CHAPTER 7

DISCUSSION AND FUTURE PERSPECTIVES

ABBREVIATIONS

CKD	=	Chronic kidney disease
HFpEF	=	Heart failure with preserved ejection fraction
HFrEF	=	Heart failure with reduced ejection fraction
IV	=	Intravenous
LVH	=	Left ventricular hypertrophy
NT-proBNP	=	N-terminal pro-brain type natriuretic peptide
NYHA	=	New York Heart Association
sTfR	=	Soluble transferrin receptor
TSAT	=	Transferrin saturation

Despite substantial improvements in heart failure treatment over the years, its prevalence is rising throughout the world and only small prolongations in survival are observed.1 There is growing evidence that the presence of comorbidities is one of the factors that has a negative influence in patients with heart failure on multiple levels.2 Both common in acute and chronic heart failure and established risk factor for morbidity and mortality, anemia has received considerable attention over the past decade.3 Although the correction of anemia was thought of as an attractive and novel treatment approach in heart failure, the neutral results of the large pivotal RED-HF trial suggest that anemia might not be a mediator of outcome, but more likely a marker of the severity of cardiac dysfunction.4 Specifically, it is not known whether it is the presence of anemia that contributes to an adverse prognosis or whether it is one of the causal factors of anemia that contributes to a poor prognosis. Aside from being the most common nutritional disorder worldwide, iron deficiency is also the most frequent cause of anemia in heart failure.5 Furthermore, data from a number of randomized controlled trials showed that supplementation of iron deficiency appears to be beneficial in patients with heart failure on multiple endpoints, even in the absence of anemia.6-8 Therefore, the prevalence and potential importance of iron deficiency per se, irrespective of hemoglobin levels, merits more clinical awareness and has generated interest as a potential therapeutic target in heart failure.

The primary aims of this thesis are:

- To study the prevalence and clinical correlates of iron deficiency in patients with chronic heart failure.
- To assess whether iron deficiency is independently related to clinical outcome in chronic heart failure.
- To investigate the relationship between hematological markers and markers of iron homeostasis on the development of heart failure and cardiovascular events in the general population.

PART 1: IRON DEFICIENCY IN CHRONIC HEART FAILURE.

Prevalence and clinical predictors of iron deficiency in chronic heart failure

In the past decade, the presence of iron deficiency in chronic heart failure has often only been explored in the context of anemia. Iron deficiency plays an important part in the pathophysiology of anemia in both chronic heart

failure and chronic kidney disease, a vicious circle described by Silverberg *et al* as the cardiorenal-anemia syndrome.9 However, information on iron deficiency as a separate comorbidity and its role within this complex interplay of pathologies in chronic heart failure is unknown. In **Chapter 2**, we evaluate the prevalence of iron deficiency in a large European multicenter cohort consisting of 1506 chronic heart failure patients. Iron deficiency, defined as a ferritin level < 100 ug/L or 100 – 299 ug/L in combination with a transferrin saturation (TSAT) < 20%, was common in this chronic heart failure cohort, affecting exactly 50% of the study population. Furthermore, the presence of iron deficiency increased with the severity of the disease, as assessed by New York Heart Association (NYHA) functional class. Interestingly, patients with iron deficiency less often had heart failure with a reduced ejection fraction (HFrEF), suggesting that the prevalence of iron deficiency in heart failure with a preserved ejection fraction (HFpEF) might be even higher. However, given the small number of patients with HFpEF in this chronic heart failure cohort, these findings should be interpreted with caution.

Chapter 3 additionally explores the role of iron deficiency, both with and without anemia and/or chronic kidney disease (CKD), using data from the same international heart failure cohort. We observed that in patients without chronic renal impairment or anemia, iron deficiency was still present in approximately 24%. As expected, anemic patients were more often iron deficient (61.2%) compared to non-anemic patients (45.6%). Likewise, iron deficiency was more pronounced in patients with versus without CKD (56.4% vs. 47.4%, respectively). When stratified by NYHA functional class, the prevalence of combined comorbidities rose, although only comorbidities incorporating iron deficiency. It has been known that the number of comorbidities increases with disease severity.2 Combined with our observations in **chapter 2**, these results show that iron deficiency is frequently observed in a wide range of patients from several countries, even when disease severity is low. Furthermore, an increase in disease severity may lead to the subsequent development of other comorbidities, like anemia and/or renal dysfunction.

