumoxytol) a decrease in $1,25\ (OH)_2$ Vitamin D has been found that was more pronounced following FCM administration (155,157,162,163). Where FDI was the comparator, a smaller and more transient decrease in $1,25\ (OH)_2$ Vitamin D was also seen, but this was not observed with ferumoxytol (155,162,163). In terms of

metabolites, both Wolf and colleagues and Emrich and colleagues explored the differential trends produced by FCM and FDI with conflicting results. In PHOSPHARE-IDA FCM produced a significantly greater increase in both $25(OH)_2$ Vitamin D and $24(R),25(OH)_2$ Vitamin D, whereas in the smaller HOMe aFers trial FDI caused a significant increase in $25(OH)_2$ Vitamin D, with no resultant significant differential effect identified (162,163). In addition in PHOSPHARE-IBD, a significantly greater increase in both $25(OH)_2$ Vitamin D and $24(R),25(OH)_2$ Vitamin D following FCM was witnessed (157).

In the current study a statistically significant differential effect was noted in terms of change in $1,25\ (OH)_2$ Vitamin D associated with FCM when compared to FDI. This was seen between baseline and visits 3, and 6, highlighting a significantly greater decrease noted with FCM when compared to FDI. This was also reflected by the absolute values in $1,25\ (OH)_2$ with no significant trend identified in the group given FDI, whereas a significant trend was seen in those receiving FCM. Nadir $1,25\ (OH)_2$ Vitamin D concentration in the FCM group was seen in visit 6, potentially suggesting an additive effect of repeat infusion. Additionally, a significant intergroup change was seen following administration of FCM ($p=0.026$), but not in the FDI group, which is also indicative of a differential effect related to FCM compared to FDI in patients with ND-CKD.

There were no trends noted for Vitamin D metabolites $25(OH)_2$ Vitamin D and $24(R),25\ (OH)_2$ Vitamin D. No differential effect was identified between the two treatments in terms of either absolute values or change caused, whilst no significant intergroup trends were seen. There was no alignment between minimum and maximum values of $1,25\ (OH)_2$ Vitamin D and the metabolites, however this may be due to the difference in distribution of values between the three variables.

The results observed in the current study are congruent with the results discussed in Appendix table 13 , whereby FCM and other similar compounds appear to produce a significant decrease in 1,25 $(OH)_2$ Vitamin D. The results discussed are consistent with reports encompassing the CKD spectrum, involving patients with ND-CKD and HD-CKD individuals. These changes in 1,25 $(OH)_2$ vitamin D within the FCM group were related to iFGF23 as noted by the coinciding significant difference in differential effect at baseline and visit 3 and baseline and visit 6 between FCM and FDI. The amplified effect on 1,25 $(OH)_2$ Vitamin D upon second infusion is a novel finding that warrants further research, especially given the increased risk of mortality and morbidity that reduced vitamin D confers in patients with CKD (334). Moreover, the decrease in active vitamin D appears to be one of the drivers behind CKD-MBD, leading to hypocalcaemia and secondary hyperparathyroidism (429). The absence of a significant FDI-induced effect may indicate that any transient, small and non-significant hypophosphataemia associated with FDI does not appear to operate on the bone axis or be related with the hormonal control of mineral metabolism.

The changes in 1,25 $(OH)_2$ Vitamin D did not necessarily relate to either the storage or degradation products of vitamin D, despite our knowledge of enzymatic induction secondary to an increase in iFGF23. This is a feature in a number of studies, and one of the potential reasons why it is important to always consider that active circulating vitamin D is usually far less than both its storage and degradation products. It is possible that the inhibitory competing effect that 1,25$(OH)_2$ Vitamin D has on 24-hydroxylase may obscure the iatrogenically induced iFGF23 changes. The chronically raised FGF23 in CKD and reduced 1,25$(OH)_2$ Vitamin D levels may filter any sudden changes, further highlighting the need for a large number of recruited participants in order to adequately identify any changes. Moreover, this may reflect the limited numbers included in this exploratory study.

The present results suggest a differential effect between the two medications and underline the potential for certain elements of the 6H syndrome to exist even within ND-CKD, even in the absence of phosphaturia. It is therefore essential to consider the effects of these changes on other markers of the 6H syndrome, namely calcium and PTH.

4.2.2: Calcium

Calcium metabolism is intricately intertwined with phosphate as part of several feedback mechanisms. Calcium metabolism is tightly regulated by vitamin D, PTH and calcitonin; in brief, a decrease in ionised serum calcium causes an increase in PTH, effecting both kidneys and bones (430). Parathyroid hormone amplifies bone resorption and promotes phosphaturia, which in turn augments the calcaemic effect of PTH on bones; as such serum calcium concentration increases. In addition, it promotes further secretion of 1,25 $(OH)_2$ Vitamin D from the kidneys leading to greater intestinal calcium absorption whilst also increasing kidney re-absorption of calcium at the distal convoluted tubule (431). In CKD, the increased phosphate and decreased 1,25 $(OH)_2$ Vitamin D can lead to changes in calcium concentration, especially in the face of secondary hyperparathyroidism characterised by low calcium concentration (431). Fibroblast growth factor 23 is a key player in the homeostasis of phosphate and calcium and its concentration has effects on both PTH and 1,25 $(OH)_2$ Vitamin D. An increase in FGF23 could potentially lead to a decrease in calcium through diminished calcium reabsorption in the intestine which is potentially augmented by a direct inhibition of PTH (239). Fibroblast growth factor 23 also promotes re-absorption of calcium from the distal convoluted tubule; therefore, any potential

increase in iFGF23 may lead to mild hypocalcaemia partly due to its action on vitamin D metabolism (236). This mechanism may explain the results of certain studies suggesting mild hypocalcaemia following the administration of particular intravenous iron preparations such as FCM (308).

At baseline, calcium concentrations were within normal reference values (2.20-2.60 mmol/L) in both groups. Serum calcium trends were different between the two groups, albeit not reaching statistical significance. A decrease in calcium was noted within 2 weeks of administration of FCM, reaching the minimum of 2.29 (0.06) mmol/L at 2 weeks. While some normalisation was seen 1 month following infusion, the second infusion led to a significantly differential change effect within 1-2 days of administration. At no point in the study was there a negative change linked to the administration of FDI, whilst this was not the case in the group receiving FCM. Minimum calcium concentration in the FCM group coincided with the greatest percentage decrease in 1,25 $(OH)_2$ Vitamin D and the greatest percentage increase in PTH. There was no statistically significant intergroup trend.

The results of the present study indicate the potential for a hypocalcaemic effect to exist in patients with advanced CKD, without however giving rise to any events of actual hypocalcaemia in the population. The trend displayed is similar to that observed when comparing FCM to FDI, ferumoxytol and low molecular weight iron dextran, whereby the initial increase in iFGF23 was accompanied by a mild and transient decrease in calcium following the administration of FCM (155,162,187). A similar mild decrease was noted within the FCM group in PHOSPHARE-IBD, which actually reached significant difference with FDI on week 2 following administration (157). In addition, a greater requirement of calcium supplementation was noted

in the FCM group when compared to the FDI group through the trial (157). Nevertheless, previously, a study in patients with ND-CKD administered FCM failed to show any significant trend in serum calcium (226). It is possible that the intervals at which serum calcium was assessed did not correspond to the actual timeline of metabolic events associated with FGF23-driven changes. As discussed previously, maximum changes in serum calcium concentration have been seen between 1-2 weeks following administration of iron. Additionally, it is important to consider what form of calcium was measured in each study, especially given the fact that most studies detecting a trend utilised ionised calcium measurements. Ionised calcium represents the “free” calcium within the blood plasma, which is metabolically active, and potentially more “reactive” to changes in vitamin D and PTH (432,433). As such, measurements of ionised calcium may be more relevant to PTH-related calcium disorders, and lead to greater detection of fluctuations in calcium (433). In the present study, adjusted calcium was used despite the potential advantages of ionised calcium, as per international guidelines relevant to the topic of CKD-MBD that highlight the appropriateness of adjusted calcium measurements in patients with CKD (434). Despite the use of adjusted calcium, a definite but not statistically significant trend was observed in “Iron and Phosphaturia”, highlighting that elements of 6H syndrome can exist with advanced CKD not requiring dialysis.

As suggested, the potential for hypocalcaemia exists. A possible amplification in effect was witnessed following the second administration. Given the intertwined nature of PTH involvement in calcium and vitamin D metabolism, it is necessary to explore the relevance of the observations made in PTH and consider how any iatrogenic rise in FGF23 associated with differing intravenous iron preparations may be detrimental in terms of CKD-MBD and overall bone health.

4.2.3: Parathyroid hormone

Parathyroid hormone is considered amongst the master regulators of mineral metabolism with implicit involvement in the regulation of both calcium and phosphate. It is secreted from the parathyroid glands and regulated by changes in extracellular calcium – low levels of calcium stimulate the release of PTH, whereas increased levels cause a decrease in PTH secretion (435). Parathyroid hormone exerts effects on the bones, kidneys and intestine, stimulating bone turnover and leading to greater calcium release from the skeleton, enhancing calcium reabsorption from the kidneys whilst limiting phosphate reabsorption and increasing active 1,25 $(OH)_2$ Vitamin D, leading to augmented intestinal calcium absorption (435).

Parathyroid hormone can be also considered a uraemic toxin as it is heavily integrated in the pathophysiology of CKD-MBD. As previously explored, progressive CKD represents a hyperphosphataemic state, which in combination with reduced glomerular function and protein-associated tubular injury leads to reduced Klotho expression and increased FGF23. These lead to a tropic effect on PTH, leading to further stimulation of PTH and the manifestation of secondary hyperparathyroidism impacting bone turnover (436). Despite the potential inhibitory effects of FGF23 on PTH release, it appears that the resultant decrease in active vitamin D, combined with a possible FGF23 resistance in parathyroid tissue on high values of FGF23, a phenomenon witnessed in advanced CKD, could be triggers associated with secondary hyperparathyroidism (437). Consequently, alterations in FGF23 metabolism and a resultant increase in iFGF23 could yield an increase in PTH, an occurrence observed in studies involving FCM and other similar compounds as indicated in Appendix

table 13; this however has not been universal and not all studies concluded significant trends.

In the present study, both groups displayed a raised PTH compared to normal reference values (1.3 – 9.3 pmol/L), potentially suggesting an element of secondary/tertiary hyperparathyroidism linked to the advanced stage of CKD in the participants. No differential effect between the two compared agents was noted either in terms of absolute values, or change caused throughout the study, however a different trend was observed. In patients receiving FCM, a positive change was noted almost in all the visits suggesting a possible increase in PTH potentially secondary to FCM, which was not seen in those administered with FDI. This became increasingly evident between baseline and visits 4 and 5. It is important to note that the trends observed appeared to resolve by the end of the study as PTH concentration approached baseline values. Upon considering intergroup trends, no significance was detected despite the changes seen in the FCM group. Nonetheless, it is important to note that statistical significance in this case may be partly determined by the small sample size of the study.

The results of the study, albeit not reaching statistical significance, do not contradict the literature surrounding the use of intravenous iron and hypophosphataemia. Recent evidence pertaining to the topic and presented in Appendix table 13 indicates that FCM appears to induce an increase in PTH release, mimicking secondary hyperparathyroidism. As per the results of comparative RCTs between FDI and FCM, “Iron and Phosphaturia” displayed a maximum in PTH within one month of administration of FCM (155,157,162,163). These results were also mirrored in studies involving ND-CKD populations, as in the observational study by Huang and colleagues, where maximum concentration of PTH was seen at day 42 (218). The trends

indicated here highlight that it is possible for patients with advanced CKD but not requiring dialysis, even where PTH was already above normal reference range, to present with features of worsening transient hyperparathyroidism secondary to FCM. The fact that the residual change appears to be smaller compared to that of larger RCTs may be associated with the advanced stage of CKD in the participants. The results could lead to the speculation that the iFGF23 driven suppression of renal production of 1,25 $(OH)_2$ Vitamin D and the augmentation of its degradation may be less pronounced in CKD, hence leading to a lesser effect on PTH secretion. Moreover, as previously mentioned, CKD represents a state of resistance of parathyroid tissue to FGF23. The results displayed warrant further research, especially on the potential resultant injury to bone metabolism secondary to FCM, taking a step away from the iatrogenically induced phosphaturia and hypophosphataemia, into effects associated with CKD-MBD.

4.2.4: Correlations between iFGF23, phosphate and markers of the 6H syndrome

As discussed in section 1.2.2, the potential of a Kidney-Bone-Haematopoiesis axis exists. As such, anaemia and declining kidney function represent variables that could affect the strength of any potential correlation. Correlation analysis nonetheless can be used to assess the presence and strength of associations between linear variables, and that can be applied to knowledge of physiology in deducing possible reasons (438).

Indeed, taking into consideration the above possible association, a correlation analysis was performed between absolute values of eGFR and haemoglobin with phosphate and iFGF23 at baseline. A significant negative correlation between eGFR and phosphate and

iFGF23, and haemoglobin concentration and phosphate and iFGF23 was noted. Previous cross-sectional studies in patients with CKD have highlighted an inverse relationship between iFGF-23 and haemoglobin, similar to the results discussed (285,439,440). Similarly, evidence from large observational studies including patients with CKD suggests an inverse relationship between both haemoglobin and eGFR and phosphate, and a greater probability of anaemia in patients with increased serum phosphate concentration (232,285,441,442). These results imply that anaemia and kidney function were important factors at baseline that may affect the strength of any residual confounding relationships, but also indicate that despite the small size, the sample was representative in terms of Kidney-Bone-Haematopoiesis relationships.

Additionally, at baseline the correlation between iFGF23 and phosphate and other markers associated with the 6H syndrome was analysed, signifying an association between iFGF23 and serum phosphate concentration, FEPi and 1,25 $(OH)_2$ Vitamin D. The results displayed above (section 3.2.3) are consistent with evidence discussed by Isakova and colleagues using data from the CRIC study (n=332). Direct significant correlations were observed between FGF23 and phosphate (Spearman's rho: 0.35; $p < 0.001$), PTH (Spearman's rho: 0.37; $p < 0.001$), and FEPi (linear regression rho: 0.25; $p < 0.001$) whilst an inverse correlation existed between 1,25 $(OH)_2$ Vitamin D and FGF23 (Spearman's rho: -0.23; $p < 0.001$) (285). The absence of significance between iFGF23 and PTH in the current sample may be explained by the fact that the CRIC utilised cFGF23 and not iFGF23; indeed, a later observational study in participants with variable eGFR failed to reveal a correlation between PTH and iFGF23, while the previously discussed associations (iFGF23, phosphate, 1,25 $(OH)_2$ Vitamin D) were observed (411).

The current study assessed the association between concurrent changes in serum phosphate and serum iFGF23 to other important markers relevant to the 6H syndrome throughout the study period, through Spearman's correlation coefficients.

For iFGF23, there was a statistically significant moderately strong positive correlation with phosphate concentration change from baseline to visit 5. In addition, a moderately strong negative correlation existed between the change in iFGF23 and 1,25 $(OH)_2$ Vitamin D between baseline and visit 3 and between baseline and visit 6. A moderately strong positive correlation was noted in terms of change in iFGF23 and FEPi between baseline and visit 4 (2 weeks following administration of first intravenous iron dose). Finally, a significant negative correlation was witnessed in terms of change in phosphate and FEPi between baseline and visits 3 and 6.

Previous evidence in comparative studies have suggested an inverse correlation between changes in iFGF23 and phosphate. In both RCT (iron deficiency anaemia - ferumoxytol vs. FCM) and observational studies (bariatric surgery – FCM only) a tendency for decreasing phosphate as iFGF23 increased was witnessed (155,213). This however, appeared to be attenuated by decreasing eGFR. In the present study, a positive correlation that reached significance between baseline and visit 5 was documented, which may appear conflicting with the results indicated. Nonetheless, research in HD-CKD has revealed significant strong positive correlation between changes in phosphate concentration (secondary to iatrogenic phosphate control) and iFGF23 (443). In addition, in patients with CKD the administration of FCM did not lead to any significant correlations between the decline in phosphate and increase in iFGF23 levels (226). This may suggest that in advanced CKD the main driver in the FGF23-phosphate relationship could be serum phosphate concentration, or that the

statistical weight in terms of correlation analysis of each variable may differ in advanced CKD status. The witnessed correlation may also be secondary to the independent trajectory of each variable; it is evident from a review of the absolute concentrations that at visit 5 both variables were on a downward trajectory (either from baseline or from a maximum previous value). Physiologically, as phosphate decreases one would expect a decrease in iFGF23 and this may be captured at visit 5 (when compared to baseline), therefore implying a significant association.

In addition, an absence in correlation between changes in phosphate and other markers of 6H (albeit fractional excretion of phosphate) was observed throughout the study. A significant negative correlation between FEPi and serum phosphate was witnessed between baseline and visit 3, which coincidentally represented the period of the largest increases in iFGF23 in the FCM group. This may highlight an increase in phosphaturia (thereby phosphate decrease) secondary to iFGF23 increase, despite the advanced CKD stage of the participants and the possibility of FGF23 tubular resistance/absence of Klotho. The correlation observed in terms of %change of iFGF23 and 1,25 $(OH)_2$ Vitamin D and FEPi may reflect the potential for expression of the 6H phenotype following administration of FCM, even in patients with severely reduced kidney function, and complements the results discussed previously. Trends relevant to 1,25 $(OH)_2$ Vitamin D changes and concentrations throughout studies appear to be irrespective of kidney function, and the association observed in “Iron and Phosphaturia – ExplorIRON-CKD” seems to verify this association, and indeed further fuel the possibility of FGF23 driven side-effects in patients with CKD, even in the absence of explicit hypophosphataemia. Intriguingly, the decrease in 1,25 $(OH)_2$ Vitamin D coincided with nadir serum phosphate concentrations in FCM treated individuals which was predated by an increase in iFGF23. This may be related to decreased intestinal phosphate absorption, despite

the attenuated effect on phosphaturia, and may suggest that even in patients with CKD a hypophosphataemic propensity may exist.

It is important to emphasise that correlation or its absence do not imply causation or dismiss any pathophysiological concept. Taking in consideration the number of potential confounders including the ones described above, the lack of bivariate correlation amongst all analyses appears to be understandable. Multivariate analysis could pose a potential answer, but one needs to reflect on the sample size and the potential different statistical weight contributed by each variable independently, including the dose/type of iron provided. The possibility of heteroscedasticity also exists within the study limiting the generalisability of the correlation analysis (444). Nonetheless, the explored correlations suggest that the potential of iatrogenically driven FGF23 increase secondary to the use of particular iron compounds may lead to consequences that are not necessarily associated to phosphate but play an intricate part in bone and cardiovascular metabolism, whilst also having an effect on quality of life and functional status. Therefore, it is key to explore the significance of the results of the study in these areas. Moreover, outside the differential effect of iron on iFGF23, it is important to consider the differential effect of treatment on markers of clinical response and kidney function further.

4.3: Markers of bone turnover (ALP, BALP, P1NP, CTx)

Upon considering the potential bone implications of intravenous iron alterations of FGF23 metabolism, it is important to reflect on the disease patterns that can be associated with increased FGF23, in particular tumour induced osteomalacia and autosomal dominant hypophosphatemic rickets (239). Both disease processes are

associated with bone turnover derangements as displayed by markers of bone turnover, including ALP, BALP, P1NP and CTx (445).

Fibroblast growth factor 23 appears to affect bone remodelling, with effects associated with both bone mineralisation and bone resorption (236,261). The exact mechanisms behind the association of bone turnover and FGF23 remain controversial and largely unknown, nonetheless it appears that FGF23 destabilises the equilibrium between osteoblastic and osteoclastic activity (Sirikul et al., 2022). Murine models have revealed conflicting results that appear to be dependent on concentration (physiological and supraphysiological) of FGF23 and the potential induction of both canonical (Klotho-dependent) and non-canonical (Klotho-independent) pathways. In essence, FGF23 is linked with decreased and defective bone mineralisation leading to a compensatory increase in ALP, aiming to mitigate the deficiency of phosphate (254,261,446,447). As a result, bone formation associated markers such as ALP, BALP and P1NP may increase (448). In vivo, both autosomal dominant hypophosphataemic and x-linked hypophosphataemic rickets are associated with raised ALP and BALP concentration, with further evidence indicating an increase in P1NP as well (448,449). Beyond bone formation and mineralisation, FGF23 may be also related to bone resorption with in vitro evidence on human osteoclasts revealing early inhibition of osteoclastogenesis accompanied by increased osteoclast-mediated bone resorption (258). Such evidence provides potential mechanistic reasons behind the results of a recent cross-sectional controlled study revealing raised CTx in patients with x-linked hypophosphataemia (448). States of raised iFGF23 may represent a bone disequilibrium with deranged bone turnover and possible negative skeletal effects for the patients in the form of reduced bone mineral density. It is therefore necessary to discuss how the results displayed through “Iron and Phosphaturia – ExplorIRON-CKD” study relate to any potential differential effect between such markers.

At baseline, both groups had concentrations of ALP and BALP that were within the normal reference values (reference values: ALP: 30.0 – 125.0 iU/L; BALP: age and gender dependent: Males: 15.0 – 41.3 U/L; Females (pre-menopause): 11.6 – 29.6 U/L; Females (post-menopause): 14.2 – 42.7 U/L). No statistically significant difference was noted in terms of median absolute concentration values for either ALP or BALP between the two groups. Nonetheless, a different trajectory was noted, whereby a statistically significant difference was observed within the FCM group but not the FDI group. Monitoring the trajectories, it is suggested that the second administration of FCM may have caused further detriment in bone mineralisation. On the other hand, commenting on P1NP, another bone marker associated with bone formation, baseline concentrations in both groups were within expected values (reference values: 26.0 – 110.0 µg/L). No differential effect between the two groups was identified, and no statistically significant trend was noted in either group. Upon reviewing the median concentrations of both groups at different stages of the study, it is difficult to conclude on one particular trend. Hence, in the current study there was no significant effect observed in terms of concentration of P1NP associated with FDI or FCM use. Bone resorption appears to also have been affected based on the results in terms of CTx concentration. At baseline concentrations of CTx were raised in both groups (reference values: 0.1 – 0.5 µg/ml), potentially reflecting the advanced CKD stage of the participating cohort (450). Cross sectional studies indicate increased CTx in advanced CKD as a result of both reduced renal clearance and distortions in bone metabolism (451–453). Despite a lack of differential effect between the absolute concentration values of the two groups, a statistically significant trend of change was noted in the FCM but not the FDI-allocated group. These results are suggestive of a potential alteration and reduction in bone resorption following administration of FCM as indicated by the decreasing trend following the first infusion.

The results presented are seminal in terms of the CKD population and the use of such high doses of intravenous iron in this group. Previously, Wolf and colleagues revealed that upon comparing FCM to FDI, patients treated with FCM experienced a significant increase in BALP and a significant decrease in P1NP one week following administration of iron and from then onwards until the end of study (day 35) (162). Carboxy-terminal collagen crosslinks concentration also significantly decreased in the group receiving FCM when compared to FDI, reaching nadir concentrations on day 8. The significant difference in effect was eliminated by day 35. This pattern exhibited in the PHOSPHARE-IDA trials was similar to that observed in “Iron and Phosphaturia – ExplorIRON-CKD” in terms of CTx; the second administration of iron infusion also appeared to display a similar tendency in terms of BALP. Similarly, markers of altered bone metabolism (ALP, BALP, P1NP) significantly changed with FCM but not FDI as Zoller and colleagues reported in PHOSPHARE-IBD (157). These changes further support the notion of altered bone metabolism secondary to FCM compared to FDI. One observational study investigating the administration of FCM in patients undergoing bariatric surgery, showed a significant increase noted in BALP from baseline to week one and week two (1.0 ± 1.3 μg/l, $p = 0.009$ and 2.5 ± 2.3 μg/l, $p < 0.001$ respectively) (211); these results also verify the trend exhibited following administration of second FCM dose in the current patient cohort. Moreover, other studies utilising intravenous iron compounds with similar carbohydrate moieties to FCM (saccharated ferric oxide, iron polymaltose) highlighted the potential of alteration of other bone biomarkers such as osteocalcin and tartrate-resistant acid phosphatase – 5b, indicating the potential for alterations in bone turnover secondary to certain preparations (297,419). Such results indicate biochemical findings consistent with the patterns observed in patients with osteomalacia and other hypophosphataemic rickets syndromes (445,454).

