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Iron deficiency in chronic heart failure with severely depressed systolic left ventricular function

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Contents

1. Introduction

1.1. Objective of the study

Recently it has been recognized that patients with heart failure (HF) may be prone to the development of iron deficiency (ID) as a result of depletion of iron stores, or due to mechanisms present in anaemia of chronic disease (Opasich et al. 2005, Nanas et al. 2006). ID with or without anaemia diminishes aerobic performance, precipitates fatigue and exercise intolerance (Haas and Brownlie 2001) and consequently worsens the clinical symptoms of patients with HF. A recent randomized, double-blind study (Anker et al. 2009) showed that intravenous iron supplementation in ID patients with or without anaemia and chronic heart failure (CHF) exerts favourable effects on functional status and quality of life. Therefore ID lately acquired a unique role in the field of HF and is the subject of novel studies.

In the HF department of the University Heart Centre Hamburg, about 120 new patients with severely progressed or terminal HF are being referred annually for further diagnostic investigation and specialised care, including optimised medical therapy, device therapy or initiation of terminal therapy options, such as implantation of ventricular assist devices or heart transplantation.

The purpose of this dissertation was to determine the prevalence of ID in a selected CHF population with severely depressed left ventricular (LV) systolic function, defined as an ejection fraction (EF) ≤ 30%. A further purpose of this study was to assess independent predictors of the prevalence of ID in CHF with severely depressed left ventricular systolic function.

1.2. Pathophysiology of iron metabolism

Iron plays a crucial role in oxygen uptake and transport [as part of the ferrous ring of haemoglobin (Hb)], oxygen storage (as a component of myoglobin), oxygen metabolism, and energy production (as a component of oxidative enzymes and respiratory chain proteins) and is involved in erythropoiesis (Fairbanks and Beutler 2001, Dunn et al. 2007). Therefore, ID, with or without concomitant anaemia, may be associated with reduced functional capacity and is often accompanied by subjective complaints of poor physical condition with objective indices of exercise intolerance such as diminished oxygen consumption, and attenuated submaximal exercise performance (Figure 1). (Haas and Brownlie 2001, Dallman 1989, Anker 2009).

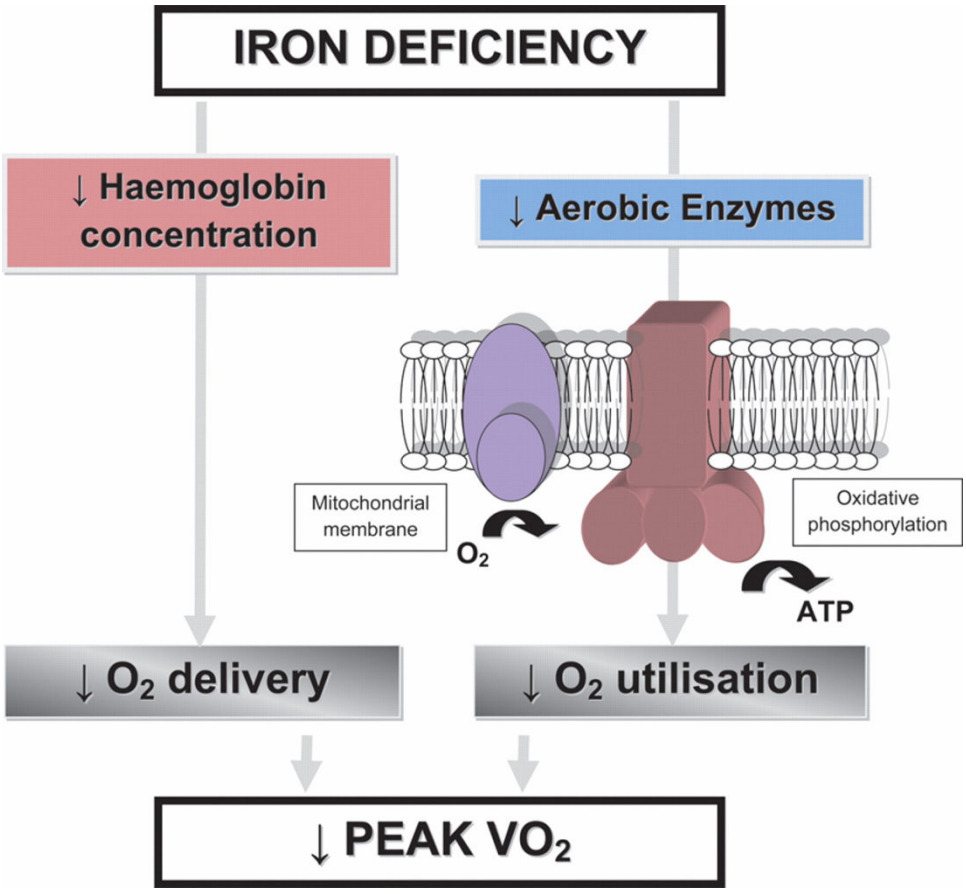

Figure 1. Effect of iron deficiency on erythropoiesis and oxygen metabolism. (from Anker et al. Eur J Heart Fail. 2009;11:1085)

In erythroid cells, iron moves mainly into the mitochondria to be used in haem-synthesis for the subsequent formation of haemoglobin outside the mitochondria and in non-erythroid cells, iron is stored as ferritin and haemosiderin (Andrews 1999).

1.3. Chronic heart failure, anaemia and iron deficiency

The epidemiology of HF is continuously being studied (Mc Kee et al. 1971, Cowie et al. 1997 and 1999, Mosterd and Hoes 2007, Levy et al. 2002). According to the latest heart failure guidelines of the European Society of Cardiology (ESC) based on data collected until 2008 (Task Force for Diagnosis and Treatment of Acute and Chronic Heart Failure 2008 of European Society of Cardiology), the ESC represents countries with a population of 900 million, and there are at least 15 million patients with HF in those 51 countries. The prevalence of asymptomatic ventricular dysfunction is similar, so that HF or asymptomatic ventricular dysfunction is evident in ~4% of the population. The prevalence of HF is between 2 and 3% and rises sharply at ~75 years of age, so the prevalence in 70- to 80-year-old people is between 10 and 20%. In younger age groups, HF is more common in men because of coronary heart disease, which is the most common cause. In the elderly, the prevalence is equal between the sexes. The prevalence of HF is increasing because of the ageing of the population, the success in prolonging survival in patients sustaining coronary events, and the efforts concerning primary or secondary prevention (Murdoch et al. 1998, Senni et al. 1999). In some countries the age-adjusted mortality from HF is falling at least in part due to modern treatment (Murdoch et al. 1998, MacIntyre et al. 2000, Blackledge et al. 2003, Schaufelberger et al. 2004). The mean age of patients with HF in the

community in developed countries is 75 years. Heart failure with normal ejection fraction (HFNEF) is more common in the elderly, in women, and patients with hypertension or diabetes. HF is the cause of 5% of acute hospital admissions, is present in 10% of patients in hospital beds, and accounts for ~2% of national expenditure on health, mostly due to the cost of hospital admissions (Stewart et al. 2002). The prognosis in general is poor, although some patients survive for many years (Mc Kee et al. 1971, Senni et al. 1999, Stewart et al 2001, Cowie et al 2000). Overall, 50% of patients have died by 4 years. Forty per cent of patients admitted to hospital with HF either die or are being readmitted within 1 year. Studies show that the efficacy of diagnosis of HF by clinical means alone is often inadequate, particularly in women, the elderly, and the obese (Remes et al. 1991, Wheeldon et al. 1993).

The reported prevalence of anaemia in CHF varies significantly, from 4 to 61%, due to lack of consensus in the definition of anaemia and varying clinical characteristics of the studied populations (Anker et al 2009, Tang et al 2008, von Haehling 2011). Approximately 20 to 33% of patients with HF may experience anaemia. The prevalence may rise to more than 50% in the setting of severe HF (Tang and Yeo, 2010). Even mild anaemia is associated with worsening of symptoms, increased New York Heart Association (NYHA) class and impaired functional capacity, quality of life, and survival (Szachniewicz et al. 2003).

Several potential causes of anaemia in CHF have been identified: i) decreased production of erythropoietin (EPO) due to impaired renal function (Eschbach 2002) and due to pro-inflammatory cytokines such as interleukin-1 (IL-1) and

tumour necrosis factor α (TNFα) (Opasisch et al. 2005, Ghali 2009). ii) Depression of haematopoiesis due to elevated levels of cytokines (IL-1, TNF-α, interferones) in HF, which directly inhibit the formation of mature erythropoietic cells from erythropoietic progenitors in the bone marrow (Weiss and Goodnough 2005). Moreover, elevated levels of N-acetyl-Ser-Asp-Lys-Pro, a negative regulator of hematopoietic stem cells are documented in HF (Van der Meer et al. 2004). iii) Fluid retention due to impaired renal perfusion in CHF can result in expansion of plasma volume and consequent haemodilution which may cause pseudo-anaemia (Westenbrink et al. 2007). iv) Medication: Angiotensin converting enzyme inhibitors (ACEi) and Angiotensin receptor blockers (ARB) abolish the stimulating actions of Angiotensin II in the proliferation of erythroid progenitor cells through activation of Angiotensin II type I receptors. Additionally ACEi prevent the degradation of the tetra-peptide N-acetyl-Ser-Asp-Lys-Pro which is a natural inhibitor of the proliferation of erythroid progenitor cells (van der Meer P et al. 2005). Antiplatelet therapy or treatment with anticoagulation can also lead to blood loss and iron deficiency.