In a secondary analysis, we examined possible predictors of iron deficiency or combined syndromes incorporating iron deficiency. **Chapter 2** revealed clinical predictors of iron deficiency to be women, the presence of anemia, worse NYHA functional class, and higher levels of N-terminal pro-brain-type natriuretic peptide (NT-proBNP). Additional results from **chapter 3** demonstrated older age and inflammation to be associated with all combinations of comorbidities. This might suggest, to some degree, that the underlying pathophysiological interplay between iron deficiency,

anemia and CKD may be age-related and has an inflammatory origin (e.g. hepcidin overproduction and subsequent iron deficiency). Supporting this hypothesis is the fact that inflammation (hs-CRP levels) was not associated with anemia, CKD or iron deficiency when present as a sole comorbidity.

Iron deficiency and survival in chronic heart failure

Over the years, both CKD and anemia have been extensively described as prognostic risk factor in chronic heart failure.[3-10] At the start of this thesis, information regarding prognosis of iron deficiency in chronic heart failure was limited and only a few studies had reported on iron deficiency as a predictor of outcome.[11-13] Unfortunately, conflicting results were observed between studies, encouraging the need for more research on iron deficiency-related mortality, independent of hemoglobin levels or renal function.

Using data from the same multicenter international study of chronic heart failure patients, we additionally analyze the role of iron deficiency and its relation to all-cause mortality in **chapter 2**. After 8 years of follow-up (median follow-up 1.92 years), 440 of the initial 1506 patients died. Eight-year survival rates were worse in iron deficient patients compared to patients without iron deficiency. Furthermore, iron deficiency remained independently associated with an increased risk for mortality, whereas the presence of anemia did not. Finally, iron deficiency added significant prognostic information on outcome on top of established clinical risk factors (including age, sex, renal function, NYHA functional class, levels of NT-proBNP and the presence of anemia).

Chapter 3 further amplifies the prognostic role of iron deficiency, either as a separate comorbidity or with concomitant anemia and/or CKD. With an increasing number of comorbidities, 8-year event-free survival rates dropped from 58% (1 comorbidity present) to 18.4% (all 3 comorbidities present). Subsequent analyses revealed that iron deficiency, both as individual comorbidity and in combination with anemia, CKD or both, was associated with increased mortality. These findings advocate a possible prognostic role of iron deficiency, both within and beyond the process of erythropoiesis, in patients with chronic heart failure.

PART 2: HEMOGLOBIN, IRON STATUS AND NEW ONSET HEART FAILURE OR CARDIOVASCULAR EVENTS IN THE GENERAL POPULATION.

Hemoglobin and risk for new onset heart failure

The second part of this thesis focused on the role of hemoglobin and markers of iron homeostasis and their association with the development of cardiovascular disease and heart failure in the general population. Previous findings from large health insurance databases in the United States indicate that the presence of anemia may be associated with the development and prognosis of heart failure.14,15 However, these studies mainly focused on the elderly population14 or subjects with an impaired renal function15 and may therefore not accurately represent the general population. In **chapter 4** we aim to determine whether hemoglobin is associated with new onset heart failure in subjects free of this condition. For this objective, we used data from the Prevention of REnal and Vascular ENd-stage Disease (PREVEND) study, a large prospective, well-characterized, observational cohort with a long-term follow-up period. This study was primarily designed to assess the impact of elevated urinary albumin excretion (UAE) on future renal and cardiovascular disease in subjects aged 28 – 75 years in the general population.16

Of the initial 8592 subjects, 6744 had data on hemoglobin measurements and outcome. A total of 217 subjects were newly diagnosed with heart failure during a median follow-up of 8.3 years. Interestingly, in subjects with slightly elevated hemoglobin levels higher annual heart failure incidence rates were detected compared to normal hemoglobin concentrations. On the other side of the distribution, a higher annual heart failure incidence was only observed in severe anemia, indicating a "U-shaped" relationship between hemoglobin and risk for new onset heart failure. In consecutive regression analyses, the association between hemoglobin (as a continuous variable) and risk for new onset heart failure appeared to be indeed "U-shaped", even after adjustment for well-established cardiovascular and heart failure risk factors such as age, gender, heart rate, smoking, the presence of diabetes, hypertension, history of myocardial infarction, and renal function.

