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Prevalence of absolute iron deficiency in chronic heart failure population at an urban academic centre in South Africa

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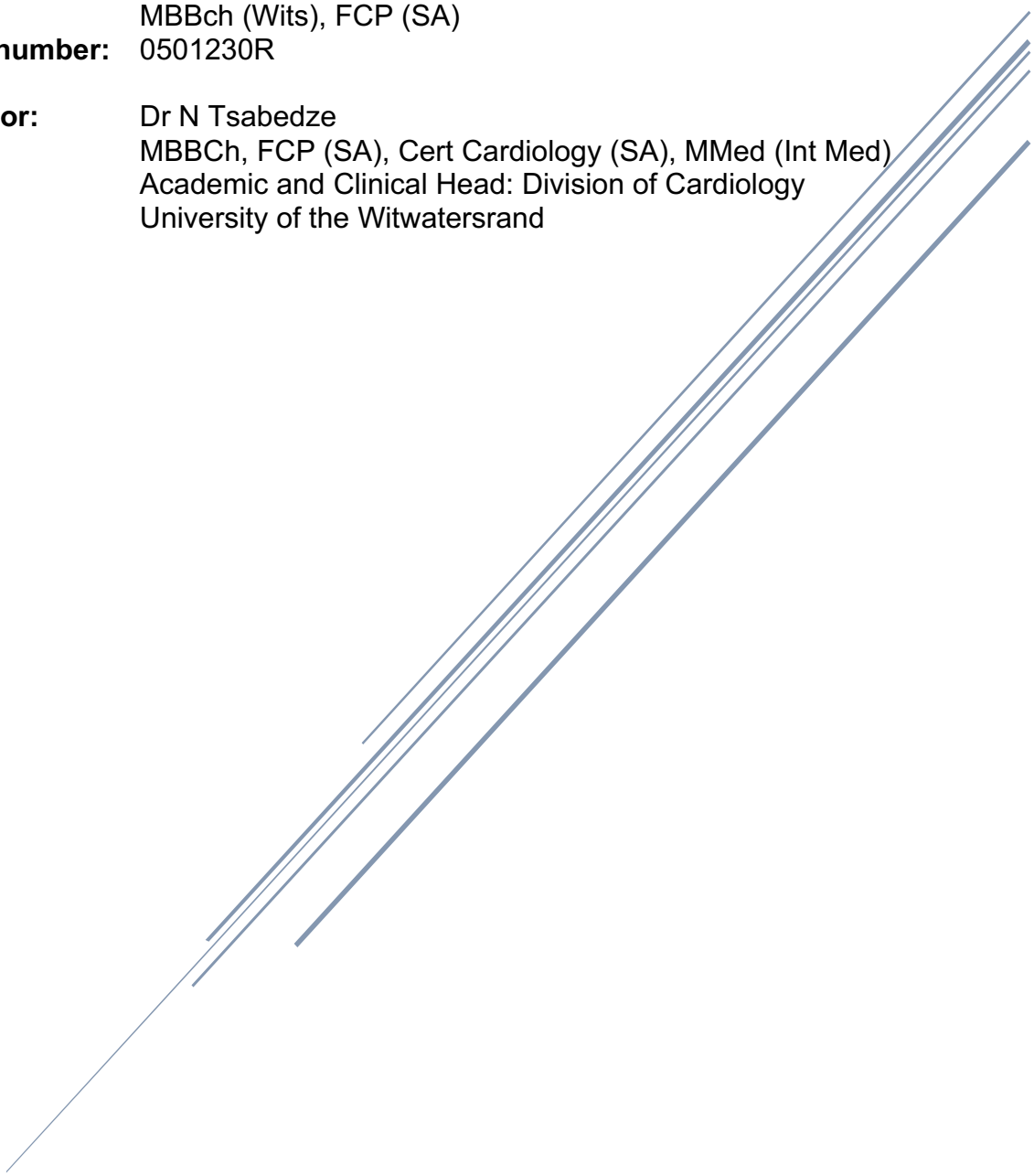
Prevalence of absolute iron deficiency in chronic heart failure population at an urban academic centre in South Africa

A research report submitted to the University of Witwatersrand, Johannesburg in fulfilment for the requirements of the degree of Master of Medicine, 2019

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DECLARATION

I, Karishma Singh; hereby declare that this research is of my own work. I am submitting this research report for the degree Master of Medicine (in the submissible format with my protocol and extended literature review) in the branch of Internal Medicine at the University of the Witwatersrand, Johannesburg. This research report has not been submitted before for any degree or examination at this or any other university

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I would like to thank my family for their support in completing this research report.