Recently, it has been recognized that patients with CHF may be also prone to the development of iron deficiency as a consequence of defective iron absorption and reduced availability of iron recycled in the reticulo-endothelial system, in other words due to the mechanisms described for anaemia of chronic disease as well (Opasisch et al. 2005, Nanas et al. 2006). CHF is an inflammatory state with elevated levels of pro-inflammatory cytokines, which in turn block the intestinal absorption of iron and the release of iron, derived from phagocytised senescent red blood cells from the reticulo-endothelial system,

causing a ‘reticulo-endothelial block’ (Weiss and Goodnough 2005).

An important aspect in anaemia of chronic disease is that despite seemingly adequate iron stores assessed by serum iron and ferritin, up to 73% of patients with anaemia, normal kidney function, and advanced CHF had ID as assessed by bone marrow aspiration in a study by Nanas et al. This study demonstrated that neither serum iron nor ferritin levels proved to be reliable markers of ID. Anaemia of chronic disease is characterized by a marked dysregulation of iron metabolism, in particular by a reduced level of iron available for erythropoiesis. Since the main feature of this type of anaemia is an impaired mobilization of iron from cells, it may occur despite adequate iron stores in the body and it is named ‘functional ID’ in contrast to absolute ID, when body iron stores are significantly depleted (Anker et al. 2009).

Therefore, true ID in CHF is defined as ferritin ≤100 µg/l, normally accompanied by high transferrin and low transferrin saturation (Tsat) while functional ID is defined as ferritin between 100 and 299 µg/l, and a Tsat ≤20% (Anker et al. 2009).

1.4. Iron supplementation in CHF

According to a review by Anker et al. (2009) introducing the FAIR-HF study on this topic, four small studies showed that the correction of iron deficiency with the use of intravenous iron in patients with CHF may result in clinical benefits (Bolger et al. 2006, Okonko et al. 2008, Toblli et al. 2007, Usmanov et al. 2008). In one of these studies (Okonko et al. 2008) the symptomatic benefit was similar in patients with anaemia and those without anaemia.

Bolger et al. showed that iron given intravenously as iron sucrose over 5–17

days improved Hb, reduced symptoms and improved exercise capacity over a 3-month follow-up period in anaemic patients with CHF. Toblli et al. further demonstrated that 5 weeks of intravenous (i.v.) iron sucrose treatment in anaemic CHF patients (baseline NYHA class III or IV and LVEF ≤35%) improved quality of life, 6-minute walk distance, and LVEF and reduced the number of hospitalizations compared with controls. Plasma levels of N-terminal pro-brain natriuretic peptide and C-reactive protein were also decreased. Another study by Okonko et al. confirmed benefits of i.v. iron loading with iron sucrose in CHF patients. The authors randomized 35 (18 anaemic and 17 non-anaemic) patients with symptomatic CHF and abnormal iron metabolism to 16 weeks of i.v. iron sucrose or no treatment. Iron therapy was well tolerated and improved exercise capacity and symptoms. Benefits were more evident in anaemic patients. Increments in peak oxygen consumption related only to increments in TSAT, a putative marker of circulating iron status, but not to changes in Hb levels. Finally, in patients with severe CHF, anaemia and chronic kidney insufficiency, Usmanov et al. demonstrated that correction of anaemia using i.v. iron sucrose over a prolonged period (26 weeks) resulted in beneficial effects on electrocardiographic indices of cardiac remodelling, including improvement in cardiac hypertrophy, cardiac dilation, and ejection fraction. An improvement in NYHA status was observed in 47% of NYHA class III patients, but no improvement was seen in NYHA class IV patients.

FAIR-HF is an international, multi-centre, randomized, placebo controlled study (Anker et al. 2009) which provided important efficacy and safety information on the impact of i.v. iron supplementation in CHF. Intravenous treatment with ferric carboxymaltose for 24 weeks in patients who had CHF and iron deficiency with

or without anaemia improved symptoms, functional capacity, and quality of life. The study showed improvement with ferric carboxymaltose in the two primary end points: the self-reported Patient Global Assessment and the NYHA class at 24 weeks. The benefit was evident after 4 weeks and was maintained throughout the study period. These results were confirmed by the observed improvements in distance on the 6-minute walk test distance and in scores on the health-related quality-of-life questionnaires (Gonzalez-Costello and Comin-Colet. 2010).

The treatment with ferric carboxymaltose was beneficial to both patients with anaemia and those without anaemia. This suggests that iron deficiency is a valid independent therapeutic target (Gonzalez-Costello and Comin-Colet 2010). The results were consistent with those from the four small studies already mentioned.

2. Material and Methods

2.1. Patients

Recruitment was performed among patients with systolic CHF visiting the heart failure outpatient clinic of the University Heart Centre Hamburg between January and October 2010. The inclusion criteria were: (i) a known history of CHF of ≥6 months; (ii) LVEF ≤ 30% as assessed by echocardiography (performed at the time of recruiting, using Simpson's planimetric method to calculate LVEF); (iii) unchanged medications for ≥1 month preceding the study (with exception of diuretics) and clinical stability with New York Heart Association functional class II or III;

Exclusion criteria included: (i) Surgery of any kind during the previous 6 months; (ii) Iron supplementation, erythropoietin therapy or blood transfusion during the last 30 days. (iv) Bleeding of any kind within the last 30 days or history of clinically significant repetitive bleedings. (v) Any acute/chronic illness known to influence iron metabolism (e.g. malignancy, infection, severe renal disease requiring dialysis); (vi) C-reactive protein (CRP) ≥10mg/l. (vii) Alanine aminotransferase or aspartate aminotransferase >3x the upper limit of normal.

The screening for this project included retrospective clinical interrogation for all of the above. Additionally, all iron deficient patients included into the study underwent a routine clinical evaluation in order to detect any potential secondary causes of iron deficiency such as an occult malignancy or gastrointestinal bleeding.

2.2. Blood sampling and laboratory, echocardiographic and cardiopulmonary exercise testing measurements.

Blood Sampling

In all patients, venous blood samples were taken in the morning between 09:00 and 11:00. This way there were no substantial influences of the circadian rhythm on iron-status indices.

Laboratory measurements

Haematological values were assessed from fresh venous blood with EDTA. The following values were measured using an automatic system Coulter LH 750 Hematology Analyzer (Beckman Coulter, Krefeld): haemoglobin concentration (Hb, g/dl), mean corpuscular volume (MCV, fl), mean corpuscular haemoglobin (MCH, pg), and reticulocytes (%).

The following biomarkers of iron status were assessed directly from serum: concentrations of iron (µmol/l), ferritin (µg/l) and transferrin (g/l); transferrin saturation (Tsat) was calculated automatically from the formula: [(serum iron (µmol/l) x 50/ transferrin (g/l) x 12.98)] x 0.87. Serum iron measurement was performed with calorimetric assay and transferrin was assessed using an immunoturbidimetric assay on a modular automated clinical chemistry analyzer. Serum ferritin was measured using immunoassay based on electrochemiluminescence. All measurements were performed with Modular E170 (Roche/Hitachi).

Iron deficiency was defined as serum ferritin <100 µg/l, or serum ferritin ≥100 µg/l and ≤300 µg/l with Tsat <20%. Anaemia was defined using the World

Health Organization's criteria (haemoglobin <12 g/dl in women and <13 g/dl in men).

Serum creatinine (mg/dl) measurement was based on the compensated Jaffe method and serum concentration of sodium (mmol/l) on indirect potentiometry and both were performed with the Roche/Hitachi Modular system.

CRP measurements were also performed using the Roche/Hitachi Modular System. Briefly, the anti-CRP antibodies coupled to latex microparticles react with antigen to form antigen-antibody complexes. After agglutination, the complex formation was measured turbidimetrically.

Plasma concentration of pro-type B natriuretic peptide (proBNP, ng/l) was measured using immunoassay based on electrochemiluminescence on the Modular system.

