1 Introduction

HEART FAILURE

Heart failure (HF) is a state in which cardiac functioning fails to meet the oxygen and nutrient demands required by the body to maintain its normal function. It is a clinical syndrome characterized by symptoms like shortness of breath, leg swelling and exercise intolerance. For a person aged 55 years, the lifetime risk of developing HF is about 30%.[1] In the adult population, 1-2% has HF, but it mainly affects the elderly; 6-10% of people aged 65 or older have HF.[2] Due to the aging population in (Western) countries, the prevalence is expected to double within the next 40 years.

Despite modern treatments, the condition usually worsens over time. Unfortunately, 30 to 40% of patients diagnosed with HF die within one year after receiving the diagnosis and 60-70% die within 5 years.[3] This makes HF more lethal than some common forms of cancer.[4] HF patients die from worsening HF or sudden cardiac death due to ventricular arrhythmia's.

Heart failure currently has no cure; treatment focuses on improving symptoms and preventing acute decompensation or disease progression. This is achieved by addressing reversible causes such as valvular anomalies, providing lifestyle education and treatments using pharmacological agents or devices.[5] It is recommended that all HF patients with a reduced ejection fraction receive first-line therapy consisting of angiotensin converting enzyme inhibitors (ACE inhibitors), or when not tolerated angiotensin II receptor blockers (ARBs), and β-blockers. In still symptomatic patients, mineralocorticoid receptor antagonists are added to this regime. Diuretics are used to treat symptoms and congestion.[5] There are far less options for patients with HF with a preserved ejection fraction. No treatment has yet been shown, convincingly, to reduce morbidity or mortality in this population. Currently, treatment consist of diuretics to alleviate signs and symptoms, and the screening and treatment of comorbidities.

Comorbidities

An essential part of the management of HF, either with a reduced or preserved ejection fraction, concerns the diagnosis and treatment of comorbidities. Of all HF patients older than 65 years, 98% have at least one comorbidity.[6-8] Two prevalent comorbidities contribute directly to the already impaired peripheral oxygen delivery: iron deficiency and anemia. First considered cause and consequence, recent results, including ours, have shown that both have to be considered as separate entities.

ANEMIA

Anemia is defined by the WHO as a hemoglobin level of <12g/dL (~7.5mmol/L) in women and <13g/dL (~8.1mmol/L) in men.[9] Worldwide, the prevalence of anemia is 32.9%, with especially high prevalence's in sub-Saharan Africa and south-east Asia.[10] In **chapter 2**, we discuss anemia in the context of the HF patient; we describe the pathophysiology and focus on the results of clinical trials aimed at correcting anemia and promising future therapies. The majority of therapies developed for anemia in HF comprise drugs targeting erythropoiesis, mainly through its hormonal stimulation, for instance by the administration of recombinant erythropoietin (EPO).

Endogenous EPO

Erythropoietin is the primary regulator of erythropoiesis; in the bone marrow, EPO promotes the proliferation of erythroid progenitor cells and increases the production of red blood cells.[11,12] While normally erythropoiesis takes place at a low basal rate, EPO is capable of enhancing production as much as eightfold compared to the baseline rate. Eighty percent of EPO is produced in the kidney in reaction to impaired oxygen delivery, the remaining mostly being produced in the liver.[13] To allow for interpretation of EPO levels, we were interested in the normal values, physiological function and regulation of human EPO. In **chapter 3**, we study endogenous EPO levels in a large sample of the general population of Groningen.

High levels of endogenous EPO are frequently observed in patients with HF.[14-16] The etiology of the elevated EPO levels in HF is multifactorial, but includes direct stimulation of EPO synthesis by renal hypoxia, bone marrow resistance to EPO and increased angiotensin II concentrations.[17-19] Previous studies showed that the endogenous EPO level is a prognostic marker in patients with chronic HF.[14,20] It is currently unknown if EPO levels are also associated with the incidence of new onset HF. We study the association of endogenous EPO levels with the development of new onset HF and other cardiovascular events in **chapter 4**.

Exogenous EPO

Anemia can successfully be corrected in the majority of HF patients using a recombinant form of EPO.[21,22] One of these drugs, darbepoetin-alfa was studied in the randomized, blinded clinical trial the **R**eduction of **E**vents with **D**arbepoetin alfa in **H**eart **F**ailure trial (RED-HF).[21] In this trial, 2,278 patients with symptomatic chronic HF (LVEF ≤ 40%) and anemia (HB level 9.0 to 12.0 g/dL) were treated with darbepoetin-alfa with a target hemoglobin level of 13.0 to 14.5 g/dL or placebo. Disappointingly, this approach did not result in a better prognosis. In contrast, rates of stroke and thromboembolic events were

increased in patients treated with darbepoetin-alfa, halting the clinical use of recombinant EPO in HF patients. Despite these consequences, patients with chronic kidney disease still often use recombinant EPO to prevent the need for blood transfusion. It is known that approximately 25% of chronic kidney disease patients are poor responders, that is a low hemoglobin increase in response to darbepoetin-alfa.[23] This low response is associated with increased mortality.[23] Since the syndromes of HF and chronic kidney disease show large overlap, we study the hematological response to darbepoetin-alfa in the large randomized controlled RED-HF trial in **chapter 5**.

