Iron deficiency in heart failure: the importance of a definition

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Summary

Iron deficiency (ID) is present in around half of patients with heart failure, is associated with more advanced symptoms, worse quality of life and poorer outcomes and may be a risk factor for the development and progression of heart failure. However, how ID is defined by blood markers in heart failure remains a debate. Treatment of ID with intravenous iron in those with heart failure improves symptoms and quality of life, but it remains unclear if benefits extend to reducing hospitalisations for heart failure and cardiovascular death. These gaps in research will form the focus of my thesis.

Through a detailed literature review, chapter one provides an overview of ID in heart failure including prevalence, diagnosis, associations with outcomes and effects of iron therapy in each phenotype of heart failure.

Chapters three, four and five present results from retrospective analysis of a large prospective cohort of ambulatory patients with heart failure from the Hull LifeLab. Chapter six reports pooled data from available randomised clinical trials of patients with heart failure and serum iron deficiency assessing the efficacy of intravenous iron to reduce cardiovascular deaths and hospitalisations for heart failure. Chapter seven reports data obtained from the comprehensive Glasgow-wide SafeHaven, comprising primary and secondary care data of patients with and at risk of heart failure within Greater Glasgow and Clyde.

Data from patients with heart failure of different phenotypes enrolled in the Hull LifeLab was analysed to determine how varying definitions of ID affected clinical characteristics, prevalence of ID and associations with mortality. Irrespective of how it is defined, ID is more common in women, in those with anaemia and in those with heart failure with preserved ejection fraction. A low serum iron (≤13μmol/L) and a low transferrin saturation (TSAT) (<20%) were independently associated with higher mortality whereas definitions of ID using ferritin, including the current guideline directed definition of ID, were not.

From the Hull LifeLab, 906 patients with heart failure who had two available blood results one year apart were investigated for incidence and recovery of ID and anaemia according to various definitions. Most patients with heart failure

have or will develop ID over the course of one year, and a large proportion of these will also have anaemia. Those with persistent ID have a worse outcome compared to those in whom it resolves but anaemia is associated with higher mortality irrespective of whether it resolves or persists. Iron deficiency when defined by a low transferrin saturation (TSAT <20%), or a low serum iron (≤13µmol/L), is more strongly associated with prognosis than the current guideline definition of ID.

Transferrin saturation reflects both serum iron and serum transferrin. Further analysis of patients from the Hull LifeLab with confirmed heart failure was undertaken to analyse the relation between serum transferrin concentrations and other markers of serum iron status and mortality. Patient characteristics were also assessed according to quartiles of serum transferrin. Over one third of patients with low transferrin (Quartile 1; ≤2.3 g/L) and evidence of ID (low serum iron, low haemoglobin) had a normal TSAT (>20%) meaning they would be falsely classified as being iron replete. Similarly, if transferrin is high (Quartile 4; ≥3.0g/L), TSAT is often <20% even if serum iron is normal. Having a low transferrin is associated with higher mortality, particularly if accompanied by a low serum iron. A low serum iron is associated with higher mortality irrespective of serum transferrin or TSAT.

From a meta-analysis comprising data from seven randomized trials, in 2,166 patients with heart failure and evidence of serum ID treated with intravenous (IV) iron or usual care, IV iron reduced the primary composite endpoint of cardiovascular deaths or hospitalisations for heart failure. This result was driven primarily by the reduction in hospitalisations for heart failure.

From the Glasgow wide SafeHaven, 197,152 patients with, or at increased risk of heart failure, were investigated for trends and results of testing for ID and anaemia according to whether heart failure was prevalent, newly incident, or not present. Haemoglobin was widely tested but iron indices were not. Testing of iron indices was more common in those with lower haemoglobin concentrations and testing was related more closely to haemoglobin result than the presence or absence of heart failure. Very high, but particularly, very low levels of haemoglobin were associated with higher mortality and incident heart

failure. Low serum iron and TSAT but not low ferritin was associated with higher mortality.

These analyses emphasize the clinical importance, both locally and more widespread, of ID and anaemia in patients with heart failure. Improving awareness of the high incidence and prevalence, and the negative consequences of ID may change clinician behaviour in favour of testing for ID in those with heart failure. This is particularly pertinent as when treated with IV iron, hospitalisations for heart failure fall. However, current diagnostic criteria for both ID and anaemia are outdated and require revision. The research presented here suggests that a low serum iron (≤13μmol/L) may be the simplest, most prognostically important marker of ID in patients with heart failure. Current guideline definitions of ID may result in many patients who stand to gain from IV iron therapy missing out on its potential benefits. Future targeted prospective research and sub-group analysis of outcomes according to various definitions of ID in ongoing large clinical trials of iron repletion in patients with heart failure will help corroborate these findings.

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Preface

The work enclosed in this thesis was planned, prepared, and completed over a three and a half-year period from August 2018-April 2022. The latter half of this period overlapped with the global COVID-19 pandemic. Due to significant interruptions to non-COVID related research activities in Glasgow, and prior delays with local research and development, previously planned prospective research (details of which are presented in the Final Discussion) was unable to be included in this thesis. Funding in the form of a project grant (£300,000) from the British Heart Foundation was successfully awarded to undertake this research and recruitment is due to start very soon. Instead, retrospective analysis of two databases and a meta-analysis of clinical trials was conducted.

In addition to completion of the research within this thesis, I have been involved with trial recruitment and study visits for various cardiovascular trials including DAPA-HF (NCT03036124), RELIEHF (NCT04142788), PONTIAC-II (NCT02817360) and IRONMAN (NCT02642562). I also am a member of the Clinical Endpoints Committee in IRONMAN, tasked with adjudication of clinical endpoints in trial participants. During the first wave of the pandemic, I was also involved with trials of the Oxford/AstraZeneca and Novovax COVID vaccine trials as a sub-investigator in Glasgow.

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Author declaration

The concept and design of the work presented herein is of that of the author and his supervisors. This thesis is a record of the author's own work. All analyses were performed by the author, except for statistical support from Ms Jocelyn Friday in Chapter 7, Dr Gabriele Masini in Chapter 3, and Professor Ian Ford in Chapter 6. Dr Gabriele Masini provided insight and support in the production of Figures and Tables in Chapter 3. This thesis has not previously been submitted nor accepted for consideration of a higher degree to any educational institution

Publications arising from this thesis

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Graham FJ, Masini G, Pellicori P, Cleland JGF, Greenlaw N, Friday J, Kazmi S, Clark AL. Natural history and prognostic significance of iron deficiency and anaemia in ambulatory patients with chronic heart failure. Eur J Heart Fail. 2021. doi: 10.1002/ejhf.2251.

Masini G*, Graham FJ*, Pellicori P, Cleland JGF, Cuthbert JJ, Kazmi S, Inciardi RM, Clark AL. Criteria for Iron Deficiency in Patients With Heart Failure. J Am Coll Cardiol. 2022;79(4):341-351. doi: 10.1016/j.jacc.2021.11.039.

*Equal contribution to the manuscript

Abbreviations

ACC	American College of Cardiology
BB	Beta blocker
CHF	Chronic heart failure
CI	Confidence interval
CV	Cardiovascular
CVM	Cardiovascular mortality
eGFR	Estimated glomerular filtration rate
Hb	Haemoglobin
HF	Heart failure
HFrEF	Heart failure with reduced ejection fraction
HFmrEF	Heart failure with mid-range ejection fraction
HFpEF	Heart failure with preserved ejection fraction
HHF	Hospitalisation for heart failure
HR	Hazard ratio
hsCRP	High sensitivity C-reactive protein
ID	Iron deficiency
IV	Intravenous

LV	Left ventricle
LVEF	Left ventricular ejection fraction
NT-proBNP	N-terminal-pro brain natriuretic peptide
NYHA	New York Heart Association functional class
OR	Odds ratio
sTfR	Soluble transferrin receptor
TSAT	Transferrin saturation
WHO	World Health Organisation

Aims and Objectives

Aims

Review of the literature has demonstrated a lack of data exploring alternative definitions of ID in patients with heart failure. Little is known on the effect that varied definitions has on prevalence, clinical associations, and prognostic significance of various markers of ID in patients with heart failure. Potential differences between patients with heart failure of various phenotypes (reduced ejection fraction (HFrEF); mid-range ejection fraction (HFmrEF); preserved ejection fraction (HFpEF)) is also yet to be examined in appropriate detail. I aim to investigate and report associations and differences, if present, in a large cohort of patients diagnosed with heart failure of varying phenotypes representative of clinical practice.

There is little to no longitudinal data reporting incidence, predictors and natural history of both anaemia and ID, according to various definitions, in those with heart failure. I will explore this in a dataset of patients with confirmed heart failure attending a secondary care specialist heart failure clinic over a period of 1 year.

Transferrin saturation is used in the definition of ID in patients with heart failure. Yet, the influence of serum transferrin which, along with serum iron, comprises transferrin saturation, has not been explored. I will explore the relations between serum transferrin and various clinical characteristics and markers of iron status and mortality in a cohort of patients with confirmed heart failure.

In the form of an updated meta-analysis, I will determine the current pooled evidence for use of intravenous iron in patients with heart failure and evidence of ID to reduce hospitalisations for heart failure and cardiovascular mortality.

Locally, there is no data on how often ID or anaemia are tested in patients with heart failure. Prognostic implications of both, including the associations between anaemia and incident heart failure, are also unknown. Using data linked to hospital admissions and deaths from NHS Greater Glasgow & Clyde, I

will explore patterns of testing for ID and anaemia, the associations between anaemia and incident heart failure and the associations between ID, anaemia, and mortality in those with established heart failure, those with new onset heart failure and in those at risk of heart failure.

In summary, the aim of this thesis is to improve our understanding of the burden of anaemia and ID in heart failure, including their incidence, prevalence, and associations with clinical outcomes, and to investigate what blood test best identifies those with ID at highest risk of mortality. I will also investigate whether treatment with IV iron in those with heart failure and evidence of serum ID can reduce hospitalisations for heart failure and cardiovascular mortality. I will do this using data from secondary care databases both locally (SafeHaven) and from different parts of the UK (Hull LifeLab), and by conducting a meta-analysis of available randomised clinical trials assessing the use of IV iron in patients with heart failure and evidence of ID.

Objectives

Chapter 3

By analysing de-identified data from the Hull LifeLab in patients with heart failure of different phenotypes, I will:

1. Describe how different definitions of ID affect its prevalence and relationship to patient characteristics.

2. Describe iron status, including prevalence of ID defined by various criteria, according to heart failure phenotypes.

3. Investigate all-cause and cardiovascular (CV) mortality in patients with heart failure according to iron status and heart failure phenotype

    i. Using continuous variables

    ii. By dichotomized thresholds of each variable/biomarker

4. Investigate the best diagnostic cut-off with regards to prognostic significance for each biomarker in the population as a whole.

Chapter 4

By analysing de-identified data from the Hull LifeLab in patients with heart failure of varying phenotypes, I will:

1. Describe the natural history of anaemia and ID.

2. Explore the differences in natural history according to how ID is defined.

3. Investigate predictors of incident ID and anaemia.

4. Examine the association with all-cause and CV mortality according to changes in anaemia and iron status over 1-year.

Chapter 5

By analysing de-identified data from the Hull LifeLab in patients with heart failure of varying phenotypes, I will:

1. Investigate the relations between serum transferrin concentrations and clinical characteristics and serum iron biomarkers (TSAT, serum iron, ferritin)

2. Examine the associations between serum transferrin concentrations and all-cause and CV mortality with serum transferrin as both:

    i. A continuous variable, and

    ii. Dichotomized into quartiles

Chapter 6

By performing a meta-analysis of data from available randomised trials that investigate the use of intravenous iron in patients with heart failure and evidence of iron deficiency, I will:

1. Present the pooled evidence for use of intravenous iron to reduce hospitalisations for heart failure and CV mortality.

2. Present the pooled evidence for use of intravenous iron to reduce hospitalisations for heart failure alone.

3. Present the pooled evidence for use of intravenous iron to reduce CV mortality alone.

Chapter 7

By analysing de-identified electronic health records via the Glasgow SafeHaven of patients with established, new onset or those at increased risk of heart failure, I will:

1. Investigate how often haemoglobin and iron indices were checked and in whom.

2. Report the prevalence of anaemia and ID, by various definitions, according to heart failure status.

3. Examine the association between haemoglobin and incident heart failure in those without confirmed heart failure.

4. Examine the association between haemoglobin, iron indices and mortality.

Chapter 1 Introduction

1.1 Background

Iron is a key element in human nutrition and is required for oxygen-transport (haemoglobin (Hb)) and storage (myoglobin), electron transport (mitochondrial ATP production) and many enzymatic functions (1–3). Iron deficiency (ID) is the most common nutrient deficiency worldwide (4). The main cause of ID is suboptimal dietary intake, but physiological (ie menstruation in young women) or pathological (for example, gastrointestinal bleeding or malabsorption due to diseases or medications) conditions can also induce ID. Anaemia is a common but late manifestation of severe, prolonged ID. However, the consequences of ID extend beyond iron-deficient erythropoiesis. Alone or in combination with anaemia, ID is associated with a range of signs and symptoms such as fatigue and lethargy, poorer quality of life, impaired physical performance and cognitive function (5–11). ID in both skeletal and myocardial muscle has been demonstrated at a cellular level in some conditions and is likely to contribute to symptomatic and functional complaints common with ID (1–3,12).

Patients with cardiovascular (CV) disease may be at increased risk of ID due to increased gastro-intestinal (GI) blood loss due to antiplatelet or anticoagulant medications (13,14) and reduced absorption due to increased inflammatory mediated secretion of hepcidin (15) and widespread use of agents that reduce gastric acidity (16).

In patients with heart failure, ID is also associated with higher mortality (17–20). However, investigations for ID are rarely done in the absence of anaemia. If they are done and they do suggest ID, iron supplements are still often not prescribed (21), perhaps because of the limited evidence, until recently, that doing so intravenously is beneficial. Iron can be replaced orally or intravenously (IV). The latter route of administration will correct ID rapidly and reliably. The evidence in favour of oral supplementation in heart failure is weak. However, some caution in administering iron intravenously is required because it bypasses the complex biological systems that have evolved to prevent iron overload (22).

Fundamentally, how ID is diagnosed by blood tests in patients with heart failure is not universally accepted nor is it validated by gold-standard bone marrow histology. Currently used criteria to diagnose ID in heart failure (23,24) use a low ferritin (<100µg/L) as the main biomarker to define ID. Due to its extracellular release in times of cellular damage and inflammation (22,25), it may not be suited to principally guide diagnosis of ID in heart failure, a condition where low-grade inflammation is common (26). If currently used guideline criteria to diagnose ID via blood tests are incorrect, this would have major implications on trial recruitment and clinical practice.

1.2 Iron absorption and homeostasis

Iron uptake occurs in the duodenum and proximal jejunum (27). Most dietary iron is in an oxidised ferric form (Fe^{3+}) which requires chelation by organic acids or reduction to ferrous iron (Fe^{2+}) to be absorbed (28,29). Gastric acidity promotes iron absorption (28,30) by gut enterocytes, which export iron via ferroportin into the circulation where it binds to serum transferrin. The iron-transferrin complex is ferried to transferrin-receptors on the surface of target cells (28), internalized and then conveyed to mitochondria for formation of iron-sulphur clusters or haem (31). Excess iron is stored in cells, particularly hepatocytes, as ferritin or haemosiderin (29). Very little ferritin escapes into the circulation, unless cells are damaged, for instance, due to inflammation or infection.

Daily absorption of iron (~1-2mg when healthy and iron replete but increasing up to 10-fold if there is ID and normal absorption) is similar to daily losses of iron, mainly from minor gastro-intestinal blood loss or menstruation in pre-menopausal women (28). The vast majority of daily iron needs comes from recycled red cells, released from macrophages, at the end of their life span (27). Absorption, storage, and recovery of iron are under tight control to avoid deficiency or overload. Hepcidin, produced and released from the liver, has a key role in iron uptake and availability. Plasma concentrations of hepcidin fall (increasing iron absorption) in response to hypoxia or low serum iron and increase (blocking iron absorption) in response to infection, inflammation or iron overload (15,29,31). Hepcidin also binds to and blocks ferroportin (32), preventing iron absorbed by enterocytes or stored in the reticulo-endothelial

system from entering the circulation. The hepcidin-ferroportin complex locks iron inside enterocytes (32), which are continuously sloughed into the faeces (28). At any time, in replete states, there is between 4-6g of iron within the body (33) (**Figure 1**).

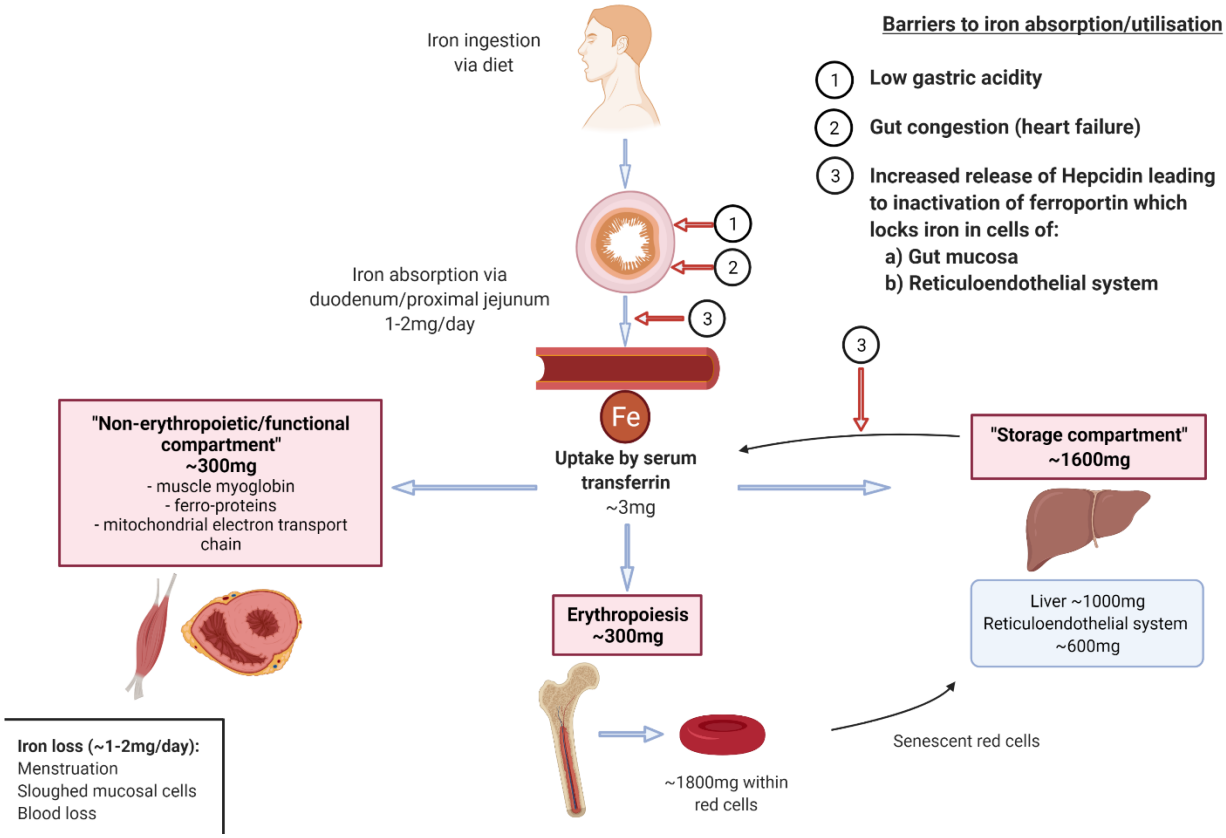

Figure 1: Iron absorption, storage, and utilisation

1.3 Prevalence of iron deficiency

The prevalence of ID (defined by serum biomarkers) in men and post-menopausal women increases with age even in the absence of overt disease (19). To what extent this indicates occult disease (cancer or cardiovascular) or physiological changes with age is uncertain, but it is associated with an increase in all-cause and cardiovascular mortality (34). Patients with CV disease probably have a higher prevalence of ID than the general population even after adjusting for age, either due to the disease or its treatment. In people with heart failure, the prevalence of ID ranges widely, from 12% to 78%, depending on heart failure phenotype, clinical presentation, and definition of ID (**Table 1**) (5,8,18–20,35–44).

Table 1: Definition and prevalence of iron deficiency (ID) for different cardiovascular conditions.

Study	Year	Study design	N=	Women (%)	Age (years)	Prevalence of ID	Definition of ID used
HFrEF							
Klip T et al	**2013**	**PC**	**1,506**	**26%**	**64**	**50%**	**Ferritin <100µg/L or 100-299µg/L+TSAT <20%**
Grote Beverborg et al	2018	PC	42	24%	68	40%	BM: Gale scale 0-1 + ≤10% erythroblasts containing iron
Okonko DO et al.	2011	PC	157	28%	71	43%	TSAT <20%
Jankowska EA et al.	**2010**	**PC**	**546**	**12%**	**55**	**37%**	**Ferritin <100µg/L or 100-300µg/L+TSAT <20%**
Tkaczyszyn M et al.	**2017**	**PC**	**1,821**	**29%**	**66**	**52%**	**Ferritin <100µg/L or 100-299µg/L+TSAT <20%**
Sierpinski et al.	2020	PC	30	7%	63	83%	BM: Gale scale 0-1
HFpEF							
Bekfani T et al.	**2019**	**PC**	**190**	**33%**	**71**	**58%**	**Ferritin <100µg/L or 100-299µg/L+TSAT <20%**
Beale A et al.	**2019**	**MA**	**1,877**	**42-70%**	**54-80**	**59%**	**Ferritin <100µg/L or 100-299µg/L+TSAT <20%**
HFmrEF							
Martens P et al.	**2018**	**PC**	**229**	**29%***	**70***	**61%**	**Ferritin <100µg/L or 100-300µg/L+TSAT <20%**
Mini P et al.	**2018**	**PC**	**37**	**56%***	**88***	**78%**	**Ferritin <100µg/L or 100-299µg/L+TSAT <20%**
HFrEF/HFpEF/HFmrEF							
Cleland JGF et al	**2016**	**PC**	**1,513**	**36%**	**74**	14% / 39%	Serum iron ≤8 or ≤12µmol/L
						24% / 45%	TSAT ≤15 or ≤ 20%
						12% / 54%	**Ferritin ≤30 or ≤100µg/L**
Moliner P et al	**2017**	**PC**	**1,821**	**21-39%**	**62-70**	46%	TSAT <20%
						33%	**Ferritin <100µg/L**
						52%	**Ferritin <100µg/L or 100-299µg/L+TSAT <20%**
Hospitalised HF							
Jankowska EA et al	**2014**	**PC**	**165**	**19%**	**65**	**65%**	**Ferritin <100µg/L or 100-299µg/L+TSAT <20%**
						37%	Hepcidin <14.5ng/ml + sTfR ≥1.59mg/L
Núñez J et al	**2016**	**PC**	**626**	**48%**	**73**	**74%**	**Ferritin <100µg/L or 100-299µg/L+TSAT <20%**
Cohen-Solal A et al.	**2014**	**PC**	**832**	**51%**	**78**	**72%**	**Ferritin <100µg/L or 100-299µg/L+TSAT <20%**

*Data from whole study population, not just cohort with HFmrEF
Definitions including ferritin in bold with prevalence aligned left. Definitions not using ferritin not in bold and aligned right.

Abbreviations: ID: iron deficiency; PC: prospective cohort; MA: meta-analysis; HFrEF: heart failure with reduced ejection fraction; HFpEF: heart failure with preserved ejection fraction; HFmrEF: heart failure with mid-range ejection fraction; BM: bone marrow; TSATs: transferrin saturations; sTfR: soluble transferrin receptor.

1.4 Diagnostic criteria for Iron Deficiency

There is no international consensus as to which biomarker/s, and which diagnostic threshold of these biomarker/s, should be used to define ID. Criteria differ considerably between guidelines intended for the general population compared to patients with heart failure (**Table 2**).

Table 2: International recommended diagnostic definitions and gold-standard bone marrow validation of serum markers of iron deficiency within various cardiovascular conditions and in the general population

	Recommended in international guideline?	Recommendation if present	Evidence base for recommendation?	Assessed in bone marrow?	Suggested tests and thresholds based on bone marrow data
HFrEF	Yes *ESC 2021* [1]	Ferritin <100µg/L or TSAT <20% if ferritin 100-299µg/L	Yes (**Table 4**)	Yes (n=42) [3] Age (years) = 68±10 Women (%) = 24%	TSAT <20% or serum iron ≤13µmol/L
	Yes *ACC/AHA/HFSA 2017 focused update* [2]	Ferritin <100µg/L or TSAT <20% if ferritin 100-300µg/L	Yes (**Table 4**)		
HFpEF	Yes *ESC 2021* [1]	Ferritin <100µg/L or TSAT <20% if ferritin 100-299µg/L	No	No	N/A
	Yes *ACC/AHA/HFSA 2017 focused update* [2]	Ferritin <100 µg/L or TSAT <20% if ferritin 100-300µg/L	No		
HFmrEF	Yes *ESC 2021* [1]	Ferritin <100 µg/L or TSAT <20% if ferritin 100-299µg/L	No*	No	N/A
	Yes *ACC/AHA/HFSA 2017 focused update* [2]	Ferritin <100 µg/L or TSAT <20% if ferritin 100-300µg/L	No*		
Hospitalised HF	No	N/A	N/A	No	N/A
General population	Yes *WHO* [4]	Ferritin <15µg/L	Yes [5, 6]	Yes (n=54) [6] Age (years) = Unknown Women (%) = Unknown	Ferritin ≤30µg/L

*Degree of overlap in some trials of patients with HFrEF

Abbreviations: CHD: coronary heart disease; ACS: acute coronary syndrome; HFrEF: heart failure with reduced ejection fraction; HFpEF: heart failure with preserved ejection fraction; HF: heart failure; TSATs: transferrin saturations; sTfR: soluble transferrin receptor; ESC: European Society of Cardiology; ACC: American College of Cardiology; AHA: American Heart Association; HFSA: Heart Failure Society of America; CRP: c-reactive protein.

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The arguments for and against current criteria are summarised in **Figure 2** and discussed below.

Possible markers of reduced intra-cellular iron

Ferritin

Pros:
•Inexpensive
•Widely available
•Specific for ID if <15µg/L

Cons:
•Poor specificity if >30µg/L
•Increased in inflammation including:
- Infection
- CV disease (incl. heart failure)
- Renal disease
•May be affected by volume status

sTfR

Pros:
•Direct marker of cellular ID
•Not directly affected by inflammation

Cons:
•Expense
•Limited clinical experience and validation
•Not widely available

Possible markers of reduced plasma iron availability

Serum iron

Pros:
•Inexpensive
•Widely available
•Less affected by inflammation than ferritin

Cons:
•Diurnal variation in healthy people - less obvious with disease/ID
•Acute increase after ingestion of iron supplements in healthy people

TSAT

Pros:
•Inexpensive
•Widely available
•Less affected by inflammation than ferritin

Cons:
•Requires measuring two indices
•May vary diurnally and be affected by volume status

Typical results of common iron biomarkers during various clinical conditions

Biomarker \ Condition	Iron deficiency - Yes Inflammation - No	Iron deficiency - Yes Inflammation - Yes	Iron deficiency - No Inflammation - Yes
Ferritin	↓	Highly variable	↑
Serum iron	↓	↓	~↓
Transferrin	↑	↓	↓
TSAT	↓	↓	~↓
sTfR	↑	↑	Normal
Haemoglobin	↓/ Normal	↓/ Normal	↓/ Normal

(~↓) = little or no decline
(↓) = small decline

Abbreviations: Fe: iron; ID: iron deficiency; TSAT: transferrin saturation; sTfR: soluble transferrin receptor

Figure 2: Pros and cons of serum biomarkers of iron deficiency. Typical results of iron biomarkers during various clinical conditions also presented.

1.4.1 The Standard Haematology Profile

Epidemiological studies indicate that the mean haemoglobin concentration declines only a little with age and for healthy men is about 14.5g/dL and for healthy women about 13.5g/dL at age 65-79 years (45,46). There is a large difference between these values and the WHO definition of anaemia (47). If tested, people with a normal haemoglobin (>14g/dL) rarely have other haematological measures suggesting ID. Patients with ID are also likely to have a lower MCV, MCHC and greater RDW, which may all increase the suspicion that a low haemoglobin is due to ID (19). However, for reasons not quite clear, patients with heart failure may not have these classical reductions in MCV, MCHC or greater RDW yet have evidence of serum ID (43).

1.4.2 Ferritin

Intracellular iron that is not immediately required is stored as ferritin, with large amounts found in the liver, spleen, and bone marrow. In the absence of inflammation, serum ferritin correlates with hepatic ferritin content (48). Most clinical laboratories can measure serum ferritin. The WHO defines a low serum ferritin as <15μg/L in the absence of infection or inflammation or, if either is present, <70μg/L (49). However, many clinical laboratories use a threshold of 30μg/L (50–54). Values <15μg/L and perhaps <30μg/L are specific for ID (55). Plasma volume expansion might transiently reduce serum ferritin but probably not to such low values (56). Some have defined ID as a serum ferritin <100μg/L but values between 30-100μg/L, or even higher, appear common in patients who have normal amounts of iron on bone marrow histology (20,57). Cell damage due to infection or inflammation releases ferritin into the circulation (58,59) and therefore serum ferritin may be normal or increased even in the presence of ID (59). Importantly, in patients with heart failure, lower serum ferritin appears to be associated with a better prognosis, probably because it reflects reduced inflammation rather than ID in the setting of CV disease (19).

1.4.3 Serum iron

Serum iron is a measure of the total amount of iron in serum and is almost exclusively transferrin bound. The test is inexpensive and widely available. In those without CV disease, there is a diurnal variation of 8-15% (60,61) and

concentrations increase transiently after oral iron ingestion (62). Low serum iron is associated with bone marrow iron depletion (20). In contrast to ferritin, there is an inverse relationship between serum iron and markers of inflammation and infection (19), probably due to hepcidin-mediated reductions in iron absorption (leading to absolute ID) and sequestration in the reticulo-endothelial system (leading to functional ID) (63).

1.4.4 Transferrin and Total Iron Binding Capacity (TIBC)

Transferrin is secreted by liver cells, binds iron in the blood and ferries it to cells (28). Serum transferrin increases in response to ID but declines with chronic inflammation, although it may transiently increase with infection (64). TIBC is the amount of iron that is required to fully saturate serum transferrin (65). It is a surrogate measure of transferrin. High transferrin concentrations suggest ID although it is not considered accurate enough to diagnose ID alone.

1.4.5 Transferrin Saturation (TSAT)

Transferrin saturation (TSAT) is the percentage of transferrin that is bound to iron. Measurement of TSAT requires measurement of both serum iron and serum transferrin or TIBC. A fall in the availability of iron is a major stimulus to the secretion of transferrin (4). Serum iron and TSAT are highly correlated (r values >0.9) in patients with chronic stable heart failure (19). This throws doubt on the value of measuring TSAT and, therefore, transferrin. TSAT, like serum iron, is inversely related to inflammation (63,66), but with chronic low-grade inflammation, theoretically, serum transferrin may fall and TSAT may be normal despite a low serum iron. If serum iron is the better marked of ID, this may lead to some cases of ID being missed due to a falsely normal TSAT.

1.4.6 Soluble Transferrin Receptor (sTfR)

Cellular uptake of iron occurs when the transferrin-receptor binds transferrin-bound iron. ID leads to intensified expression of TfR on the cell surface to increase iron avidity, which leads to increased shedding into the circulation in a soluble form (sTfR). High concentrations reflect intracellular iron depletion (18,67). sTfR is unaffected by inflammation (31,68). In anaemic patients with chronic non-CV inflammatory disease (such as rheumatoid arthritis), it

discriminates between anaemia due to ID and other causes of defective erythropoiesis associated with chronic disease (69). A high sTfR-log ferritin index has also been used to indicate ID (59,70). More research is required to establish values for sTfR that are diagnostic of ID in clinical practice.

1.4.7 Others

Zinc protoporphyrin, reticulocyte haemoglobin content, percentage of hypochromic red cells and hepcidin have also been used to assess ID (18,51,71–73) but have rarely been applied to populations with CV disease (59).

1.4.8 Bone marrow histology

Assessment of stained iron content of erythroid bone marrow remains the gold standard test for diagnosis of ID (22,64). It is rarely performed due to it being invasive - usually requiring percutaneous sampling -, painful, expensive, time consuming and requiring specialist expertise in sampling and assessment. It also has limitations in that its assessment remains largely subjective: stained iron granules are counted and graded according to their abundance and/or the magnification required (74).

1.5 Inflammation

Inflammation plays a key role in the pathogenesis and progression of many CV conditions (26,75). Inflammation causes sequestration of iron in enterocytes and macrophages and results in cellular damage, leading to release of ferritin (**Figure 3**). Inflammatory biomarkers, such as C-reactive protein (CRP), are associated with disease progression and prognosis in various cardiovascular conditions (76,77). In heart failure, serum concentrations of CRP correlate strongly and directly with serum ferritin (19) but inversely (and more weakly) with TSAT and serum iron (13,19). Concentrations of IL-6, a pro-inflammatory cytokine up-regulated in heart failure, are inversely associated with serum iron (78), consistent with hepcidin-related reduction in iron absorption and absolute ID and/or, functional ID due to iron being locked in the RE system.

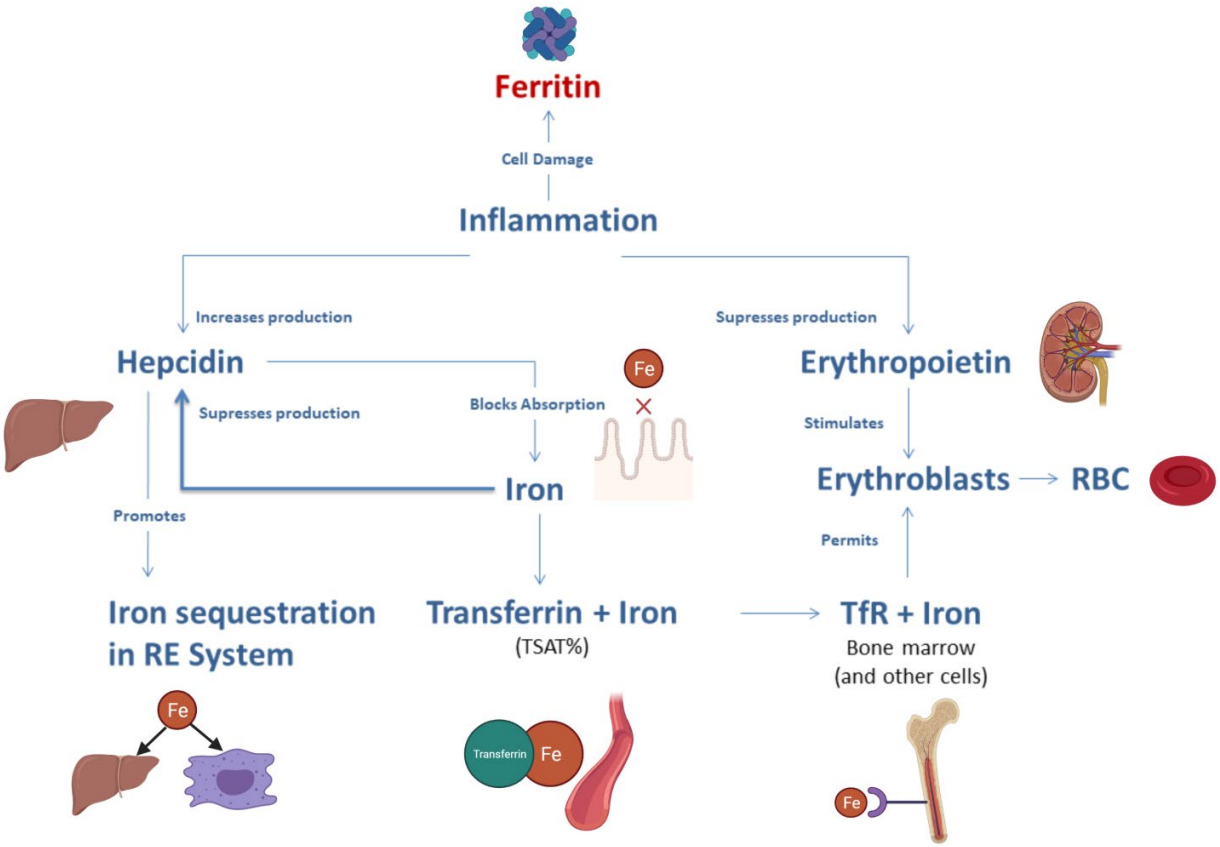


Figure 3: The effect of inflammation on serum ferritin and pathways of iron absorption and utilisation. Abbreviations – RE: reticuloendothelial; Fe: Iron; RBC: Red blood cells; TfR: Transferrin receptor.

1.6 Iron replacement therapy

By the time someone develops anaemia due to ID, the iron deficit is likely to exceed 1,000mg (79). Oral iron therapy is currently the first-line treatment of ID in primary care. In otherwise healthy individuals, ID can lead to increased absorption of iron to ~20mg/day (80). Accordingly, assuming no further loss of iron, it takes two or more months for supplements to achieve iron repletion (22). Iron absorption is reduced by some foods, such as tea and coffee, by commonly prescribed medicines, such as proton pump inhibitors and H2 antagonists (16,22), and as a consequence of many diseases. Up to a third of patients have gastrointestinal side effects with oral iron (81) which may reduce compliance and therefore the success of therapy.

Intravenous (IV) iron quickly replenishes iron stores, leading to a rapid and robust correction of anaemia (81). Adverse events were common with first-generation IV iron formulations, attributed to the rapid release of free iron into the circulation. Modern preparations use carbohydrate-encapsulated iron that is taken up by macrophages, which gradually release iron, avoiding large amounts of free, non-transferrin-bound iron (82). They can be given as a single dose over 15-30 minutes. Third generation formulations of IV iron used in clinical trials (iron sucrose (IS), ferric carboxymaltose (FCM), iron isomaltoside (aka ferric derisomaltose (FDI)) and ferrumoxytol) are safe with adverse reactions reported in fewer than 1 in 200,000 infusions (83). No instances of severe allergic reaction or anaphylaxis was reported in any recent heart failure treatment trials (84–87). FCM may cause reduction in serum phosphate (88) that may have adverse consequences on bone metabolism (89) when given repeatedly to people with normal renal function (impaired renal function prevents phosphate wasting). FDI appears to be associated with less hypersensitivity reactions than IS or FCM and a more rapid haematological response than IS (90). There do not appear to be any other clinically significant differences between the formulations (90,91).

Chronic iron overload is toxic, augmenting oxidative stress (22) and potentially predisposing to infection (92). Although no difference was reported in rates of infection over 2 years of follow-up in the PIVOTAL trial (n=2,141) using iron sucrose in patients having haemodialysis (93) or in the recent AFFIRM-AHF trial using FCM in patients (n=1,108) with heart failure (87), a more recent meta-

analysis including 64 trials of various patient groups suggests that IV iron is associated with a small increased risk of infection (94).

1.7 Iron deficiency in Heart Failure

1.7.1 Heart failure with reduced ejection fraction

Background and prevalence

The study of ID in heart failure initially began as a result of its important role in causing anaemia. Anaemia is common in patients with heart failure (~30%) and associated with worse symptoms, exercise capacity, and prognosis (19,95,96). The reported prevalence of ID (European Society of Cardiology (ESC) definition (see below): n=3,327) is approximately 50% (41,43) and even higher if patients are anaemic (5,41,43). The prevalence of ID on bone marrow histology may be lower in ambulatory patients (40%) but this is based on many fewer individuals (n=42) (20).

ID is also associated with fatigue, poor quality of life and exercise intolerance as well as higher mortality even in the absence of anaemia (5,17,41,42). Trials of erythropoietin that aimed to correct anaemia showed little evidence of clinical benefit (97), whereas trials of IV iron, in those with or without anaemia, have shown improvements in symptoms and exercise capacity and reduced hospitalisations for heart failure (84–86). The benefit of IV iron appears to occur whether or not anaemia is present, although IV iron does appear to cause small increases in haemoglobin by around 0.5g/L over 24 weeks (84). This has led to speculation that most of the benefits of IV iron are mediated by mechanisms other than on the erythron (7,12,98,99) .

Iron depletion is present in cardiomyocytes from patients with HFrEF (1–3,100) but there is a only a weak relation between tissue iron and serum markers of ID (3,100). Myocardial iron depletion exacerbates mitochondrial dysfunction already present in those with heart failure (2,98), impairing myocardial contractility (98), which may contribute to the progression of the heart failure syndrome (101). In both a retrospective study of 547 patients and a prospective study of 77 patients undergoing cardiac resynchronisation therapy (CRT), those with ID (ESC definition) were less likely to have either symptomatic benefit or

recovery in LV systolic function (102,103). Correcting iron depletion can reverse both mitochondrial structural and functional defects and, *in vitro*, improve contractility in human cardiomyocytes (98).

The European Society of Cardiology (ESC) and American College of Cardiology (ACC) define ID as a serum ferritin <100μg/L or TSAT <20% if ferritin 100-299 (ESC) or 100-300 (ACC) μg/L (23,24). These criteria are based on the inclusion criteria for several completed trials of IV iron described below (84,85,87).

The definitions suggested by the ESC and ACC have not been validated against a gold-standard bone marrow diagnosis of ID. A serum iron of ≤13μmol/L and a TSAT ≤19.8% were both found to be highly sensitive and specific, and more accurately reflect bone marrow ID, than the suggested ESC definition in a recent study of patients with HFrEF (n=42) (20). Another study including 30 patients found that a sTfR of ≥1.25mg/L was the best serum marker of ID on bone marrow histology (104). In a study of 37 patients with anaemia and advanced heart failure, 73% were found to have no iron on bone marrow histology. Serum iron concentrations were markedly reduced (mean values <10μmol/L). Mean serum ferritin was 75μg/L for those with iron absent in the marrow compared to 212μg/L in those where it was present, but the authors commented that serum ferritin was 'not a reliable marker of ID' (57).

Iron biomarkers and outcome

In patients with heart failure, amongst potential measures of ID, serum iron and TSAT are highly correlated (r >0.9) and, along with anaemia, strong predictors of prognosis (19). The relation between ferritin and mortality is inverse with higher serum concentrations generally associated with a worse prognosis.

Dichotomized, serum iron ≤13μmol/L and TSAT ≤19.8% independently identified those with HFrEF at heightened risk of mortality (n=387) whereas ferritin <100μg/L did not (20). Similarly, a TSAT of <20%, alone or in combination with a ferritin <100μg/L, independently predicted death in 1,821 patients with heart failure, the majority with HFrEF, whereas an isolated low ferritin (<100μg/L) did not (37). Applying the definition of TSAT <20%, ID without accompanying

anaemia had double the risk of death of those with anaemia not due to ID (n=157) (42).

The association between ID using the ESC/ACC definitions (based on a composite of ferritin and TSAT) and prognosis is less clear (17,37,41). ID by these definitions has been reported as an independent predictor of death (n=546; n=1,506) (17,41) and heart failure hospitalisations following cardiac resynchronization therapy (CRT) implantation (n=547) (102). However, alternative biomarker definitions using serum iron or TSAT alone were not explored in these studies and in general, are rarely explored in the literature.

In one study, serum sTfR of ≥1.41mg/L was the only marked of ID that was an independent predictor of all-cause death in younger patients (mean 58 years) with HFrEF (n=791) (104).

Effect of iron therapy

Intravenous iron

For patients with heart failure and ID, IV iron improves symptoms, quality of life and exercise capacity (84,85) and appears to reduce hospitalisations for heart failure (87,105). However, uncertainty remains about the effect of IV iron on all-cause and CV mortality (87,105).

Some smaller studies provide mechanistic insights. In a study of 40 patients with HFrEF who also had ID by the ACC-definition, IV iron isomaltoside led to a more rapid recovery of skeletal muscle phosphocreatine following exercise as assessed by MRI, suggesting improved mitochondrial function (99). In an observational study of 60 patients with HFrEF, chronic kidney disease and ID (ferritin <100µg/L or TSAT <20%), a 5-week course of IV iron sucrose (total 1000mg) was associated with improvement in echocardiographic LV function and a fall in plasma NT-proBNP over the following 6 months (106). A study of eight patients with ID (ESC definition), similar changes were seen on cardiac MRI a median of 43 days after a single dose of 1000mg IV ferric carboxymaltose (107), together with an increase in a marker of myocardial iron. In a randomized trial of 75 patients with ID (ACC-defined) and persistently reduced LVEF (<45%) despite CRT device implantation

(≥6-months), IV FCM improved LVEF and cardiac contractility compared to placebo (108).

Oral iron

In a study of 225 patients with HFrEF, oral iron did not appear effective in replenishing iron stores, improving quality of life or exercise tolerance over 16 weeks (73) (**Table 3**). This may be explained by reduced absorption of oral iron due to a combination of increases in hepcidin and low gastric acidity, the latter potentially as a result of prescriptions of proton-pump inhibitors (16,28,31). However, many of the patients enrolled in this study (73) may not have had ID; the median TSAT was 19% and a quarter of patients had a TSAT >24%; the median serum iron was 13μmol/L with a quarter having values ≥16μmol/L. Oral sucrosomal iron may hold future promise given its lower GI side-effect profile and alternative route of enteric absorption (109), yet current evidence for its use in patients with heart failure is poor (110).

Ongoing and future research

There are three large scale randomized trials of IV iron that should report in the next two years that have enrolled patients with chronic HFrEF and ID (by various definitions). Endpoints include hospitalisations for heart failure and mortality (**Table 4**).

Table 3: Completed outcome trials of iron therapy including >100 patients with heart failure and iron deficiency

	FAIR-HF	CONFIRM-HF	EFFECT-HF	IRON-OUT	AFFIRM-AHF
Year	2009	2014	2017	2017	2020
Country	10 countries in Europe and Argentina	9 European countries	8 European countries and Australia	USA	15 countries (International)
Number of patients	459 (2:1 IV FCM vs placebo)	301 (1:1 FCM vs placebo)	172 (1:1 FCM vs placebo)	225 (1:1 oral iron vs placebo)	1108 (1:1 FCM vs placebo)
Definition of ID	Ferritin < 100µg/L or 100-299µg/L if TSAT <20%	Ferritin < 100µg/L or 100-300µg/L if TSAT <20%	Ferritin < 100µg/L or 100-300µg/L if TSAT <20%	Ferritin 15-100µg/L or 100-299µg/L with TSAT<20%	Ferritin < 100µg/L or 100-299µg/L if TSAT <20%
Inclusion criteria	• NYHA II & LVEF ≤40% • NYHA III & LVEF≤45% • Hb 9.5-13.5 g/dL	• NYHA II or III • LVEF ≤45% • Hb < 15g/dL • BNP >100pg/ml / NT-proBNP >400pg/ml	• NYHA II or III • LVEF ≤45% • Hb < 15g/dL • BNP >100pg/ml / NT-proBNP >400pg/ml • pVO^2 of 10-20ml/kg/min	• NYHA II-IV • LVEF ≤40% • Hb 9-15g/dL (men); • Hb 9-13g/dL (women)	• LVEF <50% • Hospitalised with HF • NT-proBNP ≥1600pg/ml (SR) or ≥2400pg/ml (AF) • ≥40mg IV Furosemide or equivalent • Hb 8-15g/dL
Age (mean, unless otherwise stated)	67	69	64	63 (median)	71
Sex (women)	244 (53%)	141 (47%)	43 (25%)	80 (36%)	494 (45%)
Form of iron therapy; dose (mean or dosage)	IV FCM; n/a	IV FCM; 1500mg	IV FCM; 1204 mg	Oral iron polysaccharide; 150mg BD for 16 weeks	IV FCM; 1352mg

Follow-up	24 weeks	52 weeks	24 weeks	16 weeks	52 weeks
Primary endpoint	Patient Global Assessment and NYHA class at week 24	Change in 6MWT from baseline to week 24	Change in pVO^2 from baseline to week 24	Change in pVO^2 from baseline to week 16	Composite of total HF hospitalisations and CV death
Outcome	• Improvement in PGA (OR 2.51 (1.75-3.61); p<0.001) • Improvement in NYHA (by one class: OR 2.40; 1.55-3.71; p<0.001)	• Increase in distance at week 24 (33±11 metres, p<0.002) • Extending out to week 52 (p<0.001)	• Increase in pVO^2 (1.0±0.4ml/kg/min; p=0.02) • Significance lost without imputation for deaths	• No significant difference in pVO^2 at 16 weeks (21ml/min) p=0.46✝	• Trend to improvement (RR 0.79 (0.62-1.01); p=0.059) • Reduction in total HF hospitalisation with (RR 0.74 (0.58-0.94); p=0.013)

Abbreviations: HF: heart failure; HFrEF: heart failure with reduced ejection fraction; TSAT: transferrin saturation; Hb: haemoglobin; LVEF: left ventricular ejection fraction; NYHA: New York Heart Association class; BNP: brain natriuretic peptide; NT-proBNP: N-terminal pro-brain natriuretic peptide; ID: iron deficiency; pVO^2: peak VO^2: ferric carboxymaltose; 6MWT: 6-minute walk test; BD: twice daily.✝ = many patients had a TSAT >20% at baseline.

Table 4: Currently recruiting trials assessing efficacy of IV iron in ≥200 patients with iron deficiency and various cardiovascular conditions

Chronic HFrEF - Outcome trials				HFpEF
	FAIR-HF2	IRONMAN	HEART-FID	FAIR-HFpEF
ClinicalTrials.gov Identifier	NCT03036462	NCT02642562	NCT03037931	NCT03074591
Design	Prospective, DB, randomized, parallel-group; placebo controlled	Prospective, single-blind, randomized, open-label	Prospective, DB, randomized, parallel-group; placebo controlled	Prospective, single-blind, randomized, parallel-group; placebo controlled
Location	Europe (8 countries)	UK (50 centres)	USA, Australia, Canada, New Zealand, Poland	Germany (10 centres)
Number of patients	1200	1300	3014	200
Randomisation	Unknown	1:1	1:1	1:1
Definition of ID	Ferritin < 100µg/L or 100-299µg/L if TSAT <20%	TSAT <20% and/or ferritin <100µg/L but <400µg/L	Ferritin < 100µg/L or 100-300µg/L if TSAT <20%	Ferritin < 100µg/L or 100-299µg/L if TSAT <20%
Key inclusion criteria	• HFrEF for at least 12 months • Hb 9.5-14.0 g/dL	• LVEF<45% • NYHA II-IV • NT-proBNP>250ng/L (sinus) or >1000ng/L (AF); or, current or recent HFH (6-months) • Hb 9-13g/dL (women); 9-14g/dL (men)	• LVEF ≤40% • NYHA II-IV • NT-proBNP>600pg/ml (sinus) or >1000pg/ml (AF); or, current or recent HFH (12-months) • Hb 9-13.5g/dL (women), 9-15g/dL (men)	• LVEF ≥45% • NYHA II-III • NT-proBNP >300pg/ml (SR), or >600pg/ml (AF), or HFH within 12-months • DD on ECHO • Hb 9-14g/dL
Formulation of IV iron	FCM	Iron (III) derisomaltose 1000	FCM	FCM
Dosing schedule	1) 1000mg 2) +/-500-1000mg within 4 weeks	1) 1000-2000mg based on Hb and weight	1) Two doses 1 week apart (max of 1500mg combined)	1) 750mg

	3) Then 500mg every 4-months (unless Hb>16g/dL or ferritin >800µg/L)	2) Reassessed with subsequent dose within 4 weeks and at 4-monthly intervals if TSAT<25% (+ ferritin <400 µg/L) and/or ferritin<100 µg/L	2) Repeated every 6 months indicated by TSAT, ferritin and Hb	2) No further details on dosing schedule reported
Primary end-point	Composite of rate of recurrent HFH and CV death	Composite of CV mortality or HFH	1) All-cause mortality 2) HFH 3) Change in 6MWT	Difference in 6MWT
Follow-up	12 months	Event driven	12 months	12 months
Estimated completion date	December 2021	February 2021	June 2022	July 2021

*Number specifically with iron deficiency being treated with IV iron not reported

Abbreviations: HFrEF: heart failure with reduced ejection fraction; HF: heart failure; HFpEF: heart failure with preserved ejection fraction; DB: double-blind; TSAT: transferrin saturation; Hb: haemoglobin; ID: iron deficiency; HFH: heart failure hospitalisation; LVEF: left ventricular ejection fraction; NYHA: New York Heart Association class; NT-proBNP: N-terminal pro-brain natriuretic peptide; AF: atrial fibrillation; AHF: acute heart failure; CXR: chest X-ray; FCM: ferric carboxymaltose; CV: cardiovascular; 6MWT: 6-minute walk test

1.7.2 Heart failure with preserved ejection fraction

Background and prevalence

Compared to those with HFrEF, patients with HFpEF are typically older, more likely to be women and to have comorbidities, such as chronic kidney disease and atrial fibrillation, which increase the risk of developing anaemia and ID (111,112). ID in HFpEF is associated with poorer QOL and reduced exercise capacity (38,44).

A meta-analysis of studies in 1,877 patients with HFpEF reported a prevalence of ID (ESC/ACC-definitions) of 42-70% (8). A large cohort study (19), showed that 41% of those without and up to 71% of those with anaemia had a TSAT <20%.

Many of the comorbidities common in HFpEF, such as diabetes, are associated with abnormalities of skeletal muscle function and mitochondrial metabolism (12). In 8 patients with HFpEF and serum ID, concentrations of cellular TfR, obtained from endomyocardial biopsies, were higher than in a control group without ID (113), suggesting tissue ID at cardiomyocyte level.

Based on very limited evidence, the most recent ESC guidelines suggest that patients with HFpEF be screened for ID, adopting the definition used in those with HFrEF: ferritin <100µg/L or TSAT <20% if ferritin 100-299µg/L (23). Similarly, the most recent update to the 2013 ACC guidelines do not discriminate between HFrEF and HFpEF and suggest adopting the same cut-offs as for HFrEF: ferritin <100µg/L or TSAT <20% if ferritin 100-300µg/L (24).

No criteria for ID based on serum biomarkers have been validated against bone marrow histology in this group of patients.

Iron biomarkers and outcome

There is little evidence of an association between ID and higher mortality or heart failure hospitalisations in HFpEF (8). A recent meta-analysis of four studies comprising 711 patients with HFpEF found no significant association between ID and death or hospitalisation (8). However, analysis of a large cohort including more than 1,000 patients with HFpEF found that a low serum iron was associated

with an increase in the risk of sudden death as well as death from heart failure and infection (19).

Effect of iron therapy

There are no completed randomised trials in patients with HFpEF and ID.

Ongoing and future research

The FAIR-HFpEF trial aims to enrol 200 patients with HFpEF (LVEF ≥45%) and ID (ESC definition) to determine the effects of IV FCM on symptoms and exercise capacity {NCT03074591}. Heart failure hospitalisations and mortality at 1-year is a secondary outcome (**Table 4**).

1.7.3 Heart failure with mid-range ejection fraction

Background

Given heart failure with mid-range ejection fraction (HFmrEF) is the newest described phenotype of heart failure and that it comprises proportionally the least number of patients, research in ID in this group is limited. One recent report from a single centre study in Belgium described significant limitations in exercise capacity in those with HFmrEF and ID compared to those without ID (n=177). The prevalence of ID (ESC/ACC) is similar to HFpEF with rates between 61% in a mostly male population (n=229; mean age 70 years) (38), and 78% in an older, mostly female population (n=37; mean age 88 years) (39).

As is the case for HFpEF, and based on less evidence, the most recent ESC guidelines suggest that patients with HFmrEF be screened for ID, adopting the definition used in those with HFrEF: ferritin <100µg/L or TSAT <20% if ferritin 100-299µg/L (23). The most recent update to the 2013 ACC guidelines do not discriminate between HFrEF and HFmrEF and suggest: - ferritin <100µg/L, or, TSAT <20% if ferritin 100-300µg/L (24).

No criteria using serum biomarkers have been validated against the bone marrow gold-standard in this group of patients.

Iron biomarkers and outcome

Unadjusted analysis from a single centre study reported mortality and hospitalisations for heart failure were higher for patients with HFmrEF and ID compared to those without ID (n=229) (38).

Effect of iron therapy

There are currently no completed prospective trials assessing the potential benefits of iron therapy in patients with HFmrEF alone.

Ongoing research

To my knowledge, there are no trials specifically investigating ID in this cohort of patients. IRONMAN uses a definition of HFrEF as an ejection fraction of <45% for trial inclusion whilst the FAIR-HFpEF trial employs an LV ejection fraction cut-off of ≥45% for inclusion. Both trials overlap phenotypes of HFmrEF (40-49%) and HFpEF (≥50%) as defined by 2016 ESC criteria (114).

1.7.4 Hospitalised heart failure

Background and prevalence

Among patients hospitalised for heart failure, the prevalence of ID using the ESC definition varies from 65% in younger patients, mostly with HFrEF (18), to 72-74% in older patients, many of whom have HFpEF (35,36). An alternative definition (hepcidin <14.5 ng/ml and sTfR ≥1.59mg/L) reported a lower prevalence of 37% (18). Iron indices, as well as blood haemoglobin, can be low due to plasma volume expansion (115). In the placebo arm of the AFFIRM-AHF trial, serum ferritin and TSAT increased by ~25µg/L and ~5% respectively within 6 weeks after discharge following an episode of hospitalised heart failure (87), which may be attributed to resolution of congestion. Others have made similar observations (56).

The new ESC guidelines recommend using the same criteria for ID in both the acute and chronic setting. The ACC guidelines do not comment (23,24).

Unsurprisingly, no definition has been validated against the bone marrow gold standard.

Iron biomarkers an outcome

In a study of 832 patients, a low serum ferritin (<100µg/L) predicted a high risk of early readmission following an episode of hospitalised heart failure (35). Amongst potential measures of ID, only the proposed definition of a low hepcidin/high sTfR ratio predicted mortality in a study of 165 patients, most of whom had HFrEF (18).

Effect of iron therapy

In a study of 49 patients with ID (serum ferritin <300µg/L if TSAT <20%), a single dose of IV iron (1000mg) given prior to discharge did not improve 6-minute walk distance compared to placebo at 12 weeks (116). In the AFFIRM-AHF trial (n = 1,108, mean age 71 years, 56% men, mean LVEF 33%) of patients randomised to IV FCM (mean total dose 1352mg) or placebo following an episode of hospitalised heart failure, IV FCM came close to exerting a statistically significant reduction in a composite of recurrent heart failure hospitalisations and cardiovascular death at 52 weeks (RR 0.79; (0.62-1.01); p=0.059) (87) (**Table 3**). The total number of hospitalisations for heart failure was reduced by 26% in those who received IV FCM rather than placebo (p=0.013). Quality of life also improved by, on average, a small amount (87). Dosing of FCM could be repeated up to 24 weeks after inclusion if ID persisted, with the majority (>80%) receiving either one or two doses. The subgroup of patients with coronary artery disease might have derived more benefit (p=0.015).

Ongoing research

I am not aware of other trials targeted to those hospitalised with heart failure. Further analysis may identify criteria for ID that identifies a subgroup with greater benefit. Many admissions for heart failure (up to 25%) are caused or complicated by infection (117), which may confound interpretation of serum ferritin and cause concern about the safety of IV iron (contra-indicated in acute infection).

1.8 Conclusion

Many older people, especially those who also have CV disease, have evidence of ID, which may cause or exacerbate fatigue and breathlessness, impair quality of life and exercise capacity, and is associated with a worse prognosis. For some groups of patients, correction of ID improves each of these problems. However, the majority of studies of ID evaluate only one pre-determined definition of ID which may be inaccurate. The diagnostic criteria for ID in clinical practice should be re-evaluated, both diagnostically and prognostically, to ensure appropriate selection for iron therapy. The impact of recovery from ID and anaemia on clinical outcomes, both spontaneously and by treatment with IV iron, according to various definitions also warrants investigation.

Chapter 2 Methods

Introduction

The following describes statistical analyses common to some, or all chapters of the thesis. Detail regarding what analysis is used and its application are described in specific detail in respective chapters.

Descriptive statistics

Unless stated otherwise, descriptive statistics are presented as median with 25th and 75th centiles if continuous or count and (%) if categorical.

Independent t-test

A parametric test that is used to compare continuous measures between two groups. The test assumes that data is approximately normally distributed. The null hypothesis (a hypothesis in statistical tests that proposes that there is no difference in the tested variable between groups) is tested and, as a result, the presence or absence of statistically significant differences between means of groups are determined.

One-way Analysis of variance (ANOVA)

One-way analysis of variance is a parametric test used to assess if there are differences between three or more groups of continuous, independent variables. The tests assume that data is approximately normally distributed. The null hypothesis is tested and, as a result, the presence or absence of statistically significant differences between means of groups are determined.

Kruskal-Wallis test

This is the non-parametric equivalent of the one-way ANOVA. The Kruskal-Wallis test does not rely on the assumption that data are normally distributed.

Chi squared test (X^2)

The Chi-squared tests compares the distribution of categorical data in two samples. It tests the null hypothesis and determines if there are statistically

significant differences between expected and observed frequencies of data between the two groups.

Linear regression/correlation analysis

This tests the extent to which two continuous variables are related. The strength and direction of the relationship between two variables is presented via a correlation coefficient.

Restricted cubic splines

These are a way of transforming and presenting the relationship between continuous data and a given outcome when the relationship is non-linear.

Logistic regression

This is used to describe the relationship between a binary dependent variable and one or more continuous or categorical independent variables. It is a generalised linear model that applies the logit link function. It also allows adjustment of other factors to produce a multivariable model. Output from models are in the form of Odds Ratios: the measure of association between a given covariate and the dependent outcome.

Kaplan-Meier plots

Kaplan-Meier analysis is a simple, validated way to calculate and present the probability of an event occurring, typically survival or death of participants in a given analysis over time. The x-axis represents time and the y-axis represents the cumulative probability of the occurrence of an event.

Log-rank test

The log-rank test compares the survival distribution of two or more independent Kaplan-Meier survival curves. The null hypothesis is that there is no difference between the survival distributions. If the log-rank test is significant, this demonstrates that there is a statistically significant difference between all survival curves in a given analysis.

Cox proportional hazards regression

Cox proportional hazards regression is used to determine the effect of one (univariable), or several (multivariable) continuous or categorical variables on survival – the hazard rate. The model assumes that covariate effects on the hazard rate are constant over time and the output from the model gives the instantaneous risk of death or event occurrence over time.

Cumulative incidence plots

These illustrate the cumulative proportion of subjects with a specific outcome over time but account for the competing risk of death.

Fine-Gray sub-distribution hazards model is a form of cumulative incidence function used to take account of competing risks.

Receiver operating characteristic (ROC) analysis

This analysis plots sensitivity (the true positive) against 1- specificity (the true negative) of a continuous independent variable and its prediction of a definitive binary outcome, such as diagnosis. The analysis produces a ROC curve with an associated area-under-the-curve (AUC) value. An AUC value of 0.5 is the 'null hypothesis'. The aim of the analysis is to provide an optimal diagnostic threshold of a continuous variable. The AUC provides a measure of the performance of a variable at predicting a definitive binary outcome: the higher the AUC value, the better the variable performance. Values derived from AUC of different variables can be compared.

Meta-analysis

Meta-analysis is a statistical method of investigating the pooled effect, with data from two or more studies, of an intervention on a given outcome.

Fixed effects meta-analysis

This method of meta-analysis provides an estimate of the average treatment effect in the studies included in the statistical model. Use of this assumes that effect size across studies does not vary substantially.

Random effects meta-analysis

This method of meta-analysis assumes that studies included in the model have underlying treatment effects due to a random distribution. It provides estimates of the average of and variation in the treatment effect in a given distribution of studies.

Statistical software

Statistical analysis for chapters 3, 4 and 5 were conducted with SPSS versions 26 and 27 (IBM), with limited analysis in chapter 3 also conducted with STATA version 17 (STATA corp.). Analysis for chapter 4 was conducted with Review Manager (RevMan) Version 5.4 (The Cochrane Collaboration, 2020) and for Chapter 7, with R version 3.6.3.

Chapter 3 Criteria for iron deficiency in patients with heart failure

Support in statistical analysis, production of tables and figures and concept in this Chapter was provided by Dr Gabriele Masini.