Echocardiographic measurements

The echocardiographic measurements were performed with the GE Vivid E9 Ultrasound from General Electrics. The supportive EchoPAC analysing system was used, with standardised evaluations regarding the following parameters could be assessed: Left ventricular ejection fraction (LVEF), left ventricular diameter at end-diastole (LVEDD), mitral regurgitation grade (MR), tricuspid regurgitation grade (TR), tricuspid annular plane systolic excursion (TAPSE), basal right ventricular diameter at end-diastole (RV basal) and right atrial diameter at end-systole (RA).

LVEF was assessed with the biplane Simpson's method with tracing of the endocardial borders at end-diastole and end-systole in apical four- and two-

chamber views.

LVEDD measurements were made from the parasternal long-axis views at the mitral valve leaflet tips using 2D-targeted M-mode echocardiography. In cases of poor visualization of the endocardial border, 2D direct measurement was applied.

Mitral regurgitation was assessed with two methods. Firstly the mitral regurgitation jet was assessed using Doppler colour flow imaging for semi-quantitative assessment of central regurgitant jets. In colour flow imaging of mitral regurgitation, the area relative to the left atrial size is predictive of regurgitant severity when compared with angiography (Helmcke et al. 1987). In addition, the vena contracta was measured, i.e., the narrowest cross-sectional diameter of the jet.

Grading the severity of tricuspid regurgitation is in principle similar to mitral regurgitation. The measurement of the vena contracta was feasible in most of the patients and applied to quantify TR.

A vena contracta width ≥7 mm defines severe and a width ≥ 3mm and < 7mm defines moderate MR and TR (Lancellotti et al. 2010).

Measurement of TAPSE was performed using the EchoPAC supporting analysing system by M-mode echocardiography of the lateral tricuspid annulus in apical 4-chamber view with cursor placed at the junction of the tricuspid annulus and right ventricular free wall. TAPSE is measured as the maximal systolic excursion from the end-diastole after atrial contraction to end of systole (Komajda et al. 2006).

Quantitative assessment of RV size was performed in the apical four-chamber view. Measurement of the basal RV diameter in the apical four-chamber view at end-diastole (RV basal) is a simple method to quantify RV size (Lang et al. 2006).

For the measurement of RA dimension, the minor axis dimension was taken in a plane perpendicular to the long-axis of the RA at end-systole and the measurement was performed from the lateral border of the RA to the interatrial septum according to the chamber quantification recommendations of the European Society of Echocardiography (Lang et al. 2006).

The collection of the echocardiographic data was unbiased, as the laboratory results were only collected after echocardiographic evaluation of the study cohort.

Cardiopulmonary testing measurements

Cardiopulmonary exercise testing was performed with ZAN 600 CPET (nSpire Health Group, Germany), using a bicycle ergometer ramping protocol. Ventilation, oxygen uptake, and carbon dioxide production were monitored continuously using a respiratory mass spectrometer. The patients were exercised to the limit of their symptoms. Peak oxygen uptake (Peak VO2) was the highest rate of oxygen uptake achieved after reaching the anaerobic threshold, defined as the point where respiratory exchange ratio (RER) exceeds the cut-off value of 1.0 (Dickstein et al. 1990). Patients who did not reach the anaerobic threshold were not included in the documentation.

The VE/VCO2 slope was calculated by using data to the point of peak exercise (Arena and Humphrey 2002). Strong scientific support for an optimal VE/VCO2

slope expression is, however, lacking and both methods of calculation (to the point of ventilatory threshold and to maximal exercise) have produced a measure of prognostic value (Arena et al. 2003).

2.3 Medication

The medication of the patients was also taken into consideration. Beta-blockers, angiotensin-converting-enzyme inhibitors (ACEi), angiotensin-II receptor type I antagonists (ARB), aldosterone receptor antagonists, diuretics, including thiazide and loop diuretics, acetylsalicylic acid, thienopyridines and the combination of these antiplatelets, anticoagulants, statins, calcium antagonists, proton pump inhibitors (PPi), histamine H2 receptor antagonists and amiodarone were also audited for a potential association with iron deficiency.

2.4 Statistical analyses

Continuous variables are presented as mean ± SD for normal distributions, or as median plus interquartile range for skewed distributions. Categorical variables are presented as counts and percentages. Comparisons of continuous variables were performed by Student's *t* test or Mann-Whitney's *U* test. Comparisons of categorical variables were performed by chi-square test or Fisher's exact test. The impact of pertinent variables on the presence of iron deficiency was assessed by multivariate logistic regression analysis.

A two-tailed p-value < 0.05 was regarded as statistically significant.

All statistical analyses were performed using IBM SPSS Statistics 15.0.

3. Results

3.1. Clinical patient characteristics

A total of 125 patients were initially recruited in this study; 22 patients were excluded according to the exclusion criteria. Thus, the final study sample comprised 103 patients. The mean age was 56 ± 3 (interquartile range [IQR], 47-67) years; 85 patients were men [83%] and 18 women [17%]. The mean body mass index (BMI) was 27 ± 5 (IQR, 24-30) kg/m^2; 44 patients suffered from ischaemic cardiomyopathy (ICM) [43%] and 59 from non-ischaemic cardiomyopathy [57%]; 29 patients were mildly symptomatic with NYHA class II [28%] and 74 patients were severely symptomatic with NYHA class III [72%]; 46 patients were provided with an implantable cardioverter-defibrilator (ICD) [45%], 31 with a cardiac resynchronisation therapy/defibrilator device (CRT-D) [30%] and the rest had no device or a simple pacemaker at the time of study. In terms of frequent heart failure comorbidities, insulin-dependent diabetes was present in 24 patients [23%] and chronic obstructive pulmonary disease (COPD) in 21 patients [21%].

3.2. Iron status of the study sample and iron deficiency prevalence

In the 103 patients ID was present in 60 patients (58%). Of these, 43 patients (73%) showed an absolute ID with serum ferritin values <100 µg/l and 17 patients (27%) had functional ID with serum ferritin values ≥100 µg/l and ≤300 µg/l but Tsat <20%. The remaining 43 patients had a normal iron status (Figure 2).

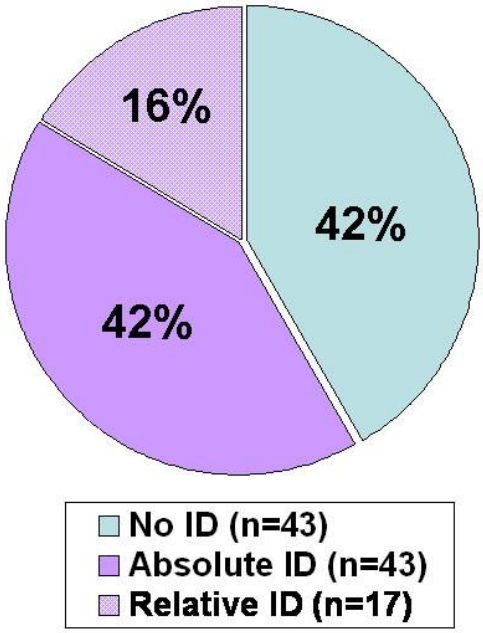

Figure 2. Prevalence of iron deficiency (ID) in the patients studied

Baseline patient characteristics in the two patient groups are given in Table 1.

Table 1. Baseline patient characteristics

	Patients without ID (n=43)	**Patients with ID (n=60)**	**P value no ID vs. ID**
Age	59.4 ± 11.4	54.3 ± 13.3	**0.045**
BMI [kg/m^2]	27.1 ± 5.6	27.6 ± 4.9	0.59
BSA [m^2]	2.00 ± 0.3	2.04 ± 0.3	0.47
Men	36 (83.7%)	49 (81.7%)	0.79
Diabetes	13 (30.2%)	11 (18.3%)	0.16
COPD	9 (20.9%)	12 (20%)	0.91
ICM DCM HCM	16 (37.2%) 27 (62.8%) 0 (0%)	28 (46.7%) 30 (50%) 2 (3.3%)	0.354
NYHA II NYHA III	15 (34.9%) 28 (65.1%)	14 (23.3%) 46 (76.7%)	0.199

Age was significantly lower in ID patients, with 54.3 ± 13.3 years vs. 59.4 ± 11.4 years in patients with no ID ($p < 0.05$). The remaining variables showed no intergroup differences.

Baseline values of haematological variables in the two groups are given in Table 2.

Table 2. Baseline haematological variables

	Patients without ID (n=43)	**Patients with ID (n=60)**	**Normal range**
Hb [g/dl]	14.2 ± 1.2 (13.3-15.2) n=35 13.3 ± 1.0 (12.7-13.9 n=8	13.3 ± 1.8 (12.4-14.4) n=49 12.9 ± 0.9 (12.3-13.2) n=11	Males: >13 Females: >12
MCH [pg]	33.9 ± 14 (29.7-32.5)	29.9 ± 8.3 (27.2-31.2)	28-34
MCV [fl]	88.5 ± 13.3 (87.8-93.1)	86.6 ± 10 (82.6-92.2)	78-94
Transferrin [g/l]	2.8 ± 0.4 (2.6-3.1)	3.3 ± 1.4 (2.8-3.5)	2.0-4.0
Transferrin saturation [%]	26.9 ± 6.2 (22-29)	15 ± 6.6 (10-18)	>20
Ferritin [µg/ l]	204 ± 89 (141.7-232)	82.6 ± 65.2 (35.4-104.5)	>100

Summarizing these results, the prevalence of ID was 58% (n = 60, 95% CI, 48%-68%).

