CHAPTER 1

GENERAL INTRODUCTION AND AIMS OF THIS THESIS

INTRODUCTION

Heart failure is considered a heterogeneous syndrome and is characterized by abnormalities in cardiac structure or function. This results in a decline of the heart's pumping capacity and subsequent inability to meet the body's circulatory demands.1 In recent decades, our understanding of the heart failure syndrome has led to remarkable advances in terms of prevention, diagnosis and treatment possibilities. Despite this progress, heart failure prevalence is rising along with the ageing of the general population and is reaching epidemic proportions.2 Furthermore, heart failure remains one of the leading causes of hospitalization in the Western world and prognosis remains dismal.2 Finally, normal daily activities of many heart failure patients remain restricted, which adversely affects their quality of life.3

Comorbidities in heart failure.

Patients with heart failure often present with concomitant diseases that have a negative effect on on multiple levels.4 Analyses from the European Society of Cardiology Heart Failure Survey indicate that 74% of chronic heart failure patients have at least one comorbidity, the most common of which are diabetes, renal dysfunction and anemia.5 During the past decade, anemia has received a lot of attention in the setting of heart failure. As a frequently observed comorbidity in both acute and chronic heart failure, its presence complicates treatment and affects clinical outcome.6,7 A large meta-analysis in 2009 showed an overall prevalence of 37.2%, increasing with severity of the disease.8 Furthermore, an adjusted 46% increased risk for all-cause mortality in anemic heart failure patients was observed. However, recent speculations suggest that anemia might not be a mediator of outcome but merely a marker of disease severity. Similarly, the mechanism by which the presence of anemia contributes to an adverse prognosis is a matter of ongoing debate. The pathogenesis of anemia in heart failure patients is considered to be complex and multifactorial, consisting of elements such as renal dysfunction, decreased erythropoietin production and resistance, hemodilution, chronic inflammation, and in particular iron deficiency.9–11

Iron homeostasis and the role of hepcidin.

In addition to being the most common nutritional deficiency and frequently observed medical disorder in everyday clinical practice, iron deficiency is one of the leading risk factors for disability and death worldwide.12 Iron is a metabolically active micronutrient needed in all mammalian cells and

generally linked to anemia and the process of erythropoiesis.13 Beyond its pivotal role for optimal hematopoiesis, iron is crucial in oxygen uptake and transport (as a component of hemoglobin), oxygen storage (as part of myoglobin), and oxygen metabolism in both skeletal and heart muscle (as part of the oxidative enzymes and respiratory chain proteins).14,15 Furthermore, iron is involved in the synthesis and degradation of many other products, such as lipids, carbohydrates, DNA, and RNA. Whilst the human body prioritizes the metabolic use of iron in several ways, erythropoiesis has priority over other functions. Therefore, hematopoiesis often remains undisturbed until late in the course of iron deficiency, leading to the development of anemia (*Figure 1*).16

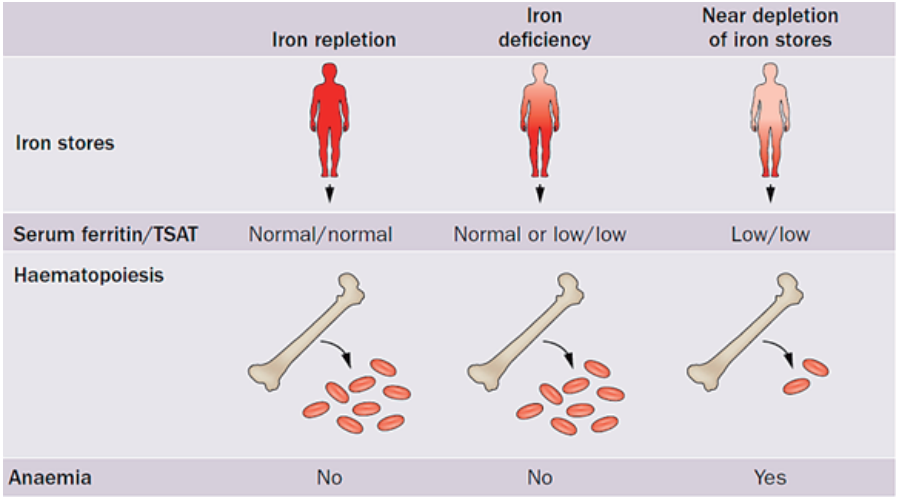

Figure 1. A comparison of different levels of iron depletion in the human body and their effects on serum iron markers and hematopoiesis. Adapted with permission from von Haehling *et al.*17
TSAT = Transferrin saturation.