3.1 Introduction

Evidence of iron deficiency (ID) in blood serum is common in chronic heart failure (CHF) and, in the presence or absence of anaemia, is associated with poorer quality of life, exercise capacity and prognosis (5,41,44,118). Many definitions of serum ID have been proposed, but consensus is lacking on which should be used in clinical practice for patients with CHF.

The World Health Organization defines ID as a serum ferritin <15μg/L (52) and in most clinical laboratories as <30μg/L. However, international guidelines on CHF define ID as a serum ferritin <100μg/L or a transferrin saturation (TSAT) <20% if ferritin is 100-299μg/L (23,24). These criteria were based on consensus opinion mainly among nephrologists (119) and on the selection criteria for successful clinical trials of intravenous (IV) iron in CHF, such as the FAIR-HF trial (84). However, a definition based primarily on ferritin has several limitations. Most ferritin resides in cells, where it binds to iron to prevent potentially toxic free radical production. Any cell damage, including activation of inflammatory pathways, may cause ferritin to be released; an increase in serum ferritin may occur even in the presence of bone marrow defined ID (20,57). Observational studies suggest that serum iron concentration and TSAT may be more strongly associated with prognosis than serum ferritin and might be a better guide to which patients benefit from IV iron (19,20,37,42,105).

Accordingly, we decided to investigate the prevalence, associations, and prognostic significance of ID using diverse criteria in a large cohort of patients attending a heart failure clinic.

3.2 Methods

3.2.1 Study population

Patients referred between December 2001 and June 2019 with suspected or confirmed heart failure to a regional heart failure clinic (Hull LifeLab), serving a community of ~550,000 individuals, were included. All patients gave written informed consent for their data to be electronically stored and used for research. Demographic data, medical history, symptoms and signs and an electro- and echocardiogram were recorded. Blood samples were obtained for haematological and biochemical tests, including N-terminal-pro brain natriuretic peptide (NT-proBNP), serum iron, transferrin, TSAT and ferritin. The study was approved by the Hull and East Yorkshire Local Research Ethics Committee.

3.2.2 Definitions

Heart failure was defined as the presence of symptoms and signs and **either** 1) a left ventricular ejection fraction (LVEF) of <40% (HFrEF) or by a raised NT-proBNP (≥125 ng/L). Those with a raised NT-proBNP were further classified as those with an LVEF of 40-49% (mid-range ejection fraction (HFmrEF)) or ≥50% (preserved ejection fraction (HFpEF)). If NT-proBNP was elevated but information on LV function was not available, patients were grouped as HF-↑NT-proBNP. Patients with an LVEF of ≥40% who had no available measurement of NT-proBNP or an NT-proBNP <125 ng/L or those who lacked echocardiographic data were excluded from this analysis.

Anaemia was defined by the World Health Organization (W.H.O.) criteria as a haemoglobin <12.0g/dL in women and <13.0g/dL in men (47). Serum ferritin, TSAT and serum iron were used as biomarkers of ID. Serum ferritin and iron were directly measured and TSAT was calculated using: TSAT (%) = serum iron (µmol/L)/(transferrin [g/L] x 25.2) x 100 (20). Only patients with full results of these iron biomarkers and haemoglobin were included in the analysis. Patients were followed up clinically and by electronic records until June 3, 2019. The cause of death was adjudicated based on available clinical and electronic records, following a protocol described elsewhere (120).

3.2.3 Statistical analysis

Continuous variables are presented as median with 25th and 75th centiles and compared using one-way ANOVA or Kruskal-Wallis test. Categorical variables are presented as numbers and percentages and compared using chi squared tests. Pearson's or Spearman's ρ correlations were used, for parametric and non-parametric variables, respectively. Natural logarithmic or square root (SqR) transformation were used for non-normally distributed variables. No imputation was performed for missing data. Variables with a high percentage of missing values were not included in multivariable analyses to reduce uncertainty.

Cox proportional hazards models were used to identify variables associated with 5-year all-cause and cardiovascular (CV) mortality. For guideline ID definition, the Fine-Gray sub-distribution hazard model for CV mortality was used, considering non-CV mortality as a competing event. Restricted cubic splines were constructed for each continuous iron biomarker. Uni- and multivariable interaction analyses for HFrEF vs HFmrEF, HFpEF and HF-↑NT-proBNP were done. Multivariable models were built including only those variables associated with outcome (p value ≤0.1) in univariable analysis. Hazard ratios (HR) with 95% confidence intervals are reported. Kaplan-Meier cumulative mortality curves for all-case death within 5 years were produced to compare survival among patients grouped by different ID definitions. Differences between groups were compared using the log-rank test.

Iron biomarkers were tested both as continuous and as categorical variables. As categorical variables, different thresholds were used: 1) international guideline criteria (ferritin <100µg/L or TSAT <20% if ferritin 100-299µg/L); 2) ferritin <100µg/L; 3) TSAT <20%; 4) serum iron ≤13µmol/L. Serum iron ≤13µmol/L was examined based on the results of a study using bone marrow iron staining as gold standard in patients with heart failure (20).

As continuous variables, ferritin, TSAT and serum iron were divided into deciles. The HR for all-cause mortality of each decile was determined by univariable Cox proportional hazards. Receiver-operating-characteristic (ROC) analysis was also performed for each biomarker to determine the best cut-off values to predict 1-year all-cause mortality, excluding patients with ≤12 months follow-up. The

optimal threshold for prediction was defined as the value on the ROC curve closest to the upper left corner: d^2= (1-sensitivity)^2 + (1-specificity)^2. P-values and 95% CIs presented in this report have not been adjusted for multiplicity; therefore, inferences drawn from these statistics may not be reproducible. All analyses were performed with SPSS statistical software, version 27 (IBM) and STATA statistical software, version 17 (STATA corp.). The two-tailed level of statistical significance was set at $p< 0.05$.

3.3 Results

Of the 9,321 patients evaluated in the clinic, 7,160 were diagnosed with heart failure. Of these, 4,422 (62%) had all required iron and haematological indices available (Figure S1). Patients with heart failure enrolled after 1st January 2009 (n= 4,182), subsequent to a revision of the clinical pathway, were more likely to have all the required iron indices (n=2,959; 71%). Compared to those with HFrEF, patients with HFpEF were older, more likely to be women and were more likely to have hypertension and atrial fibrillation but, despite these differences, they had lower median plasma NT-proBNP and similar renal function (**Table 5**).

Serum iron and TSAT were highly correlated (r= 0.92; p<0.001); correlations between ferritin and serum iron (r= 0.27; p<0.001) or TSAT (r= 0.41; p<0.001) were weaker.

3.3.1 Prevalence of ID and anaemia and association with patient characteristics

In the total population, the prevalence of ID ranged from 46-70% depending on the definition (**Table 5**). Of 3,011 patients who met guideline criteria for ID, 2,506 (83%) had a ferritin <100µg/L. Many patients fulfilled one definition of ID but not others (**Figure 4**). Of 3,011 patients with ID according to guideline criteria, 36% had a TSAT ≥20% and 39% a serum iron >13µmol/L; whilst of those who did not have ID by guideline criteria (n=1,411), 20% had a TSAT <20% or a serum iron ≤13µmol/L.

Table 5: Characteristics of patients according to heart failure phenotype

Variable	HFrEF n=1,429 (32%)	HFmrEF n=820 (19%)	HFpEF n=1,832 (41%)	HF ↑NT-proBNP n=341 (8%)	P- value
Demographics					
Age (years)	72 (64;79)	76 (68;82)	77 (71;83)	75 (68;83)	< 0.001
Sex (women)	376 (26)	262 (32)	978 (53)	147 (43)	< 0.001
BMI (kg/m^2)	27 (24;31)	28 (25;33)	29 (25;33)	28 (24;33)	< 0.001
IHD	812 (57)	465 (57)	494 (27)	105 (31)	< 0.001
Hypertension	602 (42)	460 (56)	1,222 (67)	171 (50)	< 0.001
Diabetes	351 (25)	222 (27)	506 (28)	73 (21)	0.04
COPD	134 (9)	61 (8)	176 (10)	40 (12)	0.10
eGFR (ml/min/1.73m^2)	59 (44;74)	61 (46;74)	60 (45;76)	59 (44;77)	0.45
Atrial Fibrillation/Flutter	366 (27)	303 (38)	714 (39)	124 (44)	< 0.001
Symptoms and Signs					
NYHA III/IV	533 (37)	255 (31)	446 (25)	86 (27)	< 0.001
Oedema (≥ankle)	322 (25)	227 (30)	576 (34)	93 (34)	< 0.001
Blood results					
Serum iron (µmol/L)	14 (10;19)	14 (10;18)	13 (10;17)	13 (10;18)	< 0.001
Serum iron ≤13 µmol/L	635 (44)	377 (46)	918 (50)	171 (50)	<0.01
TSAT (%)	22 (16;30)	22 (16;29)	21 (15;26)	21 (15;28)	< 0.001
TSAT <20 %	621 (44)	361 (44)	890 (49)	159 (47)	0.02
Ferritin (µg/L)	102 (54;184)	94 (46;171)	71 (38;135)	78 (38;158)	< 0.001
Ferritin <100 (µg/L)	697 (49)	432 (53)	1,175 (64)	202 (59)	< 0.001
FAIR-HF ID criteria	872 (61)	534 (65)	1,373 (75)	232 (68)	< 0.001
Hemoglobin (g/dL)	13.4 (12.2;14.6)	13.3 (12.0;14.5)	12.9 (11.8;14.1)	12.7 (11.8;13.8)	< 0.001
Anaemia	473 (33)	267 (33)	670 (37)	136 (40)	0.02
NT-proBNP (ng/L)	1,935 (841;4,333)	1,164 (497;2,587)	865 (332;1,825)	1,329 (490;2,766)	< 0.001

hs-CRP (mg/L)	4.2 (1.7;8.8)	4.0 (1.7;8.5)	3.9 (1.6;8.4)	4.2 (1.8;11.0)	0.22
Medications					
Loop diuretic	1,104 (77)	532 (65)	977 (54)	212 (74)	< 0.001
ACEi or ARB	1,161 (81)	608 (75)	1,063 (58)	196 (68)	< 0.001
MRA	547 (38)	176 (22)	172 (9)	89 (31)	< 0.001
BB	990 (69)	567 (70)	957 (53)	214 (74)	< 0.001
Anticoagulant	429 (30)	261 (32)	528 (29)	120 (35)	0.08
Antiplatelet	707 (50)	399 (49)	722 (39)	100 (29)	< 0.001

Continuous variables presented as median and (25^{th}-75^{th} centiles). Categorical variables presented as count and (%). P values provided from Chi-square tests for categorical variables and Kruskal-Wallis tests for continuous variables.

Abbreviations: - BMI: body mass index; IHD: ischaemic heart disease; COPD: chronic obstructive pulmonary disease; eGFR: estimated glomerular filtration rate; NYHA: New York Heart Association; TSAT: transferrin saturation; ID: iron deficiency; NT-proBNP: N-terminal pro B-type natriuretic peptide; hsCRP: high sensitivity C-reactive protein; ACRi: angiotensin converting enzyme inhibitor; ARB: angiotensin receptor blocker; MRA: mineralocorticoid receptor antagonist; BB: beta-blocker.

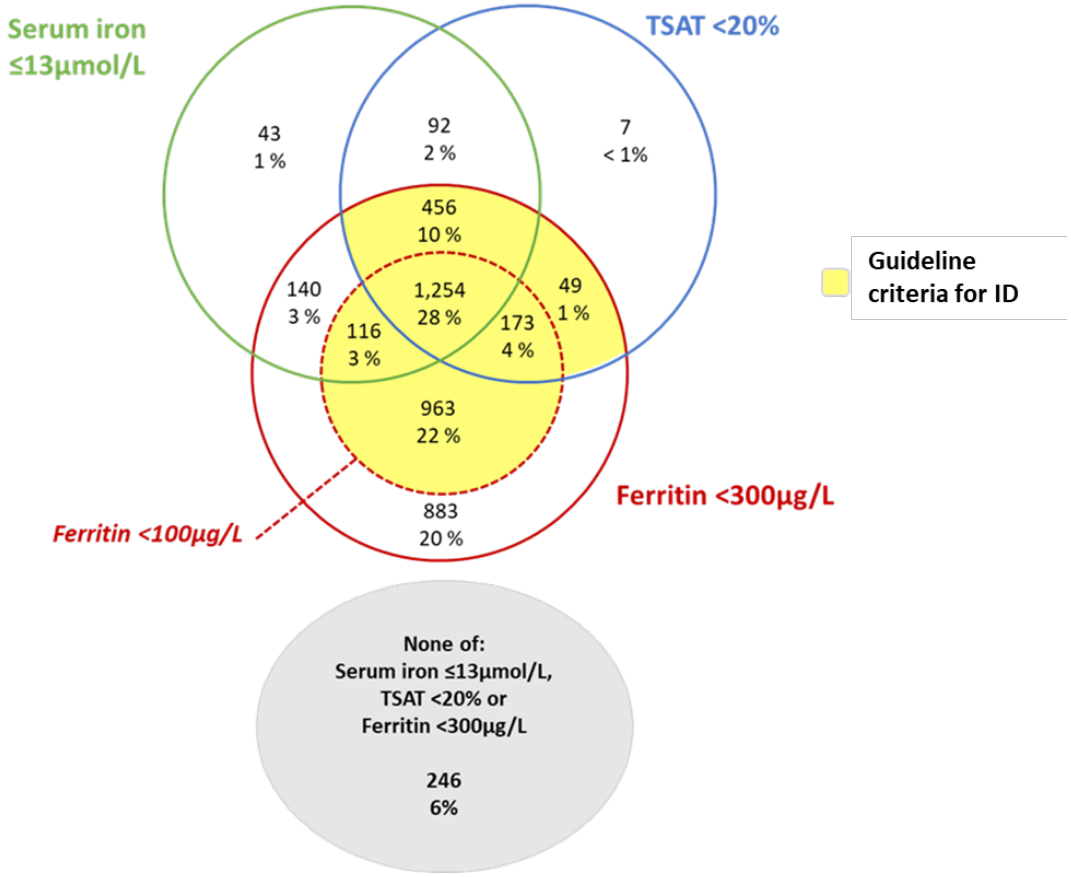


Figure 4: Venn diagram demonstrating the relationship of serum iron biomarkers (serum iron, TSAT, ferritin) by various pre-specified thresholds. Guideline criteria for ID (ferritin <100µg/L or TSAT <20% if ferritin 100-299µg/L) highlighted in yellow.

Figure 5 shows the prevalence of ID defined by TSAT, serum iron and guideline criteria in various clinical subgroups. Using any of these definitions, ID was more common in women, in those with more severe symptoms, and in those who did not have HFrEF. Compared to those in the lowest quintile, those in the highest quintiles of NT-proBNP and high sensitivity-CRP (hs-CRP) were more likely to have a low serum iron or TSAT but not ID using guideline criteria. Patients with HFpEF were more likely to have a low serum iron, TSAT and ferritin compared to those with other heart failure phenotypes (**Table 5**).

Anaemia was present in 1,543 (35%) patients. Compared to those without anaemia, those with anaemia had a higher prevalence of ID, irrespective of the ID criteria used (**Figure 5**). **Figure 6** shows the haemoglobin concentration (median, 25th and 75th centile) and 5-year mortality according to TSAT above or below 20% and by different concentrations of ferritin (<30; 30-99; 100-299 and ≥300µg/L). Compared to those with a higher TSAT, those with a TSAT <20% had a lower haemoglobin concentration, which was similar across the range of serum ferritin. Patients with a TSAT <20% and a serum ferritin ≥300µg/L had the highest prevalence of anaemia (Table S1). These patients were more likely to be men, had lower body mass index, higher median NT-proBNP and hsCRP and worse renal function compared to those with ferritin <300µg/L.

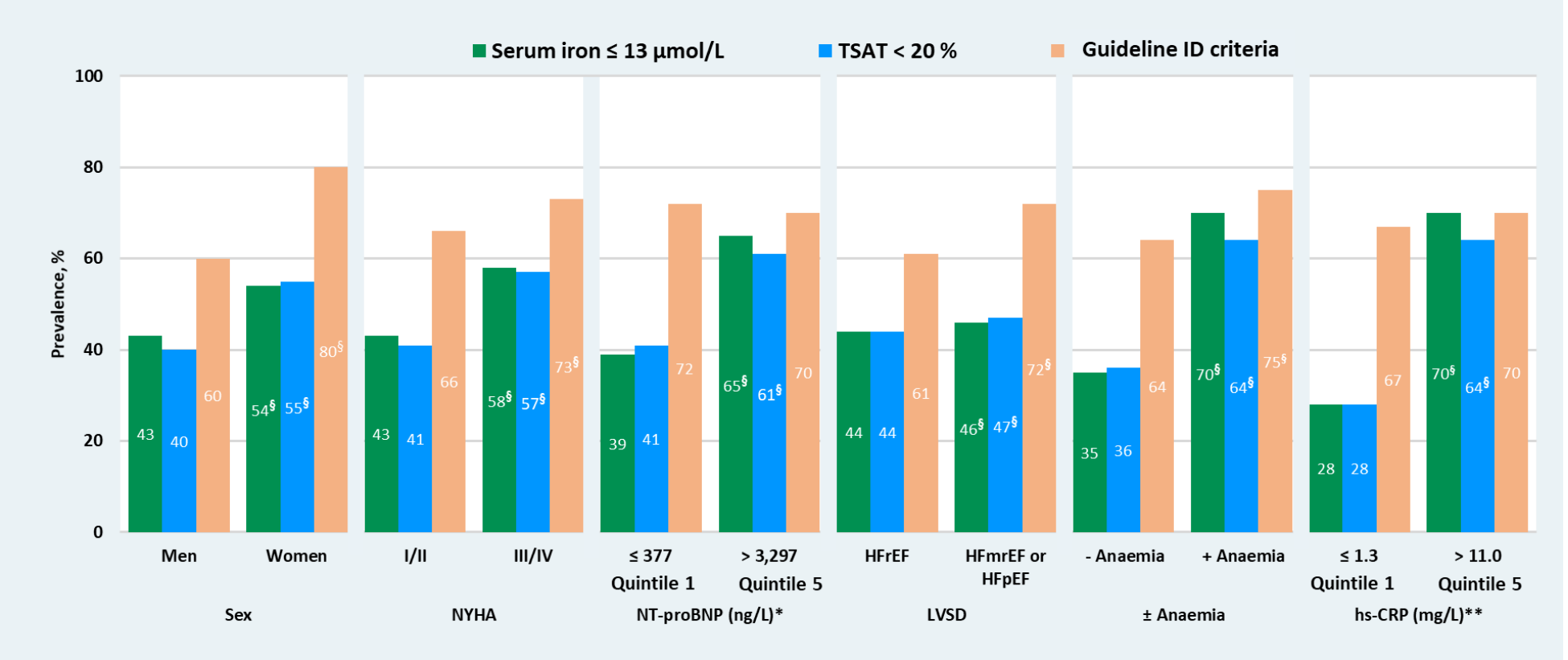


Figure 5: Prevalence (%) of various definitions of iron deficiency (serum iron ≤13 µmol/L; TSAT <20%; Guideline definition of ID) according to clinical subgroups and upper and lower quintiles of plasma NT-proBNP (*) and hs-CRP (**). Significant differences ($p<0.05$) in prevalence of ID between individual patient characteristics displayed by '§'.

3.3.2 Outcomes by different ID definitions

The median duration of follow-up was 49 (18-89) months. In total, 2,321 (53%) patients died. The 5-year mortality was 35%. Mortality was lowest for those with a serum ferritin <100µg/L and a TSAT ≥20% and highest for those with a ferritin >100µg/L with a TSAT <20% (**Figure 6**). In univariable analysis, lower TSAT and serum iron, but higher ferritin, were associated with a higher all-cause and CV mortality (**Figure 7** and **Table 6**).

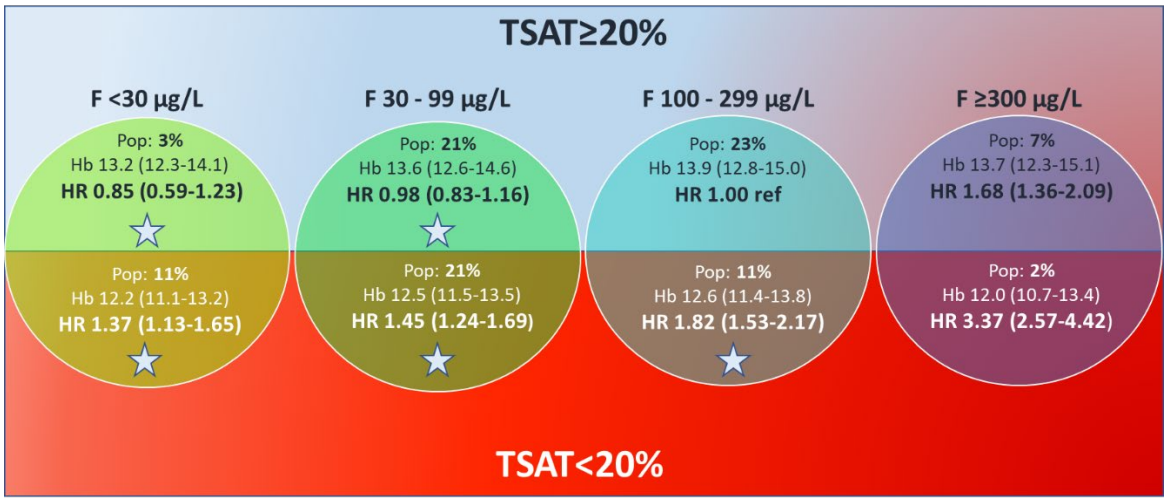


Figure 6: Percentage of patients (Pop), haemoglobin concentration (median (25^{th}-75^{th} centiles)) (g/dL) and adjusted 5-year mortality (HR (95%CI)) according to serum ferritin (F) and TSAT (%).
Hazard ratios of 5-year mortality adjusted for age (/5 years), sex and heart failure phenotype. ☆ = fulfilling definition of iron deficiency by guideline criteria.

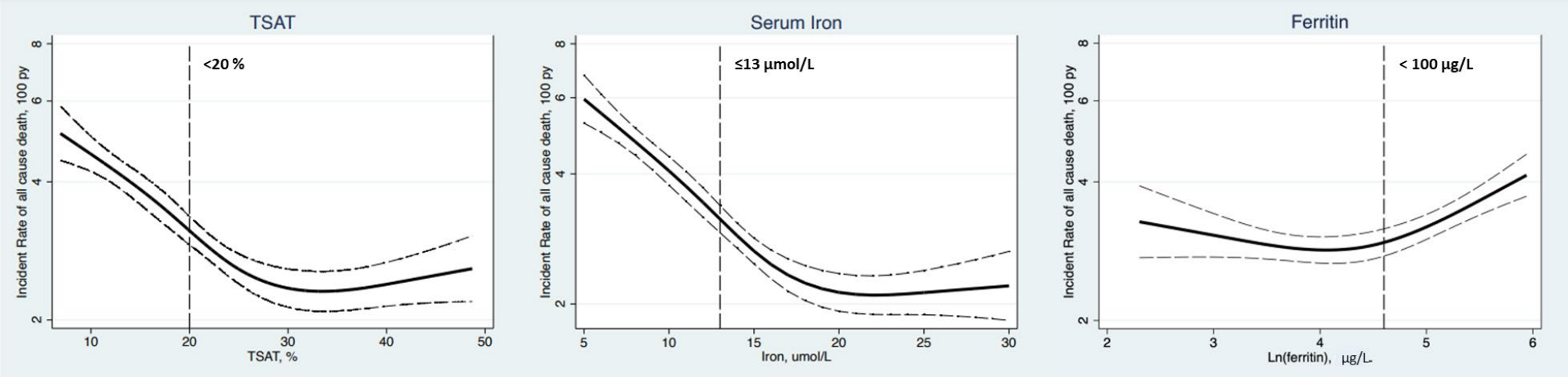


Figure 7: Restricted cubic splines detailing the association between concentrations/% of serum iron biomarkers and risk of all-cause mortality (by 100 patient years).

In multivariable analysis that included demographics, co-morbidities, vital signs, electro- and echocardiography and biochemical testing, a higher serum iron and TSAT were associated with a lower all-cause mortality: [HR 0.84 (0.78-0.91); p<0.001; HR 0.83 (0.74-0.92); p<0.01] while a higher ferritin was associated with a higher all-cause [HR 1.09 (1.02-1.16); p<0.01] and CV mortality [HR 1.11 (1.02-1.20); p=0.02] (**Table 6**). After including haemoglobin in the models, serum iron and TSAT were no longer associated with all-cause mortality, but the associations with ferritin and outcomes did not change (Table S2). Full multivariable models excluding haemoglobin are present in Tables S3 & S4.

Table 6: Uni- and Multi-variable associations (HR (95% CI) and P values) between iron biomarkers, definitions of iron deficiency and all-cause and cardiovascular mortality within 5 years.

	All-cause mortality				Cardiovascular mortality			
	Univariate		Multivariable		Univariate		Multivariable	
Variable	HR (95% CI)	P-value	HR (95% CI)	P-value	HR (95% CI)	P-value	HR (95% CI)	P-value
Serum iron biomarkers								
SqR s.iron (µmol/L)	0.66 (0.62-0.71)	<0.001	0.84 (0.78-0.91)	<0.001	0.73 (0.67-0.80)	<0.001	0.97 (0.87-1.07)	0.50
Ln [TSAT] (%)	0.63 (0.58-0.70)	<0.001	0.83 (0.74-0.92)	<0.01	0.68 (0.60-0.78)	<0.001	0.93 (0.80-1.09)	0.36
Ln [ferritin] (ng/mL)	1.11 (1.05-1.17)	<0.001	1.09 (1.02-1.16)	<0.01	1.17 (1.09-1.26)	<0.001	1.11 (1.02-1.20)	0.02
ID definitions								
Iron ≤13 µmol/L	1.81 (1.63-2.00)	<0.001	1.37 (1.22-1.54)	<0.001	1.55 (1.35-1.78)	<0.001	1.11 (0.95-1.30)	0.18
TSAT <20 %	1.56 (1.41-1.72)	<0.001	1.27 (1.14-1.43)	<0.001	1.41 (1.22-1.61)	<0.001	1.06 (0.93-1.27)	0.29
Ferritin <30µg/L	0.97 (0.84-1.12)	0.66	0.96 (0.85-1.17)	0.96	0.88 (0.72-1.09)	0.24	0.98 (0.78-1.23)	0.88
Ferritin <100µg/L	0.89 (0.81-0.99)	0.03	0.91 (0.81-1.01)	0.09	0.78 (0.68-0.89)	<0.001	0.83 (0.71-0.96)	0.02
Guideline ID criteria	1.08 (0.97-1.20)	0.16	1.02 (0.90-1.15)	0.75	1.00 (0.87-1.16)	0.98	0.96 (0.81-1.14)	0.66

Abbreviations: - SqR: square root; TSAT: transferrin saturation; ID: iron deficiency.

As categorical variables, TSAT <20% (vs ≥20%) and serum iron ≤13μmol/L (vs >13μmol/L) were associated with a higher all-cause and CV mortality in univariable models (all p<0.001) (**Table 6**) while a ferritin <100μg/L (vs ≥100μg/L) was associated with a better survival (p=0.03 for all-cause and p<0.001 for CV mortality). ID defined by guideline criteria was not associated with either all-cause (p= 0.16) or CV mortality (p= 0.98) (**Table 6** and Figure S2).

In multivariable analysis, a TSAT <20% [HR 1.27 (1.14-1.43); p<0.001] and a serum iron ≤13μmol/L [HR 1.37 (1.22-1.54); p<0.001] were independently associated with greater all-cause mortality but not with CV mortality. Ferritin <100μg/L was associated with lower CV mortality and tended to be associated with lower all-cause mortality. Ferritin <30μg/L was associated with both lower all-cause and CV mortality (**Table 6**).

Figure 8 shows the hazard ratios for all-cause death for each decile of TSAT, serum iron and ferritin. Compared to the reference range (38-50μg/L), the highest decile of ferritin (>274μg/L) was associated with a higher mortality [HR 1.67 (1.34-2.08); p<0.001). Mortality was higher for patients in deciles of TSAT <19.2% and serum iron ≤13μmol/L.

No significant interaction was found in adjusted models between definitions of ID and heart failure phenotypes for all-cause or CV mortality.

Patients with a ferritin ≥300μg/L and a TSAT <20%, who do not fulfil guideline criteria for ID, had the highest risk of death while those with a ferritin <100μg/L with a TSAT ≥20%, who do fulfil these criteria, had a similar prognosis to those with ferritin 100-299μg/L and a TSAT ≥20% (**Figure 6**).

The area-under-the-curve (AUC) for all-cause mortality at 1 year for serum iron and TSAT were 0.64 (0.61-0.67) and 0.61 (0.58-0.64) with optimal predictive values of <12.5μmol/L and <19.0% respectively. For ferritin, AUC was 0.56 (0.53-0.59) with an optimal cut-off of 143.5μg/L (Figure S3).

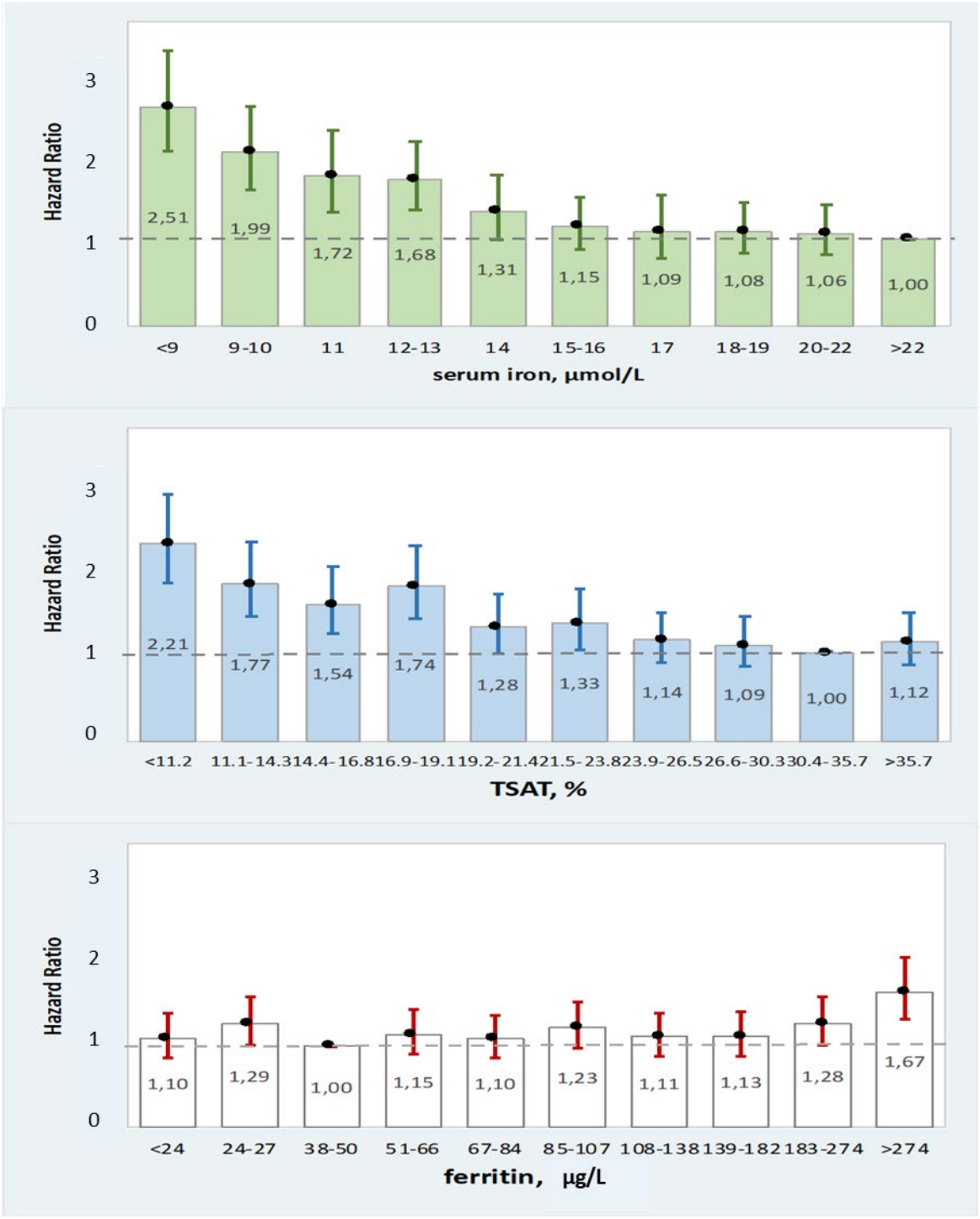


Figure 8: Unadjusted Hazard ratio (HR) and 95% CI (error bars) for mortality within 5 years by deciles of each biomarker (serum iron, TSAT, serum ferritin).

3.4 Discussion

Getting the definition of ID right is important for clinical trials and clinical practice. We found that ID is common among ambulatory patients with heart failure, but the prognostic implications differ according to definition. We did not find any association between the current guideline definition of ID and mortality; indeed, lower serum ferritin concentrations were associated with a better survival. Among other definitions of ID, TSAT <20% and serum iron ≤13μmol/L were independently associated with death, with no interaction between heart failure phenotypes. About two thirds of patients fulfilled the guideline criteria of ID but of these, around one third had a TSAT ≥20%. If this latter group of patients do not truly have ID but are being included in trials of IV iron, this could attenuate any observed benefit and might even lead to a neutral result.

To my knowledge, this is the first study to assess systematically the prevalence and prognostic implications of different definitions of ID across all heart failure phenotypes in a large population of patients with CHF. These findings confirm that ID is common in patients with heart failure, in line with previous reports (17,41,42). However, many previous studies have included only patients with HFrEF and assessed ID using only the guideline definition. I extend these findings and suggest that many patients with HFpEF also have ID, which might reflect their advanced age, the high proportion of women, and high comorbidity burden.

The relationship between serum iron biomarkers and adverse outcomes in those with ID remains uncertain. Iron deficiency defined by guideline criteria was independently associated with mortality in a study of 546 patients with HFrEF and in another study of 1,506 patients with heart failure, of whom 87% had HFrEF (LVEF ≤45%) (17,41). However, others have questioned both the diagnostic and prognostic utility of the guideline criteria in patients with CHF (19,20,37).

In a European multi-centre study of 1,821 patients with CHF (mean age 63 +/-13 years; 29% women), a TSAT <20%, but not a ferritin <100μg/L, independently predicted mortality (37). In another study, in which ID was defined by bone marrow iron staining, only a serum iron ≤13μmol/L or TSAT ≤19.8%, but not

guideline criteria, predicted bone-marrow ID in patients with HFrEF (defined by an LVEF ≤45%; n=42) undergoing coronary artery bypass. In a previous analysis of the Hull LifeLab cohort, among quintiles of ferritin, only the highest quintile was associated with the greater all-cause and CV mortality in an adjusted model. The current report includes more patients, has a longer follow-up and classifies patient phenotype in line with international guidelines on CHF.

An individual patient data meta-analysis of 4 randomised controlled trials comparing outcomes of patients treated with IV ferric carboxymaltose (FCM) vs placebo in patients with HFrEF suggested that the prognostic benefit of FCM might be limited to those with a TSAT ≤19.8%, irrespective of ferritin concentration (105). This analysis was later extended to suggest that a serum iron ≤13μmol/L also predicted benefit from IV FCM (20). These findings suggest that use of TSAT or serum iron rather than ferritin to select those more likely to benefit from IV iron. Identifying those most likely to respond to IV iron might be considered the best method for diagnosing clinically relevant ID. These considerations should be taken into account when interpreting results from randomized trials testing the benefit of IV iron in patients with heart failure. Results from the ongoing IRONMAN trial in patients with TSAT <20% or ferritin <100μg/L, and future, prespecified subgroup analyses of other ongoing trials of IV iron (121) assessing alternative diagnostic criteria such as those highlighted in our study will determine whether the current definition of ID should be revised.

Serum iron is almost entirely transferrin bound, and therefore a close association between serum iron and TSAT is not surprising. Serum ferritin increase in response to cellular damage and inflammation, highlighted by reports of extremely high serum ferritin concentrations associated with severe COVID-19 infection (122). Whilst a ferritin <15μg/L may be highly specific for diagnosing ID in patients without heart failure or inflammation (53,54), in heart failure, a higher diagnostic threshold (<100μg/L) is neither sensitive nor specific (20). Higher concentrations likely reflect a complex interplay between ID, inflammation and cell damage and do not rule out ID (58,59), rendering ferritin of limited diagnostic use in heart failure.

In the study by Beverborg and colleagues (n= 42), bone marrow ID was detected in 25% of those with a serum ferritin >300μg/L (n= 8) (20). Of those with a

ferritin >300µg/L, only those with bone marrow ID had a TSAT <20%. In our study, 26% of those with a ferritin ≥300µg/L had a TSAT <20% and a similar proportion had a serum iron ≤13µmol/L. Such patients had a lower haemoglobin and higher mortality than any other group. This may reflect more severe renal and cardiac dysfunction and congestion (123), which probably promote systemic inflammation (124,125) and hepcidin secretion, in turn leading to reduced iron absorption and increased sequestration and as such, a higher ferritin and greater mortality. However, very few patients with heart failure, perhaps none, with a serum ferritin ≥300µg/L have been included in trials of IV iron therapy because of concerns about possible iron overload.

These concerns may not be valid if ferritin is such a poor marker of iron status in heart failure. The ongoing IRONMAN trial (NCT02642562) includes patients with either a TSAT <20% or a serum ferritin <100µg/L and excludes those only when ferritin is >400µg/L. Even higher thresholds (>500µg/L or >800µg/L) have been proposed as indicating iron overload in patients with a variety of diseases (55). In the PIVOTAL trial of patients on renal dialysis, re-dosing with IV iron was encouraged provided serum ferritin was <700µg/L in the proactive high-dose intervention arm (93). Better validation of serum biomarkers of ID together with data on the efficacy and safety of IV iron in those with higher ferritin concentrations are required. New formulations of IV iron currently in use do not release large amounts of labile iron into the circulation, which may reduce the risk of iron loading, although excessive intracellular accumulation remains a concern (82).

3.4.1 Limitations

Other biomarkers of interest, such as serum soluble transferrin receptor (sTfR) or hepcidin were not measured. Although sTfR is a good marker of tissue ID (67), and may even be a better marker of bone marrow ID than TSAT or serum iron (104), heterogeneity in assays, high expense and limited clinical experience mean it is not routinely available. We did not collect information on the rate of IV iron or blood transfusions that some of our patients might have received during follow-up. However, we expect very few patients to have received IV iron as the first ESC guideline to recommend its use was published in 2016 (114) and IV iron is still not recommended by UK heart failure guidelines (126). We

enrolled patients over a period of 20 years over which time evidence-based therapies for CHF have evolved. This is a single centre study including a predominantly white British population and therefore data should be extrapolated with caution to populations with characteristics different from ours.

3.5 Conclusion

Irrespective of how it is defined, ID is common in patients with heart failure. When defined by current guideline criteria, ID was not associated with a poor outcome; indeed, lower serum ferritin concentrations were associated with a better survival. TSAT <20% and serum iron ≤13μmol/L were associated with a higher mortality, and this was independent of heart failure phenotype.

Chapter 4 Natural history and prognostic significance of iron deficiency and anaemia in ambulatory patients with chronic heart failure

4.1 Introduction

Anaemia is common in patients with chronic heart failure (127,128) and is often associated with iron deficiency (ID) (17,41,43,57). Both are thought to contribute to worsening symptoms and reduced exercise capacity (5,38,95), and both are associated with an unfavourable prognosis (17,19,42,95,128).

Several randomised trials have found that giving intravenous (IV) iron to patients with ID and chronic heart failure improves symptoms (84,129), quality of life (84) and exercise capacity (85,129). Meta-analysis data also suggest that IV iron might also improve prognosis (105). These benefits may be independent of the presence of, or correction of, anaemia. Recent expert guidance suggests that haemoglobin and, perhaps, indices of ID should be checked once, or even twice a year in patients with heart failure and ongoing symptoms (130). However, reports on the natural history of ID and anaemia in patients with heart failure that should inform these recommendations are scarce. Most research has focussed on prevalent anaemia and ID rather than their incidence or resolution. Accordingly, we studied the natural history of ID and anaemia in a cohort of patients with chronic heart failure.

4.2 Methods

4.2.1 Study Population

Between 2002 and 2014, consenting patients referred with suspected or confirmed heart failure from primary and secondary care physicians, were enrolled at a single clinic serving a local population of approximately 550,000 people (The Hull LifeLab). All patients enrolled gave written informed consent for their data to be stored electronically and used for research. Patients were reviewed by heart failure specialist nurses and doctors at regular intervals, usually at 4 and 12 months, and then annually, unless an appointment was requested sooner by the patient or a clinician. Information on demography,

symptoms and signs, haematology and biochemistry profiles and electrocardiograms were systematically collected at each visit and recorded in a dedicated electronic health record which was stored on a secure server. Echocardiograms were performed routinely at baseline only. Patients were followed until the 3rd of March 2019.

Heart failure was defined as typical symptoms and signs and **either** a measured or visually estimated left ventricular ejection fraction (LVEF) of <40% (HFrEF) **or** an elevated N-terminal-pro brain natriuretic peptide (NT-proBNP; ≥125 ng/L) following the European Society of Cardiology (ESC) guidelines (114). Those with an NT-proBNP ≥125 ng/L were further classified as those with an LVEF of 40-49% (mid-range ejection fraction (HFmrEF)) or ≥50% (preserved ejection fraction (HFpEF)). These simplified definitions were used as further echocardiographic data on structural or functional alterations were not always available. The clinic protocol indicated that all patients should have standard haematology and blood biochemistry checked, including NT-proBNP and iron indices, although this set of investigations was often incomplete. Only patients who had tests for ID and a haematology profile at baseline and one year were included in this analysis. Those treated with erythropoietin analogues or intravenous iron were excluded.

Anaemia was defined, using the World Health Organisation (W.H.O.) criteria, as a haemoglobin of <12.0g/dL in women and <13.0g/dL in men (47). Iron indices included: serum ferritin, serum iron, transferrin, and transferrin saturation (TSAT). As there are no universally accepted criteria for ID, we defined it as a serum iron ≤13μmol/L, based on a study that used bone marrow iron depletion as a diagnostic "gold-standard" (20). However, we also considered other definitions of ID including the criteria employed in the FAIR-HF trial (84) and subsequently adopted by the ESC (ferritin <100μg/L or TSAT <20% if ferritin 100-299μg/L) (114) and by a TSAT of <20% alone (**Table 7**).

Table 7: Definitions of iron deficiency and anaemia being investigated

	Iron deficiency			Anaemia
	FAIR-HF	Serum iron	TSAT	
Men	Ferritin <100 µg/L or TSAT <20% if ferritin 100-299 µg/L	≤13 µmol/L	<20%	Hb <13.0 g/dL
Women	Ferritin <100 µg/L or TSAT <20% if ferritin 100-299 µg/L	≤13 µmol/L	<20%	Hb <12.0 g/dL

Abbreviations: - TSAT: transferrin saturation.

Patients were grouped at baseline according to the presence or absence of anaemia or ID, using the serum iron criterion. Those who had neither ID nor anaemia at baseline were grouped by whether they developed ID or anaemia, respectively. Those with ID or anaemia at baseline were grouped by whether ID and anaemia did or did not resolve. These groupings were repeated separately using the other definitions of ID described above.

Deaths were adjudicated based on medical records from primary and secondary care. Deaths in patients with advanced symptoms of heart failure (New York Heart Classification IV) or recurrent hospitalizations for heart failure were classified as due to heart failure unless another cause was clear (eg:- metastatic lung cancer). Deaths due to heart failure, myocardial infarction, stroke, or other major cardiovascular insult were grouped as cardiovascular (CV) deaths. Details on adjudication have been published (120).

4.2.2 Statistical analysis

Continuous variables are presented using median, 25th and 75th percentiles and compared using one-way ANOVA if normally distributed or a Kruskal-Wallis test if not. Categorical variables are presented as numbers and percentages and compared using chi squared tests. Non-normally distributed variables were

transformed using either logarithms with base 10, or the square root as appropriate. No imputation was performed for missing data. Univariable and multivariable logistic regression analysis was used to identify predictors of incident ID at 1 year in those without ID at baseline, regardless of the presence of anaemia, and incident anaemia at 1 year, in those without anaemia at baseline, regardless of the presence of ID. Variables associated with outcome at the 10% significance level (p value ≤ 0.1) from the univariable models and/or clinically relevant variables (e.g. sex), were entered into multivariable models. Odds Ratios (ORs), corresponding 95% confidence intervals (CI) and p-values are reported.

Kaplan-Meier curves for all-cause and CV mortality within 5 years of the baseline visit were constructed for patients grouped by change in ID or anaemia status between baseline and one year. Differences between groups were evaluated using the log-rank test. Univariable and multivariable Cox proportional hazard regression models were used to assess the association between ID and anaemia groups and mortality within 5 years of the baseline visit. Those who never developed ID or anaemia respectively were used as the reference group. Further exploratory analysis compared risk of death within 5 years between those whose ID resolved against those who remained iron deficient at one year for each definition of ID. Separate multivariable models were produced using baseline and updated (1 year) haematinic values respectively. An enter method was applied for prognostic multivariable models. Hazard ratios (HRs) with 95% confidence intervals are reported. All analyses were performed with SPSS statistical software, version 26 (IBM). All tests were 2 sided and unless previously specified, used the 5% level to determine statistical significance.

4.3 Results

A complete set of blood tests was available at both baseline and follow-up for 906 (33%) of 2,763 patients with confirmed heart failure who survived 12 months (Figure S4). Those without available follow-up tests, and therefore not included in the analysis, were more likely to have HFmrEF or HFpEF (74%), be women (42%) and had lower plasma NTproBNP (818 (312-1937) ng/L) (Table S5).

4.3.1 Natural history of ID and anaemia and incident disease

Overall, the proportion of patients with anaemia and ID, whether defined by serum iron or FAIR-HF criteria, changed little over one year (**Figure 9** and Table S6) but this concealed underlying dynamic changes in incidence and recovery: 428 (47%) patients changed their classification (**Figure 10**). Only 270 patients (30%) had neither anaemia nor ID, defined as a serum iron ≤13μmol/L, measured one year apart. At either baseline or one year, 546 (60%) had ID and 376 (42%) had anaemia. Of individuals with a serum iron >13μmol/L who were not anaemic at baseline (n=425), 22% developed ID alone, 8% ID and anaemia, and 7% anaemia only

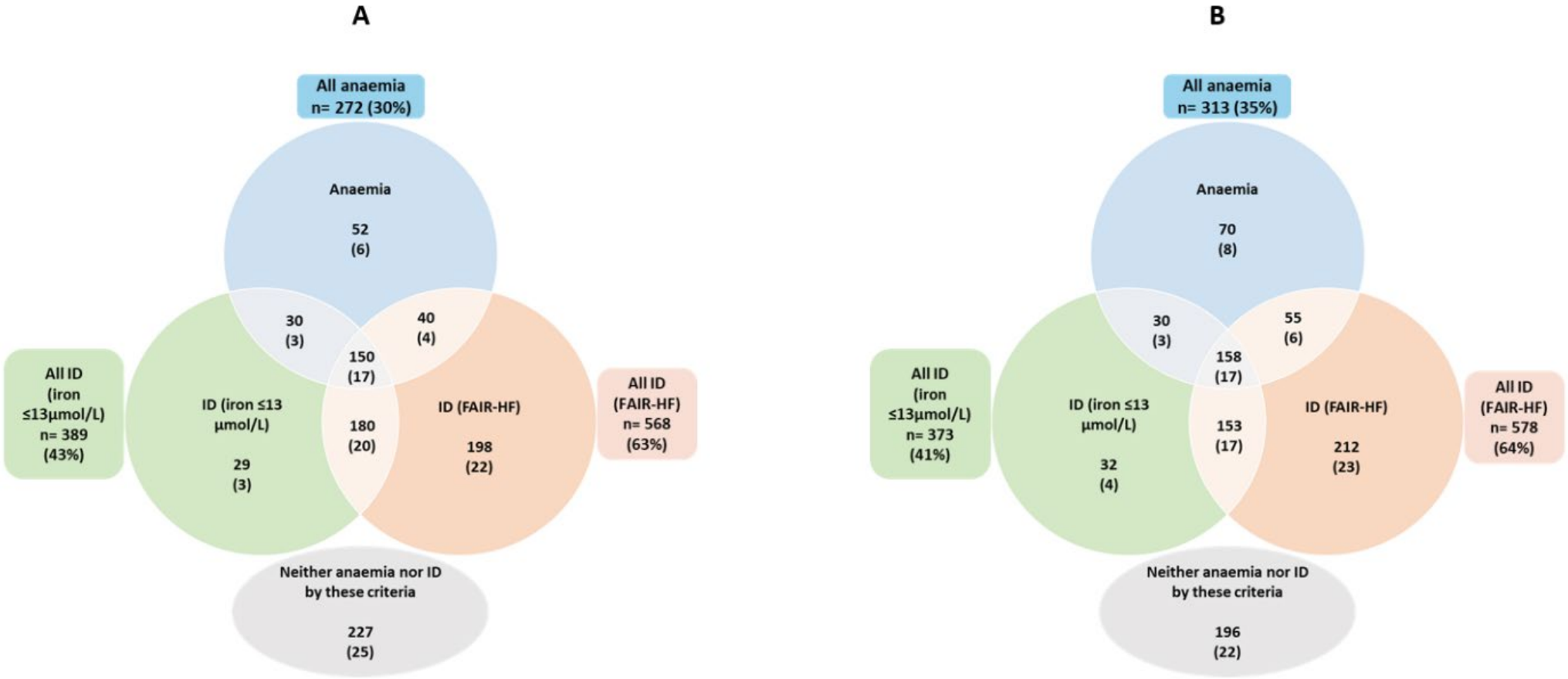


Figure 9: Venn diagram demonstrating the distribution of iron deficiency and anaemia and baseline (A) and one year (B). ID defined by both a serum iron ≤13µmol/L and the Guideline/FAIR-HF definition: ferritin <100µg/L or TSAT <20% if ferritin 100-299µg/L. Presented as number and (%).

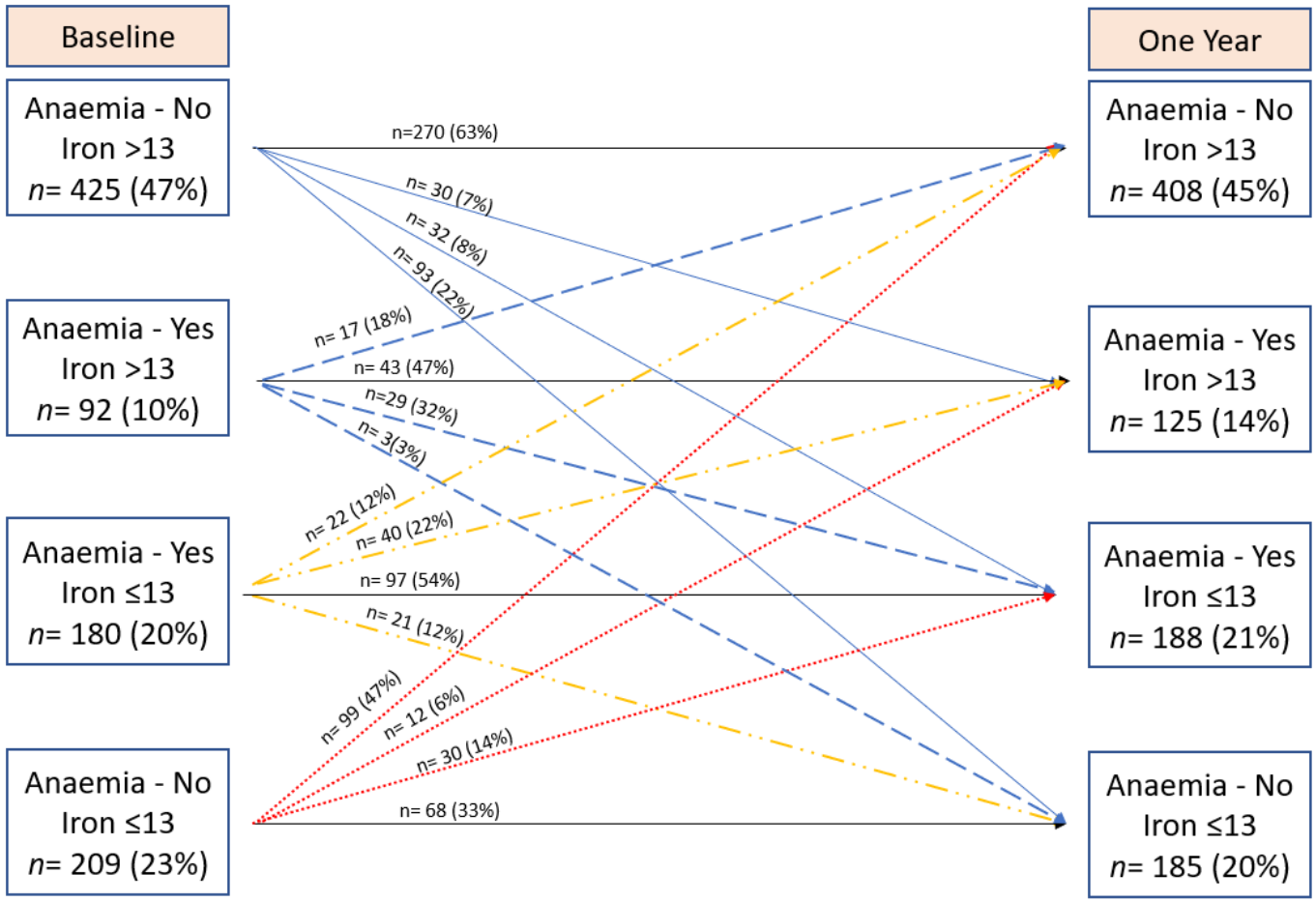


Figure 10: Classification by iron deficiency and anaemia at baseline and one year.
Iron deficiency defined by a serum iron ≤13µmol/L and anaemia by the W.H.O. definition: Hb <13.0g/dL in men and <12.0g/dL in women
Number and (%) on each line represent the count and (%) of patients moving between groups from baseline to follow-up.

Patients whose serum iron was >13μmol/L on both occasions were younger, more likely to be men, were less likely to have diabetes, had higher haemoglobin and eGFR, lower NT-proBNP and were less likely to receive iron supplements (**Table 8**).

Of 517 patients who were iron replete at baseline, serum iron had dropped to ≤13μmol/L in 157 (30%) at 1 year (**Table 8**). The rate of incident ID did not differ between those with HFrEF (31%), HFmrEF (30%) or HFpEF (32%) (p=0.84). The median (Q1, Q3) change in serum iron for those who developed ID was -5.0 (-8.5; -3.0) μmol/L (Table S7); only eight (5%) patients received oral iron supplements. In univariable analysis, the baseline variables most strongly associated with incident ID were lower haemoglobin (OR [95% CI] 0.79 [0.70-0.90]; p<0.001) and serum iron (0.39 [0.25-0.60]; p<0.001) with weaker associations for higher plasma NT-proBNP (1.66 [1.07-2.56]; p=0.02) or hsCRP (1.73 [1.15-2.61]; p=0.01) (Table S8). In multivariable analyses, only lower serum iron (0.55 [0.33-0.89]; p=0.02), or lower haemoglobin (0.83 [0.72-0.97]; p=0.02) and higher hsCRP (1.75 [1.10-2.78]; p=0.02) were significantly associated with incident ID.

ID, defined as a serum iron ≤13μmol/L, resolved in 173 (44%) of 389 patients who had ID at baseline, only 6 (4%) of whom received oral iron. Resolution of ID was more likely in the absence of anaemia at baseline (**Figure 10**). The median change of serum iron in those whose ID resolved by the serum iron criterion was +6.0 (+4.0; +9.0) μmol/L, for TSAT was +9.0 (+6.1; +14.1) % and for ferritin was +12 (-16; +59) μg/L (Table S7).

Similar patterns of prevalence, development and resolution of ID were observed when applying TSAT <20% as the definition of ID (Figure S5). Applying the FAIR-HF criteria for ID, only 338 patients (37%) were iron replete at baseline and 38% of these developed ID at 1 year (Figure S6). Of those with ID at baseline (n=568), 81% of whom had a serum ferritin <100μg/L, ID resolved in only 117 (21%) by FAIR-HF criteria. Persistent ID was substantially higher by FAIR-HF criteria (50%) compared to serum iron ≤13μmol/L (24%) (Table S6). Baseline characteristics by category of ID (persistent, incident, resolved or never) and predictors of incident ID according to the FAIR-HF criteria are presented in Tables S9 & S10.

Table 8: Characteristics at baseline according to change in iron status between baseline and one year

Variable	Persists (n=216; 24%)	Develops (n=157; 17%)	Resolves (n=173; 19%)	Never (n=360; 40%)	P-value
Demographics					
Age (years)	76 (68-82)	73 (66-78)	74 (65-80)	71 (63-78)	0.004
Sex (male)	135 (63)	105 (67)	118 (68)	275 (76)	0.003
BMI (kg/m^2)	28 (25-33)	29 (25-32)	28 (26-32)	28 (25-32)	0.79 [8]
IHD	137 (63)	92 (59)	98 (57)	209 (58)	0.52
Hypertension	120 (56)	90 (57)	80 (46)	178 (49)	0.11
Diabetes	73 (34)	40 (26)	49 (28)	68 (19)	0.001
Haemoglobin (g/dL) At baseline	12.4 (11.3-13.4)	13.6 (12.6-14.6)	13.0 (11.9-14.0)	14.2 (13.3-15.3)	<0.001 [8]
Haemoglobin (g/dL) At one year	12.1 (11.0-13.3)	13.0 (11.8-14.1)	13.5 (12.1-14.4)	14.0 (12.9-14.8)	<0.001 [8]
Iron (µmol/L) At baseline	10 (8-12)	17 (15-19)	11 (9-12)	19 (16-22)	NA
Iron (µmol/L) At one year	10 (8-12)	12 (10-13)	16 (15-18)	18 (16-21)	NA
Ferritin (µg/L) At baseline	77 (39-136)	157 (64-188)	82 (41-142)	113 (62-203)	<0.001
Ferritin (µg/L) At one year	77 (36-130)	95 (54-157)	98 (51-188)	110 (57-201)	<0.001
TSAT (%) At baseline	16 (12-18)	26 (23-31)	16 (13-19)	29 (24-35)	<0.001
TSAT (%) At one year	16 (12-18)	18 (15-21)	25 (22-30)	28 (24-36)	<0.001
eGFR ($ml/min/1.73m^2$)	53 (39-69)	61 (47-75)	63 (46-75)	65 (50-79)	<0.001 [8]

NT-proBNP (ng/L) - all	1674 (662-3555)	1309 (517-2768)	1746 (498-3078)	926 (465-1886)	<0.001
NT-proBNP (ng/L) - SR	1402 (523-3292)	1060 (342-2769)	1095 (365-2770)	756 (372-1427)	<0.001
ECG and ECHO					
AF or Flutter	56 (26)	41 (26)	53 (31)	102 (29)	0.72
Heart rate (bpm)	75 (63-85)	68 (59-81)	74 (62-89)	70 (61-81)	0.003
HFrEF	95 (44)	82 (52)	100 (58)	185 (51)	0.06
Treatment at Baseline					
Loop diuretic	154 (72)	113 (72)	122 (71)	226 (63)	0.04
ACEi or ARB	162 (76)	135 (87)	134 (78)	293 (82)	0.06
MRA	38 (18)	54 (35)	43 (25)	120 (33)	<0.001
BB	116 (55)	113 (72)	109 (63)	263 (73)	<0.001
Anticoagulant	46 (21)	51 (33)	42 (24)	102 (28)	0.07
Antiplatelet	116 (64)	80 (51)	102 (59)	179 (50)	0.23
Oral iron treatment	21 (10)	8 (5)	6 (4)	7 (2)	<0.001

Variables displayed from baseline unless otherwise stated. Continuous variables presented as median and (25^{th}-75^{th} centiles). Categorical variables presented as count and (%). P values provided from Chi-square tests for categorical variables and Kruskal-Wallis tests for non-normally distributed continuous variables, unless indicated by [8] in which case one-way ANOVA test has been used.

Patients who were not anaemic on either occasion were younger, had a higher BMI but were less likely to have diabetes, had higher serum iron and eGFR and lower NT-proBNP. They were less likely to be treated with loop diuretics or oral iron (**Table 9**). Of 634 patients who were not anaemic at baseline, 104 (16%) developed anaemia at 1 year (**Figure 10**). The rate of incident anaemia did not differ for patients with HFrEF (17%), HFmrEF (17%) and HFpEF (15%) (p=0.81). The median change in haemoglobin from baseline in those who developed anaemia was -1.5 (-0.9; -2.0) g/dL; seven (7%) patients received oral iron supplements. On univariable analysis, the baseline variables most strongly associated with incident anaemia were increasing age (1.87 [1.48-2.38]; p<0.001) and plasma NT-proBNP (2.37 [1.46-3.84]; p<0.001), lower eGFR (0.98 [0.97-0.99]; p<0.001) and haemoglobin (0.41 [0.32-0.52]; p<0.001) and treatment with loop diuretics (2.63 [1.57-4.42]; p<0.001); indices of ID at baseline were only weakly associated with incident anaemia (Table S11). On multivariable analyses, increasing age (1.67 [1.23-2.28]; p=0.001) and lower haemoglobin (0.26 [0.17-0.41]; p<0.001) were strongly associated with the risk of incident anaemia. Even when haemoglobin was excluded from the model, iron indices measured at baseline were not associated with incident anaemia.

Anaemia resolved in 63 (23%) of 272 patients with anaemia at baseline. Of those in whom anaemia resolved, 43 (68%) had ID at baseline which persisted in 21 (49%) by one year (**Figure 10**). The median change in haemoglobin in those whose anaemia resolved was +1.7 (+1.0; +2.6) g/dL. Only four patients received oral iron supplements.

In general, prescriptions of evidence-based heart failure therapies increased across all patient groups over the course of 1 year, with corresponding improvements in symptoms and plasma NT-proBNP concentrations (Tables S12 & S13).

Table 9: Characteristics at baseline according to change in anaemia status between baseline and one year

Variable	Persists (n=209; 23%)	Develops (n=104; 12%)	Resolves (n=63; 7%)	Never (n=530; 58%)	P-value
Demographics					
Age (years)	76 (70-82)	77 (70-82)	73 (69-79)	71 (61-78)	<0.001
Sex (male)	155 (74)	66 (64)	44 (70)	368 (69)	0.27
BMI (kg/m^2)	27 (24-31)	28 (25-31)	27 (24-32)	29 (25-33)	0.014 [8]
IHD	128 (61)	69 (66)	36 (57)	303 (57)	0.31
Hypertension	109 (52)	54 (52)	26 (41)	279 (53)	0.40
Diabetes	67 (32)	28 (27)	20 (32)	115 (22)	0.02
Haemoglobin (g/dL) At baseline	11.5 (10.8-12.1)	13.4 (12.7-13.9)	11.9 (11.3-12.5)	14.2 (13.5-15.2)	NA
Haemoglobin (g/dL) at one year	11.1 (10.6-11.9)	11.9 (11.4-12.4)	13.4 (13.0-14.1)	14.1 (13.4-14.8)	NA
Iron (µmol/L) At baseline	12 (9-15)	15 (12-19)	12 (8-15)	16 (12-20)	<0.001
Iron (µmol/L) At one year	12 (10-15)	13 (10-16)	15 (11-18)	16 (13-20)	<0.001
Ferritin (g/L) At baseline	90 (46-176)	111 (57-172)	85 (35-179)	100 (53-176)	0.26
Ferritin (g/L) At one year	97 (39-196)	98 (63-174)	75 (42-135)	94 (49-174)	0.23
TSAT (%) At baseline	19 (14-24)	23 (17-29)	17 (11-24)	24 (18-31)	<0.001
TSAT (%) At one year	19 (14-24)	20 (15-25)	22 (17-28)	24 (20-30)	<0.001
eGFR ($ml/min/1.73m^2$)	49 (35-64)	58 (43-70)	58 (38-72)	66 (54-80)	<0.001 [8]
NT-proBNP (ng/L) - all	1903 (745-3937)	1666 (710-2867)	2132 (729-4080)	1014 (413-2047)	<0.001

NT-proBNP (ng/L) - SR	1496 (632-3472)	1454 (604-3040)	1192 (464-3559)	656 (310-1663)	<0.001
ECG and ECHO					
AF or Flutter	45 (22)	25 (26)	18 (29)	164 (31)	0.08
Heart rate (bpm)	70 (60-83)	68 (59-80)	73 (59-92)	72 (62-84)	0.04
HFrEF	101 (48)	56 (54)	37 (59)	268 (51)	0.48
Treatment at Baseline					
Loop diuretic	162 (78)	84 (81)	45 (73)	324 (62)	<0.001
ACEi or ARB	161 (78)	86 (83)	48 (77)	429 (81)	0.59
MRA	62 (30)	30 (29)	20 (32)	143 (27)	0.77
BB	129 (62)	65 (63)	41 (66)	366 (69)	0.22
Anticoagulant	44 (21)	25 (24)	14 (22)	158 (30)	0.07
Antiplatelet	123 (59)	53 (51)	36 (57)	265 (50)	0.15
Oral iron treatment	22 (11)	7 (7)	4 (6)	9 (2)	<0.001

Variables displayed from baseline unless otherwise stated. Continuous variables presented as median and (25^{th}-75^{th} centiles). Categorical variables presented as count and (%). P values provided from Chi-square tests for categorical variables and Kruskal-Wallis tests for non-normally distributed continuous variables, unless indicated by [8] in which case one-way ANOVA test has been used.