Prevalence of anaemia

In our study population the prevalence of anaemia, defined as haemoglobin < 12g/dl in women and < 13 g/dl in men was 22% (n = 23, 95% CI 15-32%) (Figure 3).

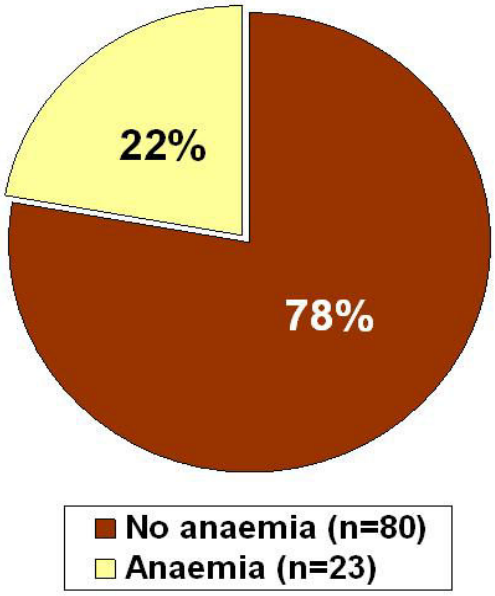

Figure 3. Prevalence of anaemia in the patients studied.

In the anaemic population, we found an ID prevalence of 83% (n = 19/23, 95% CI 61-95%), whereas in the non-anaemic population the ID prevalence was assessed at 51% (n = 41/80, 95% CI 40-63%; p = 0.008) (Figure 4)

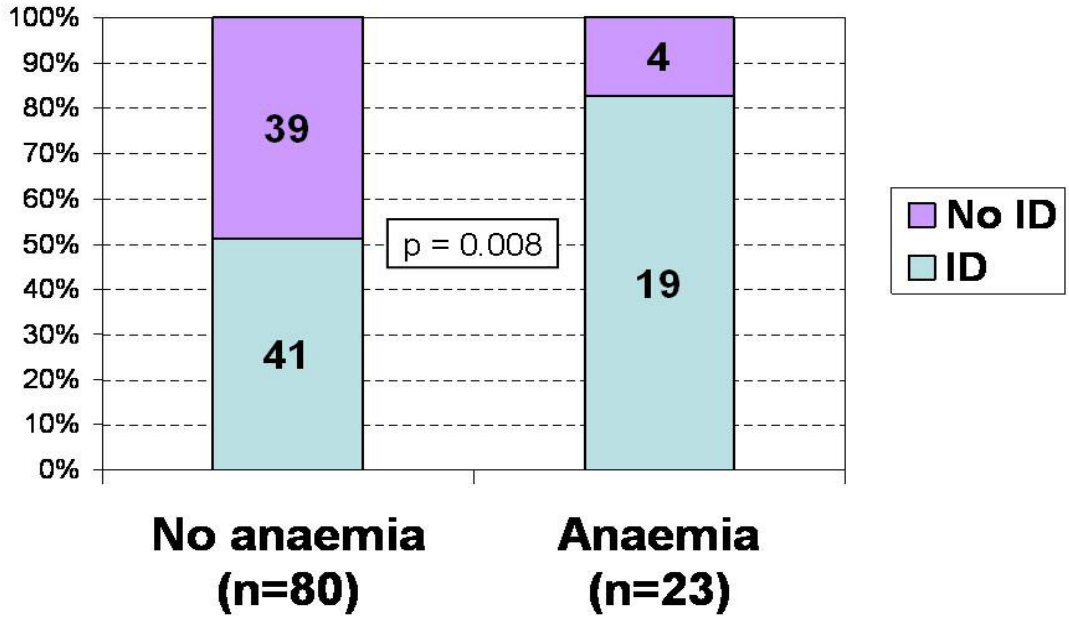

Figure 4. Prevalence of iron deficiency (ID) in anaemic and non-anaemic patients.

3.3. Descriptive statistics and intergroup differences of echocardiographic variables

The study cohort was characterised by the severely depressed left ventricular systolic function with a mean LVEF of 22 ± 5% and the severe dilatation of the left ventricle with a mean LVED of 6.7 ± 0.9 cm. Right heart variables were compromised as well, with an end-diastolic diameter of the basal right ventricle (RVbasal) of 4.6 ± 0.9 cm and a tricuspid annular plane systolic excursion (TAPSE) of 15.4 ± 4.5 mm (Table 3).

Table 3. Echocardiographic variables		
	All patients studied (n=103)	**Normal range**
LVEF [%]	22 ± 5 (20-25)	≥ 55%
LVED [cm]	6.7 ± 0.9 (6.1-7.1)	3.9-5.3
LVED / BSA [cm/ m^2]	3.3 ± 0.5 (3.0-3.6)	2.4-3.2
MR grade	MR ≤1: n=56 (54%) MR ≥2: n=47 (46%)	≤ 1
RVbasal [cm]	4.6 ± 0.9 (4.0-5.1)	2.0-2.8
RA [cm]	4.6 ± 0.9 (4.0-5.2)	2.9-4.5
RA/BSA [cm/m^2]	2.3 ± 0.5 (1.9-2.6)	1.7-2.5
TAPSE [mm]	15.4 ± 4.5 (13-18)	2.4-2.6
TR grade	TR ≤1: n=74 (72%) TR ≥2; n=29 (28%)	≤ 1

The left ventricular parameters concerning the geometry, contractility and valve function showed no difference between the ID and no-ID group. In contrast, echocardiographic parameters concerning the right ventricle were significantly different in ID patients. ID patients had a higher prevalence of severely dilated right ventricles, severely depressed longitudinal right ventricular function and moderate to severe tricuspid regurgitation (Table 4, Figure 5).

Table 4. Descriptive statistics and intergroup differences of echocardiographic variables			
	Patients without ID (n=43)	**Patients with ID (n=60)**	**P value no ID vs. ID**
LVEF [%]	22.9 ± 4.8 (20-25)	21.6 ± 5.8 (17.2-25)	0.21
LVED [cm]	6.7 ± 0.8 (6.1-7.0)	6.7 ± 0.9 (6.1-7.2)	0.927
LVED/BSA [cm/m^2]	3.4 ± 0.4 (3.0-3.6)	3.3 ± 0.5 (2.9-3.6)	0.61
TAPSE [mm]	15.6 ± 3.6 (14-17)	15.3 ± 5.0 (12-19)	0.74
RVbasal [cm]	4.4 ± 0.7 (4.0-4.8)	4.7 ± 0.9 (4.1-5.4)	0.091
RA [cm]	4.4 ± 0.9 (3.8-4.8)	4.7 ± 0.9 (4.2-5.4)	0.089
RA/BSA [cm/m^2]	2.2 ± 0.5 (1.8-2.5)	2.3 ± 0.5 (2.0-2.7)	0.28
MR grade ≤1 MR grade ≥2	21 (49 %) 22 (51 %)	35 (58 %) 25 (42 %)	0.423
TR grade ≤1 TR grade ≥2	36 (84 %) 7 (16 %)	38 (63 %) 22 (37 %)	**0.023**
TAPSE ≤13 mm	10 (23.3 %)	27 (45 %)	**0.023**
RVbasal ≥ 5 cm	6 (14 %)	23 (38.3 %)	**0.007**