Body iron content is approximately 3–5 grams, of which two-thirds is incorporated in hemoglobin.18 Since the human body does not possess any known mechanisms of active iron excretion, mammalian iron balance is maintained by limiting the intestinal uptake of iron and continuous recycling and reusing of cellular iron via hepatocytes and macrophages of the reticuloendothelial system.12

Physiologically, we can distinguish two major iron pools in the body: stored iron (in hepatocytes, bone marrow sideroblasts, and reticuloendothelial cells) and utilized iron (circulating iron bound mainly to transferrin and intracellular iron in virtually all hematopoietic and extra-hematopoietic

cells). Circulating iron is mainly bound to transferrin.19 The relative amount of iron bound to transferrin, or transferrin saturation (TSAT), reflects the amount of iron available for metabolizing hematopoietic and non-hematopoietic target cells. Iron is stored in the liver, bone marrow and spleen in a non-toxic form as ferritin, where its soluble form is excreted to the circulation.20 Under normal circumstances, serum ferritin is a reliable surrogate of storage iron as it closely correlates with body iron stores.21

Both circulating and cellular iron levels are tightly regulated by mechanisms that control iron absorption, storage, recycling and release. At the systemic level, iron homeostasis is orchestrated by the liver derived protein hepcidin, which has emerged as the master regulator of systemic iron homeostasis.22 By binding and degrading to the sole cellular iron exporter ferroportin, hepcidin restricts the amount of iron delivered to the circulation and target tissues.23 Whereas depleted iron stores, hypoxia or ineffective erythropoiesis decrease systemic hepcidin expression, iron overload and (low-grade) inflammation cause the opposite effect (*Figure 2*).

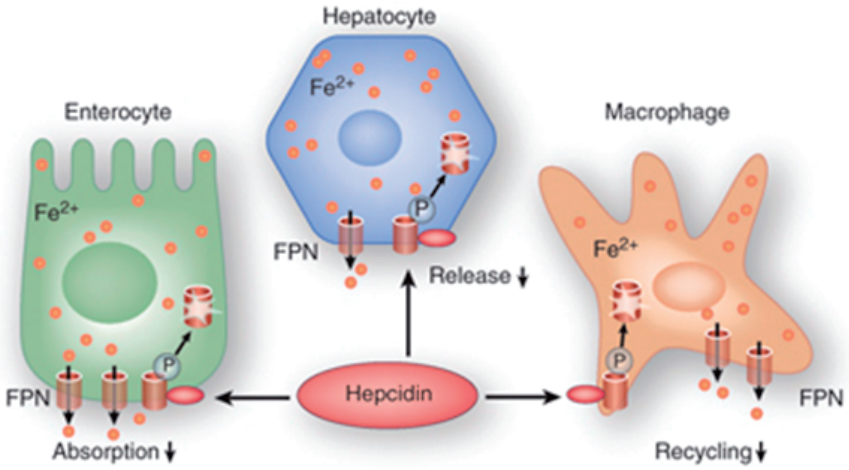

Figure 2. Hepcidin downregulates ferroportin expression on the cell surface of enterocytes, hepatocytes and macrophages of the reticuloendothelial system. The binding of hepcidin results in subsequent phosphorylation, internalization and degradation of ferroportin, leading to the reduction of iron absorption from the gut and lower iron release from hepatocytes of recycling macrophages. Adapted with permission from Cui *et al.*23

Iron deficiency in heart failure.

Recently, it has been proposed that patients with heart failure may be prone to develop iron deficiency. Traditionally, iron deficiency has been considered to only have clinical consequences in heart failure when anemia is present.