4.3.2 Survival

Within 5 years, 274 (30%) patients had died: 58% from CV causes, 39% from non-CV causes and 3% from uncertain causes.

4.3.2.1 Iron deficiency

In univariable analysis, higher serum iron measured at one year was associated with a better subsequent survival, both for all-cause (HR [95% CI] 0.69 [0.58-0.83]; p<0.001) and CV mortality (0.68 [0.53-0.85]; p=0.001) but not in multivariable models (Tables S14 & S15). Analysis by category of ID (persistent, incident, resolved or never), unadjusted for other variables, found significant differences in all-cause mortality (p<0.001). Compared to those who never had ID, patients with persistent ID had the highest all-cause mortality (2.37 [1.76-3.20]; p<0.001) (**Figure 11a**).

After adjusting for age, sex, diabetes, ischaemic heart disease, NYHA class, BMI, AF/flutter, heart rate, systolic blood pressure, left ventricular phenotype, NT-proBNP, hsCRP, eGFR, baseline haemoglobin and therapy with loop diuretics, differences persisted in mortality related to ID (p=0.02) (**Figure 11a**). Compared to those who never had ID, those with persistent ID had the greatest risk of death (1.81 [1.23-2.67]; p=0.003). There was a similar pattern for CV mortality in both unadjusted and adjusted models (Figure S7). Regardless of baseline values, patients who had a serum iron >17µmol/L at one year had a better prognosis than those who had a value <10µmol/L (**Figure 12a**, Figures S8a & S8b).

Serum ferritin measured at one year was not associated with mortality on univariable or multivariable analyses but an increase in serum ferritin between baseline and one year was associated with a higher all-cause mortality (1.01 [1.00-1.03]; p=0.04) (Table S14). When the FAIR-HF criteria were used to classify patients, mortality was similar for patients who had persistent, incident or resolved ID compared to those that never had ID in both unadjusted and adjusted models (Figures S9 & S10).

A higher TSAT at one year was associated with a lower subsequent all-cause (0.98 [0.97-0.99]; p=0.02) and CV mortality (0.98 [0.96-0.99]; p=0.03) in

univariable analysis but not in multivariable models. The association between the absence, persistence, development, or resolution of ID defined by a TSAT <20% and all-cause and CV mortality was similar to that for ID defined as a serum iron ≤13μmol/L (Figures S11 & S12), although these relationships were no longer statistically significant after adjustment.

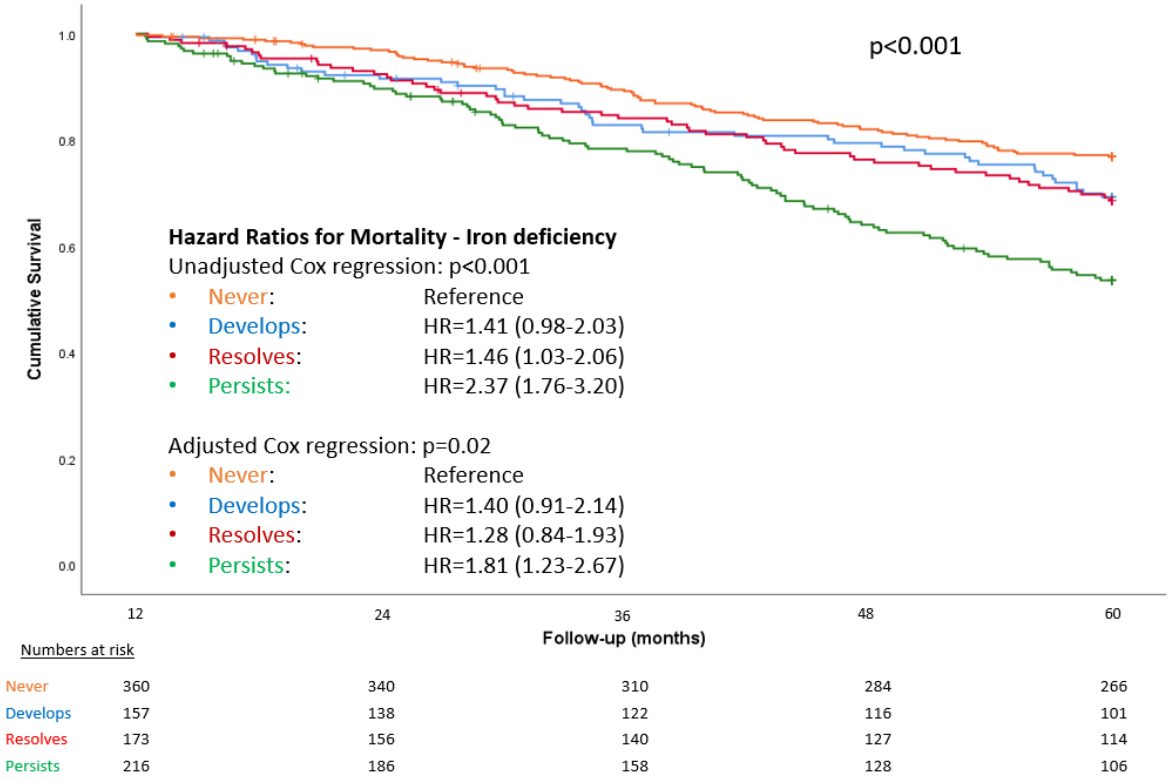
Compared to patients with persistent ID, resolution of ID according to the serum iron criterion was associated with better survival (0.61 [0.44-0.86]; p=0.004). In contrast, resolution of ID defined by either of the other two criteria was not associated with a better prognosis (Table S16 & Figure S13).

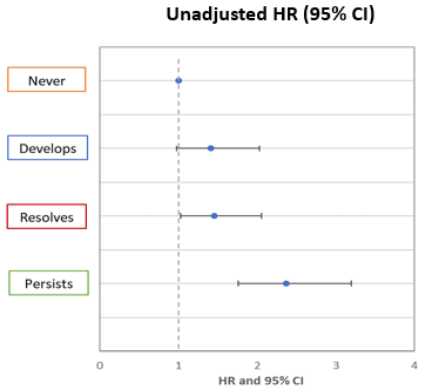
4.3.2.2 Anaemia

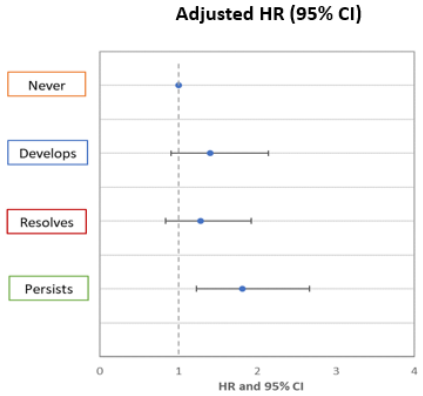
Higher haemoglobin measured at one year was associated with lower all-cause (0.82 [0.77-0.89]; p<0.001) and CV mortality (0.81 [0.73-0.89]; p<0.001) in univariable analysis, but not in multivariable models. (Tables S14 & S15).

In univariable models, analysis by category of anaemia (persistent, incident, resolved or never), unadjusted for other variables, found significant differences in all-cause mortality (p<0.001). Anaemia at baseline, regardless of whether it persisted (2.32 [1.77-3.06]; p<0.001) or resolved (2.47 [1.65-3.71]; p<0.001), was associated with higher all-cause mortality compared to those who were neither anaemic at baseline nor follow-up (**Figure 11b**). New-onset anaemia was associated with an intermediate outcome (1.72 [1.19-2.49]; p=0.004), but mortality was higher for those whose anaemia was more severe at follow-up (**Figure 12b**). Adjusting for the 15 baseline variables specified above, but with baseline iron and ferritin rather than haemoglobin in the model, similar trends were observed but statistical significance was lost. There was a similar pattern for CV mortality in both unadjusted and adjusted models (Figure S14). Patients who were neither anaemic at baseline nor follow-up had the best survival (**Figure 12b**). The survival of individual men and women according to haemoglobin at baseline and 1 year are shown in Figures S15.

A


p<0.001
Cumulative Survival
1.0
0.8
0.6
0.4
0.2
0.0
Hazard Ratios for Mortality - Iron deficiency
Unadjusted Cox regression: p<0.001
Never: Reference
Develops: HR=1.41 (0.98-2.03)
Resolves: HR=1.46 (1.03-2.06)
Persists: HR=2.37 (1.76-3.20)
Adjusted Cox regression: p=0.02
Never: Reference
Develops: HR=1.40 (0.91-2.14)
Resolves: HR=1.28 (0.84-1.93)
Persists: HR=1.81 (1.23-2.67)
12
24
36
48
60
Follow-up (months)
Numbers at risk
Never 360 340 310 284 266
Develops 157 138 122 116 101
Resolves 173 156 140 127 114
Persists 216 186 158 128 106


Unadjusted HR (95% CI)
Never
Develops
Resolves
Persists
0
1
2
3
4
HR and 95% CI


Adjusted HR (95% CI)
Never
Develops
Resolves
Persists
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4
HR and 95% CI

B

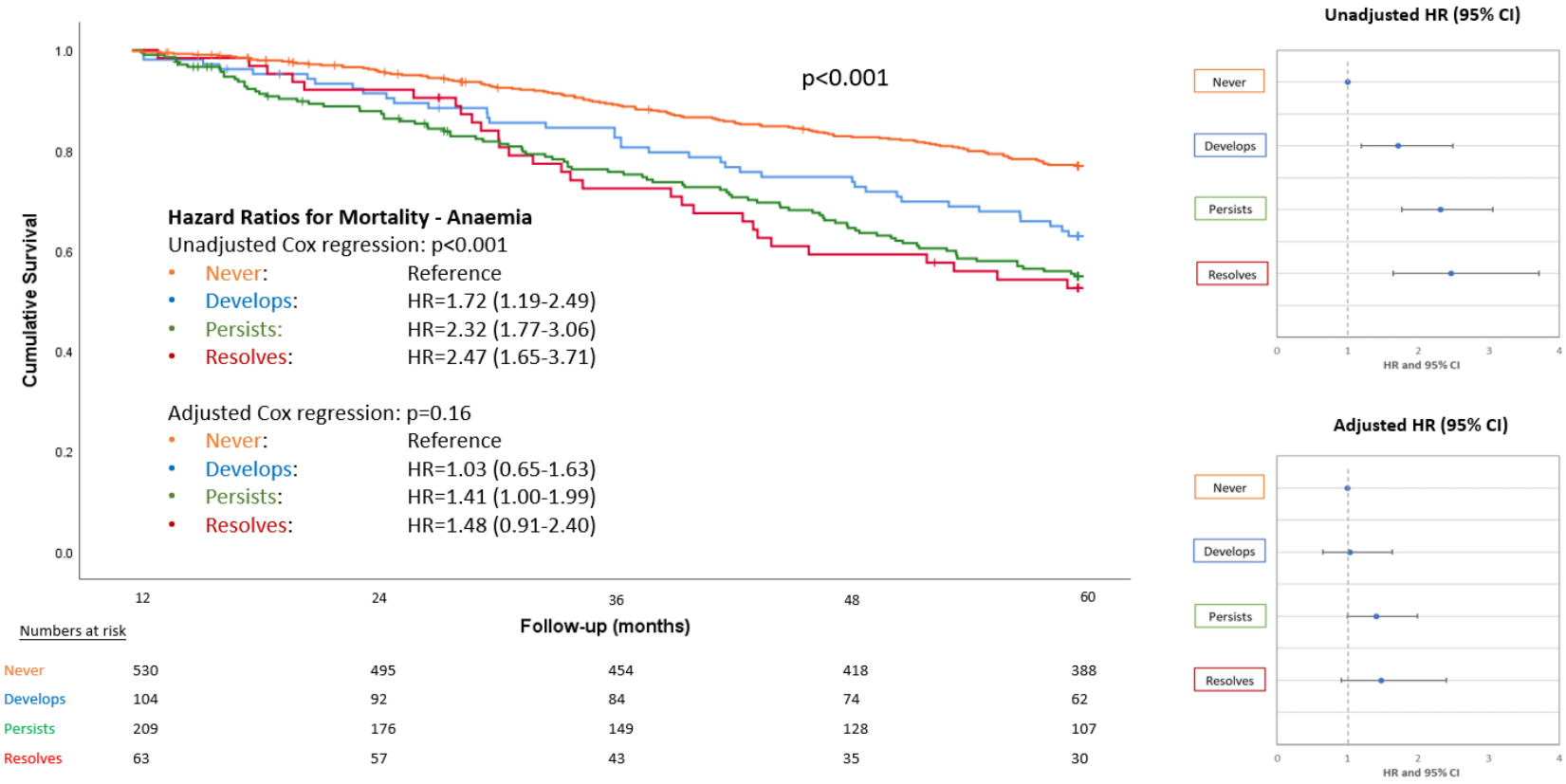


Figure 11: Kaplan-Meir survival analysis of all-cause mortality 5 years from baseline visit according to change in iron deficiency (a) or anaemia status (b).
Differences between changes in status compared using the log-rank test. Unadjusted and adjusted hazard ratios with (95% confidence intervals) also reported. Models adjusted for age, sex, diabetes, ischaemic heart disease, NYHA class, BMI, AF/flutter, heart rate, systolic

blood pressure, left ventricular phenotype, log NT-proBNP, log hsCRP, eGFR, therapy with loop diuretics and baseline haemoglobin (3a) or baseline iron and ferritin (3b). Forest plots of hazard ratios with 95% confidence intervals for each group in unadjusted and adjusted models also presented.

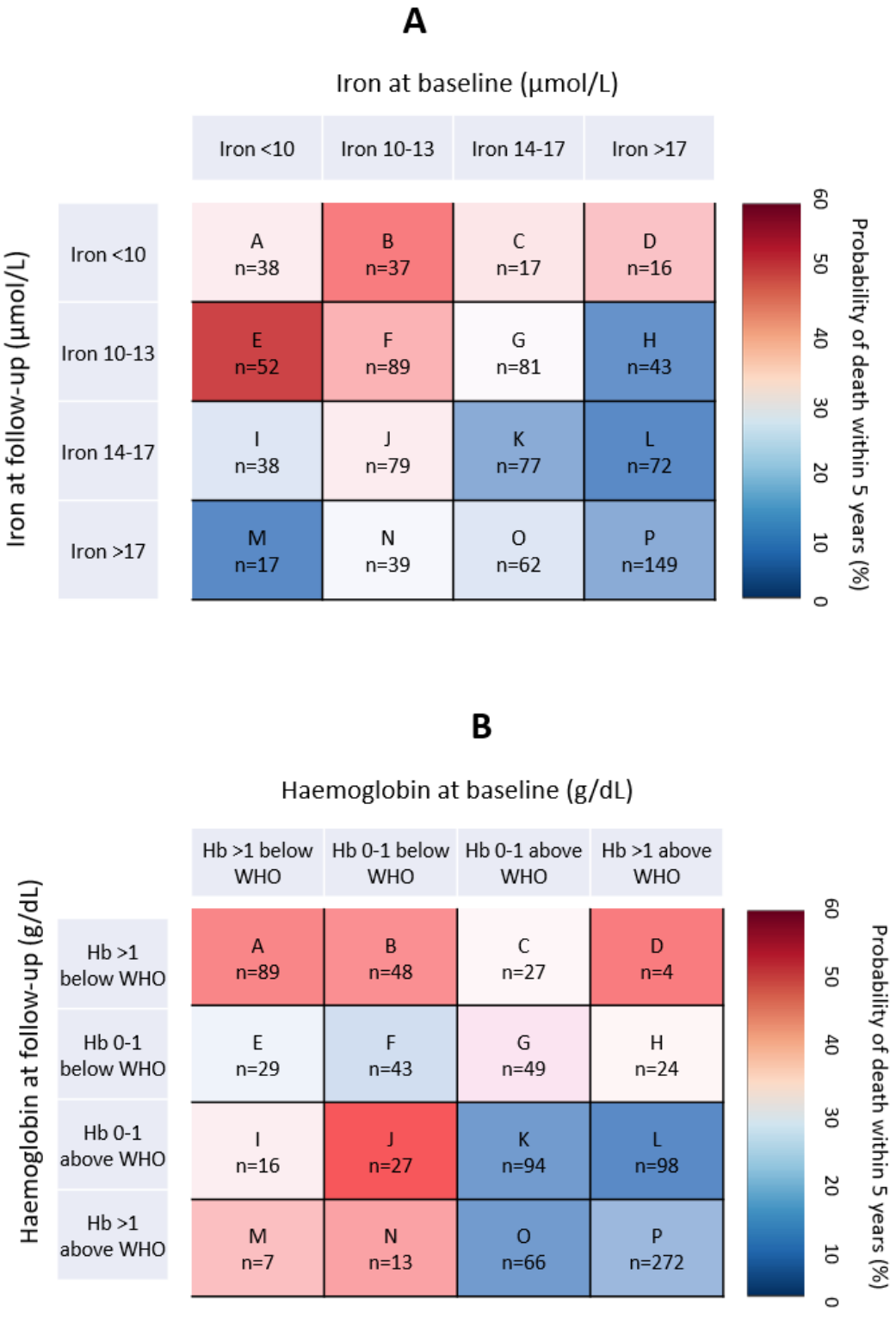


Figure 12: Heat maps depicting survival 5 years from baseline.
Classified by baseline and one year follow-up measurements of (A) serum iron (µmol/L) and (B) haemoglobin (g/dL). Number of patients within each cell reported. WHO = World Health Organization definition of anaemia: Hb <13.0 g/dL in men and <12.0 g/dL in women.

4.4 Discussion

This is the first analysis, to our knowledge, to provide a comprehensive report of the natural history of ID and anaemia in patients with heart failure representative of clinical practice. The picture is highly dynamic. Most patients with heart failure (70%) have or develop ID, anaemia, or both, with 47% changing their status within one year. There was little difference according to left ventricular phenotype (HFrEF, HFmrEF or HFpEF). The rate of new-onset anaemia (16%) and incident ID (30%) were both individually substantial. Current ESC and ACC guidelines give no indication as to how frequently iron indices or haemoglobin should be checked (24,114), but, in keeping with more recent expert advice (130), our study suggests that it would be prudent to re-check haemoglobin and markers of ID at least once a year.

We chose to define ID as a serum iron ≤13μmol/L based on a recent study of patients with HFrEF, which reported a strong relationship between low serum iron and the absence of iron on bone-marrow histology (n=42) and with mortality (20), whilst a low ferritin, in the absence of a low serum iron, was associated with neither. In our analysis, the prevalence of ID (defined as a serum iron ≤13μmol/L) at baseline (43%) was similar to previous reports despite differences in the criteria for ID (20,41). Persistent ID was associated with a poor prognosis and resolution of ID, based on serum iron measurements, was associated with an improved survival. In contrast, patients who had anaemia at any time, even if it resolved, had a worse long-term outcome, perhaps because they were more likely to also have ID or because anaemia indicates other underlying risk, including worse renal function and more severe heart failure.

The current ESC guideline recommended definition of ID, based on the inclusion criteria of the FAIR-HF trial (84), has not been universally accepted, nor validated against the gold-standard of bone-marrow histology. When applying the guideline (or the “FAIR-HF”) definition to our cohort, the prevalence of ID was 63% but it was not associated with a higher mortality, nor was it associated with an improved survival when it resolved. Data previously published from the Hull LifeLab reported that serum iron and TSAT are highly correlated ($r > 0.9$), with strong, linear associations between lower levels and worse outcome. In

contrast, the relation between ferritin and mortality was non-linear and weaker in both the Hull LifeLab and other cohorts (19,37,43).

Serum ferritin concentrations increase as a result of inflammation and cell damage, including that associated with heart failure (22,58). The W.H.O. requires a ferritin <15 µg/L to diagnose ID and many clinical laboratories define it as <30 µg/L (55). The FAIR-HF criteria for ID may have been successful not because it is either sensitive or specific but because ID is so common in patients with heart failure that testing for ID might not be essential. However, an individual-patient-data meta-analysis of trials of ferric carboxymaltose compared the effect of IV iron on outcome according to terciles of haemoglobin, ferritin and TSAT. Only patients in the lowest tercile of TSAT (which is highly correlated with serum iron) benefitted from IV iron. (105). Serum iron is a measure of the total amount of transferrin-bound iron in blood serum. Although there are reports of diurnal variation of measured values within healthy populations due to post-prandial surges (61), serum concentrations appear to be fairly stable in heart failure (20), perhaps because iron absorption is impaired (73). Despite the high correlation between serum iron and TSAT, a low serum iron may have performed better than either the FAIR-HF criteria or TSAT in prognostic models because serum iron might represent a summary-measure of iron deficiency and inflammation, which both cause a fall in serum iron (63). Transferrin concentrations may decrease with chronic disease (64). Because TSAT is calculated from transferrin and serum iron ((iron/transferrin x 25.2) x 100)), a decline in transferrin may attenuate the decline in TSAT as iron deficiency develops.

Predictors of incident ID at one year included, as might be expected, a lower serum iron or haemoglobin, reflecting borderline iron repletion. A higher hsCRP was also associated with incident ID. Heart failure and many of its co-morbid conditions are associated with chronic low-grade inflammation which may increase hepatic secretion of hepcidin (15). Hepcidin binds to and inactivates ferroportin, which is involved both in the translocation of iron from the gut epithelium to the circulation and in iron release from macrophages, thus reducing iron absorption and availability (28).

In our analysis, changes in TSAT amongst patients whose ID resolved were of similar magnitude to those observed in the treatment arms (IV ferric carboxymaltose) of the FAIR-HF (84) and CONFIRM-HF (85) trials. In our cohort, few patients were prescribed oral iron therapy at baseline (n=42; 5%). This suggests that many patients improve iron absorption following optimisation of treatment for heart failure. How iron stores are replenished may be of fundamental importance to clinical outcomes. Short-term randomized trials favour intravenous (84,85) rather than oral iron (73) in patients with heart failure. In health, the body contains about 4,000 mg of iron, with about 1,800 mg in red blood cells, 300 mg in muscle and other tissues and 2,000 mg stored in the bone marrow, liver and reticulo-endothelial system (33). Clinical ID will occur only once stores are exhausted or unavailable. In an otherwise healthy person with ID, the maximum absorption of iron is about 20 mg/day (80). Assuming no iron losses and adequate iron ingestion, it would take 2-3 months to correct ID fully. For a patient with heart failure, absorption will be lower, and losses may be greater and therefore oral supplements may be ineffective. However, once ID is corrected, good treatment of heart failure, with or without oral supplements, may reduce the need for further intravenous iron.

Prevalent ID and anaemia were both associated with a worse mortality in patients with heart failure (17,95). Similar trends were observed for incident ID or anaemia. Some patients required only a small change in values in order to be classified as incident cases. The lesser severity of ID and anaemia and greater chance of later resolution may account for the weaker relationship with outcome of incident compared to prevalent ID or anaemia. Also, some patients may have developed ID or anaemia between these visits and died before one year.

Resolution of ID was associated with a lower mortality, but resolution of anaemia was not. In contrast to our findings, a retrospective cohort study (n=1,393) reported better survival for those whose anaemia resolved (127). Differences may reflect the play of chance, variations in the population studied, covariates used for statistical modelling or frequent relapse after recovery. Patients with resolution of anaemia still had a lower haemoglobin at one year than those who never had anaemia, suggesting only partial recovery. A high prevalence of persistent ID in patients whose anaemia had recovered compared to those who had a normal haemoglobin throughout, might also explain their

poor outcome. Changes in haemoglobin concentration could also reflect changes in plasma volume or red cell mass or perhaps splenic sequestration and thus the severity of heart failure rather than ID. Additionally, despite increasing haemoglobin concentrations, treatment of anaemia with erythropoietin-analogues does not seem to improve prognosis (97).

Several trials are exploring whether IV iron in those with ID, defined by the FAIR-HF diagnostic criteria, improves morbidity and mortality in patients with HFrEF or, for HFpEF, symptoms and exercise capacity (131). The recently reported AFFIRM-AHF trial failed to demonstrate a reduction in CV mortality with intravenous iron in patients admitted with worsening heart failure, an LV ejection fraction <50% and ID defined by the FAIR-HF criteria (37). Intravenous iron was, however, associated with a lower rate of first and recurrent hospitalisations for heart failure. No significant differences were reported in subgroup analysis for those with a ferritin above or below 100µg/L, those with a TSAT above or below 20% or for those with a haemoglobin above or below 12.0g/dL. No subgroup analysis based on serum iron concentrations was reported. Given our results, exploring the effects of IV iron in subgroups with and without a low serum iron or TSAT and in those with and without anaemia will be of particular interest in future trials.

4.4.1 Limitations

This is an analysis of a clinical service. Only 906 of 2,763 patients with a follow-up visit at one year had a full set of measurements at both time-points. The main reason for a lack of repeat measurements was deviation from the clinic protocol, which may have been more likely in those who were clinically stable. Patients were recruited over a period of 18 years, during which time treatments for heart failure have evolved. However, other than intravenous iron, none has been shown to have a substantial influence on haemoglobin or iron status. Patients prescribed oral iron (<5% of the population studied) were not excluded as this reflects common clinical practice. Most of the enrolment to the study and follow-up was done before guidelines recommended IV iron for treating symptoms in patients with HFrEF and ID (class IIa, Level A) in 2016. No data on blood loss or blood transfusion was available and we did not implement a 'minimum-change' rule to define resolution of anaemia or ID (e.g. requirement

for haemoglobin to change by at least 0.5 g/dL to allow reclassification), as implemented by Tang et al (127). This might have ensured a more definitive change in status and avoided patients being reclassified when close to classification thresholds. However, such a criterion might be difficult to implement in clinical practice. Some curves on Kaplan-Meier analysis cross each other suggesting that risk in these groups may not be proportional over time. Yet the only sub-group that is of any concern is also the smallest in number which is unlikely to have an influence.

4.5 Conclusions

Most patients with heart failure have or will develop ID and a substantial proportion of these will also have anaemia. Patients with persistent ID have a worse outcome compared to those in whom it resolves. However, anaemia is associated with a poor outcome whether or not it resolves. Serum iron ≤13μmol/L is more strongly associated with prognosis than ID defined by current guideline criteria.

Chapter 5 Prognostic implications of serum transferrin and iron concentrations as determinants of transferrin saturation (TSAT) in chronic heart failure

5.1 Introduction

For patients with chronic heart failure (CHF), timely identification and treatment of iron deficiency (ID) can improve symptoms and exercise capacity, and reduce CHF-related hospitalisations (105). How iron deficiency (ID) should be defined in patients with heart failure without measuring iron stores in bone marrow is uncertain. In clinical practice, serum ferritin is most commonly used, although with highly variable diagnostic thresholds. Other blood markers (such as low serum iron, low transferrin saturation (TSAT) or more novel biomarkers such as high soluble transferrin receptor) may correlate better with bone marrow iron stores (20,104).

Transferrin saturation is the ratio of serum iron and serum transferrin (20,132). Transferrin, produced mainly in the liver, is the main molecule transporting iron in the blood. A TSAT <20%, irrespective of ferritin concentration, identifies patients with CHF at greater risk of heart failure rehospitalisation (13) and death (20,37,42,133). Serum iron may be even more strongly related to adverse outcomes (19,134) – see Chapter 4.

TSAT is susceptible to variations in both serum iron and transferrin. Serum transferrin falls with inflammation and chronic disease (64,132), and, therefore, TSAT may be >20% when serum transferrin is low even when serum iron is low, masking a possible diagnosis of ID. Also, when serum transferrin is high, TSAT may be <20% even when serum iron is normal.

Accordingly, we investigated the relationships between serum transferrin and patient characteristics, other markers of ID, inflammation, and outcomes, in patients with CHF.

5.2 Methods

5.2.1 Study population

Between December 2001 and July 2019, consenting patients with suspected or confirmed heart failure referred for assessment at a community heart failure clinic serving a local population of about 550,000 people were enrolled in a registry conducted in parallel with routine NHS clinical activities, the Hull LifeLab. Blood samples were obtained on the same day as clinical examination, electro- and echocardiography, and included serum transferrin, serum iron, serum ferritin and transferrin saturation. TSAT was calculated using the formula: TSAT (%) = (serum iron (μmol/L)/transferrin (g/L) x25.2) x100 (20).

Heart failure was defined as typical symptoms and signs and **either** a measured or visually estimated left ventricular ejection fraction (LVEF) of <40% (HFrEF) **or,** if the LVEF was >40%, a raised N-terminal pro-brain natriuretic peptide (NTproBNP) (≥ 125ng/L) (114). Those with an elevated NTproBNP without HFrEF were divided into those with an LVEF of 40-49% (heart failure with mid-range ejection fraction (HFmrEF)) or ≥50% (heart failure with preserved ejection fraction (HFpEF)). Some patients had a raised NT-proBNP but no available LVEF (n=341) and were included in the analysis as HF-NTproBNP. The W.H.O. definition of anaemia was used (haemoglobin of <12.0g/dL in women and <13.0g/dL in men) (47). Only those with confirmed heart failure and complete haemoglobin and iron indices were included in the analysis.

Two primary definitions of iron deficiency were used: serum iron ≤13μmol/L; and TSAT <20% (20). The definition of ID currently adopted by the ESC (ferritin <100μg/L or TSAT <20% if ferritin 100-299μg/L) (23) was also used for some analyses.

Follow-up completed on the 3rd of March 2019. Deaths were adjudicated based on available medical records: the adjudication process has been described previously (120).

5.2.2 Statistical analysis

Continuous variables are presented as medians with 25th and 75th percentiles and were compared using one-way ANOVA if normally distributed or by the Kruskal-Wallis test if not. Categorical variables, presented as numbers and percentages, were compared using chi-squared tests. Variables were transformed using either the square root or logarithms with base 10 as appropriate (19). No imputation was performed for missing data. Patient characteristics are displayed and compared with the patients divided by quartiles of transferrin. Differences in transferrin between heart failure phenotypes were also examined. Uni- and multi- variable linear regression analysis was used to identify correlates of transferrin. Variables associated with transferrin at the 10% significance level ($p \leq 0.1$) in univariable models were entered into the multivariable model. The strength and direction of effect between each variable and transferrin is reported using standardized beta coefficients and P values. After dividing patients into quartiles of transferrin, we arbitrarily defined a low transferrin as the lowest quartile (≤2.3g/L). Characteristics of patients with a low transferrin further classified by the presence or absence of a low serum iron (≤13μmol/L) and/or a low TSAT (<20%) were explored.

Kaplan-Meier survival curves for all-cause and CV mortality at 5 years were constructed with the patients divided by quartiles of serum transferrin and, separately, in the group with low transferrin, divided by serum iron ≤13μmol/L and/or TSAT <20%. Further survival curves were generated combining the lower two and upper two quartiles of transferrin divided by serum iron and TSAT status. Supplementary curves for all-cause mortality at 5 years for patients in each quartile of transferrin by serum iron concentration (<10μmol/L; 10-13μmol/L; 14-17μmol/L; >17μmol/L) were also produced.

Cox proportional hazard regression models were used to assess the associations between clinical variables and both all-cause and CV mortality within 5 years. A limited number of predictors for mortality, previously validated in two cohorts (age, blood urea, plasma NT-proBNP, haemoglobin and prescription (or failure to prescribe) a beta-blocker) (135), were entered into multivariable models with the variables of interest and adjusted for serum iron status. Prescription of a loop diuretic was also added to multivariable models as it is a strong predictor of

mortality (19,136). The impact of entering additional variables into the pre-existing multivariable model were also explored. Hazard ratios (HRs) with 95% confidence intervals are reported. Multivariable interaction analyses were performed according to whether patients had HFrEF or not. If the analysis suggested there was a significant interaction for a variable of interest, multivariable models were repeated separately for those with or without HFrEF.

All analyses were performed with SPSS statistical software version 27 (IBM). All tests were 2-sided and unless previously specified, used a p-value of <5% to determine statistical significance.

5.3 Results

5.3.1 Patient characteristics

Of 9,321 patients enrolled between 1st December 2001 and July 2019, 7,160 (77%) had a confirmed diagnosis of heart failure, of whom 4,422 (62%) had full iron indices available for assessment. Those with heart failure and incomplete haemoglobin or iron indices not included in this analysis (n= 2,738; 38%) were more likely to be enrolled prior to 2009, were younger and were more likely to have HFrEF than those with complete results but there was no difference in sex distribution or plasma concentrations of NT-proBNP (Table S17). More than half of those included had either HFmrEF (19%) or HFpEF (41%) whilst 32% had HFrEF. A small number (n=341; 8%) had echocardiographic data but no record of LVEF (HF-NT-proBNP). Differences in clinical characteristics between patients of various heart failure phenotypes are available in **Table 1** of Chapter 3.

Patients in the lowest quartile of serum transferrin (≤2.3g/L: n= 1,195 (27%)) were older, more likely to be men and to have anaemia. They had a lower haemoglobin, eGFR and albumin and higher serum ferritin, TSAT, hsCRP and plasma NT-proBNP (**Table 10** & Table S18). The prevalence of ID by the ESC criteria was lower in those with low transferrin. Serum transferrin concentration was similar amongst heart failure phenotypes (Figure S16).

Table 10: Characteristics according to quartiles of serum transferrin

Variable	Q1 (≤ 2.3 g/L) (n=1195 ;27 %)	Q2 (2.4-2.6 g/L) (n= 1159; 26%)	Q3 (2.7-2.9 g/L) (n= 1003; 23%)	Q4 (≥ 3.0 g/L) (n= 1065; 24%)	P-value
Demographics					
Age (years)	77 (70-83)	75 (67-82)	74 (67-80)	75 (67-81)	<0.001
Sex (female)	354 (30)	437 (38)	435 (43)	537 (50)	<0.001
Hypertension	637 (53)	640 (55)	564 (56)	614 (58)	0.21
Diabetes	284 (24)	285 (25)	280 (28)	303 (29)	0.02
IHD	537 (45)	477 (41)	421 (42)	441 (41)	0.22
COPD	120 (10)	102 (9)	82 (8)	107 (10)	0.35
Signs and symptoms					
NYHA III or IV	348 (30)	295 (26)	303 (31)	374 (36)	<0.001
ECG and ECHO					
AF/flutter	337 (29)	385 (34)	360 (38)	425 (41)	<0.001
Heart rate (bpm)	71 (61-82)	73 (63-84)	74 (63-87)	76 (65-90)	<0.001
HFrEF	382 (32)	390 (34)	322 (32)	335 (32)	0.71
Bloods					
Haemoglobin (g/dL)	12.8 (11.5-14)	13.4 (12.2-14.5)	13.4 (12.2-14.6)	13.1 (11.9-14.4)	<0.001*
Anaemia	556 (47)	345 (30)	284 (28)	361 (34)	<0.001
MCV (fL)	91 (87-95)	90 (87-94)	90 (87-93)	88 (84-92)	<0.001*
MCH (pg)	30 (29-31)	30 (29-31)	29 (28-31)	29 (27-30)	<0.001*
MCHC (g/dL)	33 (32-34)	33 (32-34)	33 (32-34)	33 (32-34)	<0.001*
RDW (%)	14.2 (13.4-15.1)	14.0 (13.4-15.0)	14.2 (13.5-15.3)	14.6 (13.7-16.1)	<0.001
Iron (µmol/L)	13 (10-18)	15 (11-19)	15 (11-18)	13 (9-17)	<0.001
Iron ≤13µmol/L	624 (52)	488 (42)	423 (42)	566 (53)	<0.001
TSAT (%)	25 (18-34)	23 (17-29)	21 (16-26)	16 (11-21)	<0.001
TSAT <20%	385 (32)	428 (37)	467 (47)	751 (71)	<0.001
TSAT <20% & Iron >13µmol/L	0 (0)	0 (0)	44 (4)	185 (17)	<0.001
Ferritin (µg/L)	147 (82-261)	95 (57-159)	73 (40-136)	41 (22-76)	<0.001
Ferritin <30µg/L	20 (2)	72 (6)	145 (15)	391 (37)	<0.001
Ferritin <100µg/L	393 (33)	609 (53)	628 (63)	876 (82)	<0.001
ESC definition of ID	568 (48)	761 (66)	727 (73)	955 (90)	<0.001
eGFR (ml/min/1.73m^2)	55 (38-71)	60 (46-75)	61 (48-77)	63 (49-77)	<0.001

hsCRP (mg/L)	5.4 (2.2-15.0)	3.9 (1.5-8.3)	3.5 (1.5-7.1)	3.8 (1.6-7.4)	<0.001
NTproBNP (ng/L): SR	966 (417-2567)	724 (321-1745)	727 (288-1784)	600 (273-1829)	<0.001
NTproBNP (ng/L): AF/Flutter	2334 (1309-4383)	1919 (1055-3331)	1824 (1073-3298)	1888 (1115-3190)	<0.001
Albumin (g/L)	36 (33-38)	38 (36-40)	38 (36-40)	38 (36-40)	<0.001
ALT (u/L)	18 (14-24)	20 (15-26)	20 (16-26)	20 (16-26)	<0.001
ALP (u/L)	77 (62-100)	76 (61-96)	74 (59-92)	80 (65-101)	<0.001
Medication					
Loop diuretic	755 (64)	702 (62)	647 (66)	721 (69)	0.005
ACEi or ARB	825 (70)	807 (71)	672 (68)	724 (69)	0.60
MRA	259 (22)	271 (24)	225 (23)	229 (22)	0.67
BB	747 (64)	708 (62)	609 (62)	664 (63)	0.83
Anticoagulants	305 (26)	353 (31)	315 (31)	365 (34)	<0.001
Antiplatelets	553 (46)	501 (43)	440 (44)	434 (41)	0.07

Continuous variables presented as median and (25^{th}-75^{th} centiles). Categorical variables presented as count and (%).

*Variables normally distributed and compared using one-way ANOVA.

Abbreviations - IHD: ischaemic heart disease; COPD: chronic obstructive pulmonary disease; NYHA: New York Heart Association; AF: atrial fibrillation; HFrEF: heart failure with reduced ejection fraction; MCV: mean corpuscular volume; MCH: mean corpuscular haemoglobin; MCHC: mean corpuscular haemoglobin concentration; RDW: red cell distribution width; TSAT: transferrin saturation; ESC: European Society of Cardiology; ID: iron deficiency; eGFR: estimated glomerular filtration rate; hsCRP: high-sensitivity CRP; NTproBNP: N terminal pro-natriuretic peptide; ALT: alanine aminotransferase; ALP: alkaline phosphatase; SR: sinus rhythm; ACEi: angiotensin converting enzyme inhibitor; ARB: angiotensin receptor blocker; MRA: mineralocorticoid receptor antagonist; BB: beta-blocker.

For patients with low transferrin, just over half had a low (≤13µmol/L) serum iron (**Figure 13**). Compared to those with a low transferrin and normal serum iron (>13µmol/L), those with a low serum iron were older, more likely to be anaemic, more likely to be women and had more severe symptoms of heart failure with higher plasma NT-proBNP (Table S19). In the absence of a low serum iron, no patient had a TSAT <20%. Of those with a low serum iron, over a third (38%, n= 239) had a TSAT ≥20% because transferrin was low. These patients, despite a high prevalence of anaemia and low serum iron, would not be considered iron deficient by current ESC criteria unless ferritin was also low (114). Characteristics of patients with a low serum iron divided by TSAT above or below 20% are described in Table S20. Of patients in the highest quartile of transferrin (n=1,065), 17% (n=185) had a normal serum iron (>13µmol/L) but a TSAT <20% because transferrin was high.

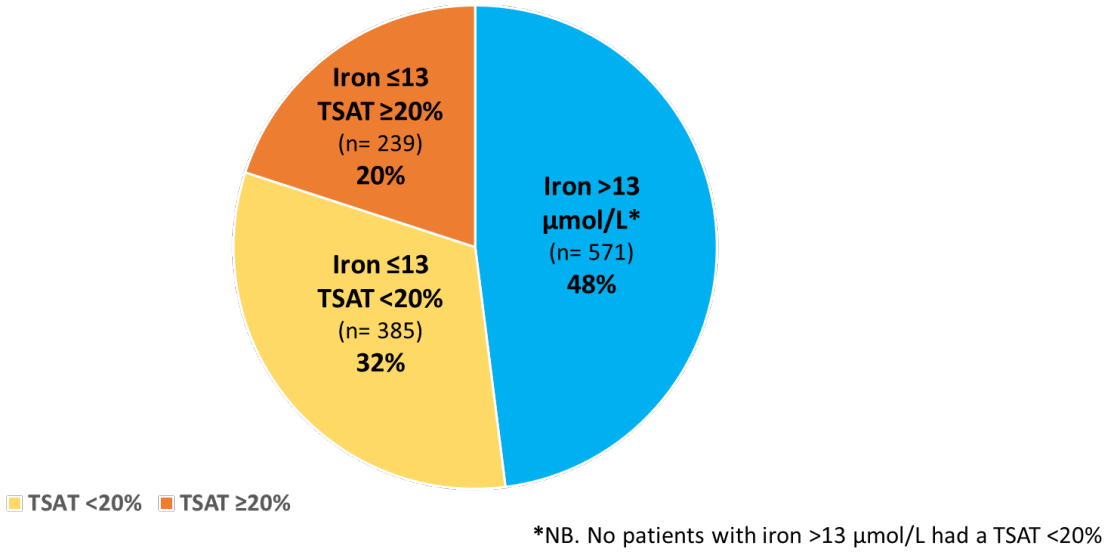


Figure 13: Measures of iron status in patients in the lowest quartile of transferrin (≤2.3 g/L) (n=1,195).
Serum iron in µmol/L.

5.3.2 Variables associated with serum transferrin

In univariate analysis there was an inverse relation between serum transferrin and ferritin and much weaker inverse relations with serum iron, NT-proBNP, age, and hsCRP. There were positive correlations between serum transferrin and haemoglobin, eGFR, and serum albumin (Figure S17).

In multivariable analysis, there were inverse associations between transferrin and age (r= -0.11; p<0.001) and serum ferritin (r=-0.52; p<0.001) and a positive correlation with serum albumin (r=0.29; p<0.001) (Table S21). NT-proBNP interacted with eGFR, albumin and hsCRP. Despite repeating the multivariable model with these variables removed, there was no correlation between NT-proBNP and transferrin.

5.3.3 Survival

Median follow-up was 49 (18 - 89) months, during which 2,321 (53%) patients died; 5 year mortality was 35% (n=1,526). In univariate analysis, transferrin, as a continuous variable, was inversely associated with all-cause mortality (HR [95% CI]: HR 0.75 [0.67-0.83] per 1 g/dL; p<0.001) (Table S22). Compared to those with a normal transferrin (>2.3g/dL), those in the lowest quartile of transferrin had a higher all-cause (HR 1.41 [1.27-1.57]; p<0.001), cardiovascular (HR 1.31 [1.13-1.52]; p<0.001) and non-cardiovascular (HR 1.57 [1.34-1.84]; p<0.001) mortality (**Figure 14a** & **Figure 15a**). A greater proportion of deaths in the lowest quartile of transferrin was due to cancer, with lung cancer being most common (**Table 11**).

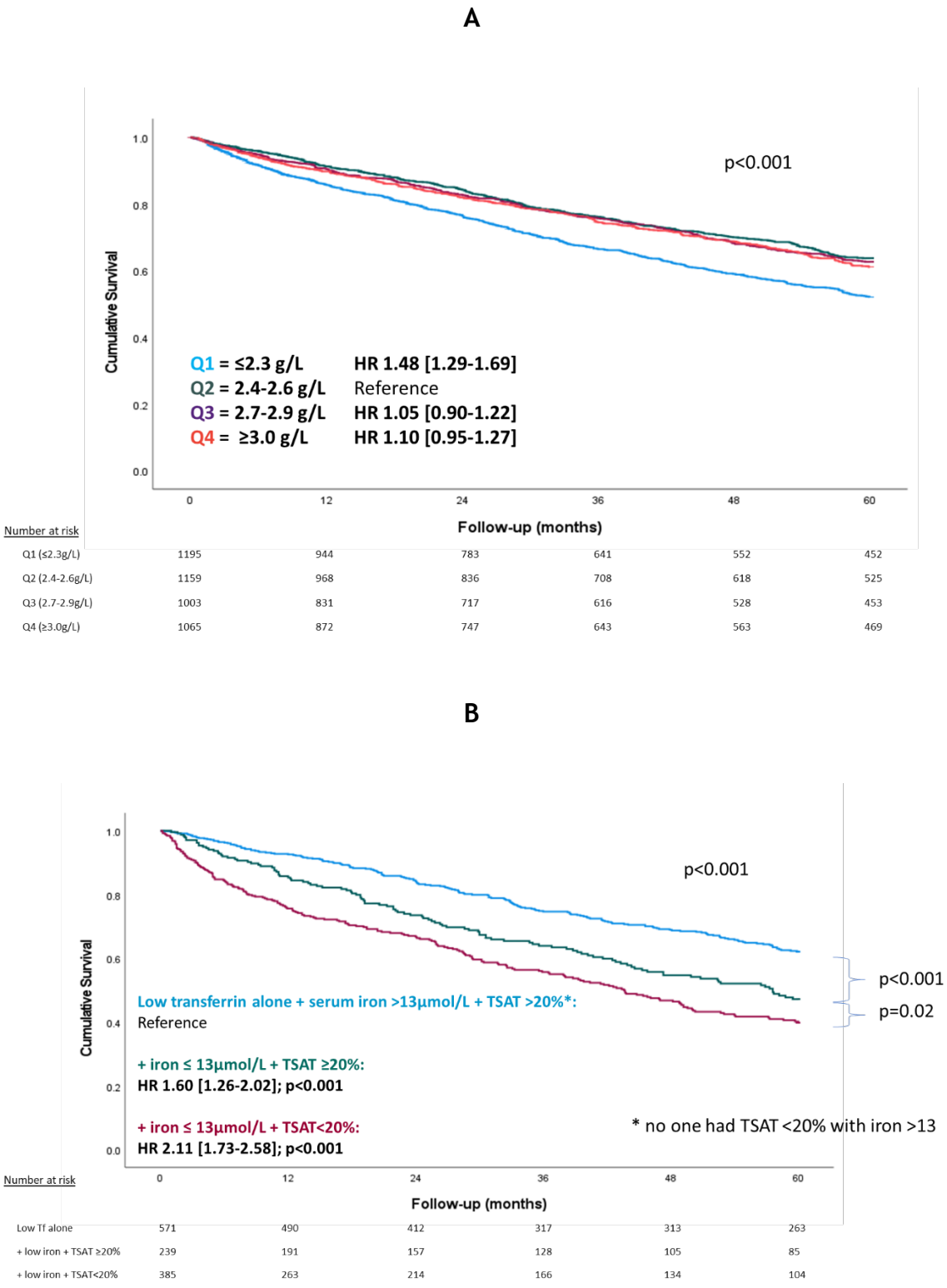


Figure 14: Kaplan-Meier survival curves for all-cause mortality in (A) all patients (n=4,422) by quartiles of serum transferrin and (B) in those with a transferrin (Tf) ≤2.3g/L (n=1,195) according to serum iron and TSAT. Log-rank p-value provided. Hazard ratios and 95% confidence intervals for each quartile of transferrin provided with quartile two (2.4-2.6g/dL) as reference in (A) and low transferrin alone with normal serum iron and TSAT used as the reference group in (B).

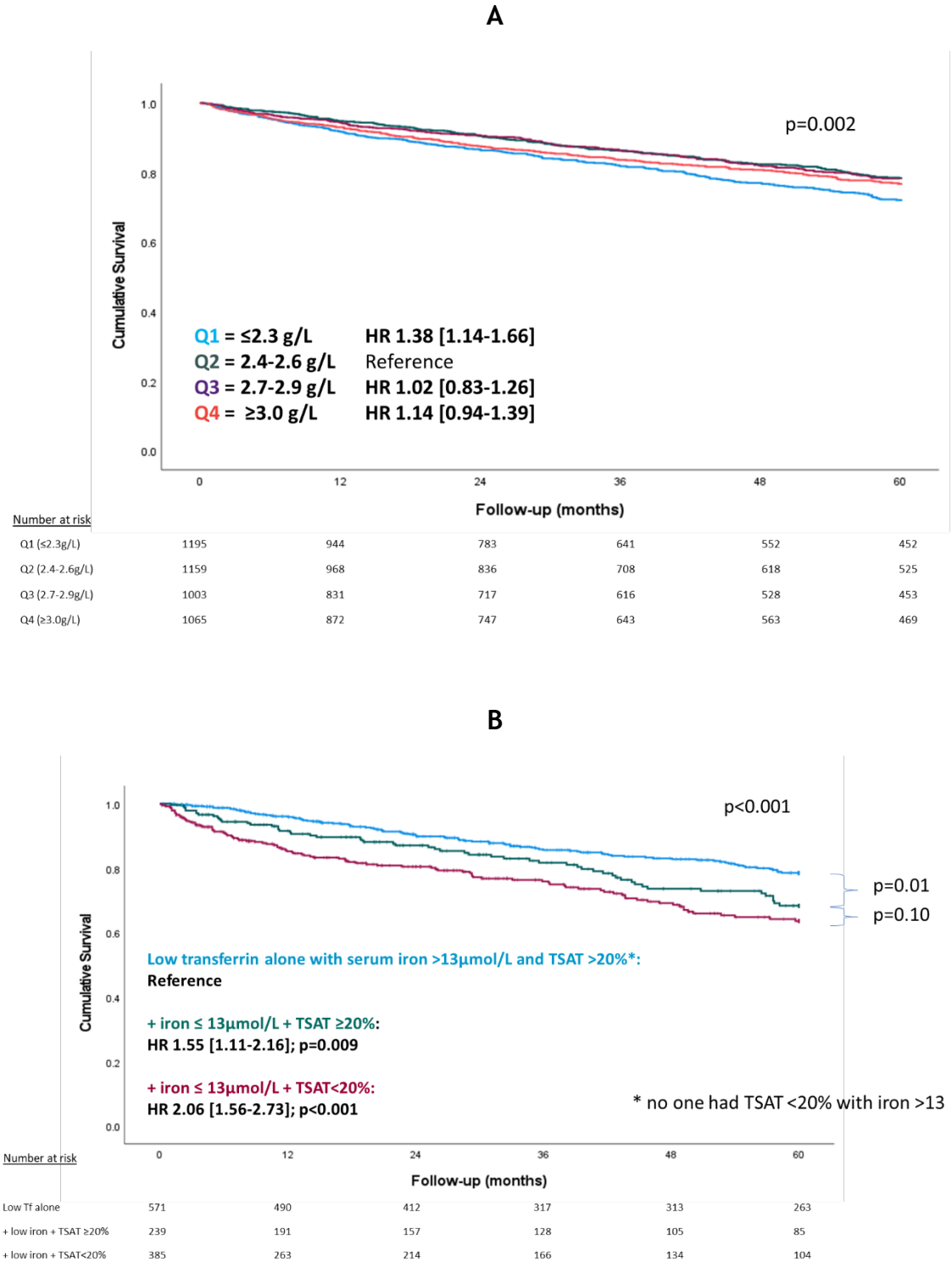


Figure 15: Kaplan-Meier survival curves for cardiovascular mortality in (A) all patients (n=4,422) by quartiles of serum transferrin and (B) in those with a transferrin (Tf) ≤ 2.3g/L (n=1,195) according to serum iron and TSAT. Log-rank p-value provided. Hazard ratio and 95% confidence intervals for each quartile of transferrin provided with quartile two (2.4-2.6g/dL) as reference in (A) and low transferrin alone with normal serum iron and TSAT used as the reference group in (B).

Table 11: Deaths in participants by quartiles of transferrin.

Table 2: Deaths in participants by quartiles of transferrin					
	Q1 (≤ 2.3 g/L) (n=1195 ;27 %)	**Q2 (2.4-2.6 g/L) (n= 1159; 26%)**	**Q3 (2.7-2.9 g/L) (n= 1003; 23%)**	**Q4 (≥ 3.0 g/L) (n= 1065; 24%)**	**P-value**
Deaths (1 year)	155 (14%)	91 (9%)	87 (10%)	103 (11%)	<0.001
CV death (as % of deaths)	87 (56%)	54 (59%)	52 (60%)	69 (67%)	0.38
Non-CV deaths (as % of deaths)	64 (41%)	35 (39%)	32 (37%)	32 (31%)	0.42
Unknown cause (as % of deaths)	4 (3%)	2 (2%)	3 (3%)	2 (2%)	N/A
Cancer deaths (as % of deaths)	27 (17%)	11 (12%)	5 (6%)	10 (10%)	0.05
Deaths (2 years)	243 (24%)	154 (16%)	144 (17%)	167 (18%)	<0.001
CV deaths (as % of deaths)	133 (55%)	91 (59%)	80 (56%)	113 (68%)	0.05
Non-CV deaths (as % of deaths)	104 (43%)	59 (38%)	63 (44%)	53 (32%)	0.09
Unknown cause (as % of deaths)	6 (2%)	4 (3%)	1 (<1%)	1 (<1%)	N/A
Cancer deaths (as % of deaths)	42 (17%)	23 (15%)	15 (10%)	16 (10%)	0.09
Deaths (5 years)	497 (42%)	356 (31%)	321 (32%)	352 (33%)	<0.001
CV death (as % of deaths)	252 (51%)	193 (54%)	170 (53%)	199 (57%)	0.39
Non-CV death (as % of deaths)	233 (47%)	152 (43%)	140 (44%)	144 (41%)	0.35
Unknown cause (as % of deaths)	12 (2%)	11 (3%)	11 (3%)	9 (3%)	N/A
Cancer deaths (as % of deaths)	79 (16%)	51 (14%)	36 (11%)	35 (10%)	0.048

Types of cancer deaths at 5 years (as % of cancer deaths)					
Upper GI	4 (5%)	2 (4%)	4 (11%)	2 (6%)	0.53
Lower GI	4 (5%)	3 (6%)	2 (6%)	5 (14%)	0.32
Lung	25 (32%)	14 (28%)	9 (25%)	14 (40%)	0.52
Breast	2 (3%)	3 (6%)	5 (14%)	2 (6%)	0.13
Prostate	10 (13%)	4 (8%)	1 (3%)	0 (0%)	0.07
Other solid organ	15 (19%)	8 (16%)	8 (22%)	6 (17%)	0.88
Haematological	13 (16%)	9 (17%)	5 (14%)	3 (9%)	0.66
Unknown	6 (8%)	8 (16%)	2 (6%)	3 (9%)	0.35

Presented as count and (%).

Abbreviations: - CV: cardiovascular; GI: gastrointestinal.

A low serum iron was associated with greater all-cause mortality (Table S22) regardless of transferrin concentration (Figure S18), but rates of death were highest in those who had both low serum iron and low transferrin (**Figure 16**).

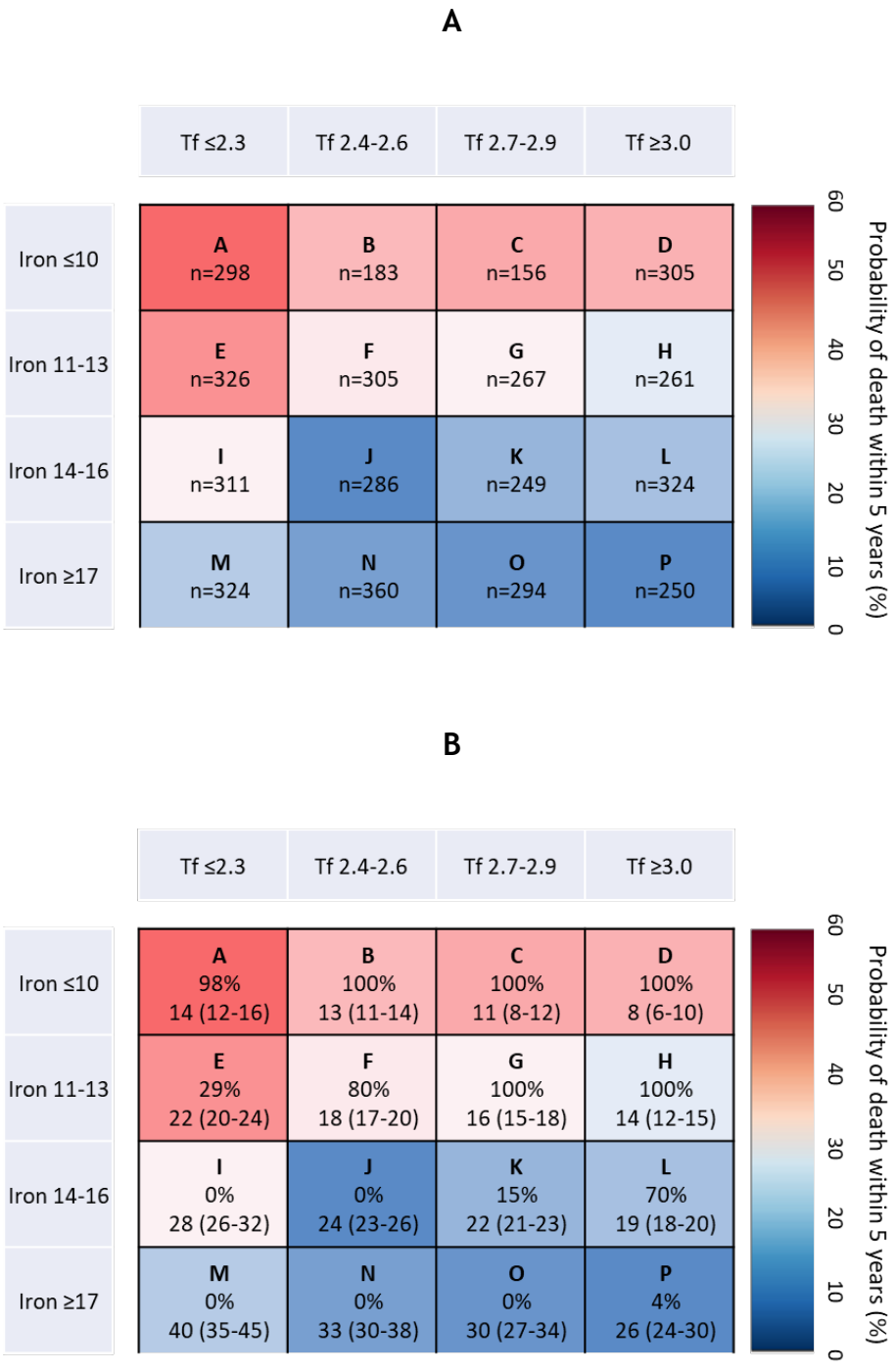


Figure 16: Heat maps detailing all-cause mortality within 5 years by concentrations of serum iron and transferrin. Number of patients (A) and proportion (%) of patients with TSAT <20% and median and (Q1-Q3) TSAT (B) shown.

In analyses restricted to those with low transferrin, patients with a low serum iron had higher all-cause and CV mortality than those with a normal serum iron (**Figure 14b** & **Figure 15b**). Examining outcomes by the lowest two (Q1 & Q2) and highest two quartiles (Q3 & Q4) of transferrin, a low serum iron identified those at higher risk of death better than a low TSAT, irrespective of transferrin (Figure S19).

Overall, in multivariable analysis, transferrin as a continuous variable was inversely related to all-cause mortality (HR 0.82 [0.73-0.91] p<0.001) (**Table 12**). A transferrin in the lowest quartile was also associated with higher all-cause mortality (HR 1.24 [1.11-1.39]; p<0.001). However, in multivariable analyses, neither transferrin as a continuous variable nor the lowest quartile of transferrin as a categorical variable were associated with CV mortality. Analysis by quartiles of transferrin showed interactions by heart failure phenotype for all-cause mortality (p=0.008). In patients with an LVEF >40%, the lowest quartile of transferrin was associated with higher all-cause mortality (HR 1.24 [1.11-1.39]; p<0.001). Repeating the analysis with transferrin as a continuous variable did not confirm significant interactions between heart failure phenotype and all-cause mortality.

Table 12: Multivariable Cox regression analysis of all-cause and cardiovascular mortality

Variable	All-cause mortality		Cardiovascular mortality	
	HR (95% CI)	p-value	HR (95% CI)	p-value
Age (/10 years)	1.44 (1.35-1.53)	<0.001	1.33 (1.23-1.45)	<0.001
Urea (/5mmol/L)	1.16 (1.11-1.21)	<0.001	1.16 (1.10-1.23)	<0.001
NT-proBNP (Log10 ng/L)	1.97 (1.76-2.21)	<0.001	2.73 (2.33-3.20)	<0.001
Haemoglobin (g/dL)	0.93 (0.89-0.96)	<0.001	0.92 (0.87-0.96)	<0.001
Beta blocker (Yes/No)	0.78 (0.70-0.87)	<0.001	0.76 (0.65-0.88)	<0.001
Loop diuretic (Yes/No)	1.44 (1.26-1.63)	<0.001	1.47 (1.23-1.77)	<0.001
Serum iron (sq. root 1µmol/L)	0.87 (0.81-0.95)	<0.001	1.02 (0.91-1.13)	0.75
Transferrin (1g/L)	0.82 (0.73-0.91)	<0.001	0.95 (0.82-1.10)	0.51
The following was added to the above model				
Transferrin ≤2.3g/L*	1.24 (1.11-1.39)	<0.001	1.15 (0.98-1.34)	0.09
The following continuous variables were added individually to the above model excluding serum iron				
TSAT (Log10) (%)	0.61 (0.45-0.83)	0.001	1.08 (0.70-1.65)	0.73
Ferritin (Log 10) (1µg/L)	1.19 (1.01-1.40)	0.03	1.45 (1.16-1.81)	0.001
The following categorical variables were added individually to the above model excluding serum iron				
Serum iron ≤13µmol/L	1.30 (1.15-1.46)	<0.001	1.04 (0.88-1.22)	0.67
TSAT <20%	1.26 (1.12-1.42)	<0.001	0.99 (0.84-1.17)	0.93
Ferritin <30 µg/L	1.03 (0.87-1.22)	0.71	0.90 (0.71-1.15)	0.41
Ferritin <100 µg/L	0.95 (0.84-1.07)	0.37	0.79 (0.67-0.93)	0.004
ESC Definition of ID	1.06 (0.94-1.20)	0.35	0.93 (0.79-1.11)	0.43

*Results for transferrin ≤ 2.3g/L were obtained from separate models with all above variables minus transferrin. Other variables (eg: TSAT) were added to the full model excluding only variables named within the table subheadings.

Abbreviations – NTproBNP: N terminal pro-natriuretic peptide; TSAT: transferrin saturation; ESC: European Society of Cardiology; ID: iron deficiency.

5.4 Discussion

Low transferrin is, in health, associated with being iron replete (63,137). By contrast, we have found that in a large, unselected population of patients with CHF, low serum transferrin was associated with low serum iron, low haemoglobin and high serum ferritin. In addition, we found that lower transferrin was associated with higher mortality particularly if accompanied by a low serum iron and/or low TSAT.