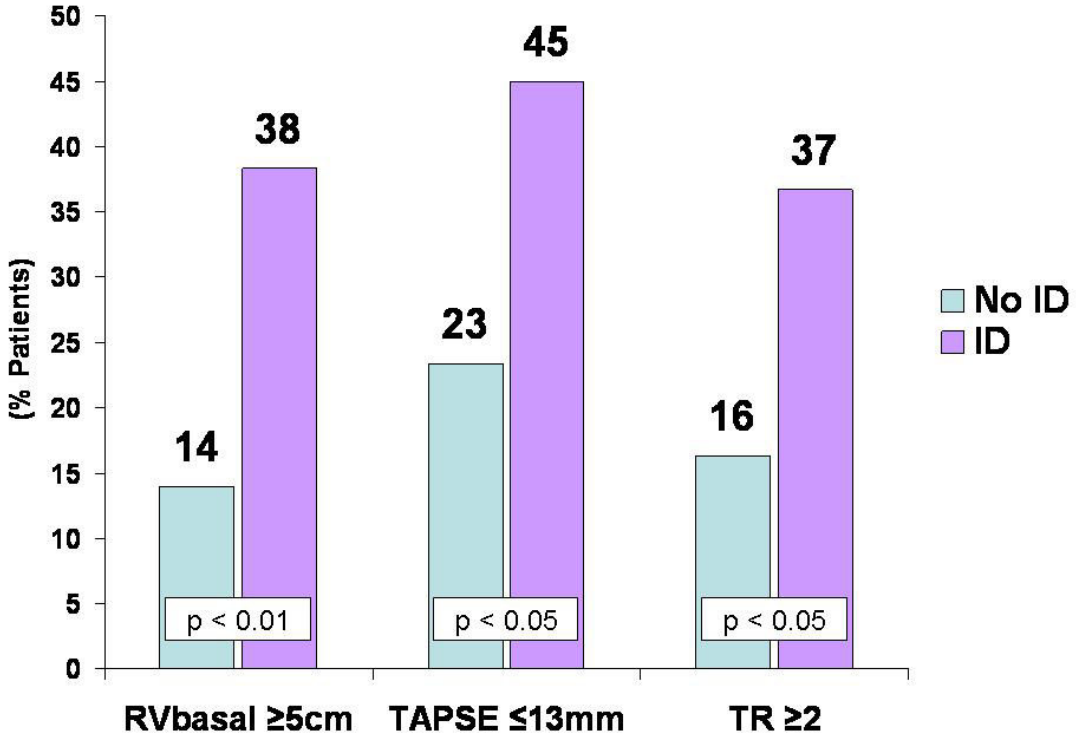

Figure 5. Right heart variables with statistically significant intergroup differences.

3.4. Descriptive statistics and intergroup differences of laboratory variables

The laboratory variables examined in our study were creatinine, sodium and proBNP. Creatinine and proBNP showed skewed distributions and were therefore log transformed in order to normalise their distribution (Figure 6).

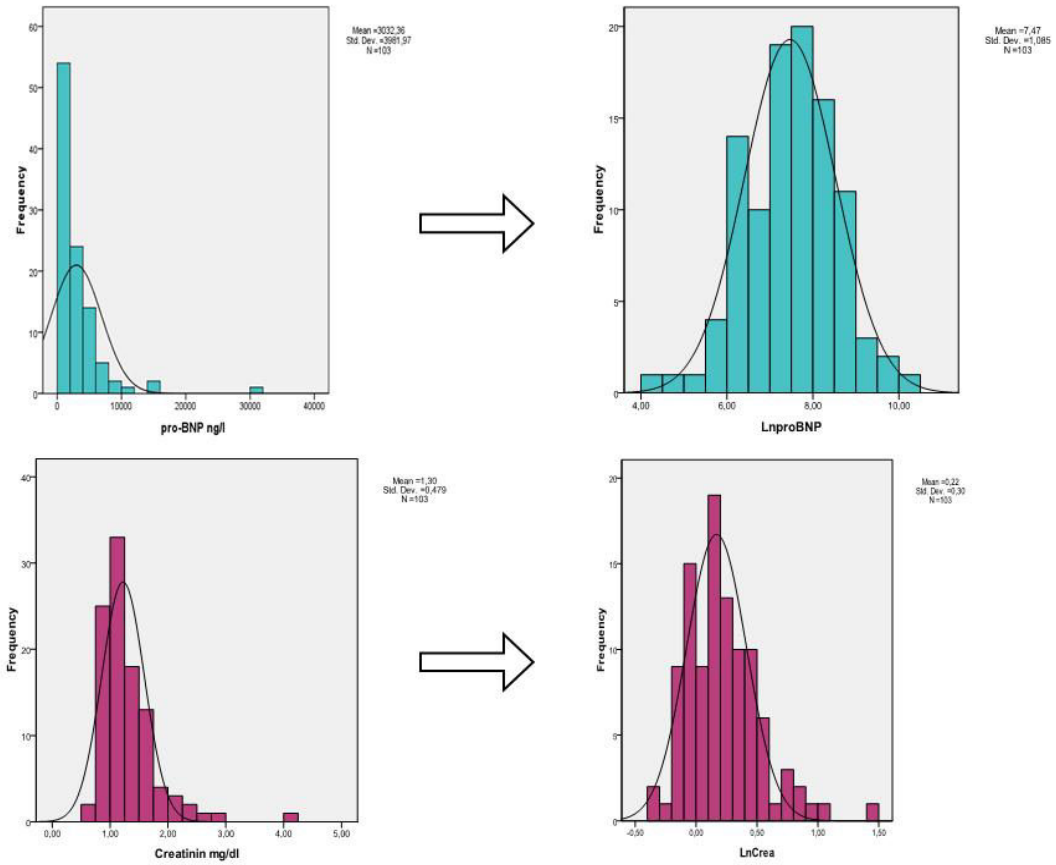

Figure 6. Logarithmic transformation and normalisation of creatinine and proBNP distributions.

Laboratory variables of the patients associated with heart failure were taken into consideration. The study cohort exhibited proBNP values with a median of 1886 (IQR, 788-3882) ng/l and creatinine values with a median of 1.2 (IQR, 1.0-1.5) mg/dl, whereas the mean sodium was 141 mmol/l. None of these variables showed significant intergroup differences between the ID and non-ID group (Table 5).

Table 5. Descriptive statistics and intergroup differences of laboratory variables

	Patients without ID (n=43)	Patients with ID (n=60)	P value no ID vs. ID
proBNP [ng/l]	1886 (980-3502)	1849 (743-4444)	0.976
Creatinine [mg/dl]	1.1 (1.1-1.4)	1.2 (1.0-1.5)	0.499
Sodium [mmol/l]	141.1 ± 2.8	141.2 ± 3.4	0.754

3.5. Descriptive statistics and intergroup differences of medication

Table 6 summarises the patients' medication, both for the whole group as well as for patients with and without ID. For the whole group, 99% were treated with betablockers, 66% received ACEis, 42% ARBs, 87% aldosterone antagonists, and 86% were on diuretics. There were no intergroup differences in medication except for statins: the percentage of patients treated was significantly higher in patients with ID than in those without (68 vs. 49%, $p<0.05$, Figure 7)

Table 6. Descriptive statistics and intergroup differences of medication

Medication	All patients (n=103)	Patients without ID (n=43)	Patients with ID (n=60)	P value no ID vs. ID
Beta-blockers	102 (99%)	42 (97.7%)	60 (100%)	0.235
ACEi	68 (66%)	33 (76.7%)	35 (58.3%)	0.052
ARB	42 (40.8%)	14 (32.6%)	28 (46.7%)	0.151
Aldosterone receptor blockers	90 (87.4%)	37 (86%)	53 (88.3%)	0.730
Diuretics	89 (86.4%)	38 (88.4%)	51 (85%)	0.622
ASS or thienopyridine	46 (44.7%)	17 (39.5%)	29 (48.3%)	0.376
Combined antiplatelet therapy	9 (8.7%)	4 (9.3%)	5 (8.3.%)	0.864
Anticoagulation	55 (53.4%)	22 (51.2%)	33 (55%)	0.700
Statins	62 (60.2%)	21 (48.8%)	41 (68.3%)	**0.046**
Ca antagonists	5 (4.9%)	3 (7%)	2 (3.3%)	0.396
Ppi	49 (47.6%)	18 (41.9%)	31 (51.7%)	0.326
H_2 antihista-minics	4 (3.9%)	1 (2.3%)	3 (5%)	0.488
Amiodarone	18 (17.5%)	7 (16.3%)	11 (18.3%)	0.787

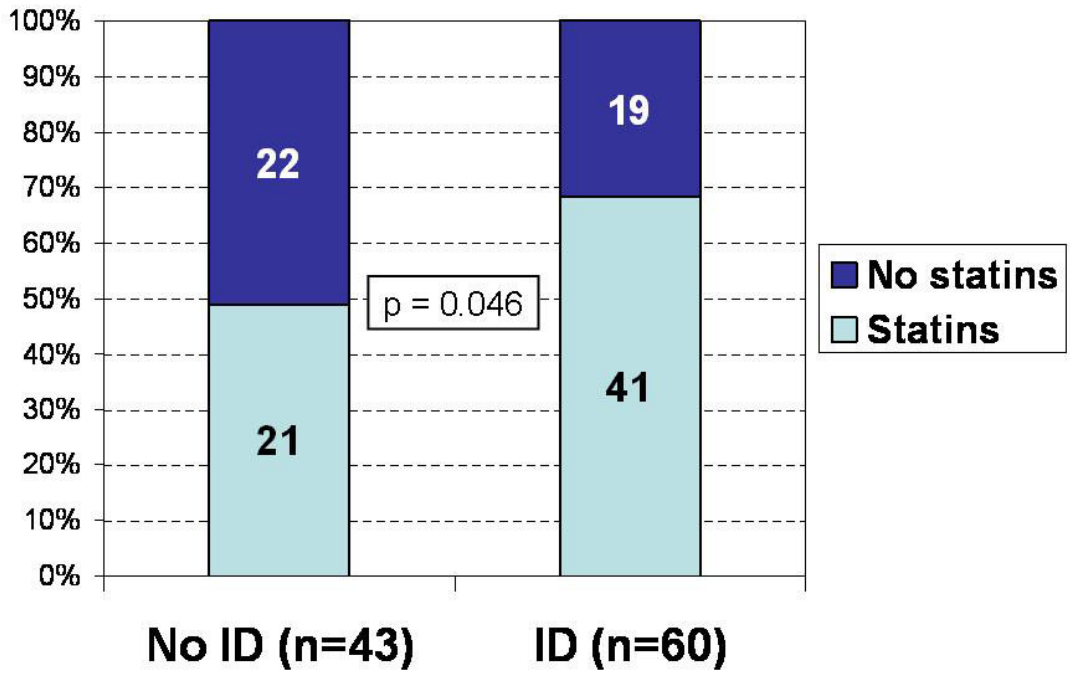

Figure 7. Prevalence of statin therapy in No-ID and ID patients.