Unlike these studies, other trials failed to indicate a significant bone turnover effect secondary to administration of FCM or other similar compounds. In the smaller comparative RCT HOMe aFers, no differential effect was identified in terms of BALP when comparing FCM with FDI. In addition, participants administered with either comparator developed a significant decrease in P1NP . Similarly in an observational study in patients with ND-CKD (n=35; eGFR: 22.9±10.4 ml/min/1.73 m^2) given saccharated ferric oxide (total dose 500 mg over 5 days), despite an increase in iFGF23, noted no change in BALP concentration (303). Nonetheless, both studies carry limitations which could explain the lack of significant effect: as the former mentioned, the number of recruits could severely limit the power to detect any statistical change, whilst the latter focused on a very short period of time (6 days), which is outside the period in which an effect was noted in all the aforementioned trials. The small numbers may represent a limitation in assigning any statistically significant importance in the results of the present study, in terms of differential effect, alongside the extent of renal disease that can be a limiting factor in relating such results to clinically relevant consequences. However, the results discussed and the accompanying literature review are suggestive of a differential effect in terms of bone turnover following administration of certain intravenous iron products, and may explain how repeated dosing of some compounds such as FCM is associated with osteomalacia and fractures (455–457). Therefore, the possibility of iatrogenically induced osteomalacia and fractures becomes increasingly important in patients with CKD, as they may already experience a state of altered bone turnover in CKD-MBD. The current study provides important and relevant results especially considering both the advanced CKD stage of the participants and the dynamic doses administered which reflect current clinical practice.

Beyond the potential differential effects of intravenous iron compounds in patients with CKD in terms of 6H and bone turnover markers, the results provided by the present study require further discussion in terms of patient reported outcome measures, especially as FGF23 and phosphate may be implicated in reduced functional status and quality of life.

4.4: Functional status and patient eported outcome measures

Anaemia (with/without iron deficiency) and CKD are associated with reduced quality of life, with an impact on both functional status and patient-reported outcome measures. Functional status appears to be increasingly important in determining both management, effect of treatment and prognosis in CKD, and encompasses both physical and cognitive function (458,459). Patient reported outcome measures include recording of the patients' response to variables that are important to them, outside clinical values such as improvement in haemoglobin or increase in kidney function (460). These include the utilisation of health-related quality of life associated questionnaires, which can be seen as means to improve personalised care, and a method of assessment of what can truly affect health and disease perception in patients with chronic disease (461). Such measures are progressively more imperative in patients with CKD, as indicated by the "Standardized Outcomes in Nephrology" initiative whereby patients with end-stage renal disease ranked self-reported quality of life above long-term survival (462). Truly, the incorporation of functional status assessment and patient-reported measures in research can be viewed as an attempt to incorporate patient-related variables of interest into clinical research and potentially help accelerate the development of more patient-centred care in CKD (461). Previous large-scale evidence has suggested an improvement in functional status in patients with iron deficiency and heart failure, however there is paucity in the CKD population (101,463,464). In order

to counter this phenomenon and aid in further development of research on the topic of improved functional status and health-related quality of life, the current study has provided results relevant to both these important factors, both for the full cohort but also each group individually. In addition, taking into consideration the potential differential effects arising due to the effect on FGF23 metabolism and the 6H syndrome, a differential analysis also took place which was reported in section 3.5.

4.4.1: Functional status: DASI and 1-minute-sit-to-stand

The DASI score can be used to assess functional status. It has been previously used in patients with CKD and has demonstrated good reliability and reproducibility. The results can be reported as estimated peak oxygen uptake (VO_2) or maximum metabolic equivalent of tasks (METs). The lower the MET scored, the poorer the exercise capacity of the individual, with a score of less than 5.0 likely to contribute to a poorer prognosis (465).

At baseline there was no significant difference between the two groups; however, the median scores of the FDI and FCM groups were close to or lower than the 5.0 METs cut-off previously mentioned (FDI: 5.1 METs; FCM: 4.1 METs). Comparison with previous studies in CKD incorporating the DASI score, revealed that both groups had a worse DASI score both in terms of VO_2 peak and total score, indicating a sample potentially affected by the comorbid status of iron deficiency, or the extent of kidney disease (398,466,467). No differential effect between the two groups (FCM vs. FDI) was noted throughout the study. A trend to an increase in DASI score was noted in the whole cohort of the study that did not reach statistical significance. There was no significant trend noted in either group, however a numerical

increase in terms of median scores was noted in the FDI group. It should be pointed out that the DASI score throughout the study remained relatively low.

The 1-minute-sit-to-stand test was also used to assess exercise tolerance and functional capacity. No reference values relevant to expected 1-minute-sit-to-stand tests exist, however the number of repetitions can be affectedby age and gender (466,468,469). The 1-minute-sit-to-stand test has been used in a number of studies relevant to CKD, and alongside other sit-to-stand tests has good validity and reliability in this patient group (468).

Baseline mean values both for the cohort and each individual group were below previously observed reference values based on cross-sectional studies for elderly individuals above 75 years of age (male: 30/min, female: 27/min). In addition, they were lower than other reported repetition scores in patients with CKD, but not necessarily anaemia, as seen in studies of validation of the particular test (466,468). The performance in both groups and the whole cohort improved significantly throughout the study and reached maximum at visit 8. No differential effect was noted between the two groups in relation to completed 1-minute-sit-to-stand cycles.

Patients with CKD often have a heavy symptom burden, which is associated with frailty and poor prognosis (470). Iron deficiency with or without anaemia further complicates the fragile disease burden and therefore its alleviation may be an attractive therapeutic strategy (471). Evidence in studies on patients with chronic heart failure have suggested a beneficial response in quality of life and exercise tolerance measures even in the absence of absolute anaemia (122,464). Further studies are currently in progress. Intriguingly, it

appears that anaemia defined as haemoglobin deficiency is not the only cause of persistent exercise intolerance in CKD, and elements of mitochondrial dysfunction and sub-optimal oxygen extraction exist (471). Iron is important for both oxygen-carrying capacity and a component of the mitochondrial machinery; therefore, theoretically one would expect improvement in functional status secondary to alleviation of any such deficiency. Ferric carboxymaltose has been shown to improve exercise tolerance (assessed by the 6-minute-walking test) in patients with heart failure and chronic obstructive pulmonary disease (463,472). Mechanistic evidence in heart failure patients also points towards improved skeletal energy metabolism upon repletion of iron deficiency (107). Administration of FDI improved phosphocreatinine half-life, a marker of improved mitochondrial function (107). Additionally, improved, albeit not statistically significantly, mean 6-minute-walk-test values have been noted in a recently completed RCT in patients with heart failure receiving FDI (baseline: 252.9 (13.7) m vs. 20-months: 288.8 (13.9) m; p=0.068) (473). It is important to acknowledge the effect of COVID-19 pandemic on these results as suggested by the authors. Moreover, murine models of CKD induced through one-stage subtotal nephrectomy have demonstrated a significant improvement in skeletal tissue oxidative stress and antioxidant activity following administration of intravenous iron (equivalent dose adults: 510 mg) (474). These findings may suggest improved skeletal dynamics upon intravenous iron infusion that may be associated with the results exhibited. Nonetheless, a multicentre prospective double-blinded RCT comparing FDI with placebo in iron deficient but non-anaemic patients with ND-CKD (n=54; FDI dose: 1000 mg; mean eGFR: 31.1 ml/min/1.73m^2) failed to identify a statistically significant difference between the two arms in terms of 6-minute-walking-test (106). An increase was however witnessed, and the authors mentioned as a potential limitation, the small nature of the study in terms of participants, duration, high initial 6-minute-walking-test values and the lack of statistical power.

The present study incorporating patients with iron deficiency with/without anaemia indicated a statistically significant improvement in terms of 1-minute-sit-to-stand transfers, which was evident following administration of first dose that was maintained until the end of the study. No differential effect in terms of 1-minute-sit-to-stand cycles was seen and the lack of absolute phosphate changes in-between groups, an important mineral within the mitochondrial apparatus, alongside the absence of clinically significant hypophosphataemia may explain this. The improving trajectory associated with 1-minute-sit-to-stand cycles was not reflected on the DASI score, where there was no significant trend within the whole-cohort, and no differential effect between the two compounds throughout the study The lack of significant improvement in DASI may be associated with the CKD status and advanced disease of the participants, as kidney function appears to significantly affect DASI (466).

Certain limitations exist and therefore the findings should be interpreted with caution. Beyond the lack of statistical power to detect changes, the potential of improved fatigability/exercise capacity secondary to other activities of the individual participant outside the remits of the trial need to be considered. Furthermore, the results are not indicative of any correlation or association between the 6H syndrome and markers of bone metabolism with functional status; hence the findings observed cannot be directly linked to any witnessed changes and previously discussed. Additionally, an improvement in functional status/exercise capacity does not necessarily infer an improvement in quality of life especially in patients with CKD, and sit-to-stand tests should be ideally interpreted as proxy indicators of muscle power (475).

Nonetheless, the current results indicate an improvement in functional status as assessed by exercise capacity and fatigability and will be hopefully complemented by the results of the larger Iron and Muscle study that are eagerly awaited (476). Such trends highlight the need for further research on the topic of exercise capacity and functional status in patients with iron deficiency and ND-CKD, alongside further comparator studies that are adequately powered to detect potential differential effects secondary to derangements in phosphate and FGF23, something absent from current literature.

4.4.2: Quality of life and fatigue

As noted, iron deficiency is associated with implications on exercise capacity, and the present results indicate some improvement following administration in patients with ND-CKD. The commonest and most consistent symptom associated with iron deficiency and anaemia is that of fatigue (477). Fatigue as an entity is not restricted within physical restrictions but it is also associated with activity both physical and mental. As suggested by Strauss and Auerbach, fatigue and its potential resolution or improvement is better monitored when considered as a continuum between vitality and fatigue (104). Therefore, the combination of the SF-36, which covers a multitude of different variables, and the FSS can provide useful information relevant to the effect of intravenous iron compounds in this patient group.

At baseline, quality of life across seven of the eight variables associated with the SF-36 was poor and below that of 50%, the normative value for all scales, alluding to the severe impact that CKD and iron deficiency can have on this patient group (478). Compared to previous analysis of SF-36 results in ND-CKD and HD-CKD and

anaemia (n=194), the two groups formed in the present study had lower baseline scores in most domains except bodily pain, general health and social functioning, than their counterparts as noted on Appendix table 23 (390). Unlike the aforementioned analysis, the patients in the present cohort experienced both physical and mental impact secondary to their background condition.

Numerically, most variables (with the exception of general health) improved throughout the study both in the whole cohort but also in the individual groups formed. A significant improvement was noted in terms of physical function within the whole cohort and the FDI group, with similar findings in the domain pertaining to energy. Limitations due to emotional reasons improved significantly within the whole cohort and the FCM group, while a significant improvement in pain severity was witnessed in the total population of participants. No differential effect was noted between the two comparator groups in terms of domains of the SF-36.

The FSS was reported as both a total score and a visual analogue scale score. An improvement was noted in both components from baseline to last visit in the whole cohort; a statistically significant improvement was seen in the visual analogue scale scores. Upon review of baseline scores it is evident that fatigue was a major problem for all participants of the study, with scores suggestive of worse fatigue when compared to other small-scale evidence incorporating patients with CKD and the FSS (479). In addition, this is verified by reviewing the baseline scores against the cut-off (total score: 36) indicative of severe fatigue suggested by Krupp and colleagues at their pivotal work on FSS and patients in multiple sclerosis (480). A significant difference existed in the total score at baseline between FCM and FDI, with patients randomised to FDI having scores consistent with greater fatigue; this was not present for visual analogue scale scores.

Nonetheless, no further differential effect between the two groups existed in any remaining visits, both for total score and visual analogue scale score. Total fatigue scores remained similar throughout the study in both groups and the whole population, however visual analogue scale scores improved in both groups and in the whole cohort, and this trend was statistically significant within the whole population and the FDI-allocated group. Indeed, on considering the numerical trends it is evident that FDI caused a larger improvement in visual analogue scale score when compared to FCM, throughout the study, and this maintained momentum following administration of both doses of iron. This was not the case in patients administered with FCM, which reached the maximum visual analogue scale score on visit 5 and not upon full repletion (assessed on visits 7 and 8).

The beneficial effect of iron suggested in the present study on physical function, fatigue, emotional restriction and pain domains of the SF-36 and the FSS visual analogue can be related to the improved iron availability. Studies in patients with iron deficiency anaemia comparing intravenous iron preparations (ferumoxytol and iron sucrose) against placebo (blinded) noted improvement in energy and fatigue scores through the SF-36 – energy subscale and the FACIT-Fatigue Scale (481,482). They also noted that the baseline scores were much lower than the reference population, similar to the current cohort, reflecting how broadly iron deficiency anaemia may affect both mental and physical aspects of quality of life. The observed improvement was above the minimally clinically important difference in these studies, a vital parameter to consider when discussing and reporting health related quality of life questionnaire (104). The enhancement in quality of life can be put down to increasing haemoglobin concentration, however this may be a simplistic approach and this was not seen in “Iron and Phosphaturia – ExplorIRON-CKD” (discussed in section 4.5.1). Both in patients with heart failure or cancer statistically significant changes in health-related quality of life were either not

statistically significantly different between anaemic and non-anaemic iron deficient patients or preceded increase in haemoglobin respectively, therefore highlighting the potential link between iron deficiency and quality of life extending beyond haemoglobin (101,483). Interestingly, Zoller and colleagues have recently identified a slower, lesser improvement in FACIT-fatigue where FCM was administered compared to FDI, with analysis suggesting that this could be phosphate dependent (157). This was also suggested in the present study in terms of the FSS visual analogue scale.

It is important to comment on the differential effects already noted; these included changes in iFGF23 and 1,25 $(OH)_2$ Vitamin D. In the present study, an improvement in domains relevant to fatigue such as physical function and energy was suggested in participants administered with FDI – importantly FDI-treated participants did not exhibit statistically significant changes in markers of 6H syndrome or bone metabolism. Studies previously focusing on the FGF23 pathway, iron administration and quality of life are mainly observational in nature and encompass patients with gastrointestinal disorders. In such studies no significant changes were observed in phosphate or FGF23 secondary to FDI, but FCM displayed hypophosphataemic tendencies stemming from alterations in FGF23 (204). Moreover, no significant differences were noted between patient groups with or without hypophosphataemia at any time point of the study, contradicting results later noted by Zoller and colleagues of an inverse relationship between fatigue and phosphate concentration (157,204). The authors mentioned (in agreement with the results of the current study) that baseline recordings were low within their population reflecting both their chronic disease status and iron deficiency (204). Interestingly, supplementaton with Vitamin D in CKD has not caused previously significant changes in quality of life as that was measured through SF-36 (484). As such, the reasons behind any potential difference in improved fatigue status warrant further research, with

greater focus on other variables that may be associated with quality of life.

On the other hand, despite the described trends and potential reasons behind them, one should consider the fact that the changes identified do not always necessarily reflect on clinically measurable outcomes. Previous research attempted to define clinically relevant changes in SF-36 in CKD anaemia with some success, however any meaningful change was based on alleviation of anaemia and not markers of iron deficiency and was based on longer-term changes. Therefore, the findings of that study cannot be used to extrapolate the present results into clinically meaningful changes (390). No correlations were explored between markers of iron metabolism, bone metabolism and quality of life; as such, no distinct associations can be inferred given the results. In addition, as “Iron and Phosphaturia – ExplorIRON-CKD” was a pilot study it was not not statistically powered to detect significance within changes; as such, the ability to detect statistically significant changes may have been limited. The significant difference in age at baseline between the two groups may have also been implicated. Consideration should also be placed on the possibility of a placebo effect associated with the performance of any health-related quality of life questionnaire, however the double-blinded nature of the intervention could aid in alleviating this possible confounder. Additionally, as the study period was during the COVID-19 pandemic, it is possible that the effect of repeated lockdown strategies and restrictions in movement could have affected the results as previously described in pregnant women, university students and people with chronic neurological conditions (485–487). The impact of the COVID-19 pandemic on older individuals with advanced CKD but not COVID-19 associated pathology (n=82; eGFR < 20 ml/min/1.73 m^2; mostly not on dialysis (97%), has been recently explored (488). An important depressive and anxiety inducing sentiment was observed, alongside a

concurrent significant decline in physical health related quality of life as defined by the Short Form (12) Questionnaire (488).

In conclusion, the trends exhibited are promising and indicate potential improvement in quality of life associated with the use of intravenous iron, which may be more prominent following the use of FDI in terms of fatigue and physical function. These tendencies may be related to FGF23 but further research is required on the topic, especially given the absence of persistent changes in markers of 6H or bone metabolism following administration of FCM. As no clinical hypophosphataemia was detected within the population, it is unlikely that any differential effect secondary to hypophosphataemia exhibited in other studies not incorporating patients with CKD, can be relevant to the present population. Given the findings that seem to complement the resolution of iron deficiency in heart failure and the long-awaited Iron and Muscle study incorporating patients with CKD and iron deficiency, further studies are needed to verify whether resolution of CKD-associated iron deficiency can replicate the beneficial effects displayed in other disease processes.

4.5: Clinical measures (haematinic, kidney function and inflammatory markers)

4.5.1: Haematinic response (haemoglobin, serum ferritin, transferrin saturation)

Treatment of anaemia aims to restore haemoglobin concentration to levels appropriate for each individual population, maximising quality of life and prognosis and minimising toxicity and side effects. The same

principle applies in iron deficiency anaemia of CKD, with iron enhancing compounds presenting an attractive option, especially in minimising administration of erythropoiesis stimulating agents (489). This is reflected through the guidelines of the Renal Association which specifically mention that "*the aim of iron treatment targets is to optimise anaemia therapy while minimising potential toxicity*" while "*minimising the erythropoiesis stimulating agents dose required to maintain target haemoglobin levels in patient*" and "*maximising the haemoglobin concentrations and minimising the need to initiate erythropoiesis stimulating agents therapy to achieve target-range haemoglobin levels*" (56). Therefore, and taking into consideration the potential side-effects of intravenous iron that were explored in the section 1.1.6, it is important for therapy to be regularly evaluated, through markers of haematinic response, using haemoglobin and markers of iron homeostasis such as serum ferritin and transferrin saturation.

At baseline, both groups had a haemoglobin concentration consistent with anaemia (at least <120 g/L in both males and females). No statistical difference existed at baseline between the number of participants requiring erythropoiesis stimulating agent therapy between the two groups. Both groups had one participant that was non-anaemic at baseline, with similar mean haemoglobin concentrations and no statistically significant difference between the two groups. A statistical improvement in haemoglobin concentration occurred in both groups from baseline until visit 7. A clinically significant change (>10 g/L) was not achieved in mean values in either group. Three participants in the FDI group (21.4%) and five participants in the FCM group (41.7%) attained such a change. There was no differential effect in haemoglobin response to iron between the two groups at any point of the study.

Most patients displayed absolute iron deficiency as opposed to functional iron deficiency at baseline (61.5% vs. 38.5%), with both groups comparable in terms of markers of iron metabolism. Serum ferritin and TSAT displayed numerical improvements in both groups throughout the study. No differential effect between the two groups was observed based on serum ferritin or TSAT response at any point in the study, however both increased significantly in both groups. Transferrin saturation increased rapidly following the administration of intravenous iron in both groups, recording highest values 1-2 days following administration of iron. In the participants receiving FCM, the maximum storage effect of iron as defined by serum ferritin was seen relatively early following administration, with a more prolonged effect witnessed in the FDI group. By the end of the study most patients demonstrated effective repletion of their iron stores (defined as serum ferritin > 100 µg/L and transferrin saturation >20%) (56).

The results of two important meta-analyses demonstrated similar haemoglobin responses secondary to intravenous iron found in our study (91,92). Shepshelovich and colleagues reported that 48.0% of ND-CKD patients in five trials incorporating intravenous iron (total n=1,404; receiving intravenous iron n= 794) achieved an increase in haemoglobin of > 10 g/L, with a similar result in those on dialysis, with a mean increase of 3.6 g/L (95% CI: 2.0 – 5.1 g/L) (91). The Cochrane review by O'Lone and colleagues also noted a mean change of 4.1 g/L in haemoglobin in their study population (ND-CKD and HD-CKD; 39 trials; n=3,852); 57.3% of participants achieved an increase of 10 g/L (48.5% in ND-CKD associated studies – n=992) (92). One important difference between the meta-analyses was the inclusion of different intravenous iron compounds, with the Cochrane review including a greater number of available preparations. There was also a high grade of heterogeneity in administered doses of iron amongst the studies included in meta-analyses.

Despite alterations in markers of 6H as previously suggested through the duration of the study, and more specifically FGF23 that has been shown to be related to iron metabolism and anaemia, no differential effect in haemoglobin was observed between the two groups.

Our trial indicated that both preparations displayed efficient and effective repletion of iron in the majority of participants. Patients treated with FDI had a steady increase in ferritin throughout the study with a maximum ferritin concentration achieved at the final visit, in contrast to FCM where the maximum ferritin was achieved at visit 4, i.e.2 weeks following administration of first iron infusion. This may be due to the difference in half-life's between FDI and FCM, with FDI having a much longer half-life, potentially reflecting a more stable structure (90). Moreover, this difference in stability may be more evident through the TSAT results, especially those 1-2 days following administration of the second intravenous iron dose. The lower TSAT observed in the FCM group may represent the maximum effect of FCM taking place within 16 hours of administration, which differs to the one observed with FDI at 24 hours of administration (89). Such subtle differences may be associated with greater non-transferrin bound iron (labile plasma iron) representing the redox-active form of iron, that may lead to inflammation, oxidative stress and resultant cardiovascular side effects (89). Nonetheless, modern intravenous iron compounds represent potentially safer options compared to older compounds. A recent observational study focusing on HD-CKD patients receiving FDI, FCM and iron sucrose has complimented the pharmacokinetic abilities of the newer intravenous iron compounds, with decreased amount of liver iron deposition as identified by MRI with FDI or FCM when compared to iron sucrose (145).

The trends suggested in the present study are comparable to other RCTs and indirect treatment comparisons relevant to modern intravenous iron compounds (i.e., FDI, FCM and Ferumoxytol). The PHOSPHARE-IDA trials identified increases in all associated haematinic parameters including haemoglobin concentration, haemoglobin rise per gram of actual iron dose administered, ferritin and TSAT (162). Further comparison between the results of “Iron and Phosphaturia – ExplorIRON-CKD” and PHOSPHARE-IDA are limited by the difference in iron dosing, administration frequency, duration of follow up and inclusion criteria and therefore should be avoided. A post-hoc analysis of the trial identified low ferritin as an independent risk factor for hypophosphataemia incidence and persistence (229). As “Iron and Phosphaturia – ExplorIRON-CKD” Trial did not focus on any correlations between haematinic parameters and the 6H syndrome, no direct comparison can be made between the displayed results and the existing literature in terms of predictors of change of phosphate concentration, and this is beyond the scope of the study. Nonetheless, based on the evidence from head-to-head comparative trials and other observational evidence, it appears that both compounds are efficacious in producing an adequate haematinic response; a similar trend was observed here.