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ABBREVIATIONS

ESC: European Society of cardiology
WHO: World Health Organization
ID: Iron deficiency
CHF: Chronic heart failure
CKD: Chronic kidney disease
HFrEF: heart failure with reduced ejection fraction
HIV: human immunodeficiency virus
QOL: quality of life

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Submissible article

Prevalence of absolute iron deficiency in chronic heart failure population at an urban academic centre in South Africa.

Abstract

Background: Iron deficiency is prevalent in chronic heart failure populations from high-income countries, with associated poor quality of life measures and symptomatology. However, there is a paucity of data from sub-Saharan Africa.
Objective: To determine the prevalence and associated clinical characteristics of patients with absolute iron deficiency (AID) and heart failure with reduced ejection fraction (HFrEF), in an urban academic setting in South Africa.
Methods: This was a single-centre retrospective study in an urban academic centre in South Africa. Patients that attend the chronic heart failure with reduced ejection fraction clinic were screened for absolute iron deficiency, which was defined as a ferritin level of <100 mcg/l.
Results: A total of 208 patients fulfilling the study inclusion criteria were analysed. The cohort consisted of 120 (57.69%) females. Absolute iron deficiency was found to be present in 99 (47.60%) patients. Concurrent anaemia was noted in 32 (15.38%) patients. The mean age of the population was 56.78 (SD 14.54) years. The mean left ventricular ejection fraction was 31% (SD 10.83) and the most frequent comorbidity was hypertension, reported in 79% of the population.
Conclusions: Absolute iron deficiency was diagnosed in 47.6% of patients attending the heart failure with reduced ejection fraction clinic, in an urban academic centre in South Africa.

Introduction

Chronic heart failure (CHF) is an increasingly common problem with an estimated prevalence of 1-2% in the general population [1,2]. Globally, the burden of heart failure is on the rise and there are poor prognostic implications particularly in low-income countries [2]. Iron deficiency (ID) is the most prevalent nutritional deficiency seen in the world, affecting a third of the population in the world [3]. Iron deficiency has significant wide-ranging adverse effects, especially in the CHF population, which include unfavourable prognostic outcomes and worse symptom profiles even independent of co-existent anaemia [4-5]. Chronic heart failure may also contribute to causing iron deficiency via numerous mechanisms, including reduced gastrointestinal intake and absorption due to the pro-inflammatory state of CHF [4,5,6]. Other factors that may contribute to the development of ID in heart failure include the concurrent use of medications such as antiplatelet agents that may contribute to gastrointestinal bleeding as well as comorbid diseases such as chronic kidney disease (CKD) [4,5,6].

Recent strides in the management of heart failure include anti remodelling therapy, which has improved the morbidity and mortality associated with heart failure. However, often patients remain symptomatic, complaining of exercise intolerance and self-administered heart failure quality of life questionnaire assessments show lower scores, despite being on adequate anti remodelling therapy [3-5]. Recent research in the developed world has shown that iron deficiency even without anaemia may play a role in conferring a worse functional status, worse quality of life (QOL) scores and reduced exercise capacity [3-5]. Iron deficiency has also been implicated as a poor prognostic factor in patients admitted with acute heart failure [6-7].

Recent clinical trials that focused on intravenous iron replacement have shown benefit concerning improving symptoms in this subgroup of patients [8-11]. There is a paucity of data concerning iron deficiency in African heart failure populations, and there are many unique factors to consider in the African setting, including appropriate biomarkers, the effect of HIV on ID as well as possible treatment options [12]. Studies have focused on the incidence of iron deficiency anaemia in Africa

however there has been little work done into iron deficiency without anaemia in this context [12].

Hence, this pilot study seeks to focus on the prevalence of absolute iron deficiency (AID) in an African chronic heart failure population to determine the scope and nature of the problem as to guide possible interventions and future research in order to improve symptoms in this patient group and treatment of iron deficiency in our resource-constrained setting.

Materials and methods

This is a retrospective, single-centre, descriptive observational study conducted at a tertiary hospital in Gauteng, South Africa. The study was a retrospective review of all the out-patient files at the chronic heart failure with reduced ejection fraction clinic at the Charlotte Maxeke Johannesburg Academic Hospital (CMJAH). The study included all patients older than 18 years of age with complete clinical data from the index clinic visit recorded in their clinic file.

All patients attending the heart failure clinic between January 2010 and July 2018 with echocardiographically confirmed heart failure with a reduced ejection fraction (HFrEF) were included. At the initial visit to the heart failure clinic, all patients had a clinical examination by a doctor and a baseline blood laboratory biochemical profile was performed. At each clinic visit, all patients completed a Minnesota Living with heart failure questionnaire and performed a 6-minute walk test under standard conditions. A comprehensive transthoracic echocardiogram was also performed at the admission visit by a cardiologist. Laboratory biochemical investigations that are requested at the initial clinic visit included a ferritin level, a full blood count (FBC), urea and electrolytes (U+E), pro-brain natriuretic peptide level (pro-BNP) and a C-reactive protein (CRP). These samples were collected in standardised blood collection tubes by the laboratory phlebotomy service.