A number of consequences flow from these findings. Firstly, I have identified a group of patients with low serum iron (≤13μmol/L), low haemoglobin and a high mortality who are not currently defined as iron deficient by the ESC guideline definition (23,24). This is because, in the absence of a low serum ferritin (<100μg/L), a TSAT <20% is required to diagnose ID. When transferrin is low, TSAT may be normal (≥20%) despite a low serum iron. Serum iron ≤13μmol/L is more accurate than the current ESC definition of ID in identifying iron depletion as diagnosed from bone-marrow samples in patients with heart failure (20). It is also a better predictor of mortality than either the ESC definition of ID or a TSAT <20% (134). I have also shown that spontaneous resolution of low serum iron concentrations is associated with better survival: in contrast, recovery from ID defined by the ESC criteria or by or a TSAT <20% is not associated with improved survival (134) – see Chapter 4.

Secondly, mortality was high in those with both low serum transferrin and low iron, although more than one third of these patients had a normal TSAT. Using current definitions of ID, these patients would not have been included in trials of iron supplementation. Amongst patients in the highest quartile of transferrin concentrations, 71% had a TSAT <20% but 25% of these had a normal serum iron. Across all four quartiles of serum transferrin, prognosis was strongly associated with serum iron concentration and, because of fluctuations in serum transferrin, TSAT was inferior to serum iron at identifying those most at risk. These findings suggest that a low serum iron, rather than TSAT, is more strongly related to outcome, which might reflect a stronger association with ID.

Thirdly, there was a strong inverse correlation between transferrin and ferritin. Whilst this could reflect a reciprocal decline in transferrin and increase in

ferritin due to iron repletion, ferritin increases as a result of inflammation and cell damage common in chronic diseases, such as heart failure (22,25,64). Ferritin may be high even in the context of severe iron deficiency (20). I also found a positive correlation between transferrin and albumin and an inverse correlation with hsCRP, consistent with the hypothesis that ferritin is predominantly an inflammatory biomarker in heart failure and that inflammation might suppress the production of transferrin.

Iron deficiency is associated with worse prognosis in heart failure (19,37). Given that ID normally causes a rise in serum transferrin, higher transferrin might be expected to be associated with worse outcome, yet the opposite was true. Regardless of heart failure phenotype, higher transferrin was associated with lower mortality independent of serum iron. The association may be primarily driven by the fact that patients with lower transferrin tended to be older, and to have higher hsCRP and NTproBNP.

Patients with low transferrin were more likely to die of both cardiovascular and non-cardiovascular causes, including cancer. This was especially the case for patients with HFmrEF/HFpEF, for whom cancer accounts for a larger proportion of deaths than for HFrEF (138). This may help explain why a transferrin ≤2.3g/L was most clearly associated with a higher all-cause mortality in patients with HFmrEF/HFpEF.

Serum transferrin falls in malignant disease (137). In fact, lower transferrin can help differentiate malignant tumours from benign conditions (139,140). Cellular transferrin receptors are upregulated and overexpressed on cancer cells (141). It may be that low transferrin values reflect a high degree of internalisation of the transferrin-transferrin receptor complex.

The current ESC heart failure guideline definition of ID is neither an accurate marker of bone marrow ID nor prognosis (20,134). If the definition of ID includes many patients who do not have ID, then it will dilute the apparent benefits of iron replacement therapy in clinical trials and expose patients to unnecessary treatment in clinical practice. Prospective analyses of ongoing clinical trials may provide some answers. On the other hand, many patients with evidence of ID by other criteria, including a low iron and a low transferrin, were excluded from

randomised trials of iron therapy. Consequently, it is unclear if such patients benefit from treatment with intravenous (IV) iron. The use of IV iron in those with low transferrin and low iron should, however, be safe. Current formulations release only small amounts of unbound iron into the circulation before uptake by the reticuloendothelial system (RES) (82). The rate of subsequent release of iron from the RES depends on available iron binding sites, that is, the serum concentration of transferrin, and neither on the dose of IV iron nor the rate of infusion (142). Low transferrin concentrations in those who are iron deficient and treated with IV iron should not lead to the release of potentially toxic non-transferrin-bound iron into the circulation.

5.4.1 Limitations

Participants were from a single clinic and were mainly Caucasian, and therefore results might not be generalizable to more ethnically diverse populations. Patients were recruited over a period of nearly 20 years during which time, treatments for heart failure have improved. Novel biomarkers of iron status, such as soluble transferrin receptor, were not available to us. A proportion of those with confirmed heart failure (32%) did not have full iron indices or haemoglobin available and were therefore not included in the analysis. We did not do bone marrow biopsies to confirm the presence of ID. It is possible that serum markers are associated with mortality by pathways other than ID. Co-variates used in prognostic analysis were derived from a validated model of patients with worsening heart failure, rather than ambulatory patients as in our cohort.

5.5 Conclusion

In patients with CHF, low serum concentrations of transferrin are associated with a worse prognosis, especially if accompanied by low serum iron. Independent of serum iron, variations in transferrin lead to changes in TSAT, which may confound its utility as a marker of ID. Clinicians should consider both components of the TSAT ratio when deciding whether a patient has ID.

Chapter 6 Intravenous iron for heart failure with evidence of iron deficiency: a meta-analysis of randomised trials

6.1 Introduction

Patients with heart failure often have evidence of iron deficiency (ID), with or without anaemia, which is associated with more severe symptoms, lower exercise capacity and higher rates of hospitalisations for heart failure (HHF) and mortality (17,41). In an individual patient meta-analysis of four trials including 839 patients with heart failure with reduced ejection fraction (HFrEF) and serum markers of ID, Anker and colleagues suggested that administration of intravenous iron (IV) reduced the risk of first and recurrent HHF when compared to placebo (105). Recently, the AFFIRM-AHF trial narrowly missed its primary efficacy endpoint of recurrent HHF or cardiovascular (CV) death (87). Therefore, we produced an updated meta-analysis to investigate whether the effects of IV iron were consistent amongst the randomised trials reported so far and whether sufficient evidence had accumulated to indicate a conclusive effect on HHF and CV mortality.

6.2 Methods

We searched for English language trials from 1st January 2000 to 5th December 2020 in PubMed using pre-specified search terms (see Supplements), and from additional sources including a recent systematic review (14). Only published randomised trials investigating the effects of IV iron compared to a control group that did not receive IV iron in patients with heart failure, regardless of participants' left ventricular ejection fraction, the formulation of IV iron, concomitant therapy, or definition of ID, that reported either HHF or CV mortality were included in the main report. If mortality was not explicitly reported but HHF was, it was assumed that no deaths had occurred. An additional analysis was done including two unpublished trials, with data derived from the meta-analysis reported by Anker et al (105).

Data was extracted by two independent reviewers (FG and PP). Deaths not clearly declared as CV or non-CV were adjudicated independently by two

authors, both of whom are experienced in clinical end-point adjudication. Adjudication was based on clinical information provided by authors in the text. Disparities were resolved by discussion or by checking with a third author (JGFC). Outcomes assessed were the composite of HHF or CV mortality as first events, and HHF as a first event and CV mortality separately. Data analysed were the numbers of first events and numbers of participants in each treatment arm for each trial. Odds ratios and 95% confidence intervals for the effect of treatment with IV iron relative to control were calculated for each trial. The data were meta-analysed using both fixed effects (primary analysis) and random effects models. Forest plots with odds ratios and corresponding (95% confidence intervals) were produced and reported. A level of significance of 5% was considered statistically significant.

To assess the impact of results from the largest trial to date, additional analysis comparing odds ratios for studies excluding AFFIRM-AHF to the AFFIRM-AHF trial alone were carried out.

All analyses were conducted with Review Manager (RevMan) Version 5.4 (The Cochrane Collaboration, 2020).

6.3 Results

We identified 19 reports assessing the effect of iron therapy in patients with heart failure (**Figure 17**). After excluding 12 reports (73,99,106,143–151), mainly because they were not randomised-controlled trials or did not report relevant outcomes (Table S23), seven trials (**Table 13**) that enrolled 2,166 patients (n=1,168 assigned to IV iron; n=998 assigned to the control/placebo) were included in the primary analysis (84–87,116,129,152). The most common definition of ID was a ferritin <100µg/L and/or, if ferritin was 100-300µg/L, a TSAT of <20%. Most trials excluded patients with a very low haemoglobin (less than 8-10g/dL) or with values greater than 15g/dL (85,87). Only two trials followed patients for >6 months (85,87). Five trials used ferric carboxymaltose and two used iron sucrose (129,152).

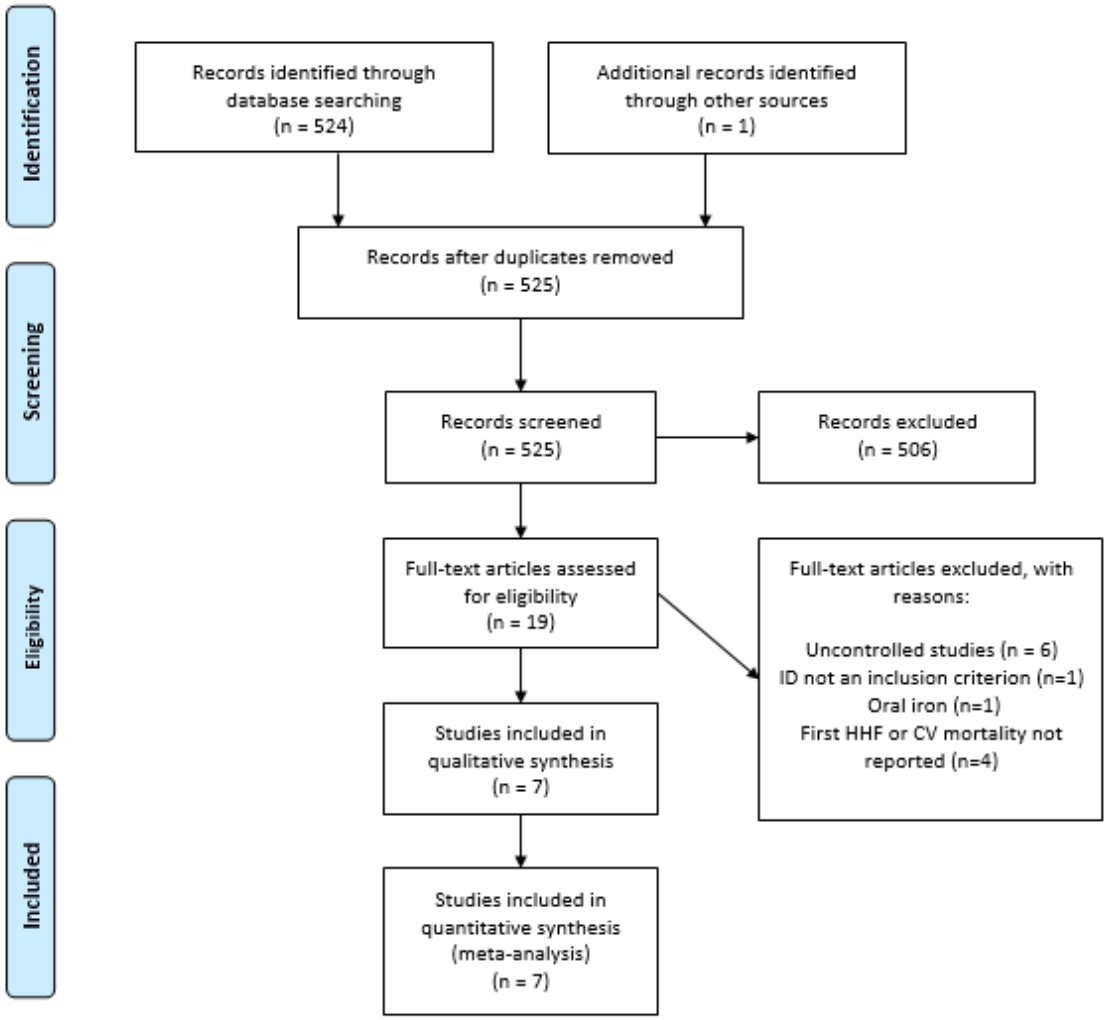


Figure 17: PRISMA diagram. Detailing the number of records identified, screened, included, and excluded, with a summary of the reasons for exclusion.
Modified from: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (29). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(7): e197. doi:1.1371/journal.pmed197.

Table 13: Characteristics of included trials

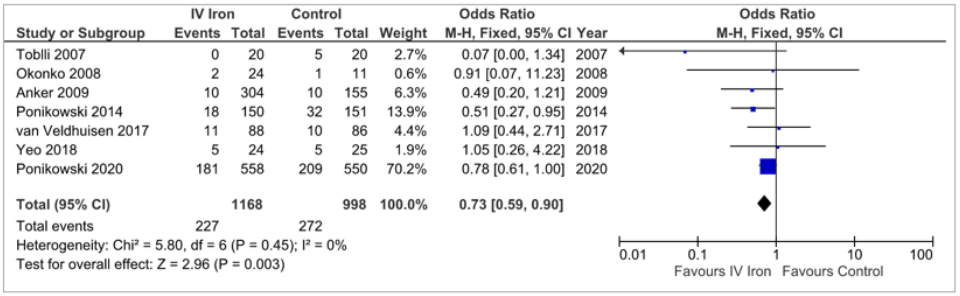
	Toblli et al.	FERRIC-HF	FAIR-HF	CONFIRM-HF	EFFECT-HF	PRACTICE-ASIA-HF	AFFIRM-AHF
Year of publication	2007	2008	2009	2014	2017	2018	2020
Country	Argentina	UK and Poland	Europe & Argentina	Europe	Europe and Australia	Singapore	15 countries (International)
Number of patients (IV iron: control)	40 (1:1)	35 (2:1)	459 (2:1)	301 (1:1)	174 (1:1)	49 (1:1)	1108 (1:1)
Double-Blind	Yes	No	Yes	Yes	No	No	Yes
Definition of ID	F <100 and/or T ≤20%	F <100 or T <20% + F100-300	F <100 or T <20% + F100-299	F <100 or T <20% + F100-300	F <100 or T <20% + F100-300	T <20% and F <300	F <100 or T <20% + F 100-299
Main Inclusion criteria (Hb: g/dL)	• LVEF ≤35% • NYHA II-IV • Anaemia	• LVEF ≤45% • NYHA II-III • Hb ≤14.5	• LVEF ≤45% • NYHA II-III • Hb 9.5-13.5	• LVEF ≤45% • NYHA II/ III • Hb <15	• LVEF ≤45% • NYHA II or III • Hb <15	• HF Hosp. • Hb <14	• LVEF <50% • HF Hosp. • NT-proBNP↑ • Hb 8-15
Age (years)	75	63	68	70	64	63	71
Women (%)	---	29	54	47	25	22	45

Ischaemic aetiology (%)		63	74	80	83	---	---	47
LVEF (%)		31 ± 4	30 ± 7	32 ± 6	37 ± 8	33 ± 9	39 ± 18	33 (10)
NT-proBNP (pg/ml)		256 ± 125	---	---	2511 ± 5006	1576*	---	4743 (2781-8128)*
eGFR (ml/min/1.73m^2)		---	---	64	66	52	---	---
Haemoglobin (g/dL)		10.3 ± 0.6	12.6 ± 1.2	11.9 ± 1.3	12.3 ± 1.4	12.9 ± 1.3	11.6 ± 1.9	12.3 ± 1.6
Ferritin (µg/L)		73 ± 30	62 ± 37	53 ± 55	57 ± 48	48*	91 ± 80	84 ± 62
TSAT (%)		20 ± 1	20 ± 8	18 ± 13	20 ± 18	17*	16 ± 10	15 ± 8
Form of iron therapy (mean dose)		iron sucrose; 1,000mg	iron sucrose; 1,433mg	FCM; n/a	FCM; 1,500mg	FCM; 1,204 mg	FCM; 1,000mg	FCM; 1,352mg
Follow-up		24 weeks	18 weeks	24 weeks	52 weeks	24 weeks	12 weeks	52 weeks
Outcomes reported	HHF	+	+	+	+	+	+	+
	CVM	-	+	+	+[8]	+	-	+

Data shown are for the active group only, but this is also representative of the control group. Data presented as mean +/- SD or count and (%) unless otherwise stated. If data not available/reported, cell filled (---). *Median and (Q1-Q3) reported. [8]Not specifically reported but derived from reported outcomes in the paper and from the individual-patient-data meta-analysis by Anker et al.

In the primary analysis, IV iron reduced the composite outcome of HHF or CV death: OR 0.73 [0.59-0.90]; p=0.003 (**Figure 18a**). HHF occurred in 175 (15%) patients administered IV iron and 227 (23%) assigned to control: OR 0.67 [0.54-0.85]; p=0.0007) (**Figure 18b**). CV deaths occurred in 93 (8%) patients administered IV iron and in 98 (10%) assigned to control: OR 0.89 [0.66-1.21]; p=0.47) (**Figure 18c**). Adding data from the two unpublished trials to the main analysis did not substantially alter these results (Figure S20).

A



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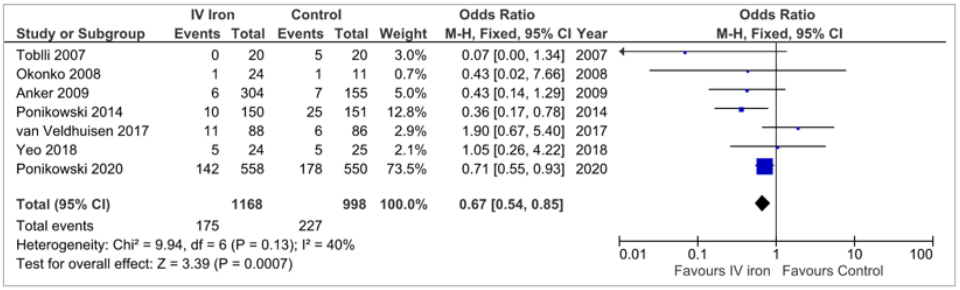

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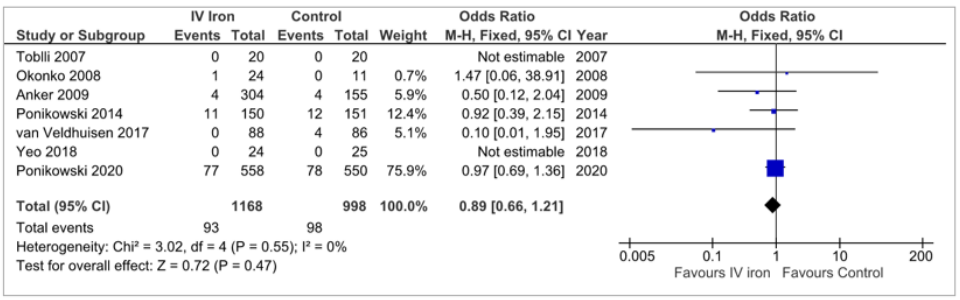


Figure 18: Fixed-effects meta-analysis model of all included trials. Model details the pooled effect of intravenous iron on the composite of first hospitalisations for heart failure or cardiovascular mortality (A), and first hospitalisation for heart failure (B) and cardiovascular mortality (C) alone. Abbreviations: IV: intravenous; CI: confidence interval.

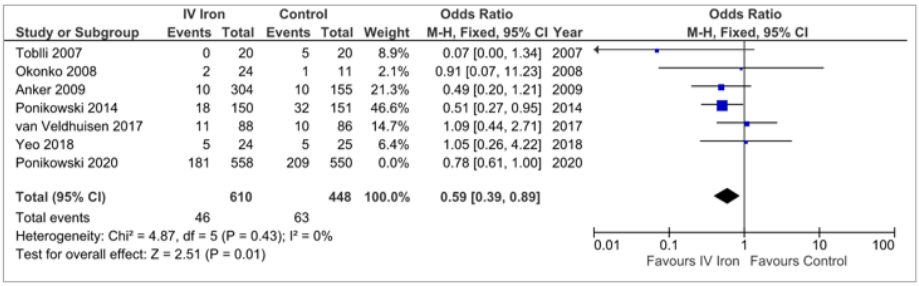
When AFFIRM-AHF was excluded from the model, the point estimates for the effect of IV iron were OR 0.59 [0.39-0.89]; p=0.01 for the composite outcome, OR 0.57 [0.36-0.90]; p=0.02 for HHF and OR 0.66 [0.34-1.28]; p=0.22 for CV mortality (**Table 14** & **Figure 19**). The odds ratios for all outcomes were not significantly different for the pooled data excluding AFFIRM-AHF compared to AFFIRM-AHF alone (**Table 15**).

Table 14: Summary of results from meta-analysis models. Includes models with and without AFFIRM-AHF, and AFFIRM-AHF alone, assessing the effect of IV iron on outcomes

AFFIRM-AHF Excluded								
Outcome	IV iron		Controls		Fixed Effect		Random effect	
	Events	Patients	Events	Patients	OR (95% CI)	p	OR (95% CI)	p
CVM or HHF	46	610	63	448	0.59 (0.39, 0.89)	0.01	0.62 (0.41, 0.93)	0.02
HHF	33	610	49	448	0.57 (0.36, 0.90)	0.02	0.60 (0.28, 1.28)	0.19
CVM	16	610	20	448	0.66 (0.34, 1.28)	0.22	0.72 (0.36, 1.43)	0.35
AFFIRM-AHF								
CVM or HHF	181	558	209	550	0.78 (0.61, 1.00)	---	0.78 (0.61, 1.00)	---
HHF	142	558	178	550	0.71 (0.55, 0.93)	---	0.71 (0.55, 0.93)	---
CVM	77	558	78	550	0.97 (0.69, 1.36)	---	0.97 (0.69, 1.36)	---
All Trials								
CVM or HHF	227	1168	272	998	0.73 (0.59, 0.90)	0.003	0.74 (0.60-0.91)	0.005
HHF	175	1168	227	998	0.67 (0.54, 0.85)	0.0007	0.64 (0.40, 1.04)	0.07
CVM	93	1168	98	998	0.89 (0.66, 1.21)	0.47	0.91 (0.67, 1.24)	0.56

Abbreviations: - IV: intravenous; CVM: cardiovascular mortality; HHF: hospitalisation for heart failure.

A



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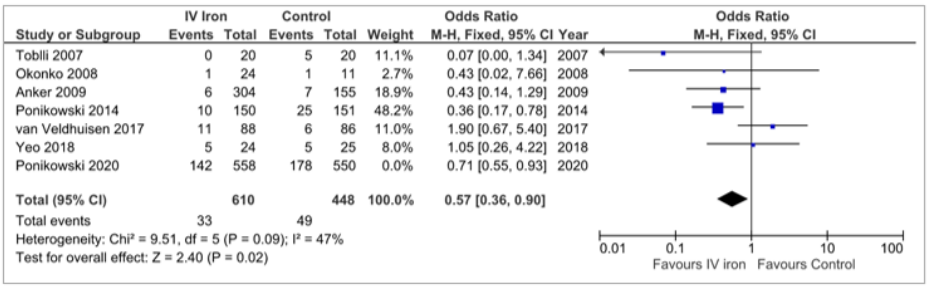

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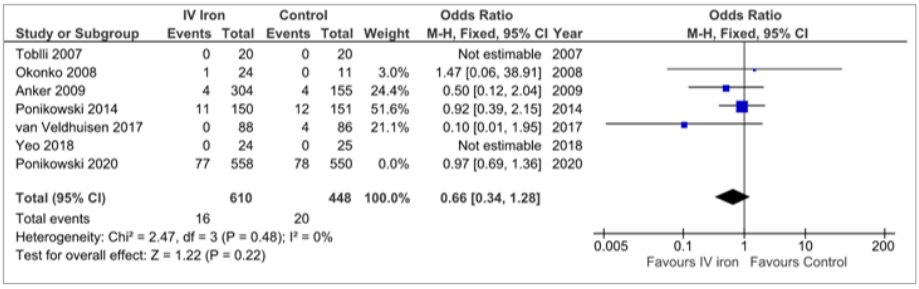


Figure 19: Fixed effects meta-analysis model of all trials excluding AFFIRM-AHF, detailing the pooled effect of intravenous iron on the composite of first hospitalisations for heart failure or cardiovascular mortality (A), and first hospitalisation for heart failure (B) and cardiovascular mortality (C) alone. Although not included in the pooled analysis, Odds Ratios and (95% Confidence Intervals) are presented for AFFIRM-AHF for comparison.

In random effects models, IV iron reduced the composite outcome (OR 0.74 [0.60-0.91]; p=0.005) but neither HHF (OR 0.64 [0.40-1.04]; p=0.07) nor CV mortality (OR 0.91 [0.67-1.24]; p=0.56) (Figure S21). Results were similar when AFFIRM-AHF was excluded (**Table 14** & Figure S22).

Table 15: Comparison of fixed-effects Odds ratios (ORs) between pooled trials prior to AFFIRM-AHF and AFFIRM-AHF

Composite endpoint			
	Odds ratio (95% Confidence Interval)	P for comparison	OR (95% Confidence Interval) of comparison
All trials except AFFIRM-AHF	0.59 (0.39, 0.89)	0.26	0.76 (0.47, 1.22)
AFFIRM-AHF	0.78 (0.61, 1.00)		
Hospitalisation for heart failure			
All trials except AFFIRM-AHF	0.57 (0.36, 0.90)	0.41	0.80 (0.47, 1.36)
AFFIRM-AHF	0.71 (0.55, 0.93)		
Cardiovascular mortality			
All trials except AFFIRM-AHF	0.66 (0.34, 1.28)	0.31	0.68 (0.32, 1.43)
AFFIRM-AHF	0.97 (0.69, 1.36)		

6.4 Discussion

This meta-analysis suggests that IV iron reduces the risk of the composite outcome of first HHF or CV death for patients with serum markers of ID and heart failure. This result was driven predominantly by an effect on HHF with no convincing evidence of a reduction in CV mortality. Because HHF is associated with a higher risk of CV mortality, the effect of IV iron for each outcome might be expected to be rather similar. The relatively small number of deaths, the short duration of follow-up and the play of chance might explain this possible anomaly. A longer duration of follow-up might show a greater effect on CV mortality, but the trial with the longest follow-up to date, albeit only one year, showed little effect on this outcome (87). The AFFIRM-AHF trial suggests that the reduction in hospitalisations for heart failure is not observed until 8-12 weeks after administration of IV iron, consistent with its benefits being mediated through the synthesis of new red blood cells, myoglobin and other metalloproteins. Accordingly, large effects observed in some small trials lasting three months or less may reflect chance effects.

The effects of IV iron appeared somewhat greater in a pooled analysis of trials excluding AFFIRM-AHF, although differences were not statistically significant. IV iron might be more effective in clinically stable populations. Differences in study design, inclusion criteria, iron dosing and length of follow-up may affect outcome. The small size of some trials confounded statistical assessment of heterogeneity. Instead, we produced both fixed and random effects models which, for the composite outcome, yielded similar results, although less secure for effects on HHF in the random effects model.

Whether the definition of ID used in these trials is optimal is uncertain. Using a TSAT <20% alone might be a better guide to ID than one based on ferritin (19,20,37). This is important, because giving IV iron to patients who are not iron deplete is unlikely to be beneficial. Fortunately, ID appears common in patients with heart failure and therefore an effect might be detected even if the diagnostic accuracy of the test for ID is poor. Perhaps most patients with heart failure have ID and the key question is how severe it is, rather than whether it is present; ID should not be a binary, all-or-nothing classification.

We did not conduct subgroup analyses, which are best left to an individual-patient-data (IPD) meta-analysis that can adjust for confounding variables. In an IPD meta-analysis (105), lower TSAT but not lower serum ferritin predicted greater benefit from IV iron. In AFFIRM-AHF it appeared that lower serum ferritin or a lower TSAT were associated with greater benefit from IV iron, but >80% of participants had a TSAT <20%. Further analyses are required. Haemoglobin concentration has not predicted benefit but, because women have lower concentrations than men, such analyses may be confounded by participants' sex. The AFFIRM-AHF trial enrolled patients with new-onset heart failure, which is unusual for trials of heart failure; these patients may have had somewhat less benefit from IV iron, possibly because they were less likely to have true ID or because the determinants of outcome in such patients is different. In AFFIRM-AHF patients with ischaemic cardiomyopathy appeared to have greater benefit; the reasons for this are unclear. The reduction in events with IV iron, compared to control, might have been underestimated because treatments for heart failure might have been more likely to be intensified in the control group who did not receive the symptomatic benefits of iron therapy. This possibility should be explored in future analysis of substantial long-term trials.

Results from three other large ongoing trials should clarify the effects of IV iron on morbidity and mortality in patients with HFrEF and ID and provide further insights into the possible predictors of response (131). Trials in heart failure with preserved ejection fraction are also underway but limited data currently exist (116).

6.4.1 Limitations

We did not investigate the effect of IV iron on all-cause mortality as this is not yet reported for AFFIRM-AHF. The composite outcome reported for CONFIRM-HF (85) was HHF and all-cause mortality, which included one non-CV death amongst patients assigned to iron and two to placebo. This would not materially alter our overall results. An analysis of recurrent HHF rather than just the first event would make the result more robust but requires access to IPD. An IPD meta-analysis has many advantages when exploring the interaction amongst variables (153–155). In particular, an IPD would have allowed analysis of the potential interaction between sex and the effects of IV iron. However, aggregate data has

the advantage that it includes all the published data rather than the proportion where IPD is available to the authors. Each type of meta-analysis has advantages, and they are complimentary. All meta-analyses should be interpreted cautiously, particularly in analyses involving a number of small studies where there will be little power to detect heterogeneity. Fixed effects meta-analysis provides an estimate of an average treatment effect in the studies conducted but uncertainty about heterogeneity may make it difficult to extrapolate that effect to a particular clinical context. Random effects analyses assume that studies have underlying treatment effects arising from a random distribution and provide estimates of the average of, and variation in, the treatment effect in that distribution. However, if the variation is systematic and not random then the random effects analysis may not be helpful in extrapolating a treatment effect to a new situation. In the context of this analysis, length of follow-up, clinical status of patients at recruitment and IV iron dosing strategy are systematically different amongst the studies. Whether these factors systematically impact the treatment effect is difficult to determine with the data available.

6.5 Conclusion

In a meta-analysis of seven trials, administration of IV iron to patients with heart failure and ID reduced the risk of the composite outcome of heart failure hospitalisation or cardiovascular mortality in the following 12 months. To date, this outcome is driven predominantly by an effect on HHF. Longer-term effects of repeated administration of IV iron are unknown. More evidence is desirable.

Reviewers:

FG: Fraser Graham; PP: Pierpaolo Pellicori; JGFC: John GF Cleland

Chapter 7 Testing patterns of haemoglobin and serum markers of iron deficiency in people with and without heart failure

7.1 Introduction

In the general population, low, but also very high levels of haemoglobin are associated with an increase in cardiovascular morbidity and death (156,157). Very high concentrations of haemoglobin are infrequent, and usually reflect smoking habits, severe chronic lung disease or more rarely, myeloproliferative disorders. Low blood concentrations of haemoglobin are much more common. Although they might be due to physiological blood losses in pre-menopausal women, in older individuals they are usually a marker of co-morbid conditions or their treatments, that might reduce erythropoiesis, or predispose to malabsorption and/or blood loss (158,159). The most common cause of anaemia worldwide is iron deficiency (4), for which treatment is readily available, but it is unclear how often cases of anaemia are investigated for iron deficiency and which tests are most commonly used.

Much of the research on anaemia and iron deficiency has focussed on cohorts of patients who have consented to participate in a registry or trial, where case selection bias is inevitable, and used only a binary definition of anaemia based on World Health Organisation (W.H.O.) criteria that may not be robust. Although widely used for epidemiological studies, the definition of anaemia suggested by the W.H.O. is based on research conducted more than 50 years ago, using out-dated laboratory practices, on young, otherwise healthy individuals with dietary iron deficiency; therefore, it should be extrapolated with caution to contemporary cohorts of patients with cardiovascular disease (46). Currently, the availability of a large volume of highly granular, routinely collected, electronic health records (EHR) provide an opportunity to study, longitudinally, a broad population of people to address important research questions and audit quality of care.

Accordingly, we used de-identified data to investigate the distribution of haemoglobin concentrations and their associations with outcome in a large

cohort of adults with a broad range of cardiovascular diseases, including hypertension, atherosclerotic disease and heart failure. We also assessed how often diagnostic investigations for anaemia and iron deficiency were done, and the link between haemoglobin concentrations and subsequent incidence of heart failure, cancer and death.

7.2 Methods

7.2.1 Study population

The Glasgow SafeHaven, managed jointly by NHS Greater Glasgow and Clyde (GG&C) and the Robertson Centre for Biostatistics, University of Glasgow, links and provides secure, anonymised, routinely collected administrative EHR for people managed within the NHS GG&C health board; a population of approximately 1.1 million. Linked data include demographics, blood tests (conducted in primary and secondary care), electrocardiography in secondary care, community prescription records, hospital admissions and related diagnoses, and deaths. The project was approved by the SafeHaven Local Privacy Advisory Committee; reference GSH/18/CA/002.

For this analysis, we requested and obtained authorisation for access to anonymised patient information for adults aged ≥50 years who, between 1st January 2010 and 1st April 2018, had a new or existing diagnosis of coronary or peripheral arterial disease or heart failure or with repeated prescriptions of treatments such as angiotensin-converting-enzyme inhibitors (ACEi), angiotensin II receptor blockers (ARBs), beta-blockers, mineralocorticoid receptor antagonists (MRA), or loop diuretics. These criteria were designed to capture a broad range of cardiovascular problems, including many patients with hypertension as the sole manifestation of CV disease. Data from 2010-2012 was used to provide a medical history and identify prevalent anaemia. Patients with less than 12 months of data were excluded as were patients with end-stage renal disease (ie: estimated glomerular filtration rate of <20 mL/min/1.73 m2, chronic kidney disease stage 5 or on renal dialysis) (n = 16,372)) who are well known to have a high prevalence of anaemia and a poor prognosis.

From 1st January 2013 to 31st December 2014 (testing period) we collated blood test results to identify how often haemoglobin, ferritin and transferrin saturations (TSAT) were measured. Most measurements were taken by primary care physicians or at out-patient clinics. Measurements of haemoglobin obtained during admissions for gastro-intestinal haemorrhage or those due to trauma were excluded. Otherwise, only the first blood test during a hospital admission was used for this analysis to avoid confounding due to blood loss from surgery or other procedures. The nadir value for each test during this period was used to classify patients.

Patients were also classified according to a known diagnosis of heart failure prior to 1st January 2013 (prevalent heart failure), incident heart failure between 1st January 2013 and 31st December 2014 and no recorded diagnosis of heart failure prior to 31st December 2014.

Patients within each diagnostic group were then stratified according to haemoglobin concentration into seven groups relative to the W.H.O. definition: severe anaemia (more than 2g/dL below); moderate anaemia (1-2g/dL below); mild anaemia (0-1g/dL below); borderline (0-1g/dL above), 1-3 g/dL above, 3-4g/dL above and greater than 4g/dL above W.H.O. Four definitions of iron deficiency were considered: ferritin <30µg/L; ferritin <100µg/L, serum iron ≤13µmol/L and a TSAT <20%.

From 2015-2018, patients were followed to identify incident cases of heart failure and cancer and mortality, including causes of death.

7.2.2 Statistics

Descriptive data are shown as numbers and percentage when categorical and as median with 1st and 3rd quartiles if continuous. Mortality from 1st January 2015 until 31st March 2018 (last day of follow-up) was calculated for patients according to heart failure diagnosis and nadir of haemoglobin or iron deficiency categories described above. All Multivariable Cox models were adjusted for age and sex. No imputation was performed for missing data. Associations between haemoglobin and mortality are presented using Kaplan-Meier cumulative events curves and/or forest plots and between haemoglobin and incident heart failure

diagnoses using cumulative events curves. Patient groups with high haemoglobin concentrations (>3g/dL above W.H.O.) were combined in some mortality analyses due to small patient numbers. All statistical analysis was conducted with ‘R’ version 3.6.3.

7.3 Results

From an initial population of 364,785 individuals, after excluding mis-linked data (n=1,176), those aged younger than 50 years (n=123,143) or censored before 1st January 2013 (n=21,844), those with missing data (n=5,098) and those with end-stage renal disease (n=16,372), a total of 197,152 patients were included in this analysis (**Figure 20**).

Prior to 2013, 10,678 (5%) patients were reported to have heart failure and a further 3,657 (2%) developed heart failure in 2013/14. Patients with heart failure were older, more likely to be men and more likely to have ischaemic heart disease, diabetes, hypertension, atrial fibrillation, chronic obstructive airways disease and have a lower eGFR than those without (**Table 16**).

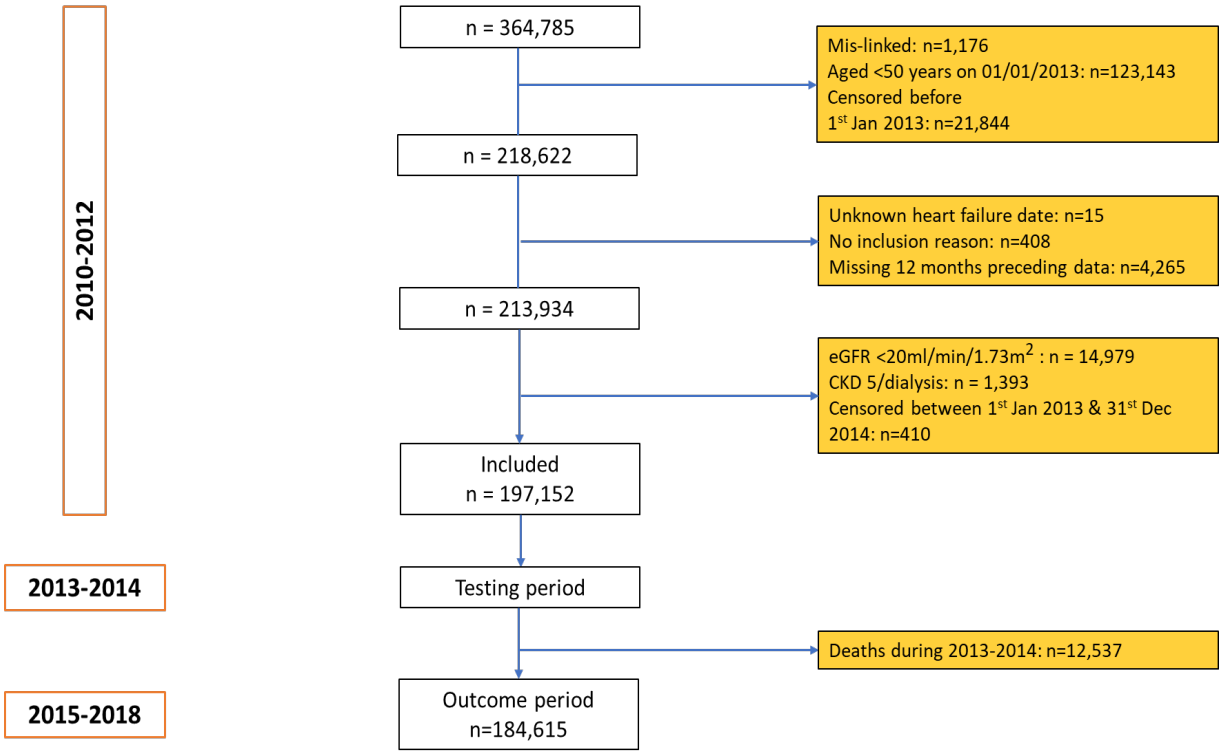


Figure 20: STROBE diagram of included patients.
Exclusions with number and (%) of each are presented in boxes on the right-hand panel. Panels on the left indicate the year(s) of each part of the study.

Table 16: Characteristics of survivors and those who died according to whether heart failure was prevalent (Prevalent), newly developed (Incident), or did not develop (Not) between 1st January 2013 and 31st December 2014.

	Not Heart Failure		Incident Heart Failure		Prevalent Heart Failure	
Demographics and co-morbidities on or before 01/01/2013 unless stated otherwise						
	Surviving at 31/12/2014	**Died before 31/12/2014**	**Surviving at 31/12/2014**	**Died before 31/12/2014**	**Surviving at 31/12/2014**	**Died before 31/12/2014**
N= (%)	172,940	9,877	2,776	881	8,899	1,779
Age (years)	65 (58 - 74)	79 (70 - 86)	74 (65 - 81)	82 (75 - 87)	72 (64 - 80)	82 (75 - 88)
Sex (women)	95,280 (55%)	5,351 (54%)	1,291 (47%)	452 (51%)	3,478 (39%)	903 (51%)
Hypertension	57,633 (33%)	3,009 (30%)	1,281 (46%)	281 (32%)	4,848 (54%)	851 (48%)
Diabetes or Hypoglycaemic Therapy	28,244 (16%)	1,767 (18%)	553 (20%)	156 (18%)	2,122 (24%)	421 (24%)
IHD	32,400 (19%)	2,556 (26%)	984 (35%)	291 (33%)	6,568 (74%)	1,151 (65%)
COPD	17,168 (10%)	1,894 (19%)	531 (19%)	169 (19%)	2,035 (23%)	588 (33%)
eGFR (last available prior to 2013)	82 (71 - 94)	75 (59 - 91)	78 (65 - 91)	72 (58 - 86)	75 (61 - 90)	65 (49 - 82)
GI disease	5,360 (3%)	907 (9%)	130 (5%)	60 (7%)	462 (5%)	167 (9%)
Any cancer prior to 2013	10,780 (6%)	2,127 (22%)	216 (8%)	123 (14%)	756 (8%)	327 (18%)
Any incident cancer 2013/14	3,376 (2%)	2,119 (21%)	116 (4%)	114 (13%)	215 (2%)	208 (12%)
ECG (last result available between 2010 and 31/12/2014)						
ECG available	49,022 (28%)	4,534 (46%)	1,992 (72%)	549 (62%)	4,010 (45%)	917 (52%)
AF/Flutter	3,890 (8%)	848 (19%)	588 (30%)	169 (31%)	961 (24%)	314 (34%)
Haemoglobin Results						
Test prior to 2013 (yes/no)	137,812 (80%)	9,047 (92%)	2,339 (84%)	811 (92%)	8,195 (92%)	1,719 (97%)
Anaemia prior to 2013 (% of those tested)	37,804 (27%)	5,450 (60%)	959 (41%)	468 (58%)	3,714 (45%)	1,235 (72%)
Test during 2013/14 (yes/no)	132,200 (76%)	8,411 (85%)	2,704 (97%)	873 (99%)	7,806 (88%)	1,515 (85%)
Anaemia 2013/14 (% of those tested)	35,310 (27%)	5,651 (67%)	1,418 (52%)	604 (69%)	3,265 (42%)	1,051 (69%)
Incident anaemia 2013/14	13,992 (11%)	1,825 (22%)	666 (25%)	210 (24%)	887 (11%)	197 (13%)
Hb (median / quartiles)	13.3 (12.2-14.4)	11.4 (9.8-12.9)	12.3 (10.7-13.6)	11.2 (9.7-12.7)	12.9 (11.5-14.2)	11.4 (9.8-12.8)
Prescriptions (anytime in 2013 or 2014)						
Iron (oral)	13,817 (8%)	1,750 (18%)	584 (21%)	233 (26%)	1,377 (15%)	435 (24%)
B12	7,689 (4%)	680 (7%)	203 (7%)	71 (8%)	593 (7%)	132 (7%)
Folate	12,137 (7%)	1,601 (16%)	365 (13%)	172 (20%)	1,028 (12%)	312 (18%)
Loop diuretics	21,431 (12%)	3,517 (36%)	1,814 (65%)	551 (63%)	4,744 (53%)	1,255 (71%)
ACEi/ARB	94,839 (55%)	4,089 (41%)	2,161 (78%)	468 (53%)	7,186 (81%)	965 (54%)
BB	64,274 (37%)	3,529 (36%)	2,005 (72%)	401 (46%)	6,392 (72%)	906 (51%)
MRA	1,737 (1%)	391 (4%)	511 (18%)	77 (9%)	1,216 (14%)	255 (14%)
Antiplatelets	66,090 (38%)	5,021 (51%)	2,015 (73%)	547 (62%)	6,238 (70%)	1,124 (63%)
OAC	9,639 (6%)	822 (8%)	897 (32%)	164 (19%)	2,396 (27%)	387 (22%)

NSAID	48,991 (28%)	1,232 (12%)	524 (19%)	90 (10%)	1,201 (13%)	95 (5%)
Insulin	4,115 (2%)	309 (3%)	108 (4%)	33 (4%)	426 (5%)	94 (5%)
Other hypoglycaemic agents	23,015 (13%)	1,293 (13%)	470 (17%)	119 (14%)	1,625 (18%)	258 (15%)
PPI/H2 antagonist	87,992 (51%)	5,939 (60%)	1,799 (65%)	547 (62%)	5,447 (61%)	1,151 (65%)
Deaths 2013-2014						
Age at death	NA	80 (71-87)	NA	83 (76-88)	NA	83(76-89)
All	0 (0%)	9,877 (100%)	0 (0%)	881 (100%)	0 (0%)	1,779 (100%)
Cancer	NA	3,288 (33%)	NA	118 (13%)	NA	306 (17%)
GI Cancer	NA	877 (9%)	NA	28 (3%)	NA	77 (4%)
CVD	NA	2,666 (27%)	NA	401 (46%)	NA	755 (42%)
Neurological	NA	1,087 (11%)	NA	37 (4%)	NA	116 (7%)
Chronic Respiratory	NA	1,018 (10%)	NA	123 (14%)	NA	244 (14%)
Infection	NA	799 (8%)	NA	104 (12%)	NA	181 (10%)
Other	NA	1,022 (10%)	NA	99 (11%)	NA	178 (10%)

Continuous variables presented as median and (25^{th}-75^{th} centiles). Categorical variables presented as count and (%).

Abbreviations – IHD: ischaemic heart disease; COPD: chronic obstructive pulmonary disease; eGFR: estimated glomerular filtration rate; GI: gastrointestinal; ECG: electrocardiogram; AF: atrial fibrillation; ACEi/ARB: angiotensin converting enzyme inhibitor/angiotensin receptor blocker; BB: beta-blocker; MRA: mineralocorticoid receptor antagonist; OAC: oral anticoagulant; NSAID: non-steroidal anti-inflammatory drugs; PPI: proton pump inhibitors; CVD: cardiovascular disease

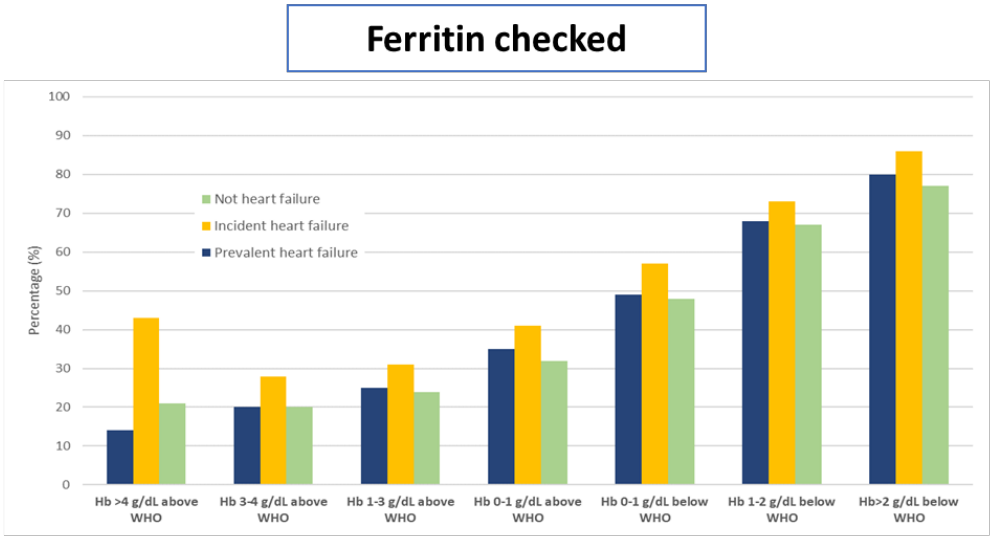
Sodium-glucose Co-transporter 2 inhibitors were used in ≤1% of individuals in all categories

Blood results reported are the nadir result unless otherwise stated. The presence of any of anaemia or AF/flutter are reported.

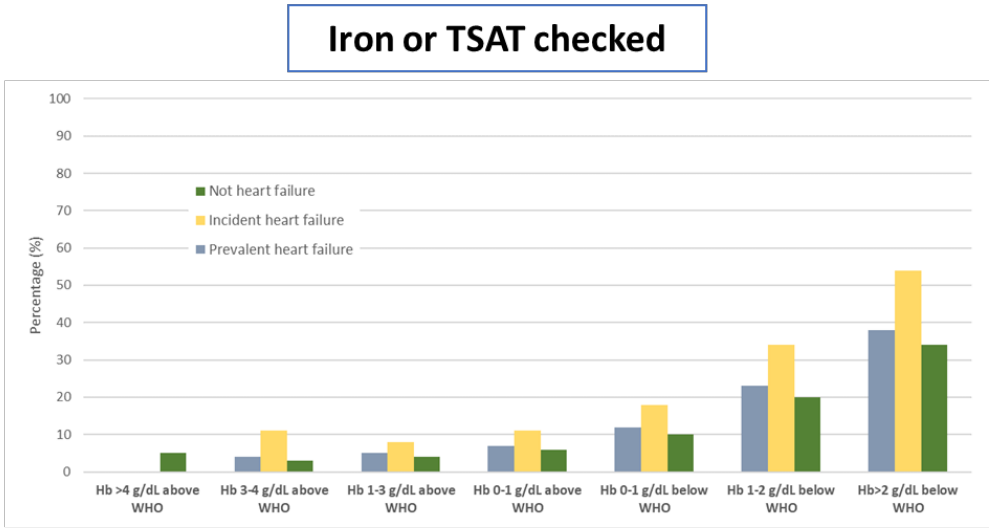
7.3.1 Testing patterns and results of testing

Most patients had haemoglobin measured both before and between 2013 and 2014. Patients with heart failure were more likely to be tested and more likely to have anaemia (**Table 16**). Most of those with anaemia in 2013/14 already had anaemia prior to 2013, and more than one in ten developed anaemia between 2013/14; new onset anaemia was common (25%) in those newly diagnosed with heart failure during this time period. Of those without anaemia prior to 2013, those closest to the W.H.O. threshold (Hb 0-1g/dL above) were most at risk of developing anaemia (Tables S24-S26).

Rates of testing of iron indices increased as severity of anaemia increased, with around 80% of those with Hb >2g/dL below the W.H.O. definition for anaemia having at least one test for iron deficiency. However, serum ferritin was also measured in >20% of those with Hb >1g/dL above the W.H.O. criteria for anaemia (**Figure 21**). Serum iron or TSAT were tested much less frequently than ferritin. Blood tests for iron deficiency were done slightly more often amongst those with incident heart failure compared to other patients (**Figure 21**).



Not HF	310	1,316	13,733	11,422	9,125	7,130	8,742
Inc HF	9	23	217	316	350	396	742
Prev HF	11	57	638	752	815	797	1,176



Not HF	68	204	2,105	2,037	1,912	2,142	3,805
Inc HF	<6	9	58	84	112	187	469
Prev HF	0	11	117	143	203	272	562

Figure 21: Bar charts detailing testing patterns of iron biomarkers according to haemoglobin concentration and heart failure group. Abbreviations: - Hb: haemoglobin; TSAT: Transferrin saturation; WHO: World Health Organization; HF: heart failure; Inc: Incident; Prev: Prevalent.

When investigated, iron deficiency (by all definitions) was more common as haemoglobin decreased (**Table 17**). Of those with anaemia and investigated for iron deficiency (**Table 17**), >50% had a ferritin <100µg/L, a serum iron ≤13µmol/L or a TSAT <20%. A large proportion of those without anaemia, even when haemoglobin was >1g/dL above the W.H.O. definition, also had blood tests suggesting iron deficiency.

Table 17: Haematology profile and iron measurements according to heart failure group.

	Hb by WHO Grade	N=	Ferritin done (Yes vs No)	F <30 µg/L (% of all/ % of those tested)	F <100 µg/L (% of all/ % of those tested)	S. iron done (Yes vs No)	S. iron ≤13 µmol/L (% of all/ % of those tested)	TSAT done (yes v no)	TSAT <20 % (% of all/ % of those tested)	MCV <80 fl
Not HF	ND	42,206 (23%)	184 (0%)	16 (<1%/9%)	82 (<1%/45%)	39 (0%)	19 (<1%/49%)	38 (0%)	11 (<1%/29%)	0 (NA%)
	>4	1,447 (1%)	310 (21%)	13 (1%/4%)	93 (6%/30%)	68 (5%)	14 (1%/21%)	67 (5%)	10 (1%/15%)	6 (0%)
	>3 to 4	6,469 (4%)	1,316 (20%)	59 (1%/4%)	480 (7%/36%)	204 (3%)	47 (1%/23%)	204 (3%)	40 (1%/20%)	29 (0%)
	>1 to 3	56,341 (31%)	13,733 (24%)	1,370 (2%/10%)	7,006 (12%/51%)	2,105 (4%)	691 (1%/33%)	2,085 (4%)	593 (1%/28%)	596 (1%)
	≥0 to 1	35,393 (19%)	11,422 (32%)	2,087 (6%/18%)	6,968 (20%/61%)	2,037 (6%)	1,002 (3%/49%)	2,018 (6%)	838 (2%/42%)	971 (3%)
	<0 to 1	18,938 (10%)	9,125 (48%)	2,486 (13%/27%)	5,902 (31%/65%)	1,912 (10%)	1,249 (7%/65%)	1,900 (10%)	1,060 (6%/56%)	1,226 (6%)
	<-1 to 2	10,706 (6%)	7,130 (67%)	2,492 (23%/35%)	4,753 (44%/67%)	2,142 (20%)	1,655 (15%/77%)	2,128 (20%)	1,436 (13%/67%)	1,303 (12%)

	<-2	11,317 (6%)	8,742 (77%)	3,554 (31%/41%)	5,692 (50%/65%)	3,805 (34%)	3,129 (28%/82%)	3,789 (33%)	2,778 (25%/73%)	3,018 (27%)
Incident HF	ND	80 (2%)	<6 (NA%)	0 (0%/0%)	0 (0%/0%)	0 (0%)	0 (NA%/NA%)	0 (0%)	0 (0%/0%)	0 (NA%)
	>4	21 (1%)	9 (43%)	0 (0%/0%)	<6 (NA%/NA%)	<6 (NA%)	<6 (NA%/NA%)	<6 (NA%)	<6 (NA%/NA%)	0 (0%)
	>3 to 4	83 (2%)	23 (28%)	0 (0%/0%)	<6 (NA%/NA%)	9 (11%)	<6 (NA%/NA%)	9 (11%)	<6 (NA%/NA%)	<6 (NA%)
	>1 to 3	689 (19%)	217 (31%)	10 (1%/5%)	87 (13%/40%)	58 (8%)	34 (5%/59%)	58 (8%)	33 (5%/57%)	11 (2%)
	≥0 to 1	762 (21%)	316 (41%)	46 (6%/15%)	166 (22%/53%)	84 (11%)	59 (7%/70%)	84 (11%)	54 (7%/64%)	35 (5%)
	<0 to 1	614 (17%)	350 (57%)	82 (13%/23%)	191 (31%/55%)	112 (18%)	95 (15%/85%)	110 (18%)	77 (13%/70%)	56 (9%)
	<-1 to 2	545 (15%)	396 (73%)	93 (17%/23%)	242 (44%/61%)	187 (34%)	162 (30%/87%)	186 (34%)	146 (27%/78%)	65 (12%)
	<-2	863 (24%)	742 (86%)	234 (27%/32%)	489 (57%/66%)	469 (54%)	417 (48%/89%)	467 (54%)	383 (44%/82%)	228 (26%)
Prevalent HF	ND	1,357 (13%)	16 (1%)	<6 (NA%/NA%)	<6 (NA%/NA%)	7 (1%)	<6 (NA%/NA%)	7 (1%)	<6 (NA%/NA%)	0 (NA%)

	>4	78 (1%)	11 (14%)	0 (0%/0%)	<6 (NA%/NA%)	0 (0%)	0 (NA%/NA%)	0 (0%)	0 (0%/0%)	0 (0%)
	>3 to 4	284 (3%)	57 (20%)	<6 (NA%/NA%)	23 (8%/40%)	11 (4%)	<6 (NA%/NA%)	11 (4%)	<6 (NA%/NA%)	<6 (NA%)
	>1 to 3	2,503 (23%)	638 (25%)	43 (2%/7%)	287 (11%/45%)	117 (5%)	58 (2%/50%)	116 (5%)	46 (2%/40%)	29 (1%)
	≥0 to 1	2,140 (20%)	752 (35%)	103 (5%/14%)	394 (18%/52%)	143 (7%)	87 (4%/61%)	142 (7%)	71 (3%/50%)	63 (3%)
	<0 to -1	1,675 (16%)	815 (49%)	181 (11%/22%)	470 (28%/58%)	203 (12%)	134 (8%/66%)	203 (12%)	119 (7%/59%)	93 (6%)
	<-1 to -2	1,180 (11%)	797 (68%)	226 (19%/28%)	500 (42%/63%)	272 (23%)	213 (18%/78%)	270 (23%)	181 (15%/67%)	119 (10%)
	<-2	1,461 (14%)	1,176 (80%)	404 (28%/34%)	766 (52%/65%)	562 (38%)	488 (33%/87%)	561 (38%)	435 (30%/78%)	346 (24%)

Variables presented as count and (%).

Abbreviations – Hb: haemoglobin; WHO: World Health Organization; HF: heart failure; F: ferritin; TSAT: transferrin saturation; MCV: mean cell volume

7.3.2 Associations between haemoglobin concentrations, treatments, and incident heart failure or cancer

Patients with lower haemoglobin concentrations were older and more likely to have diabetes, IHD, COPD and gastrointestinal (GI) diseases, and a lower eGFR, than those without. Prescriptions of oral iron, folate and B12 therapies, loop diuretics and particularly proton-pump-inhibitors/H2-receptor antagonists increased amongst patients with lower haemoglobin concentrations. For all patients with heart failure, those with lower haemoglobin concentrations were less likely to receive beta-blockers and angiotensin converting enzyme-inhibitors or angiotensin receptor blockers (Tables S24-S26).

An inverse relation was present between rates of both prevalent and incident cancer diagnoses and haemoglobin concentration, regardless of heart failure. Between 2013 and 2014, rates of incident cancer were highest in those with severe anaemia (7-11%).

In those without prior heart failure, a non-linear relationship was present between haemoglobin and rates of new onset heart failure (after 1st January 2015) (Figure S23): those with Hb concentrations >2g/dL below W.H.O. definition of anaemia had the highest risk of developing heart failure, despite an increase in the competing risk of death.

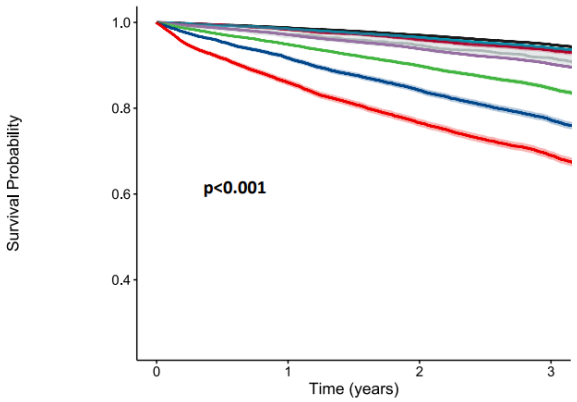
7.3.3 Associations with mortality

Of the 197,152 patients included, 12,537 died between 1st January 2013 and 31st December 2014. Rates of death were highest for those with incident heart failure (24%) followed by those with prevalent heart failure (17%) and lowest for those without heart failure (5%) (**Table 16**).

From 2015 onwards, 11% of those without heart failure and 25% of those with heart failure died (Tables S24-S26). Most patients with heart failure died from cardiovascular causes irrespective of haemoglobin concentration. Cancer was the most common cause of death in those who did not develop heart failure (n=5,313; 29% of deaths) followed by cardiovascular events (n=4,884; 27% of deaths). Approximately 10% of patients died due to an infection with similar rates whether or not patients had heart failure.

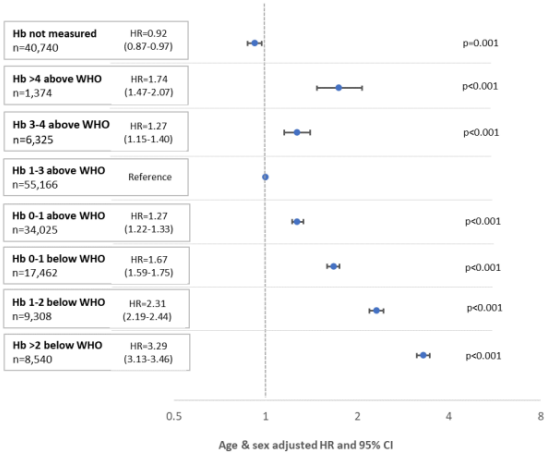
Of 184,615 patients alive at the end of the 2013/14 testing period, those in whom haemoglobin had not been measured had the best and those with severe anaemia (>2g/dL below W.H.O.) the worst outcomes across all diagnostic groups (**Figure 22**). There was a U-shaped relationship between haemoglobin and all-cause mortality, most marked in those without a history of heart failure. Compared to those with a haemoglobin of 1-3g/dL above the W.H.O. definition of anaemia, mortality was greater both for those with borderline anaemia (Hb 0-1 g/dL above W.H.O.) (**Figure 22**) and those with a haemoglobin >3g/dL above the W.H.O. definition of anaemia. Patients with higher haemoglobin had a greater proportion of deaths due to chronic respiratory diseases compared to those with normal or low haemoglobin concentrations (Tables S24-S26).