The results discussed, however, and their interpretation are limited by the small sample size and the non-powered nature of the study. The lack of significant clinically relevant haemoglobin rise may reflect the multifaceted nature of the pathophysiology of CKD-associated anaemia, alongside the presence of confounding factors such as on-going occult pathology, need for haemodialysis and other invasive procedures associated with blood loss.

4.5.2: Kidney function and injury

Concerns surrounding the use of intravenous iron in patients with CKD extend to the topic of nephrotoxicity. Indeed, as mentioned in section 1.1.6, concerns were raised both through in vitro and in vivo studies, suggesting transient induction of proteinuria following administration of iron sucrose in a crossover trial incorporating 12 patients with ND-CKD (118).

In summary, the kidneys play a crucial role in iron homeostasis through a reduction in erythropoietin from the peritubular cells leading to increased glomerular leakage and tubular absorption of iron, and greater inflammation therefore leading to greater hepcidin concentration and limiting absorption of iron and iron concentration (114). The cascade described above, is disease state dependable with CKD affecting both erythropoietin production and the hepcidin pathway leading to functional deficiency or absolute iron deficiency, whilst certain glomerulopathies can have a distinct effect on urinary iron loss (114). Van Swelm and colleagues have recently summarised the iron impact on the kidneys, with iron overload and other iron genetic disorders having detrimental consequences on the kidneys and leading to kidney injury (114). Iron itself may increase oxidative stress, leading to increased free radicals and labile redox cycling within the organs, directly affecting the kidney endothelium and the mesangial and glomerular cells (48). In addition, any cellular dysregulation may lead to pyroptosis of kidney cells, and therefore further injury (114). Furthermore, iron may lead to ferroptosis, which itself leads to greater inflammation and oxidative stress leading to further necroptosis and pyroptosis and additional potential renal injury (114).

It is important to recognise the pro-inflammatory state of iron deficiency, and therefore consider the potential benefit of intravenous iron in alleviating iron deficiency and by-proxy attenuating any resultant oxidative stress. Murine models have suggested that activation of the oxidant-dependent NRF2 pathway through iron use may lead to renal pre-conditioning with eventual anti-oxidant and renoprotective effects (490). The same group demonstrated that iron sucrose was able to induce an increase in urinary ferritin, with increase in albuminuria, in healthy volunteers and patients with ND-CKD which according to the authors could be an indication of renal preconditioning to future acute insults (491). Iron sucrose was also linked with increased renal hepcidin production in both healthy and ND-CKD participants that could have beneficial effects and cytoprotective renal effects (492). Putting these results into clinical context, a retrospective review of renal allograft recipients (n=169) identified that increased serum ferritin at baseline was linked with an improved outcome for the transplanted patients even following haemoglobin adaptation (493). These results further stimulated murine experiments identifying an antioxidant response secondary to iron through action of NRF2 in macrophages and a reduction in their mobilisation (493). Additionally, the FAIR-HF RCT in patients with heart failure and iron deficiency revealed a trend for improvement in eGFR from baseline to end of study (122). This phenomenon may be the result of improved cardiac function or secondary to decreased hypoxic proximal renal tubular cell injury as suggested by Zager and colleagues (494). However, as previously discussed intravenous iron preparations have different pharmacodynamics and pharmacokinetic properties, with murine evidence suggesting a differential renal response to different iron preparations, such as iron sucrose, iron gluconate and ferumoxytol (113). Ferumoxytol, unlike the other three, showed bio-neutrality not invoking any cyto/nephrotoxic or “cytoresistant” effects (113). As FCM and FDI belong in the same group of compounds as ferumoxytol it is paramount that the two compounds are compared further in the ND-CKD population to identify any subtle differences that may be

potentially related to their differential effect on FGF23, especially given their increasing use.

At baseline, the median eGFR was 18.0 (IQR11.3) ml/min/1.73 m^2, lower than previous studies encompassing any of the two compounds in patients with ND-CKD. Most patients in the study had CKD stage 4 (i.e., 15-29 ml/min/1.73 m^2) and multifactorial CKD was the commonest cause of CKD. At baseline, both eGFR and creatinine were comparable between the groups. During the study, creatinine concentration remained largely unaffected in both groups, with no statistically significant trend displayed and no differential effect at any time-point. Median eGFR values displayed some increase in the FDI group, and remained largely unaffected in the FCM group, with no statistically significant trend displayed within group and no differential effect witnessed between the two groups. In terms of proteinuria, a higher baseline protein: creatinine ratio was seen in the FDI group, when compared to the FCM group but this did not reach statistical significance. This may be explained by the difference in composition of underlying renal pathologies between the two groups, with a greater proportion of participants in the FDI group having either membranous nephropathy or diabetic nephropathy which are conditions associated with a greater degree of proteinuria. Nevertheless, the numerical difference between the two groups at baseline did not reach statistical significance. There was no particular trend identified in terms of proteinuria in either group, as this was exhibited by the lack of statistical significance following the within-group analysis; in addition, there was no differential effect caused between the two compounds. Second administration of intravenous iron did not lead to any changes in the trends already presented.

No comparative evidence in kidney function and injury has been previously published relevant to FCM and FDI in the ND-CKD

population. The RCTs focusing on the comparison between FCM and FDI did not provide results associated with markers of kidney function (157,162,163) Shifting focus to other studies incorporating modern intravenous iron compounds such as FCM and FDI, one can note no clinically relevant or statistically significant detriment following administration of these preparations. The aforementioned FIND-CKD multicentre RCT did not identify any change in eGFR within 12 months of initial administration (1000 mg vs. 200 mg FCM) (121) . Less than 3% (2.6%) of the study group progressed to dialysis by the end of the study, with no difference between the two groups (121). In the present study 7.6% (n=2) patients required dialysis, all within the FDI group – however this may be related to the greater eGFR of the participants in the FIND-CKD trial (mean eGFR: >30.0 ml/min/1.73 m2, median eGFR in "Iron and Phosphaturia – ExplorIRON-CKD": 18.0 ml/min/1.73 m2) with a greater proportion of patients at CKD stage 3. Moreover, iron deficiency resolution in a prospective observational study in ND-CKD was associated with a significant increase in eGFR in patients (n=78; mean eGFR at baseline: 27.7 (17.3)) 24 weeks following 1000 mg of FCM, similar to trends previously demonstrated in heart failure with reduced ejection fraction (122,495).

Regarding FDI-relevant studies, two small scale RCTs (Iron-CKD and Iron & the Heart) have provided re-assuring data surrounding the use of high dose intravenous FDI in patients with ND-CKD. In an RCT (n=40) evaluating the potential for oxidative injury and stress secondary to administration of high dose FDI, participants were divided into four groups according to iron preparation received and dose administered (iron sucrose 200 mg vs. low molecular weight iron dextran 200 mg vs. FDI 200 mg vs. FDI 1000 mg) (87). Analysis of variance indicated no statistically significant change within each group or any differential effect in eGFR in the participants irrespective of iron or dose administered (87). Additionally, in the Iron & the Heart RCT, participants with ND-CKD were randomised to receive 1000 mg of FDI or placebo (n=54; FDI=26; placebo=28) – over a course of 3 months

there were no statistically significant changes in terms of cystatin C, creatinine, eGFR, proteinuria (urinary albumin:creatinine ratio) or neutrophil-associated lipocalin (124). This pilot study provided valuable insight to the potential effect of high dose intravenous iron preparations in ND-CKD especially considering the array of markers used which encompassed renal function (both creatinine and cystatin C) and injury (proteinuria as evidence of glomerular damage and neutrophil-associated lipocalin as evidence of tubular damage) (124). The authors suggested that the neutral results of this study could be secondary to the iron preparation used, which could further highlight the potential safety of modern intravenous iron compounds in ND-CKD (124). One major drawback however of all the studies documented is that kidney function deviations were not a primary outcome, and especially in the case of the evidence surrounding FDI, the studies were not statistically powered to examine the outcomes discussed. In addition, the median eGFR at baseline of the current study was lower than that of the other studies, alongside a different approach to total intravenous iron administered, potentially limiting the extrapolation and association of their results to the current study. Nevertheless, the data reported here and in combination with the conclusions of previous studies encompassing the comparator iron preparations, appear to support that no gross detriment occurs to renal function following their administration and may be consistent with previous evidence indicating bio-neutrality with ferumoxytol.

The main limitation of the present study is the small number of recruited patients, therefore leading to a non-statistically powered analysis. The method of analysis incorporates intention-to-treat, and approaches of this kind carry with them a number of limitations: more specifically two patients in the FDI group progressed to requiring haemodialysis during monitoring of kidney function, therefore affecting the reported indices incorporated in the study. Moreover, not all participants provided urinary samples at all times, further affecting the

numbers involved in the analysis. Considering the proposals put forward by Agarwal and colleagues regarding the potential nephrotoxic nature of intravenous iron (i.e., proteinuria within 15 minutes of administration of iron sucrose), the present study cannot rebut any theory of a possible ultra-acute kidney effect (118). In addition, given the possible acute detrimental effect that intravenous iron may have on the kidneys, other biomarkers could be incorporated that would be able to better isolate any early evidence of renal injury such as neutrophil gelatinase-associated lipocalin and N-acetyl-β-D-glucosaminidase that are associated with tubular damage (496).

Despite the limitations discussed, the present study provided some useful results to further reassure researchers and clinicians on the safety of modern intravenous iron products in terms of clinically relevant markers of kidney function. Moreover, the repeated administration of iron did not relate to any detrimental effect monitored during the study. Furthermore, it is worth noting that in the three cases of haemodialysis initiation, none of them was related to the administration of FDI. On the contrary, one case was planned but not adequately communicated prior to enrolment in the study whilst the other two arose secondary to an emergency admission to the hospital due to an infected renal cyst leading to anuria and interstitial renal injury secondary to sepsis. The differential effect on markers of the 6H syndrome displayed in the two groups did not appear to exert any effect on kidney function or injury. Inflammation, secondary to increased oxidative stress is one other potential source of kidney injury secondary to intravenous iron, and another area of concern relevant to long-term complications and is later explored.

4.5.3: Inflammatory response (CRP)

Long-term inflammation has been closely associated with several factors including aging, cardiovascular disease and malignancy (497). Iron can be related to oxidative stress, inflammation and regulated cell death mechanisms (114). Concerns remain regarding a potential pro-inflammatory response secondary to intravenous iron administration, with a vicious cycle surrounding the association between inflammation and oxidative stress (8). These concerns stem from the ability of intravenous iron to induce oxidative stress and subsequent biological damage secondary to labile plasma iron release that is available to be involved in the Fenton reaction, leading to the creation of reactive oxygen species and free radicals whilst playing a major role in the initiation and propagation of lipid peroxidation (116,498).

Both CKD and iron deficiency are increasingly recognised states of inflammation. Tinti and colleagues have recently described CKD as a “systemic inflammatory syndrome”, with abnormally increased oxidative stress exerting a pathogenetic effect (499). Cytokines such as CRP, interleukin 6, tumour necrosis factor, fibrinogen, adhesion molecules and pentraxin-3 appear, amongst others, to play key roles in the persistent inflammatory state of CKD (500–503). This pro-inflammatory interplay can be seen as key in the progression of CKD, leading to numerous complications as discussed previously (figure 1), whilst a number of complications such as CKD-MBD, anaemia, uraemia, and sympathetic overactivity can be perceived as pro-inflammatory themselves, therefore enabling a vicious circle (504,505). A study including 9,586 individuals with ND-CKD independently linked the presence of a high CRP and metabolic syndrome with increased prevalence of CKD, highlighting the relationship between the two, with the direction of association remaining controversial (506). These findings indicate that the

relationship between inflammation and CKD are key in the development of iron deficiency, especially when considering the effect of inflammation on hepcidin and iron absorption. Despite the propensity for excess iron to be involved in the evolution of inflammation and oxidative stress, iron deficiency itself may represent a pro-inflammatory state. Evidence from in vitro models indicates enhanced inflammation in cultured monocytes and macrophages upon induction of iron deficiency; in addition, diminished anti-inflammatory production of lipopolysaccharide-induced itaconic acid production has been reported in iron deficient alveolar macrophages (507,508). A post-hoc observational analysis of the DO-HEALTH RCT (n=2,141) reported an association between iron deficiency and interleukin-6 and CRP levels. Iron deficiency was found to be associated with a statistically significant greater CRP and interleukin-6 level increase over the three years of follow up. Moreover, no interaction was displayed between anaemia and iron deficiency, potentially indicating that the pro-inflammatory status was secondary to iron deficiency and not anaemia (509). Results of in vitro studies and observational analyses cannot verify a causative link, and further research is required on the topic.

In the present study, the two groups displayed comparable CRP concentrations at baseline, with neither group displaying evidence of acute inflammation (CRP normal reference range: <8.0 mg/L). There were no significant changes in CRP within each group, and no differential effect between therapies throughout the present study. No large changes in median concentration were observed between different time points in the study. The results suggest that at the time-specific points where CRP was monitored, no residual inflammation was caused by any of the comparator drugs administered; no lasting increase between administration and one month later was seen, even following the second administration.

Previous comparative RCTs including FDI, FCM and ferumoxytol did not target inflammation as one of their primary or secondary outcomes, and on the topic of inflammation/infection opted to monitor clinically relevant events. As previously discussed, a significant amount of data associated with the topic can be extracted from non-clinical models which verify significant differences in the generation of oxidative stress which are preparation, tissue and dose-dependent (510,511). However, such results did not reflect in clinical studies encompassing patients with various underlying co-morbidities following administration of either FCM, FDI or ferumoxytol. In patients with inflammatory bowel disease and iron deficiency but no anaemia (n=105), administration of FCM (500 mg) led to no change in CRP concentration after 1 month (512). Likewise, administration of FCM in a prospective multicentre non-interventional post-marketing study of patients with inflammatory bowel disease (mean dose: 1,139 mg; n=224) led to a significant decrease in CRP during the observation period of twelve months (513). Intriguingly the same author group employing a similar methodology with FDI and inflammatory bowel disease (n=197; mean dose: 1,307 mg) indicated a higher proportion of patients with a higher CRP by the end of the study (514). In data pertaining to ND-CKD, the use of such compounds and impact on inflammation can be obtained from a prospective observational study (n=50 - mean eGFR: 26.1 ml/min/1.73 m^2) following infusion with FCM (15mg/kg – max dose: 1000 mg) (226). C-reactive protein and interleukin-6 were not statistically significantly affected by the administration of iron either acutely (within 1 hour), or sub-acutely (3 weeks or 3 months). In haemodialysis, administration of ferumoxytol (total dose: 1020 mg) in individuals with iron deficiency anaemia was not related to any changes in CRP over a 28-day period of observation (515). Interpretation of these studies is limited by their observational nature, which are liable to bias and confounding (516). Kassianides and colleagues reported two pilot RCTs assessing the potential

resultant oxidative stress and inflammation that intravenous iron could have on this patient group (total n=94). Ferric derisomaltose was not associated with an increase in CRP or interleukin-6 irrespective of dose administered (87,123). These results are comparable to our current study, whereby no significant effect on CRP was seen at any point of follow-up, even though two administrations of iron were provided. This is important as ND-CKD patients may receive numerous infusions of iron throughout the year at high doses, with concerns regarding inflammation and infection severely limiting the amount of iron administered (105).

As previously discussed, a differential effect was noted between the two comparators in FGF23, phosphate and 1,25 $(OH)_2$ Vitamin D, potentially leading to a different trend exhibited in terms of markers of bone metabolism. The two comparators, however, exhibited no differential effect relevant to patient reported outcome measures, functional status and other important markers of clinical response such as haematinics and kidney function. Markers of inflammation did not exhibit a dissimilar trend, as no differential effect at any point of the study was witnessed. Previous evidence extracted from the CRIC study (n=3,879) concluded a significant association through univariate and multivariable-adjusted linear regression analyses between FGF23 and the natural log of CRP (517). However, the cross-sectional design adopted, does not allow for any causal interpretation, especially when considering the nature of confounders surrounding the study. Furthermore, the use of cFGF23 instead of iFGF23 limits the generalisability of results in terms of biologically active FGF23 in response to inflammation. As later studies suggest in murine models, in the case of CKD inflammation appears to increase FGF23, which is further amplified through the presence of iron deficiency in CKD (294). Fascinatingly, FGF23 appears to further enhance inflammation through elevated cytokine production, which in itself acts as a promoter to enhanced FGF23 transcription by osteocytes (251,518).

Smaller observational studies incorporating HD-CKD patients (n=219) failed to identify any significant correlation between cFGF23 and CRP (519). As noted by Lewerin and colleagues who correlated iFGF23 with iron stores but failed to identify a correlation with inflammation, and as alluded by the results of a number of animal studies and multivariable regression analyses, the potential of a complex interplay between bone-inflammation-anaemia exists within CKD, with a number of variables involved that do not necessarily express a unidirectional effect (520). Interventional studies failed to identify any correlation between inflammatory markers such as CRP and FGF23, both with oral and intravenous iron both in children with normal renal function and patients with ND-CKD (303,521).

There are certain limitations worth exploring in terms of inflammation in the present study. This was a pilot study which was not statistically powered to identify a significant differential effect between the two, however was set up to identify a potential differential trend. Indeed, data analysis and review of numerical values led to the conclusion that the two compounds did not have a differential effect or exhibited a specific trend. During the study period, there were instances of infection necessitating hospital admission within the FDI group, which have potentially altered the results, as infection is a cause of increasing CRP. In addition, the study did not focus on an ultra-acute/acute effect in terms of inflammation, as the study points where CRP was measured was 2 weeks following initial administration, 1 month following initial administration and 2 months following initial administration. Nevertheless, no sub-acute differential or within-group effect was noted, potentially alleviating some concern surrounding the topic of inflammation and intravenous iron use. In addition, upon monitoring the effect of therapeutic interventions on inflammation, an arsenal composed of several biomarkers can be employed, to maximise scientific yield and ensure capture of the related inflammatory pathway, which was not present during “Iron and

Phosphaturia – ExplorIRON-CKD". However, CRP is a clinically relevant marker of inflammation, which despite concerns on whether it can be employed as an indicator of chronic inflammation has been previously shown to be appropriate for patients with kidney disease (522,523). Finally, the discussed lack of association between FGF23 and CRP should not be directly extrapolated and generalised without caution, as no appropriate statistical test was employed as that was beyond the scope of the trial.

Administration of high dose intravenous iron compounds over two doses in patients with ND-CKD and iron deficiency with/without anaemia did not lead to any statistically significant, or numerically evident trend, or indeed a differential effect. Taking into consideration the potential impact of chronic low-grade inflammation of CKD and iron deficiency, alongside the concerns regarding direct pro-oxidant effect of high dose intravenous iron compounds, it is important to mechanistically consider whether infusion of such compounds that have a differential effect in terms of FGF23 may have cardiovascular impact. The next section reviews and associates the results of "Iron and Phosphaturia – ExplorIRON-CKD" relevant to cardiovascular variables ranging from blood investigations to vascular reactivity and electrocardiography both in the whole cohort and each group.

4.6: Cardiovascular impact (mechanistic study: cardiac markers, ECG and pulse wave velocity)

Nephrologists have used intravenous iron for over three decades throughout the CKD spectrum. The use of intravenous iron nonetheless, has gained momentum in cardiovascular medicine following evidence supporting its use in patients with heart failure and

iron deficiency, given its improvement in functional status and hospitalisation rates (101,102,524,525). Considering the extra-haematopoietic effects of iron deficiency, lack of iron can lead to cellular effects of myoblasts and cardiomyocytes including mitochondrial dysfunction, reduced myoglobin and increased apoptosis, causing a switch from aerobic to the less effective anaerobic metabolism. Additionally, muscle strength and cardiomyocyte contractility can be affected by iron deficiency (526). These detrimental changes secondary to iron deficiency in cardiac muscle may be amplified in uraemia – a cardinal feature of patients with CKD (527). Chronic kidney disease can be related to the uraemic cardiomyopathy phenotype, characterised by contractile dysfunction (both systolic and diastolic), and alterations to left ventricular dimensions, with both hypertrophy and dilatation present. The development of this phenotype is the result of mitochondrial dysfunction secondary to uraemia and other cardiotoxic metabolites related to CKD and chronic inflammation, hypertension, volume overload, CKD-MBD and iron deficiency (527,528). Indeed, optimal iron deficiency anaemia resolution using intravenous iron has been associated with improved cardiovascular mortality and morbidity in both ND-CKD and HD-CKD (96,97). Moreover, a safety analysis of the FERWON-Nephro RCT comparing patients based on presence of heart failure, deduced a greater absolute benefit in terms of cardiovascular risk reduction in patients with heart failure compared to those without (529).

Nevertheless, intravenous iron administration can be considered as potentially cardiotoxic, as discussion in section 1.1.6. Theories suggest that intravenous iron may express a deleterious effect on the cardiovascular system through labile iron and the resultant oxidative stress, with an impact especially on the vasculature, but also through accelerated ferroptosis of cardiomyocytes (530,531). Doxorubicin associated cardiomyopathy appears to be ferroptosis-mediated, whilst

in patients with haemochromatosis cardiomyocyte dysfunction is related to oxidative stress and reactive oxygen species formation (532–534). Furthermore, earlier murine models of uraemia have previously concluded the presence of increased oxidative stress markers within cardiac tissue within minutes of administration of low molecular weight iron dextran (535). Iron accumulation is also linked to atherosclerosis and provokes atheromatous plaque formation through action on vascular cell adhesion molecules and selectins (536–538). In patients with CKD, iron sucrose has been associated both with the induction of an increased concentration of vascular cell adhesion molecules, whilst in haemodialysis there was evidence of a dose-related increased intima-media thickness (539,540).

A number of common pathways appear to exist between CKD and cardiac dysfunction, especially in terms of inflammation, iron deficiency and disorders of bone metabolism. The treatment of iron deficiency appears safe and efficacious in both populations, with potential positive implications for quality of life, prognosis and functional status. However, concerns remain, and these become intriguing when considering the differential effect that different preparations have on bone metabolism. As previously noted (sections 1.2.3 and 1.4.3) FGF23 displays a wide array of potentially harmful cardiovascular effects, therefore assessing any provocation to such, secondary to the aforementioned differential effect, is necessary. The scope of the mechanistic cardiovascular aspect of “Iron and Phosphaturia – ExplorIRON-CKD” was to further explore any such phenomena.

In the present study baseline NT-proBNP for whole-cohort and each group of participants was above diagnostic limit for presence of heart failure/heart strain (>300.0 ng/L); NT-proBNP was non-significantly higher in the FCM group than the FDI group. These concentrations

and differences between the groups may be explained by the presence of severe CKD in the population alongside heart failure (26.9% of participants) and a statistically significant greater incidence of heart failure in the FCM group compared to the FDI group ($p<0.026$). No significant trends were exhibited throughout the study in terms of NT-proBNP either in the whole-cohort, or within each group, or as a differential effect.

Median troponin T concentration at baseline was higher than the normal cut-off for myocardial injury according to the high-sensitivity Troponin T assay used (reference range < 5.0 ng/L). In the general population, a significant rise in Troponin T above the 99th percentile of the population's reference value can alert the clinician to acute myocardial injury, with 14 ng/L defined as that point (383). High-sensitivity Troponin T appears to be raised in patients with CKD chronically, and the reasons behind this extend beyond renal clearance, into chronic low-grade myocardial injury and strain, and impaired cardiac dynamics (541,542). Studies have suggested the need for higher cut-off values in the CKD population, alongside the importance of serial troponins to identify any important myocardial injury (541,543). There was no identifiable trend within the whole-cohort, and within each group throughout the study. Importantly, administration of iron did not contribute to any acute myocardial injury observed within 1 hour of infusion, with a small decrease was noted in most patients in the study. No differential effect between the two comparators was observed, however a decreasing trend was seen within 2 weeks of administration of FDI. There was no statistically significant trend in terms of Troponin T concentrations within the whole cohort or within each group.