The biochemical data was then recorded in relevant data sheets that are retained in the patient record. Two hundred and eight patients were included in the study. The data extracted from the clinic files was then entered into a Redcap database

(Appendix 1) by the study investigator and then submitted for statistical analysis. Anaemia was defined as haemoglobin level <13 g/dL for males and less than 12g/dL for females as per the Acute and Chronic Heart Failure Guideline of the European Society of Cardiology 2016 (ESC Clinical Practice Guidelines) [13]. Absolute iron deficiency was defined as a ferritin level of less than 100mg/dL as per Acute and Chronic Heart Failure Guideline of the European Society of Cardiology 2016 (ESC Clinical Practice Guidelines) [13]. This value was determined by literature review of patients with heart failure and iron deficiency [6-8,13]. Files were excluded if the data regarding ferritin was missing or incomplete. Ethical approval was granted by the University of the Witwatersrand Human Ethics Committee and the permission to conduct the study was obtained from the CMJAH.

Statistical analysis

The analysis was conducted with STATA version 15 Software, and statistical significance was considered at two-sided p values of <0.05. Categorical variables were expressed in numbers and percentages, whereas normally distributed data are presented as means and standard deviation. Chi-square test and Student's t-test were used to calculate p-values as appropriate. The Student's T-test for equality of means was used to compare means of normally distributed variables. Non-normally distributed variables were described using the median and interquartile range (IQR). The two-sample Wilcoxon rank-sum (Mann-Whitney) test was used to compare medians for non-normal data. For categorical variables, Pearson's chi-squared test was used to compare proportions. If n<5 in any of the cells, the Fischer's exact test for comparison of proportions was used. Variables with p value <0.2 for T-tests/Rank-sum/Chi-squared/Fischer's exact were considered for further exploration in logistic regression models. Variables with p<0.1 were included in the multivariable logistic regression model. Odds ratios (OR) are represented with their 95% confidence intervals (CI).

Results

Five hundred and twenty-five files were screened for entry into the study. However, 317 files were excluded due to incomplete data. Two hundred and eight files from the heart failure clinic were included in the study as they had the full data profile under investigation (See Figure 1). There were 120 (57.69%) females in the study population. The mean age of the study participants was 56.78 (SD 14.54) years. Females were more likely than males to be iron deficient in our population.

Absolute iron deficiency (ferritin < 100mcg/l) was present in 99 (47.60%) of the study population. Iron deficiency anaemia was found in only 32 (15.32%) of the population. Other characteristics of our cohort are illustrated in table 1.

The most prevalent comorbidity found in our cohort was hypertension which was found in 55.9% of our population, and other comorbidities are summarised in Table 2. The most frequent aetiology of heart failure seen in our study was also attributed to hypertension which was found in 79 (37.98%) patients followed by ischaemic heart disease which was found in 41 patients (19.71%). Aetiology is further illustrated in Table 3.

Patients with absolute iron deficiency had a higher Minnesota scores (43.24 (SD 38.47) vs 34.42 (SD 25.18) p-value = 0.011). Peripartum cardiomyopathy was identified as an independent predictor of absolute iron deficiency (OR: 3.55, 95% CI: 1.07 – 11.73, p-value = 0.038) in the multivariable regression model.

The median *mean* cell haemoglobin level was lower in the iron-deficient group 29.1 (IQR: 27.1 – 30.6) as compared to the non-iron deficient group vs 29.9 (IQR: 28.6 – 32.4) (p-value <0.001). Pro- BNP was found to be higher in the iron-deficient group 2509.5 (IQR: 166 – 5390) as opposed to the non-iron deficient group 2509.5 (IQR: 166 – 5390) vs 1724.5 (IQR: 294 – 6255) (p-value = 1.000). The median estimated glomerular filtration rate in the entire study population was 60 (IQR: 50 – 65) ml/min.

The mean left ventricular ejection fraction in the study population was 31% (SD 10.50), and this did not differ between the iron deficient and non-iron deficient

groups. The study population was also found to be on adequate anti remodelling therapy including beta-blockade in 204 (98.0%) patients, RAAS inhibition in 167 (80.29%) and mineralocorticoid antagonists in 146 (70.19%) patients.