3.6. Univariate logistic regression analysis

All variables were examined with a univariate logistic regression model with the following results

Clinical variables

Table 7. Univariate logistic regression analysis of clinical variables			
	Odds ratio	**95% CI**	**P**
Age	0.967	0.936-1.000	**0.049**
Sex	1.155	0.408-3.269	0.787
BMI	1.021	0.946-1.103	0.587
BSA [m^2]	1.762	0.381-8.142	0.468
NYHA III vs. II	1.607	0.682-3.787	0.278
DCM vs. ICM	0.635	0.284-1.420	0.269
Diabetes	0.518	0.206-1.303	0.162
COPD	0.944	0.358-2.490	0.908

Echocardiographic variables

Table 8. Univariate logistic regression analysis of echocardiographic variables			
	Odds ratio	**95% CI**	**P**
LVEF [%]	0.954	0.886-1.027	0.210
LVED [cm]	1.022	0.647-1.614	0.926
LVED/ BSA [cm/m^2]	0.805	0.352-1.842	0.608
MR ≤1 vs MR ≥2	0.682	0.310-1.499	0.341
TAPSE mm	0.985	0.903-1.075	0.739
TAPSE ≥13 mm	2.7	1.130-6.453	**0.025**
RVbasal [cm]	1.499	0.935-2.405	0.093
RVbasal ≥ 5cm	3.833	1.400-10.498	**0.009**
TR ≤1 vs TR ≥2	2.977	1.134-7.815	**0.027**
TR ≥ II°	2.977	1.134-7.815	**0.027**
RA [cm]	1.462	0.940-2.272	0.092
RA/BSA [cm/m^2]	1.537	0.706-3.343	0.279

Laboratory variables

Table 9. Univariate logistic regression of laboratory variables			
	Odds ratio	**95% CI**	**P**
Ln(proBNP)	0.958	0.666-1.378	0.818
Ln(Crea)	1.672	0.436-6.411	0.453
Sodium [mmol/l]	1.011	0.891-1.146	0.868

Medication

Table 10. Univariate logistic regression of medication			
	Odds ratio	**95% CI**	**P**
ACEi	0.424	0.177-1.017	0.055
AT1 antagonists	1.812	0.802-4.094	0.153
ARB	1.228	0.382-3.950	0.731
Diuretics	0.746	0.231-2.405	0.623
ASS or thienopyridine	1.431	0.647-3.164	0.376
Combined antiplatelet therapy	0.886	0.224-3.514	0.864
Anticoagulation	1.167	0.532-2.558	0.700
Statins	2.261	1.007-5.073	**0.048**
Ca antagonist	0.460	0.073-2.878	0.406
PPi	1.485	0.674-3.270	0.327
H2 antihistaminics	2.211	0.222-22.004	0.499
Amiodarone	1.155	0.408-3.269	0.787

In the univariate logistic regression models (Tables 7-10), we found the following variables to be associated with ID: age, RVbasal ≥5 cm, TAPSE ≤13 mm, TR grade ≥2 and statin therapy; ACE inhibitor tharapy showed only a tendency to association (Figure 8).

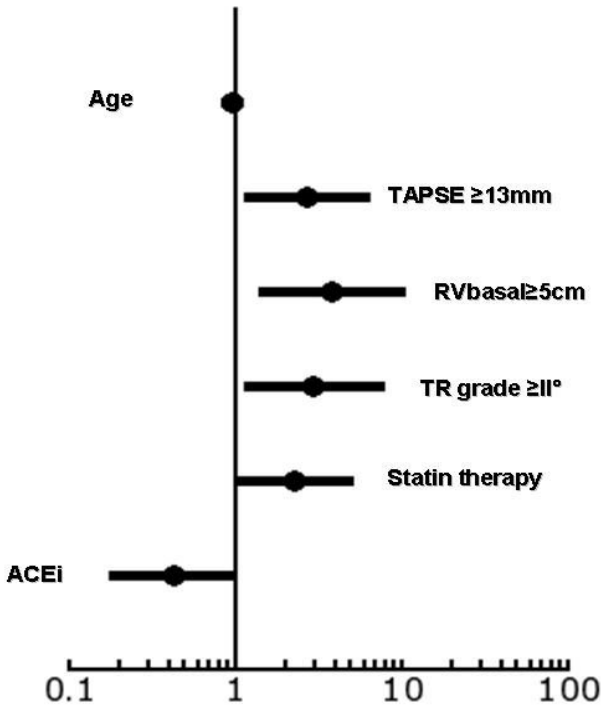

Figure 8. Forest plot of univariate logistic regression analysis.

3.7. Multivariate logistic regression analysis

In the multivariate logistic regression analysis all the variables that showed a significant association in the univariate analysis and variables that could possibly have an impact on the prevalence of iron deficiency – based on the current scientific knowledge, even if they presented in the univariate analysis as non significant – were taken into consideration. For the multivariate regression analysis a stepwise model was used. Table 11 presents the final variables included.

Table 11. Multivariate logistic regression analysis			
	Odds ratio	**95% CI**	**P**
Age	0.939	0.899-0.980	**0.004**
TAPSE ≥13 mm	1.513	0.508-4.501	0.457
TR ≥ II°	1.807	0.545-5.994	0.333
RVbasal ≥ 5 cm	3.579	1.12-11.520	**0.033**
ACE inhibitors	0.360	0.124-1.044	0.060
Statins	4.812	1.670-13.863	**0.004**

The following independent clinical associates with ID in patients with CHF were established: Age, RVbasal ≥5 cm and statins (Figure 9).

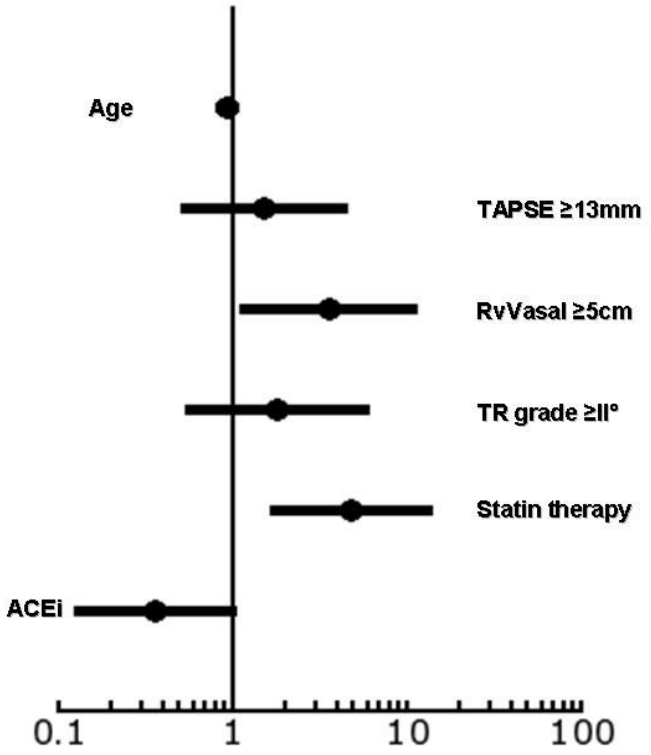

Figure 9. Forest plot of multivariate logistic regression analysis.