Possible explanations for this U-shaped relationship might be the effect of hemoglobin on nitric oxide and subsequent effects on vascular resistance.17,18 Decreasing hemoglobin levels, developing hypoxia and enhanced nitric oxide (NO) activity may lead to a reduced vascular resistance, vasodilatation and an increased cardiac output. Eventually, this process may result in progressive cardiac enlargement and left ventricular hypertrophy (LVH).18

On the other side of the spectrum, higher hemoglobin and hematocrit levels lead to increased viscosity and reduced nitric oxide bioavailability, resulting in impaired flow-mediated dilatation and increased peripheral resistance. Peripheral resistance, the hallmark of established hypertension, alters wall stress in the left ventricle, which may consequently lead to LVH.17 However, as with other studies that link have linked hemoglobin levels with outcome (in this case new onset heart failure), the question rises whether hemoglobin levels itself or underlying diseases, causing alterations in hemoglobin concentration, influence outcome.

Markers of iron homeostasis and risk for new onset heart failure or cardiovascular events

Cardiomyopathy is a common manifestation in patients with iron overload.19 In contrast, animal studies show that severe iron deficiency is also associated with abnormalities in systolic and diastolic cardiac function,20 suggesting an U-shaped relationship between iron stores and the process leading to heart failure (similar to the observation we made for hemoglobin and new onset heart failure in **chapter 4**). Prior epidemiological studies have proposed an association between increasing body iron stores (expressed as serum ferritin levels) and risk for cardiovascular disease in the general population, whereas others have found conflicting results.21-23 Likewise, the role of hepcidin as a risk modifier in cardiovascular disease is also controversial.24 In **chapter 5,** we investigated whether markers of iron status, either depleted and/or in overload, or iron-regulatory hormone hepcidin predict the risk of new onset heart failure or cardiovascular events among healthy individuals. For these analyses, we used data from the previously mentioned PREVEND cohort. At the time of analyses, the follow-up of cardiovascular disease had been extended to level with the heart failure follow-up data, giving us the opportunity to additionally explore the association with the development of cardiovascular disease.

In 6386 subjects measurements of ferritin, hepcidin, and data on outcome were available. During follow-up, 199 subjects were newly diagnosed with heart failure and 456 experienced a cardiovascular event. Unlike the previously suggested U-shaped relationship, the association between ferritin levels and new onset heart failure appeared to best described as linear. To our surprise, the annual adjusted heart failure incidence rose per increasing ferritin quartile in women only. Likewise, multivariable analyses revealed increasing ferritin levels only to remain predictive for new onset heart failure in women. This association persisted within strata defined by markers of the metabolic syndrome, markers of inflammation or other markers of iron

homeostasis, including hepcidin. No relationship between body iron stores or hepcidin levels and the development of cardiovascular disease or all-cause mortality was observed (neither in men nor women) in multivariable analyses.