Discussion

Our study found a high prevalence of absolute iron deficiency of 47.6%. This is in keeping with other studies on the prevalence of iron deficiency in CHF patients [5-6,8, 13-14]. The prevalence percentage value found was also high for absolute ID, as most studies included both functional and absolute measures of iron deficiency. Absolute iron deficiency was also found to be prevalent in patients without anaemia as concurrent anaemia was only found in 32 (15.38%) patients. Again, these findings are consistent with other published studies in CHF populations. However, since our study focused on absolute iron deficiency (AID), the prevalence of ID in the population may have been higher if functional iron deficiency measures were also included in the study [5-6,8, 13-15].

Due to budgetary constraints, full iron studies are not carried out on all patients at the CMJAH. Ferritin is therefore used as a screening test at the clinic where the study is based and hence AID is the measure that is utilised in this study. This study may indicate a role for a full iron study profile to be undertaken at the first clinic visit to better characterise the iron deficiency and identify those patients that require further workup. Functional iron deficiency may also prove to be prevalent as suggested by other studies [5-6,8, 13-15], and this may be missed by not doing a full iron study profile.

Nevertheless, these study findings may designate a role for aggressively screening for iron deficiency even when haemoglobin levels are normal. The causes of absolute iron deficiency in our population are likely to be multifactorial. These could include inadequate intake, reduced absorption, as well as increased losses of iron via the urogenital or gastrointestinal tracts [6,13,16]. Reduced intake could be related to the fact that CHF is a pro-inflammatory state, and this leads to reduced appetite and consequently reduced dietary intake [6,13,16]. Absorption may be further compromised due to gut congestion and increased hepcidin production due to inflammation [6,13,16].

Increased losses could be due to the use of antiplatelet agents, causing occult blood loss in the gastrointestinal tract [6,13,16]. Iron deficiency is prevalent in the general South African population, and this is also related to socioeconomic and hence, nutritional factors [17].

The median age in the study population was 56 years, and patients had multiple comorbidities. The high level of AID could indicate a role for aggressive screening where appropriate for concurrent gastrointestinal malignancies, especially in the elderly and comorbid smokers [18].

In our study, AID in patients was also associated with worse QOL measures with higher Minnesota Living with Heart Failure scores, more clinical signs of heart failure and more reported symptoms. These findings are in keeping with the published findings on ID in CHF populations both in the developed and developing world [5-8, 13-16]. These symptoms are unlikely to be due to suboptimal therapy as most patients in the clinic were already on optimal anti remodelling therapy (see figure 3) and hence possibly could be contributed to the presence of iron deficiency.

A significant proportion of our patients had AID even without anaemia, indicating that iron deficiency should be screened for in patients with heart failure, despite being anaemic. Even without anaemia, patients with AID had a more inferior functional status once again suggesting that ID could be one of the causes responsible for the poorer functional status [5-8] as iron is physiologically responsible for oxygen-carrying capacity and transport of oxygen. It also plays a role in the energetics of cardiac myocytes and skeletal muscle due to its role in the electron transport chain and mitochondria [4,13]. Hence AID has wide-ranging consequences in HF that are not limited to anaemia alone, and this may help to explain the associations with a poorer functional and clinical status in these patients [4,13].

Regarding our gender-based analysis, logistic regression analysis showed that being female and having peripartum cardiomyopathy were significant risk factors for having AID. Females were found to be more affected by anaemia and iron deficiency in our population, and this again correlates with other studies on ID [5-8, 13-16]. Traditionally the peripartum period was thought to be the safest for iron-deficient states however

studies now indicate that this may be an emerging problem, especially when iron deficiency anaemia was present during the gestational period [20,21]. Iron deficiency in this population can be postulated to be related to genitourinary losses during menstruation and the intrapartum period.

The study could indicate a role for further research into ID in these patients that present with peripartum cardiomyopathy. ID is a common problem in females of menstrual age, but this is usually diagnosed when co-existing anaemia is present. Iron deficiency is only considered in the context of anaemia in the postpartum period in published guidelines [20-21]. This could indicate an area for further study and possible therapeutic intervention. However, there is a well-established correlation between females, CHF and ID regardless of age in the published data [5-8, 13-16].

Ischaemic heart disease was also found to be a statistically significant risk factor for AID in our study. This could be because patients with ischaemic heart disease tend to be older and hence may also have a concomitant poor nutritional status, multiple medical comorbidities including CKD as well the use of antiplatelet agents in the group could contribute to occult GI bleeding [22 -24]. Ischaemic heart disease is also associated with a pro-inflammatory state, and it is plausible that this too could be contributing to the iron deficiency. Ferritin is protective against iron deficiency, and hence there may be a role that iron deficiency plays in the development of ischaemic heart disease, however, these associations would need to be further explored [22-24].