3.8. Iron deficiency and cardiopulmonary exercise testing parameters

Cardiopulmonary exercise testing parameters, such as peak oxygen consumption and the relationship between minute ventilation and carbon dioxide production (VE/VCO_2 slope), where acquired during the latest 3 months from recruitment in 49 patients. 37 patients were exercised to the limit of their symptoms and reached the anaerobic threshold, defined as the point where respiratory exchange ratio (RER) exceeds the cut-off value of 1.0 These surrogate parameters of functional capability and prognosis have been subject of interrogation in order to see if ID has an impact on them. In this subgroup of patients no difference in cardiopulmonary exercise testing parameters was found for patients with vs. without ID (Table 12).

Table 12. Cardiopulmonary exercise testing parameters in relation to iron deficiency

	All patients (n=37)	**Patients w/o ID (n=21)**	**Patients with ID (n=16)**	**P value No ID vs ID**
VO_2 max [ml/min/kg]	13.4 ± 4.6	14.0 ± 5.2	12.5 ± 3.6	0.343
VE/VCO_2 [l/min]	37.3 ± 9.1	35.1 ± 4.1	40.1 ± 12.7	0.104

4. Discussion

4.1. Prevalence of iron deficiency

It has recently been recognized that the presence of ID constitutes a predictor of unfavourable outcome, regardless of the coincidence of anaemia, in patients with CHF and depressed left ventricular systolic function (Jankowska et al. 2010). Furthermore, it has recently been published that treatment of ID in patients with systolic CHF with intravenous iron supplementation had beneficial effects, with improvement of functional capacity in both patients with or without anaemia (Anker et al, 2009). These findings lead to the conclusion that ID should be considered an indispensable parameter of interrogation because it might represent a potential treatment option in patients with severe systolic CHF, irrespective of the presence of anaemia.

The prevalence of ID in our cohort was found to be 58% (95% CI, 48%-68%) and thus represents the highest reported until now. In the study of Jankowska et al. which was published in the phase of collecting the data for this thesis and which is the only one documenting the prevalence of ID regardless of the presence of a concomitant anaemia, the study sample exhibited a 37% (95% CI, 33%-41%) ID prevalence using the same ID definition values (absolute ID: serum ferritin <100 µg/l, or functional ID: serum ferritin ≥100 µg/l and ≤300 µg/l with Tsat <20%). Comparing the prevalence of ID in both studies, it is obvious that the prevalence of ID in our study was significantly higher.

An important difference in the study sample of this thesis is the investigation of ID on patients with severely depressed systolic left ventricular function. Inclusion LVEF was ≤30% (mean LVEF 22.2 ± 5.5%), while Jankowska et al.

established as inclusion criterion an LVEF ≤45% (mean LVEF 26 ± 7%). The LVEF was significantly lower in the study sample of the current thesis ($p<0.001$).

Consecutive to the more depressed left ventricular systolic function, the symptomatology of the patients in the current study was also significantly worse (NYHA class ≥III, $p<0.001$). Thus according to the data of the present study, a highly symptomatic heart failure population with severely depressed systolic function, an average LVEF of only 22% and predominantly NYHA III is associated with an markedly higher ID prevalence in relation to the data published before on less symptomatic patients with less depressed systolic function.

Apart from the study of Jankowska et al. and the current thesis, the only data referring to the presence of ID in CHF derive from studies where ID was evaluated only in subgroups of patients who presented with anaemia. The prevalence of ID in the anaemic patients varied from 10 to 60% (Witte et al. 2004, de Silva et al. 2006, Anand 2008). Nanas et al. (2006) were the first to show that ID assessed by bone marrow aspiration was present in 73% of patients with anaemia despite seemingly adequate iron stores assessed by serum iron and ferritin. This study caused a change in the definition of ID in heart failure by Okonko et al., including now the functional form of ID. The study sample examined in this thesis showed a prevalence of anaemia of 22% (n=23; 95% CI, 15-32%). Focusing on the anaemic population, we calculated an ID prevalence of 82% (n=18; 95% CI, 60-95%) which is also the highest documented to date. In the non-anaemic population, the ID prevalence was estimated at 52% (n=42; 95% CI, 40-63%). In the study by Jankowska et al.

(2010), the prevalence of ID in the anaemic patients was 57 ± 10% vs. 32 ± 4% in non-anaemic patients. Thus the prevalence of iron deficiency in the anaemic and non-anaemic subgroup in the present study was significantly higher than in the study of Jankowska et al. At this point it is important to again underline the different inclusion criteria, which result in a much more ailing population studied.

The prevalence of ID in the anaemic population studied by Nanas et al. was estimated on the basis of different anaemia criteria (haemoglobin <11.5 g/dl in women and <12 g/dl in men). Using these criteria we estimated an anaemia prevalence of 11% (n=11; 95% CI, 5-18%) and an ID prevalence of 91% in this small anaemia group (n=10; 95% CI, 59-100%), which is in concordance with the ID prevalence of 73% (95% CI, 56-86%) of the study of Nanas et al. In the study of Nanas et al. the mean LVEF was 22.5 ± 5.9% and thus not significantly different from the anaemic population of this thesis, which exhibited a mean LVEF of 19.5 ± 4.5%. These findings support our thesis that the prevalence of ID is determined by the severity of heart failure.

4.2 Interpretation of the results and detection of independent prevalence predictors of iron deficiency

In the present study, one of the main foci was to pursue a comprehensive analysis of diverse variables, which could possibly interfere with the iron status of the patients; thus, a vast variety of clinical, echocardiographic and laboratory variables was included. In addition, the medication of the patients was contemplated in the total of variables.

Clinical characteristics

In the baseline clinical characteristics of the study group, age was significantly

lower in ID patients compared to patients without ID (54. vs. 59.years ID). The relation was confirmed by univariate and multivariate analysis which demonstrated that lower age is an independent clinical predictor of ID (odds ratio 0.94). This might in part be a bias due to the exclusion criterion in this study. Patients with a history of bleeding, known to be the main cause of ID in the elderly (Mukhopadhyay and Mohanaruban 2002) were excluded. In our study, nine patients over 60 years where excluded from the study sample due to a history of haemorrhage or iron supplementation. Another explanation for the correlation between age and presence of ID is that with advancing age there is a progressive decrement of marrow haematopoesis and consecutive iron demand (Lipschitz et al. 1981). This could explain why all the patients under 40 years in our study belong to the iron deficiency group. The remaining clinical parameters showed no significant differences between the ID and no-ID groups and no association with iron deficiency in univariate and multivariate analysis. Jankowska et al. (2010) in a differently structured study found no correlation of age with ID; however, female gender and NYHA functional class showed an association with ID. The aetiology of heart failure and the presence of diabetes were also of no significance.

Echocardiographic characteristics

Since in our study population the mean left ventricular ejection fraction was 22% and there was severe LV enlargement, with a mean LVED of 6.7 mm, our patients represent a study sample with severely depressed systolic left ventricular function and dilation. Neither parameter nor the severity of mitral regurgitation as an additional parameter of LV function showed any intergroup

differences or association with iron deficiency.

In contrast, right heart variables such as RVbasal, TR grade and TAPSE after using cut-off values indicative of moderate to severe right ventricular dilatation and dysfunction (RVbasal ≥5 cm, TR grade ≥2. TAPSE ≤13 mm), illustrated a significantly higher prevalence in the ID group, and an association with ID in univariate analysis. In a multivariate logistic regression model only RVbasal ≥5 cm was established as independently associated with ID (odds ratio [OR] 3.6) whereas TR grade ≥2 and TAPSE ≤13 mm exhibited no independent association. Thus, an end-diastolic right ventricular basal diameter ≥5 cm was associated with a 3.6-fold increased risk for ID.

The association of RVbasal with ID is plausible. In cases of right heart congestion, bowel wall oedema and, more specifically, proximal duodenum oedema lead to reduced iron absorption and consecutively to iron deficiency. It had been speculated about this association before, but it has not been directly evaluated. After thorough literature research no scientific papers were found that explored the potential relationship between right heart failure and iron deficiency.

However, in patients with pulmonary arterial hypertension a high prevalence of iron deficiency has also been documented (Ruiter at al. 2011, Soon et al. 2011) which reinforces our result that right heart congestion or dilatation correlates with ID.

Laboratory characteristics

It is known that haemodilution is common in patients with CHF (Androne et al. 2003). Sodium was measured because a haemodilutional hyponatraemia could

interfere with the iron status values. The sodium values in the overall study sample were not different in the ID group and the no-ID group; thus, there were no signs of haemodilution in the ID group and sodium did not associate with ID.

Elevated natriuretic peptide levels are directly correlated with prognosis, NYHA class, intraventricular pressure, pulmonary pressure, and are inversely proportional to cardiac output (Silver et al. 2004). The proBNP values of the study sample had a skewed distribution. There were no intergroup differences in our study sample [1886 (980-3502) in the non-ID group vs. 1849 (743-4444) in the ID group] and thus no association of proBNP to iron deficiency in contrast with Jankowska et al. (2010).