The reason why increasing ferritin levels are associated with the development of heart failure is not clear-cut. The observations made in **chapter 5** suggest that iron maldistribution among organs; tissues or cellular compartments may play an indirect or direct role in the development of heart failure in apparently healthy individiuals. Also known as dysmetabolic hyperferritinemia, this form of iron maldistribution has been associated with diabetes, obesity, and hypertension, all strong predictors of new onset heart failure.25 During circumstances of subclinical systemic inflammation, iron efflux in organs may be halted due to increased hepcidin production and subsequent internalization and degradation of its receptor, iron exporter ferroportin.Thus, elevated ferritin levels (and hepcidin) might reflect a low-grade inflammatory response to another pathophysiological process, causally responsible for the development of HF. Supporting this hypothesis is the fact that we observed a positive trend in blood pressure, BMI, and levels of glucose, cholesterol and hs-CRP per increasing ferritin quartile. Additionally, strong associations between ferritin and levels of hepcidin, hs-CRP, and glucose in women were observed. Still, the exact mechanism linking increased ferritin levels, iron-regulatory hormone hepcidin and the development of heart failure is still not completely understood and merit more exploration.

Interestingly, the association of serum ferritin and new onset heart failure was far stronger in women compared to men. It has been advocated that some traditional cardiovascular risk factors may have a different impact on males compared to females.26 Additional literature also suggests that the link between ferritin, hepcidin and dysmetabolic features may be particularly relevant in women.27 Hormones might also be partially responsible for the sex-specific differences observed in the present study. Yet, although we adjusted for menstrual status in multivariable analyses and performed sensitivity analyses in postmenopausal women, levels of ferritin remained associated with incident heart failure. Nevertheless, these results need to be reproduced in other population-based cohorts and warrant further investigation.

Chapter 6 of this thesis is a review on our current understanding on iron deficiency in heart failure. We describe recent advantages made in this field

in terms of pathophysiology, prevalence, diagnostics, prognostic implications, and both current and emerging therapeutic approaches.

WHAT HAVE WE LEARNED FROM READING THIS THESIS?

This thesis has provided an overview of the prevalence, clinical predictors and prognosis of iron deficiency in chronic heart failure, either as a separate comorbidity or in combination with other comorbidities, such as anemia and CKD. We conclude from the first part of this thesis that iron deficiency is frequently observed in patients with chronic heart failure. Secondly, disease severity (assessed by NT-proBNP levels and NYHA functional class) seems to be a strong predictor of iron deficiency, while inflammation is associated with iron deficiency when accompanied by anemia, CKD, or both. Finally, using a wide range of chronic heart failure patients from several countries with different cultural characteristics and geographic environments, our results confirm that a depleted iron status has detrimental effect on survival in chronic heart failure, regardless of hemoglobin concentration or renal function. Furthermore, we studied the role of hemoglobin and markers of iron homeostasis and their association with new onset heart failure or cardiovascular events in the general population. Summarizing from the second part, the association between levels of hemoglobin and the risk for new onset heart failure appears to be best described as "U-shaped". Interestingly, higher hemoglobin levels, already within the high-normal range, are associated with an increased heart failure incidence. This was in contrast to anemia, where a higher annual HF incidence was only observed for severe anemia. The relationship between serum ferritin levels and new onset heart failure appears to be linear. Increasing ferritin levels independently amplify the risk for new onset heart failure in apparently healthy women from the general population and might be directly or indirectly involved in the pathogenesis of heart failure.

FUTURE PERSPECTIVES

In recent years, the role of iron deficiency in patients with heart failure has received considerable attention. Recent heart failure guidelines of the European Society of Cardiology now recommend a diagnostic work-up for iron deficiency in all patients suspected to have heart failure.28 However, many

questions, that merit the need for further investigation and exploration, remain unanswered.

How should iron deficiency be defined in patients with heart failure?

Although frequently observed in heart failure, there is no clear-cut definition for iron deficiency, leading to a wide variation in reported prevalence. To date, the definition of iron deficiency in heart failure has been based primarily on measurements of serum ferritin levels and TSAT and includes both absolute and functional iron deficiency. However, in heart failure or other chronic diseases (e.g. CKD), both markers may be influenced by multiple factors such as low-grade inflammation, concomitant liver disease or malnutrition.[29,30] This makes the interpretation of ferritin levels or TSAT more challenging in these patients.[30,31]