A

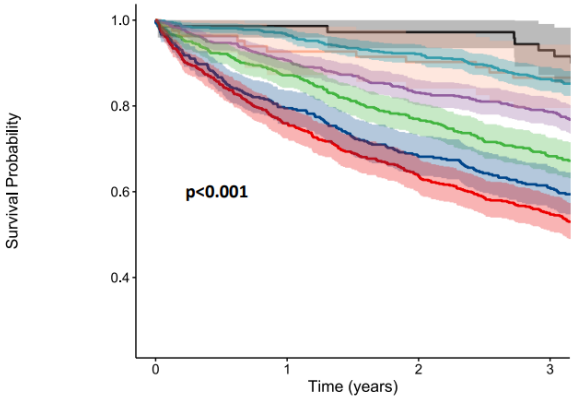


Number at risk

Hb not measured	40740	40079	39094	36599
> 4 above	1374	1332	1293	1221
(3, 4] above	6325	6209	6037	5734
(1, 3] above	55166	54295	53036	50648
[0, 1] above	34025	33018	31860	30248
[-1, 0)	17462	16552	15660	14619
[-2, -1)	9308	8524	7823	7128
< -2	8540	7342	6535	5860

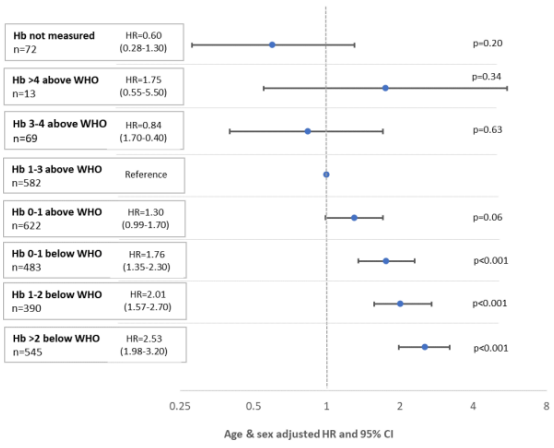


B



Number at risk

Hb not measured	72	71	69	66
> 3 above	82	76	74	69
(1, 3] above	582	562	536	498
[0, 1] above	622	564	516	486
[-1, 0)	483	420	371	328
[-2, -1)	390	309	265	237
< -2	545	414	347	299



c

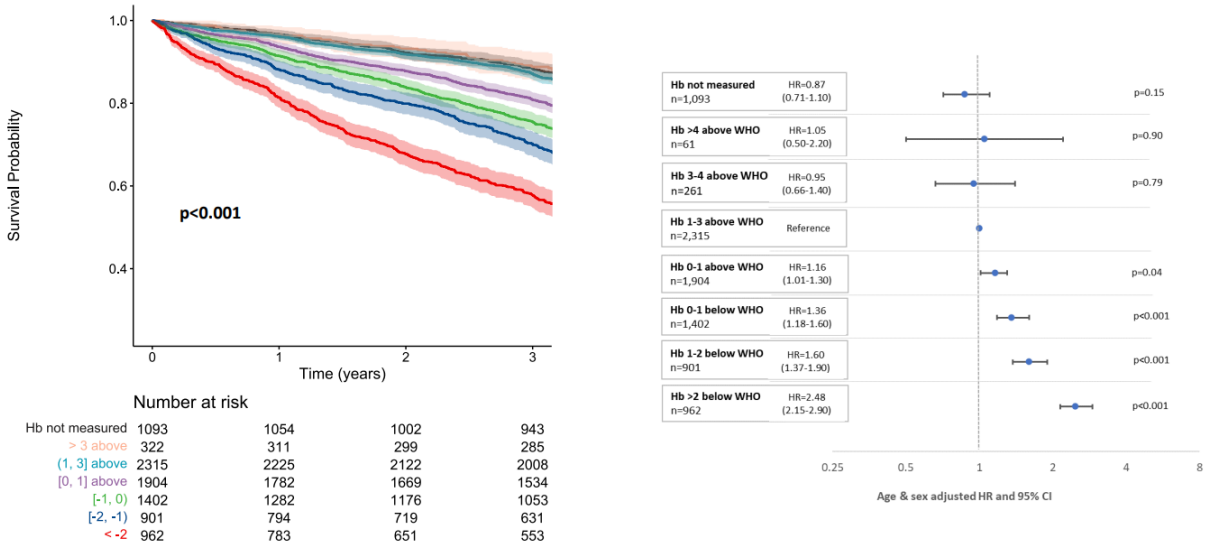


Figure 22: Kaplan-Meier survival curves and corresponding forest plots showing associations between haemoglobin concentrations and mortality from 1st January 2015 to 31st March 2018.
In patients (a) without heart failure recorded at any time, patients (b) with incident heart failure between 1st January 213 and 31st December 2014, and in patients (c) with prevalent heart failure prior to 1st January 213. Numbers at risk presented with each Kaplan-Meier and Hazard Ratios with corresponding 95% Confidence Intervals also presented.

Neither a ferritin <30µg/L nor a ferritin 30-99µg/L were associated with a higher mortality in any patient group (Figure S24-S26). A ferritin >300µg/L was associated with greater mortality in those without a history of heart failure (Figure S24) and in those with prevalent heart failure (Figure S26).

A U-shaped relationship between both serum iron and TSAT was observed for patients without heart failure, with a nadir at a serum iron between 17 and 30µmol/L and of TSAT between 30-39% (Figure S24). For patients with heart failure, serum iron and TSAT were rarely measured precluding meaningful analysis (Figures S25 & S26).

7.4 Discussion

This study has several important findings: 1) many adults with cardiovascular disease living in the West of Scotland have their haemoglobin checked routinely and often have anaemia, 2) blood tests for iron deficiency are rarely done unless anaemia is severe, 3) ferritin is measured more often than TSAT, 4) anaemia is associated with a higher incidence of cancer and heart failure, 5) both high and low haemoglobin concentrations are associated with an increased risk of mortality, with the nadir of risk at levels 1-3 g/dL above WHO criteria for anaemia, 6) a low serum ferritin is associated with a better prognosis but a low TSAT or serum iron with a worse prognosis.

In keeping with previous reports of patients with (128,160–162) and without overt cardiovascular disease, (156,157,163) lower haemoglobin concentrations were associated with greater mortality in our cohort. An important finding was that the risk of mortality increases for concentrations that would be considered as normal by current W.H.O. criteria. This is especially telling given the reference haemoglobin used for each patient was the lowest recorded over the two-year testing period. In other words, all other haemoglobin results for a given patient were either higher or equal to the reference haemoglobin result in our analysis. Similar associations between haemoglobin concentrations and mortality have been demonstrated in other large cohorts (156,163), and the present data add further fuel to ongoing debate (46) on whether current W.H.O. criteria to diagnose anaemia are appropriate for contemporary clinical practice in older patients. My findings suggest that the threshold to define anaemia should be raised, at least for adults with cardiovascular disease, by about 1g/dL.

Approximately one fifth of our patients had a haemoglobin of 0-1g/dL above the W.H.O. reference. This has important implications on health service capacity to deal with an increase in referrals for further investigations to detect and manage or prevent serious treatable diseases. In contrast to the present findings, some researchers have postulated that the haemoglobin concentration used to define anaemia should be even lower than that suggested by the W.H.O. in healthy, younger populations (164,165). These studies defined ‘anaemia’ as below the 5th percentiles for age and sex rather than relating haemoglobin concentrations to clinical outcomes. The causes and relative impact of anaemia will vary between

healthy individuals and those with chronic cardiovascular disease. My data show that patients with cardiovascular disease have a greater mortality even when haemoglobin concentration are only slightly higher than what is currently considered normal.

Anaemia in patients with, or at risk of CV disease is commonly multifactorial in origin and may be a marker of significant co-morbidity and risk, rather than a distinct therapeutic target (158). In this cohort, as haemoglobin decreased, patients were more likely to have diabetes and renal dysfunction, increasing the risk of iron deficiency and defective erythropoiesis (166). Rates of GI disease were also higher in those with anaemia. GI disease can provoke anaemia in several ways, including malabsorption, increased blood loss and inflammation (50). However, it might also be that patients with anaemia are more thoroughly investigated than those without, leading to more diagnoses of GI disease and cancers. Interestingly, I found that a substantial proportion of patients – almost 80% of those with incident heart failure – were prescribed proton-pump inhibitors (PPI). PPI prescriptions are increasing in the general population in the United Kingdom (167), often at higher doses and for longer durations than guidelines suggest (168,169). Adequate absorption of iron in the duodenum and proximal jejunum requires an acidic gastric environment to convert ferric (Fe^{3+}) to ferrous iron (Fe^{2+}) that enables absorption by divalent metal transporter-1 (28). PPIs reduce gastric acid secretion, which in turn will reduce enteral iron absorption. Although PPIs may reduce GI bleeding in those prescribed antiplatelet or non-steroidal anti-inflammatory drugs (NSAIDs), our findings suggest that PPI might increase the risk of iron deficiency and anaemia, supporting the concerns of others about appropriate use (16,168–170).

The incidence of heart failure in our cohort was markedly higher in those with lower haemoglobin concentrations compared to those without anaemia. Low haemoglobin concentration reduces the oxygen carrying capacity of blood. In order to maintain delivery of oxygen to tissues, cardiac output rises, which may increase cardiac work and oxygen demand and may eventually lead to deleterious myocardial remodelling (128,158). These physiological adaptations, coupled with the higher level of co-morbidity associated with lower haemoglobin, may help explain our findings (171).

Optimisation of treatments for heart failure whilst improving survival may, with the exception of SGLT2i (172), reduce haemoglobin (173,174). Haemoglobin is reported as a concentration, which will reflect changes in plasma volume as well as red cell mass. Patients with decompensated heart failure will often have increased plasma volume, correction of which will cause haemoglobin to rise (175). During longer-term follow-up of patients with chronic heart failure, anaemia will often resolve, either spontaneously or as a consequence of treatment (87,97). Effective treatment of heart failure may reduce plasma volume or improve iron absorption. Iron deficiency can be rapidly corrected by intravenous administration of iron, which increases haemoglobin concentrations, improves symptoms and exercise capacity and reduces hospitalisations for heart failure (12,85).

Although an association between higher haemoglobin concentrations and greater mortality has not been consistently demonstrated (7,8), the weight of evidence from multiple epidemiological reports of mostly older patients mirrors our findings (1,2,9–11). High haemoglobin concentrations will increase viscosity which may potentiate ischaemic or embolic events (12,13) or aggravate hypertension (15). High haemoglobin concentrations may also be secondary to chronic lung disease or myeloproliferative disorders, which are associated with an increased risk of morbidity and mortality (14).

This analysis also throws further doubt on the utility of serum ferritin for diagnosing iron deficiency (23,24) in patients with cardiovascular disease. Most patients had values <100µg/L even in the absence of anaemia, many patients with profound anaemia had values >30µg/L and low rather than high values of ferritin were associated with a good prognosis. Ferritin binds iron inside cells, protecting them from its toxic effects. In health, very little ferritin is shed into the circulation, but inflammation and cell damage can release large amounts of ferritin (25,122). Thus, serum ferritin appears to be a marker of inflammation rather than iron deficiency in patients with CV disease, which might increase and normalise serum ferritin even in the presence of iron deficiency. Serum iron and TSAT were less often measured but were usually low in patients with moderate or severe anaemia, although often low even when haemoglobin was normal. However, unlike serum ferritin, a low serum iron and TSAT were associated with a worse prognosis. Ultimately, bone marrow histology for iron

deposits and the clinical response to correction of iron deficiency are the most reliable ways of defining which test should be used to identify iron deficiency (22,176).

Iron deficiency - due to medications predisposing to bleeding (antiplatelets) and/or malabsorption of iron (proton-pump inhibitors), reduced dietary intake, or the failure to utilise appropriate body iron stores (e.g., due to inflammation) - is a common cause of anaemia in patients with CV disease (13,158,177). In patients with heart failure, iron deficiency with or without anaemia is associated with worse symptoms, quality of life and greater mortality (19,38,44). Regular testing of iron indices in all patients with heart failure, regardless of their haemoglobin concentration, is suggested in updated European guidelines (23). However, in our cohort, testing for iron deficiency was driven primarily by haemoglobin result – being much more common in those with moderate or severe anaemia. Many of those with heart failure and borderline anaemia who were tested in this cohort also had evidence of iron deficiency. In contemporary data of patients with heart failure, the rate of testing of iron indices may be as low as 1-2% (21) or as high as 27% (178). If iron markers are not routinely tested in patients with heart failure, those with iron deficiency may miss out on the benefits of intravenous iron repletion.

This analysis investigates associations that may or may not be causal on a large population of patients with a broad range of cardiovascular conditions. The nadir result for each test was used to classify patients rather than their average result, which may have led to different results. Assessment of the relation between some measures of iron deficiency and mortality was limited by low numbers and selective testing. Models were adjusted only for age and sex, as other important clinical information such as systolic blood pressure or body mass index was not available for many of our patients.

7.5 Conclusion

In patients with a broad range of cardiovascular disorders, including heart failure, haemoglobin is commonly measured but, even when anaemia is profound, iron indices are often not. Haemoglobin <13g/dL for women or <14g/dL for men, thresholds that are 1g/dL higher than the W.H.O. definition of

anaemia, are associated with a worse prognosis, predominantly from cancer and cardiovascular disease. A low serum iron and TSAT are also associated with a worse prognosis but, a serum ferritin <100µg/L is associated with a better prognosis.

Chapter 8 Final Discussion

In the work enclosed I have sought to improve our understanding of ID and its definition by blood tests in patients with heart failure by examining three different populations: a contemporary database of consenting patients referred to a regional UK heart failure clinic, a large electronic database comprising primary and secondary care data of patients locally in Greater Glasgow and Clyde and in a meta-analysis of randomised trials.

I have demonstrated that the prevalence, incidence and associations between ID and mortality is highly dependent on how ID is defined. There remains no universally accepted and validated diagnostic criteria using blood tests to define ID in patients with heart failure. The current ESC guideline recommended definition (ferritin <100μg/L or TSAT <20% if ferritin 100-299μg/L) (23) is largely based on the presence of a low serum ferritin. Ferritin is wholly unsuited to diagnose ID in patients with heart failure. It is a mostly intracellular protein that is released from cells in the context of cell damage or lysis due to inflammation (25). Measured concentrations therefore increase in chronic inflammation, even in the presence of ID (22,58). Bone-marrow studies in patients with heart failure and in those at risk of heart failure show that it is a poor marker of bone-marrow histology defined ID (20,40). In keeping with previous reports (19,20,37), I have demonstrated its limitations as a prognostic marker. Indeed, results from the Hull LifeLab and SafeHaven databases showed that a high ferritin, rather than a low ferritin, is associated with a poor prognosis, further emphasizing its primary role as an inflammatory biomarker in heart failure.

Inexpensive and readily available serum measures of iron and transferrin saturation provide more robust, linear associations with mortality than ferritin. A TSAT <20% and, in particular, a serum iron ≤13μmol/L are strongly associated with higher mortality in the present data. Together with ROC analysis performed in Chapter 3, I have helped to validate these biomarker thresholds that were previously reported to be sensitive and specific to diagnose bone-marrow ID in patients with heart failure (20). A large proportion of patients who, by current definitions (23,24), are not considered iron deficient have evidence of serum ID with low haemoglobin concentrations, and higher mortality than most patients

who are considered iron deficient by current criteria. In particular, patients with high ferritin (>300µg/L), who have a low TSAT or low serum iron, or those with a low transferrin (≤2.3g/L) who have low serum iron but normal TSAT, have bad outcomes yet are defined as being iron replete by current definitions. Using a low serum iron alone to define ID in heart failure may simplify things a great deal yet whether or not treatment with IV iron in these patients is safe and can reduce morbidity and mortality remains to be seen. Unless inclusion criteria in clinical trials of iron repletion broaden for these patients to be included, a definitive answer will not be found.

Serum iron and TSAT also have unique limitations which must be considered. Inflammation reduces serum concentrations of available iron and may also affect serum concentrations of transferrin (64,132). Indeed, the strong, independent prognostic performance of a low serum iron may be due, at least in part, to this coupled reduction due to both ID and inflammation.

Screening for ID and anaemia in patients with heart failure at least yearly is warranted. As I have reported, the vast majority of patients with heart failure, or at risk of heart failure, have haemoglobin tested but much less are tested for ID. If iron markers are tested a year apart, the incidence of ID at one year is between 30-38%. In addition, similar to previous reports (179), those with ID but without anaemia had a greater chance of developing anaemia at one year and as such adopting a higher risk of death compared to those without. My research is the first to show that spontaneous recovery from ID may lessen risk of death but only if defined by a low serum iron (≤13µmo/L). Once patients become anaemic however, this risk may not be as modifiable. Analysis of subgroups in the ongoing IV iron trials of those with and without a low serum iron or TSAT alone will yield interesting results.

Treatment with intravenous iron for those with heart failure and evidence of serum iron deficiency improves symptoms, quality of life and exercise tolerance. (84,85). Data from Chapter 6 reaffirms that IV iron also appears to reduce hospitalisations for heart failure in the 6-12 months following treatment. Reasons behind this remain unclear. Iron replenishment probably targets cellular energetic deficiencies first and improvements in energetics and substrate handling probably explain the relatively early benefit (within 4-6 weeks) of IV

iron in earlier trials (84,85). Improvements were present in those even without anaemia, adding more weight to this hypothesis. In vitro and in vivo studies report that iron replacement improves both skeletal and cardiac energy utilization and production translating into improved myocyte contractility (98,99). However, in the only outcome trial of IV iron to date, benefits of IV iron in terms of reductions in hospitalisations were only seen from 8-12 weeks, suggesting that this may be mainly due to its effects on oxygen storage and carrier proteins rather than muscle energetics (87). Unfortunately, changes in haemoglobin status in AFFIRM-AHF throughout the trial period are not available, although haemoglobin increased by a mean 0.8g/dL in the treatment group at the end of the trial (52 weeks) compared to only 0.3g/dL in the placebo group.

Despite having a noticeable impact on reducing hospitalisations for heart failure, the question remains why IV iron does not appear to improve survival. Intravenous iron protects against more marked decline in renal function (180), is associated with reductions in plasma NT-proBNP (152) and may also promote positive remodelling in exploratory cardiac MRI and 3-Dimensional echocardiography studies in patients with heart failure (107,108,144). It is therefore slightly surprising that no major mortality benefit has as yet been identified. It may be, as stated previously, that the wrong patients are being selected. All patients with heart failure have muscle energetic insufficiencies (12). Although IV iron might improve muscle mitochondrial function even in those not truly iron deficient, to have a more resounding impact on mortality, only a truly iron deficient patient may benefit from treatment. Additionally, the potential improvements in survival mediated by IV iron may take longer than one year. Three large trials of IV iron, still currently recruiting, with more patients and with longer follow-up, will help to answer these questions.

Data on longer term safety of repeated IV iron dosing in patients with heart failure is lacking and requires more attention. Given the promise of newer formulations of oral iron such as sucrosomial iron (110), a strategy of initial IV iron, optimised medical therapy and longer-term oral iron in those still deficient may be the way forward in the future. Again, well conducted trials are necessary to provide guidance.

If ID is not investigated, which, as I have shown, is common, anaemia will develop. Certain commonly prescribed medications in patients with CV disease such as antiplatelets and PPIs may exacerbate ID and potentially even hasten the development of anaemia. Occurring together, anaemia and ID worsen cellular energetic deficiencies and put heightened anaerobic demand on the cardiovascular system and result in adverse cardiac remodelling (128,158). Similar to previous research groups (156,163), my data show that defining anaemia by current W.H.O. diagnostic criteria (47) in patients with CV disease, including heart failure, is not optimal at identifying those at risk. If diagnostic thresholds are raised, patients would be investigated for potential reversible causes, such as ID, earlier and appropriate treatments commenced. Treatment of ID in heart failure with IV iron can improve outcomes and, although some of our data from ambulatory patients with heart failure in the community show otherwise, in a clinical trial setting, optimizing treatments for heart failure can improve haemoglobin concentrations which may translate into better outcomes (172). It must be appreciated however, that raising diagnostic thresholds would lead to large swathes of patients being diagnosed with anaemia. The potential pressures on healthcare systems to deal with this change needs to be considered prior to widespread implementation.

8.1 Future directions

The central theme in this thesis, and where I think research in ID in patients with heart failure should focus, is in accurate diagnosis of ID. Without correct patient selection, interpretation of results from large intervention trials will be problematic. By conducting comparative serum and bone marrow trials in patients with heart failure and other cardiovascular diseases, we can help validate serum biomarkers and diagnostic thresholds. This will aid patient selection for clinical trials and allow a more reliable interpretation of results.

To do this, we have successfully received funding from the British Heart Foundation to conduct a study in patients with established CV disease, including heart failure, to determine:

1) The prevalence of ID before elective cardiac surgery by various serum definitions and by bone marrow biopsy,

2) To describe the predictive accuracy of various pre-operative blood tests, alone or in combination, for ID as defined by the bone marrow gold-standard, and,

3) To conduct a randomised controlled trial in patients with ID awaiting elective cardiac surgery, investigating the impact of IV iron, compared to standard care, on pre-operative blood markers of ID and bone marrow iron stores.

This work will pave the way for a future trial investigating whether the benefits of detecting and correcting ID outweigh the potential risks of giving IV iron.

8.2 Limitations

More novel biomarkers including sTfR or plasma hepcidin were not measured in these retrospective datasets. However, plans are in place for collaboration with other local, national, and international research teams to test stored samples from the Hull LifeLab and our own prospective bone marrow study for sTfR and plasma hepcidin.

Supplementary Tables

Chapter 3

Table S1: Characteristics of patients with TSAT <20 % according to ferritin ≥300µg/L and <300µg/L.

Variable	F < 300 ng/ml N=1,932	F ≥ 300 ng/ml N=99	p-value
Demographic/Comorbidities			
Age (years)	77 (70-82)	75 (69-81)	0.32
Sex (women)	945 (49)	32 (32)	<0.01
BMI (kg/m^2)	28 (25-33)	27 (24-31)	<0.01
Ischaemic heart disease	799 (41)	42 (42)	0.83
Hypertension	1,103 (57)	42 (42)	<0.01
Diabetes	613 (32)	26 (26)	0.25
COPD	195 (10)	12 (12)	0.52
Atrial Fibrillation/Flutter	689 (37)	39 (41)	0.44
Signs, symptoms and Echo			
NYHA III/IV	710 (37)	38 (38)	0.84
Oedema (≥ ankle)	681 (38)	35 (38)	0.98
HFrEF (vs. HFmrEF/HFpEF)	593 (33)	28 (30)	0.57
Laboratory			
Serum Iron ≤ 13µmol/L	1,710 (86)	92 (93)	0.18
Serum Iron	10 (8-12)	9 (6-10)	<0.001
TSAT, %	15 (11-18)	16 (13-18)	<0.01
Ferritin, µg/L	55 (29-103)	389 (326-523)	NA
Hemoglobin, g/dL	12.5 (12.4-13.5)	12.0 (10.7-13.4)	0.02
Anaemia	922 (48)	61 (62)	<0.01
eGFR (ml/min/1.73m^2)	57 (43-72)	45 (31-71)	<0.01
NT-proBNP, ng/L	1,508 (566-3,354)	2,692 (979-5,746)	<0.001
hs-CRP, mg/L	5.8 (2.6-13.0)	16.0 (5.8-53.0)	<0.001
Medications			
Loop diuretic	1,288 (68)	75 (76)	0.07
ACEi or ARB	1,256 (66)	58 (59)	1,16
MRA	348 (18)	27 (28)	0.02
BB	1,117 (59)	61 (63)	0.49
Anticoagulant	581 (30)	28 (28)	0.71
Antiplatelet	843 (44)	41 (41)	0.66
Outcomes			
Cancers	124 (6)	17 (17)	<0.001
Deaths within 5 years	755 (39)	64 (65)	<0.001
Non-CV deaths within 5 years	345 (18)	33 (33)	<0.001

Values expressed as count and (%) or median and (25th – 75th centile) as appropriate. BMI: body mass index; IHD: ischaemic heart disease; COPD: chronic obstructive pulmonary disease; NYHA: New York heart association; HFrEF: heart failure with reduced ejection fraction; HFmrEF: heart failure with mid-range ejection fraction; HFpEF: heart failure with preserved ejection fraction; TSAT: transferrin saturation; eGFR: estimated glomerular filtration rate; NT-proBNP: N-

terminal pro-natriuretic peptide; hs-CRP: high sensitive C-reactive protein; ACEi: angiotensin converting enzyme inhibitor; ARB: angiotensin receptor blocker; MRA: mineralocorticoid receptor antagonist; BB: beta blocker; CV: cardiovascular.

Table S2: Basic and hierarchical Cox regression models for 5-years mortality

Model	**All-Cause Mortality HR (95% CI)**	**Cardiovascular Mortality HR (95% CI)**
Basic*		
+ Hb	0.89 (0.86-0.93)	0.91 (0.87-0.95)
+ SqR [serum iron]	0.84 (0.78-0.91)	0.97 (0.87-1.07)
+ Ln [TSAT]	0.83 (0.74-0.92)	0.93 (0.80-1.09)
+ Ln [ferritin]	1.09 (1.03-1.16)	1.11 (1.02-1.20)
Basic* + Hb		
+ SqR [serum iron]	0.93 (0.86-1.01)	1.07 (0.96-1.19)
+ Ln [TSAT]	0.96 (0.85-1.08)	1.06 (0.90-1.26)
+ Ln [ferritin]	1.12 (1.05-1.19)	1.13 (1.04-1.23)
Basic* + Hb + SqR [serum iron]		
+ Ln [ferritin]	1.14 (1.07-1.21)	1.12 (1.03-1.22)
Basic* + Hb + Ln [TSAT]		
+ Ln [ferritin]	1.16 (1.08-1.24)	1.14 (1.04-1.25)
	AUC	AUC
Basic* Model	0.72	0.74
+ Hb	0.72	0.74
+ SqR [serum Iron]	0.72	0.74
+ Ln [TSAT]	0.72	0.74
+ Ln [ferritin]	0.72	0.74
+ Hb and SqR [Iron]	0.72	0.74
+ Hb and Ln [TSAT]	0.72	0.74
+ Hb and Ln [ferritin]	0.72	0.74
+ Hb and SqR [Iron] and Ln [ferritin]	0.73	0.74
+ Hb and Ln [TSAT] and Ln [ferritin]	0.73	0.74

*Model adjusted for age (/5), sex, body mass index (/5), ischaemic heart disease, diabetes, systolic blood pressure (/5), New York heart association class III or IV, heart rate (/5), atrial fibrillation or atrial flutter, HF phenotype, natural log N-terminal pro-natriuretic peptide, estimated glomerular filtration rate (/5). Hb: haemoglobin; SqR: square root; Ln: natural log; TSAT: transferrin saturation

Table S3: Cox regression models for 5-year all-cause mortality

		HR	95% C.I.	p-value	HR	95% C.I.	p-value
Variable	**N missing (%)**	**Univariate**			**Multivariable Model**		
Demographic/Comorbidities							
Age (/5 years)	0 (0)	1.31	1.27-1.35	<0.001	1.25	1.20-1.29	<0.001
Sex (female)	0 (0)	0.87	0.79-0.97	0.01	0.69	0.62-0.78	<0.001
BMI (/5 kg/m^2)	85 (2)	0.81	0.77-0.85	<0.001	0.90	0.85-0.94	<0.001
IHD	0 (0)	1.05	0.95-1.16	0.32	1.02	0.91-1.15	0.69
Diabetes	0 (0)	1.15	1.02-1.28	0.02	1.30	1.15-1.48	<0.001
Signs and symptoms							
Systolic BP (/5 mmHg)	80 (2)	0.97	0.96-0.98	<0.001	0.97	0.93-0.98	<0.001
NYHA III or IV	57 (1)	1.98	1.78-2.19	<0.001	1.62	1.45-1.82	<0.001
ECG and Echo							
Heart rate (/5 bpm)	102 (2)	1.04	1.02-1.05	<0.001	1.02	1.003-1.04	0.02
AF/Atrial Flutter	151 (3)	1.29	1.15-1.43	<0.001	0.76	0.67-0.86	<0.001
HF phenotypes	0 (0)						
HFmrEF vs. HFrEF		0.85	0.74-0.98	0.03	1.17	0.94-1.30	0.22
HFpEF vs. HFrEF		0.86	0.77-0.97	0.01	1.49	1.25-1.69	<0.001
HF ↟NT-proBNP vs. HFrEF		0.92	0.73-1.15	0.44	0.87	0.95-1.56	0.12
Laboratory							
Hb (g/dL)	0 (0)	0.79	0.77-0.81	<0.001			
Ln [NT-proBNP] (ng/L)	152 (3)	1.62	1.55-1.69	<0.001	1.41	1.33-1.49	<0.001
eGFR (/5 ml/min/1.73m^2)	74 (2)	0.90	0.89-0.91	<0.001	0.97	0.96-0.98	<0.001
Medications							
Loop diuretic	73 (2)	2.00	1.77-2.25	<0.001			
ACEi or ARB	73 (2)	0.83	0.74-0.92	0.001			
BB	73 (2)	0.79	0.72-0.88	<0.001			
MRA	73 (2)	1.13	1.003-1.27	0.04			
Anticoagulant	0 (0)	0.99	0.86-1.11	0.86			

Antiplatelet	0 (0)	1.03	0.93-1.14	0.59	

BMI: body mass index; IHD: ischaemic heart disease; BP: blood pressure; NYHA: New York heart association; AF: atrial fibrillation; HF: heart failure; HFmrEF: heart failure with mid-range ejection fraction; HFpEF: heart failure with preserved ejection fraction; HFrEF: heart failure with reduced ejection fraction; Hb: haemoglobin; SqR: square root; Ln: natural log; TSAT: transferrin saturation; NT-proBNP: N-terminal pro-natriuretic peptide; eGFR: estimated glomerular filtration rate; ACEi: angiotensin converting enzyme inhibitor; ARB: angiotensin receptor blocker; MRA: mineralocorticoid receptor antagonist; BB: beta blocker. Non-significant results in the multivariate models are not reported.

Table S4: Cox regression model for 5-years cardiovascular mortality

		HR	95% C.I.	p-value	HR	95% C.I.	p-value
Variable	**N missing (%)**	**Univariate**			**Multivariable Model**		
Demographic/Comorbidities							
Age (/5 years)	0 (0)	1.27	1.22-1.32	<0.001	1.21	1.15-1.27	<0.001
Sex (female)	0 (0)	0.79	0.68-0.91	0.001	0.68	0.58-0.80	<0.001
BMI (/5 kg/m^2)	85 (2)	0.79	0.74-0.84	<0.001	0.89	0.83-0.96	<0.01
IHD	0 (0)	1.28	1.11-1.46	0.001	1.15	0.97-1.35	0.10
Diabetes	0 (0)	1.14	0.98-1.32	0.10	1.31	1.10-1.55	<0.01
Signs and symptoms							
Systolic BP (/5 mmHg)	80 (2)	0.95	0.94-0.96	<0.001	0.97	0.96-0.99	<0.001
NYHA III or IV	57 (1)	2.08	1.81-2.39	<0.001	1.57	1.34-1.84	<0.001
ECG and Echo							
Heart rate (/5 bpm)	102 (2)	1.04	1.02-1.06	<0.001	1.02	0.99-1.04	0.14
AF/Atrial Flutter	151 (3)	1.33	1.15-1.53	<0.001	0.82	0.70-0.98	0.03
HF phenotypes	0 (0)						
HFmrEF vs. HFrEF		0.74	0.61-0.89	0.001	1.01	0.82-1.25	0.92
HFpEF vs. HFrEF		0.61	0.52-0.71	<0.001	1.16	0.94-1.43	0.16
HF ↑NT-proBNP vs. HFrEF		0.59	0.34-0.82	<0.01	0.78	0.53-1.15	0.21
Laboratory							
Hb (g/dL)	0 (0)	0.80	0.77-0.83	<0.001			
Ln [NT-proBNP] (ng/L)	152 (3)	1.80	1.70-1.91	<0.001	1.52	1.40-1.64	<0.001
eGFR (/10 ml/min/1.73m^2)	74 (2)	0.88	0.87-0.90	<0.001	0.96	0.94-0.98	<0.001
Medications							
Loop diuretic	73 (2)	2.28	1.92-2.70	<0.001			
ACEi or ARB	73 (2)	0.92	0.79-1.06	0.25			
BB	73 (2)	0.83	0.72-0.95	<0.01			
MRA	73 (2)	1.49	1.28-1.74	<0.001			
Anticoagulant	0 (0)	0.99	0.85-1.15	0.90			

Antiplatelet	0 (0)	1.09	0.95-1.25	0.22	

BMI: body mass index; IHD: ischaemic heart disease; BP: blood pressure; NYHA: New York heart association; AF: atrial fibrillation; HF: heart failure; HFmrEF: heart failure with mid-range ejection fraction; HFpEF: heart failure with preserved ejection fraction; HFrEF: heart failure with reduced ejection fraction; Hb: haemoglobin; SqR: square root; Ln: natural log; TSAT: transferrin saturation; NT-proBNP: N-terminal pro-natriuretic peptide; eGFR: estimated glomerular filtration rate; ACEi: angiotensin converting enzyme inhibitor; ARB: angiotensin receptor blocker; MRA: mineralocorticoid receptor antagonist; BB: beta blocker. Non-significant results in the multivariate models are not reported.

Chapter 4

Table S5: Characteristics of patients in baseline study cohort (n= 4023) according to exclusions and compared to those with follow-up visit with full-iron and haemoglobin assessment available (n=906)

Variable	Not HF (n= 938)	HF; dead <1 year (n=322)	HF; ≥1-year follow-up, missing iron/Hb tests (n= 1856)	HF; ≥1-year follow-up, full iron/Hb tests (n=906)	P value (HF; ≥1-year follow-up and full tests vs those with missing tests)
Demographics					
Age (years)	69 (61-76)	78 (72-83)	74 (67-81)	73 (65-79)	0.003
Sex (male)	495 (53)	204 (63)	1068 (58)	633 (70)	<0.001
BMI (kg/m^2)	30 (26-34)	26 (23-31)	28 (25-32)	28 (25-32)	0.15 [8]
IHD	280 (30)	166 (52)	809 (44)	536 (59)	<0.001
Hypertension	543 (58)	138 (43)	1073 (58)	468 (52)	0.002
Diabetes	258 (28)	86 (27)	472 (25)	230 (25)	0.98
eGFR (ml/min/1.73m^2)	74 (58-87)	46 (32-67)	62 (49-77)	61 (46-76)	0.27 [8]
NT-proBNP (ng/L)	66 (39-93)	2913 (1169-6333)	818 (312-1937)	1217 (510-2626)	<0.001
Iron (µmol/L)	15 (12-19)	11.0 (8.0-15.0)	14.0 (11.0-18.0)	14.0 (11.0-19.0)	0.22
Ferritin (µg/L)	81 (41-145)	112 (56-238)	83 (44-155)	98 (50-176)	<0.001
TSAT (%)	23 (17-29)	18 (12-24)	22 (16-29)	23 (17-29)	0.04
Haemoglobin (g/dL)	13.8 (12.8-14.7)	12.2 (10.8-13.7)	13.3 (12.2-14.4)	13.5 (12.3-14.6)	0.07 [8]
ECG and ECHO					
AF or Flutter	74 (8)	118 (37)	535 (29)	252 (28)	0.84
Heart rate (bpm)	71 (62-82)	75 (66-89)	72 (62-84)	71 (61-84)	0.14

HFrEF	0 (0)	129 (40)	478 (26)	462 (51)	<0.001
Treatment					
ACEi or ARB	488 (55)	204 (64)	1245 (69)	724 (80)	<0.001
MRA	76 (9)	94 (30)	339 (19)	255 (28)	<0.001
BB	305 (34)	184 (58)	1043 (58)	601 (67)	<0.001
Loop diuretic	342 (39)	257 (81)	1079 (60)	615 (68)	<0.001
Anticoagulant	82 (9)	79 (25)	462 (25)	241 (27)	0.33
Antiplatelet	400 (43)	161 (50)	856 (46)	477 (53)	0.001
Iron treatment	29 (3)	29 (9)	88 (5)	42 (5)	0.90

Values expressed as count and (%) or median and (Q1-Q3) as appropriate.

Abbreviations: - ID: iron deficiency; BMI: body mass index; IHD: ischaemic heart disease; eGFR: estimated glomerular filtration rate; NT-proBNP: N-terminal pro-natriuretic peptide; AF: atrial fibrillation; HFrEF: heart failure with reduced ejection fraction; ACEi: angiotensin converting enzyme inhibitor; ARB: angiotensin receptor blocker; MRA: mineralocorticoid receptor antagonist; BB: beta blocker.

P values provided from Chi-square tests for categorical variables and Kruskal-Wallis tests for non-normally distributed continuous variables, unless indicated by [8] in which case one-way ANOVA test has been used.

Table S6: Count and (%) of patients with or without iron deficiency (ID) at baseline and change at 1 year. ID defined by serum iron ≤13μmol/L and by the FAIR-HF definition (ferritin <100μg/L or TSAT <20% if ferritin 100-299 μg/L)

Serum iron				
		1 year		
		No ID	ID	
Baseline	No ID	360 (40)	157 (17)	
	ID	173 (19)	216 (24)	P = 0.41
FAIR-HF				
		1 year		
		No ID	ID	
Baseline	No ID	211 (23)	127 (14)	
	ID	117 (13)	451 (50)	P = 0.57

P-value obtained via McNemar's test

Table S7: Change in iron biomarkers between baseline and follow-up visits according to changes in iron deficiency status (serum iron ≤13 µmol/L). For comparison, change in TSAT % at week 24 in patients treated with intravenous ferric carboxymaltose in FAIR-HF and CONFIRM-HF trials are shown.

Patient group	**Serum iron (µmol/L)** **Median (Q1; Q3)**	**TSAT (%)** **Median (Q1; Q3)**	**Serum Ferritin (µg/L)** **Median (Q1; Q3)**
ID Develops	-5.0 (-8.5; -3.0)	-8.2 (-13.4; -4.3)	-9 (-52; 20)
ID Resolves	6.0 (4.0; 9.0)	9.0 (6.1; 14.1)	12 (-16; 59)
ID Persists	0.0 (-2.0; 2.0)	0.1 (-3.6; 3.5)	-2 (-30; 23)
Never ID	-1.0 (-3.5; 2.0)	-1.3 (-6.3; 3.9)	-2 (-42; 29)
Trial		**Delta TSAT (%)** **Mean (SD)**	
FAIR-HF		11.3 (no SD quoted)	
CONFIRM-HF		8.9 (± 1.1)	

Table S8: Statistical associations between baseline variables and incident iron deficiency (serum iron ≤13 µmol/L) at follow-up in those without iron deficiency at baseline

		Univariate analysis		**Multivariable analysis 1**		**Multivariable analysis 2**	
	Unit change/ category	**OR (95% CI)**	**P value**	**OR (95% CI)**	**P value**	**OR (95% CI)**	**P value**
Age	10 years	1.17 (0.97-1.40)	0.10	1.01 (0.80-1.28)	0.93	1.04 (0.82-1.32)	0.74
Sex	Female	**1.60 (1.06-2.42)**	**0.03**	1.36 (0.84-2.19)	0.21	1.38 (0.85-2.21)	0.19
Diabetes	Yes/No	1.47 (0.94-2.29)	0.09	1.04 (0.60-1.78)	0.90	1.11 (0.65-1.89)	0.71
IHD	Yes/No	1.02 (0.70-1.50)	0.91				
NYHA	I/II vs III/IV	1.50 (0.99-2.27)	0.05	1.11 (0.68-1.83)	0.68	1.13 (0.69-1.84)	0.64
BMI	1 kg/m^2	1.02 (0.98-1.05)	0.37				
AF/Flutter	Yes/No	0.90 (0.59-1.39)	0.64				
HR	5 bpm	0.96 (0.90-1.02)	0.17				
Systolic BP	5mmHg	1.03 (0.99-1.08)	0.12				
HFrEF	Yes/No	1.03 (0.71-1.51)	0.86				
eGFR	1 ml/min/1.73 m^2	0.99 (0.99-1.00)	0.22	1.01 (0.99-1.02)	0.17	1.01 (0.99-1.02)	0.21
Log NT-proBNP	(Log 10) 1 ng/L	**1.66 (1.07-2.56)**	**0.02**	1.26 (0.74-2.15)	0.39	1.32 (0.78-2.25)	0.30
Log hsCRP	(Log10) 1 mg/L	**1.73 (1.15-2.61)**	**0.01**	1.60 (0.99-2.58)	0.05	**1.75 (1.10-2.78)**	**0.02**
Loop diuretic	Yes/No	**1.55 (1.03-2.33)**	**0.04**	1.44 (0.88-2.35)	0.15	1.34 (0.83-2.17)	0.24
Haemoglobin	1 g/dL	**0.79 (0.70-0.90)**	**<0.001**	0.87 (0.74-1.02)	0.08	**0.83 (0.72-0.97)**	**0.02**
Ferritin	(Log10) 1 µg/L	0.85 (0.51-1.44)	0.55				
Iron	(Sq root) 1 µmol/L	**0.39 (0.25-0.60)**	**<0.001**	**0.55 (0.33-0.89)**	**0.02**	**Removed from model**	

Anaemia *	Yes/No	1.28 (0.79-2.06)	0.31	0.89 (0.49-1.60)	0.69	0.97 (0.54-1.72)	0.90
Antiplatelet treatment	Yes/No	1.05 (0.72-1.53)	0.80	1.30 (0.78-2.14)	0.31	1.37 (0.84-2.26)	0.21
Anticoagulant therapy	Yes/No	1.22 (0.81-1.83)	0.34	1.54 (0.90-2.64)	0.12	1.57 (0.92-2.67)	0.10
Iron therapy	Yes/No	2.71 (0.96-7.60)	0.06	1.78 (0.57-5.55)	0.32	1.69 (0.55-5.21)	0.36

Abbreviations: - BMI: body mass index; HR: heart rate; NT-proBNP; N-terminal pro-brain natriuretic peptide; hsCRP: high-sensitivity C-reactive protein; eGFR: estimated glomerular filtration rate; IHD: ischaemic heart disease; ACEi: angiotensin converting enzyme inhibitor; ARB: angiotensin receptor blocker; MRA: mineralocorticoid receptor antagonist.

*Multivariable results obtained from separate models with baseline haemoglobin excluded from each model.

Table S9: Characteristics at baseline of patients according to change in iron deficiency status (FAIR-HF definition) between baseline and 1 year

Variable	Persists (n=451; 50%)	Develops (n=127; 14%)	Resolves (n=117; 13%)	Never (n=211; 23%)	P-value
Demographics					
Age (years)	74 (66-80)	73 (66-79)	75 (66-80)	71 (64-79)	0.11
Sex (male)	276 (61)	98 (77)	80 (68)	179 (85)	<0.001
BMI (kg/m^2)	29 (25-32)	28 (25-31)	29 (26-33)	28 (25-32)	0.04[8]
IHD	280 (62)	78 (61)	68 (58)	110 (52)	0.10
Hypertension	241 (53)	64 (50)	68 (58)	95 (45)	0.10
Diabetes	128 (28)	26 (21)	39 (33)	37 (18)	0.002
Haemoglobin (g/dL)	13.2 (12.1-14.2)	13.7 (12.3-14.7)	13.4 (12.0-14.4)	14.1 (13.1-15.2)	<0.001[8]
Iron (µmol/L)	13 (10-17)	17 (14-20)	12 (10-14.0)	18 (15-21)	<0.001
Ferritin (µg/L)	56 (35-83)	157 (119-225)	86 (57-139)	221 (154-347)	<0.001
TSAT (%)	18 (14-24)	26 (23-32)	18 (15-21)	29 (25-36)	<0.001
eGFR (ml/min/1.73m^2)	61 (46-75)	62 (46-77)	56 (43-72)	64 (48-78)	0.09[8]
NT-proBNP (ng/L) - all	1111 (418-2324)	1058 (570-2579)	2156 (896-4126)	1213 (565-2444)	<0.001
NT-proBNP (ng/L) - SR	792 (334-1964)	871 (370-2224)	1833 (630-4062)	1040 (484-2017)	0.002
ECG and ECHO					
AF or Flutter	106 (25)	30 (25)	46 (41)	70 (34)	0.002

Heart rate (bpm)	71 (60-83)	69 (60-88)	75 (62-90)	71 (62-82)	0.19
HFrEF	208 (46)	69 (54)	68 (58)	117 (56)	0.03
Treatment at Baseline					
Loop diuretic	291 (65)	93 (74)	81 (69)	150 (71)	0.14
ACEi or ARB	355 (79)	110 (88)	90 (77)	169 (81)	0.12
MRA	89 (20)	54 (43)	28 (24)	84 (40)	<0.001
BB	269 (60)	98 (78)	81 (69)	153 (79)	<0.001
Anticoagulant	104 (23)	29 (23)	36 (31)	72 (34)	0.01
Antiplatelet	247 (55)	73 (58)	62 (53)	95 (45)	0.07
Oral iron treatment	16 (4)	6 (5)	13 (11)	7 (3)	0.004

Iron deficiency (ID) defined as ferritin <100 µg/L or TSAT <20% if ferritin 100-299 µg/L. Values expressed as count and (%) or median and (Q1-Q3) as appropriate.

Abbreviations: - ID: iron deficiency; BMI: body mass index; IHD: ischaemic heart disease; eGFR: estimated glomerular filtration rate; NT-proBNP: N-terminal pro-natriuretic peptide; SR: sinus rhythm; AF: atrial fibrillation; HFrEF: heart failure with reduced ejection fraction; ACEi: angiotensin converting enzyme inhibitor; ARB: angiotensin receptor blocker; MRA: mineralocorticoid receptor antagonist; BB: beta blocker.

* P values provided from Chi-square tests for categorical variables and Kruskal-Wallis tests for non-normally distributed continuous variables, unless indicated by (ϑ) in which case one-way ANOVA test has been used.

Table S10: Statistical associations between baseline variables and incident iron deficiency (FAIR-HF) at follow-up in those without iron deficiency at baseline

		Univariate analysis		**Multivariable analysis 1**		**Multivariable analysis 2**	
	Unit change/ category	**OR (95% CI)**	**P value**	**OR (95% CI)**	**P value**	**OR (95% CI)**	**P value**
Age	10 years	1.17 (0.95-1.44)	0.13	1.03 (0.77-1.39)	0.82	1.09 (0.83-1.44)	0.53
Sex	Female	1.66 (0.95-2.90)	0.08	1.36 (0.66-2.80)	0.41	1.61 (0.83-3.18)	0.17
Diabetes	Yes/No	1.21 (0.69-2.12)	0.50				
IHD	Yes/No	1.46 (0.93-2.29)	0.10	1.18 (0.62-2.25)	0.62	1.15 (0.63-2.11)	0.66
NYHA	I/II vs III/IV	1.42 (0.87-2.32)	0.16	1.53 (0.81-2.88)	0.19	1.37 (0.75-2.49)	0.31
BMI	1 kg/m^2	0.97 (0.93-1.02)	0.20				
AF/Flutter	Yes/No	0.64 (0.39-1.06)	0.08	0.78 (0.35-1.75)	0.54	0.87 (0.40-1.89)	0.72
HR	5 bpm	0.99 (0.93-1.06)	0.82				
Systolic BP	5mmHg	0.99 (0.94-1.04)	0.61				
HFrEF	Yes/No	0.96 (0.61-1.49)	0.84				
eGFR	1 ml/min/1.73m^2	0.99 (0.98-1.01)	0.52	1.00 (0.99-1.02)	0.43	1.00 (0.99-1.02)	0.63
Log NT-proBNP	(Log 10) 1 ng/L	0.84 (0.52-1.36)	0.48	0.72 (0.39-1.37)	0.32	0.75 (0.42-1.36)	0.35
Log hsCRP	(Log10) 1 mg/L	1.21 (0.80-1.85)	0.37	1.34 (0.79-2.26)	0.28	1.06 (0.65-1.72)	0.83
Loop diuretic	Yes/No	1.16 (0.70-1.92)	0.56				
Haemoglobin	1 g/dL	**0.86 (0.75-0.98)**	**0.02**	**0.83 (0.70-0.99)**	**0.049**	0.89 (0.75-1.06)	0.18
Ferritin	(Log10) 1 µg/L	**0.04 (0.01-0.12)**	**<0.001**	**0.03 (0.01-0.13)**	**<0.001**	Removed from model	
Iron	(Sq root) 1 µmol/L	0.79 (0.56-1.10)	0.17				

Anaemia *	Yes/No	1.52 (0.92-1.51)	0.11	1.83 (0.94-3.55)	0.08	1.51 (0.81-2.83)	0.20
Antiplatelet treatment	Yes/No	**1.65 (1.06-2.57)**	**0.03**	0.99 (0.48-2.03)	0.97	1.27 (0.66-2.47)	0.48
Anticoagulant therapy	Yes/No	**0.57 (0.35-0.94)**	**0.03**	0.59 (0.26-1.38)	0.22	0.62 (0.28-1.36)	0.23
Iron therapy	Yes/No	1.45 (0.48-4.40)	0.52				

Abbreviations: - IHD: ischaemic heart disease; NYHA: New York heart association functional class; BMI: body mass index; AF: atrial fibrillation; HR: heart rate; NT-proBNP; N-terminal pro-brain natriuretic peptide; hsCRP: high-sensitivity C-reactive protein; eGFR: estimated glomerular filtration rate; IHD: ischaemic heart disease

*Multivariable results obtained from separate models excluding baseline haemoglobin

Table S11: Statistical associations between baseline variables and incident anaemia at follow-up in those without anaemia at baseline

		Univariate analysis		Multivariable analysis 1		Multivariable analysis 2	
	Unit change/category	**OR (95% CI)**	**P value**	**OR (95% CI)**	**P value**	**OR (95% CI)**	**P value**
Age	10 years	**1.87 (1.48-2.38)**	**<0.001**	**1.67 (1.23-2.28)**	**0.001**	**1.70 (1.27-2.29)**	**<0.001**
Sex	Female	1.31 (0.84-2.03)	0.23	**0.29 (0.14-0.62)**	**0.001**	1.25 (0.72-2.15)	0.43
Diabetes	Yes/No	1.33 (0.82-2.15)	0.25				
IHD	Yes/No	1.48 (0.95-2.30)	0.08	1.33 (0.69-2.53)	0.39	1.50 (0.82-2.73)	0.19
NYHA	I/II vs III/IV	1.29 (0.83-2.03)	0.26	0.91 (0.51-1.64)	0.76	0.97 (0.56-1.67)	0.91
BMI	1 kg/m^2	0.97 (0.93-1.01)	0.11				
Atrial fibrillation	Yes/No	0.73 (0.44-1.19)	0.21				
HR	5 bpm	**0.92 (0.86-0.99)**	**0.02**	0.92 (0.85-1.01)	0.06	**0.89 (0.82-0.97)**	**0.005**
Systolic BP	5 mmHg	0.99 (0.95-1.04)	0.85				
HFrEF	Yes/No	1.14 (0.5-1.74)	0.54				
eGFR	1 ml/min/1.73m^2	**0.98 (0.97-0.99)**	**<0.001**	1.00 (0.99-1.02)	0.82	0.99 (0.98-1.01)	0.59
Log NT-proBNP	(Log 10) 1 ng/L	**2.37 (1.46-3.84)**	**<0.001**	1.51 (0.81-2.80)	0.20	1.28 (0.71-2.29)	0.42
Log hsCRP	(Log10) 1 mg/L	**1.62 (1.06-2.49)**	**0.03**	**1.90 (1.06-3.40)**	**0.03**	1.52 (0.87-2.65)	0.14

Loop diuretic	Yes/No	**2.63 (1.57-4.42)**	**<0.001**	**2.58 (1.36-4.90)**	**0.004**	**2.24 (1.28-4.34)**	**0.01**
Iron	(Sq root) 1 µmol/L	**0.70 (0.52-0.95)**	**0.02**	1.34 (0.85-2.09)	0.21	0.79 (0.53-1.20)	0.27
TSAT *	1%	**0.98 (0.96-1.00)**	**0.048**	1.01 (0.98-1.04)	0.63	0.98 (0.96-1.01)	0.27
Ferritin	(Log10) 1 µg/L	1.15 (0.65-2.02)	0.64				
ID (iron ≤13 µmol/L) *	Yes/No	1.47 (0.96-2.27)	0.08	0.72 (0.40-1.30)	0.27	1.16 (0.67-1.99)	0.60
ID (TSAT <20%) *	Yes/No	**1.60 (1.04-2.47)**	**0.03**	0.90 (0.50-1.61)	0.71	1.43 (0.84-2.45)	0.19
ID (FAIR-HF) *	Yes/No	1.16 (0.75-1.78)	0.51	1.02 (0.57-1.82)	0.94	1.18 (0.69-2.03)	0.64
Haemoglobin	1 g/dL	**0.41 (0.32-0.52)**	**<0.001**	**0.26 (0.17-0.41)**	**<0.001**	**Removed from model**	
Antiplatelet treatment	Yes/No	1.04 (0.68-1.58)	0.86	0.70 (0.37-1.32)	0.27	0.64 (0.35-1.19)	0.16
Anticoagulant therapy	Yes/No	0.75 (0.46-1.21)	0.24	0.66 (0.32-1.32)	0.24	**0.47 (0.24-0.91)**	**0.03**
Oral iron therapy	Yes/No	**4.18 (1.52-11.48)**	**0.01**	1.81 (0.50-6.57)	0.37	2.20 (0.67-7.27)	0.20

Table S12: Symptoms, blood results and treatments at baseline and 1 year according to change in iron deficiency status (serum iron ≤13 μmol/L)

Variable		Persists (n=216; 24%)	Develops (n=157; 17%)	Resolves (n=173; 19%)	Never (n=360; 40%)	P-value
Symptoms						
NYHA (III or IV)	B/L	91 (42)	51 (33)	63 (36)	87 (24)	<0.001
	1 year	60 (29)	38 (24)	34 (20)	60 (17)	0.01
Blood results						
Haemoglobin (g/dL)	B/L	12.4 (11.3-13.4)	13.6 (12.6-14.6)	13.0 (11.9-14.0)	14.2 (13.3-15.3)	<0.001 [8]
	1 year	12.1 (11.0-13.3)	13.0 (11.8-14.1)	13.5 (12.1-14.4)	14.0 (12.9-14.8)	<0.001 [8]
Iron (μmol/L)	B/L	10 (8-12)	17 (15-19)	11 (9-12)	19 (16-22)	NA
	1 year	10 (8-12)	12 (10-13)	16 (15-18)	18 (16-21)	
Ferritin (μg/L)	B/L	77 (39-136)	157 (64-188)	82 (41-142)	113 (62-203)	<0.001
	1 year	77 (36-130)	95 (54-157)	98 (51-188)	110 (57-201)	<0.001
TSAT (%)	B/L	16 (12-18)	26 (23-31)	16 (13-19)	29 (24-35)	<0.001
	1 year	16 (12-18)	18 (15-21)	25 (22-30)	28 (24-36)	<0.001
eGFR (ml/min/1.73m^2)	B/L	53 (39-69)	61 (47-75)	63 (46-75)	65 (50-79)	<0.001 [8]
	1 year	47 (35-64)	56 (42-71)	54 (42-72)	60 (47-76)	<0.001[8]
NT-proBNP (ng/L) - all	B/L	1674 (662-3555)	1309 (517-2768)	1746 (498-3078)	926 (465-1886)	<0.001
	1 year	1286 (529-2977)	1023 (413-2283)	956 (404-2055)	674 (308-1690)	<0.001
NT-proBNP (ng/L) - SR	B/L	1402 (523-3292)	1060 (342-2769)	1095 (365-2770)	756 (372-1427)	<0.001
	1 year	919 (436-2392)	803 (313-1674)	610 (269-1296)	448 (240-1250)	<0.001
Treatments						
Loop diuretic	B/L	154 (72)	113 (72)	122 (71)	226 (63)	0.04
	1 year	168 (78)	110 (70)	116 (67)	230 (64)	0.006
ACEi or ARB	B/L	162 (76)	135 (87)	134 (78)	293 (82)	0.06

	1 year	181 (84)	131 (83)	146 (84)	308 (86)	0.91
MRA	B/L	38 (18)	54 (35)	43 (25)	120 (33)	<0.001
	1 year	68 (32)	67 (43)	76 (44)	143 (40)	0.049
BB	B/L	116 (55)	113 (72)	109 (63)	263 (73)	<0.001
	1 year	159 (74)	123 (78)	129 (75)	288 (80)	0.26
Anticoagulant	B/L	46 (21)	51 (33)	42 (24)	102 (28)	0.07
	1 year	61 (28)	62 (40)	57 (34)	126 (35)	0.14
Antiplatelet	B/L	116 (64)	80 (51)	102 (59)	179 (50)	0.23
	1 year	108 (50)	73 (47)	94 (54)	177 (49)	0.54
Oral iron treatment	B/L	21 (10)	8 (5)	6 (4)	7 (2)	<0.001
	1 year	19 (9)	10 (6)	16 (9)	18 (5)	0.19

Iron deficiency (ID) defined as serum iron ≤13µmol/L. Values expressed as count and (%) or median and (Q1-Q3) as appropriate.

Abbreviations: - B/L: Baseline; ID: iron deficiency; eGFR: estimated glomerular filtration rate; NT-proBNP: N-terminal pro-natriuretic peptide; SR: sinus rhythm; ACEi: angiotensin converting enzyme inhibitor; ARB: angiotensin receptor blocker; MRA: mineralocorticoid receptor antagonist; BB: beta blocker.

Variables displayed from baseline unless otherwise stated.

P values provided from Chi-square tests for categorical variables and Kruskal-Wallis tests for non-normally distributed continuous variables, unless indicated by $^{(8)}$ in which case one-way ANOVA test has been used.

Table S13: Symptoms, blood results and treatments at baseline and 1 year according to change in anaemia status

Variable		Persists (n=209; 23%)	Develops (n=104; 12%)	Resolves (n=63; 7%)	Never (n=530; 58%)	P-value
Symptoms						
NYHA (III or IV)	B/L	77 (37)	35 (34)	31 (49)	149 (28)	0.002
	1 year	59 (28)	31 (29)	21 (33)	81 (15)	<0.001
Blood results						
Haemoglobin (g/dL)	B/L	11.5 (10.8-12.1)	13.4 (12.7-13.9)	11.9 (11.3-12.5)	14.2 (13.5-15.2)	NA
	1 year	11.1 (10.6-11.9)	11.9 (11.4-12.4)	13.4 (13.0-14.1)	14.1 (13.4-14.8)	
Iron (µmol/L)	B/L	12 (9-15)	15 (12-19)	12 (8-15)	16 (12-20)	<0.001
	1 year	12 (10-15)	13 (10-16)	15 (11-18)	16 (13-20)	<0.001
Ferritin (µg/L)	B/L	90 (46-176)	111 (57-172)	85 (35-179)	100 (53-176)	0.26
	1 year	97 (39-196)	98 (63-174)	75 (42-135)	94 (49-174)	0.23
TSAT (%)	B/L	19 (14-24)	23 (17-29)	17 (11-24)	24 (18-31)	<0.001
	1 year	19 (14-24)	20 (15-25)	22 (17-28)	24 (20-30)	<0.001
eGFR (ml/min/1.73m^2)	B/L	49 (35-64)	58 (43-70)	58 (38-72)	66 (54-80)	<0.001 [8]
	1 year	42 (32-54)	47 (35-60)	54 (39-69)	63 (51-77)	<0.001
NT-proBNP (ng/L) - all	B/L	1903 (745-3937)	1666 (710-2867)	2132 (729-4080)	1014 (413-2047)	<0.001
	1 year	1379 (651-2926)	1206 (576-1387)	1254 (418-2720)	690 (307-1749)	<0.001
NT-proBNP (ng/L) - SR	B/L	1496 (632-3472)	1454 (604-3040)	1192 (464-3559)	656 (310-1663)	<0.001
	1 year	990 (533-2325)	880 (499-2283)	744 (261-2087)	425 (222-1085)	<0.001
Treatments						
Loop diuretic	B/L	162 (78)	84 (81)	45 (73)	324 (62)	<0.001

	1 year	157 (75)	88 (85)	49 (78)	330 (62)	<0.001
ACEi or ARB	B/L	161 (78)	86 (83)	48 (77)	429 (81)	0.59
	1 year	169 (81)	93 (89)	49 (78)	455 (86)	0.07
MRA	B/L	62 (30)	30 (29)	20 (32)	143 (27)	0.77
	1 year	79 (38)	45 (43)	27 (43)	203 (38)	0.70
BB	B/L	129 (62)	65 (63)	41 (66)	366 (69)	0.22
	1 year	158 (76)	82 (79)	50 (79)	409 (77)	0.89
Anticoagulant	B/L	44 (21)	25 (24)	14 (22)	158 (30)	0.07
	1 year	55 (26)	32 (31)	24 (38)	195 (37)	0.04
Antiplatelet	B/L	123 (59)	53 (51)	36 (57)	265 (50)	0.15
	1 year	116 (56)	49 (47)	34 (54)	253 (48)	0.23
Oral iron treatment	B/L	22 (11)	7 (7)	4 (6)	9 (2)	<0.001
	1 year	36 (17)	8 (8)	10 (16)	8 (2)	<0.001

Anaemia defined by haemoglobin <12.0 g/dL in women, or <13.0 g/dL in men.
Values expressed as count and (%) or median and (Q1-Q3) as appropriate.

Abbreviations: - B/L: Baseline; ID: iron deficiency; eGFR: estimated glomerular filtration rate; NT-proBNP: N-terminal pro-natriuretic peptide; SR: sinus rhythm; ACEi: angiotensin converting enzyme inhibitor; ARB: angiotensin receptor blocker; MRA: mineralocorticoid receptor antagonist; BB: beta blocker.

Variables displayed from baseline unless otherwise stated.

P values provided from Chi-square tests for categorical variables and Kruskal-Wallis tests for non-normally distributed continuous variables, unless indicated by [8] in which case one-way ANOVA test has been used.

Table S14: Statistical associations between baseline and updated (1-year) variables and all-cause mortality subsequent to the one-year follow-up

		Univariate analysis		Multivariable analysis - Baseline Haematinics		Multivariable analysis - Updated Haematinics	
	Unit Change/ Category	**HR (95% CI)**	**P value**	**HR (95% CI)**	**P value**	**HR (95% CI)**	**P value**
Age	10 years	**1.76 (1.53-2.01)**	**<0.001**	**1.58 (1.32-1.89)**	**<0.001**	**1.58 (1.32-1.88)**	**<0.001**
Sex	Men	1.22 (0.93-1.59)	0.15	**1.62 (1.18-2.24)**	**0.003**	**1.61 (1.17-2.22)**	**0.004**
Diabetes	Yes / No	1.04 (0.80-1.36)	0.76	1.01 (0.73-1.40)	0.95	1.01 (0.73-1.40)	0.94
IHD	Yes/No	1.07 (0.84-1.36)	0.60	1.07 (0.79-1.45)	0.65	1.07 (0.79-1.45)	0.65
NYHA	I/II vs III/IV	**1.78 (1.40-2.26)**	**<0.001**	1.28 (0.95-1.73)	0.11	1.28 (0.95-1.73)	0.11
BMI (kg/m^2)	$1kg/m^2$	**0.95 (0.93-0.97)**	**<0.001**	0.98 (0.95-1.01)	0.14	0.98 (0.95-1.01)	0.13
AF/Flutter	Yes/No	1.18 (0.91-1.53)	0.22	0.76 (0.54-1.07)	0.11	0.76 (0.54-1.07)	0.11
Heart rate	5 bpm	**1.03 (1.00-1.07)**	**0.048**	1.03 (0.99-1.07)	0.21	1.03 (0.99-1.07)	0.21
Systolic BP	5 mmHg	**0.97 (0.94-0.99)**	**0.01**	0.98 (0.95-1.01)	0.14	0.98 (0.95-1.01)	0.13
HFrEF	Yes/No	**1.43 (1.13-1.82)**	**0.003**	1.05 (0.77-1.43)	0.83	1.05 (0.77-1.43)	0.77
eGFR	1 $ml/min/1.73m^2$	**0.98 (0.97-0.99)**	**<0.001**	0.99 (0.98-1.00)	0.33	0.99 (0.98-1.00)	0.33
Log NT-proBNP	(Log 10) 1 ng/L	**2.77 (2.14-3.59)**	**<0.001**	**1.46 (1.01-2.08)**	**0.046**	**1.44 (1.01-2.07)**	**0.047**
Log hs-CRP	(Log 10) 1 mg/L	**1.57 (1.25-1.97)**	**<0.001**	1.23 (0.92-1.64)	0.17	1.23 (0.93-1.64)	0.16
Loop diuretic	Yes/No	**2.25 (1.66-3.05)**	**<0.001**	**1.54 (1.07-2.22)**	**0.02**	**1.53 (1.06-2.20)**	**0.02**
Haemoglobin - Baseline	1 g/dL	**0.83 (0.77-0.89)**	**<0.001**	0.93 (0.84-1.04)	0.22		
Haemoglobin - 1 year	1 g/dL	**0.82 (0.77-0.89)**	**<0.001**			0.94 (0.84-1.04)	0.23

Delta Hb	1 g/dL	1.01 (0.92-1.12)	0.81	1.02 (0.90-1.16)	0.72	1.10 (0.96-1.25)	0.17
Iron - Baseline	(Sq root) 1 µmol/L	**0.71 (0.60-0.83)**	**<0.001**	0.80 (0.59-1.08)	0.14		
Iron - 1 year	(Sq root) 1 µmol/L	**0.69 (0.58-0.83)**	**<0.001**			0.78 (0.58-1.06)	0.11
Delta Iron	1 µmol/L	1.01 (0.99-1.03)	0.42	0.97 (0.94-1.00)	0.07	1.00 (0.97-1.03)	0.97
TSAT – Baseline*	1%	**0.98 (0.97-0.99)**	**0.005**	0.99 (0.97-1.01)	0.36		
TSAT - 1 year*	1%	**0.98 (0.97-0.99)**	**0.02**			0.99 (0.96-1.01)	0.19
Delta TSAT*	1%	1.00 (0.99-1.02)	0.51	0.98 (0.97-1.00)	0.06	0.99 (0.98-1.02)	0.82
Ferritin - Baseline	(Log 10) 1 µg/L	1.12 (0.82-1.53)	0.49	1.14 (0.74-1.67)	0.60		
Ferritin - 1 year	(Log 10) 1 µg/L	1.31 (0.97-1.77)	0.08			1.14 (0.77-1.68)	0.51
Delta Ferritin	10 µg/L	1.01 (0.99-1.02)	0.08	**1.01 (1.00-1.03)**	**0.04**	1.01 (0.99-1.03)	0.12

Abbreviations: - ID: iron deficiency; IHD: ischaemic heart disease; NYHA: New York heart association functional classification; BMI: body mass index; AF: atrial fibrillation; eGFR: estimated glomerular filtration rate; NT-proBNP: N-terminal pro-brain natriuretic peptide; hsCRP: high-sensitivity C-reactive protein; Hb: haemoglobin; Sq: square; TSAT: transferrin saturation.