Electrocardiography measurements in the population were within normal parameters in terms of PR and QRS intervals (PR < 200.0 ms;

QRS: 98.0 ms). Comparative analysis of QTc in terms of normal reference values should not be attempted, as the population was composed of both male and female participants, with different cut-offs applied. There was a statistically significant difference in terms of QRS at baseline between the two groups, which can be explained by the presence of pacemaker devices in a larger proportion of the participants, which can lead to a prolonged QRS interval (544). There was no statistically significant trend in each interval within the whole population or each comparator group. A decrease in median QRS interval was noted in the FCM group between baseline and visit 5 (1-month following first intravenous iron administration), but this may be explained by the absence of ECG in one of the patients within the FCM group with a prolonged QRS interval. Median QTc remained relatively constant throughout the study, with a higher median concentration in the FCM group, compared to the FDI group, which may be secondary (similarly to QRS interval) to the presence of pacemaker devices and prolonged QRS (545). No differential effect was observed in PR and QTc intervals between the two groups; a statistically significant difference between comparator groups in terms of QRS was noted at visit 3, likely associated with the baseline difference described relevant to the difference in composition of the two groups.

Baseline PWV(cf) mean values were within the reference values described by the manufacturer enverdis® (7,0 m/s > and < 9,7 m/s). Pulse wave velocity and AIX(ao) values decreased throughout the study, both in the whole-cohort but also within each comparator group. No differential effect existed between the two groups. The decreasing trend in terms of pulse wave velocity did not reach statistical significance at any point in the study, however the trend exhibited in terms of augmentation index within the whole group of participants reached statistical significance. It is noteworthy that mean PWV(cf) measurements by the end of the study, both within each group, and

the whole population reached the optimal level reported by the manufacturer as delineated in the operational manual (< 7.0 m/s).

Despite an abundance in evidence suggestive of benefits of intravenous iron in patients with heart failure in terms of performance, quality of life and improved prognosis, only a limited number of studies addressed any cardiovascular effect using NT-proBNP. Indeed, the pivotal work by Toblli and colleagues in patients with renal insufficiency, anaemia and heart failure with reduced ejection fraction (left ventricular ejection fraction ≤35%) has provided useful information on NT-proBNP trends following administration of iron. In a randomised placebo-controlled trial 40 patients (1:1 randomisation) were allocated to receive either iron sucrose (total dose: 1000 mg) or placebo. Over the six-month period of review, NT-proBNP was reduced significantly between baseline and end-point, whilst improvements were also seen in terms of ejection fraction (100). In the randomised placebo controlled trial “Iron and Heart” focusing on patients with CKD and iron deficiency but not anaemia, NT-proBNP levels decreased from 422.5 (881.9) pg/ml to 242.5 (209.1) pg/ml within one month of 1000 mg of FDI. This was not a significant change compared to that exhibited in patients treated with placebo, however as the authors concluded the study was not designed to power to detect a significant change, and no within group analysis was made (106). A similar decrease in NT-proBNP was witnessed following administration of 1000 mg of FCM in patients with heart failure with reduced ejection fraction and iron deficiency, with no statistical significance reported (Baseline: 4829 ± 9573 pg/ml to 1-month: 2271 ± 4856 pg/ml) (546). The absence of within-group effect in the present study may be secondary to several reasons, such as the development of haemodialysis need in 3 patients in the FDI group which is associated with raised NT-proBNP, the low eGFR that also leads to raised NT-proBNP, and the statistically significant difference in composition of the two groups in terms of heart failure incidence. The relatively stationary NT-proBNP concentration,

mimics the trajectory exhibited in a recently completed RCT (FCM vs. placebo) in patients with iron deficiency and stable heart failure (n=53), whereby NT-proBNP remained stable in patients receiving FCM, but worsened in patients receiving placebo within one month of administration (FCM dose: 1000 mg) (547). The study commented on improved ejection fraction following administration of iron (which was not statistically significant) and myocardial iron repletion that appeared to be associated with improvements in terms of the Kansas City Cardiomyopathy Questionnaire score and the New York Heart Association grade described by the patients (547). Nonetheless, similar to this study, the results exhibited in the present study may be secondary to insufficient statistical power, and cannot exclude outside interference such as the increase of diuretic therapy in certain patients.

It is important to underline that no differential effect was observed despite the changes discussed in terms of iFGF23 and 1,25 $(OH)_2$ Vitamin D; this is in line with findings from IRON-Turtle, whereby despite an increase in iFGF23 induced by FCM in patients with heart failure, NT-proBNP displayed a decreasing tendency (546). Nonetheless, neither IRON-Turtle nor the present study can exclude a hyperacute/acute effect or can truly assess the potential of repeated administrations beyond a full repletion regime on NT-proBNP (546). In the previously discussed HOMeaFers RCT, comparing FDI and FCM in individuals with iron deficiency anaemia that displayed a statistically significant differential response in terms of iFGF23, there was no differential effect between the two treatments between baseline and 7 days following infusion relevant to echocardiographical values (163). Such results indicate that the differential effect observed here and in other RCTs may not provoke a clinically significant change in terms of markers of cardiac strain, however further fully powered RCTs are warranted in order to reach definitive conclusions.

Similarly, studies focusing on or exploring the effect of intravenous iron on troponin T are scarce. Previous evidence from an observational study in HD-CKD (n=78) concluded an association between elevated troponin T and iron dose (patients with elevated troponin T received significantly more intravenous iron sucrose (3692 ± 1771 mg vs. 1761 ± 1595 mg; $p < 0.001$)) even following adjustment for time on dialysis (548). These results should be interpreted with caution, given the observational nature of the study, the large interquartile range between iron doses, the absence of association to time of infusion, and the small amount of patients with raised troponin T (n=18), compared to the other participants to analysis (n=60) (548). In the absence of directly comparable results on troponin T following intravenous iron, one needs to consider potential causative mechanisms that could be related to myocardial injury, with acute increase in oxidative stress representing a potential pathological pathway. An acute dose-related increase in labile plasma iron in patients with CKD and iron deficiency with/without anaemia within 2-hours of administration has been noted, which however did not translate to any significant trends in terms of clinical events or markers of oxidative stress (87). Within the same time period (2-hours following infusion of iron), no increase in troponin T was noted in the current study, possibly indicating that the rising labile plasma iron previously noted with FDI at a dose of 1000 mg does not acutely affect the myocardium. Additionally, animal models in renal injury related mice (acute and chronic) and iron deficient mice demonstrated improved cardiac mitochondrial function with attenuation of oxidative/nitrosative stress within a potential pre-conditioned/primed oxidant status existing in iron deficiency and renal disease (527,549,550). The results of “Iron and Phosphaturia – ExplorIRON-CKD” are promising for the safety of intravenous iron in patients with CKD in terms of acute cardiac injury, given the absence of any effect on troponin T. Nonetheless, it is important to note the underpowered nature of the study, alongside potential external factors negatively affecting the trajectory of troponin T concentration (i.e., maintaining a relatively high troponin T

throughout the study), such as haemodialysis initiation, arrhythmias and infections. Moreover, the absence of a differential effect between the two comparators further complements safety and correlates with recent murine models indicating a Klotho insufficiency-related myocardial injury, rather than an FGF23 driven process (266). Nonetheless, as no correlation analysis was performed, any further associations between FGF23, 1,25 $(OH)_2$ Vitamin D, phosphate and other markers of the 6H syndrome with myocardial injury based on the discussed results should be performed with caution.

Electrocardiography was not affected throughout the study, irrespective of intravenous iron compound provided. Monitoring of intervals allows an understanding of pathophysiological implications to cardiac electrical conductance secondary to electrolyte disturbance that may predispose to arrhythmias. Indeed, hypophosphataemia has been associated with the presence of arrhythmias especially in the early stages of sepsis and cardiomyopathy (150,551). Additionally, FGF23 (that has been differentially induced by FCM in the current study) has been associated with induction of ventricular arrhythmias and prolongation of QTc in murine models (552). The HOMe aFers study, similar to the present study, did not demonstrate any significant changes in terms of QTc or QT dispersion, between FCM and FDI (163). The safety suggested by the results of “Iron and Phosphaturia – ExplorIRON-CKD” in lack of electrophysiological changes should not be underestimated, as iron deficiency even in the absence of anaemia, appears to be related to prolongation of QRS and impact on the QTc interval (553).

One disadvantage to the employed methodology was the absence of electrocardiography monitoring at every visit. Another potential problem with the approach of utilising 12-lead electrocardiograms and not telemetry/holter-monitoring is the old adage: “an ECG is a

snapshot in time", therefore not necessarily capturing any detriment occurring outside visits.

The trends exhibited in PWV(cf) and AIx(ao) are noteworthy as they highlight a potential mechanistic safety in terms of intravenous iron at high doses despite concerns regarding oxidative stress and endothelial damage. The results are consistent with previous studies conducted by the group responsible for "ExplorIRON-CKD" in patients with ND-CKD, whereby a decreasing trend was noted at least over the period of one month following administration of iron, which did not reach statistical significance (Appendix table 24) (87,106). However, upon generalising this comparison, it is important to consider the difference in iron compounds and doses provided (ExplorIRON-CKD: total 1000-2000 mg over 2 doses; Iron-CKD: 200-1000 mg over 1 dose; Iron and the Heart: 1000 mg over 1 dose). The results reported are in agreement with previous evidence in patients on peritoneal dialysis (n=38), whereby vascular reactivity was not affected by the administration of intravenous iron sucrose (300 mg) (554). Evidence from such interventional studies in patients with kidney disease and iron deficiency suggest either a lack of or an improvement of arterial stiffness in these cases, which contradicts observational evidence in patients whereby hyperferritinaemia or increased haemoglobin is associated with greater vascular resistance in the general population, patients with Type II diabetes mellitus and haemodialysis (555–559) Nonetheless, it is important to consider that such observational studies are not proof of concept and are open to certain areas of bias. Ferritin, beyond the role of iron store marker, is also affected by inflammation, with inflammatory markers not routinely monitored in those studies. In addition, iron deficient patients were not included, thereby not assessing the impact that iron deficiency has on arterial stiffness in terms of PWV, and how its potential resolution may be beneficial. Intriguingly, further solidifying the potential of either a neutral or a positive effect of iron replenishment on arterial stiffness, severity of

pulmonary arterial hypertension has been associated with greater degree of iron deficiency (560).

No differential effect was noted between the two preparations in terms of measurements of arterial stiffness, despite the effects discussed on FGF23, phosphate and 1,25 $(OH)_2$ Vitamin D. Studies and observations discussed in Appendix table 15 have failed to conclude a significant correlation between iFGF23 and arterial stiffness as measured by pulse wave velocity, despite a clearer signal in terms of cFGF23. The absence of detrimental differential effect may be multifactorial: a short-lasting increase in iFGF23 may not be able to mount a clinically relevant vascular response; additionally, the associations between cFGF23 and arterial stiffness may represent severity of disease and the presence of progressive and chronic inflammation. In addition, the absence of deterioration following a single episode of repletion dosing does not exclude the potential of impact on arterial stiffness following numerous infusions. Interestingly, the use of lanthanum carbonate, a phosphate binder that has been shown through a meta-analysis of RCTs (Trials: 10; n=687) to lower iFGF23 in HD-CKD and coronary artery calcification, was not associated with any change in PWV in a 96-week lasting randomised placebo-controlled trial in patients with ND-CKD (n=278) (561,562). This, however, may be explained by the absence of a significant iFGF23 reducing response in patients with ND-CKD as recently displayed in an RCT comparing nicotinamide, lanthanum carbonate, and placebo in 205 patients (563).

The present study has provided useful information on the cardiovascular effects of two modern intravenous irons, indicating a lack of differential effect and highlighting the potential of improved arterial stiffness with no detriment to myocardial strain or induction of clinically relevant injury. The results mechanistically complement the

cardiovascular safety of the two comparators and contribute to a further discussion on safety monitoring throughout the present study.

4.7: Safety

In total, there were eight serious adverse events, which were adjudicated as non-related to the comparator drug administration. All episodes took place at least 2 weeks after administration of each drug, and in several cases, these were related to infection. Most adverse events recorded were infections, whereby any association outside pure observation cannot be made with any comparator, at least using the evidence from other studies and the current results.

There was one death during the study (in a patient receiving FCM) which was caused by intestinal perforation. The event was arbitrated as non-related to the administration of intravenous iron secondary to the timing of the event (more than one month from second iron administration), and absence of evidence suggesting a possible mechanistic pathway for iron-inflicted intestinal wall injury. Local tissue injury secondary to intentional iron overdose via ingestion has previously been reported, however the blood results during the current episode were not indicative of raised iron (564). Moreover, intravenous iron is regularly used in patients with inflammatory bowel disease, with no reports of intestinal perforation in safety reviews in such patients (565).

On the topic of cardiovascular safety, one episode of atrial fibrillation requiring hospitalisation was witnessed in the FDI group. This episode was adjudicated as non-related to the study medication due to the circumstances surrounding the development of atrial fibrillation which

included recent hospitalisation with infected liver cyst and the initiation of haemodialysis which is a pro-arrhythmogenic environment, alongside the fact that more than one month had elapsed from administration of intravenous iron.

Review of both PHOSPHARE-IDA and HOMe aFers RCTs, showed no report of infections as adverse events, with greater focus placed on the presence of hypophosphataemia and the clinical derangement of any of the markers of 6H (162,163). In the present study, only two episodes of mild hypophosphataemia occurred, which spontaneously resolved with no associated symptomatology. Interestingly, Wolf and colleagues, as part of PHOSPHARE-IDA, monitored serum ferritin increase beyond the pre-specified limits for second administration of iron and reported a 2.6% incidence of that phenomenon in patients receiving FCM (162). Here, 1 patient (7.1%) in the FDI group could not be administered with a second dose of intravenous iron secondary to high serum ferritin.

There were no hypersensitivity reactions throughout the study, causing cessation of treatment. Previous evidence pertaining to the use of modern intravenous iron compounds such as FCM and FDI in patients with iron deficiency anaemia indicate low and comparable rates in patients with/without kidney dysfunction (8,109).

On considering the results discussed, it is important to acknowledge that the study was not designed to attribute and quantify safety patterns and signals. The results represent the safety monitoring that took place during the study period as part of the regulations set by the HRA and the MHRA. In addition, there was no standardised definition of infection with diagnosis made using clinical acumen and blood investigations (CRP, full blood count)/urine dip. The lack of

standardisation in definition of safety measures can only allow for association but not causation. Nonetheless, an adverse event does not equate to reaction – throughout the study, not all adverse events were reported as relevant to the medication by the investigators. It is also noteworthy that a separate committee within the sponsor institution, provided adjudication of causation in terms of serious adverse events, therefore negating the presence of investigator-led bias.

4.8: Limitations and the impact of the COVID-19 pandemic

4.8.1: Limitations

The study design was exploratory in nature and aimed to address questions that have not been previously studied in depth in the ND-CKD population. As such, it was not designed to provide conclusive evidence, but was able to offer further results to stimulate a better understanding of intravenous iron supplementation in patients with ND-CKD. Therefore, it is important to acknowledge that the results presented are not conclusive and should be interpreted with caution prior to any generalisability given the small number of participants with no full sampling/complete outcome data at all time-points. This limits the within-group statistical analysis. Moreover, there was no statistical power calculations.

Additionally, an intention-to-treat analysis was employed in the present study given the exploratory nature of the methodology. Intention-to-treat analysis represents a practical clinical scenario including elements of protocol deviation and non-compliance, therefore allowing

a more realistic representation of clinical practice (566). In addition, it preserves sample size. Nonetheless, intention-to-treat analysis is prone to type II error and is a potential source of heterogeneity (566).

Furthermore, the recruitment pool was small, based within the area of the East Riding of Yorkshire, Hull and Northern Lincolnshire, an area that is predominantly inhabited by White British individuals. The results discussed are relevant to a population of patients with ND-CKD stages 4-5; the low kidney function predominant in the study can be seen both as a limitation (i.e. the effects are restricted to low eGFR) and a strength, as no previous comparator study included participants with such glomerular filtration rates.

In addition, the study focused on biomarker status – despite the utility and usefulness of biomarkers and the rise of “liquid biopsy” as a theory enabling a quicker and less invasive understanding of bone metabolism, no bone density scan or biopsy took place. Similarly, no echocardiography or functional imaging of the heart or vessels occurred during the study. Certain biomarkers can be affected by diurnal variation, and despite the best attempts of the researching group to standardise visit times, this was not possible for all patients at all study points.

The present study has suggested the possibility of osteomalacia related changes in bone metabolism profile in patients receiving FCM. Nevertheless, this is not based on any objective symptomatology or imaging – future studies including observational evidence could potentially focus on further isolating long-term clinical consequences of iron administration on bone health.

In terms of functional status and quality of life, the tools employed have been previously utilised in research in CKD. However, the results observed may not be only secondary to intravenous iron infusion but also relevant to lifestyle (including: exercise, diet, etc.) and deterioration/alterations in health status outside the scope of the study. In addition, the significant difference at baseline in terms of age and heart failure may have also affected the results.

4.8.2: The impact of the COVID-19 pandemic

The study proceedings relevant to execution and data collection took place during the COVID-19 pandemic. The COVID-19 pandemic, secondary to the SARS-COV2 virus originating from Wuhan, China in late 2019 has reached 616,906,811 cases and 6,543,423 deaths (as of September 2022), and had significant impact on healthcare provision, and by-proxy research activity. Emergency guidelines through the MHRA were put into action, limiting research activity in terms of both recruitment and data collection, diverting emphasis to redeployment of healthcare professionals in support of front-line work or research associated with the COVID-19 pandemic (567). A number of studies were impacted due to the swift and necessary changes in policy; however earlier phase trials (such as the present study) appeared to be affected at a greater degree than larger counterparts (568).

Given the above, both recruitment and execution of the “Iron and Phosphaturia – ExplorIRON-CKD” study suffered throughout the pandemic, leading to two complete halts (specifically March 2020-August 2020; November 2020-December 2020) and slowing down of the rate of recruitment throughout the study. Given the previous experience of the Academic Renal Research Department of HUTH

NHS Trust in studies focusing on a similar topic and revolving around iron administration, rate of recruitment has been historically noted as between at least 3-5 patients per month. In the present study, the recruitment rate was between 1-3 patients per month, possibly secondary to research-associated, patient-associated, and provider-associated reasons. Redeployment of staff limited severely the ability to perform the study timely, whilst the healthcare provider (HUTH NHS Trust) shifted focus on provision of urgent medical care, thus restricting availability for elective interventions such as intravenous iron administration. Furthermore, the commonest reason for patients' refusal to attend screening were COVID-19 related, either directly (43.4%) or due to disruptions in travelling that arose due to the ongoing pandemic (36.7%). A potential apprehension towards participation can be understandable, especially provided that COVID-19 disease was associated with worse outcomes in patients with CKD when compared to other comorbidities as evident by cohort, systematic reviews and meta-analyses (569–571). Other research groups within rheumatology and oncology, incorporating patients that also have a degree of immunosuppression, reported similar apprehension (572,573).

To mitigate the impact of the ongoing pandemic, changes to the protocol took place enabling certain remote visits (in particular visit 8) and allowing emphasis on the use of personalised protective equipment, enhanced cleaning, and decontamination during the study. Such changes alleviated a degree of the issues relevant to recruitment and retention of participants, however increased the time demands on each individual visit. Furthermore, it led to greater attention being placed on digital enhancement of the study, with remote monitoring and recording of data permitted. The strains placed on the implementation and execution and implementation of the protocol by the COVID-19 pandemic related restrictions were met through proactive and reactive actions, in order to facilitate the delivery of the study and guarantee the safety of the participants.

Moreover, beyond the logistic and study implementation barriers posed by the pandemic, COVID-19 could also affect certain outcomes investigated throughout the study, namely functional status and health related quality of life measures. Higher psychological stress has been reported in patients with CKD during the period of the pandemic, as noted through depression, anxiety and insomnia related questionnaires (488,574).

It is important to acknowledge that trial execution suffered in a number of studies and this was perceived as a significant cause of worry for a number of researchers and investigators throughout the pandemic (575). Additionally, praise and consideration should be paid to the research staff and healthcare professionals associated with the study as they aided in the facilitation of this study despite the negative impact that COVID-19 had on clinical academics, with negative impact previously reported based on qualitative evidence on workload, career aspirations and mental health (576).

4.9: Future directions

The results of this study highlight the need for further studies within the field of intravenous iron treatment in patients with ND-CKD. It is important to mention that no significant hypophosphataemia was detected in the present study, however a differential effect was seen – it could therefore be advisable for further research to focus on hypophosphataemia incidence in order to provide a more definitive answer, encompassing and stratifying different grades of CKD. Nonetheless, given the extremely low incidence of hypophosphataemia in the present study (1.64 vs. 1.92%) an adequately powered study at 80% would require a very large sample

size (>70,000). Even taking into consideration a higher incidence of hypophosphataemia as reported by Charytan and colleagues in patients with ND-CKD and HD-CKD of 4.3% following administration of FCM, more than 1,200 patients would need to be randomised in a 1:1 fashion to detect any statistically significant difference between the two compounds (185). Given the above, the most feasible solution would be that of a multicentre trial, encompassing a population with varying kidney function and different ethnic backgrounds that could also allow for potential stratification. It is also important to consider that a clinically meaningful change in phosphate and iFGF23 does not necessarily exist, especially in the case of the latter. Indeed, true clinical significance is attached in the presence of symptomatic hypophosphataemia, or at least moderate hypophosphataemia. Despite this, the differential effect identified in terms of iFGF23, phosphate, 1,25 $(OH)_2$ Vitamin D and markers of bone metabolism warrants more research, potentially focusing on the long term implications of repeated intravenous iron infusion or using clinically relevant and objective markers of bone mineral density such as DEXA.

Upon considering the potential hypophosphataemic effect of FCM, which appears to be attenuated by decreasing kidney function, research could also be useful in the use of such agents in hyperphosphataemic patients with CKD, as a useful tool in reducing phosphate. However, the side effects on vitamin D and PTH concentration, alongside the potential impact on bone metabolism need to be closely monitored and considered where repeated administration takes place.

Some improvement was suggested in terms of functional status and quality of life following administration of intravenous iron. Indeed, certain differential results were noted, however any congruent associations between markers and changes cannot be made, given

the exploratory and not definitive nature of the study. Thus, considering the increasing interest in patient tailored care and the importance of patient reported outcome measures, further studies evaluating both the impact of iron deficiency resolution and the potential differential effect between compounds could be beneficial.

In addition, this study has complemented evidence surrounding improved arterial stiffness following administration of intravenous iron in patients with ND-CKD; this field may warrant and yield further interest with an improved understanding of potential positive implications of iron deficiency resolution and iron supplementation in vascular dynamics. Previously, intravenous iron was perceived as potentially deleterious to vascular and endothelial dynamics. Studies dedicated to vascular dynamics that are adequately powered, monitoring the administration of intravenous iron could be helpful.

As this has been an exploratory pilot study, any further studies stemming from primary and secondary outcome analyses necessitate both power calculations and further consideration to identify targets whereby further exploration prior to definitive research is needed. The latter is especially true in terms of the secondary outcomes monitored in this study, encompassing patient reported outcome measures and functional status, and cardiovascular health. Given the experience faced during the COVID-19 pandemic it is also important that any further study designed is dynamic and versatile, with maximisation of output even with limited interaction.