In our study, patients with ID had higher scores on the Minnesota questionnaire indicating a poorer functional status. Patients with iron deficiency were also found to have higher serum sodium levels and NT proBNP levels correlating with a more severe clinical HF picture. These patients with ID were also more likely to have physical signs of heart failure, such as peripheral oedema and raised jugular venous pressure.

Interestingly, in our study, in contrast to the other studies on ID in CHF – there were some changes detected in the red cell parameters of the full blood count, which were statistically significant including changes in mean corpuscular volume and mean cell haemoglobin concentration [16]. This could suggest an avenue again for further

research as changes in the full blood count even without anaemia could be indicative of a reason to screen a patient for iron deficiency in this setting. It is uncertain why this is a finding in this specific population; however, it may also indicate a more advanced iron deficiency in our population.

Based on the available literature, patients with lower eGFR are less likely to have absolute iron deficiency, possibly as a result of higher circulating ferritin levels caused by the reduction in renal function and as such, functional iron deficiency measures may be more helpful in this group of patients [10]. In our study, the median estimated GFR in patients with and without iron deficiency was 60 ml/min. Therefore, definitive conclusions regarding those with CKD and AID cannot be reached in our study.

A type 2 error may exist, as the heart failure clinic where the study is based is a referral centre for the sickest patients that other facilities with lower levels of care could not manage appropriately. Hence, the sickest patients with heart failure have been selected for the study inadvertently. Despite these confounding factors, these findings do correlate with other studies as iron deficiency is commonly found in patients with advanced heart failure [5-8, 13-16]. In keeping with other published data, we did not find a significant difference between the ejection fractions of patients with or without ID [5-8, 13-16]. Our cohort had a mean ejection fraction of 31%, indicating the severity of their heart failure and an HFrEF population.

Limitations

Our study limitations included the observational as well as the retrospective nature of our study. There was a relatively small sample size, and the study was conducted in a single tertiary referral facility. Since full iron study profiles are not conducted routinely, this was a further limiting factor; therefore, we were unable to explore the prevalence of functional iron deficiency in this population. Our study population also had a very low HIV prevalence, and hence we were unable to conclude this sub-

group of patients. Since this was a retrospective study, we were unable to comment on prognostic factors and mortality status of patients.

Conclusions

Our study highlights the high prevalence of absolute iron deficiency, reported in 46.60% of patients attending the chronic heart failure with a reduced ejection fraction (HFrEF) clinic at a tertiary academic centre in South Africa.