* Multivariable results obtained from separate models as TSAT is highly correlated with serum iron.

Table S15: Statistical associations between baseline and updated (1-year) variables and cardiovascular mortality subsequent to the one-year follow-up

		Univariate analysis		Multivariable analysis - Baseline Haematinics		Multivariable analysis - Updated Haematinics	
	Unit Change/ Category	**HR (95% CI)**	**P value**	**HR (95% CI)**	**P value**	**HR (95% CI)**	**P value**
Age	10 years	**1.79 (1.49-2.14)**	**<0.001**	**1.49 (1.18-1.89)**	**0.001**	**1.50 (1.18-1.89)**	**0.001**
Sex	Men	1.37 (0.96-1.97)	0.09	**1.94 (1.26-3.00)**	**0.003**	**1.90 (1.23-2.94)**	**0.004**
Diabetes	Yes / No	0.82 (0.56-1.19)	0.29	0.89 (0.58-1.38)	0.61	0.89 (0.58-1.39)	0.62
IHD	Yes/No	1.17 (0.85-1.61)	0.35	1.21 (0.81-1.82)	0.34	1.21 (0.81-1.81)	0.35
NYHA	I/II vs III/IV	**1.89 (1.39-2.58)**	**<0.001**	1.31 (0.89-1.94)	0.19	1.33 (0.90-1.97)	0.16
BMI (kg/m^2)	$1kg/m^2$	**0.93 (0.90-0.96)**	**<0.001**	0.99 (0.95-1.03)	0.53	0.99 (0.95-1.03)	0.53
AF/Flutter	Yes/No	1.25 (0.89-1.76)	0.20	0.73 (0.47-1.14)	0.17	0.73 (0.47-1.14)	0.16
Heart rate	5 bpm	1.04 (0.99-1.08)	0.11	1.04 (0.98-1.10)	0.19	1.04 (0.98-1.10)	0.17
Systolic BP	5 mmHg	**0.95 (0.92-0.98)**	**0.004**	0.98 (0.94-1.02)	0.34	0.98 (0.94-1.02)	0.36
HFrEF	Yes/No	**2.02 (1.46-2.81)**	**<0.001**	1.44 (0.95-2.19)	0.09	1.45 (0.95-2.20)	0.08
eGFR	1 $ml/min/1.73m^2$	**0.97 (0.96-0.98)**	**<0.001**	0.99 (0.98-1.00)	0.06	0.99 (0.98-1.00)	0.07
Log NT-proBNP	(Log 10) 1 ng/L	**3.94 (2.80-5.55)**	**<0.001**	**2.18 (1.35-3.50)**	**0.001**	**2.12 (1.32-3.41)**	**0.002**
Log hs-CRP	(Log 10) 1 mg/L	**1.43 (1.06-1.92)**	**0.02**	1.01 (0.69-1.47)	0.82	0.99 (0.69-1.44)	0.97
Loop diuretic	Yes/No	**2.31 (1.55-3.46)**	**<0.001**	1.26 (0.78-2.06)	0.28	1.25 (0.77-2.04)	0.37
Haemoglobin - Baseline	1 g/dL	**0.81 (0.74-0.89)**	**<0.001**	0.93 (0.81-1.07)	0.32		
Haemoglobin - 1 year	1 g/dL	**0.81 (0.73-0.89)**	**<0.001**			0.93 (0.81-1.07)	0.29

Delta Hb	1 g/dL	1.01 (0.88-1.15)	0.89	1.06 (0.90-1.24)	0.48	1.15 (0.97-1.37)	0.11
Iron - Baseline	(Sq root) 1 µmol/L	**0.76 (0.61-0.93)**	**0.01**	0.84 (0.56-1.25)	0.39		
Iron - 1 year	(Sq root) 1 µmol/L	**0.68 (0.53-0.85)**	**0.001**			0.83 (0.56-1.22)	0.34
Delta Iron	1 µmol/L	0.99 (0.97-1.02)	0.76	0.96 (0.92-1.00)	0.05	0.98 (0.95-1.02)	0.41
TSAT – Baseline *	1%	0.99 (0.97-1.00)	0.14	0.99 (0.97-1.02)	0.77		
TSAT - 1 year*	1%	**0.98 (0.96-0.99)**	**0.03**	1.00 (0.99-1.02)	0.69	0.99 (0.96-1.02)	0.44
Delta TSAT*	1%	0.99 (0.98-1.01)	0.64	0.99 (0.95-1.00)	0.06	0.98 (0.96-1.01)	0.17
Ferritin - Baseline	(Log 10) 1 µg/L	1.11 (0.74-1.67)	0.61	0.87 (0.50-1.50)	0.62		
Ferritin - 1 year	(Log 10) 1 µg/L	1.24 (0.83-1.84)	0.29			1.06 (0.64-1.74)	0.83
Delta Ferritin	10 µg/L	1.00 (0.99-1.02)	0.75	1.00 (0.98-1.02)	0.87	1.00 (0.99-1.02)	0.78

Abbreviations: - ID: iron deficiency; IHD: ischaemic heart disease; NYHA: New York heart association functional classification; BMI: body mass index; AF: atrial fibrillation; eGFR: estimated glomerular filtration rate; NT-proBNP: N-terminal pro-brain natriuretic peptide; hsCRP: high-sensitivity C-reactive protein; Hb: haemoglobin; Sq: square; TSAT: transferrin saturation.

* Multivariable results obtained from separate models with iron excluded as TSAT is highly correlated with serum iron.

Table S16: Cox regression analysis assessing survival within 5 years in those whose iron deficiency resolved against those with persistent iron deficiency (reference) according to each definition
Presented as HR, (95% confidence intervals) and p-value.

Persistent ID	Resolution of ID
Serum iron ≤13 µmol/L	
Reference	0.61 (0.44-0.86); p=0.004
FAIR-HF	
Reference	1.22 (0.87-1.73); p=0.25
TSAT <20%	
Reference	0.77 (0.54-1.09); p=0.14

Chapter 5

Table S17: Key characteristics of those with heart failure and all iron and haemoglobin results available vs those with missing tests

Variable	Full tests (n= 4,422)	Missing tests (n= 2,738)	P value
Enrolled prior to 2009	1635 (52)	1537 (48)	<0.001
Enrolled after 2009	2787 (70)	1201 (30)	<0.001
Age (years)	75 (68-82)	75 (67-81)	0.01
Sex (female)	1763 (40)	1060 (39)	0.33
HFrEF	1429 (32)	1079 (39)	<0.001
NTproBNP (ng/L)	1199 (482-2667)	1117 (448-2649)	0.16

Presented as count and (%) or median (Q1-Q3) as appropriate.

Abbreviations – HFrEF: heart failure with reduced ejection fraction; NTproBNP: N terminal pro-natriuretic peptide.

Table S18: Characteristics according to serum transferrin concentration

Variable	Transferrin ≤ 2.3 g/L (lowest quartile) (n=1195 ;27 %)	Transferrin > 2.3 g/L (upper three quartiles) (n= 3,227; 73%)	P value
Demographics			
Age (years)	77 (70-83)	75 (67-81)	<0.001
Sex (female)	354 (30)	1409 (44)	<0.001
Hypertension	637 (53)	1818 (56)	0.07
Diabetes	284 (24)	868 (27)	0.04
IHD	537 (45)	1339 (42)	0.04
COPD	120 (10)	291 (9)	0.29
Signs and symptoms			
NYHA III or IV	348 (30)	972 (31)	0.51
ECG and ECHO			
AF/flutter	337 (29)	1170 (38)	<0.001
Heart rate (bpm)	71 (61-82)	74 (64-87)	<0.001
HFrEF	382 (32)	1047 (32)	0.76
Bloods			
Haemoglobin (g/dL)	12.8 (11.5-14)	13.3 (12.1-14.5)	<0.001*
Anaemia (WHO definition)	556 (47)	990 (31)	<0.001
MCV (fL)	91 (87-95)	90 (86-93)	<0.001*
MCH (pg)	30 (29-31)	30 (28-31)	<0.001*
MCHC (g/dL)	33 (32-34)	33 (32-34)	0.02*
RDW (%)	14.2 (13.4-15.2)	14.2 (13.5-15.4)	0.02
Iron (µmol/L)	13 (10-18)	14 (10-18)	0.001
Iron ≤ 13µmol/L	624 (52)	1477 (46)	<0.001
TSAT (%)	25 (18-34)	20 (14-26)	N/A
TSAT <20%	385 (32)	1646 (51)	N/A
Ferritin (µg/L)	147 (82-261)	68 (36-127)	<0.001
Ferritin <30µg/L	20 (2)	608 (19)	<0.001
Ferritin <100µg/L	393 (33)	2113 (66)	<0.001
ESC definition of ID	568 (48)	2443 (76)	<0.001

eGFR (ml/min/1.73m^2)	55 (38-71)	61 (47-76)	<0.001*
hsCRP (mg/L)	5.4 (2.2-15.0)	3.7 (1.5-7.6)	<0.001
NTproBNP (ng/L) - SR	966 (417-2567)	692 (288-1781)	<0.001
NTproBNP (ng/L) - AF/Flutter	2334 (1309-4383)	1882 (1073-3281)	<0.001
Albumin (g/L)	36 (33-38)	38 (36-40)	<0.001
Medication			
Loop diuretic	755 (64)	2070 (65)	0.52
ACEi or ARB	825 (70)	2203 (69)	0.65
MRA	259 (22)	725 (23)	0.56
BB	747 (64)	1981 (62)	0.51
Anticoagulants	305 (26)	1033 (32)	<0.001
Antiplatelets	553 (46)	1375 (43)	0.03
Deaths			
Deaths (1 year)	155 (14%)	281 (10%)	<0.001
CV death (as % of deaths)	56%	62%	0.21
Non-CV deaths (as % of deaths)	41%	35%	0.21
Unknown cause (as % of deaths)	3%	3%	N/A
Cancer deaths (as % of deaths)	17%	9%	0.01
Deaths (5 years)	497 (42%)	1029 (32%)	<0.001
CV death (as % of deaths)	51%	55%	0.15
Non-CV death (as % of deaths)	47%	42%	0.10

Unknown cause (as % of deaths)	2%	3%	N/A
Cancer deaths (as % of deaths)	16%	12%	0.03

Presented as count and (%) or median (Q1-Q3) as appropriate.

Abbreviations - IHD: ischaemic heart disease; COPD: chronic obstructive pulmonary disease; NYHA: New York Heart Association; AF: atrial fibrillation; HFrEF: heart failure with reduced ejection fraction; MCV: mean corpuscular volume; MCH: mean corpuscular haemoglobin; MCHC: mean corpuscular haemoglobin concentration; RDW: red cell distribution width; TSAT: transferrin saturation; ESC: European Society of Cardiology; ID: iron deficiency; eGFR: estimated glomerular filtration rate; hsCRP: high-sensitivity CRP; NTproBNP: N terminal pro-natriuretic peptide; SR: sinus rhythm; ACEi: angiotensin converting enzyme inhibitor; ARB: angiotensin receptor blocker; MRA: mineralocorticoid receptor antagonist; BB: beta-blocker.

Table S19: Characteristics of patients with serum transferrin in the lowest quartile (n= 1,195) according to serum iron values

Variable	Iron ≤13 µmol/L (n=624)	Iron >13 µmol/L (n=571)	p-value
Demographics			
Age (years)	78 (72-83)	76 (69-81)	<0.001
Sex (female)	217 (35)	137 (24)	<0.001
Hypertension	333 (53)	304 (53)	0.97
Diabetes	181 (29)	103 (18)	<0.001
IHD	290 (47)	247 (43)	0.26
COPD	71 (11)	49 (9)	0.11
Symptoms and Signs			
NYHA III or IV	208 (34)	140 (25)	0.001
ECG and ECHO			
AF/flutter	177 (29)	160 (29)	0.87
Heart rate (bpm)	73 (62-83)	69 (60-80)	0.005
HFrEF	180 (28)	202 (35)	0.01
Bloods			
Haemoglobin (g/dL)	12.0 (10.8-13.1)	13.6 (12.5-14.6)	<0.001*
Anaemia	393 (63)	163 (29)	<0.001
MCV (fL)	89 (87-94)	92 (88-96)	<0.001*
MCH (pg)	29 (28-31)	31 (30-32)	<0.001*
MCHC (g/dL)	32 (31-33)	33 (32-34)	<0.001*
RDW (%)	14.6 (13.7-15.6)	13.8 (13.2-14.7)	<0.001
Iron (µmol/L)	10 (7-12)	18 (16-21)	N/A
TSAT (%)	19 (14-22)	34 (29-40)	N/A
Ferritin (µg/L)	132 (78-231)	157 (89-284)	0.002
Ferritin <30µg/L	15 (2)	5 (1)	0.04
Ferritin <100µg/L	229 (37)	164 (29)	0.003
ESC definition	404 (65)	164 (29)	<0.001
eGFR (ml/min/1.73m^2)	48 (33-65)	61 (45-76)	<0.001*

hsCRP (mg/L)	10.0 (4.2-29.0)	2.9 (1.5-5.9)	<0.001
NTproBNP (ng/L) SR	1301 (517-3726)	799 (338-1795)	<0.001
NTproBNP (ng/L) AF/Flutter	3110 (1707-5738)	1837 (1130-2937)	<0.001
Albumin (g/L)	35 (32-37)	37 (35-39)	<0.001
Medication			
Loop diuretic	412 (67)	343 (61)	0.04
ACEi or ARB	394 (64)	431 (77)	<0.001
MRA	95 (15)	164 (29)	<0.001
BB	362 (59)	385 (69)	0.001
Anticoagulants	158 (25)	147 (26)	0.87
Antiplatelets	293 (47)	260 (46)	0.62
Deaths			
Deaths (1 year)	116 (19%)	39 (7%)	<0.001
CV deaths (as % of deaths)	58%	51%	0.48
Non-CV deaths (as % of deaths)	40%	46%	0.48
Unknown cause (as % of deaths)	2%	3%	N/A
Cancer deaths (as % of deaths)	17%	15%	0.79
Deaths (5 years)	312 (50%)	185 (32%)	<0.001
CV deaths (as % of deaths)	50%	51%	0.82
Non-CV deaths (as % of deaths)	47%	47%	0.89

Unknown cause (as % of deaths)	3%	2%	
Cancer deaths (as % of deaths)	16%	16%	0.92

Presented as count and (%) or median (Q1-Q3) as appropriate.

Abbreviations - IHD: ischaemic heart disease; COPD: chronic obstructive pulmonary disease; NYHA: New York Heart Association; AF: atrial fibrillation; HFrEF: heart failure with reduced ejection fraction; MCV: mean corpuscular volume; MCH: mean corpuscular haemoglobin; MCHC: mean corpuscular haemoglobin concentration; RDW: red cell distribution width; TSAT: transferrin saturation; ESC: European Society of Cardiology; ID: iron deficiency; eGFR: estimated glomerular filtration rate; hsCRP: high-sensitivity CRP; NTproBNP: N terminal pro-natriuretic peptide; SR: sinus rhythm; ACEi: angiotensin converting enzyme inhibitor; ARB: angiotensin receptor blocker; MRA: mineralocorticoid receptor antagonist; BB: beta-blocker.

Table S20: Characteristics of patients with the lowest quartile of transferrin (n=1,195) according to serum iron and TSAT (there were no patients with serum iron >13 µmol/L)

Variable	Transferrin ≤ 2.3 g/L + iron >13 µmol/L + TSAT ≥20% (n= 571; 48%)	Transferrin ≤ 2.3 g/L + iron ≤ 13µmol/L + TSAT ≥20% (n=239; 20%)	Transferrin ≤ 2.3 g/L + iron ≤ 13µmol/L + TSAT<20% (n= 385; 32%)	P-value
Demographics				
Age (years)	76 (69-81)	78 (72-83)	79 (72-83)	<0.001
Sex (female)	137 (24)	70 (29)	147 (38)	<0.001
Hypertension	304 (53)	131 (55)	202 (53)	0.85
Diabetes	103 (18)	67 (28)	114 (30)	<0.001
IHD	247 (43)	115 (48)	175 (46)	0.43
COPD	49 (9)	25 (11)	46 (12)	0.23
Symptoms and Signs				
NYHA III or IV	140 (25)	63 (27)	145 (38)	<0.001
ECG and ECHO				
AF/flutter	160 (29)	59 (26)	118 (31)	0.35
Heart rate (bpm)	69 (60-80)	69 (59-80)	75 (65-85)	<0.001
HFrEF	202 (35)	68 (29)	112 (29)	0.05
Bloods				
Haemoglobin (g/dL)	13.6 (12.5-14.6)	12.6 (11.5-13.7)	11.6 (10.6-12.7)	<0.001*
Anaemia	163 (29)	124 (52)	269 (70)	<0.001
MCV (fL)	92 (88-96)	92 (88-95)	89 (86-93)	<0.001*
MCH (pg)	31 (30-32)	30 (29-31)	29 (28-30)	<0.001*
MCHC (g/dL)	33 (32-34)	33 (32-34)	32 (31-33)	<0.001*
RDW (%)	13.8 (13.2-14.7)	14.2 (13.4-15.0)	14.8 (13.9-15.8)	<0.001
Iron (µmol/L)	18 (16-21)	12 (11-13)	8 (6-9)	N/A

TSAT (%)	34 (29-40)	23 (22-25)	16 (12-18)	N/A
Ferritin (µg/L)	157 (89-284)	131 (82-237)	134 (77-231)	0.006
Ferritin <30µg/L	5 (1)	2 (1)	13 (3)	0.01
Ferritin <100µg/L	164 (29)	84 (35)	145 (38)	0.01
ESC definition	164 (29)	84 (35)	320 (83)	<0.001
eGFR (ml/min/1.73m^2)	61 (45-77)	52 (36-66)	46 (32-64)	<0.001
hsCRP (mg/L)	2.9 (1.5-5.9)	6.3 (2.6-14.0)	15.0 (6.1-39.0)	<0.001
NTproBNP (ng/L) SR	799 (338-1795)	989 (389-2699)	1538 (612-4604)	<0.001
NTproBNP (ng/L) AF/Flutter	1837 (1130-2937)	2405 (1281-4000)	3476 (1895-6485)	<0.001
Albumin (g/L)	37 (35-39)	36 (34-38)	34 (30-36)	<0.001
Medication				
Loop diuretic	343 (61)	152 (65)	260 (68)	0.08
ACEi or ARB	431 (77)	165 (71)	229 (60)	<0.001
MRA	164 (29)	37 (16)	58 (15)	<0.001
BB	385 (69)	137 (59)	225 (59)	0.002
Anticoagulants	147 (26)	60 (25)	98 (26)	0.98
Antiplatelets	260 (46)	117 (49)	176 (46)	0.65
Deaths				
Deaths (1 year)	39 (7%)	31 (13%)	85 (22%)	<0.001
CV deaths (as % of deaths)	51%	61%	57%	0.70
Non-CV deaths (as % of deaths)	46%	35%	41%	0.67
Unknown cause (as % of deaths)	3%	4%	2%	N/A

Cancer deaths (as % of deaths)	15%	16%	18%	0.95
Deaths (5 years)	185 (32%)	112 (47%)	200 (51%)	<0.001
CV deaths (as % of deaths)	51%	50%	51%	0.97
Non-CV deaths (as % of deaths)	46%	47%	47%	0.99
Unknown cause (as % of deaths)	3%	3%	2%	N/A
Cancer deaths (as % of deaths)	16%	18%	15%	0.80

Presented as count and (%) or median (Q1-Q3) as appropriate.

Abbreviations - IHD: ischaemic heart disease; COPD: chronic obstructive pulmonary disease; NYHA: New York Heart Association; AF: atrial fibrillation; HFrEF: heart failure with reduced ejection fraction; MCV: mean corpuscular volume; MCH: mean corpuscular haemoglobin; MCHC: mean corpuscular haemoglobin concentration; RDW: red cell distribution width; TSAT: transferrin saturation; ESC: European Society of Cardiology; ID: iron deficiency; eGFR: estimated glomerular filtration rate; hsCRP: high-sensitivity CRP; NTproBNP: N terminal pro-natriuretic peptide; SR: sinus rhythm; ACEi: angiotensin converting enzyme inhibitor; ARB: angiotensin receptor blocker; MRA: mineralocorticoid receptor antagonist; BB: beta-blocker.

Table S21: Univariate and multi-variable associations with serum transferrin

Variable	Unit/ Category	Univariable analysis			Multivariable analysis 1			Multivariable analysis 2		
		ß	B (95% CI)	P value	ß	B (95% CI)	P value	ß	B (95% CI)	P value
Age	/10 years	**-0.09**	**-0.04 (-0.06; -0.03)**	**<0.001**	**-0.11**	**-0.05 (-0.06; -0.04)**	**<0.001**	**-0.14**	**-0.06 (-0.08; -0.05)**	**<0.001**
Sex	Women	**0.17**	**0.17 (0.14; 0.19)**	**<0.001**	**0.07**	**0.07 (0.04; 0.09)**	**<0.001**	**0.09**	**0.09 (0.06; 0.12)**	**<0.001**
IHD	Yes/No	-0.02	-0.02 (-0.05; 0.01)	0.13						
Hypertension	Yes/No	0.02	0.02 (-0.01; 0.05)	0.11						
Diabetes	Yes/No	**0.05**	**0.05 (0.02; 0.08)**	**0.002**	0.02	0.02 (-0.01; 0.05)	0.12	0.02	0.03 (-0.003; 0.06)	0.08
COPD	Yes/No	-0.004	-0.01 (-0.06; 0.04)	0.79						
NYHA	III/IV	**0.06**	**0.06 (0.03; 0.09)**	**<0.001**	**0.05**	**0.06 (0.03; 0.09)**	**<0.001**	**0.04**	**0.05 (0.02; 0.07)**	**0.002**
BMI	5 kg/m^2	**0.09**	**0.04 (0.03; 0.05)**	**<0.001**	**0.04**	**0.01 (0.003; 0.03)**	**0.01**	**0.02**	0.001 (-0.004; 0.017)	0.23
Systolic BP	5 mmHg	**0.06**	**0.006 (0.003; 0.008)**	**<0.001**	-0.01	-0.001 (-0.003; 0.002)	0.48	0.01	0.001 (-0.002; 0.003)	0.69
AF/flutter	Yes/No	**0.10**	**0.11 (0.08; 0.14)**	**<0.001**	**0.11**	**0.11 (0.08; 0.14)**	**<0.001**	**0.12**	**0.13 (0.10; 0.16)**	**<0.001**
HR	10 bpm	**0.12**	**0.03 (0.02; 0.04)**	**<0.001**	**0.08**	**0.02 (0.01; 0.03)**	**<0.001**	**0.06**	**0.02 (0.01; 0.03)**	**<0.001**
HFrEF	Yes/No	0.001	0.001 (-0.03; 0.03)	0.92						
eGFR	10ml/min /1.73m^2	**0.12**	**0.02 (0.01; 0.03)**	**<0.001**	0.03	0.06 (0.00; 0.01)	0.05			
NT-proBNP	(Log 10) 1ng/L	**-0.07**	**-0.06 (-0.09; -0.03)**	**<0.001**	**0.06**	**0.06 (0.03; 0.09)**	**<0.001**	0.01	0.01 (-0.02; 0.04)	0.47
hsCRP	(Log 10) 1mg/L	**-0.17**	**-0.15 (-0.17; -0.12)**	**<0.001**	-0.004	-0.003 (-0.03; 0.03)	0.82			

Albumin	1 g/L	**0.28**	**0.036 (0.032; 0.040)**	**<0.001**	**0.29**	**0.037 (0.033; 0.041)**	**<0.001**			
Haemoglobin	1 g/dL	**0.05**	**0.01 (0.005; 0.021)**	**0.001**	0.03	0.007 (-0.001; 0.016)	0.09	**0.09**	**0.02 (0.02; 0.03)**	**<0.001**
Ferritin	(Log 10) 1µg/L	**-0.52**	**-0.61 (-0.64; -0.58)**	**<0.001**	**-0.52**	**-0.62 (-0.65; -0.59)**	**<0.001**	**-0.55**	**-0.65 (-0.69; -0.62)**	**<0.001**
Iron	(Sq root) 1µmol/L	**-0.05**	**-0.03 (-0.05; -0.01)**	**<0.001**	-0.01	-0.008 (-0.03; 0.01)	0.42	**0.06**	**0.04 (0.02; 0.06)**	**<0.001**

Due to interactions, multivariable model 2 removes eGFR/10, albumin and log 10 hsCRP from the original model.

Bivariate correlations with log10 NT-proBNP: eGFR/10: r=-0.37; albumin: r= -2.9; log10 hsCRP: r=-0.24, all p<0.001

Abbreviations – IHD: ischaemic heart disease; COPD: chronic obstructive pulmonary disease; NYHA: New York Heart Association; BMI: body mass index; BP: blood pressure; HR: heart rate; HFrEF: heart failure with reduced ejection fraction; eFR: estimated glomerular filtration; NT-proBNP: N terminal pro-natriuretic peptide; hsCRP: high sensitivity C-reactive protein.

Table S22: Univariable Cox regression analysis for all-cause and cardiovascular mortality

Variable	Unit/category	All-cause mortality		Cardiovascular mortality	
		HR (95% CI)	p-value	HR (95% CI)	p-value
Age	10 years	1.71 (1.62-1.81)	<0.001	1.60 (1.48-1.73)	<0.001
Sex	Female	0.87 (0.79-0.97)	0.01	0.79 (0.68-0.91)	0.001
Diabetes	Yes/No	1.15 (1.02-1.28)	0.02	1.14 (0.98-1.32)	0.10
IHD	Yes/No	1.05 (0.95-1.16)	0.32	1.28 (1.12-1.46)	<0.001
COPD	Yes/No	1.58 (1.36-1.84)	<0.001	1.33 (1.07-1.66)	0.01
NYHA	I/II vs III/IV	1.98 (1.78-2.19)	<0.001	2.08 (1.81-2.39)	<0.001
BMI	5 kg/m^2	0.81 (0.77-0.85)	<0.001	0.79 (0.74-0.84)	<0.001
Systolic BP	5 mmHg	0.97 (0.96-0.98)	<0.001	0.95 (0.94-0.96)	<0.001
HR	10 bpm	1.07 (1.04-1.10)	<0.001	1.08 (1.04-1.12)	<0.001
AF/flutter	Yes/No	1.28 (1.15-1.43)	<0.001	1.33 (1.15-1.53)	<0.001
HFrEF	vs non-HFrEF	1.17 (1.05-1.30)	0.005	1.54 (1.34-1.78)	<0.001
eGFR	10 $ml/min/1.73m^2$	0.81 (0.79-0.83)	<0.001	0.78 (0.76-0.81)	<0.001
Urea	5 mmol/L	1.38 (1.34-1.42)	<0.001	1.41 (1.35-1.46)	<0.001
Log NT-proBNP	(Log 10) 1 ng/L	3.02 (2.74-3.33)	<0.001	3.87 (3.38-4.44)	<0.001
Log hsCRP	(Log 10) 1 mg/L	1.81 (1.65-1.99)	<0.001	1.58 (1.39-1.80)	<0.001

Albumin	1 g/L	0.88 (0.87-0.89)	<0.001	0.90 (0.89-0.92)	<0.001
Haemoglobin	1 g/dL	0.79 (0.77-0.81)	<0.001	0.80 (0.77-0.83)	<0.001
RDW	1 (%)	1.20 (1.18-1.23)	<0.001	1.20 (1.17-1.24)	<0.001
Ferritin	(Log 10) µg/L	1.27 (1.12-1.44)	<0.001	1.44 (1.21-1.71)	<0.001
Iron	(Sq root) 1 µmol/L	0.67 (0.62-0.71)	<0.001	0.73 (0.67-0.80)	<0.001
Transferrin	1 g/L	0.75 (0.67-0.83)	<0.001	0.85 (0.73-0.99)	0.03
Loop diuretic	Yes/No	1.96 (1.74-2.21)	<0.001	2.23 (1.92-2.70)	<0.001

Presented as count and (%) or median (Q1-Q3) as appropriate.

Abbreviations– IHD: ischaemic heart disease; COPD: chronic obstructive pulmonary disease; NYHA: New York Heart Association; BMI: body mass index; BP: blood pressure; HR: heart rate; AF: atrial fibrillation; HFrEF: heart failure with reduced ejection fraction; eFR: estimated glomerular filtration; NT-proBNP: N terminal pro-natriuretic peptide; hsCRP: high sensitivity C-reactive protein; RDW; red cell distribution width.

Chapter 6

Search terms

Pre-specified search terms: ((Heart failure) and (iron deficiency) OR (iron repletion) OR (intravenous iron) OR (ferric carboxymaltose) OR (iron sucrose) OR (iron supplementation) OR (iron therapy))

Filters: English Language; Clinical trial; Randomized trial

Date range: 1st January 2000 – 5th December 2020

Table S23: Excluded Trials and reasons for exclusion

Reason for exclusion	Trial
Uncontrolled studies (n= 6)	Silverberg DS, Wexler D, Blum M, Tchebiner JZ, Sheps D, Keren G, Schwartz D, Baruch R, Yachnin T, Shaked M, Schwartz I, Steinbruch S, Iaina A. The effect of correction of anaemia in diabetics and non-diabetics with severe resistant congestive heart failure and chronic renal failure by subcutaneous erythropoietin and intravenous iron. Nephrol Dial Transplant. 2003;18(1):141-6. doi: 10.1093/ndt/18.1.141. Silverberg DS, Wexler D, Blum M, Schwartz D, Keren G, Sheps D, Iaina A. Effect of correction of anemia with erythropoietin and intravenous iron in resistant heart failure in octogenarians. Isr Med Assoc J. 2003;5(5):337-9. Bolger AP, Bartlett FR, Penston HS, O'Leary J, Pollock N, Kaprielian R, Chapman CM. Intravenous iron alone for the treatment of anemia in patients with chronic heart failure. J Am Coll Cardiol. 2006;48(6):1225-7. doi:10.1016/j.jacc.2006.07.015. Epub 2006 Aug 28. Usmanov RI, Zueva EB, Silverberg DS, Shaked M. Intravenous iron without erythropoietin for the treatment of iron deficiency anemia in patients with moderate to severe congestive heart failure and chronic kidney insufficiency. J Nephrol. 2008;21(2):236-42. Reed BN, Blair EA, Thudium EM, Waters SB, Sueta CA, Jensen BC, Rodgers JE. Effects of an accelerated intravenous iron regimen in hospitalized patients with advanced heart failure and iron deficiency. Pharmacotherapy. 2015;35(1):64-71. doi: 10.1002/phar.1525. Epub 2014 Dec 29.

	Mirdamadi A, Arefeh A, Garakyaraghi M, Pourmoghadas A. Beneficial effects of the treatment of iron deficiency on clinical condition, left ventricular function, and quality of life in patients with chronic heart failure. Acta Biomed. 2018;89(2):214-218. doi: 10.23750/abm.v89i2.5020.
Iron deficiency not an inclusion criterion (n= 1)	Silverberg DS, Wexler D, Sheps D, Blum M, Keren G, Baruch R, Schwartz D, Yachnin T, Steinbruch S, Shapira I, Laniado S, Iaina A. The effect of correction of mild anemia in severe, resistant congestive heart failure using subcutaneous erythropoietin and intravenous iron: a randomized controlled study. J Am Coll Cardiol. 2001;37(7):1775-80. doi: 10.1016/s0735-1097(01)01248-7.
Oral iron (n= 1)	Lewis GD, Malhotra R, Hernandez AF, McNulty SE, Smith A, Felker GM, Tang WHW, LaRue SJ, Redfield MM, Semigran MJ, Givertz MM, Van Buren P, Whellan D, Anstrom KJ, Shah MR, Desvigne-Nickens P, Butler J, Braunwald E; NHLBI Heart Failure Clinical Research Network. Effect of Oral Iron Repletion on Exercise Capacity in Patients With Heart Failure With Reduced Ejection Fraction and Iron Deficiency: The IRONOUT HF Randomized Clinical Trial. JAMA. 2017;317(19):1958-1966. doi: 10.1001/jama.2017.5427.
First HFH and/or CV mortality not reported (n= 4)	Beck-da-Silva L, Piardi D, Soder S, Rohde LE, Pereira-Barretto AC, de Albuquerque D, Bocchi E, Vilas-Boas F, Moura LZ, Montera MW, Rassi S, Clausell N. IRON-HF study: a randomized trial to assess the effects of iron in heart failure patients with anemia. Int J Cardiol. 2013;168(4):3439-42. doi: 10.1016/j.ijcard.2013.04.181. Epub 2013 May 13. Toblli JE, Di Gennaro F, Rivas C. Changes in Echocardiographic Parameters in Iron Deficiency Patients with Heart Failure and Chronic Kidney Disease Treated with Intravenous Iron. Heart Lung Circ. 2015;24(7):686-95. doi: 10.1016/j.hlc.2014.12.161. Epub 2015 Jan 21.

	Charles-Edwards G, Amaral N, Sleigh A, Ayis S, Catibog N, McDonagh T, Monaghan M, Amin-Youssef G, Kemp GJ, Shah AM, Okonko DO. Effect of Iron Isomaltoside on Skeletal Muscle Energetics in Patients With Chronic Heart Failure and Iron Deficiency. Circulation. 2019;139(21):2386-2398. doi: 10.1161/CIRCULATIONAHA.118.038516. Núñez J, Miñana G, Cardells I, Palau P, Llàcer P, Fácila L, Almenar L, López-Lereu MP, Monmeneu JV, Amiguet M, González J, Serrano A, Montagud V, López-Vilella R, Valero E, García-Blas S, Bodí V, de la Espriella-Juan R, Lupón J, Navarro J, Górriz JL, Sanchis J, Chorro FJ, Comín-Colet J, Bayés-Genís A; Myocardial-IRON Investigators. Noninvasive Imaging Estimation of Myocardial Iron Repletion Following Administration of Intravenous Iron: The Myocardial-IRON Trial. J Am Heart Assoc. 2020;9(4):e014254. doi: 10.1161/JAHA.119.014254. Epub 2020 Feb 13.

Chapter 7

Table S24: Characteristic of patients without heart failure (Not heart failure) - Survivors at 31/12/2014
(All data listed on or before 31/12/2014 unless stated otherwise)

WHO anaemia Class (Nadir 2013 to 2014)	No Hb measured	>4 above	3-4 above	1-3 above	0-1 above	0-1 below	1-2 below	>2 below
N=	40,740 (24%)	1,374 (1%)	6,325 (4%)	55,166 (32%)	34,025 (20%)	17,462 (10%)	9,308 (5%)	8,540 (5%)
Age (years) in 2013	62 (56-70)	62 (55-69)	62 (56-69)	64 (57-72)	67 (59-76)	71 (62-79)	73 (64-80)	73 (65-81)
Sex (women)	20,606 (51%)	448 (33%)	2,498 (39%)	29,812 (54%)	20,848 (61%)	10,927 (63%)	5,592 (60%)	4,549 (53%)
Diabetes or Hypoglycaemic Therapy	4,671 (11%)	239 (17%)	1,090 (17%)	9,847 (18%)	7,019 (21%)	4,313 (25%)	2,569 (28%)	2,411 (28%)
IHD	4,741 (12%)	230 (17%)	1,108 (18%)	11,232 (20%)	8,589 (25%)	4,973 (28%)	2,861 (31%)	2,692 (32%)
COPD	2,430 (6%)	214 (16%)	818 (13%)	6,524 (12%)	4,729 (14%)	2,927 (17%)	1,767 (19%)	1,765 (21%)
eGFR (last available)	82 (72-93)	82 (71-94)	82 (72-93)	81 (70-92)	78 (67-90)	74 (62-87)	72 (57-87)	73 (58-88)
GI disease	679 (2%)	38 (3%)	158 (2%)	1,609 (3%)	1,370 (4%)	923 (5%)	700 (8%)	1,071 (13%)
Any cancer prior to 2013	1,472 (4%)	58 (4%)	246 (4%)	2,960 (5%)	2,377 (7%)	1,555 (9%)	1,011 (11%)	1,101 (13%)
Any incident cancer 2013/14	49 (0%)	11 (1%)	44 (1%)	577 (1%)	630 (2%)	618 (4%)	537 (6%)	910 (11%)
ECG (last result prior to 31/12/2014)								
AF/Flutter	222/3,271 (7%)	45/361 (12%)	138/1,536 (9%)	1,118/15,526 (7%)	887/11,776 (8%)	604/7,276 (8%)	410/4,413 (9%)	466/4,863 (10%)
Haemoglobin prior to 2013								
Hb 4g/dL above WHO	296 (1%)	271 (26%)	266 (5%)	197 (0%)	35 (0%)	18 (0%)	<6 (NA%)	13 (0%)
Hb 3-4g/dL above WHO	1,439 (6%)	360 (35%)	1,401 (29%)	1,813 (4%)	162 (1%)	96 (1%)	30 (0%)	31 (0%)

Hb 1-3g/dL above WHO	12,165 (52%)	324 (31%)	2,794 (57%)	29,661 (65%)	7,586 (25%)	1,866 (12%)	810 (9%)	718 (9%)
Hb 0-1g/dL above WHO	5,835 (25%)	28 (3%)	250 (5%)	10,049 (22%)	13,954 (47%)	4,799 (30%)	1,537 (18%)	1,168 (15%)
Anaemia prior to 2013 (% of those tested)	3,726 (16%)	53 (5%)	203 (4%)	4,243 (9%)	8,265 (28%)	9,226 (58%)	6,238 (72%)	5,850 (75%)
Haematinic profile (Between 01/01/2013 and 31/12/2014)								
Ferritin (median (Q1-Q3))	107 (62-197)	158 (86-291)	128 (75-217)	96 (52-171)	74 (38-141)	61 (26-133)	47 (18-123)	35 (12-120)
Iron (median (Q1-Q3))	16 (12-20)	18 (15-23)	19 (14-24)	16 (12-20)	14 (10-18)	11 (8-16)	9 (6-13)	7 (4-12)
Transferrin (median (Q1-Q3))	2.4 (2.1-2.7)	2.3 (2.1-2.6)	2.5 (2.1-2.7)	2.4 (2.2-2.7)	2.4 (2.0-2.7)	2.3 (2.0-2.7)	2.3 (2.0-2.8)	2.3 (1.8-2.8)
TSAT (median (Q1-Q3))	28 (22-34)	29 (26-37)	30 (22-38)	26 (20-33)	22 (15-31)	19 (12-27)	16 (10-22)	12 (6-21)
B12 (median (Q1-Q3))	313 (234-433)	335 (249-446)	350 (265-464)	344 (259-456)	336 (252-461)	328 (244-447)	320 (238-438)	316 (230-449)
Folate (median (Q1-Q3))	5.7 (4.0-7.8)	3.9 (2.7-6.0)	4.4 (3.1-6.7)	4.9 (3.3-7.4)	5.0 (3.5-7.8)	4.9 (3.3-7.4)	4.7 (3.2-7.5)	4.4 (2.9-7.0)
Serum sodium	139 (137-140)	138 (137-140)	138 (137-140)	138 (137-140)	138 (136-139)	137 (134-139)	136 (133-138)	135 (132-137)
Albumin	38 (36-40)	38 (36-40)	38 (36-40)	37 (36-39)	36 (34-38)	35 (33-37)	34 (31-36)	31 (27-35)
Prescriptions (on 01/01/2015)								
Iron (oral)	149 (0%)	<6 (NA%)	26 (0%)	493 (1%)	887 (3%)	1,314 (8%)	1,717 (18%)	2,901 (34%)
B12	259 (1%)	18 (1%)	72 (1%)	941 (2%)	1,054 (3%)	825 (5%)	632 (7%)	704 (8%)
Folate	272 (1%)	59 (4%)	189 (3%)	1,807 (3%)	1,889 (6%)	1,663 (10%)	1,370 (15%)	1,690 (20%)
Loop diuretics	1,632 (4%)	87 (6%)	382 (6%)	4,197 (8%)	3,843 (11%)	2,593 (15%)	1,775 (19%)	1,911 (22%)

ACEi/ARB	18,629 (46%)	681 (50%)	3,285 (52%)	28,782 (52%)	18,284 (54%)	9,578 (55%)	5,076 (55%)	4,242 (50%)
BB	11,367 (28%)	412 (30%)	1,991 (31%)	18,206 (33%)	12,061 (35%)	6,092 (35%)	3,265 (35%)	2,962 (35%)
MRA	115 (0%)	14 (1%)	35 (1%)	337 (1%)	311 (1%)	188 (1%)	142 (2%)	248 (3%)
Antiplatelets	8,303 (20%)	417 (30%)	1,907 (30%)	18,245 (33%)	13,503 (40%)	7,892 (45%)	4,439 (48%)	3,986 (47%)
OAC	890 (2%)	95 (7%)	312 (5%)	2,813 (5%)	2,043 (6%)	1,210 (7%)	751 (8%)	746 (9%)
NSAID	4,889 (12%)	182 (13%)	863 (14%)	8,641 (16%)	5,647 (17%)	2,793 (16%)	1,436 (15%)	1,140 (13%)
Insulin	559 (1%)	14 (1%)	86 (1%)	942 (2%)	874 (3%)	666 (4%)	422 (5%)	394 (5%)
Other hypoglycaemics	2,985 (7%)	148 (11%)	685 (11%)	6,491 (12%)	4,897 (14%)	3,217 (18%)	1,914 (21%)	1,746 (20%)
PPI/H2 antagonists	12,745 (31%)	540 (39%)	2,769 (44%)	27,565 (50%)	19,986 (59%)	11,350 (65%)	6,565 (71%)	6,472 (76%)
Deaths 2015-2018								
All	2,340 (6%)	134 (10%)	454 (7%)	3,631 (7%)	3,648 (11%)	2,940 (17%)	2,286 (25%)	2,827 (33%)
Age at death	79 (70-87)	74 (66-81)	75 (67-83)	78 (70-86)	82 (74-88)	83 (76-89)	83 (76-89)	82 (74-88)
Cancer	702 (30%)	32 (24%)	136 (30%)	1,126 (31%)	991 (27%)	751 (26%)	598 (26%)	977 (35%)
GI cancer	195 (8%)	7 (5%)	36 (8%)	266 (7%)	253 (7%)	176 (6%)	132 (6%)	302 (11%)
CVD	692 (30%)	41 (31%)	122 (27%)	1,022 (28%)	1,016 (28%)	780 (27%)	598 (26%)	613 (22%)
Neuro	288 (12%)	8 (6%)	31 (7%)	448 (12%)	575 (16%)	515 (18%)	368 (16%)	355 (13%)
Chronic Resp	182 (8%)	26 (19%)	77 (17%)	408 (11%)	408 (11%)	315 (11%)	250 (11%)	277 (10%)
Infection	197 (8%)	15 (11%)	38 (8%)	272 (7%)	289 (8%)	272 (9%)	214 (9%)	282 (10%)
Other	281 (12%)	12 (9%)	50 (11%)	357 (10%)	373 (10%)	307 (10%)	260 (11%)	326 (12%)

Presented as count and (%) or median (Q1-Q3) as appropriate.

Abbreviations – IHD: ischaemic heart disease; COPD: chronic obstructive pulmonary disease; eGFR: estimated glomerular filtration rate; GI: gastrointestinal;

ECG: electrocardiogram; AF: atrial fibrillation; ACEi/ARB: angiotensin converting enzyme inhibitor/angiotensin receptor blocker; BB: beta-blocker; MRA: mineralocorticoid receptor antagonist; OAC: oral anticoagulant; NSAID: non-steroidal anti-inflammatory drugs; PPI: proton pump inhibitors; CVD: cardiovascular disease

Sodium-glucose Co-transporter 2 inhibitors were used in ≤1% of individuals in all categories

Blood results reported are the nadir result unless otherwise stated. The presence of any of anaemia or AF/flutter are reported.

Table S25: Characteristic of patients with new onset heart failure (Incident heart failure) - Survivors at 31/12/2014
(All data listed on or before 31/12/2014 unless stated otherwise)

WHO anaemia Class (Nadir 2013 to 2014)	No Hb measured	>4 above	3-4 above	1-3 above	0-1 above	0-1 below	1-2 below	>2 below
N=	72 (3%)	13 (0%)	69 (2%)	582 (21%)	622 (22%)	483 (17%)	390 (14%)	545 (20%)
Age (years) in 2013	68 (62-76)	68 (59-75)	66 (56-74)	67 (60-75)	72 (63-80)	75 (67-82)	77 (70-84)	77 (70-83)
Sex (women)	23 (32%)	7 (54%)	15 (22%)	226 (39%)	301 (48%)	252 (52%)	218 (56%)	249 (46%)
Diabetes	17 (24%)	<6 (NA%)	<6 (NA%)	107 (18%)	140 (23%)	120 (25%)	112 (29%)	175 (32%)
IHD	52 (72%)	<6 (NA%)	34 (49%)	359 (62%)	405 (65%)	316 (65%)	246 (63%)	344 (63%)
COPD	13 (18%)	6 (46%)	16 (23%)	144 (25%)	191 (31%)	134 (28%)	107 (27%)	183 (34%)
eGFR (last available)	80 (66-89)	78 (68-89)	77 (65-96)	76 (65-89)	72 (59-86)	67 (52-81)	62 (49-78)	62 (47-77)
GI disease	<6 (NA%)	0 (0%)	<6 (NA%)	28 (5%)	36 (6%)	23 (5%)	21 (5%)	70 (13%)
Any cancer prior to 2013	<6 (NA%)	0 (0%)	<6 (NA%)	31 (5%)	50 (8%)	33 (7%)	37 (9%)	59 (11%)
Any incident cancer 2013/14	0 (0%)	0 (0%)	0 (0%)	<6 (NA%)	17 (3%)	14 (3%)	18 (5%)	62 (11%)
ECG (last result prior to 31/12/2014)								
AF/Flutter	<6/20 (NA%)	<6/8 (NA%)	15/49 (31%)	139/408 (34%)	115/426 (27%)	104/362 (29%)	77/277 (28%)	130/442 (29%)
Haemoglobin prior to 2013								
Hb 4g/dL above WHO	<6 (NA%)	NA	<6 (NA%)	10 (2%)	<6 (NA%)	NA	NA	NA
Hb 3-4g/dL above WHO	<6 (NA%)	<6 (NA%)	17 (36%)	38 (8%)	11 (2%)	<6 (NA%)	<6 (NA%)	<6 (NA%)
Hb 1-3g/dL above WHO	10 (22%)	<6 (NA%)	19 (40%)	272 (60%)	202 (39%)	111 (27%)	51 (14%)	57 (12%)
Hb 0-1g/dL above WHO	16 (35%)	NA	<6 (NA%)	79 (18%)	164 (31%)	130 (31%)	79 (22%)	78 (16%)

Anaemia prior to 2013 (% of those tested)	14 (30%)	NA	<6 (NA%)	52 (12%)	139 (27%)	170 (41%)	224 (63%)	358 (72%)
Haematinic profile (Between 01/01/2013 and 31/12/2014)								
Ferritin (median (Q1-Q3))	298 (298-298)	113 (84-252)	164 (110-211)	128 (64-243)	88 (44-178)	72 (29-178)	58 (26-142)	45 (20-116)
Iron (median (Q1-Q3))	NA	13 (12-20)	14 (9-21)	12 (9-17)	10 (6-15)	9 (6-12)	8 (5-11)	6 (4-9)
Transferrin (median (Q1-Q3))	NA	2.2 (1.9-2.8)	2.4 (2.1-3.0)	2.6 (2.3-2.8)	2.3 (2.0-2.6)	2.3 (2.0-2.7)	2.3 (2.0-2.7)	2.4 (2.0-3.0)
TSAT (median (Q1-Q3))	NA	27 (23-29)	27 (17-32)	18 (13-29)	18 (11-24)	16 (11-21)	13 (9-20)	10 (6-17)
B12 (median (Q1-Q3))	NA	412 (378-760)	368 (222-439)	338 (263-452)	361 (274-477)	352 (258-448)	328 (248-422)	331 (226-464)
Folate (median (Q1-Q3))	NA	4.1 (3.0-4.8)	3.1 (2.6-4.3)	5.0 (3.3-6.9)	4.3 (2.8-6.6)	4.8 (3.3-7.3)	4.3 (2.9-6.7)	4.1 (2.9-6.3)
Serum sodium	138 (137-140)	138 (135-140)	137 (135-138)	137 (135-139)	136 (134-138)	135 (133-138)	135 (132-137)	135 (131-137)
Albumin	38 (36 - 39)	35 (33 - 37)	35 (34 - 37)	36 (33 - 38)	34 (32 - 36)	33 (31 - 35)	32 (29 - 34)	30 (27 - 33)
Prescriptions (on 01/01/2015)								
Iron (oral)	<6 (NA%)	<6 (NA%)	0 (0%)	11 (2%)	17 (3%)	47 (10%)	77 (20%)	226 (41%)
B12	<6 (NA%)	0 (0%)	0 (0%)	8 (1%)	23 (4%)	21 (4%)	19 (5%)	44 (8%)
Folate	<6 (NA%)	<6 (NA%)	<6 (NA%)	20 (3%)	48 (8%)	42 (9%)	63 (16%)	98 (18%)
Loop diuretics	15 (21%)	6 (46%)	31 (45%)	285 (49%)	333 (54%)	308 (64%)	270 (69%)	399 (73%)
ACEi/ARB	44 (61%)	7 (54%)	48 (70%)	451 (77%)	447 (72%)	337 (70%)	265 (68%)	338 (62%)
BB	44 (61%)	<6 (NA%)	47 (68%)	423 (73%)	415 (67%)	332 (69%)	245 (63%)	349 (64%)

MRA	<6 (NA%)	<6 (NA%)	9 (13%)	88 (15%)	86 (14%)	90 (19%)	63 (16%)	97 (18%)
Antiplatelets	40 (56%)	6 (46%)	39 (57%)	336 (58%)	397 (64%)	300 (62%)	255 (65%)	331 (61%)
OAC	11 (15%)	<6 (NA%)	21 (30%)	201 (35%)	180 (29%)	143 (30%)	110 (28%)	150 (28%)
NSAID	7 (10%)	<6 (NA%)	7 (10%)	31 (5%)	41 (7%)	28 (6%)	19 (5%)	28 (5%)
Insulin	<6 (NA%)	0 (0%)	0 (0%)	10 (2%)	17 (3%)	21 (4%)	20 (5%)	33 (6%)
Other hypoglycaemics	9 (12%)	<6 (NA%)	<6 (NA%)	55 (9%)	96 (15%)	88 (18%)	71 (18%)	116 (21%)
PPI/H2 antagonists	26 (36%)	<6 (NA%)	26 (38%)	318 (55%)	374 (60%)	322 (67%)	305 (78%)	423 (78%)
Deaths 2015-2018								
All	7 (10%)	<6 (NA%)	8 (12%)	88 (15%)	147 (24%)	162 (34%)	160 (41%)	259 (48%)
Age at death	84 (83-90)	80 (76-86)	77 (74-85)	80 (73-86)	82 (75-89)	84 (77-89)	86 (79-91)	85 (79-89)
Cancer	<6 (NA%)	0 (0%)	<6 (NA%)	16 (18%)	22 (15%)	25 (15%)	24 (15%)	46 (18%)
GI cancer	0 (0%)	NA	<6 (NA%)	<6 (NA%)	<6 (NA%)	<6 (NA%)	7 (4%)	14 (5%)
CVD	<6 (NA%)	<6 (NA%)	<6 (NA%)	33 (38%)	56 (38%)	65 (40%)	58 (36%)	105 (41%)
Neuro	<6 (NA%)	0 (0%)	<6 (NA%)	9 (10%)	18 (12%)	9 (6%)	22 (14%)	24 (9%)
Chronic Resp	0 (0%)	0 (0%)	<6 (NA%)	20 (23%)	31 (21%)	27 (17%)	23 (14%)	34 (13%)
Infection	0 (0%)	0 (0%)	0 (0%)	<6 (NA%)	7 (5%)	18 (11%)	15 (9%)	28 (11%)
Other	0 (0%)	0 (0%)	0 (0%)	6 (7%)	14 (10%)	18 (11%)	18 (11%)	22 (8%)

Presented as count and (%) or median (Q1-Q3) as appropriate.

Abbreviations – IHD: ischaemic heart disease; COPD: chronic obstructive pulmonary disease; eGFR: estimated glomerular filtration rate; GI: gastrointestinal; ECG: electrocardiogram; AF: atrial fibrillation; ACEi/ARB: angiotensin converting enzyme inhibitor/angiotensin receptor blocker; BB: beta-blocker; MRA: mineralocorticoid receptor antagonist; OAC: oral anticoagulant; NSAID: non-steroidal anti-inflammatory drugs; PPI: proton pump inhibitors; CVD: cardiovascular disease

Sodium-glucose Co-transporter 2 inhibitors were used in ≤1% of individuals in all categories

Blood results reported are the nadir result unless otherwise stated. The presence of any of anaemia or AF/flutter are reported.

Table S26: Characteristic of patients with known heart failure (Prevalent heart failure) - Survivors at 31/12/2014
(all data listed on or before 31/12/2014 unless stated otherwise)

WHO anaemia Class (Nadir 2013 to 2014)	No Hb measured	>4 above	3-4 above	1-3 above	0-1 above	0-1 below	1-2 below	>2 below
N=	1,093 (12%)	61 (1%)	261 (3%)	2,315 (26%)	1,904 (21%)	1,402 (16%)	901 (10%)	962 (11%)
Age (years) in 2013	68 (61-76)	62 (56-70)	66 (59-74)	68 (61-76)	73 (65-80)	76 (68-81)	77 (69-82)	77 (70-83)
Sex (women)	350 (32%)	6 (10%)	68 (26%)	777 (34%)	857 (45%)	643 (46%)	402 (45%)	375 (39%)
Diabetes	216 (20%)	11 (18%)	63 (24%)	569 (25%)	461 (24%)	403 (29%)	286 (32%)	344 (36%)
IHD	747 (68%)	39 (64%)	200 (77%)	1,746 (75%)	1,481 (78%)	1,052 (75%)	695 (77%)	749 (78%)
COPD	172 (16%)	14 (23%)	68 (26%)	519 (22%)	526 (28%)	393 (28%)	274 (30%)	314 (33%)
eGFR (last available)	78 (65-91)	83 (69-94)	78 (64-90)	76 (64-90)	71 (57-85)	69 (53-84)	64 (49-79)	62 (47-77)
GI disease	37 (3%)	<6 (NA%)	8 (3%)	89 (4%)	117 (6%)	84 (6%)	88 (10%)	122 (13%)
Any cancer prior to 2013	55 (5%)	<6 (NA%)	15 (6%)	138 (6%)	184 (10%)	130 (9%)	116 (13%)	113 (12%)
Any incident cancer 2013/14	<6 (NA%)	<6 (NA%)	<6 (NA%)	25 (1%)	30 (2%)	41 (3%)	46 (5%)	69 (7%)
ECG (last result prior to 31/12/2014)								
AF/Flutter	41/204 (20%)	<6/16 (NA%)	23/79 (29%)	222/918 (24%)	185/867 (21%)	167/742 (23%)	142/537 (26%)	177/647 (27%)
Haemoglobin prior to 2013								
Hb 4g/dL above WHO	17 (2%)	14 (26%)	7 (3%)	10 (0%)	<6 (NA%)	<6 (NA%)	NA	<6 (NA%)
Hb 3-4g/dL above WHO	35 (4%)	20 (38%)	50 (21%)	67 (3%)	11 (1%)	<6 (NA%)	<6 (NA%)	<6 (NA%)
Hb 1-3g/dL above WHO	327 (41%)	11 (21%)	132 (55%)	1,198 (56%)	356 (20%)	121 (9%)	48 (6%)	36 (4%)
Hb 0-1g/dL above WHO	178 (22%)	<6 (NA%)	29 (12%)	508 (24%)	740 (41%)	346 (26%)	134 (15%)	72 (8%)

Anaemia prior to 2013 (% of those tested)	241 (30%)	6 (11%)	20 (8%)	365 (17%)	706 (39%)	870 (65%)	684 (79%)	822 (88%)
Haematinic profile (Between 01/01/2013 and 31/12/2014)								
Ferritin (median (Q1-Q3))	128 (87-246)	146 (62-208)	122 (72-249)	109 (57-220)	85 (40-164)	72 (31-152)	54 (24-131)	43 (16-112)
Iron (median (Q1-Q3))	18 (15-22)	NA	18 (14-23)	14 (10-20)	12 (9-16)	12 (8-16)	9 (6-13)	6 (4-10)
Transferrin (median (Q1-Q3))	2.1 (1.9-2.3)	NA	2.5 (2.2-2.7)	2.5 (2.0-2.8)	2.4 (2.0-2.7)	2.3 (2.0-2.7)	2.4 (2.0-2.8)	2.3 (1.8-2.8)
TSAT (median Q1-Q3))	33 (29-37)	NA	23 (23-36)	23 (17-33)	20 (15-26)	19 (13-27)	16 (10-23)	11 (6-19)
B12 (median (Q1-Q3))	285 (192-365)	372 (276-476)	350 (282-408)	334 (255-447)	322 (245-436)	330 (244-452)	316 (242-461)	332 (237-460)
Folate (median (Q1-Q3))	8.8 (5.4-11.0)	4.2 (2.7-5.6)	4.0 (3.0-5.8)	4.6 (3.3-6.8)	4.6 (3.2-7.2)	4.4 (3.2-7.0)	4.6 (3.2-7.0)	4.5 (3.1-7.2)
Serum sodium	139 (137-140)	138 (136-139)	138 (136-140)	138 (136-139)	137 (135-139)	136 (134-38)	136 (133-138)	135 (132-137)
Albumin	37 (36-39)	37 (35-39)	37 (35-39)	37 (35-38)	36 (33-37)	34 (32-37)	33 (30-35)	31 (28-34)
Prescriptions (on 01/01/2015)								
Iron (oral)	25 (2%)	0 (0%)	<6 (NA%)	40 (2%)	77 (4%)	126 (9%)	166 (18%)	380 (40%)
B12	15 (1%)	<6 (NA%)	<6 (NA%)	43 (2%)	66 (3%)	78 (6%)	62 (7%)	90 (9%)
Folate	26 (2%)	<6 (NA%)	8 (3%)	95 (4%)	139 (7%)	150 (11%)	122 (14%)	223 (23%)
Loop diuretics	345 (32%)	24 (39%)	94 (36%)	945 (41%)	899 (47%)	757 (54%)	521 (58%)	640 (67%)
ACEi/ARB	818 (75%)	49 (80%)	200 (77%)	1,837 (79%)	1,483 (78%)	1,051 (75%)	609 (68%)	616 (64%)
BB	747 (68%)	42 (69%)	199 (76%)	1,645 (71%)	1,316 (69%)	891 (64%)	591 (66%)	574 (60%)

MRA	73 (7%)	6 (10%)	28 (11%)	269 (12%)	216 (11%)	201 (14%)	115 (13%)	129 (13%)
Antiplatelets	684 (63%)	33 (54%)	170 (65%)	1,482 (64%)	1,289 (68%)	887 (63%)	575 (64%)	572 (59%)
OAC	192 (18%)	19 (31%)	81 (31%)	602 (26%)	456 (24%)	375 (27%)	245 (27%)	278 (29%)
NSAID	40 (4%)	<6 (NA%)	10 (4%)	134 (6%)	126 (7%)	89 (6%)	54 (6%)	57 (6%)
Insulin	29 (3%)	<6 (NA%)	8 (3%)	71 (3%)	80 (4%)	76 (5%)	66 (7%)	76 (8%)
Other hypoglycaemics	132 (12%)	7 (11%)	35 (13%)	345 (15%)	299 (16%)	286 (20%)	172 (19%)	241 (25%)
PPI/H2 antagonists	427 (39%)	31 (51%)	119 (46%)	1,294 (56%)	1,210 (64%)	955 (68%)	653 (72%)	758 (79%)
Deaths 2015-2018								
All	142 (13%)	7 (11%)	32 (12%)	345 (15%)	401 (21%)	376 (27%)	295 (33%)	432 (45%)
Age at death	84 (74 - 89)	59 (57 - 60)	74 (67 - 82)	81 (74 - 88)	83 (77 - 90)	84 (78 - 90)	84 (77 - 90)	83 (77 - 89)
Cancer	22 (15%)	<6 (NA%)	<6 (NA%)	72 (21%)	60 (15%)	55 (15%)	47 (16%)	85 (20%)
GI cancer	8 (6%)	0 (0%)	<6 (NA%)	14 (4%)	14 (3%)	12 (3%)	14 (5%)	27 (6%)
CVD	51 (36%)	<6 (NA%)	15 (47%)	129 (37%)	164 (41%)	155 (41%)	129 (44%)	149 (34%)
Neuro	22 (15%)	0 (0%)	6 (19%)	38 (11%)	47 (12%)	39 (10%)	43 (15%)	37 (9%)
Chronic Resp	15 (11%)	0 (0%)	<6 (NA%)	47 (14%)	65 (16%)	48 (13%)	32 (11%)	55 (13%)
Infection	15 (11%)	0 (0%)	<6 (NA%)	28 (8%)	37 (9%)	32 (9%)	24 (8%)	48 (11%)
Other	17 (12%)	<6 (NA%)	<6 (NA%)	31 (9%)	28 (7%)	47 (12%)	20 (7%)	60 (14%)

Presented as count and (%) or median (Q1-Q3) as appropriate.

Abbreviations – IHD: ischaemic heart disease; COPD: chronic obstructive pulmonary disease; eGFR: estimated glomerular filtration rate; GI: gastrointestinal; ECG: electrocardiogram; AF: atrial fibrillation; ACEi/ARB: angiotensin converting enzyme inhibitor/angiotensin receptor blocker; BB: beta-blocker; MRA: mineralocorticoid receptor antagonist; OAC: oral anticoagulant; NSAID: non-

steroidal anti-inflammatory drugs; PPI: proton pump inhibitors; CVD: cardiovascular disease

Sodium-glucose Co-transporter 2 inhibitors were used in ≤1% of individuals in all categories

Blood results reported are the nadir result unless otherwise stated. The presence of any of anaemia or AF/flutter are reported.