5: Conclusion

The present thesis discussed the differential effect of modern intravenous iron compounds in iron deficiency with/without anaemia in patients with ND-CKD. This has not previously explored in patients with ND-CKD comparatively. The notion of the 6H syndrome due to changes in iFGF23 was monitored. A significant differential effect of iFGF23 was noted within 1-2 days following intravenous iron administration, due to the large increase recorded in the FCM group. This change was associated with a non-significant decrease in phosphate, a significant decrease in 1,25 $(OH)_2$ Vitamin D and calcium, but no effects on parathyroid hormone. Despite no clinically relevant hypophosphataemia, the effects recorded on those markers alongside the impact on bone metabolism require further research to ascertain the clinical implications of administration of certain intravenous iron compounds in patients with ND-CKD over a longer period and after repeated dosing.

As part of the secondary outcome analysis of the current trial, no significant differential effect was noted in quality of life measures, functional status, markers of clinical response and cardiovascular biomarkers. Some particular signals were noted (larger improvement in aspects relevant to fatigue, physical function) with FDI. Similar efficacy was displayed by both compounds, with no negative signal identified relevant to clinical markers of kidney function and injury and cardiovascular variables. Further studies are required to elicit whether any differential effect exists secondary to intravenous iron compound used in this patient group, as has been demonstrated in patients with inflammatory bowel disease. The eagerly awaited “Iron and Muscle” trial could provide further answers as to the potential improvement in functional status of patients with iron deficiency and ND-CKD, a signal alluded in the present trial.

The study results are limited due to the small number of participants and therefore caution should be exercised in terms of generalizability of its findings, however the trends suggested warrant further research. This is particularly important considering the frequency of intravenous iron treatment in ND-CKD, alongside the potential implications of the ND-CKD process in terms of bone metabolism complications and the impact that it has on the functional status of the patient and cardiovascular prognosis.

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7: Appendix 1 – Tables

Appendix table 1: Summary of CKDOPPS consortium regarding mortality and anaemia		
Investigator led	Patients included and type	Conclusion
Locatelli et al., 2004 (14)	HD-dependent; n=4591	Each gram per decilitre of higher haemoglobin caused a significantly decreased relative risk for mortality (RR=0.95%; p=0.03)
Levin et al., 2006 (15)	ND-dependent; n=3028	Haemoglobin is an independent predictor of survival even after multi-level adjustment (RR:0.88%; p<0.001)
Karaboyas et al., 2020 (16)	HD-dependent; n=4604	Haemoglobin levels 1 month following dialysis initiation are inversely associated with mortality (adjusted HR: 0.89; p=0.001)

Appendix table 2: Haematopoietic and non-haematopoietic implications of iron deficiency	
Haematopoietic	Non-haematopoietic
• Impaired erythropoiesis • Iron deficiency anaemia	• Impairment in DNA cycle (replication, repair, cell-cycle) • Impaired energetic pathways (non-mitochondrial) • Potential neurohormonal abnormalities • Impaired immunity • Decreased muscular O_2 content (2^o to reduced myoglobulin) • Mitochondrial dysfunction
Adapted from Wish et al, 2021 (27)	

Appendix table 3: Causes of Anaemia of CKD
• Decreased erythropoietin production • Chronic inflammation • Iron deficiency • Blood loss • Reduced intestinal absorption of iron • Nutritional deficits • Shortened erythrocyte lifespan • Hyperparathyroidism • Haemolysis

Appendix table 4: Treatment targets associated with iron supplementation in CKD		
Category	ND-CKD	HD-CKD
Iron deficiency (absolute)	TSAT ≤ 20% and/or serum ferritin ≤100 µg/L	TSAT ≤ 20% and/or serum ferritin <200 µg/L
Iron deficiency (patients on ESA, or requiring blood transfusions)	TSAT ≤ 30% and/or serum ferritin < 500 µg/L	TSAT ≤ 30% and/or serum ferritin < 500 µg/L
Adapted from Gutiérrez, 2021 (54)		

Appendix table 5: Causes of ESA hyporesponsiveness
• Iron deficiency • Hyperparathyroidism • Blood loss • Haemolysis • Malignancy • Medications (inc. angiotensin converting enzyme inhibitors) • Long-term inflammation • Infection • Uraemia

Appendix table 6: Risks associated with blood transfusion in CKD
• Allosensitisation • Iron overload • Circulatory overload • Transfusion associated reactions • Hyperkalaemia • Transfusion associated errors

Appendix table 7: Third generation intravenous iron preparations			
Characteristics of currently available third generation intravenous iron formulations			
	Ferumoxytol	**Ferric Carboxymaltose**	**Ferric Derisomaltose**
Brand Name	Feraheme ®	Ferinject ®	Monofer ®
Maximum Single Dose	510 mg	1000 mg	20 mg/kg
Minimum administration time (minutes)	15	15	15
Replacement dose possible in a single infusion	No	Yes	Yes
Availability in UK	No	Yes	Yes
Comparison of physicochemical characteristics and pharmacokinetics of third generation IV irons			
Molecular weight (Ds)	185000	150000	150000
Carbohydrate ligand	Polyglucose sorbitol carboxymethylether	Carboxymaltose	Isomaltoside
Relative stability of iron carbohydrate complex	High	High	High
Reactivity with transferrin	Low	Low	Low
Relative labile iron release	Low	Low	Low
Plasma half-life (hours)	-15	7-12	20
Adapted from Bhandari et al, 2018 (47)			

Appendix table 8: Symptomatology and disease processes linked with hypophosphataemia

Musculoskeletal:

- Osteoporosis
- Osteopenia
- Osteoporosis
    - Fractures
    - Pain
    - Weakness

Neurological:

- Metabolic Encephalopathy
    - Altered mental state
    - Irritability
    - Paraesthesia
    - Numbness
    - Seizures
    - Coma

Cardiological:

- Arrhythmias

Gastrointestinal:

- Ileus
- Dysphagia

Respiratory:

- Respiratory compromise – diaphragmatic weakness:
    - Important in patients on ventilator

Appendix table 9: Summary of studies relevant to modern intravenous iron and hypophosphataemia								
Study	Design	Population	Participants/comparator drugs	Dosing	Duration	Hypophosphataemia definition	Incidence	Other relevant details
Wolf et al, 2018 (155)	RCT - sub-analysis of Adkinson et al	Iron deficiency anaemia	FCM:98; Ferumoxytol: 87	FCM: 2 x 750mg; Ferumoxytol: 2 x 510 mg	5 weeks	<0.64 mmol/L	FCM: 50.8% Ferumoxytol: 0.9%;p <0.001;	No
Bailie et al, 2010 (156)	RCT - crossover	Iron deficiency anaemia	FCM: 559; placebo: 559	Single infusion: 15 mg/kg, maximum 1000 mg	14 days	Not defined	16.0%	No
Zoller et al, 2022 (157)	RCT	Inflammatory bowel disease	FDI: 49; FCM: 48	FDI: 1500 mg or 2000 mg; FCM 1500 mg or 2000 mg on two sittings one month apart	70 days	<0.64 mmol/L	At 35 days: FDI: 8.3%; FCM: 51.0% - At 70 days: FDI: 12.5%; FCM: 59.2%	No severe hypophopshataemia noted
Makharadze et al, 2021 (158)	RCT	Oncology (non myeloid cancer)	FCM: 122; placebo: 122	2x 15 mg/kg 7 days apart; max dose: 750 mg per dose	18 week	<0.80 mmol/L	FCM: 16.0%; placebo: 3.0%	0.61 - 0.80 mmol/L: 3.3%; 0.3-0.6 mmol/L: 10.7%; marked decrease from baseline to day 7, and baseline to week 2 corresponding to infusion days. 70 patients with normal phosphate at baseline developed severe hypophosphataemia (<0.3 mmol/L)
Howard et al, 2021 (159)	RCT	Pulmonary arterial hypertension	FCM: 39; Iron dextran: 17	Not specified	12 weeks	Not defined	0.0% for both	No

Bhandari et al, 2021 (106)	RCT	ND-CKD	FDI: 26; placebo: 28,	1000 mg	3 months	Not defined	No events	No
Richards et al, 2021 (160)	RCT	Pre-operative iron deficiency anaemia	FCM: 244; placebo: 243	1000 mg	30 days	Not defined	0.4%	No
Drexler et al, 2020 (161)	RCT	Blood donors	FCM: 86; oral iron: 90	FCM: 1000 mg	84 days	<0.84mmol/L	FCM: 16.4%	No
Wolf et al, 2020 (162)	RCT	Iron deficiency anaemia	FCM: 122; FDI: 123	FCM: 750 mg x2; FDI: 1000 mg	35 days	<0.64 mmol/L	FCM: 74.4%; FDI:8.0% (p<0.001)	Significant difference between hypophosphataemia incidence at all time points (p<0.001).
Emrich et al, 2020 (163)	RCT	Iron deficiency anaemia	FCM: 13; FDI: 13	Single infusion: 20 mg/kg body weight (maximum:1000 mg)	37 days	<0.64 mmol/L	FCM: 75.0%; FDI: 8.0%; p=0.001	No
Auerbach et al, 2020 (164)	RCT	Iron deficiency anaemia	FDI: 972; iron sucrose: 494	FDI: 1000 mg single dose; iron sucrose: 200 mg up to 5 times	8 weeks	<0.64 mmol/L	FDI: 3.9%; iron sucrose: 2.3%	No
Bhandari et al, 2020 (96)	RCT	ND-CKD	FDI: 1,027; iron sucrose: 511	FDI: 1000 mg single dose; iron sucrose: 200 mg up to 5 times	10 weeks (2 weeks screenin g period)	<0.64 mmol/L	FDI: 3.2%; iron surose: 0.8%; p=0.004	Severe hypophosphataemia <0.32 mmol/L: 0.00% in both groups
Jankowska et al, 2020 (103)	RCT	Heart failure (acute)	FCM: 558; placebo: 550	Mean total dose: 1352 mg	52 weeks	Not defined	No events	Mean change in serum phosphate by week 6: FCM: -0.06 mmol/L; placebo: -0.03 mmol/L.

Santer et al, 2020 (165)	RCT	COPD	FCM:24; placebo: 24	FCM: maximum dose 1000 mg; 15 mg/kg	8 weeks	<0.80 mmol/L	FCM: 91.7%; placebo: 8.3% (p<0.001)	A significant mean treatment effect was seen with FCM (−0.46 (95% CI: −0.51 to −0.40); p<0.001).
Jose et al, 2019 (166)	RCT	Pregnant	FCM: 50; iron surose: 50	As per Ganzoni formula (maximal 1000 mg for FCM)	12 weeks	Not defined	FCM: 4.0%; iron sucrose: 6.0%	No
Ikuta et al, 2018 (167)	RCT	Iron deficiency anaemia	FCM: 119; iron sucrose: 119	Patients allocated on 1000 mg or 1500 mg: Where 1000 mg allocated: mean cumulative dose: FCM: 988.2 mg; iron sucrose: 980.0 mg. Where 1500 mg allocated: FCM: 1485.2 mg, iron sucrose: 1414.0 mg	12 weeks	Not defined	Not reported	Stated phosphate decrease: FCM 18.5%, iron sucrose: 20.2%
Adkinson et al, 2018 (168)	RCT	Iron deficiency anaemia	FCM: 1,000; Ferumoxytol: 997	FCM: 2 x 750mg; Ferumoxytol: 2 x 510 mg	5 weeks	<0.64 mmol/L	FCM: 38.7%; Ferumoxytol : 0.4%	Statistically significant difference in phosphate between FCM and Ferumoxytol at day 8, week 2 and week 5 (p<0.001) and FEPi (FCM>ferumoxytol) at day 8, week 2 (p<0.001) and week 5 (p<0.05). Results further explored through nested analysis by Wolf et al, 2018
Gybel-Brask et al, 2018 (169)	RCT	Female blood donors	FDI: 43; placebo: 42	FDI: 1000 mg	24 weeks	<0.64 mmol/L	FDI: 2.0%	No

Shim et al, 2018 (170)	RCT	Pregnancy	FCM: 46; oral iron: 44	FCM: 1500 mg	12 weeks	Not defined	0.0% in either arm	No
Seid et al, 2017 (171)	RCT	Iron deficiency anaemia	FCM: 1,023; standard medical care:1,022	FCM: 15 mg/kg (max 1000 mg) single dose	30 days	Not defined	FCM: 0.6% SMC: 0.0%	Greater proportion of patients had a drop in phosphate with FCM (0.9% vs 0%, p<0.001)
Breyman et al, 2017 (172)	RCT	Pregnant	FCM: 126; Oral iron: 126	FCM: 1000 - 1500 mg	12 weeks	<0.64 mmol/L	FCM: 8.1%; oral iron: 0.8%	No
Derman et al, 2017 (173)	RCT	Iron deficiency anaemia	FDI: 342, iron surose: 169	FDI: Body weight and then either as infusion of 1000 mg or 500 mg bolus until repleted. Iron sucrose: Ganzoni formula with repeated 200 mg infusions	5 weeks	Not defined	FDI: 1.5%; iron sucrose: 0.0%	No
Holm et al, 2017 (174)	RCT	Post-partum haemorrhage	FDI 97; oral iron: 99	FDI: 1200 mg	12 weeks	<0.64 mmol/L	FDI: 5.2%; oral iron: 2.0%	No
Mahey et al, 2016 (175)	RCT	Iron deficiency anaemia	FDI: 30; iron sucrose: 30	Ganzoni formula	12 weeks	Not defined	FDI: 50.0%; iron sucrose: 40.0%	No
Birgergard et al, 2016 (176)	RCT	Non-myeloid cancer	FDI: 231; Oral iron: 119	Ganzoni formula; either as twice max per week (1000 mg each time (infusion)) or once per week (500 mg (bolus))		<0.64 mmol/L	FDI 7.9%; Oral iron: 5.4%	

Kalra et al, 2016 (177)	RCT	ND-CKD	FDI: 233; Oral iron: 118	FDI: Ganzoni formula; either 1000 mg infusion or 500 mg bolus until replete	8 weeks	<0.64 mmol/L	FDI: 1.7%; Oral iron: 0.9%	No
Roberts et al, 2016 (178)	RCT	HD-CKD	FCM: 22; iron sucrose: 20	FCM: 200 mgl iron sucrose: 200 mg	42 days	Not defined	Nil stated	Significant decrease in phosphate between baseline and day 2 with FCM (p=0.03).
Johansson et al, 2015 (179)	RCT	Cardiac surgery (non-anaemic)	FDI:30 Placebo: 30	FDI: 1000 mg	4 weeks	<0.64 mmol/L	Nil identified	No
Bhandari et al, 2015 (180)	RCT	HD-CKD	FDI:234; IS: 117	FDI: either single 500 mg bolus or 500 mg split; iron sucrose: 500 mg split	8 weeks	<0.64 mmol/L	FDI: 1.3%; iron sucrose: 2.6%	No
Onken et a, 2014 (181)	RCT	Iron deficiency anaemia	FCM: 503; Oral iron: 257; standard medical care: 251	FCM: 2 x 750 mg	35 days	Not defined	FCM: 4.6%	No
Onken et al , 2014 (182)	RCT	ND-CKD	FCM: 1276 IS: 1285	FCM: 2 x750 mg; iron sucrose: 5 x 200 mg (max)	56 days	Not defined	FCM: 18.5%, iron sucrose: 0.8%	No

Macdougall et al, 2014 (183)	RCT	ND-CKD	FCM: 305; Oral iron: 308	FCM: targetting high ferritin or low ferritin. FCM high ferritin: initial single dose: 1000 mg (or 500 mg x 2 weight dependent). FCM low ferritin: 200 mg IV if ferritin <100µg/L. During Weeks 4–48: FCM high ferritin: every 4 weeks 500 mg iron if ferritin was in the range 200 to <400 µg/L, or 1000 mg iron if ferritin was <200 µg/L; FCM low ferritin: every 4 weeks 200 mg if ferritin was <100 µg/L.	52 weeks	Not defined	Nil stated	Drop in phosphate noted in figure S4 of appendix at 4, 8, 12, 24, 36, & 52 weeks with FCM
Favrat et al, 2014 (184)	RCT	Iron deficiency anaemia	FCM: 144; placebo: 146	FCM: 1000 mg	56 days	<0.80 mmol/L	86.0%	Resolved spontaneously in the majority of patients by the end of the study – 91.9%
Charytan et al, 2013 (185)	RCT	CKD (HD and ND-CKD)	FCM:204; standard medical care:259	15 mg/kg to a maximum of 1000 mg IV; if on HD (50 patients) received 200 mg bolus	30 days	Not defined	FCM: 4.3%; standard medical care: 0.1%	No

Hussain et al, 2013 (186)	RCT	Iron deficiency anaemia	FCM:82; iron dextran: 78	Single maximum dose (15 mg/kg body weight up to 750 mg) administered weekly until the total iron requirement (calculated by the Ganzoni formula) or a maximum of 2 250 mg was reached.	7 weeks	<0.64 mmol/L	FCM: 8.5% iron dextran: 0%; p<0.05	Significantly greater mean decrease from baseline to final value with FCM ($p \leq 0.001$)
Wolf et al, 2013 (187)	RCT	Iron deficiency anaemia	FCM: 25; iron dextran: 30	Single dose 15mg/kg or up to 1000 mg	35 days	<0.64 mmol/L	FCM: 40.0% iron dextran: 0.0%	No
Reinisch et al, 2013 (188)	RCT	Inflammatory bowel disease	FDI: 225; Oral iron: 113	FDI: according to Ganzoni formula	8 weeks	<0.64 mmol/L	FDI: week 2: 7.0%; week 8: 1.0%; Oral iron: week 2: 1.0%; week 8: 1.0%	No

Barish et al, 2012 (189)	RCT	Iron deficiency anaemia	FCM:360; standard medical care: 366	Multi-dose (FCM 15 mg/kg up to a single dose of 750 mg at 100 mg per minute weekly until the calculated iron deficit dose had been administered (to a maximum cumulative dose of 2,250 mg) and single dose (750 mg FCM or 15 mg/kg, whichever was smaller)	Multi-dose: 42 days; Single-dose: 30 days	<0.64 mmol/L	FCM:7.0%; standard medical care: 0.0%, p<0.001	No
Evstatiev et al, 2011 (190)	RCT	Inflammatory bowel disease	FCM:244 ; iron sucrose:239	FCM: 3 x 1000 or 500 mg; iron sucrose: 11 x 200 mg (Ganzoni based)	12 weeks	Not defined	FCM: 2.5%; iron sucrose: 0.0% p=0.03	No
Allen et al, 2011 (191)	RCT	Restless legs syndrome	FCM: 24; Placebo: 22	FCM: 1000 mg (2 x 500 mg) and where needed an extra 500 mg; Placebo: initially palcebo but at day 28 single dose of FCM (1000 mg)	24 weeks	Not defined	12.5%	
Goodnough et al, 2009 (192)	RCT	Iron deficiency anaemia	FCM: 228; Oral iron: 225	FCM: as per Ganzoni formula (mean total dose: 1568 mg); Oral iron: 325 mg TDS	42 days	<0.64 mmol/L	70.1%	Lowest phosphate recorded: 0.29 mmol/L at day 21. Median time from baseline to nadir was 15 days.

Van Wyck et al, 2007 (193)	RCT	Post-partum	FCM: 169; Oral iron: 168	Mean dose FCM: 1403.1 mg	42 days	Not defined	Not provided - stated no change in mean phosphate	FCM: Δphosphate from baseline, – 0.36 +/- 0.25 vs. oral: 0.00 +/- 0.24 mmol/L; p<0.001
Kawabata et al, 2022 (194)	Randomized uncontrolled open-label study	Iron deficiency anaemia	FDI: 40	Total: 1500 mg	14 weeks	<0.64 mmol/L	10.0%	Mean serum phosphate value above 0.80 mmol/L throughout the study except week 2. No severe hypopophosphataemia.
Kawabata et al, 2022 (195)	Randomized uncontrolled open-label study	Iron deficiency anaemia	FDI: 238; Saccharated ferric oxide: 119)	FDI: 1000 mg or 20 mg/kg; Saccharated ferric oxide: 120 mg per visit to achieve repletion	12 weeks	<0.64 mmol/L	FDI: 8.4%; Saccharated ferric oxide: 83.2%	No severe hypophosphataemia (< 0.32 mmol/L) with FDI; severe hypophosphataemia in 6.7% with saccharated ferric oxide
Malone et al, 2014 (196)	Pooled analysis (from 5 RCTs)	Iron deficiency anaemia (bariatric)	FCM: 123; standard medical are: 126	N/A	N/A	Not defined in manuscript	FCM: 4.9% ; standard medical care: 0.05	No
Auerbach et al, 2022 (197)	Pooled analysis (from 2 RCTs)	Bariatric patients	FDI: 93; iron sucrose:66	Mean dose: FDI:1119 mg; iron sucrose: 937 mg	4 weeks	<0.64 mmol/L	Week 1: FDI: 0.0%, iron sucrose: 1.6%; Week 2: FDI: 3.3%, iron sucrose: 0.0%; Week 4: 0.0% in both cases	No severe hypophosphataemia (conc < 0.32 mmol/L)
Jesus-Silva et al, 2020 (198)	Observational - real world data	HD-CKD	190 patients (doses: FDI: 4,1295 prescriptions; iron sucrose: 14,685 doses)	N/A	12 months	Not defined	No events	No

Fragkos et al, 2020 (199)	Observational - real world data	Iron deficiency anaemia	FCM: 162	Median dose: 1000 mg	90 days	<0.80 mmol/L	87.0%	Mild hypophosphataemia:50.3% ; Moderate (<0.65 mmol/L): 33.7; Severe (≤0.32 mmol/L): 3.0%
Udina et al, 2022 (200)	Observational	Iron deficiency anaemia	FCM: 8	Median dose: 18 mg/kg	Up to 40 months	Not defined	12.5%	Nil
Dashwood et al, 2021 (201)	Observational	Heart failure	FCM: 173	Maximum of 1000 mg	60 days	<0.64 mmol/L	27.0%	Severe hypophosphataemia (0.4 - <0.64mmol/L) 44 patients (25%); Extreme (<0.4 mmol/L): 3 patients (2%); identified reduced creatinine clearance as a protective factor; median time to nadir 8 days (interquartile range: 4-16 days).
Kramer et al, 2021 (202)	Observational	Pulmonary arterial hypertension	FCM: 117	Maximum of 1000 mg	18 months	Not defined	No events	No
DelRosso et al, 2021 (203)	Observational	Restless legs	FCM: 39	Maximum of 750 mg	8 weeks	No defined	No events	
Detlie et al, 2021 (204)	Observational	Inflammatory Bowel Disease	FDI: 54; FCM: 52	Extension of previous study				No
Minutolo et al, 2021 (205)	Observational	CKD (ND-CKD)	FCM: 54	At least 500 mg (single)	24 weeks	<0.65 mmol/L	3.7%	No
Cococcioni et al, 2021 (206)	Observational	Inflammatory bowel disease (paediatric)	FCM: 129	Weight dependent	12 weeks	Age dependent - severe:0.3 - 0.59 mmol/L	19.5%	No symptomatology was reported even with severe hypophosphataemia