In recent years, novel circulating markers of iron status have been identified that might aid in the diagnosis of iron deficiency in patients with heart failure and other chronic illnesses.[30] To date, the examination of bone marrow iron content is still considered the *golden standard* for determining reduced or depleted iron stores.[32] However, the invasiveness of this procedure limits its use in daily clinical practice, thereby increasing the need for studies that compare a biomarker-oriented approach to bone marrow iron content in numerous clinical scenarios. A recent study in patients with stable coronary artery disease undergoing cardiac surgery showed that levels of soluble transferrin receptor (sTfR) had the strongest association with bone marrow iron content at a cut-off level of ≥ 1.32 mg/L.[33] These results need to be reproduced in patients with heart failure. Likewise, more studies identifying novel or additional serum markers reflecting iron status in comparison to the criterion standard of bone marrow iron staining are warranted.

Iron deficiency in acute heart failure.

Studies investigating the presence of iron deficiency, with and without anemia, have been primarily performed in patients with chronic heart failure. Data on prevalence, clinical characteristics and prognosis of iron deficiency in the acute decompensated phase have, however, not been adequately described. To date, only two studies have reported on the presence and prognostic role of iron deficiency in patients with acute heart failure. Cohen-Solal and colleagues recently examined the prevalence of iron deficiency in 832 heart failure patients admitted for decompensation.[34] Of all patients, 72% was iron deficient and the presence of iron deficiency remained high during the entire length of hospitalization. In another recently published

study, Jankowska *et al* investigated the presence of iron deficiency, defined as depleted iron stores (low serum hepcidin) accompanied by increased cellular iron requirements (high sTfR), in 165 Polish patients hospitalized for acute heart failure.35 When meeting both conditions (low serum hepcidin and high serum sTfR) iron deficiency was present in 37% of all patients, whereas iron deficiency was present in 65% when based on the contemporary ferritin and TSAT measurements. Interestingly, in this patient cohort, low hepcidin and high sTfR had the highest 1-year mortality risk when compared with those with isolated high sTfR, isolated low hepcidin or a preserved iron status. These data indicate an even higher prevalence of iron deficiency in acute heart failure compared to its chronic form (reporting a prevalence of 30 – 50% in Western populations) when using the contemporary ferritin/ TSAT criteria. Furthermore, the presence of iron deficiency seems to have equally detrimental results on prognosis in patients with acute heart failure, independent from anemia. Therefore, it may be desirable to validate these findings in larger patient cohorts with acute decompensated heart failure.

Iron deficiency in heart failure with a preserved ejection fraction.

Aside from primarily being an object of studies in chronic heart failure patients, investigations on iron deficiency in heart failure have mainly focused on patients with heart failure and a reduced ejection fraction (HFrEF). However, data from large heart failure registries illustrate a similar prevalence (approximately 50%) and equally prognostic burden in patients with preserved ejection fraction (HFpEF).36–38 In recent years, HFpEF is increasingly viewed as a disease entity that might be driven, to some part, by the presence of comorbidities.39 The presence of anemia is reported to be more frequent in HFpEF compared to HFrEF. Moreover, a recent analysis from the SENIORS study demonstrated that anemia was independently associated with multiple endpoints in both HFrEF and HFpEF.40

To date, information on iron deficiency in patients with HFpEF is lacking. Given iron deficiency's relation to anemia status and its potential as a therapeutic target, this area is of particular interest. One might speculate that the results from **Chapter 2** suggest, to some degree, that the prevalence of iron deficiency in HFpEF may be even higher. Only one study by Kasner and coworkers investigated the impact of iron deficiency in 26 nonanemic patients with HFpEF.41 Iron deficiency was present in 15 of the 26 patients (57.6%), indicating that iron deficiency is also common in HFpEF without concomitant anemia. In this HFpEF population, no significant association was observed between iron deficiency and exercise capacity, whereas such an observation has been described in patients with HFrEF.42 Furthermore,

recent unpublished findings in 774 patients of the PEOPLE study showed iron deficiency to be more often present in HFpEF compared to HFrEF (64% vs. 53%, respectively).(*Fitzsimons et al, poster presentation ESC congress 2015*) However, these results need cautious interpretation and further investigation on iron deficiency in HFpEF is warranted to validate these results.