Supplementary Figures

Chapter 3

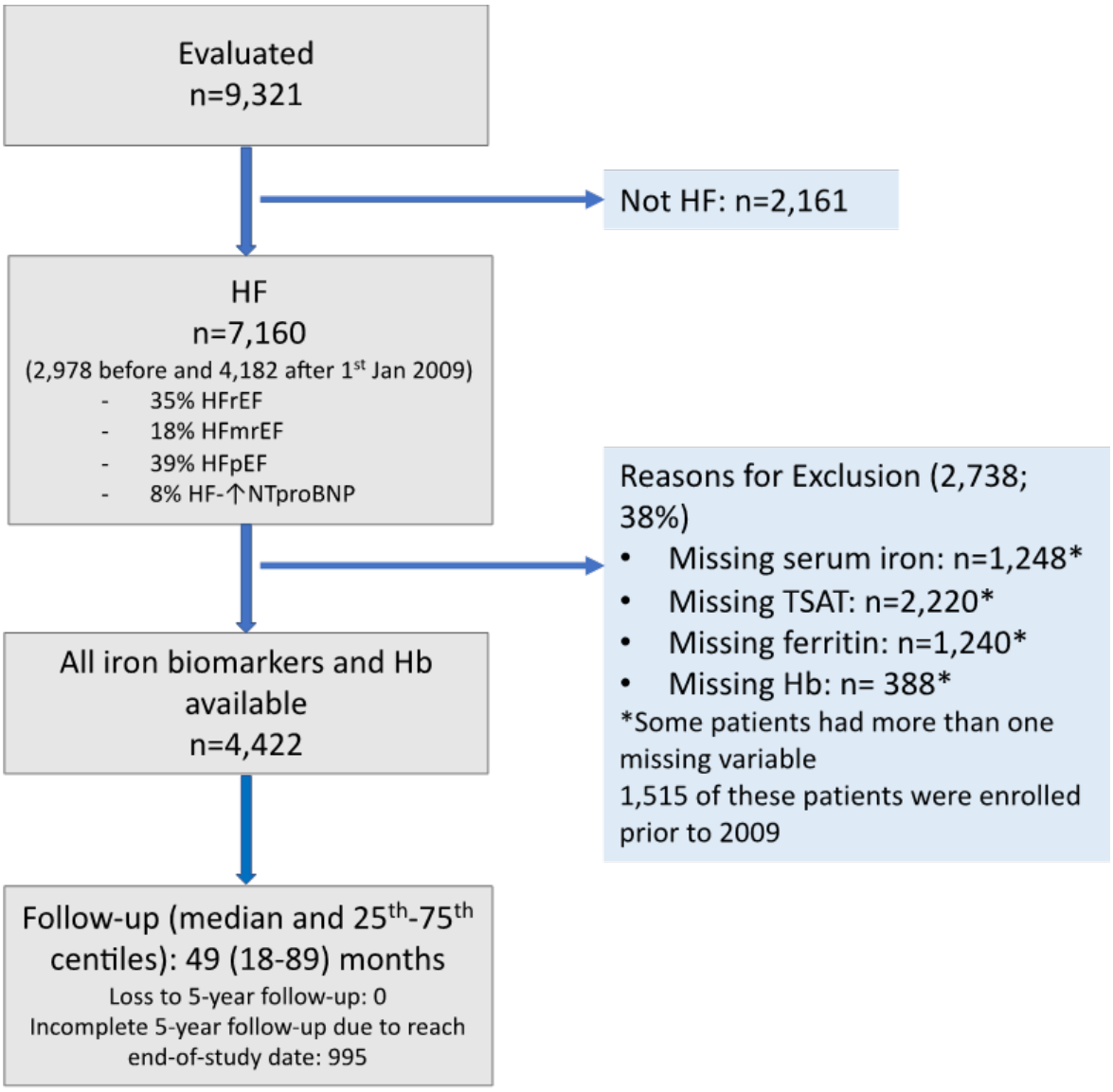


Figure S1: Flow chart detailing patients included and reasons for exclusion

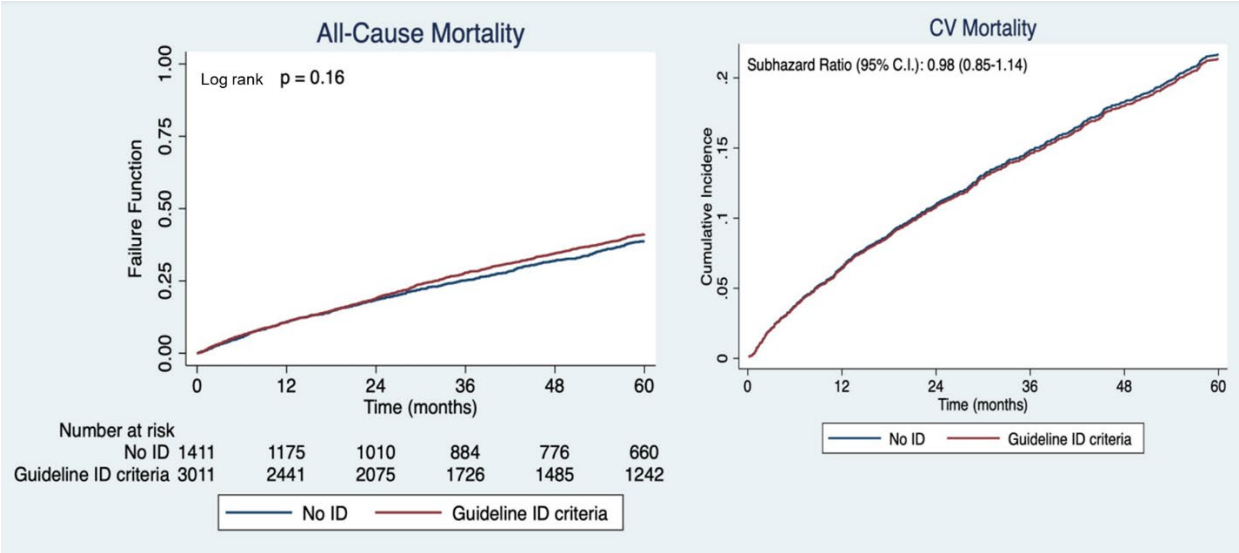


Figure S2: Kaplan-Meier survival curve for all-cause mortality and cumulative incidence of cardiovascular mortality, with log-rank and subhazard ratios respectively, by presence or absence of guideline definition of iron deficiency.

Fine-Gray method used for Cardiovascular mortality.

Abbreviations: - CV: Cardiovascular; ID: iron deficiency

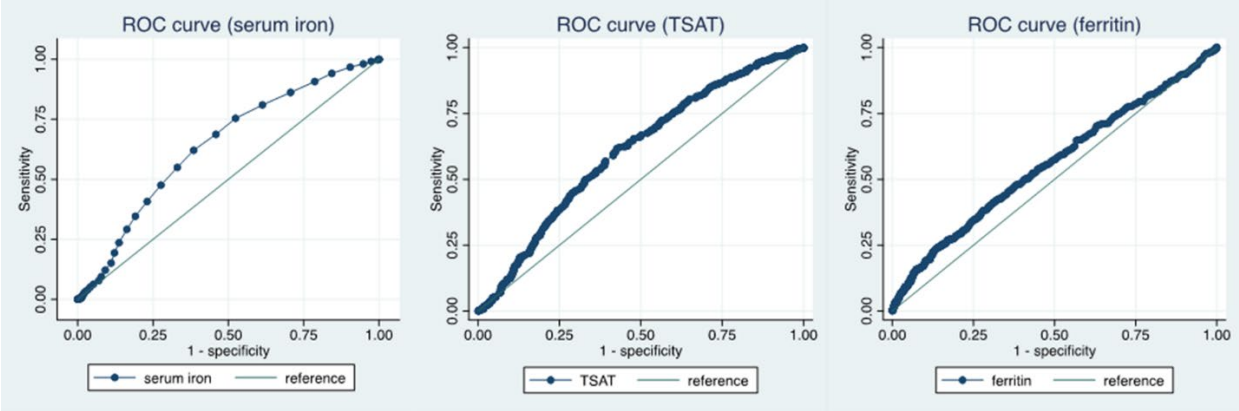


Figure S3: ROC curves (1-year mortality) for each iron biomarker.
Abbreviations: - TSAT: transferrin saturation.

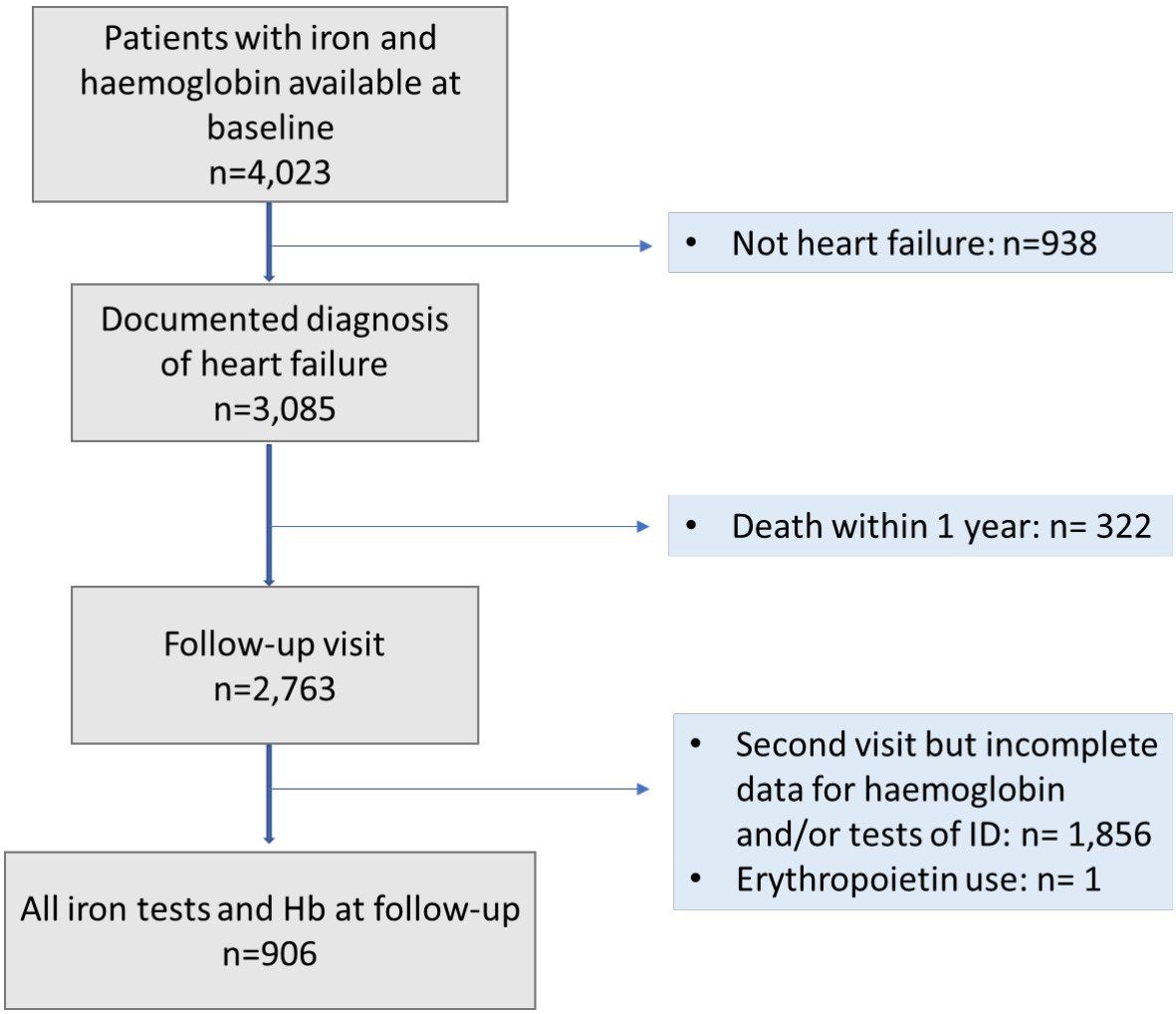


Figure S4: Study flow chart detailing patients included and those excluded including numbers and justification for exclusion

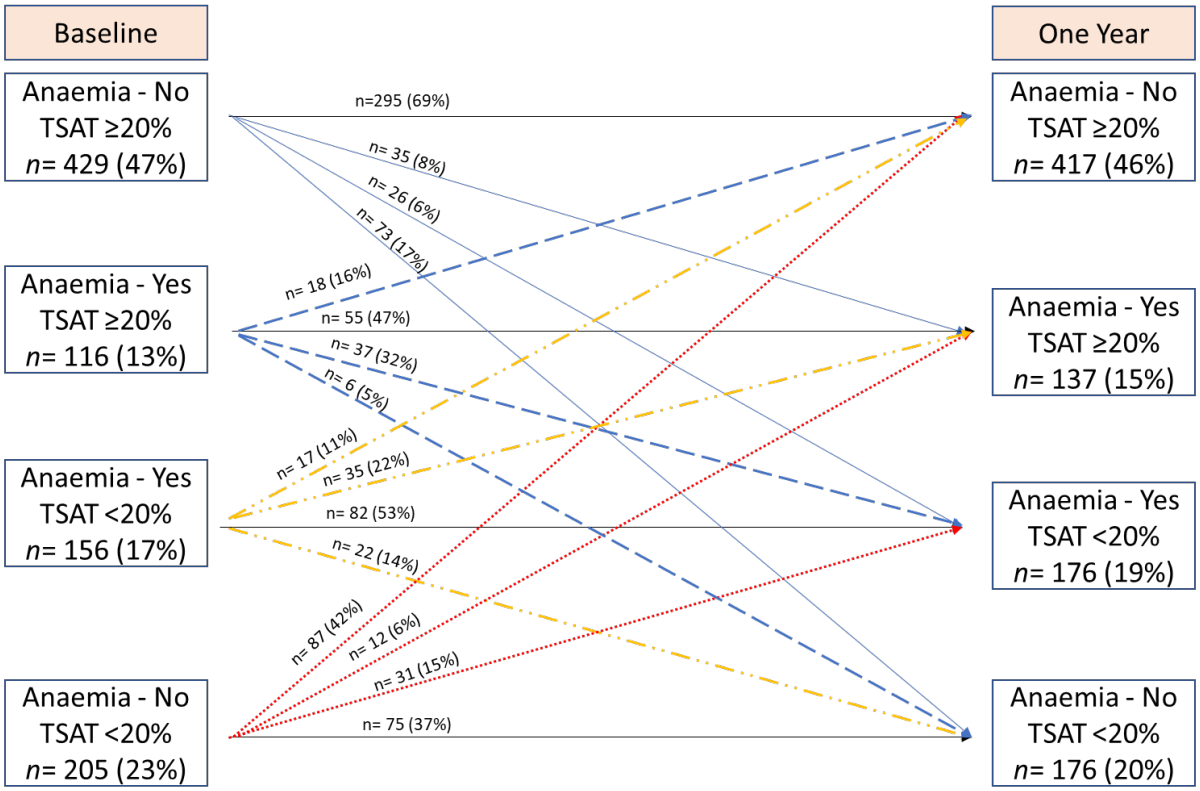


Figure S5: Classification of patients according to iron deficiency, defined by TSAT <20%, and anaemia at baseline and one year follow-up.

Number and (%) on each line represent the count and (%) of patients moving between groups from baseline to follow-up. Anaemia defined by Hb <13.0g/dL in men and <12.0g/dL in women.

Abbreviations: - TSAT: transferrin saturation.

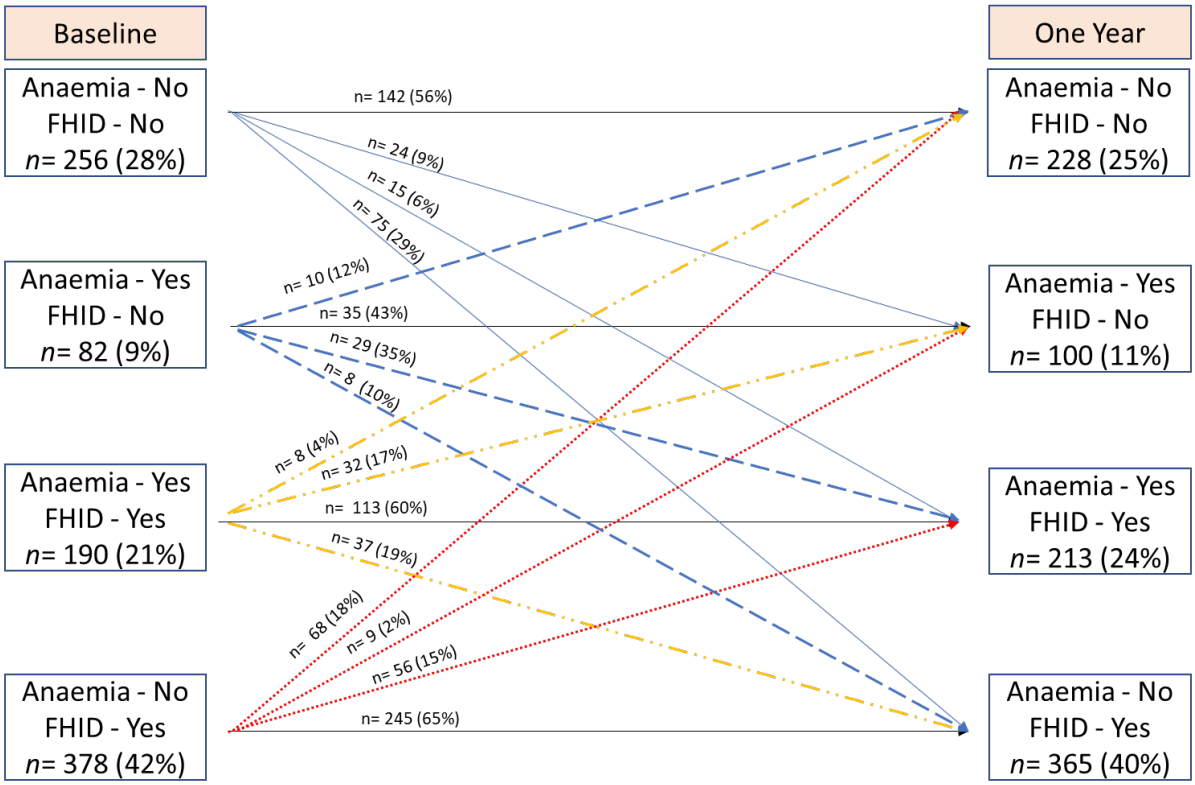
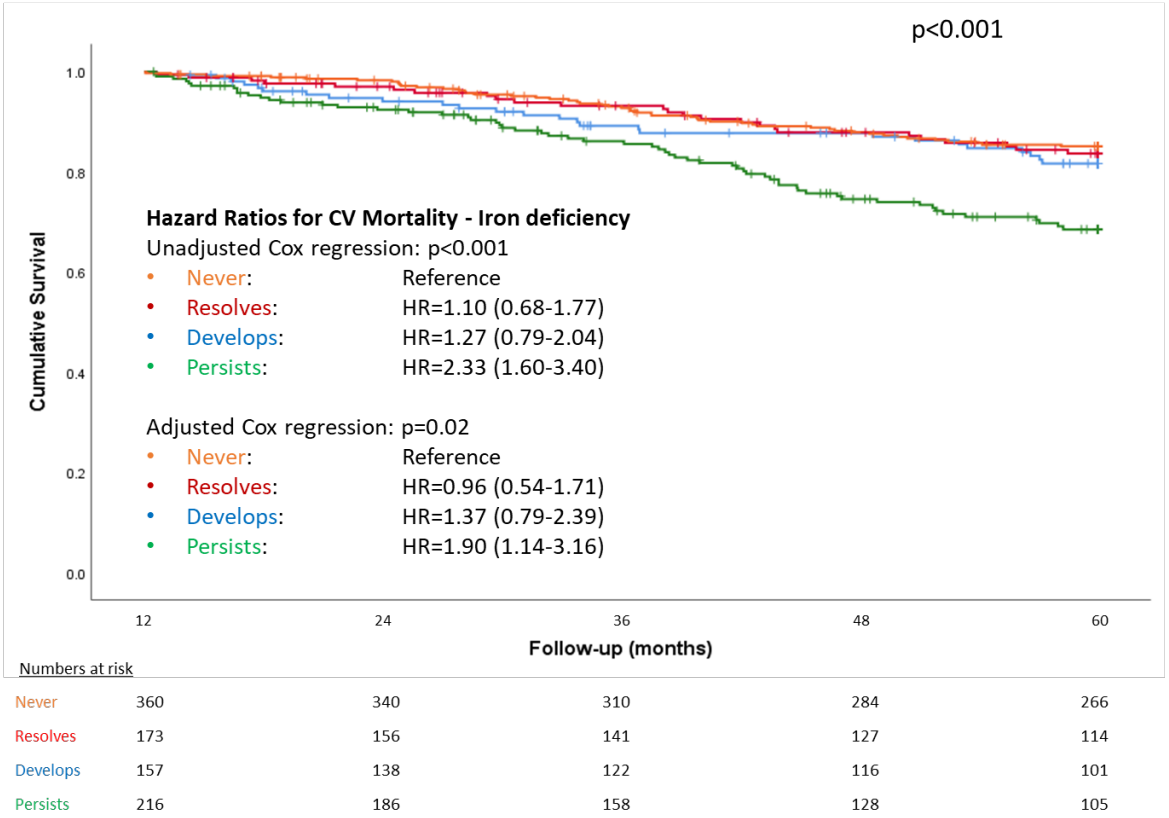


Figure S6: Classification of patients according to iron deficiency, defined by FAIR-HF/guideline criteria (FHID), and anaemia at baseline and one year follow-up. FAIR-HF criteria: ferritin <100µg/L or TSAT <20% if ferritin 100-299µg/L.

Number and (%) on each line represent the count and (%) of patients moving between groups from baseline to follow-up. Anaemia defined by Hb <13.0g/dL in men and <12.0g/dL in women.

Abbreviations: - TSAT: transferrin saturation.



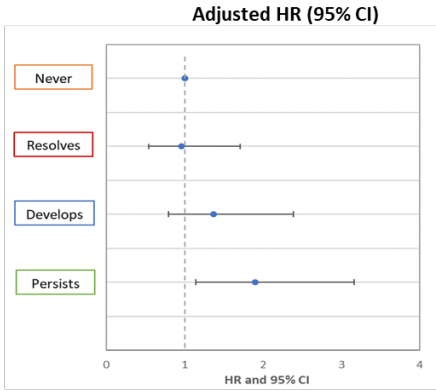


Figure S7: Kaplan-Meir survival analysis of cardiovascular mortality 5 years from baseline visit according to whether iron deficiency (serum iron ≤13 µmol/L) was never present at either baseline or one year, or whether it developed, resolved, or persisted.
Never developing iron deficiency used as the reference. Differences between changes in status compared using the log-rank test. Unadjusted and adjusted hazard ratios with (95% confidence intervals) also reported. Models adjusted for age, sex, diabetes, ischaemic heart disease, NYHA class, BMI, AF/flutter, heart rate, systolic blood pressure, left ventricular phenotype, log NT-proBNP, log hsCRP, eGFR, therapy with loop diuretics and baseline haemoglobin. Forest plots of hazard ratios with 95% confidence intervals for each group in unadjusted and adjusted models also presented.

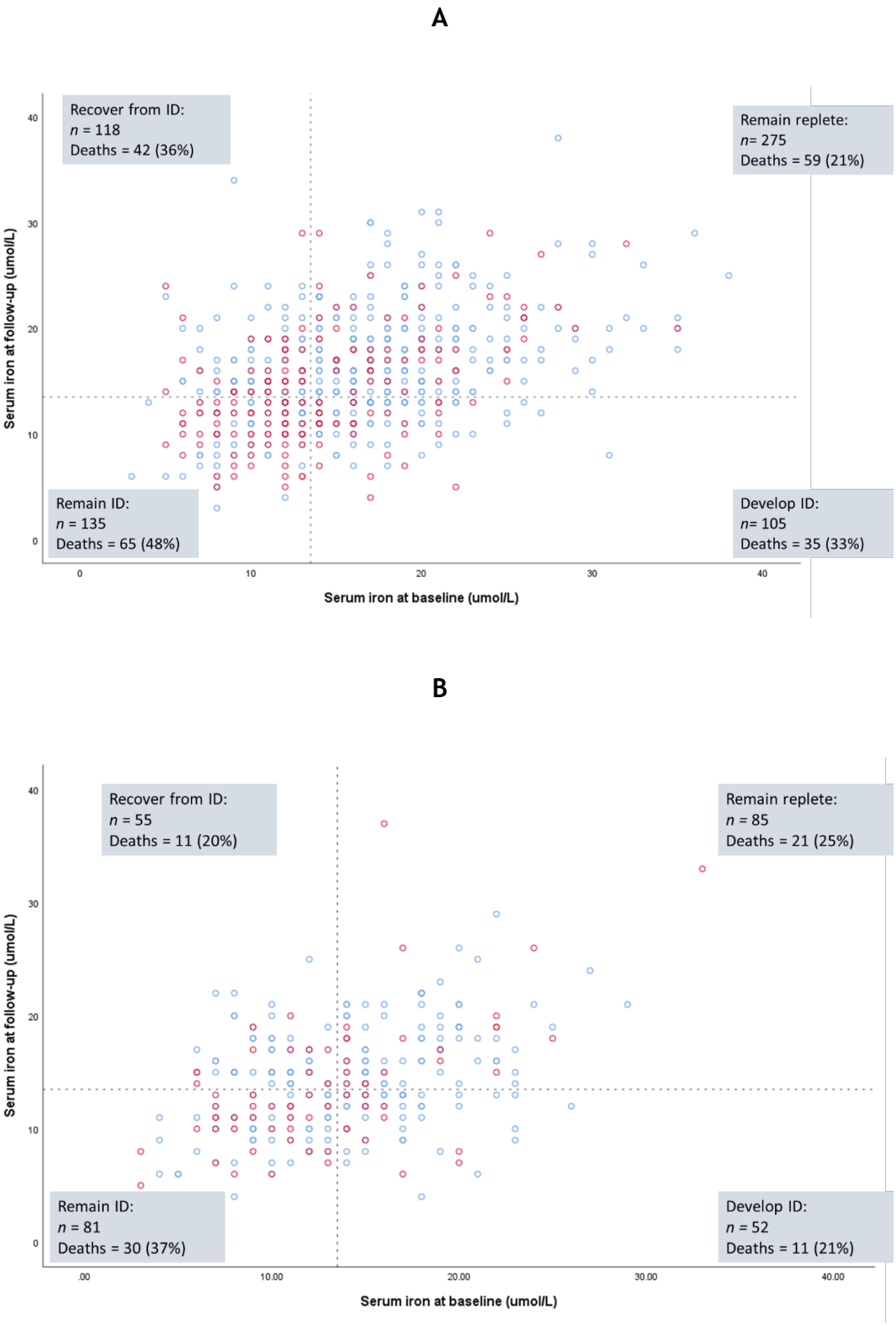
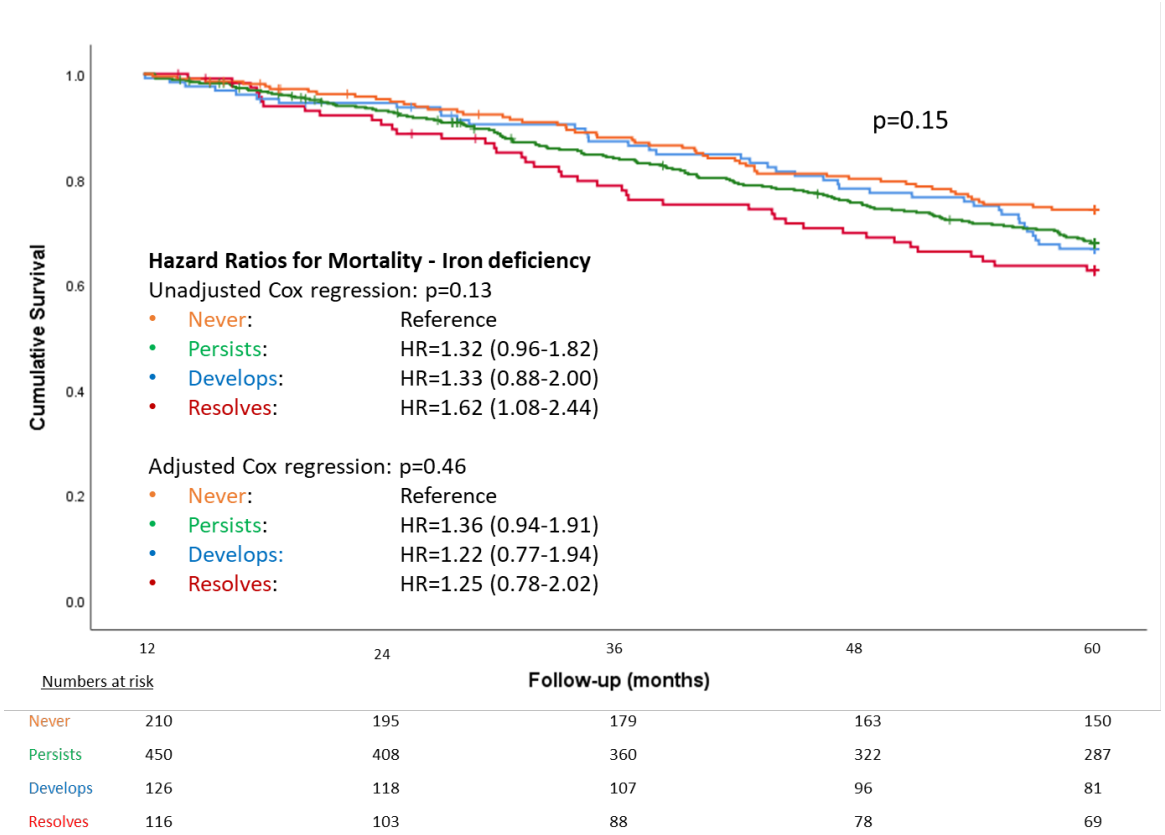
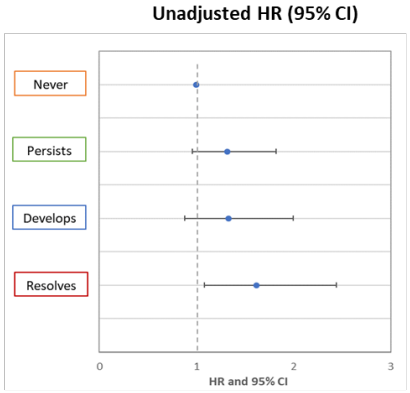


Figure S8: Sex specific scatter plots showing survival 5 years from baseline in men (a) and women (b) according to serum iron at baseline (x-axis) and one year (y-axis). Red circles = dead; Blue circles = alive.





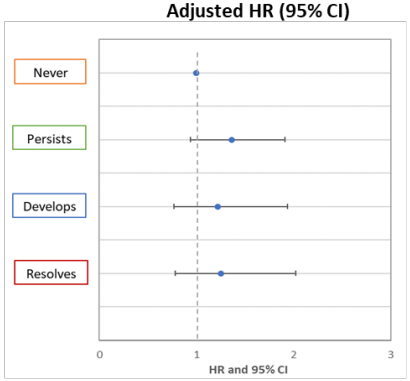
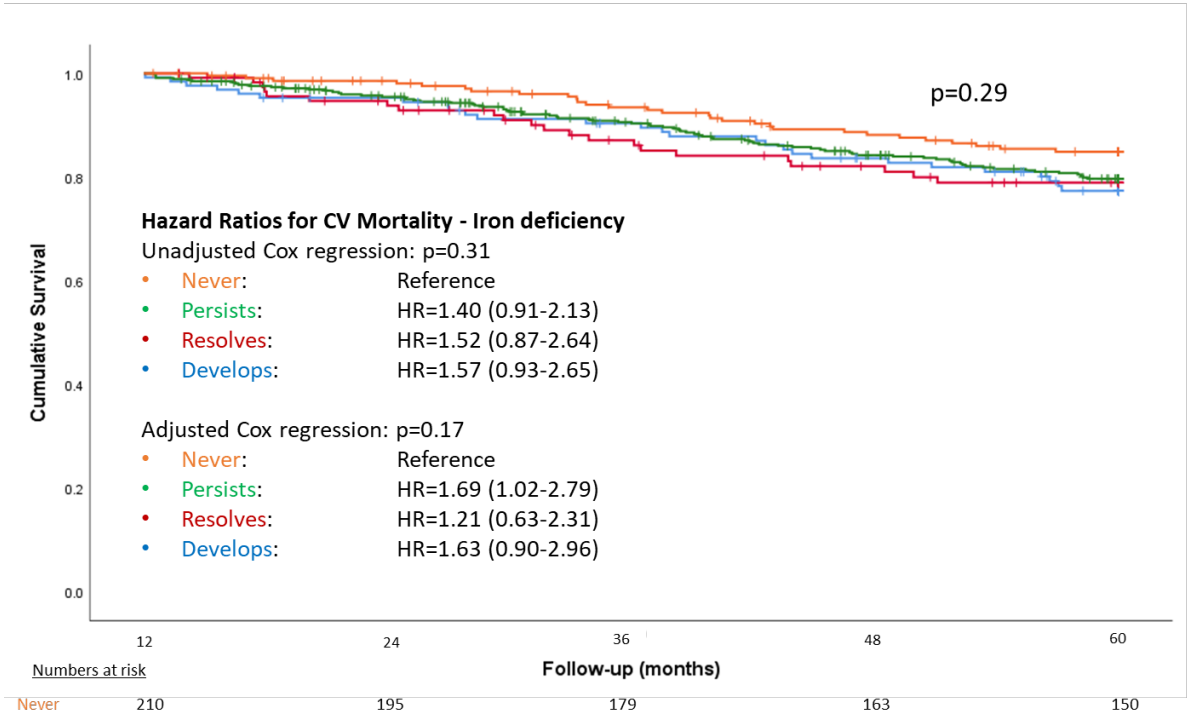


Figure S9: Kaplan-Meir survival analysis of all-cause mortality according to whether iron deficiency, defined by the FAIR-HF criteria, was never present at either baseline or one year or whether it developed, resolved or persisted. FAIR-HF criteria: ferritin <100 µg/L or TSAT <20% if ferritin 100-299 µg/L. Never developing iron deficiency used as the reference. Differences between changes in status compared using the log-rank test. Unadjusted and adjusted hazard ratios with (95% confidence intervals) also reported. Models adjusted for age, sex, diabetes, ischaemic heart disease, NYHA class, BMI, AF/flutter, heart rate, systolic blood pressure, left ventricular phenotype, log NT-proBNP, log hsCRP, eGFR, therapy with loop diuretics and baseline haemoglobin. Forest plots of hazard ratios with 95% confidence intervals for each group in unadjusted and adjusted models also presented.



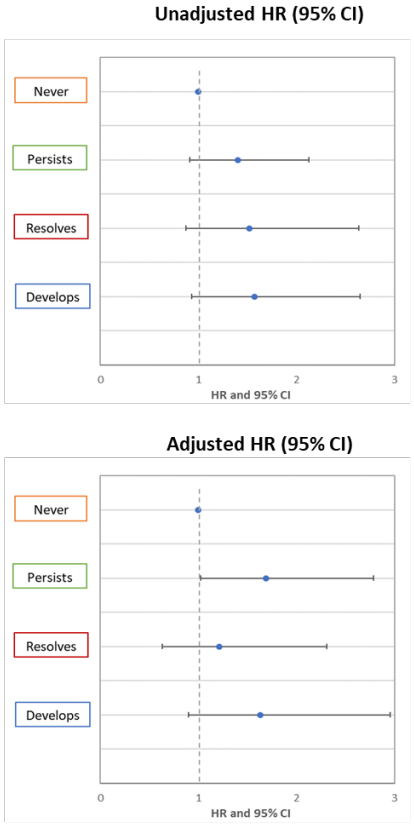
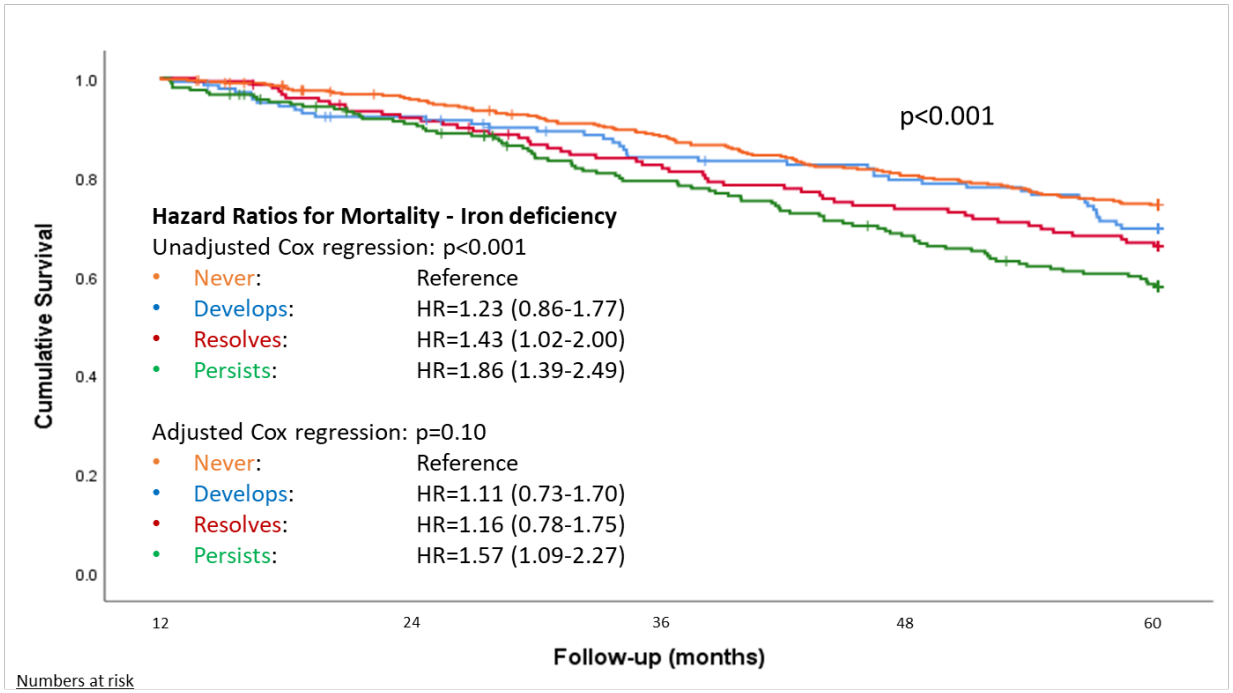


Figure S10: Kaplan-Meir survival analysis of cardiovascular mortality according to whether iron deficiency, defined by the FAIR-HF criteria, was never present at either baseline or one year or whether it developed, resolved or persisted. FAIR-HF criteria: ferritin <100 µg/L or TSAT <20% if ferritin 100-299 µg/L. Never developing iron deficiency used as the reference. Differences between changes in status compared using the log-rank test. Unadjusted and adjusted hazard ratios with (95% confidence intervals) also reported. Models adjusted for age, sex, diabetes, ischaemic heart disease, NYHA class, BMI, AF/flutter, heart rate, systolic blood pressure, left ventricular phenotype, log NT-proBNP, log hsCRP, eGFR, therapy with loop diuretics and baseline haemoglobin. Forest plots of hazard ratios with 95% confidence intervals for each group in unadjusted and adjusted models also presented.



Numbers at risk

Never	403	374	344	312	288
Develops	142	128	112	105	91
Resolves	151	137	121	108	98
Persists	210	185	157	134	112

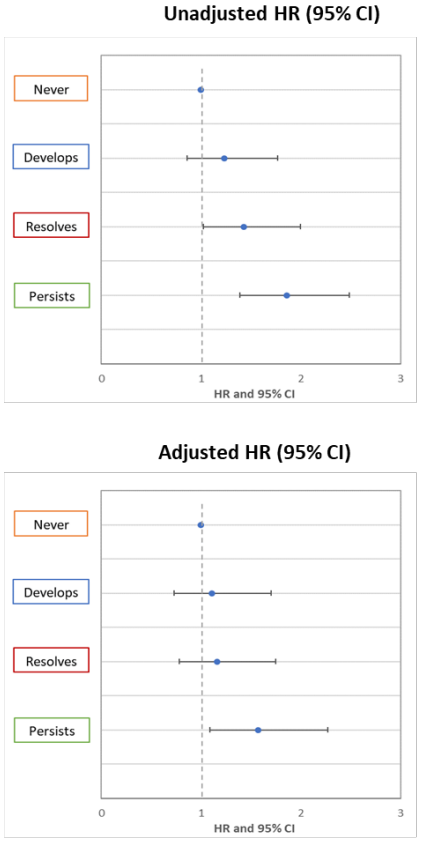
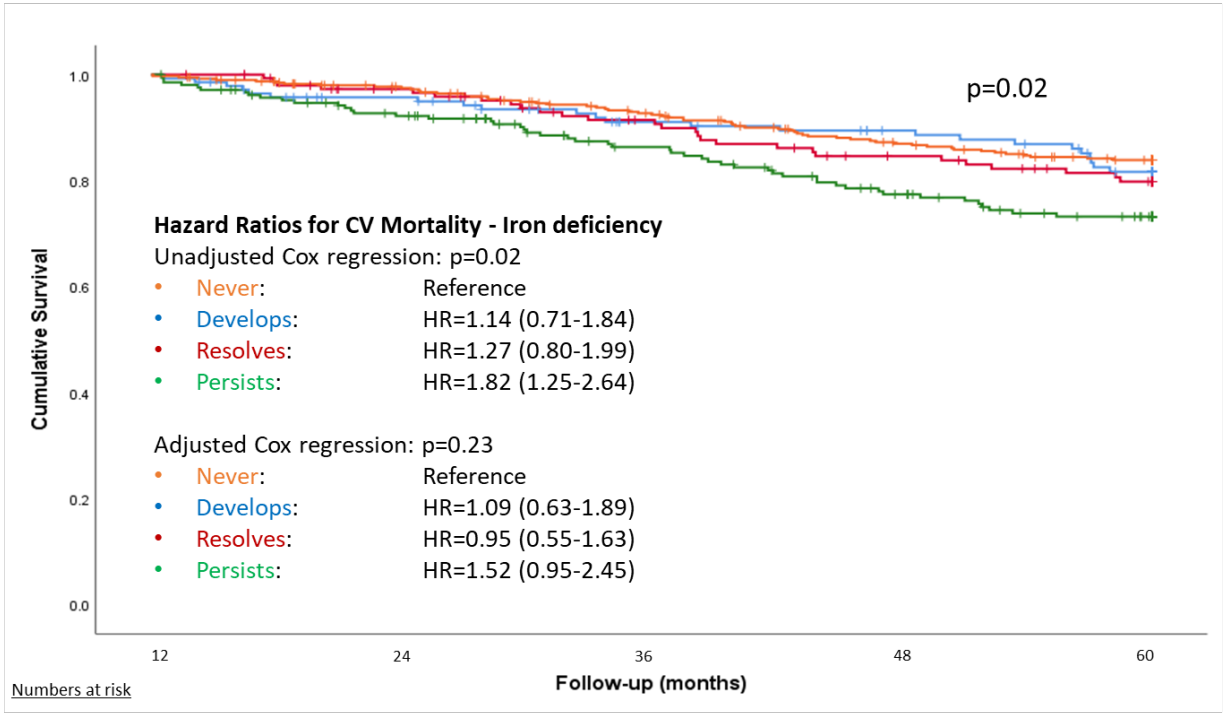
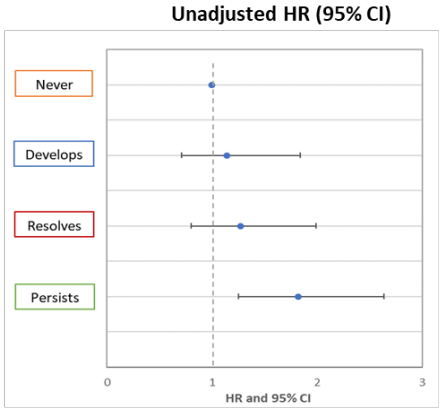


Figure S11: Kaplan-Meir survival analysis of all-cause mortality according to whether iron deficiency, defined by a TSAT <20%, was never present at either baseline or one year, or whether it developed, resolved or persisted. Never developing iron deficiency used as the reference. Differences between changes in status compared using the log-rank test. Unadjusted and adjusted hazard ratios with (95% confidence intervals) also reported. Models adjusted for age, sex, diabetes, ischaemic heart disease, NYHA class, BMI, AF/flutter, heart rate, systolic blood pressure, left ventricular phenotype, log NT-proBNP, log hsCRP, eGFR, therapy with loop diuretics and baseline haemoglobin. Forest plots of hazard ratios with 95% confidence intervals for each group in unadjusted and adjusted models also presented.





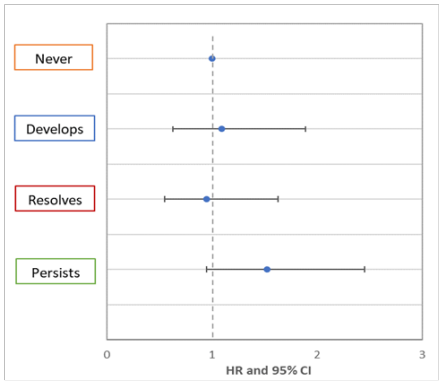


Figure S1226: Kaplan-Meir survival analysis of cardiovascular mortality according to whether iron deficiency, defined by a TSAT <20%, was never present at either baseline or one year, or whether it developed, resolved or persisted. Never developing iron deficiency used as the reference. Differences between changes in status compared using the log-rank test. Unadjusted and adjusted hazard ratios with (95% confidence intervals) also reported. Models adjusted for age, sex, diabetes, ischaemic heart disease, NYHA class, BMI, AF/flutter, heart rate, systolic blood pressure, left ventricular phenotype, log NT-proBNP, log hsCRP, eGFR, therapy with loop diuretics and baseline haemoglobin. Forest plots of hazard ratios with 95% confidence intervals for each group in unadjusted and adjusted models also presented.

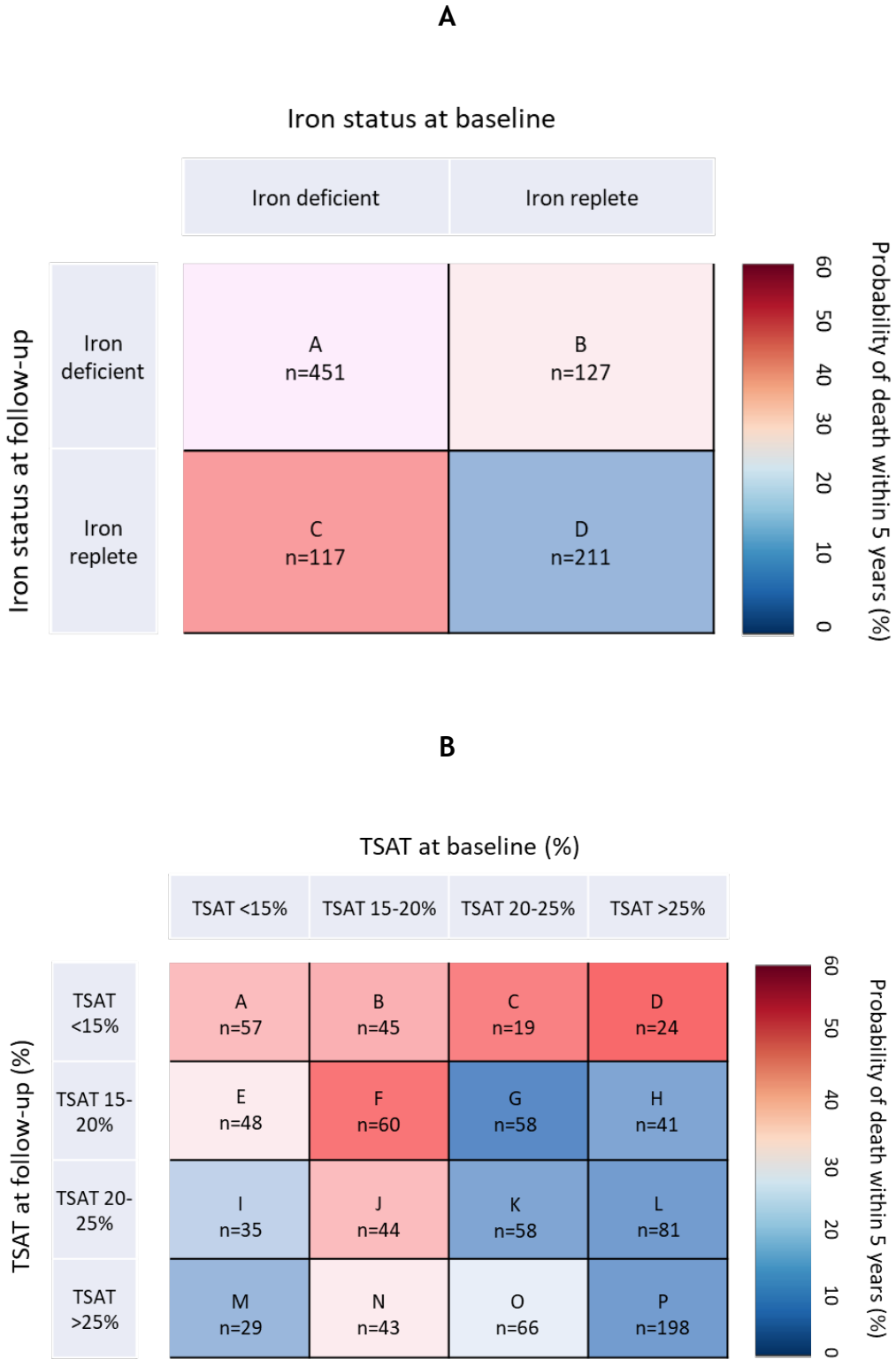


Figure S13: Heat maps depicting survival 5 years from baseline classified by (A) baseline and one-year definitions of iron deficiency using the FAIR-HF criteria (ferritin <100 µg/L or TSAT <20% if ferritin 100-299 µg/L) and (B) by baseline and one-year measurements of TSAT (%).
Number of patients within each cell reported.

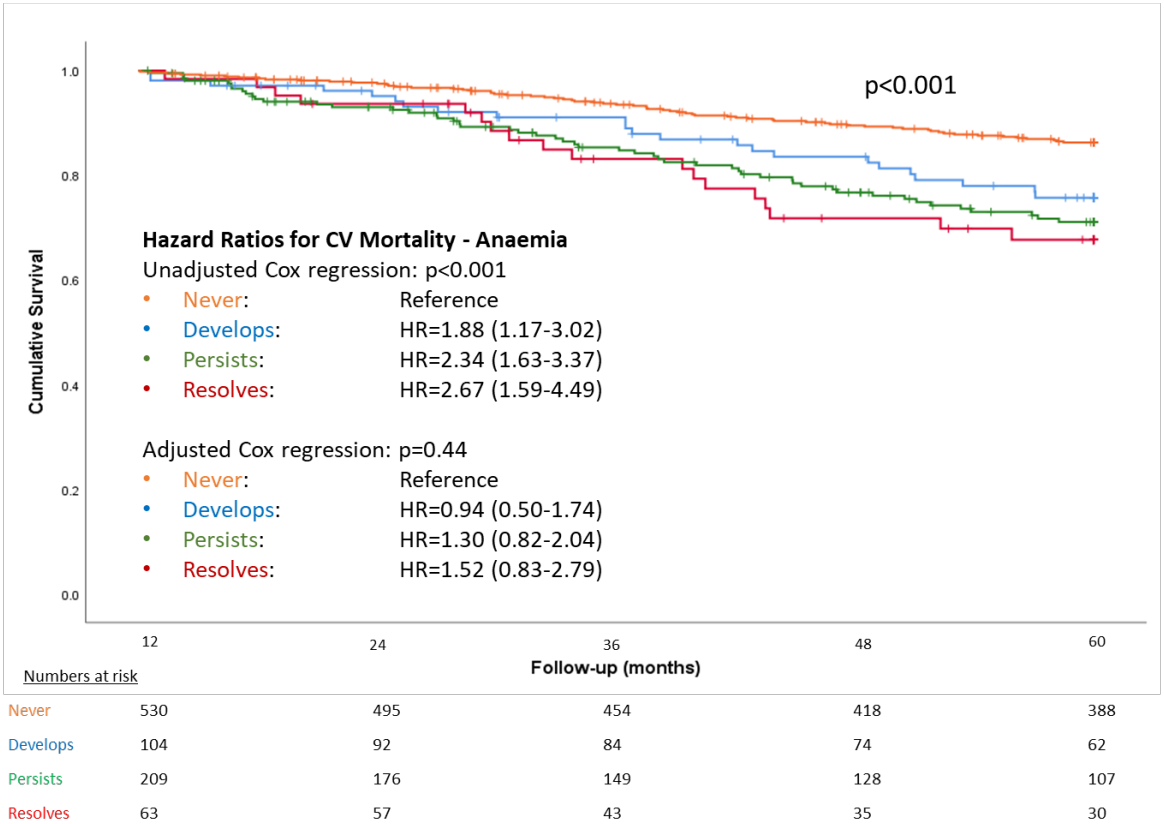


Figure S14: Kaplan-Meir survival analysis of cardiovascular mortality 5 years from baseline visit according to whether anaemia was never present at either baseline or one year, or whether it developed, resolved, or persisted.
Never developing anaemia used as the reference. Differences between changes in status compared using the log-rank test. Unadjusted and adjusted hazard ratios with (95% confidence intervals) also reported. Models adjusted for age, sex, diabetes, ischaemic heart disease, NYHA class, BMI, AF/flutter, heart rate, systolic blood pressure, left ventricular phenotype, log NT-proBNP, log hsCRP, eGFR, therapy with loop diuretics and baseline iron and ferritin. Forest plots of hazard ratios with 95% confidence intervals for each group in unadjusted and adjusted models also presented.

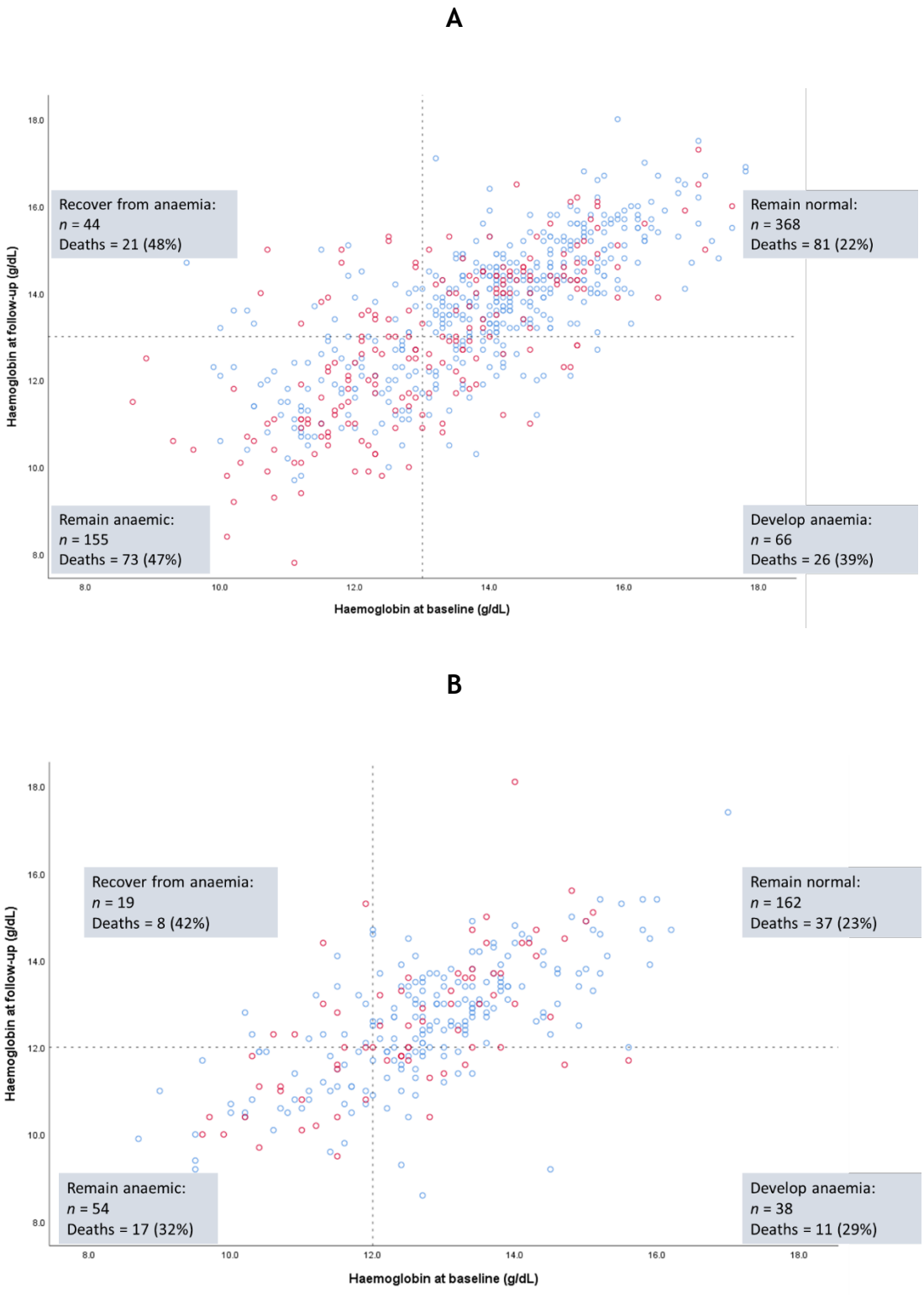


Figure S15: Sex specific scatter plots showing survival 5 years from baseline in men (a) and women (b) according to serum haemoglobin at baseline (x-axis) and one year follow-up (y-axis). Red circles = dead; Blue circles = alive.

Chapter 5

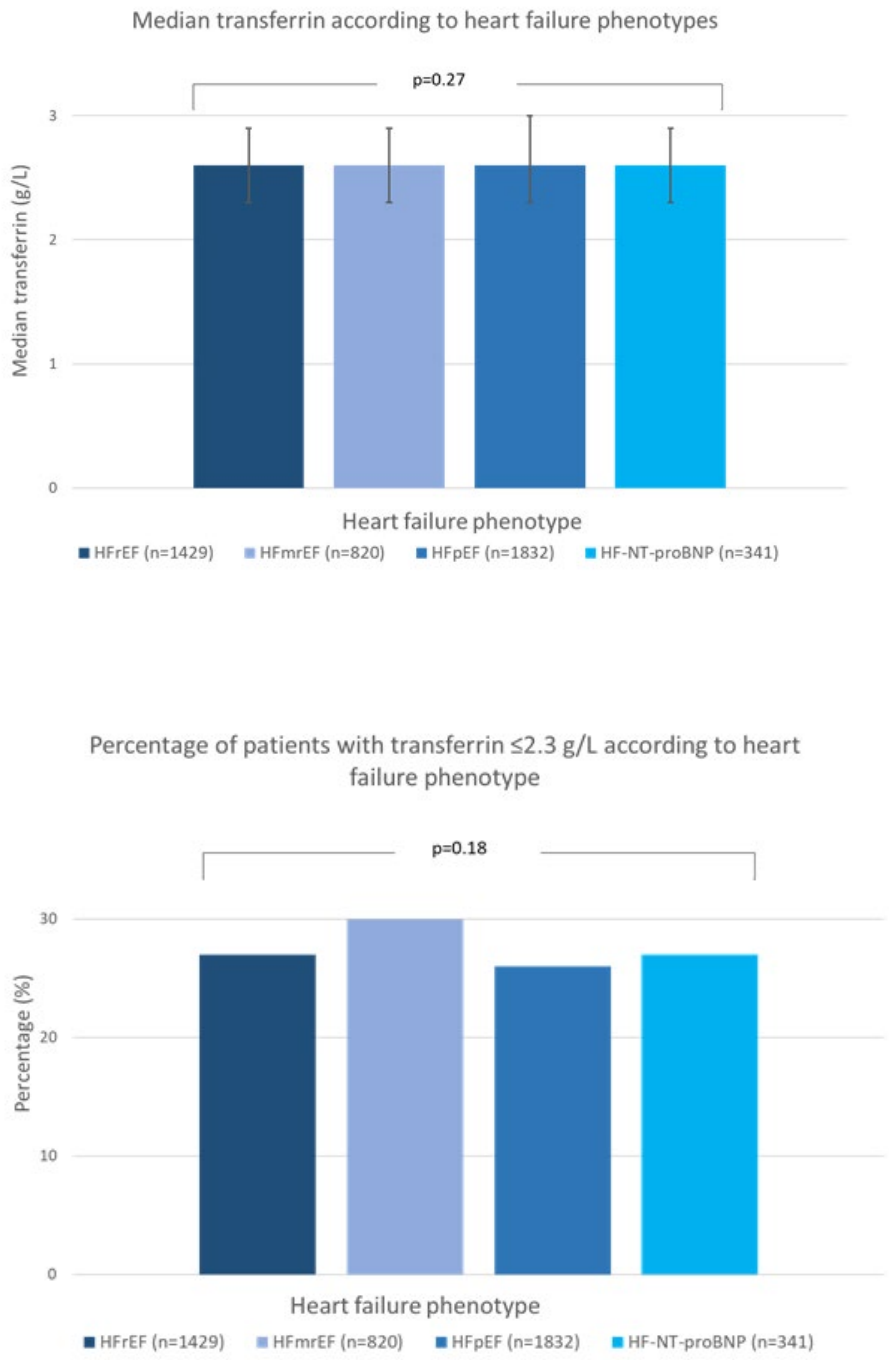


Figure S16: Bar graphs detailing median transferrin and % of patients with a low transferrin (≤2.3 g/L) according to heart failure phenotypes.
Abbreviations: HFrEF: heart failure with reduced ejection fraction; HFmrEF: heart failure with mid-range ejection fraction; HFpEF: heart failure with preserved ejection fraction; NT-proBNP: N terminal-pro brain natriuretic peptide.

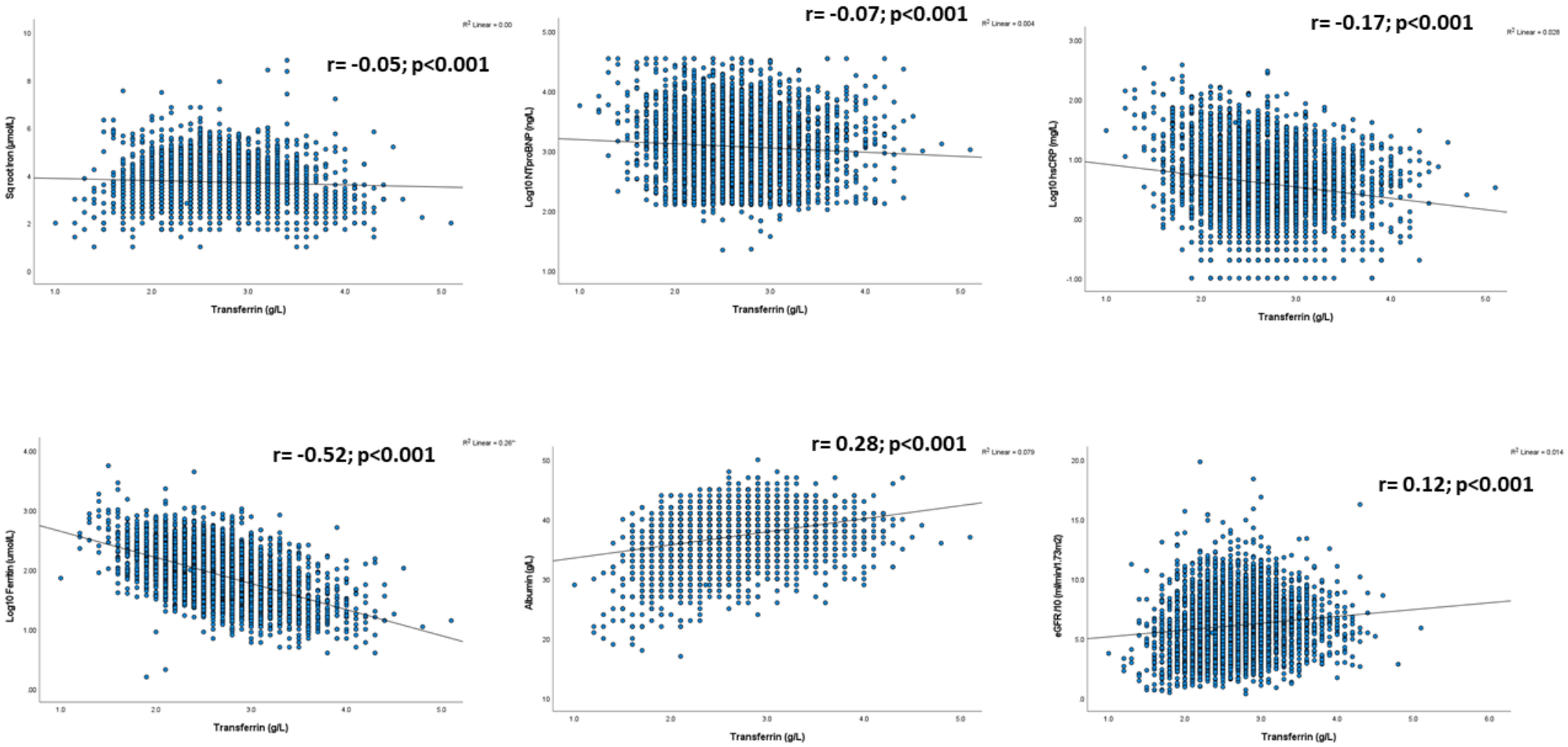
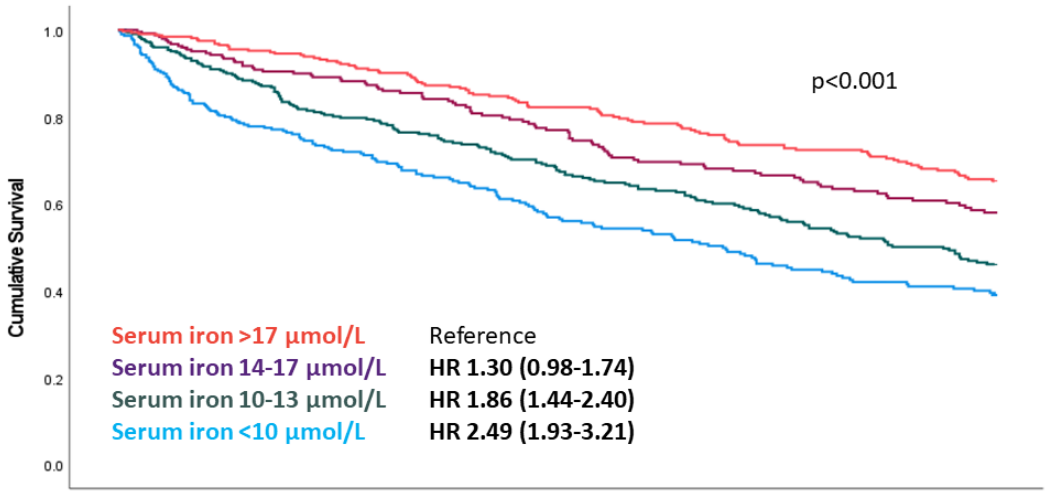


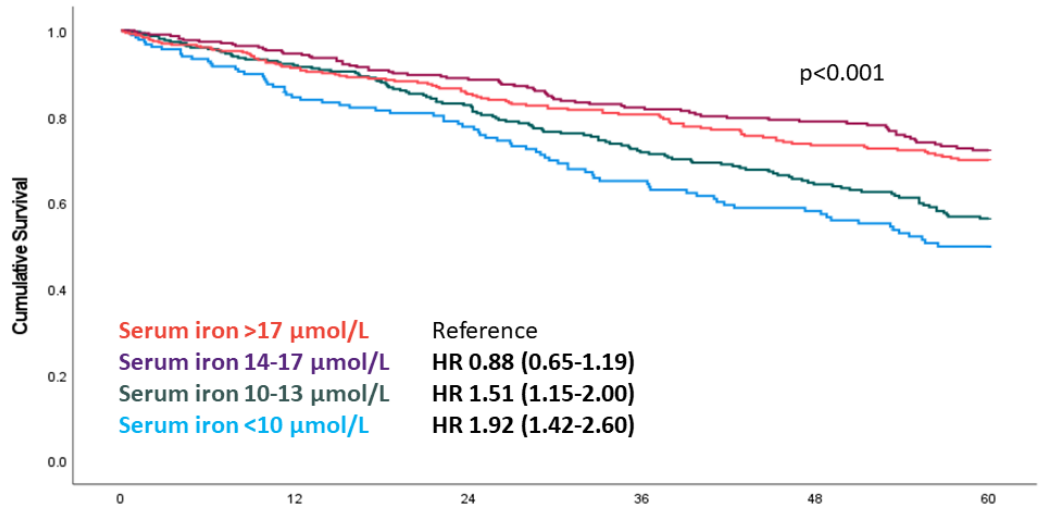
Figure S17: Scatterplots demonstrating the correlation between transferrin (x-axis) and various biomarkers (y-axis). Corresponding correlation coefficients and p-values are provided for each variable.