IRON DEFICIENCY

Next to erythropoietin, iron is an essential factor in the successful production of a red blood cell. A deficiency in iron often leads to anemia, and many of the consequences seen in patients with iron deficiency were thus attributed to anemia. However, data showed that iron deficiency is, independent from the presence of anemia, associated with more signs and symptoms and an increased morbidity and mortality in patients with HF.[24,25] New, safer, iron preparations boosted research in this area and clinical trials showed that treatment with intravenous iron improved signs and symptoms of HF in iron deficient subjects.[26-31] Iron deficiency is present in 30 – 72% of the HF population, depending on its definition and HF severity.[24,32,33] The gold standard of the diagnosis of iron deficiency is a Prussian blue staining of a bone marrow aspirate. This procedure is invasive, painful and time-consuming. In clinical care, but also in research, a definition based on serum biomarkers is therefore used but never validated. Based on data from healthy subjects and other chronic diseases a combination between ferritin and transferrin saturation (TSAT) is often used. However, ferritin is an acute phase reactant and therefore increased in varying degrees in subjects with HF, depending on the severity of the low grade inflammation. In **chapter 6**, we study bone marrow aspirates in relation with circulating biomarkers in patients with HF to provide an optimal definition of iron deficiency. We subsequently study this definition in two different clinical settings to assess effects on morbidity, mortality and treatment effect. The additional benefit of a bone marrow iron staining is the possibility to assess the pathophysiology of the iron deficit. We can quantify the amount of iron stored in the bone marrow and the actual amount of iron incorporated in the erythroblasts, the precursors of the red blood cells. Using this method, we assess if iron deficient patients have low iron stores or if they have defective iron utilization. We study these different pathophysiological mechanisms in **chapter 7**.

Iron has more functions besides hematopoiesis. Iron can switch between its ferrous (Fe^{2+}) and ferric (Fe^{3+}) form, which gives it the ability to mediate electron transfer, and play a vital role in many redox reactions. Mitochondria rely in their function of ATP production on these redox reactions. The mitochondrial electron transport chain complexes I-V contain iron-sulfur clusters, heme and cytochromes. Complexes I, III and IV facilitate the redox reactions required to pump H+ outside the inner membrane. The result is an electrochemical gradient which is used to drive complex V: an ATP synthase which generates ATP from ADP by oxidative phosphorylation. Cells that require a high energy demand, such as the cardiomyocytes and skeletal myocytes, are rich in mitochondria. Therefore, potential detrimental effects of iron deficiency are expected in these cell types. We study the effects of iron deficiency on mitochondrial function, cell metabolism and contractile function in the human cardiomyocyte in **chapter 8**.

Aims of this thesis

The aim of this thesis is to evaluate the role of the major factors in oxygen transport and utilization in patients with heart failure. These factors include erythropoietin, hemoglobin and iron.

In part I, we study erythropoietin and anemia. In **chapter 2**, we discuss the pathophysiology of anemia in heart failure, review treatments that have been studied and why some have failed and mention potential new treatments. We study erythropoietin in the general population in **chapter 3.** We provide reference values and report on clinical and biochemical correlates. Additionally, we provide a novel genetic variant associated with erythropoietin levels. **Chapter 4** elaborates on this work by assessing associations between endogenous erythropoietin levels and new onset HF and cardiovascular events. In **chapter 5**, we study the treatment of anemia with recombinant erythropoietin. We assess the increase in hemoglobin concentration in reaction to treatment with recombinant erythropoietin, define a group of hypo-responsive subjects and study its consequences.

In part II of this thesis, we focus on the role of iron, and specifically iron deficiency in HF. In **chapter 6** we start defining iron deficiency in HF by using the gold standard for ID: bone marrow aspirations. We assess the value of different circulating biomarkers of iron status in HF patients and compare them to the gold standard. Using the same bone marrow aspiration data, we asses in **chapter 7** two distinct pathophysiological mechanisms of iron deficiency: low iron stores and defective iron utilization. In **chapter 8**, we study the direct effects of iron deficiency on the human cardiomyocyte. In **chapter 9**, we assess potential causality between iron levels coronary artery disease, the most prevalent cause of HF.

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Part I

Anemia and erythropoietin