Kirk et al, 2021 (207)	Observational	Iron deficiency anaemia (paediatric)	FCM: 225	Not specified	6 weeks	<0.64mmol/L ; severe : <0.32 mmol/L	18.0%	No incidence of severe hypophosphataemia
Pasricha et al, 2021 (208)	Observational	Iron deficiency anaemia	FCM: 121	1000 mg	4 weeks	<0.60 mmol/L	32.2%	Serum phosphate dropped from 1.14 mmol/L at baseline to 0.70 mmol/L after 2 weeks
Ding et al, 2020 (209)	Observational	Iron deficiency anaemia	FCM:24	Escalation study: 12 participants: 500 mg; 12 participants: 1000 mg	Not stated	< 0.80 mmol/L	75.0%	Low dose cohort: 58.3%; high dose cohort: 91.7% One episode of severe hypophosphataemia in high-dose cohort (8.3%)
Abdel-Razeq et al, 2020 (210)	Observational	Oncology (chemotherapy)	FCM: 84	1000 - 2000 mg (single dose up to 1000 mg with subsequent dose as needed)	12 weeks	< 0.64 mmol/L	46.4%	All asymptomatic - greater incidence of hypophosphataemia in patients with absolute as opposed to functional iron deficiency anaemia (65.4% vs. 25.0%; p=0.04)
Fang et al, 2020 (212)	Observational	Inflammatory bowel disease and control	FCM: 44 (IBD: 24; control: 20)	1000 mg	28 days	<0.80 mmol/L	72.7%	Moderate to severe hypophosphataemia (<0.60 mmol/L): 55%.
Frazier et al, 2020 (211)	Observational	Iron deficiency anaemia	FCM: 16	750 mg x 2	5 weeks	<0.81 mmol/L	87.5%	Severe hypophosphataemia (<0.32 mmol/L): 25%
Schoeb et al, 2020 (213)	Observational	Bariatric patients	FCM: 52	Single dose: 500 or 1000 mg (median 500 mg)	12 weeks	<0.80 mmol/L	29.0%	Moderate to severe hypophosphataemia (<0.60 mmol/L): 21%.
Ionescu et al, 2020 (214)	Observational	Surgical with iron deficiency anaemia	FCM:329; red blood cells: 342	maximum dose 1000 mg	4 weeks	Not defined	9.4%	No

Ikuta et al, 2019 (215)	Observational	Gastroenterology	FCM: 39	500 mg per dose: dosage requirement as: 1000 mg: Hb level = 10 g/dL + body weight < 70 kg; 1500 mg: all other subjects iron for other subjects	12 weeks	<0.81 mmol/L	92.1%	Severe hypophosphataemia <0.32 mmol/L: 5.13%
Detlie et al, 2019 (216)	Observational	Inflammatory bowel disease	FCM:52; FDI: 54	Single dose: 1000 mg	6 weeks	<0.80 mmol/L	At week 2: FCM: 72.5%, FDI: 11.3% (p<0.001). At week 6: FCM: 21.6%, FDI: 3.7% (p=0.0013)	Moderate to severe hypophosphataemia (<0.65 mmol/L): At week 2: FCM: 56.9%, FDI: 5.7%; p<0.001. At week 6: FCM: 13.7%, FDI: 1.9%; p=0.054.
Sivakumar et al, 2019 (110)	Observational	ND-CKD	FDI: 708; ID: 783	Dose range: FDI:1000 – 1500 mg; ID: 750 mg - 1500 mg	182 days	Not defined	Not reported	Levels of phosphate were not significantly affected after administration of iron
Stohr et al, 2018 (217)	Observational	Heart failure	FCM: 23	Single dose: 1000 mg	28 days	<0.80 mmol/L	60.9%	More evident hypophosphataemia in non-CKD patients.
Huang et al, 2018 (218)	Observational	Female iron deficiency anaemia + CKD + control	FCM: 65 (control=20;pregnant=20;CKD=25)	Single dose: 1000 mg	42 days	Not defined	Not reported	No

Hofman et al, 2018 (219)	Observational	HD-CKD	221 (switched from iron sucrose to FCM)	Weekly doses: FDI: 48 mg/week vs IS: 55 mg/week; p=0.04	15 months	Not defined	Not reported	Non-significant drop in phosphate (0.03 mmol/L) noted
Bager et al, 2017 (220)	Observational	Gastroenterology	FCM: 192 infusions; FDI: 116 infusions; 39 patients received both types	Median dose: 1000 mg	10 weeks	<0.64 mmol/L; severe: <0.32 mmol/L	Moderate: At 2 weeks: FCM: 69 patients; FDI: 9 patients (p<0.001). At 5 weeks: FCM: 37 patients, FDI: 6 patients (p<0.001). Severe exclusively at the FCM group (13 at week 2, 4 at week 5)	Greater phosphate drop (>50%) following FCM than FDI at week 2 and week 5 (p<0.001)
Sari et al, 2017 (221)	Observational	Kidney transplant	FCM: 23 patients (+2 index cases)	Mean dose: 896 mg (median: 1000mg)	N/A	Not defined in manuscript but defined severe hypophosphataemia as <0.5 mmol/L	56.5%; severe in 34.8%	Median time to hypophosphataemia: 15 days (3-24); Median duration of hypophosphataemia: 41 days (2-99)

Dahlerup et al, 2016 (222)	Observational	Inflammatory bowel disease	FDI:21	1500 mg: 7 patients; 2000 mg: 8 patients; 2500 mg: 4 patients; 3000 mg: 2 patients	Group A: 10 weeks; Group B: 18 weeks	<0.64 mmol/L	9.5%	No
Schaefer et al, 2016 (223)	Observational	Gastroenterology	FCM: 55l FDI: 26	Dosage was divided into 500 mg, 1g and >1g	N/A	<0.8 mmol/L; severe: <0.6 mmmol/L; life-threatening: <0.3 mmol/L	FCM: 45.5%; FDI: 3.9%	Severe and life-threatening only in FCM group (29.1% and 3.6% respectively)
Toledano et al, 2016 (224)	Observational	Hematological and solid tumors	FCM: 367	Median dose: 1000 mg	N/A	Not defined	6.1%	No
Hardy et al, 2015 (225)	Observational	Iron deficiency anaemia	FCM: 78; iron sucrose: 52	FCM: mean dose: 2123 mg (quartile: 1000–2000 mg).;iron sucrose: mean dose 701 mg (quartile 200–800)	N/A	< 0.64 mmol/L	FCM: 51.0%; iron sucrose: 22.0%	Severe: <0.32 mmol/L: 13%; FCM dose was associated with hypophosphataemia. Mean hypophosphatemia duration was 6 months (2–9 months). Fatigue worsening reported in 30% of FCM-induced hypophosphatemia cases.
Prats et al, 2013 (226)	Observational	CKD (ND-CKD)	FCM: 47	Mean dose: 971.7 mg	12 weeks	Not defined	Not stated	Significant decrease in phosphate from baseline to week 3 (1.36 mmol/L to 1.16 mmol/L; p<0.0001). 74.5% experienced a change in phosphate.
Adapted from Kassianides and Bhandari 2021 (150)								

Appendix table 10: Risk factors associated with the development of intravenous iron induced hypophosphataemia
Bone-metabolism associated factors: • Low baseline phosphate • Vitamin D deficiency • Hyperparathyroidism Kidney function associated factors: • Normal renal function Alimentary canal associated factors: • Malabsorption • Bariatric surgery • Malnourishment Iron deficiency associated factors: • Anaemia severity

Appendix table 11: Factors affecting phosphate renal regulation	
Increase PO_4^{3-} absorption	Decrease PO_4^{3-} absorption
• Low phosphate diet • 1,25 $(OH)_2$ Vitamin D • Thyroid hormone	• Parathyroid hormone • Fibroblast growth factor 23 • Low potassium • Glucocorticoids • Hypertension • Oestrogen • Metabolic acidosis • Dopamine
Adapted from Blaine et al, 2015 (232)	

Appendix table 12: Stimulators of FGF23 production	
Classical	Non-classical
Parathyroid Hormone Hypercalcaemia 1,25 $(OH)_2$ Vitamin D High dietary phosphate content	Inflammation Erythropoietin Iron Deficiency

Appendix table 13: Summary of studies relevant to intravenous iron and the 6H syndrome										
Study	Design	Population	Compound used /participants	Dosing	Blood sampling	Findings related to FGF23	Association with phosphate	Association with PTH	Association with vitamin D	Association with calcium
Zoller et al, 2022 (157)	RCT	Inflammatory Bowel Disease	FCM: 48; FDI: 49	1500 - 2000 mg (Initial dose: 1000 mg)	Baseline, Day 7, Day 14, Day 35 (second infusion), Day 42, Day 49, Day 70	iFGF23: FCM: significant ↑. FDI: no change Maximum increase noted 1 day post administration. A statistically significant difference between the mean changes was noted at all points except day 70.	FCM: Significant ↓; FDI group: No change. Significant difference in change between FDI and FCM at all points.	FCM: ↑ FDI: mild ↓. Significant difference between the change cause at day 7 and day 14	1,25 $(OH)_2$ Vitamin D: FCM: ↓↓ > FDI: ↓ (both significant). Significant difference in mean change in 1,25 $(OH)_2$ Vitamin D at all points except day 35 and day 70/ . Both 24(R),25 $(OH)_2$ Vitamin D and 25$(OH)_2$ Vitamin D: FCM: ↑↑ > FDI: ↑.	FCM: ↓; FDI: no significant change; statistically significant difference in change at day 14 and day 35.
Emrich et al, 2020 (163)	RCT	Iron Deficiency Anaemia (female)	FCM: 13; FDI: 13	Single infusion: 20 mg/kg body weight (maximum:1000 mg)	Baseline, Day 1, Day 7, Day 35	iFGF23: FCM: significant ↑; FDI: no change. cFGF23 ↓in both groups. Significant difference in change at day 1 and day 7 in iFGF23 and cFGF23.	FCM significant ↓; FDI: significant ↓. Greater ↓with FCM significant at day 7. Significantly more hypophosphataemia with FCM than FDI (75% vs. 8%).	FCM: no significant change; FDI: no significant change.	1,25 $(OH)_2$ Vitamin D: FCM: ↓↓ > FDI: ↓ (both significant). Significant difference in change rate. No significant difference between the changes caused by each comparator.	FCM: no significant change; FDI: no significant change. No significant difference in change caused.

Wolf et al, 2020 (162)	RCT	Iron Deficiency Anaemia	FCM: 122; FDI: 123	FCM: 750 mg x2; FDI: 1000 mg	Baseline, Day 1, Day 7, Day 8, Day 14, Day 21, Day 35	iFGF23: FCM: significant ↑; FDI: no change. Significant difference in change caused throughout study. cFGF23: FDI ↓↓> FCM: ↓ (both significant).	FCM ↓↓ > FDI: ↓. Significant difference between changes caused by comparators.	FCM: ↓; FDI: no change. Significant difference in mean change caused at day 14, 21 and 35.	1,25 $(OH)_2$ Vitamin D: FCM: ↓↓ > FDI: ↓; Significat difference in mean change caused. 25$(OH)_2$ Vitamin D: No change in either group. 24,25 $(OH)_2$ Vitamin D: FCM ↑ > FDI: no change. Significant difference in change caused from day 7 onwards.	FCM: significant ↓; FDI: no significant change (ionised calcium measured).
Wolf et al, 2018 (155)	RCT - sub-analysis of Adkinson et al	Iron Deficiency Anaemia	FCM:98; Ferumoxytol: 87	FCM: 2 x 750mg; Ferumoxytol: 2 x 510 mg	Baseline, Week 1, Week 2, Week 5	iFGF23: FCM: significant ↑. Significant difference in percentage change between FCM and ferumoxytol. cFGF23 ↓in both groups.	Significant correlation between iFGF23 and phosphate from baseline to week 2 (ρ=-0.57; p<0.001) and baseline to week 5 (ρ= -0.33; p<0.001). Significantly more hypophosphataemia with FCM than ferumoxytol (50.8% vs. 0.9%).	FCM: ↑↑ (statistically significant); Ferumoxytol: mild ↑.	1,25 $(OH)_2$ Vitamin D: FCM: ↓↓ (statistically significant) > Ferumoxytol: ↓. 25 $(OH)_2$ Vitamin D: No significant changes in either group.	FCM: significant ↓; Ferumoxytol: mild ↓. Significant difference in change in calcium from baseline to week 2.

Fukao et al, 2018 (301)	RCT	HD-CKD	Saccharated ferric oxide: 32; Oral ferric citrate: 29	Oral: 50 mg/day; intravenous: 40 mg/week	Baseline, Week 10	iFGF23: Saccharated ferric ocide: significant ↑. cFGF23: significant ↓ both preparations. iFGF23:cFGF23: ↑both groups; saccharated ferric oxide > ferric citrate.	No significant change in either group.	No significant change in either group.	1,25 $(OH)_2$ Vitamin D: Significant ↓ in both groups.	No significant change in either group.
Roberts et al, 2016 (178)	RCT	HD-CKD	FCM: 22; iron sucrose: 20	FCM: 200 mg; iron sucrose 200 mg	Baseline, Day 2, Day 7, Day 21, Day 42	iFGF23: FCM: significant ↑; Iron sucrose: No significant change. cFGF23: FCM: significant ↓; iron sucroe: no significant change. No significant pair-wise difference at specific time points.	FCM: significant ↓; iron sucrose: No significant change. No significant pair-wise difference at specific time points.	Not recorded	Not recorded	Not recorded
Wolf et al, 2013 (187)	RCT	Iron Deficiency Anaemia (female)	FCM: 25; Iron dextran: 30	Single dose 15mg/kg or up to 1000 mg	Baseline, Day 1, Day 7, Day 14, Day 35	iFGF23: FCM: significant ↑ > Iron dextran: mild ↑. Statistical significance in percentage change at day 1 and 7. cFGF23 significant ↓ in both groups.	Hypophosphataemia was only present in the FCM group.	No significant changes in either group. FCM: mild ↑.	1,25 $(OH)_2$ Vitamin D significant ↓ in both groups. $25(OH)_2$ Vitamin D: no significant change in either group.	FCM: significant ↓; iron dextran: mild ↑. Maximal effects seen by day 7.

Detlie et al, 2021 (204)	Observational	Inflammatory Bowel Disease	FDI: 54; FCM: 52	Single dose: 1000 mg	Baseline, Week 2, Week 6	iFGF23: FCM: ↑; FDI: no change. Significant difference in iFGF23 change between baseline and week 2 (FCM>FDI). cFGF23: ↓ in both groups (FDI>FCM). No significant difference between groups.	FCM ↓; FDI: no change. Significant difference in change caused at week 2 (FCM driven).	No significant changes in either group.	1,25 $(OH)_2$ Vitamin D: FCM: significant ↓; FDI: no significant change. Significant difference in change noted. 25 $(OH)_2$ Vitamin D: no significant difference between groups.	FCM: significant ↓; FDI: no change (Ionised calcium). No statistical difference in change caused.
Schoeb et al, 2020 (213)	Observational	Bariatric patients	FCM: 52	Single dose: 500 or 1000 mg (median 500 mg)	Baseline, Week 1	iFGF23: significant ↑. cFGF23: significant ↓.	Significant ↓	No significant change	1,25 $(OH)_2$ vitamin D: significant ↓. 25 $(OH)_2$ vitamin D: no significant change.	No significant change.
Frazier et al, 2020 (211)	Observational	Iron Deficiency Anaemia (female)	FCM: 16	750 mg x 2	Baseline, Week 1, Week 2, Week 5	iFGF23: significant ↑ by week 2. cFGF23 significant ↓ by week 2.	Significant ↓	Significant ↑ (baseline to week 2 and baseline to week 5)	1,25 $(OH)_2$ vitamin D: significant ↓ (baseline and week 1 and week 2)	Significant ↓ (baseline to week 1 and week 2).
Fang et al, 2020 (212)	Observational	Inflammatory Bowel Disease	FCM: 44 (Inflammatory Bowel Disease: 24; control: 20)	1000 mg	Baseline, Day 2, Day 4, Day 7, Day 14, Day 28	FCM: iFGF23: significant ↑ within 2 days. cFGF23: significant ↓.	FCM: significant ↓.	Not recorded	No significant changes or correlations noted.	Not recorded
Honda et al, 2019 (302)	Observational	HD-CKD	No iron: 84; Ferric citrate/Sucroferric oxyhydroxide	IV iron: 40 mg/week - oral iron: daily dose (not specified)	Baseline, Day 3, Day 5, Day 7, Day 14	Saccharated ferric oxide: significantly ↑↑ iFGF23 compared to oral	No significant change.	Nil correlation with iFGF-23	No significant changes or correlations noted.	Not recorded

			(oral): 17 ; Saccharated Ferric oxide (intravenous) : 22			groups throughout study.				
Muras-Szwedziak and Nowicki, 2018 (303)	Observational	ND-CKD	Saccharated Ferric Oxide: 35	IV iron: 100mg/24h over 5 days	Baseline, 2 hours after first infusion, day 3, 2 hours after 3rd infusion and day 6	iFGF23: significant ↑; cFGF23: significant ↓.	Significant ↓	Non-significant ↓.	Not recorded.	No significant changes.
Stohr et al, 2018 (272)	Observational	Heart failure	FCM (CKD: 12; non-CKD: 11)	Single dose: 1000 mg	Baseline, Post infusion, Day 1, Day 7, Day 14, Day 28 (CKD only)	iFGF23: ↑; cFGF23: ↓ (non-CKD: significant for both; CKD: non-significant for either).	Significant ↓ (non-CKD cohort)	Not recorded	$1,25(OH)_2$ Vitamin D: Significant ↓ (both cohorts). $25(OH)_2$ Vitamin D: no significant change.	Not recorded
Huang et al, 2018 (218)	Observational	Iron Deficiency Anaemia (female) + ND-CKD + control	FCM: 65 (control=20;pregnant=20; CKD=25)	Single dose: 1000 mg	Baseline, Day 2, Day 7, Day 21 , Day 42	iFGF23: significant ↑ (all groups). cFGF-23 ↓(all groups). iFGF23:cFGF23: significant ↑ (all groups).	Significant correlation between iFGF23 and phosphate (baseline to week 2 and week 5). Significant ↓ (all groups).	Control: Significant ↑. Pregnancy: no significant change. CKD: mild ↑ (significant at day 2).	$1,25\ (OH)_2$ Vitamin D: Significant ↓ (all groups). $25(OH)_2$ Vitamin D: No significant changes.	Not recorded
Tan et al, 2017 (304)	Observational	Iron Deficiency Anaemia (HD-CKD and non-CKD)	Iron polymaltose: 24 (ID: 9 HD: 15)	ID: 500 - 1000 mg; HD: 100 mg/month	Baseline, Day 4, Day 12	HD: iFGF23 and cFGF23: Non-significant ↑; iFGF23:cFGF23: no change. Non-CKD: iFGF23: no changes; cFGF23:	No significant changes in either group.	Not recorded	Not recorded	No significant changes in either group

						significant ↓; iFGF23: cFGF23: significant ↑.				
Dahlerup et al, 2016 (222)	Observational	Inflammatory Bowel Disease	FDI: 21	1500 mg: 7 patients; 2000 mg: 8 patients; 2500 mg: 4 patients; 3000 mg: 2 patients	Group A: Baseline, Week 1, Week 4, Week 8; Group B: Week 1, week 4, week 8 week 9, week 12, week 16	iFGF23: No significant changes.	Non-significant change (mild ↓)	Not recorded	Not recorded	Not recorded
Prats et al, 2013 (226)	Observational	ND-CKD	FCM: 47	Mean total dose: 971.7 mg	Baseline, Week 3, Week 12	cFGF23: significant ↓.	Significant ↓.	No significant change (mild ↓)	Not recorded	No significant change
Hryszko et al, 2012 (305)	Observational	HD-CKD	Iron dextran: 12	Individually	Baseline, Week 1, Week 3	iFGF23: significant ↑.	No significant changes	Significant ↓.	Not recorded	No significant change
Takeda et al, 2011 (297)	Observational	HD-CKD	Saccharated ferric oxide: 27	120 mg / week	Baseline, Week 1, Week 3, Week 5	iFGF23: significant ↑.	No significant changes	Significant ↓.	No significant changes	No significant change
Schouten et al, 2009 (296)	Observational	Iron Deficiency Anaemia	Iron polymaltose: 8	500-1600 mg	Baseline, Week 1, Week 2, Week 3	iFGF23: significant ↑.	Significant ↓.	No significant change (mild ↑)	$1,25\ (OH)_2$ Vitamin D: significant ↓. $25\ (OH)_2$ Vitamin D: no significant change	No significant change (mild ↓)

Appendix table 14: Disorders of FGF23 metabolism			
Disorder	Hereditary/Acquired	Effect on phosphate	Presentation
Disorders of increased FGF23			
X-linked hypophosphataemic rickets	Hereditary	Low	Limb deformities, dental disease, osteoarthritides, enthesopathies, pseudofractures, skull malformations and neurological sequale
Autosomal dominant hypophosphataemic rickets	Hereditary	Low	Activity fluctuates – dependent on iron status and on onset of disease. Lower extremity deformities, bone pain, weakness, insufficiency fractures,
Autosomal recessive hypophosphataemic rickets	Hereditary	Low	Short stature, skeletal deformities, dental abnormalities, bone, and joint pain, enthesopathies. Ectopic brain calcification may also happen.
Jansen's metaphyseal chondrodysplasia	Hereditary	Low	Form of dwarfism due to growth abnormalities; biochemically similar to primary hyperparathyroidism in the absence of PTH increase

Osteoglophonic dysplasia	Hereditary	Low	Form of dwarfism, associated with craniofacial defects and bone lesions.
Mc-Cune Albrigh syndrome – polyostotic fibrous dysplasia	Acquired	Low (in 50% of cases)	Triad of fibrous dysplasia, café-au-lait macules, and precocious puberty. Renal phosphate wasting when high degree of bone involvement.
Tumour-induced osteomalacia	Acquired	Low	Vague, long-standing muscle weakness and bone pain, accompanied by fractures and fatigue. Mesenchymal origin tumours that can occur in both children and adults.
Epidermal naevus syndrome	Acquired	Low	Neurocutaenous syndrome with skeletal deformities. The neurological symptoms are associated with seizures hemimegalencephaly and intellectual disability.
Iatrogenic – certain intravenous iron modalities	Acquired	Low	Pain, pseudofractures, proximal myopathy, fatigue.
Disorders of decreased FGF23			
Familial tumoural calcinosis / Hyperostosis-	Hereditary	High	Pain, ulceration, vascular injury, ectopic calcifications (e.g. ocular and,

Hyperphosphataemia syndrome			subcutaneous tissue), dental problems and symptoms of systemic involvement.
Acquired tumoural calcinosis	Acquired	High	As above

Appendix table 15: Studies relevant to FGF23 and vascular calcification in humans					
Study	Population	Participants	FGF23 assay	Mode of Quantification	Result
Desjardins et al, 2012 (323)	CKD (various stages)	142	iFGF23	• Aortic calcification score (spiral CT) • Pulse wave velocity	Significant relationship following multivariate analysis between iFGF23 and Aortic calcification score but not pulse wave velocity(p=0.008)
Salam et al, 2021 (349)	ND-CKD and healthy controls	69 CKD; 68 healthy	iFGF23	Lower leg arterial calcification (CT imaging)	Significant correlation (rho=0.40, p=0.001)
Fitzpatrick et al, 2020 (350)	HD-CKD	391	iFGF23	Coronary artery score Pulse wave velocity	Coronary artery score: 1.12-fold per increase of 100 logRu/ml in FGF23; 95%CI: 1.02-1.34 No association with pulse wave velocity
Krishnasamy et al, 2017 (351)	ND-CKD	40	iFGF23	Pulse wave velocity	Using logistic regression every 1 pg/mL increase in log transformed FGF23 was associated with 2.9-fold increase in odds of abnormal pulse wave velocity
Mirza, Larsson et al, 2009 (352)	Elderly individuals (>70 years old)	967	cFGF23	• Invasive forearm technique • Pulse wave velocity	FGF23 was weakly associated with impaired endothelium-dependent (beta=-0.08, $p<0.05$) and endothelium-independent (beta=-0.08, $p<0.01$) vasodilation. The relationship was enhanced at eGFR < 60 ml/min/1.73m^2