A



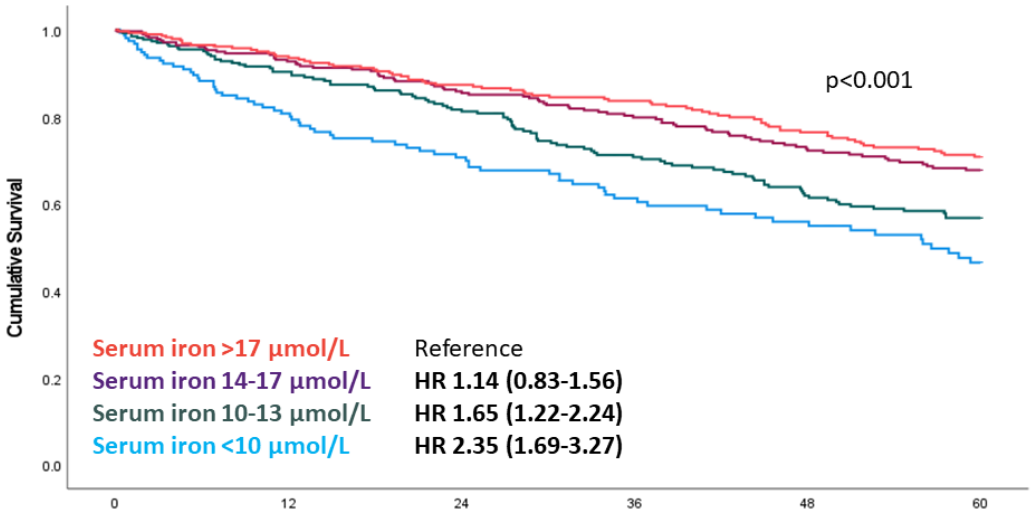
Number at risk						
Serum iron >17	324	283	241	209	189	159
Serum iron 14-17	247	207	172	139	124	104
Serum iron 10-13	326	249	213	172	142	112
Serum iron <10	298	206	157	122	97	76

B



Number at risk						
Serum iron >17	360	298	258	228	200	181
Serum iron 14-17	311	270	242	207	185	157
Serum iron 10-13	305	261	216	179	151	125
Serum iron <10	183	139	120	94	82	62

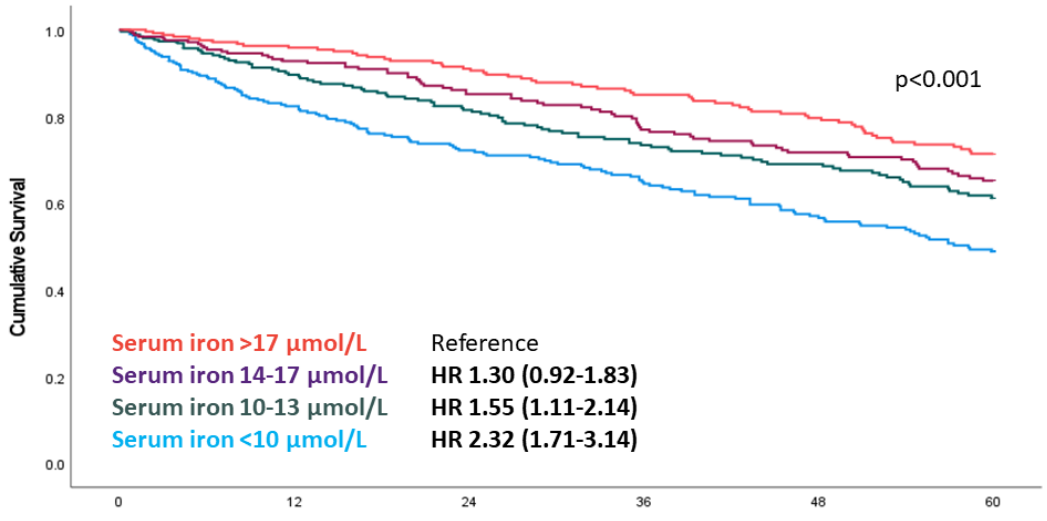
C



Number at risk

Serum iron >17	294	249	219	200	177	158
Serum iron 14-17	286	250	220	195	167	148
Serum iron 10-13	267	217	184	149	124	105
Serum iron <10	156	115	95	72	59	43

D

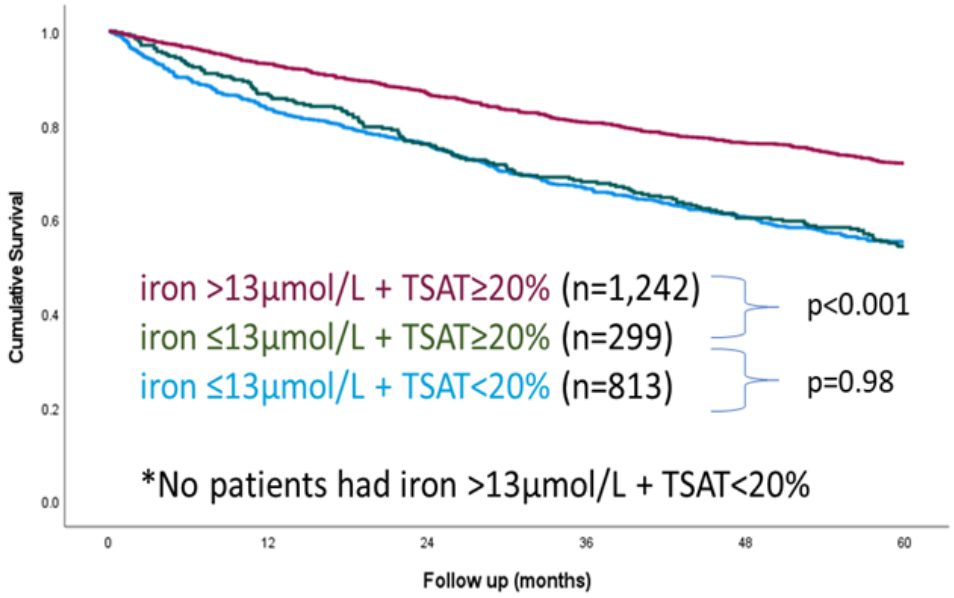


Number at risk

Serum iron >17	250	225	202	181	160	133
Serum iron 14-17	249	205	174	149	135	119
Serum iron 10-13	261	215	185	158	139	113
Serum iron <10	305	227	186	156	129	105

Figure S1827: Kaplan-Meier survival curves for all-cause mortality for patients in 1st (A; n=1,195), 2nd (B; n=1,159), 3rd (C; n=1,003) and 4th (D; n=1,065) quartiles of serum transferrin by serum iron concentration. Log-rank p-value provided. Hazard ratios and 95% confidence intervals for each level of serum iron provided. Serum iron >17µmol/L used as the reference in each.

A



B

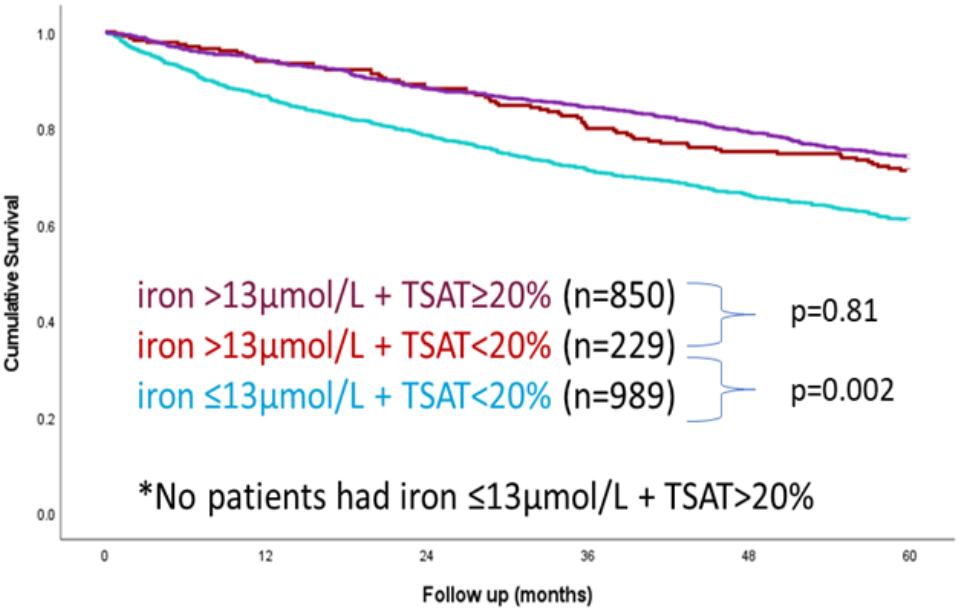
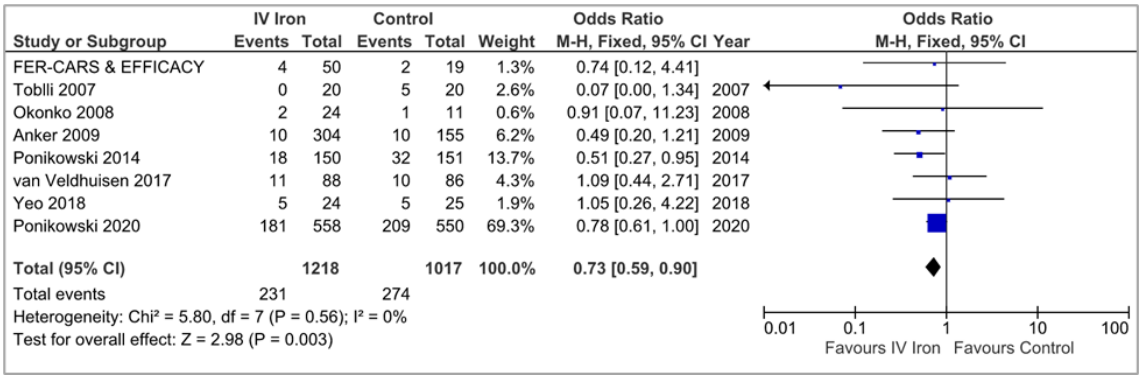


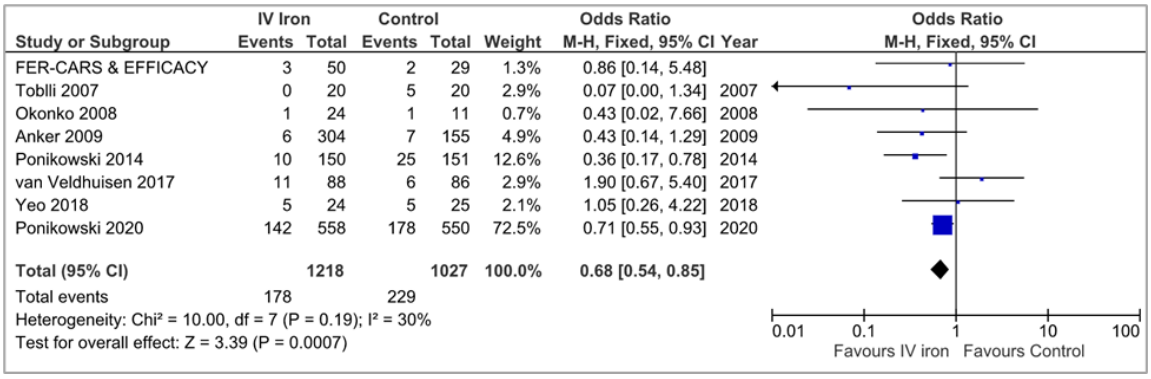
Figure S19: Kaplan-Meier survival curves for all-cause mortality for patients in lowest two quartiles (A) (n=2,364) and highest two quartiles (B) (n=2,068) of serum transferrin by serum iron concentration and TSAT (%). Log-rank p-value provided. Hazard ratios and 95% confidence intervals for each level of serum iron provided. Serum iron >13µmol/L + TSAT ≥20% used as the reference in each.

Chapter 6

A



B



C

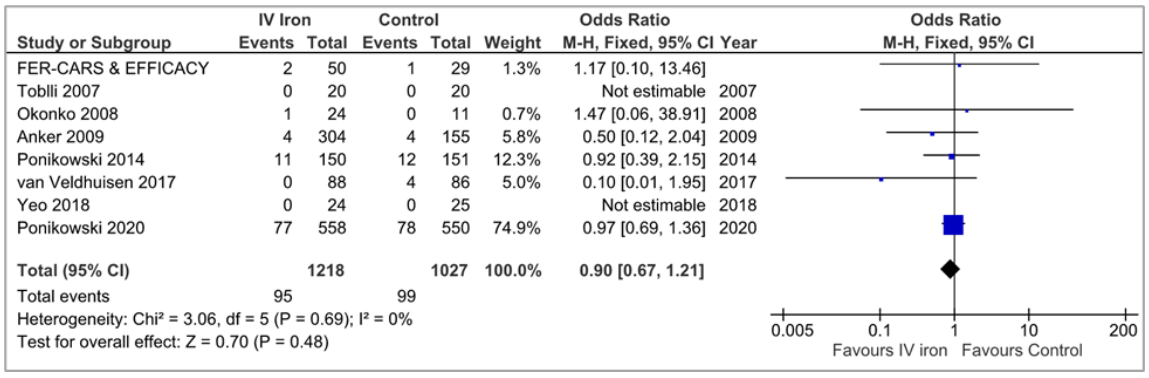
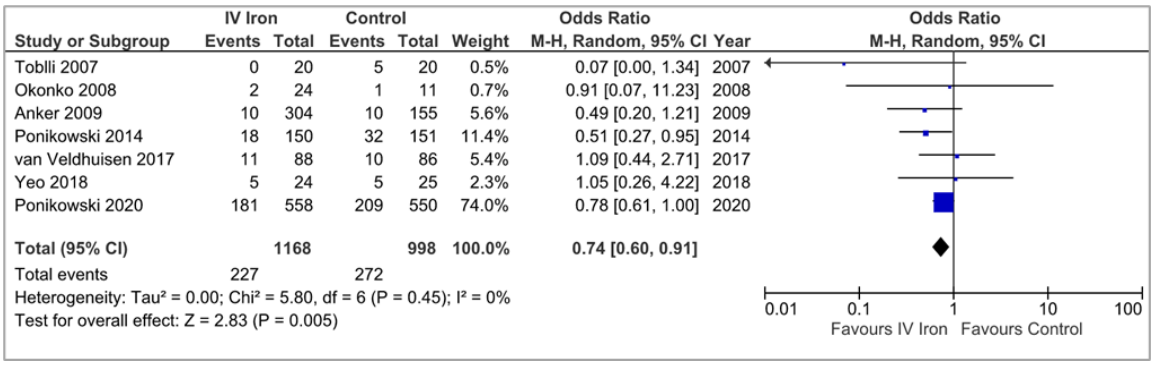


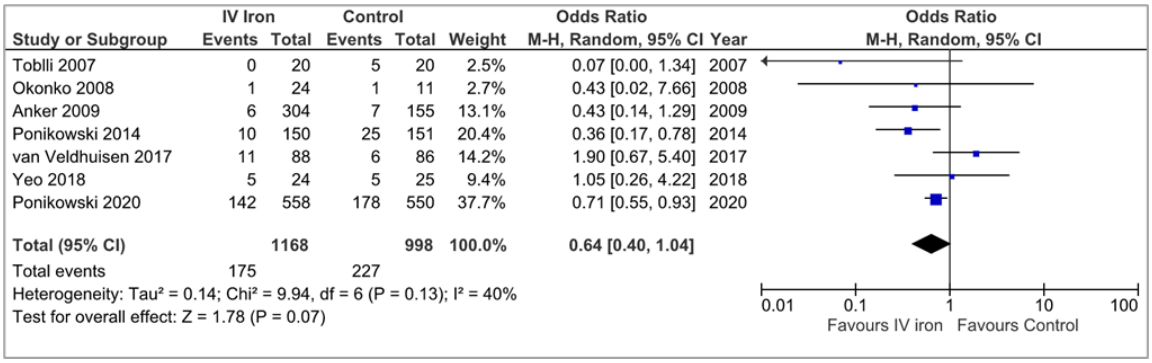
Figure S20: Fixed-effects meta-analysis model of all included trials, including additional unpublished trials FER-CARS-01 and FER-CARS-03/EFFICACY-HF, detailing the pooled effect of intravenous iron on the composite of first hospitalisations for heart failure or cardiovascular mortality (A), and first hospitalisation for heart failure (B) and cardiovascular mortality (C) alone.

Abbreviations: IV: intravenous; CI: confidence interval

A



B



C

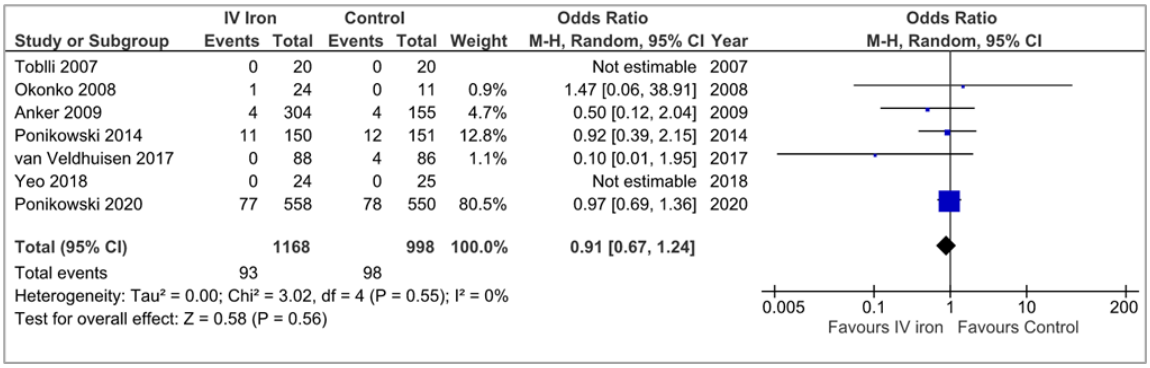
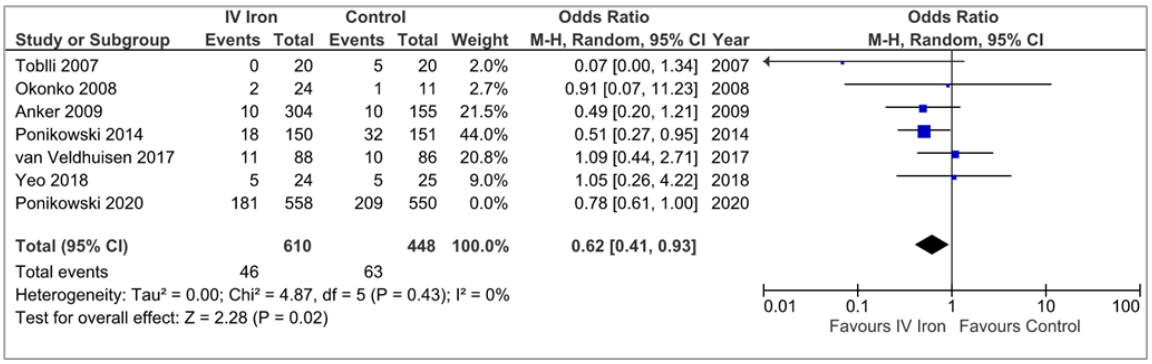


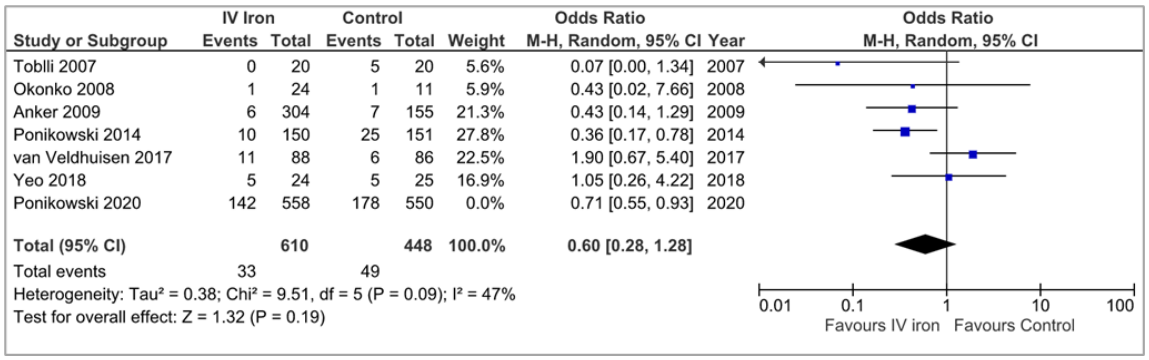
Figure S21: Random-effects meta-analysis model of all included trials detailing the pooled effect of intravenous iron on the composite of first hospitalisations for heart failure or cardiovascular mortality (A), and first hospitalisation for heart failure (B) and cardiovascular mortality (C) alone.

Abbreviations: IV: intravenous; CI: confidence interval

A



B



C

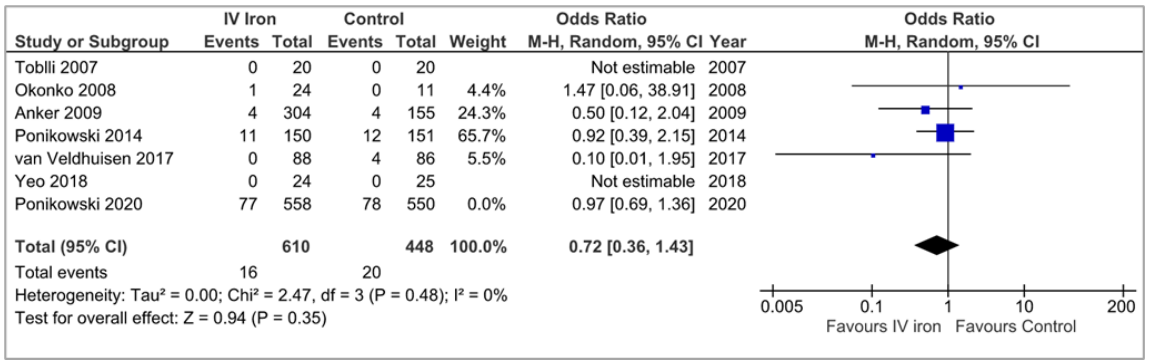
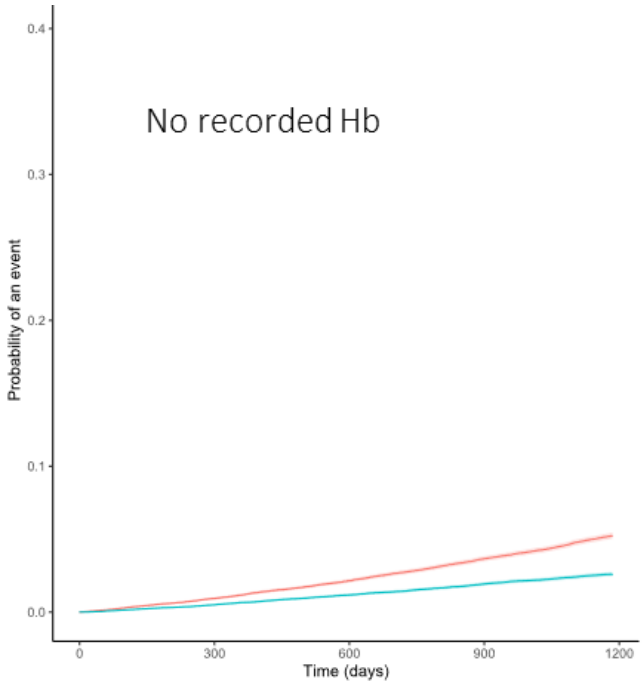
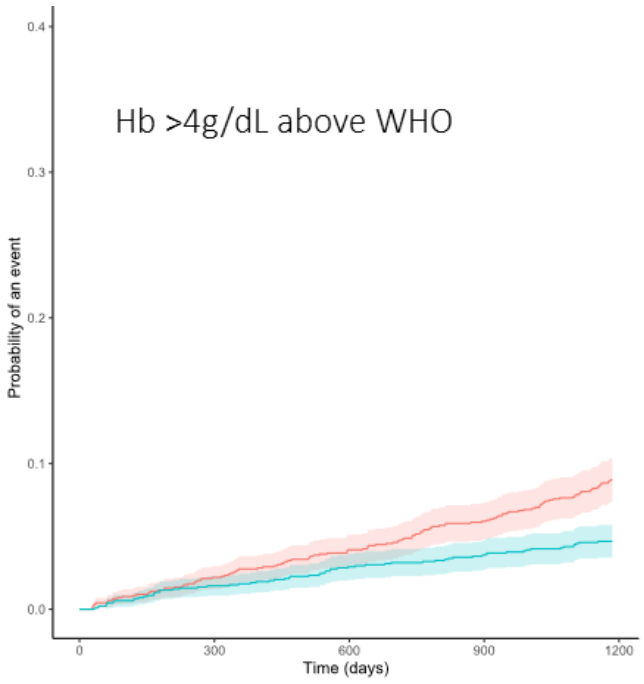


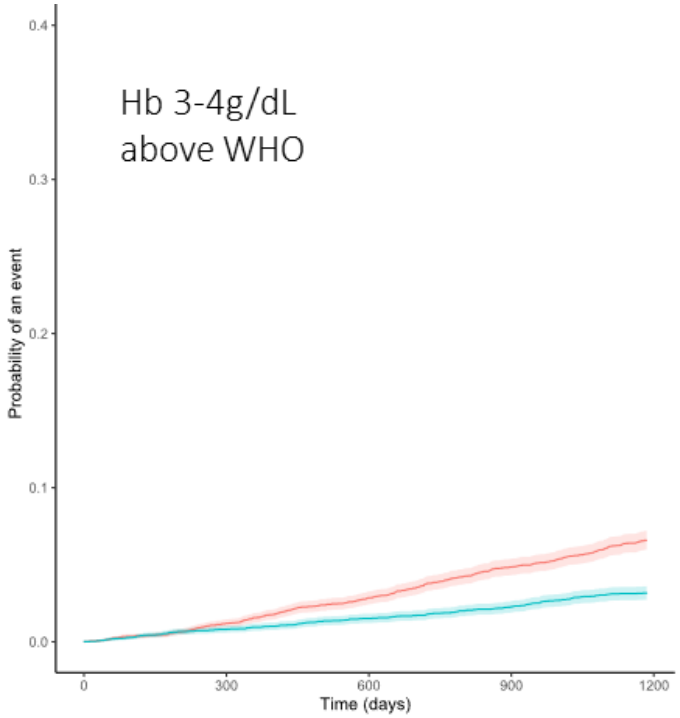
Figure S22: Random-effects meta-analysis model of all included trials, excluding AFFIRM-AHF, detailing the pooled effect of intravenous iron on the composite of first hospitalisations for heart failure or cardiovascular mortality (A), and first hospitalisation for heart failure (B) and cardiovascular mortality (C) alone. Although not included in the pooled analysis, Odds Ratios and (95% Confidence Intervals) are presented for AFFIRM-AHF for comparison.

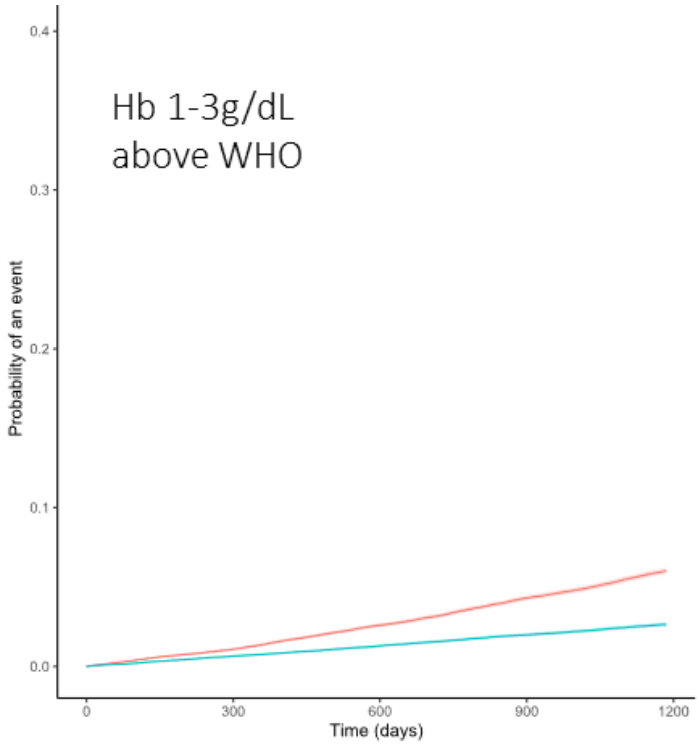
Abbreviations: IV: intravenous; CI: confidence interval

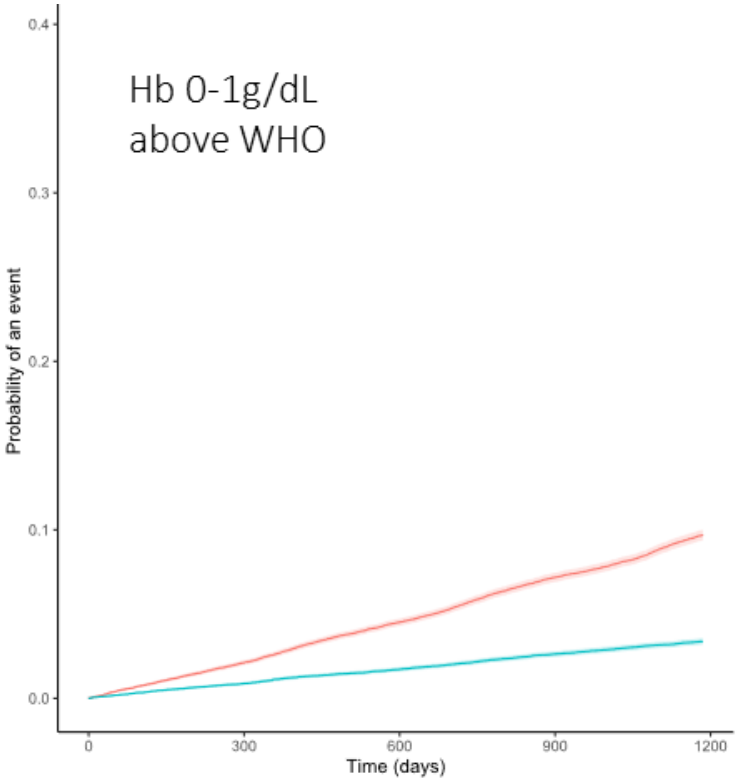
Chapter 7

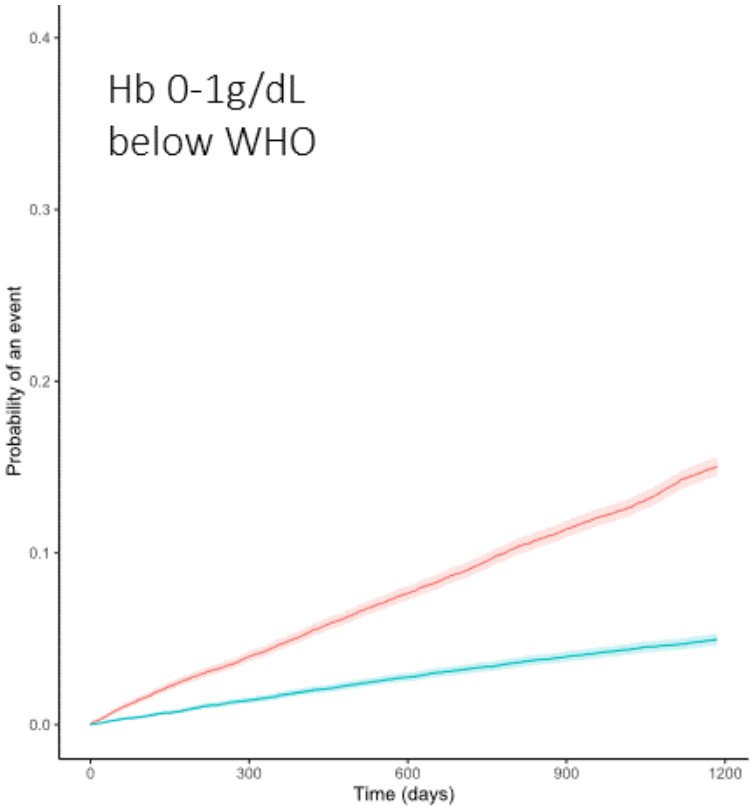

No recorded Hb
Probability of an event
0.0
0.1
0.2
0.3
0.4
0
300
600
900
1200
Time (days)


Hb >4g/dL above WHO
Probability of an event
0.0
0.1
0.2
0.3
0.4
0
300
600
900
1200
Time (days)


Hb 3-4g/dL
above WHO
Probability of an event
0.4
0.3
0.2
0.1
0.0
0
300
600
900
1200
Time (days)


Hb 1-3g/dL
above WHO
Probability of an event
0.4
0.3
0.2
0.1
0.0
0
300
600
900
1200
Time (days)


Hb 0-1g/dL
above WHO
Probability of an event
0.4
0.3
0.2
0.1
0.0
0
300
600
900
1200
Time (days)


Hb 0-1g/dL
below WHO
Probability of an event
0.4
0.3
0.2
0.1
0.0
0
300
600
900
1200
Time (days)

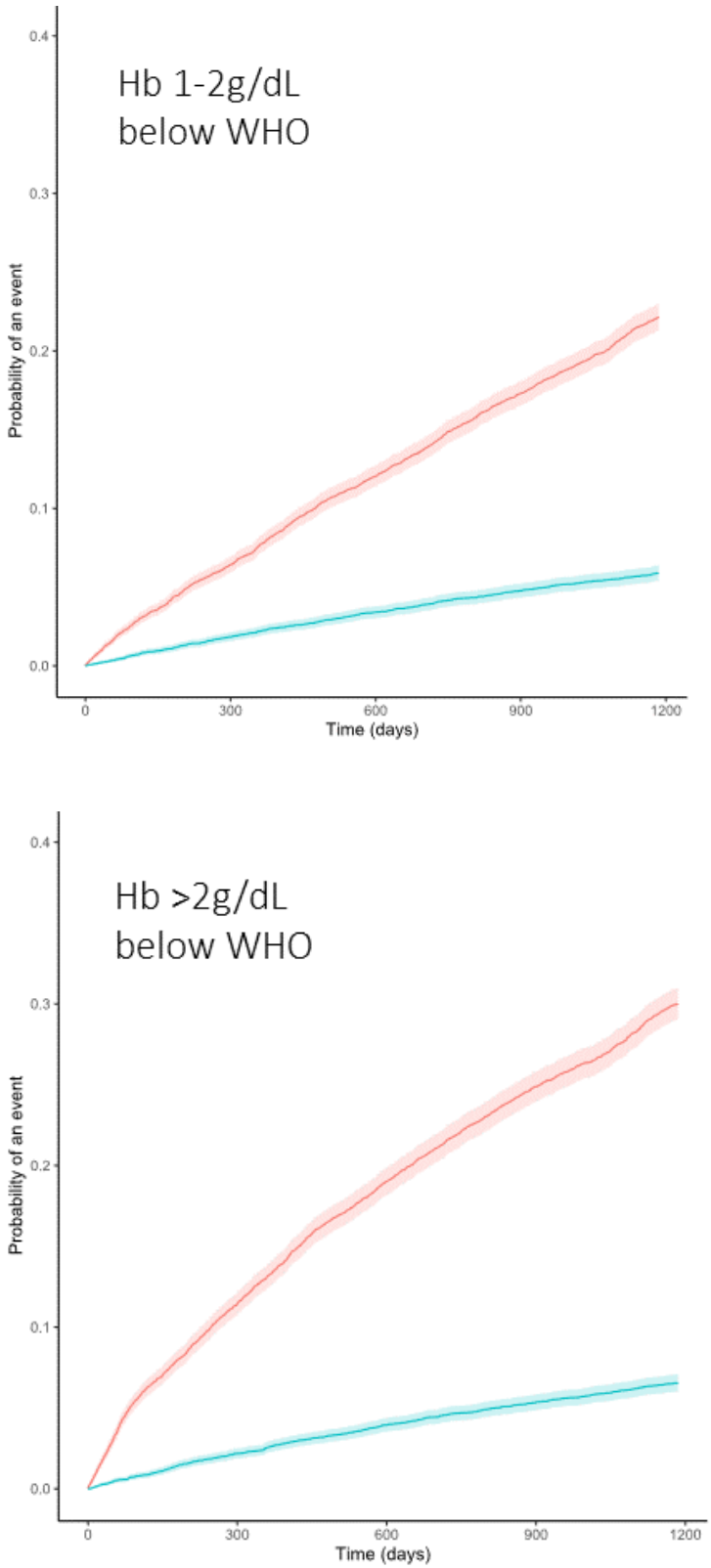
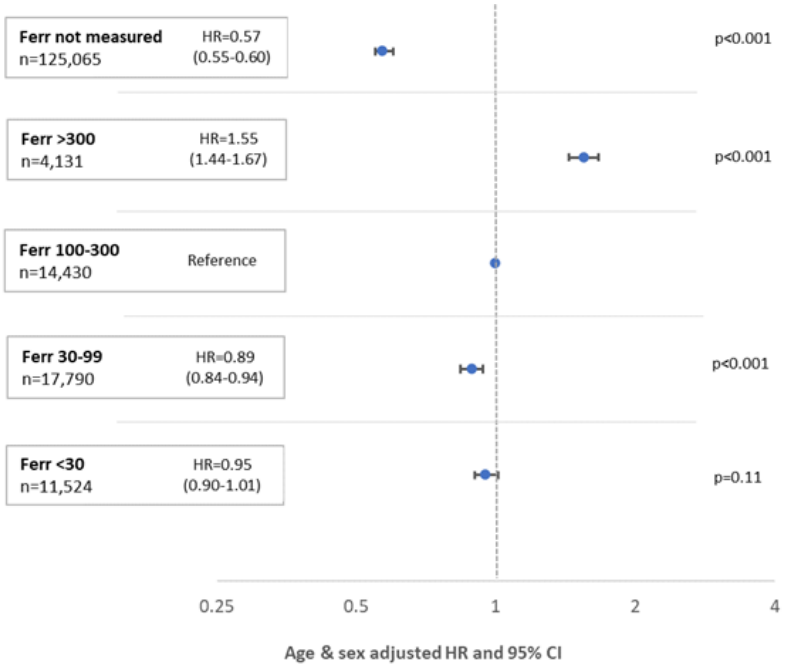
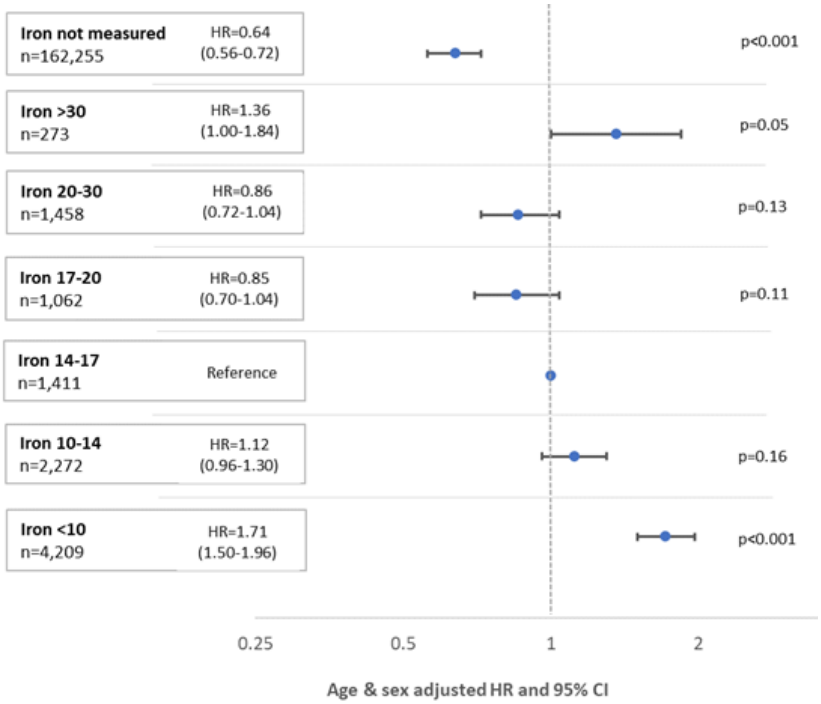


Figure S23: Cumulative incidence curves showing rates of incident heart failure (blue) and all-cause mortality (pink) with associated 95% Confidence Intervals from 1st January 2015 to 31st March 2018 by haemoglobin concentration for patients who survived, free of heart failure up to 31st December 2014. Abbreviations: Hb: haemoglobin; W.H.O.: World Health Organization

A


Ferr not measured
n=125,065
HR=0.57
(0.55-0.60)
p<0.001
Ferr >300
n=4,131
HR=1.55
(1.44-1.67)
p<0.001
Ferr 100-300
n=14,430
Reference
Ferr 30-99
n=17,790
HR=0.89
(0.84-0.94)
p<0.001
Ferr <30
n=11,524
HR=0.95
(0.90-1.01)
p=0.11
0.25
0.5
1
2
4
Age & sex adjusted HR and 95% CI

B


Iron not measured
n=162,255
HR=0.64
(0.56-0.72)
p<0.001
Iron >30
n=273
HR=1.36
(1.00-1.84)
p=0.05
Iron 20-30
n=1,458
HR=0.86
(0.72-1.04)
p=0.13
Iron 17-20
n=1,062
HR=0.85
(0.70-1.04)
p=0.11
Iron 14-17
n=1,411
Reference
Iron 10-14
n=2,272
HR=1.12
(0.96-1.30)
p=0.16
Iron <10
n=4,209
HR=1.71
(1.50-1.96)
p<0.001
0.25
0.5
1
2
Age & sex adjusted HR and 95% CI

C

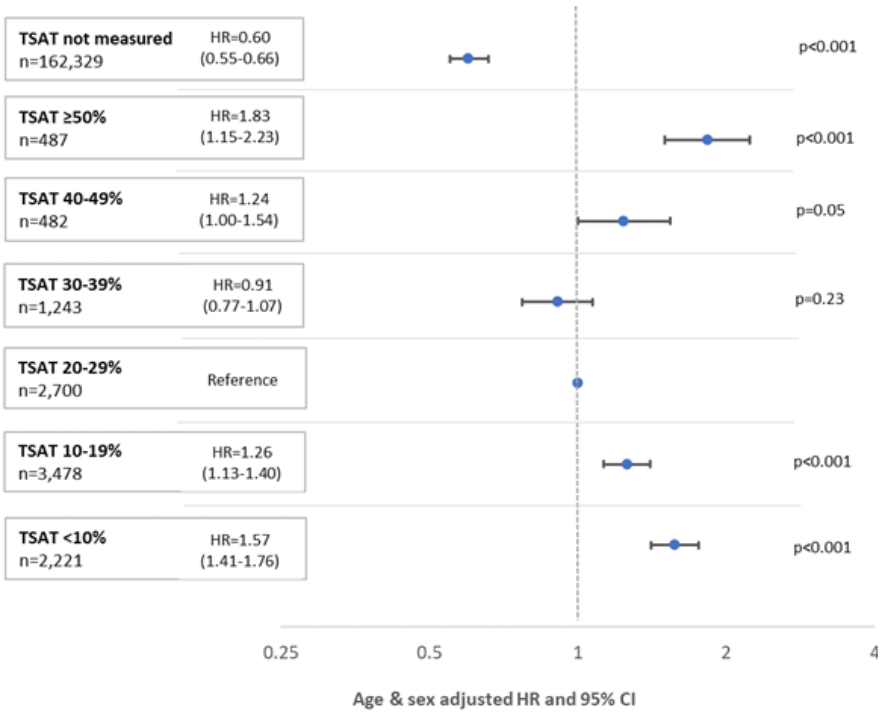
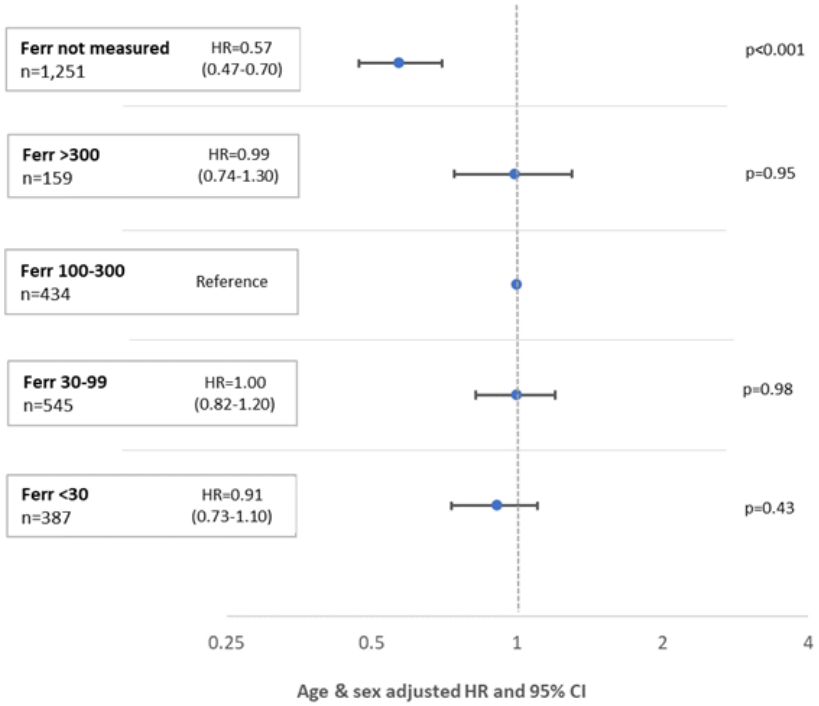


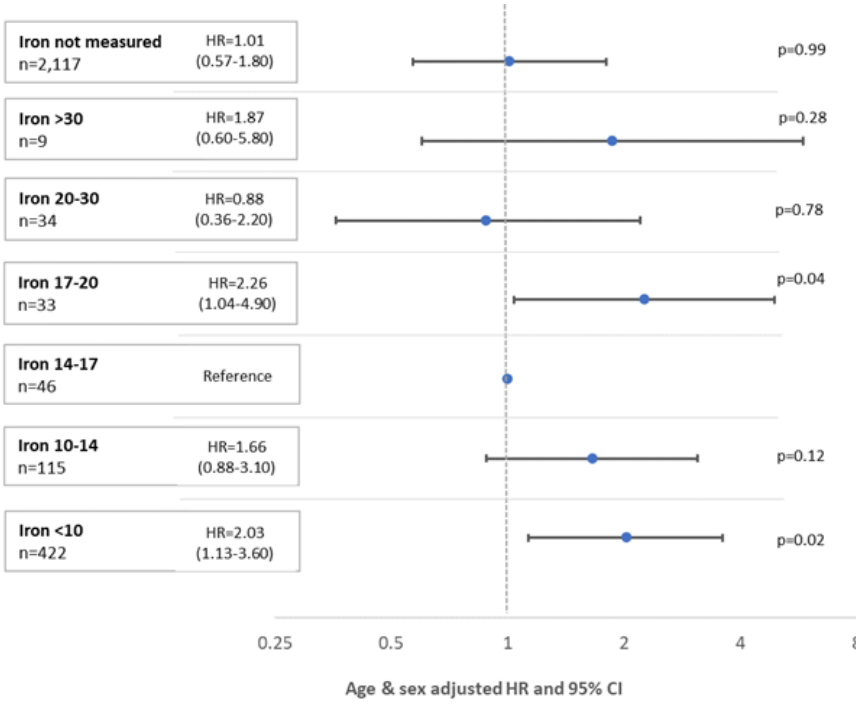
Figure S24: Forest plots showing all-cause mortality from 1st January 2015 to 31st March 2018 in surviving patients without a history of heart failure during, or prior to, 2013/14 according to concentrations of serum ferritin (A) (Not measured; >300 µg/L; 100-300 µg/L; 30-100 µg/L; <30 µg/L), serum iron (B) (Not measured; >30 µmol/L; 20-30 µmol/L; 17-20 µmol/L; 14-17 µmol/L; 10-14 µmol/L; <10 µmol/L) and transferrin saturation (TSAT) (C) (Not measured; ≥50%; 40-49%; 30-39%; 20-29%; 10-19%; <10%).

Abbreviations: - Ferr: ferritin; TSAT: transferrin saturation.

A


Ferr not measured
n=1,251
HR=0.57
(0.47-0.70)
p<0.001
Ferr >300
n=159
HR=0.99
(0.74-1.30)
p=0.95
Ferr 100-300
n=434
Reference
Ferr 30-99
n=545
HR=1.00
(0.82-1.20)
p=0.98
Ferr <30
n=387
HR=0.91
(0.73-1.10)
p=0.43
0.25
0.5
1
2
4
Age & sex adjusted HR and 95% CI

B


Iron not measured
n=2,117
HR=1.01
(0.57-1.80)
p=0.99
Iron >30
n=9
HR=1.87
(0.60-5.80)
p=0.28
Iron 20-30
n=34
HR=0.88
(0.36-2.20)
p=0.78
Iron 17-20
n=33
HR=2.26
(1.04-4.90)
p=0.04
Iron 14-17
n=46
Reference
Iron 10-14
n=115
HR=1.66
(0.88-3.10)
p=0.12
Iron <10
n=422
HR=2.03
(1.13-3.60)
p=0.02
0.25
0.5
1
2
4
Age & sex adjusted HR and 95% CI

C

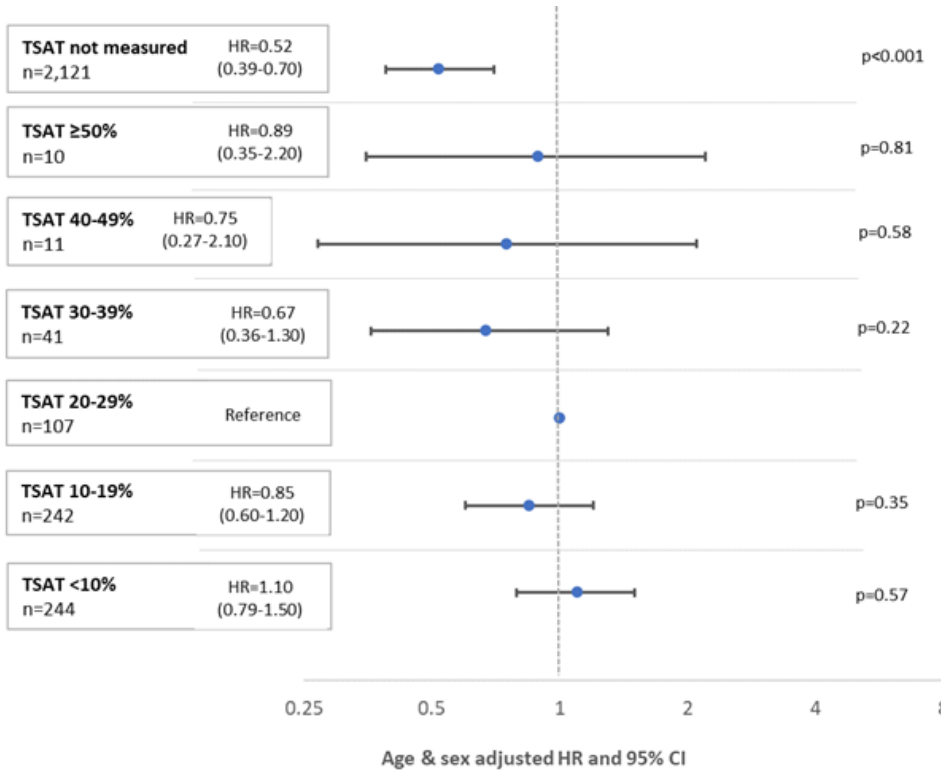
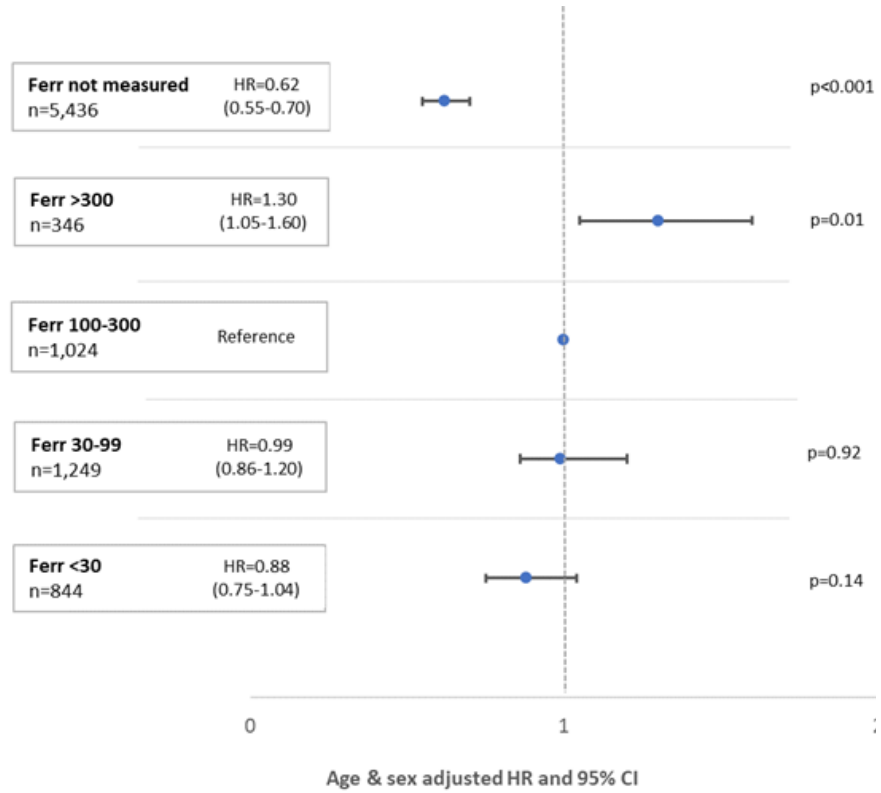


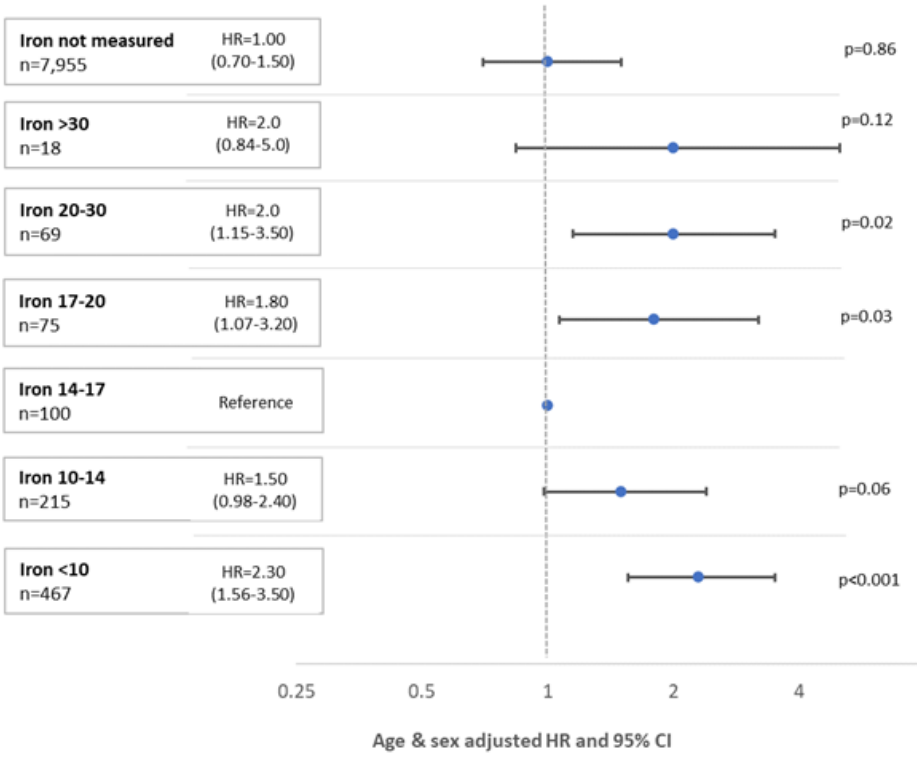
Figure S25: Forest plots showing all-cause mortality from 1st January 2015 to 31st March 2018 in surviving patients with incident heart failure during 2013/14 according to concentrations of serum ferritin (A) (Not measured; >300 µg/L; 100-300 µg/L; 30-100 µg/L; <30 µg/L), serum iron (B) (Not measured; >30 µmol/L; 20-30 µmol/L; 17-20 µmol/L; 14-17 µmol/L; 10-14 µmol/L; <10 µmol/L) and transferrin saturation (TSAT) (C) (Not measured; ≥50%; 40-49%; 30-39%; 20-29%; 10-19%; <10%).

Abbreviations: - Ferr: ferritin; TSAT: transferrin saturation.

A


Ferr not measured
n=5,436
HR=0.62
(0.55-0.70)
p<0.001
Ferr >300
n=346
HR=1.30
(1.05-1.60)
p=0.01
Ferr 100-300
n=1,024
Reference
Ferr 30-99
n=1,249
HR=0.99
(0.86-1.20)
p=0.92
Ferr <30
n=844
HR=0.88
(0.75-1.04)
p=0.14
0
1
Age & sex adjusted HR and 95% CI

B


Iron not measured
n=7,955
HR=1.00
(0.70-1.50)
p=0.86
Iron >30
n=18
HR=2.0
(0.84-5.0)
p=0.12
Iron 20-30
n=69
HR=2.0
(1.15-3.50)
p=0.02
Iron 17-20
n=75
HR=1.80
(1.07-3.20)
p=0.03
Iron 14-17
n=100
Reference
Iron 10-14
n=215
HR=1.50
(0.98-2.40)
p=0.06
Iron <10
n=467
HR=2.30
(1.56-3.50)
p<0.001
0.25
0.5
1
2
4
Age & sex adjusted HR and 95% CI

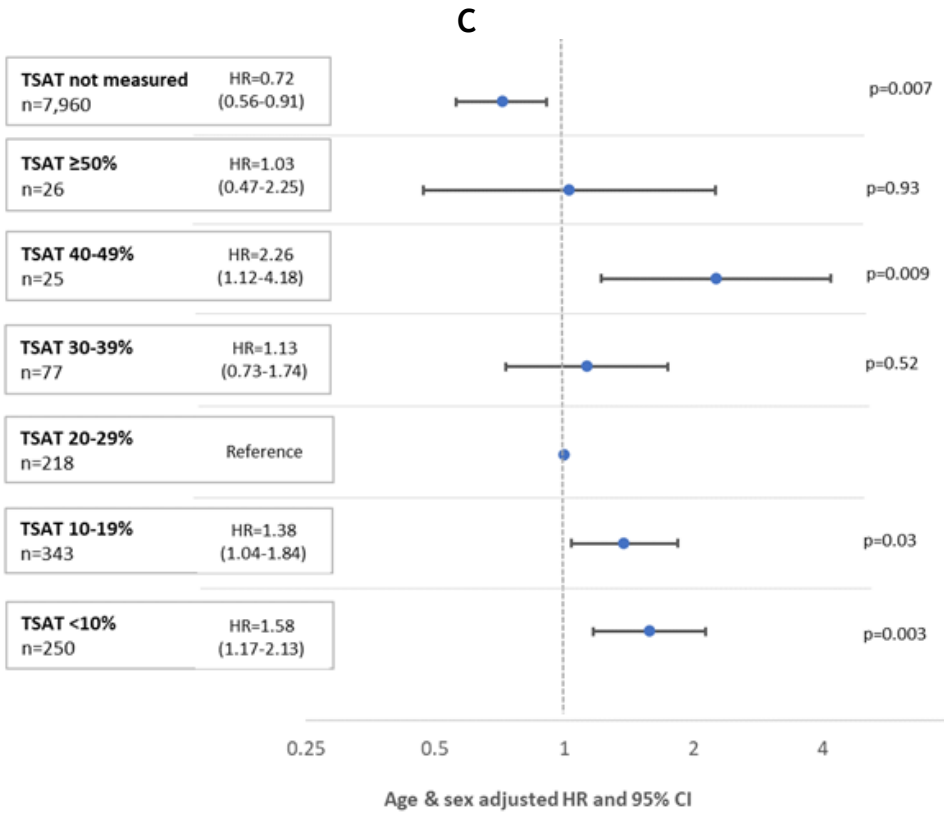


Figure S2628: Forest plots showing all-cause mortality from 1st January 2015 to 31st March 2018 in surviving patients with prevalent heart failure during 2013/14 according to concentrations of serum ferritin (A) (Not measured; >300 µg/L; 100-300 µg/L; 30-100 µg/L; <30 µg/L), serum iron (B) (Not measured; >30 µmol/L; 20-30 µmol/L; 17-20 µmol/L; 14-17 µmol/L; 10-14 µmol/L; <10 µmol/L) and transferrin saturation (TSAT) (C) (Not measured; ≥50%; 40-49%; 30-39%; 20-29%; 10-19%; <10%).

Abbreviations: - Ferr: ferritin; TSAT: transferrin saturation.

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