Aside from its prevalence in HFpEF, all randomized controlled trials investigating the treatment of iron deficiency, using IV iron supplementation, have shown clinical benefit on multiple endpoints only in patients with HFrEF.6-8,43 Whether IV iron supplementation is equally beneficial in patients with HFpEF has not yet been investigated. Expected to commence recruitment in 2015, the FAIR- HFpEF trial is the first randomized, double-blind, placebo-controlled study to examine the role of IV iron on change in exercise capacity in 260 patients with HFpEF. Hopefully the positive effects of IV iron supplementation observed in previous trials in patients with HFrEF, can be extrapolated to the iron deficient population with HFpEF.

Iron supplementation and its effect on morbidity and mortality in heart failure.

Treatment of iron deficiency, using IV iron, in chronic heart failure has shown beneficial results on endpoints such as exercise capacity, quality of life, levels of NT-proBNP, renal function and echocardiographic parameters.6-8,43-46 In addition, the positive effect of IV iron supplementation in heart failure was also observed after 1-year follow-up in the recently published CONFIRM-HF study.43 Although not initially powered to address the effect of IV iron therapy on morbidity and mortality, a significant reduction in hospital admissions due to worsening heart failure was observed. Even when pooling the 4 randomized controlled studies, using IV iron in heart failure patients with iron deficiency, the reduction in admissions due to worsening heart failure remained (*Figure 1*).47 Since no difference in the numbers of deaths between groups was described, the authors conclude that 1-year follow-up

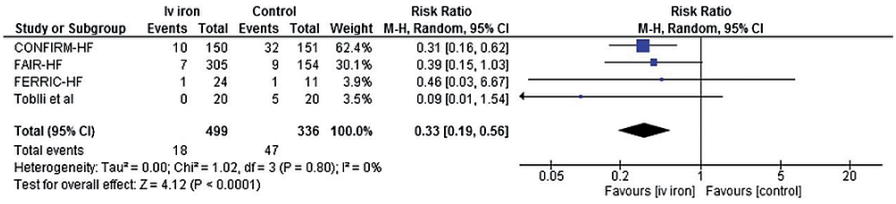

Figure 1. Forest plot of the effects of intravenous iron supplementation (using ferric carboxymaltose in the CONFIRM-HF and FAIR-HF trials; iron sucrose complex in the remaining two trials) on heart failure hospitalization. Abbreviations: CI = Confidence interval.
Adapted with permission from Brunner-la Rocca *et al.*47

may not be adequate to detect any mortality difference. Nevertheless, these results may provide a strong background for an adequately powered, long-term follow-up study to be performed in the future. Such a trial, investigating the effects of IV iron supplementation on morbidity and mortality in heart failure patients with a reduced ejection fraction, is currently underway (FAIR-HF 2).48

So far all studies in heart failure patients have used IV iron to correct iron deficiency. Whether oral iron supplementation might be equally effective in these patients remains unknown. Oral iron has the benefit of being inexpensive and therefore widely available. On the other hand, gastrointestinal side-effects due to oral iron, as well as a number of drug interactions (e.g. proton pump inhibitors) are frequently observed and may lead to limited compliance. Moreover, even if effective, achieving iron repletion using oral iron may take much longer in patients with heart failure, whereas very rapid effects (not only iron stores but also clinical effects) are seen with IV iron. Limited evidence in kidney disease suggests that IV iron is indeed more effective than oral iron.49 However, direct comparisons in heart failure are scarce50 and a definitive prospective head-to-head comparison between oral and IV iron supplementation is pending.

Hopefully, the results from previous studies and the present thesis may help clinicians to better comprehend the relative importance of iron deficiency in heart failure, irrespective of hemoglobin levels. Furthermore, biomarkers such as hemoglobin or ferritin may provide important information regarding etiology and might, to some degree, aid in the identification of subjects at risk for heart failure alongside clinical characteristics. Finally, both ongoing and future studies will confidently establish whether iron deficiency may become one of the treatable comorbidities in the heart failure syndrome.

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