Shah et al, 2015 (353)	Non-CKD	1,512	cFGF23	2-d carotid ultrasound imaging	Linear regression models indicated that log FGF23 (was associated with greater odds of plaque presence and plaque area
Holden et al, 2018 (354)	Ischaemic heart disease	204	cFGF23	2-d carotid ultrasound imaging	Significant correlation between carotid plaque burden and cFGF23 (r=0.19; p<0.01).
Rodriguez-Ortiz et al, 2020 (355)	Ischaemic heart disease	939	Not specified	2-d carotid ultrasound imaging	Carotid artery intima-media thickness strongly correlation with higher FGF23 (rho=0.160; p<0.001)
Mirza, Hansen et al, 2009 (356)	Elderly individuals (>70 years old)	306	cFGF23	Magnetic resonance imaging-based angiography	Higher FGF23 was associated with a significant increase in the odds of having a high AS (OR 1.43, CI 1.06-1.92 to OR 3.01, CI 1.52-5.99)
Scialla et al, 2013 (357)	ND-CKD	1,501	iFGF23	CT calculated coronary artery and thoracic aorta calcium score	Multivariable analysis including FGF23, phosphate, PTH, calcium and traditional risk factors – no significant association between calcium score and iFGF23
Hsu et al, 2014 (358)	Not-known cardiovascular disease	5,977	iFGF23	• Large and small artery elasticity (Radial artery pulse waveforms) • Ankle-brachial index	No significant between iFGF23 and large/small artery elasticity or ankle brachial index. This remained irrespective of kidney function.
Mert et al, 2020 (359)	ND-CKD	46	cFGF23	Cardio-ankle vascular index	No significant correlation (rho=0.066; p=0.445)

Appendix table 16: Study outcomes	
Outcome	Domain
Primary outcome:	
Percentage (%) change in iFGF23 from baseline to 1-2 days post-infusion between FDI and FCM	FGF-23, phosphate and bone metabolism
Co-primary outcome:	
Composite of change in iFGF23 and delta change in phosphate at 2 days and 2 weeks.	FGF-23, phosphate and bone metabolism
Secondary outcomes:	
% change in iFGF23 from baseline to 2 weeks post-infusion between FDI and FCM	FGF-23, phosphate and bone metabolism
Difference between the two treatments in 1,25 $(OH)_2$ Vit D at each time point	FGF-23, phosphate and bone metabolism
Difference between the two treatments in calcium at each time point	FGF-23, phosphate and bone metabolism
Difference between the two treatments in PTH at each time point	FGF-23, phosphate and bone metabolism
Difference between the two treatments in ALP at each time point	FGF-23, phosphate and bone metabolism
Difference between the two treatments in bone specific ALP at each time point and others	FGF-23, phosphate and bone metabolism
Difference between the two treatments in serum phosphate levels at each time point	FGF-23, phosphate and bone metabolism
Incidence of hypophosphataemia (<0.65 mmol/L) and severe hypophosphataemia (<0.3mmol/L) at each time point	FGF-23, phosphate and bone metabolism
% failed repeat infusion due to hypophosphataemia	FGF-23, phosphate and bone metabolism

Effect / Difference on Quality of Life (The Fatigue Severity Scale and The Short Form (36) Health Survey)	Functional status and patient reported outcome measures
Effect on Functional Status (Duke Activity Status Index Score and 1 minute-sit-to-stand test) over specific times cumulatively and between two groups	Functional status and patient reported outcome measures
Difference in the co analysis of clinical end points including: haemoglobin, ferritin response and others	Clinical measures
Difference between the two treatments in NT-proBNP	Cardiovascular effect
Difference between the two treatments in Troponin T	Cardiovascular effect
Effect of intravenous iron on Pulse wave velocity measurement	Cardiovascular effect
Difference in ECG parameters throughout the study period (PR interval prolongation; QRS prolongation, arrhythmia presence)	Cardiovascular effect

Appendix table 17: Inclusion / Exclusion criteria	
Inclusion criteria	**Exclusion**
• Men and women aged ≥18 years; • Patients with CKD stages 3a-5 (not on dialysis) • Resting BP ≤ 160/95mmHg; • Able to give written and signed informed patient consent; • Able to complete study assessments; • Ferritin level less than 200µg/L OR TSAT ≤20% and serum ferritin between 200-299µg/L; • Haemoglobin less than 150g/L; • Serum phosphate > 0.8 mmol/L; • A negative pregnancy test for females of child bearing potential; • For a female of child bearing potential (i.e. have not undergone a hysterectomy or bilateral oophorectomy or not post-menopausal (at least 12 months must have elapsed since last menstruation) or surgically sterile) must agree to acceptable birth control from	• Age < 18 years; • Pregnancy or lactation; • Patients being investigated for potential blood loss; • Dialysis patients (either peritoneal or haemodialysis) • Weight ≤ 70kg; if Haemoglobin is ≥ 100 g/L; • Bleeding (> 500 ml) or surgery in the 30 days prior to recruitment; • Known allergy to iron therapy; • Symptomatic ischaemic heart disease; • Haemochromatosis or history of acquired iron overload; • Parenteral iron therapy within the previous 6 weeks; • Inability to co-operate with study protocol; • Active infection or a CRP>50 mg/L where clinical suspicion arises • Patients with potential confounding factors - cancer, (with exception of basal cell or squamous cell carcinoma of the

	screening through to Visit 8 using one of the followiEstablished use of oral, injected or implanted hormonal methods of contraception; intrauterine device or intrauterine system; tubal ligation; barrier methods or male sterilisation (with post vasectomy documentation of absence of sperm).	skin, and cervical intraepithelial neoplasia); • Patients who are unable or do not wish to give consent; • Patients who have received blood transfusions in last 6 weeks; • Patients with known haemoglobinopathy, myelodysplasia, myeloma; • Involvement in another clinical trial of an investigational medicinal product within the past four weeks.

Appendix table 18: Dosing of FDI and FCM		
Haemoglobin	Weight: 50 – 70 kg	Weight: > 70 kg
≥ 100 g/L	N/A	1500 mg
< 100 g/L	1500 mg	2000 mg
Visit 2 - 1st administration: 1000 mg given Visit 5 - 2nd administration: remaining dose (4 weeks after initial)		

Appendix table 19: Schedule of events

	Visit 1; -30 – 0 Screening	**Visit 2; Baseline 1st IV iron**	**Visit 3; 1-2 days post Visit 2**	**Visit 4; 11-17 days post Visit 2**	**Visit 5; 25-35 days post visit 2; 2nd IV Iron (if phosphate >0.65 mmol/L**	**Visit 6; (26-37 days post visit 2**	**Visit 7: (53 to 60 days post Visit 2)**	**Visit 8: 75-105 days post visit 2**
Eligibility screening and consent	X							
Demographics								
Age	X							
Gender	X							
Ethnicity	X							
Aetiology of CKD	X							
Weight	X							
Height	X							
Co-morbidities								
Blood pressure		X	X	X	X	X	X	
Physical examination	X							X
Smoking	X							
Alcohol Intake	X							
Medical history*	X							
Concomitant medication	X	X	X	X	X	X	X	X
Study Medication / Intervention								
Intravenous iron		X			X			

Bloods								
Haemoglobin	X	X		X	X		X	
Serum ferritin	X		X	X	X	X	X	
TSAT	X		X	X	X	X	X	
Creatinine	X			X	X		X	
eGFR	X			X	X		X	
CRP	+/-	X		X	X		X	
Urine PCR		X		X	X		X	
Bone Markers								
FEPi		X	X	X	X	X	X	
Calcium		X	X	X	X	X	X	
Phosphate		X	X	X	X	X	X	
PTH		X	X	X	X	X	X	
Vitamin D (and metabolites)		X	X	X	X	X	X	
ALP		X	X	X	X	X	X	
BALP		X	X	X	X	X	X	
CTx		X	X	X	X	X	X	
P1NP		X	X	X	X	X	X	
24h urine phosphate		X	X	X	X	X	X	
iFGF23		X	X	X	X	X	X	
Safety assessment		X	X	X	X	X	X	X
Cardiac assessment								
NT-proBNP		X			X		X	X
Troponin T		X	X	X	X	X	X	X
ECG		X	X		X		X	X

Pulse wave velocity	X				X		X	X
Quality of Life Questionnaires								
FSS		X			X		X	X
SF-36		X			X		X	X
Functional Status								
DASI score		X			X		X	X
1-minute-sit-to-stand test	X				X		X	X
* Comorbidities in Medical History: including Type I/II Diabetes Mellitus, Cerebrovascular disease, Ischaemic Heart Disease, Heart failure, cancer, hypertension								

Appendix table 20: Assays used for bone / cardiovascular markers	
Measure	Assay / Method used
iFGF23	Chemiluminescence Assay (Liaison XL, DiaSorin S.p.A., Saluggia, Italy)
$1,25(OH)_2$ Vitamin D	Chemiluminescence Assay (Liaison XL, DiaSorin S.p.A., Saluggia, Italy)
$25(OH)_2$ Vitamin D and $24(R),25(OH)_2$ Vitamin D	Method used: Liquid Chromatography Tandem Mass Spectrometry as described by Tang et al, 2017 (404)
PTH	Two-site immunoenzymatic (“sandwich”) assay – Access Intact PTH Assay (Beckman Coulter, California, USA)
BALP	Enzyme-Linked Immunosorbent Assays (Quidel, San Diego, California, USA)
CTx	Electrochemiluminescence sandwich immunoassay (Roche Diagnostics, Risch-Rotkreuz, Switzerland)
P1NP	Electrochemiluminescence assay – Elecsys total P1NP assay (Roche Diagnostics, Risch-Rotkreuz, Switzerland)
NT pro-BNP	Electrochemiluminescence assay - Elecsys proBNP II immunoassay (Roche Diagnostics, Risch-Rotkreuz, Switzerland)
Troponin T	Electrochemiluminescence assay – Elecsys Troponin T assay (Roche Diagnostics, Risch-Rotkreuz, Switzerland)

Appendix table 21: Pharmacovigilance terms used	
Term	Definition
Adverse Event (AE)	Any untoward medical incident in a participant following administration of a medicinal product, including occurrences are not necessarily caused by or related to that product.
Adverse Reaction (AR)	An untoward and unintended response in a participant to an investigational medicinal product related to any dose administered to that participant, consistent with Section 4.8 of the SmPC. The phrase "response to an investigational medicinal product" means that a causal relationship between a trial medication and an AE is at least a reasonable possibility, i.e. the relationship cannot be ruled out. Any case that is reported by a medically qualified professional or the sponsor as having a reasonable suspected causal relationship to the trial product is considered as an adverse reaction.
Serious Adverse Event (SAE)	A serious adverse event is any untoward medical occurrence that: • results in death • is life-threatening o Actual risk of death was considered in such cases. As event in which hypothetically the condition would have been life-threatening if it was more severe (i.e. a pneumonia) were not considered as such. • requires inpatient hospitalisation or prolongation of existing hospitalisation • results in persistent or significant disability/incapacity • consists of a congenital anomaly or birth defect Other ‘important medical events’ may also be considered serious if they jeopardise the participant or require an intervention to prevent one of the above consequences. - Elective surgery or planned admissions to hospital, or prolongation of existing hospitalisation for pre-existing conditions are not considered under the umbrella term serious adverse events.

Serious Adverse Reaction (SAR)	An adverse event that is both serious and considered to be (with reasonable probability) related to the administration of the trial treatments, based on information available.
Suspected Unexpected Serious Adverse Reaction (SUSAR)	A serious adverse reaction, the nature and severity of which is not consistent with the information about the medicinal product in question set out: • in the case of a product with a marketing authorisation, in the SmPC for that product • in the case of any other investigational medicinal product, in the investigator's brochure relating to the trial in question
Adapted from: HUTH NHS Trust Safety Reporting SOP – R&D GCP SOP 07	

Appendix table 22: Severity, seriousness, causality and expectedness	
Severity Assessed by PI	The assessment of severity of an SAE will be based on the investigator's clinical judgement using the following definitions: **Mild:** An event that is easily tolerated by the trial subject, causing minimal discomfort and not interfering with everyday activities. **Moderate:** An event that is sufficiently discomforting to interfere with normal everyday activities. **Severe:** An event that prevents normal everyday activities.
Seriousness Assessed by PI	An event is considered serious if it meets one or more of the following criteria: (a) results in death (b) is life-threatening (c) requires hospitalisation or prolongation of existing hospitalisation (d) results in persistent or significant disability or incapacity (e) consists of a congenital anomaly or birth defect.
Causality Assessed by the PI	An assessment of whether the SAE is likely to be related to the administered medicinal product is expected according to the following definitions: 4. **Unrelated**: Where an event is not considered to be related to the IMP and does not appear in section 4.8 of the SmPC of the investigational medicinal product administered. **5.** **Possibly related**: The nature of the event, the underlying medical condition, concomitant medication or temporal relationship make it possible that the SAE has a causal relationship to the study drug or it is stated in section 4.8 of the SmPC
Expectedness	In order to assess expectedness, the R&D Director will need to check if the SAR is listed in the Reference Safety Information.

Assessed by the R&D Director (on behalf of the Sponsor) for single-site trials	The RSI for IMPs with a marketing authorization is section 4.8 (Undesirable Effects) of the MHRA approved Summary of Product Characteristics (SmPC) or for IMPs without an MA, the RSI is the relevant section in the Investigator Brochure. If the SAR is listed in the RSI then it is expected. If the event is not listed in the RSI then it is unexpected and is a SUSAR and subject to expedited reporting to the MHRA and REC.
Adapted from: HUTH NHS Trust Safety Reporting SOP – R&D GCP SOP 07	

Appendix table 23: Comparison of SF-36 scores of "Iron and Phosphaturia – ExplorIRON-CKD" study with evidence from Finkelstein et al, 2018 (390)

Domain	Finkelstein et al, 2018 (n=194) (390)	Present study: FDI (n=14)	Present study: FCM (n=12)
Physical functioning	38.1 (17.1 – 57.0)	22.5 (0.0 – 95.0)	34.4 (0.0 – 65.0)
Role limitation (physical)	39.7 (17.7 – 56.9)	0.0 (0.0 – 100.0)	0.0 (0.0 – 50.0)
Role limitation (emotional)	44.2 (9.2 – 55.9)	33.3 (0.0 – 100.0)	0.0 (0.0 – 100.0)
Energy/vitality *	49.0 (20.9 – 70.8)	26.4 (24.8)	24.6 (14.2)
Emotional well-being	52.8 (19.0 – 64.1)	66.0 (8.0 – 96.0)	68.0 (56.0 – 92.0)
Social functioning	45.9 (13.2 – 56.9)	43.8 (25.0 – 100.0)	43.8 (12.5 – 100.0)
Pain	46.1 (19.9 – 62.1)	45.0 (12.5 – 80.0)	45.0 (22.5 – 100.0)
General Health	38.6 (16.2 – 62.5)	37.5 (5.0 – 55.0)	37.5 (0.0 – 45.0)
All values reported as median (range) except * which is mean (SD)			

Appendix table 24: Pulse wave velocity and Augmentation Index in previous studies in patients with ND-CKD receiving intravenous iron

	Baseline PWV (cf) / ms	Baseline AIx (ao) / %	1-month PWV (cf) m/s	1-month AIx (ao) / %	3-month PWV (cf) / m/s	3-month AIx (ao) / %
ExplorIRON-CKD (n=26)	8.0 (2.6)	26.0 (11.8)	6.8 (2.5)	24.3 (11.8)	6.7 (1.9)	22.2 (9.5)
Iron and the Heart (n=26) (106)	8.3 (2.8)	25.4 (10.7)	8.0 (2.0)	21.0 (10.5)	8.3 (1.9)	25.5 (10.6)
Iron-CKD (n=40) (87)	7.5 (2.5)	20.9 (11.2)	6.9 (2.6)	17.9 (10.3)	6.7 (2.0)	18.4 (10.2)

All values are set as mean (SD) – PWV: pulse wave velocity; AIx: augmentation index

8. Appendix 2: Publications and presentations

8.1: Publications arising from this study:

Kassianides X, Bodington R, Bhandari S. An evaluation of ferric derisomaltose as a treatment for anemia. Expert Rev Hematol. 2021 Jan;14(1):7-29. doi: 10.1080/17474086.2021.1858406. Epub 2020 Dec 14. PMID: 33317356.

Kassianides X, Hazara AM, Bhandari S. Improving the safety of intravenous iron treatments for patients with chronic kidney disease. Expert Opin Drug Saf. 2021 Jan;20(1):23-35. doi: 10.1080/14740338.2021.1853098. Epub 2020 Nov 26. PMID: 33203251.

Kassianides X, Bhandari S. Hypophosphataemia, fibroblast growth factor 23 and third-generation intravenous iron compounds: a narrative review. Drugs Context. 2021 Jan 19;10:2020-11-3. doi: 10.7573/dic.2020-11-3. PMID: 33519940; PMCID: PMC7819638.

Kassianides X, Bhandari S. Methodology and Baseline Data of a Comparative Exploratory Double-Blinded Randomized Study of Intravenous Iron on Fibroblast Growth Factor 23 and Phosphate in Chronic Kidney Disease. Kidney Blood Press Res. 2023;48(1):151-164. doi: 10.1159/000528313. Epub 2023 May 25. PMID: 37015198.

Kassianides X, Bhandari S. Patient reported outcome measures and cardiovascular outcomes following high dose modern intravenous iron in non-dialysis dependent chronic kidney disease: secondary analysis

of ExplorIRON-CKD. Sci Rep. 2023 Oct 26;13(1):18401. doi: 10.1038/s41598-023-44578-6. PMID: 37884522; PMCID: PMC10603042.

Kassianides X, Bhandari S. The differential effect of modern intravenous iron on fibroblast growth factor 23 and phosphate in non-dialysis dependent CKD - the exploratory randomized controlled double-blind ExplorIRON-CKD study. BMC Nephrol. 2024 Feb 12;25(1):54. doi: 10.1186/s12882-023-03440-7. PMID: 38347520; PMCID: PMC10860218.

8.2: Publications arising from work relevant to intravenous iron:

Kassianides X, Gordon A, Sturmey R, Bhandari S. The comparative effects of intravenous iron on oxidative stress and inflammation in patients with chronic kidney disease and iron deficiency: a randomized controlled pilot study. Kidney Res Clin Pract. 2021 Mar;40(1):89-98. doi: 10.23876/j.krcp.20.120. Epub 2021 Mar 22. PMID: 33745264; PMCID: PMC8041632.

Kassianides X, Hazara AM, Macdougall IC, Kalra PA, Bhandari S. The Impact of Intravenous Iron on Renal Injury and Function Markers in Patients With Chronic Kidney Disease and Iron Deficiency Without Anemia. Kidney Int Rep. 2021 Nov 24;7(2):322-326. doi: 10.1016/j.ekir.2021.11.002. PMID: 35155871; PMCID: PMC8820978.

Kassianides X, Allgar V, Macdougall IC, Kalra PA, Bhandari S. Analysis of oxidative stress, inflammation and endothelial function following intravenous iron in chronic kidney disease in the Iron and

Heart Trial. Sci Rep. 2022 Apr 27;12(1):6853. doi: 10.1038/s41598-022-10717-8. PMID: 35477731; PMCID: PMC9046378.

Kassianides X, White S, Bhandari S. Markers of Oxidative Stress, Inflammation and Endothelial Function following High-Dose Intravenous Iron in Patients with Non-Dialysis-Dependent Chronic Kidney Disease-A Pooled Analysis. Int J Mol Sci. 2022 Dec 16;23(24):16016. doi: 10.3390/ijms232416016. PMID: 36555659; PMCID: PMC9787941.

8.3: Presentations to learned societies arising from this study

The effect of modern intravenous iron preparations on fibroblast growth factor 23 and phosphate in non-dialysis dependent chronic kidney disease patients with iron deficiency with/without anaemia

***Xenophon Kassianides**, Sunil Bhandari*

Oral presentation at European Iron Club 2022 in Oxford, U.K, July 2022

The differential effect of third generation intravenous iron preparations (ferric carboxymaltose, ferric derisomaltose) on cardiovascular variables in patients with non-dialysis dependent CKD and iron deficiency +/- anemia

***Xenophon Kassianides**, Sunil Bhandari*

Mini-oral presentation at the European Renal Association Congress 2022 in Paris, France, May 2022

The differential effect of third generation intravenous iron preparations (ferric carboxymaltose, ferric derisomaltose) on hematinic and renal function variables in patients with non-dialysis dependent CKD and iron deficiency +/- anemia

***Xenophon Kassianides**, Sunil Bhandari*

Mini-oral presentation at the European Renal Association Congress 2022 in Paris, France, May 2022

The differential effect of third generation intravenous iron preparations (ferric carboxymaltose, ferric derisomaltose) on patient reported outcome measures in patients with non-dialysis dependent CKD and iron deficiency +/- anemia

***Xenophon Kassianides**, Sunil Bhandari*

Mini-oral presentation at the European Renal Association Congress 2022 in Paris, France, May 2022

The differential impact of two modern intravenous iron compounds of fibroblast growth factor 23, phosphate, and other clinical and functional markers – the ExplorIRON-CKD study

Xenophon Kassianides, Sunil Bhandari

Poster presentation at the International Society of Nephrology – World Congress of Nephrology 2022 in Kuala-Lumpur, Malaysia, February 2022

9. Appendix 3: Study particulars

9.1: Consent form

ExplorIRON-CKD

Hull University Teaching Hospitals NHS Trust

INFORMED CONSENT FORM

Title of Project: Iron and Phosphaturia Trial (EXplorIRON-CKD)
Principal Investigator: Dr Xenophon Kassianides
Department: Academic Renal Research, Hull Royal Infirmary

Participant Number ☐☐☐

		Please ***Initial*** each box below
1.	I confirm that I have been given adequate time to read and understand the information sheet for the above study dated Version 6.0 dated [13/01/2021] and have had the opportunity to consider the information, ask questions and these have been answered satisfactorily.	
2.	I understand that my participation is voluntary and that I am free to withdraw at any time without giving a reason and without my medical care or legal rights being affected.	
3.	I agree that should I withdraw from the study that any data and samples collected up until the date of withdrawal can be used as part of the study results.	
4.	I understand that relevant sections of any of my medical records and/or study data may be looked at by responsible individuals from the research team, the sponsor (Hull University Teaching Hospitals NHS Trust), relevant third parties or from regulatory authorities where it is relevant to my taking part in the research. I give permission for these individuals to access my records.	
5.	I agree to my General Practitioner being informed of my participation in this study and will be advised of any significant information relating to my health that comes to light.	
6.	I consent to my blood and urine samples being taken, stored and used for analysis of biomarkers within this trial. I agree to my blood samples to be sent to external laboratories (e.g. Norfolk and Norwich University) for analysis. I understand that samples will be sent and stored unnamed.	
7.	I agree to Pharmacosmos, the funder, holding my annoymised data at the end of the trial.	
8.	I agree to take part in the study.	

OPTIONAL	I give consent that the samples used in this trial, can also be used for future research	

	Name	**Signature**	**Date**	**Time**
Participant				
Researcher *On Delegation Log*				

When completed: 1 copy for patient, 1 copy for medical notes, 1 original for Investigator Site File.

EXplorIRON Consent form – V6.0, 13/01/2021
IRAS project ID: 272279

Page **1** of **1**

9.2: Participant information sheet

Hull University Teaching Hospitals NHS Trust

Participant information sheet

Title: Iron and Phosphaturia (ExplorIRON-CKD)

Trial Doctor: Dr Xenophon Kassianides

Trial Centre: Academic Renal Research,
2nd Floor, Alderson House Residencies
Hull Royal Infirmary,
Anlaby Road
Hull
HU3 2JZ

Telephone: 01482 605260 / 674055

Sponsor: Hull University Teaching Hospitals NHS Trust

Invitation

We would like to invite you to take part in our research trial. Before you decide, we would like you to understand why the research is being done and what it will involve.