Medication

An important first observation is that the study sample was almost completely treated in accordance with current guidelines. 99% of the patients received beta-blockers. Only one patient could not tolerate beta-blockers due to severe COPD. 66% of the patients received ACEi and 41% received ARBs. 7 Patients were treated with an ACEi/ARB combination due to persistent high afterload values. 87% of the patients received aldosterone receptor antagonists. With regard to the patients' medication, only statins showed a significant intergroup difference; thus, statin therapy was established as independently associated with of ID. ACEi showed a strong tendency to association and therefore a possible involvement in the ID prevalence.

Statins demonstrated significant intergroup differences, univariate analysis showed an association with ID and, finally, multivariate analysis revealed statin therapy as independently predictive of ID with an odds ratio of 4.812. Thus,

statin therapy was associated with a 4.8-fold greater risk for ID. This association appeared also in the FEAST study, a prospective, randomized clinical trial that tested the hypothesis that reduction of iron stores using phlebotomy would influence clinical outcomes in peripheral arterial disease (PAD) patients (De Palma et al. 2010). The aim of that study was to test the hypothesis that iron-induced oxidative stress might provide inflammatory stimuli in atherosclerosis. The effects of statin administration were also examined in this study. The study showed that patients on statin therapy demonstrated lower ferritin values at the entry point, but also in the follow-up data, after adjusting for the phlebotomy treatment effect and that statins were associated with significantly lower ferritin levels.

However, there are two conflicting mechanisms induced by statins which support the association of statins with ID. Firstly, statins have been reported to reduce Interleukin-6 (IL-6) concentration (Rezaie-Majd et al. 2002, Arnaud et al. 2005, Mantuano et al. 2007) and IL-6 is an inducer of hepcidin (Wrighting and Andrews 2006), a peptide that reduces the intestinal iron absorption and favors iron retention in the reticulo-endothelial system (Weiss and Goodnough 2005); therefore, statin use should normally a be associated with an increase in ferritin levels. Secondly, statins induce heme oxygenase-1 and thereby induce heme catabolism (Chen et al. 2006, Ali et al. 2007), leading to higher ferritin levels. Heme catabolism is a key process in mobilizing macrophage iron derived from ingested erythrocytes. Alterations in the activity of heme oxygenase-1 influence the rate of clearance of haemoglobin-derived iron from macrophages. Nevertheless, the pleiotropic actions of statins which are not completely investigated and the elucidation of the same findings concerning statins and

ferritin from other authors signify the need for further investigation in this field. There are strong reasons to optimise lipid levels in heart failure patients with treatment with statins; however, physicians should be aware of all possible side-effects, particularly when these cause further deterioration of the functional capacity of the patients.

ACEi demonstrated borderline significance values in the intergroup comparison; univariate and multivariate analysis suggested that ACEi tend to reduce the risk of ID. The role of ACEi and ARB in suppressing the effect of erythropoietin in the bone marrow has been recognized in both experimental and clinical studies (Albitar et al 1998, Julian et al. 1998, Chatterjee et al. 2000) and therefore the use of this medication correlates with lower iron demands, which explains the reduced odds ratio for ID. It is thought that ACEis induce erythropoietin resistance by blocking the clearance of a hematopoietic inhibitor, N -acetyl-seryl-aspartyl-lysyl-proline (van der Meer et al. 2005). ARBs have also been reported to lower haematocrit values in HF patients, possibly through blunting the direct stimulating effect of angiotensin II on bone marrow (Marathias et al. 2004). ARBs showed no tendency to association with iron status in this study, however it has been suggested that the suppression of erythropoietin might be milder than the respective induced by ACEis (Chatterjee et al. 2000).

4.3 Impact of iron deficiency on cardiopulmonary exercise testing parameters

Okonko et al. 2008 demonstrated in a small randomized trial with 35 patients with CHF and iron deficiency that i.v. iron supplementation improves peak oxygen consumption in anaemic patients but not in non-anaemic patients.

Unfortunately, in the present study only a small number of 37 patients were adequately exercised beyond the anaerobic threshold. Among these, 16 patients had iron deficiency and only 3 of them were anaemic as well. The intergroup difference (ID vs. no ID) concerning peak VO_2 ($p = 0.343$) and VE/VCO_2 did not reach statistical significance; however VE/VCO_2 showed a slight tendency towards better function in the no-ID population. Due to the small number of anaemic iron deficient patients who were able to perform adequate cardiopulmonary exercise testing, no adequate comparison of these patients to non-ID patients was possible. The small sample size of both studies certainly limits the power to detect small differences between the two groups.

5. Synopsis

The aim of this study was to assess the prevalence of ID in a selected chronic heart failure (CHF) population with a left ventricular ejection fraction (LVEF) ≤ 30% and to determine independent predictors of ID. The final study sample comprised 103 patients (age 56 ± 3 years; 85 men [83%]; 44 with ischaemic cardiomyopathy [43%]). Mean LVEF was 22 ± 6% and the prevalence of ID was 58% (n = 60, 95% CI, 48 - 68%).

Univariate logistic regression analysis revealed the following variables to be associated with ID: age, basal right ventricular diameter at end-diastole (RVbasal) ≥ 5cm, tricuspid annular plane systolic excursion (TAPSE) ≤ 13 mm, tricuspid valve regurgitation (TR) ≥ 2 and statin therapy. By multivariate logistic regression analysis, only age (odds ratio [OR] 0.94, p = 0.004), RV basal ≥ 5cm (OR 3.58, p = 0.033), and statin therapy (OR 4.81, p = 0.004) were established as independent clinical predictors of ID.

Summarizing, the following conclusions could be drawn:

- Patients with highly symptomatic CHF and severely depressed systolic LV function, with an average LVEF of only 22%, exhibit a very high prevalence of ID, the highest documented to date. It seems that the severity of systolic heart failure plays a key role in the prevalence of ID.
- The prevalence increases with younger age, probably because of the higher iron demand of a more efficient erythropoiesis of the younger.
- Severe right ventricular compromise is related to presence of ID, indicative of the adverse impact of right heart overload or failure to the

intestinal iron absorption because of bowel wall oedema in cases of right heart congestion.

- Medication may play an interesting role in the genesis of iron deficiency and statins possibly belong in this group as they seem to correlate with reduced ferritin levels, a result which seems to be provocative, yet is acknowledged from other authors as well. Further investigation in this field due to the pleiotropic effects of statins is needed. In contrast, ACEis who are known to be associated with erythropoietin resistance seem to have a protective role against ID due to the reduced iron demand.
- In a small subgroup of patients who were able to perform adequate cardiopulmonary exercise testing beyond the anaerobic threshold, prognostic markers of heart failure and indices of functional capacity such as peakVO_2 and VE/VCO_2 were not affected by iron deficiency, however the group was too small to reach the power needed to establish small differences.

6. Abbreviations

ACEi	Angiotensin converting enzyme inhibitors
ARB	Angiotensin receptor blockers
BMI	Body mass index
BSA	Body surface area
CHF	Chronic heart failure
COPD	Chronic obstructive pulmonary disease
CRT-D	Cardiac resynchronisation therapy/defibrillator device
CRP	C-reactive protein
DCM	Dilated cardiomyopathy
DHF	Diastolic heart failure
EF	Ejection fraction
EPO	Erythropoietin
ESC	European society of cardiology
Hb	Haemoglobin
HCM	Hypertrophic cardiomyopathy
HF	Heart failure
HFNEF	Heart failure with normal left ventricular ejection fraction
ICD	Implantable cardioverter defibrilator
ICM	Ischaemic cardiomyopathy
ID	Iron deficiency
IL-1	Interleukin-1
IL-6	Interleukin-6
IQR	Interquartile range
LV	Left ventricular
LVEDD	Left ventricular diameter at end-diastole
LVEF	Left ventricular ejection fraction
MCH	Mean corpuscular haemoglobin
MCV	Mean corpuscular volume
MI	Myocardial infarction
MR	Mitral regurgitation
NYHA	Odds ratio

OR	Peripheral arterial disease
PAD	New York Heart Association
Peak VO_2	Peak oxygen uptake
PPi	Proton pump inhibitors
proBNP	pro-type B natriuretic peptide
RA	Right atrium diameter at end-systole
RER	Respiratory exchange ratio
RV basal	Basal right ventricular diameter at end-diastole
TAPSE	Tricuspid annular plane systolic excursion
Tf	Transferrin
TNFα	Tumour necrosis factor α
TR	Tricuspid regurgitation
Tsat	Transferrin saturation
VE/VCO2	Relationship of minute ventilation to carbon dioxide production
VO2	Oxygen uptake

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9. Curriculum vitae

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09.1992. – 06.1998. Second gymnasium of Kalamaria, Thessaloniki, Greece

09.1999 – 06.2005 Study of human medicine at Aristoteles University of Thessaloniki, Greece

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10. Eidesstattliche Versicherung

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