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Figures

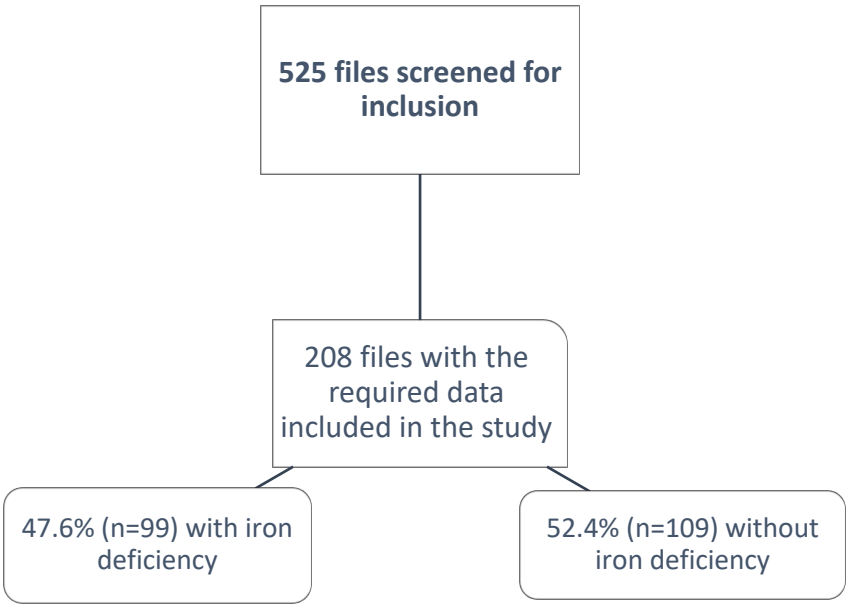


Figure 1: Aetiology of heart failure

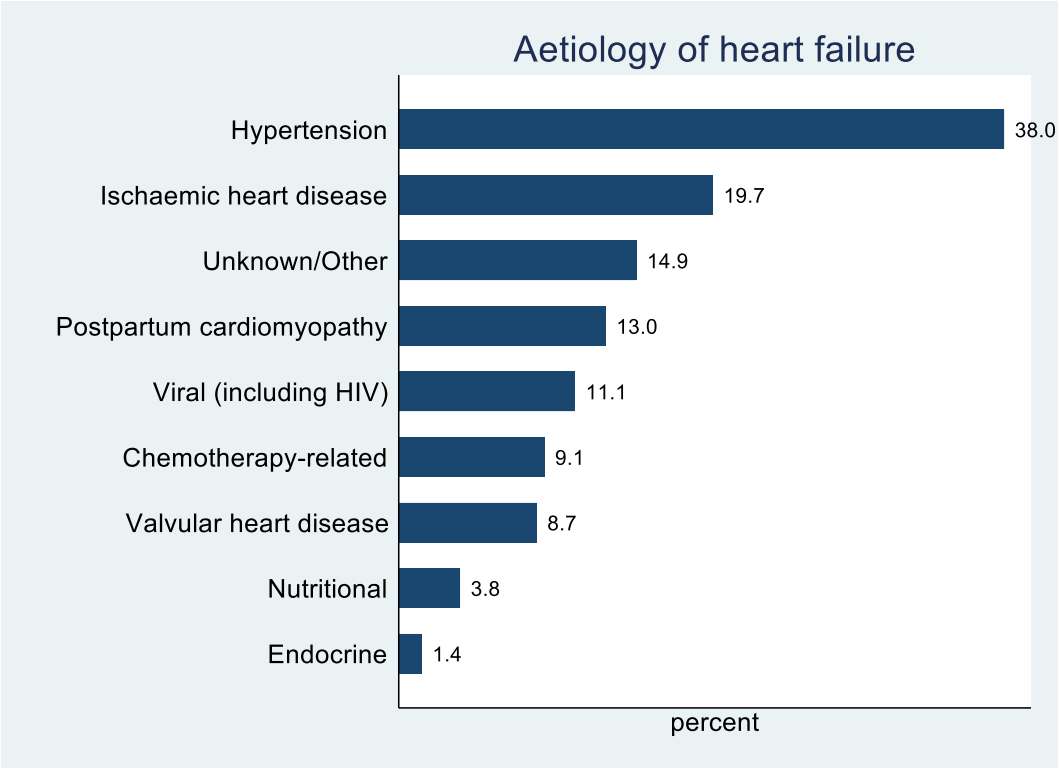


Figure 2: Medications

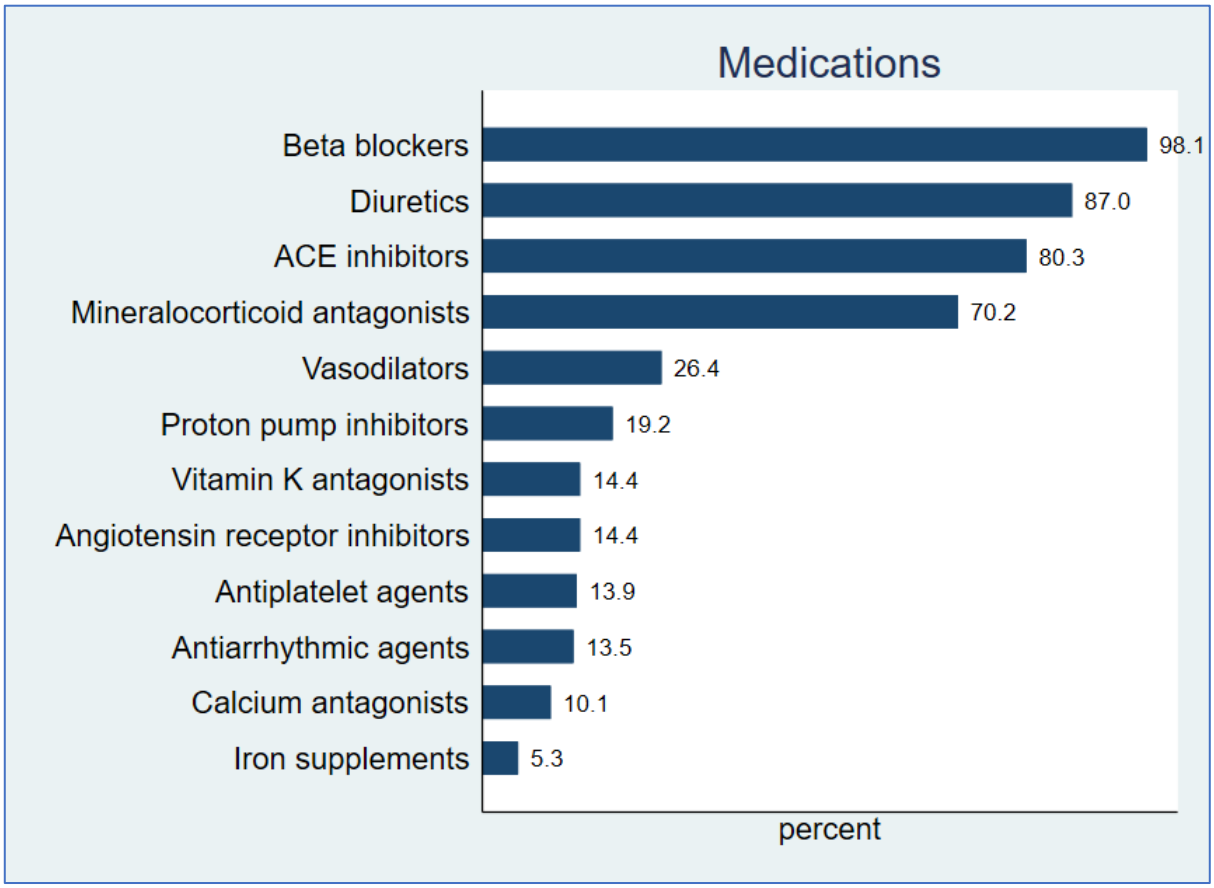
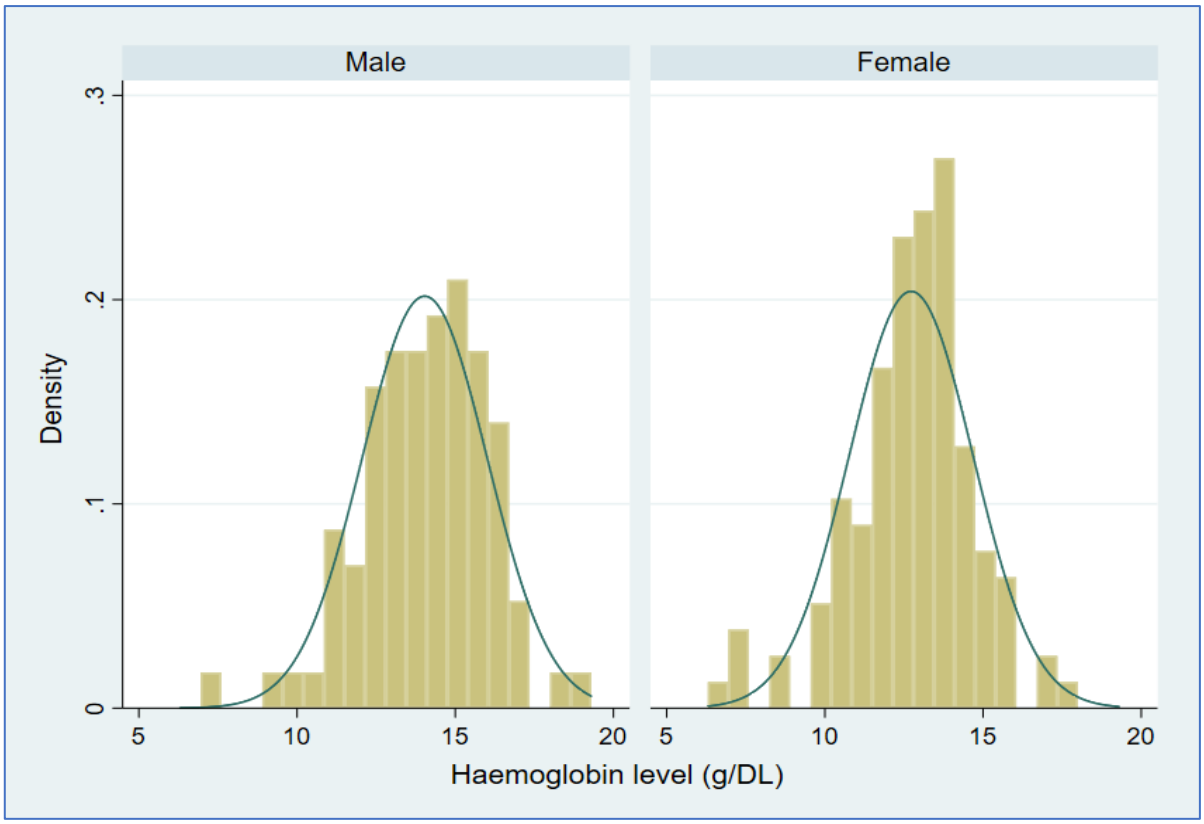


Figure 3: The haemoglobin level of the population



TABLES

Table 1: Overall characteristics of chronic heart failure patients

Variable	***Mean (SD)/ Median (IQR)**	**N (%)**
Demographics		
Age	56.78 (14.54)	
Gender Male Female		 88 (42.31) 120 (57.69)
Clinical findings		
Resting heart rate (beats/minute)	83.74 (40.31)	
6 minute walk test	318.07 (128.62)	
Minnesota Score	38.62 (24.89)	
Weight (kg)	78.60 (18.15)	
Biochemical parameters		