Please take time to read the following information carefully and discuss it with your family, friends and your doctor if you wish. Please ask if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part

- **Part 1:** tells you the purpose of this trial and what will happen to you if you take part.
- **Part 2**: gives you more detailed information about the conduct of the clinical trial.

Thank you for reading this information sheet.

Part 1

Why am I being invited to take part?

You are being asked to take part in this trial because you have a condition known as Chronic Kidney Disease and you may need iron treatment through your veins at some point in the future as identified by your renal specialist.

What is the purpose of this trial?

Individuals with advanced chronic kidney disease (CKD) often suffer with anaemia. One of the most common reasons for anaemia is low iron. Iron can be replaced using intravenous iron (iron that can be given through the vein), and a number of preparations exist. Previous trials have suggested that different intravenous iron preparations affect the bones in different ways and that there is the possibility that different iron preparations can affect the heart.

The trial will be comparing two different intravenous iron preparations. These are ferric carboxymaltose (Ferinject ®) and iron isomaltoside (Monofer ®). Both have been granted a marketing authorisation license by the MHRA (Medicines and Healthcare products Regulatory Agency) for the safe treatment of anaemia.

Participants will be randomised (a bit like tossing a coin), to receive either Ferinject or Monofer. This allows us to assess whether anything different happens to the bones and the heart depending on which iron is used. This will help us to decide which iron to use in matching individual patient's needs.

During the trial period you will receive regular medical input and investigations including blood tests, urine tests and an assessment of blood flow in your arteries. We will gather important information to study the impact of different intravenous preparations on your general health and wellbeing.

Please note that this is a pilot trial. It means that the findings of this trial alone will not be sufficient to prove if different iron preparations have different impact on the bones and the heart. Our findings will help us design a larger trial in the future to investigate the differences of intravenous iron and aid in tailoring a personalised regime for our patients with CKD and iron deficiency anaemia.

Do I have to take part?

No, taking part in the trial is entirely up to you. If you decide not to participate, it will not affect the treatment you receive now or in the future. If you do take part and change your mind once it has started, you can withdraw at any time without having to give a reason. Any data collected up to the point of your withdrawal will be used in the final data analysis.

What will happen to me if I take part?

The trial requires a total of 8 visits to the Renal Department at Hull Royal Infirmary over a period of approximately 12 weeks. If you agree to participate in this trial, you will be seen by a member of the renal research team. We will try to arrange some of the study visits to fit in with your other routine appointments. The trial doctor will discuss the trial with you and answer any questions you may have.

If you decide to go ahead with the trial, you will be asked to sign a consent form. The consent form will be explained to you by the trial doctor. After you have given your consent you will undergo the screening tests and examinations required to ensure you are eligible for the trial.

During the trial you will receive two infusions of intravenous iron as per routine practice. The intravenous iron sessions are one month apart.

Within the first ten days of the trial, you will be seen by the trial doctor four times, at your screening visit (Visit 1), the day you receive your iron infusion (Visit 2), 1 to 2 days after your infusion (Visit 3) and then 11-17 days after your infusion (Visit 4).

You will then receive your second iron infusion (Visit 5). You will be seen 1 - 2 days (Visit 6) after the second infusion.

Further study visits will follow at 53 – 60 days (Visit 7) and 75 – 105 days (Visit 8) after the first infusion.

Most visits will last approximately 30 minutes to 1 hour. Visit 2 and 5 which are the ones where you are having the intravenous iron will last 2 hours and 30 minutes.

At these visits we will conduct some further testing and assess your response to the iron you have been given. These tests include:

- Blood tests at each visit. The amount of blood taken will be the equivalent of 12 – 18 teaspoons.
- ECG, which means a simple tracing of your heart. (Visits 2, 3, 5, 7 and 8).

- Pulse Wave Velocity Measurement which takes a measurement of how fast blood flows in the arteries on screening (visit 1) and visit 5,visit 7 and your last visit (visit 8).
- 1-minute sit to stand test: We will ask you to sit on a chair (standard height) and stand up as many times as you can within one minute: this will give us valuable information on your strength and stamina. This test will take place on screening (visit 1) and visits 5,7 and 8.

You will be asked to complete a questionnaire which will ask questions about your general health, any pain you may be experiencing, your emotional well-being and your mental agility (visits 2, 5, 7 and 8).

You will also be asked to provide urine samples over 24 hours (visits 2, 3, 4, 5, 6 and 7).

Trial visits will have to be within the scheduled timeframe but where possible will be planned at your convenience.

If you have any questions about any of the tests, please feel free to ask your trial doctor, GP or your renal consultant to explain them to you.

The table below shows the timing of the trial visits and what will be carried out at each visit in more detail.

If your phosphate is too low following the first infusion, this will be picked up by the research team and a second infusion will not be given. In that case you will not need to attend visit 6 but we would use visit 5 to perform all the necessary investigations. You will be remaining in the study and expected to come at visits 7 and 8.

To minimise your risk of exposure to COVID-19 infection there is the option to have your final study visit (visit 8) over the phone or by video call.

For the study visits that require you to come to the hospital, those for treatment and to give a blood sample, all COVID-19 requirements will be in place (e.g social distancing, wearing of masks, hand sanitisers available), while all the staff are expected to adhere to the rules and regulations regarding home testing and vaccination of hospital staff.

Visit schedule								
	Visit 1 **-30-0 Screening**	**Visit 2; Baseline** **1st IV iron**	**Visit 3;** **1-2 days post Visit 2**	**Visit 4;** **11-17 days post Visit 2**	**Visit 5;** **25-35 days post visit 2;** **2nd IV Iron (if phosphate ok)**	**Visit 6;** **(1-2 days post visit 5)** **26-37 days post visit 2**	**Visit 7:** **53 to 60 days post Visit 2**	**Visit 8:** **75-105 days post Visit 2**
Screening and consent	X							
Demographic check (gender, height, weight, ethnicity)								
	X							X
Medical history (inc. smoking and alcohol), medication check and examination								
Medical history	X							
Physical examination	X							X
Blood pressure		X	X	X	X	X	X	
Medication	X	X	X	X	X	X	X	X
Bloods for anaemia and kidneys								

	X	X	X	X	X	X	X	
Bloods for bone								
		X	X	X	X	X	X	
Iron Infusion								
		X			X			
Bloods for heart								
	X	X	X	X	X	X	X	X
Urine measurement								
Simple urine	X	X		X	X		X	
24-hour collection		X	X	X	X	X	X	
Questionnaires								
		X			X		X	X
ECG (heart tracing)								
		X	X		X		X	X
Artery speed measurement								
	X				X		X	X
1 minute sit-to-stand test								
	X				X		X	X

What are the alternative treatments available?

If you decide not to participate in this trial, you will receive iron preparation and follow-up as required for your routine care.

What are the risks of the iron preparations?

Iron preparations have long been associated with allergic reactions. In this trial we will be using third generation intravenous iron preparations that are less associated with allergic reactions.

Intravenous iron can have some undesired effects; these include:

- Allergic reaction (ranging from difficulty breathing and blood pressure drop to itchiness and rash)
- Chest or back pain as hypersensitivity reaction
- Skin flushing (limited and goes away quickly)
- Mild-flu like illness (high temperature, aches and pains in joints and muscles)
- Worsening of inflammatory joint disease (worse joint pain if you are suffering from autoimmune condition – e.g. rheumatoid arthritis)
- Pain and inflammation at site of intravenous cannula (very limited and goes away quickly)(simple pain killers can be used)
- Low phosphate levels (will be monitored in study)
- Nausea (very limited and goes away quickly)(simple anti-sickness medication can be used)
- Headache (very limited and goes away quickly)(simple pain killers can be used)

The table on the next page shows how common these effects are:

Potential side effects of different Iron preparations				
	Monofer ®		**Ferinject ®**	
	How Common	Frequency	How Common	Frequency
Allergic reaction	Rare	Between 1000 and 10000 people	Rare	Between 1000 and 10000 people
Chest or back pain	Uncommon	Between 100 and 1000 people	Uncommon	Between 100 and 1000 people
Skin flushing	Uncommon	Between 100 and 1000 people	Common	Between 10 and 100 people
Mild-flu like illness	Rare	Between 1000 and 10000 people	Rare	Between 1000 and 10000 people
Worsening of inflammatory joint disease	Uncommon	Between 100 and 1000 people	Uncommon	Between 100 and 1000 people
Pain /inflammation at site of intravenous cannula	Common	Between 10 and 100 people	Common	Between 10 and 100 people
Low phosphate levels	Uncommon	Between 100 and 1000 people	Common	Between 10 and 100 people
Nausea	Common	Between 10 and 100 people	Common	Between 10 and 100 people
Rash	Uncommon	Between 100 and 1000 people	Uncommon	Between 100 and 1000 people
Headache	Uncommon	Between 100 and 1000 people	Common	Between 10 and 100 people

Throughout the study we will monitor for iron overload (manifesting as tiredness, skin pigmentation, weakness, muscle aches)

Please note that these problems potentially affect all patients receiving intravenous iron not just those taking part in the trial. Your trial doctor may also discontinue you from the trial if your safety is compromised at any time.

What measures have been put in place to manage these risks?

You will be regularly seen by a doctor for the duration of the trial that will be assessing your well-being. In addition the blood investigations we carry out will provide us with valuable information to assess any risk to you.

On both visits for intravenous iron a trained doctor will be available while you are receiving the infusion, alongside trained nurses. You will be monitored during the infusion with regular checks of your temperature, respiratory rate, heart rate and blood pressure.

What are the possible benefits of taking part?

Participants in research trials such as this receive very close monitoring. Should any other health issues be identified whilst you are in the trial you will be referred to an appropriate specialist within the hospital.

What will happen after the trial?

When the trial is complete, your future treatment will be decided by your current renal consultant.

What will happen to the data collected in the trial?

The information we get from the trial may help us to improve the treatment of people with Chronic Kidney Disease in the future.

The results from the trial may be published or presented at scientific meetings and in journals so other doctors caring for similar patients can learn from your experience.

Pharmacosmos AS, the company funding this trial, will receive an anonymised copy of the final data to upload to their internal clinical database.

Will my GP be notified of my participation in this trial?

Yes, if you agree to take part in this trial we will inform your GP. We will also contact your GP if we have any concerns about your health during the trial.

Thank you for reading so far.
If you are interested, please continue to read Part 2

Part 2

What if new information becomes available?

Sometimes during the course of a research trial, new information becomes available about the treatment that is being studied. If this happens, your trial doctor will discuss how this affects your care and participation. If you decide to withdraw, your doctor will make arrangements for your routine care to continue. If you decide to carry on in the trial you will be asked to sign an updated consent form.

What will happen if I do not want to carry on with the trial?

You are free to withdraw from the trial at any time. If you wish to withdraw, please contact your research team so that your concerns can be discussed. We ask you to consent to any information and blood samples collected up to the time of your withdrawal can still be used. Withdrawing from the study will not affect your standard of care. Once you withdraw you will not be expected to attend any further research visits.

What if there is a problem?

If you have a concern about any aspect of this trial, you should first ask to speak to a trial doctor who will do their best to answer your questions. The contact details for the trial are:

Dr Xenophon Kassianides

Tel: 01482 605260

Email: xenophon.kassianides@hey.nhs.uk

If you have any concerns or complaints about the way the researcher has carried out this trial, or any other aspects of your care, you may contact:

The Patient Experience Service

1st Floor Alderson House, Hull Royal Infirmary, Anlaby Road, Hull, HU3 2JZ

Tel: 01482 623065/675508

Email: Pals.Mailbox@hey.nhs.uk

In the unlikely event that something does go wrong and you are harmed as a result of the research trial the normal NHS complaints mechanism will still be available to you if appropriate.

How will we use information about you?

We will need to use information from your medical records and your GP for this research project.

This information will include your:

- Initials,
- NHS number,
- Name,
- Contact details
- Any other information that is relevant to the research

The information will be held by the site and sponsor for the research. People will use this information to do the research or to check your records to make sure that the research is being done properly.

People who do not need to know who you are will not be able to see your name or contact details. Your data will have a code number instead.

We will keep all information about you safe and secure.

Once we have finished the study, we will keep some of the data so we can check the results. We will write our reports in a way that no-one can work out that you took part in the study.

What are your choices about how your information is used?

•You can stop being part of the study at any time, without giving a reason, but we will keep information about you that we already have.

•We need to manage your records in specific ways for the research to be reliable. This means that we won't be able to let you see or change the data we hold about you.

•If you agree to take part in this study, you will have the option to take part in future research using your data saved from this study. Blood will be stored in anonymised form at the Hull University Teaching Hospitals secure freezers at Hull Royal Infirmary Pathology Lab.

Where can you find out more about how your information is used?

You can find out more about how we use your information

•at www.hra.nhs.uk/information-about-patients/

•our information available from https://www.hey.nhs.uk/privacy/data-protection/

•by asking one of the research team

•by sending an email to Information Governance at information.governance@hey.nhs.uk or

•by ringing Carla Ramsay, Data Protection Officer on 01482 674920.

What will happen to the blood/urine samples I give?

The blood and urine samples will, where possible, be collected at a time when you are having a blood/urine test performed for routine clinical purposes. The blood testing will focus on your iron levels, red blood cells and markers of inflammation and the function of kidneys, heart markers and bone. The urine samples will be analysed for protein and phosphate. Some of the blood you give will be stored in secure freezers at the pathology laboratory of Hull Royal Infirmary for analysis within the trial. Most of the blood taken will be analysed in Hull University Teaching Hospitals. However some tests will be performed at the Norfolk and Norwich University Hospital NHS Trust. This is because certain tests cannot be analysed in the local laboratory.

Samples that are processed immediately in the Hull Hospitals' laboratory (samples that will not be stored) will be identifiable with your name, date of birth, NHS/hospital number as per Trust policy;

access to this would only be available to the laboratory, research team and any doctor providing you with direct care.

Some samples that are tested in Hull will be frozen for a year and then destroyed. All urine samples and samples sent to Norfolk and Norwich will be destroyed as soon as they have been analysed in accordance to the Human Tissue Authority's Code of Practice.

All samples sent away from the research site (Hull) to Norfolk and Norwich University Hospital NHS Trust laboratory will be anonymised with a unique code, only known to research staff in Hull. Samples frozen in Hull will also be kept anonymised with the same unique code.

The anonymised samples stored in the laboratory in Hull Royal Infirmary may be used for future research within the course of this trial period. Any future research carried out would be in relation to findings from this trial and how iron affects the body. This will only be conducted with your prior consent and it is optional.

Any abnormal or concerning results of your blood tests will be picked up by the dedicated study doctor who will inform you and then refer you to any necessary specialist. You will be informed about this as soon as possible. Any issues with your kidneys will be discussed with your renal physician. Any problems with your heart will lead to referral to cardiology for further investigation. Any urgent findings will be discussed with Acute Medicine for urgent assessment. If it is ok with you, your general practitioner will be informed.

Who is organising and funding the research?

The Hull University Teaching Hospitals NHS Trust is taking overall responsibility for the trial. The trial has been funded by Pharmacosmos A/S. The trial's main investigator is Dr Xenophon Kassianides at Hull Royal Infirmary. The trial is conducted by NHS research staff. Your doctor will NOT be paid or receive any financial or other benefits for including you in this trial.

Who has reviewed the trial?

All research in the NHS is looked at by an independent group of people called the Research Ethics Committee to protect your safety, rights, wellbeing and dignity. This trial has been reviewed and given a favourable opinion by the (awaited)

What do I do now?

If you do not wish to take part then is no need to do anything else. If you would like take part, or feel you need more information then please speak to the member of staff who gave you this leaflet or Dr Kassianides whose contact details are on page 7 of this leaflet.

Will I be reimbursed?

You will not receive payment for taking part in this study; however, all reasonable travel expenses (i.e. car parking, taxis, and bus) will be reimbursed on request for all non-standard of care follow up visits (4 visits (1, 3, 6, and 8)).

Thank you for considering participation in this trial.

You will be given a copy of this information sheet to keep if you decide that you wish to take part in the trial.

9.3: Duke Activity Status Index

time
make it count

The DASI Score Sheet (to be used by staff)

DUKE ACTIVITY STATUS INDEX
Score sheet

Can you:	Scores are only given for 'yes' replies	
1. Take care of yourself, that is, eat, dress, bathe or use the toilet?	2.75	Yes/No
2. Walk indoors, such as around your house?	1.75	Yes/No
3. Walk a block or two on level ground?	2.75	Yes/No
4. Climb a flight of stairs or walk up a hill?	5.50	Yes/No
5. Run a short distance?	8.00	Yes/No
6. Do light work around the house like dusting or washing dishes?	2.70	Yes/No
7. Do moderate work around the house like vacuuming, sweeping floors or carrying groceries?	3.50	Yes/No
8. Do heavy work around the house like scrubbing floors or lifting or moving heavy furniture?	8.00	Yes/No
9. Do garden work like raking leaves, weeding or pushing a lawn mower?	4.50	Yes/No
10. Have sexual relations?	5.25	Yes/No
11. Participate in moderate recreational activities like golf, bowling, dancing, doubles tennis or throwing a ball?	6.00	Yes/No
12. Participate in strenuous sports like swimming, singles tennis, football, basketball or skiing?	7.50	Yes/No

(To be completed by staff) Duke Activity Status Index (DASI) = ☐

The higher the score is, the more physically active a person is according to this set of activities of daily living. The DASI score should be completed every three months and the score entered into the back of the exercise diary to monitor progress.

Supported by the NKF.
www.kidney.org.uk
Helpline: 0845 601 02 09

Supported by ANSA.
www.anaemianurse.org
Helpline: 01483 724472

9.4: Fatigue Severity Scale

FATIGUE SEVERITY SCALE (FSS)

Date _____ Name _____

Please circle the number between 1 and 7 which you feel best fits the following statements. This refers to your usual way of life within the last week. 1 indicates "strongly disagree" and 7 indicates "strongly agree."

Read and circle a number.	Strongly Disagree → Strongly Agree						
1. My motivation is lower when I am fatigued.	1	2	3	4	5	6	7
2. Exercise brings on my fatigue.	1	2	3	4	5	6	7
3. I am easily fatigued.	1	2	3	4	5	6	7
4. Fatigue interferes with my physical functioning.	1	2	3	4	5	6	7
5. Fatigue causes frequent problems for me.	1	2	3	4	5	6	7
6. My fatigue prevents sustained physical functioning.	1	2	3	4	5	6	7
7. Fatigue interferes with carrying out certain duties and responsibilities.	1	2	3	4	5	6	7
8. Fatigue is among my most disabling symptoms.	1	2	3	4	5	6	7
9. Fatigue interferes with my work, family, or social life.	1	2	3	4	5	6	7

VISUAL ANALOGUE FATIGUE SCALE (VAFS)

Please mark an "X" on the number line which describes your global fatigue with 0 being worst and 10 being normal.

0	1	2	3	4	5	6	7	8	9	10
_____										

9.5: Short form (36) Questionnaire

RAND HEALTH

RAND > RAND Health > Surveys > RAND Medical Outcomes Study > 36-Item Short Form Survey (SF-36) >

36-Item Short Form Survey Instrument (SF-36)

RAND 36-Item Health Survey 1.0 Questionnaire Items

Choose one option for each questionnaire item.

1. In general, would you say your health is:

- 1 - Excellent
- 2 - Very good
- 3 - Good
- 4 - Fair
- 5 - Poor

2. **Compared to one year ago**, how would you rate your health in general **now**?

- 1 - Much better now than one year ago
- 2 - Somewhat better now than one year ago
- 3 - About the same
- 4 - Somewhat worse now than one year ago
- 5 - Much worse now than one year ago

The following items are about activities you might do during a typical day. Does **your health now limit you** in these activities? If so, how much?

	Yes, limited a lot	Yes, limited a little	No, not limited at all
3. **Vigorous activities**, such as running, lifting heavy objects, participating in strenuous sports	○ 1	○ 2	○ 3
4. **Moderate activities**, such as moving a table, pushing a vacuum cleaner, bowling, or playing golf	○ 1	○ 2	○ 3
5. Lifting or carrying groceries	○ 1	○ 2	○ 3
6. Climbing **several** flights of stairs	○ 1	○ 2	○ 3
7. Climbing **one** flight of stairs	○ 1	○ 2	○ 3
8. Bending, kneeling, or stooping	○ 1	○ 2	○ 3
9. Walking **more than a mile**	○ 1	○ 2	○ 3
10. Walking **several blocks**	○ 1	○ 2	○ 3
11. Walking **one block**	○ 1	○ 2	○ 3
12. Bathing or dressing yourself	○ 1	○ 2	○ 3

During the **past 4 weeks**, have you had any of the following problems with your work or other regular daily activities **as a result of your physical health**?

	Yes	No
13. Cut down the **amount of time** you spent on work or other activities	1	2
14. **Accomplished less** than you would like	1	2
15. Were limited in the **kind** of work or other activities	1	2
16. Had **difficulty** performing the work or other activities (for example, it took extra effort)	1	2

During the **past 4 weeks**, have you had any of the following problems with your work or other regular daily activities **as a result of any emotional problems** (such as feeling depressed or anxious)?

	Yes	No
17. Cut down the **amount of time** you spent on work or other activities	1	2
18. **Accomplished less** than you would like	1	2
19. Didn't do work or other activities as **carefully** as usual	1	2

20. During the **past 4 weeks**, to what extent has your physical health or emotional problems interfered with your normal social activities with family, friends, neighbors, or groups?

- 1 - Not at all
- 2 - Slightly
- 3 - Moderately
- 4 - Quite a bit
- 5 - Extremely

21. How much **bodily** pain have you had during the **past 4 weeks**?

- 1 - None
- 2 - Very mild
- 3 - Mild
- 4 - Moderate
- 5 - Severe
- 6 - Very severe

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22. During the **past 4 weeks**, how much did **pain** interfere with your normal work (including both work outside the home and housework)?

- 1 - Not at all
- 2 - A little bit
- 3 - Moderately
- 4 - Quite a bit
- 5 - Extremely

These questions are about how you feel and how things have been with you **during the past 4 weeks**. For each question, please give the one answer that comes closest to the way you have been feeling.

How much of the time during the **past 4 weeks**...

	All of the time	Most of the time	A good bit of the time	Some of the time	A little of the time	None of the time
23. Did you feel full of pep?	1	2	3	4	5	6
24. Have you been a very nervous person?	1	2	3	4	5	6
25. Have you felt so down in the dumps that nothing could cheer you up?	1	2	3	4	5	6
26. Have you felt calm and peaceful?	1	2	3	4	5	6
27. Did you have a lot of energy?	1	2	3	4	5	6
28. Have you felt downhearted and blue?	1	2	3	4	5	6
29. Did you feel worn out?	1	2	3	4	5	6
30. Have you been a happy person?	1	2	3	4	5	6
31. Did you feel tired?	1	2	3	4	5	6

32. During the **past 4 weeks**, how much of the time has **your physical health or emotional problems** interfered with your social activities (like visiting with friends, relatives, etc.)?

- 1 - All of the time
- 2 - Most of the time
- 3 - Some of the time
- 4 - A little of the time
- 5 - None of the time

How TRUE or FALSE is **each** of the following statements for you.

	Definitely true	Mostly true	Don't know	Mostly false	Definitely false
33. I seem to get sick a little easier than other people	1	2	3	4	5
34. I am as healthy as anybody I know	1	2	3	4	5
35. I expect my health to get worse	1	2	3	4	5
36. My health is excellent	1	2	3	4	5

ABOUT

The RAND Corporation is a research organization that develops solutions to public policy challenges to help make communities throughout the world safer and more secure, healthier and more prosperous. RAND is nonprofit, nonpartisan, and committed to the public interest.

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