Alternatively, since hematopoiesis often remains undisturbed until late in the course of iron deficiency, a reduction in hemoglobin can also be viewed as the end results of a process beginning with the gradual depletion of iron stores.[16,21] Similar to other chronic diseases, patients with heart failure present with augmented generalized inflammation and consecutive activation and production of inflammatory cytokines and acute phase proteins, like hepcidin.[24,25] Other factors that may play a role in the development of iron deficiency in heart failure include insufficient dietary iron take, gastrointestinal malabsorption of iron and blood loss.[26-29] Therefore, the maintenance of a normal iron metabolism is crucial for cells with a high mitogenic potential as well as cells with a high energy demand (e.g. skeletal myocytes, and cardiomyocytes). This is important in heart failure, since an impaired oxidative metabolism or energy production in both myocardium and peripheral tissues are cardinal features of this syndrome.[10]

Although data on incidence of iron deficiency in heart failure is scarce, a few reports in the past 50 years already describe the occurrence of sympathetic activation, left ventricular hypertrophy, dilatation and reversible symptomatic HF with the presence of iron deficiency.[30-32] The first evidence that iron deficiency frequently co-existed with anemia in heart failure was reported with a prevalence ranging from 20–40%.[33,34] Using the gold standard of bone marrow iron staining, one study revealed that 73% of anemic patients with advanced heart failure had depleted iron stores.[35] However, recent data suggest that the presence of iron deficiency, both as a separate non-cardiac comorbidity and with concomitant anemia, may be even higher in heart failure.[36] Furthermore, a number of recent studies have shown clinical benefit on multiple endpoints when correction of iron deficiency was achieved using intravenous iron supplementation in patients with chronic systolic heart failure.[37-41] Interestingly, these observations were made in both anemic and non-anemic patients, shifting the focus for anemia in heart failure away from hemoglobin and towards iron.[41] Therefore, the prevalence and prognostic importance of iron deficiency per se, irrespective of hemoglobin levels, is currently a subject of interest in heart failure.

AIMS OF THIS THESIS

The current profile of "real-world" heart failure patients (e.g. advanced age and presence of multiple comorbidities) has generated interest in emerging comorbidities, like iron deficiency, as potential therapeutic targets. However, information on prevalence and clinical correlates of iron deficiency in heart

failure is limited. Furthermore, the role of iron deficiency with regard to morbidity and mortality in heart failure is currently unclear. Finally, interest in the use of biomarkers alongside clinical characteristics to aid in early disease identification of subjects at risk for the development of heart failure or cardiovascular events has grown in recent years. Several biomarkers have been proposed to have modest additive value on top of clinical characteristics for predicting new onset heart failure in the general population. However, only a few are actually used in daily clinical practice. Both widely available and relatively inexpensive to measure, markers of erythropoiesis and iron homeostasis could be possible potential markers for early identification of subject at risk for new onset heart failure. Their role in terms of risk prediction is currently unknown.

Part 1 of this thesis investigates the role of iron deficiency in patients with chronic heart failure. For these purposes, we use a more contemporary definition (ferritin < 100 ug/L or ferritin 100–299 ug/L with a transferrin saturation < 20 %) to define iron deficiency in heart failure. This definition has been used in numerous clinical heart failure trials and has been recently implemented in the most recent heart failure guidelines of the European Society of Cardiology. **Chapter 2** examines the prevalence of iron deficiency, its clinical associates and prognostic consequences in chronic heart failure patients, with and without concomitant anemia. The combination of anemia and renal dysfunction in chronic heart failure has been described as the cardiorenal-anemia syndrome. **Chapter 3** investigates the role of iron deficiency within this complex interplay of co-existing pathologies regarding prevalence and prognosis. **Chapters 2** and **3** are both retrospective analyses at patient level of a large international pooled patient cohort consisting of "real-life" chronic heart failure patients.

Part 2 focuses on hemoglobin and markers of iron homeostasis in terms of risk prediction for new onset heart failure and cardiovascular events. Identifying subjects at risk for the development of heart failure or cardiovascular disease is appealing and the use of (simple) biomarkers may provide important information regarding etiology and aid in clinical risk prediction. **Chapter 4** investigates hemoglobin levels and the risk for new onset heart failure in the community. **Chapter 5** describes the predictive role of ferritin (as a marker of iron status) and iron-regulatory hormone hepcidin for the development of heart failure, cardiovascular events and all-cause mortality. Both **chapters 4** and **5** are post-hoc analyses of the Prevention of REnal and Vascular ENdstage Disease (PREVEND) study, a large, prospective, well-

characterized, observational-cohort, initially aimed to assess the impact of elevated urinary albumin excretion in non-diabetic subjects on future renal and cardiovascular disease.42

Chapter 6 provides an overview of our current understanding and novel insights into the mechanisms of iron homeostasis, its global perspective, clinical implications and prognostic consequences and recent and possible future therapeutic approaches for treatment of iron deficiency in heart failure.

Finally, the findings, conclusions and relevance of this thesis and directions for future research are discussed in the **Discussion and future perspectives**.

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