Mean corpuscular volume (fL)	89.62 (11.05)	
Haemoglobin level (g/DL)	13.28 (2.06)	
Ferritin level (ug/L)	106.50 (53 – 206)	
Mean cell haemoglobin level (g/DL)	29.60 (28 – 31.30)	
Mean cell haemoglobin concentration	31.80 (1.72)	
Pro BNP (ng/L)	2054.50 (176.50 – 5822.0)	
CRP (mg/L)	10 (10 – 27.5)	
Serum sodium (mmol/L)	141.23 (3.23)	
HIV Positive		26 (12.50)
if positive CD4 count	300 (252 – 434)	
Specific factors related to heart failure		
Left ventricular ejection fraction	31.40 (10.50)	
Aetiology of heart failure		
Hypertension		79 (37.98)
Ischaemic heart disease		41 (19.71)
Nutritional		8 (3.85)
Viral (including HIV)		23 (11.06)
Peripartum cardiomyopathy		27 (12.98)
Valvular heart disease		18 (8.65)
Endocrine		3 (1.44)
Chemotherapy-related		19 (9.13)
Unknown		22 (10.58)
Other		10 (4.81)
ECG findings		
Rhythm		
Sinus		188 (90.38)
Arrhythmia		20 (9.62)

Heart Rate (beats per minute)	83.61 (17.38)	
QRS duration (milliseconds)	92 (80 – 106)	
Left bundle branch Block or Right bundle branch block present		
Right bundle branch block		4 (1.92)
Left bundle branch block		23 (11.06)
None		183 (87 .98)
PR interval (milliseconds)	167.64 (35.47)	

*Normally distributed variables were described using the mean and standard deviation; non-normally distributed variables were described using the median and interquartile range.

Table 2: Comorbidities in patients with chronic heart failure

Hypertension	115 (55.29)
CKD	69 (33.17)
Diabetes	50 (24.04)
HIV	26 (12.50)
Ischaemic heart disease	24 (11.54)
Post-chemotherapy	19 (9.13)
COPD secondary to smoking	18 (8.65)
Thyroid disease	13 (6.25)

Table 3: Aetiology of heart failure

	Number	**Percentage**
Hypertension	79	37.98
Ischaemic heart disease	71	19.71
Peripartum cardiomyopathy	27	12.98
Viral (including HIV)	23	11.06
Unknown	22	10.58
Chemotherapy-related	19	9.13
Valvular heart disease	18	8.65
Other	10	4.81
Nutritional (alcohol related)	8	8.00
Endocrine	3	1.44

Table 4: Medications in patients with chronic heart failure

Beta blockers	204 (98.08)
Diuretics	181 (87.02)
ACE inhibitors	167 (80.29)
Mineralocorticoid receptor antagonists	146 (70.19)
Vasodilators	55 (26.44)
Proton pump inhibitors	40 (19.23)
Vitamin K antagonists	30 (14.42)
Angiotensin receptor antagonists	30 (14.42)
Antiplatelet agents	29 (13.94)
Antiarrhythmic agents	28 (13.46)
Calcium antagonists	21 (10.10)
Iron supplements	11 (5.29)

Table 5: Clinical factors associated with iron deficiency in chronic heart failure patients

Clinical Feature	**Iron Deficiency absent (n=109)**	**Iron deficiency present (n=99)**	**p-value**
Clinical findings			
Weight (kg) *Mean (SD)*	78.75 (18.79)	78.43 (17.51)	0.900
Resting heart rate (beats/minute) *Mean (SD)*	81.24(18.62)	86.49 (55.09)	0.349
Minnesota score Mean (SD)	34.42(25.18)	43.24 (38.47)	0.011
Aetiology of heart failure *N (%)*			
Hypertension	37 (46.84)	42 (53.16)	0.208
Ischaemic heart disease	14 (34.15)	27 (65.85)	**0.054**
Nutritional (Alcohol related)	5 (62.50)	3 (37.50)	0.724
Viral (including HIV)	15 (65.22)	8 (34.78)	0.192
Peripartum cardiomyopathy	10 (37.04)	17 (62.96)	**0.006**
Valvular heart disease	8 (44.44)	10 (55.56)	0.479
Endocrine	2 (66.67)	1 (33.33)	1.000
Chemotherapy-related	10 (52.63)	9 (47.37)	0.983
Unknown	22 (70.97)	9 (23.03)	**0.025**
Medical conditions *N (%)*			
Diabetes mellitus	23 (46.00)	27 (54.00)	0.298
Hypertension	59 (51.30)	56 (48.70)	0.724
Ischaemic heart disease	13 (54.17)	11 (45.83)	0.854
HIV	14 (53.85)	12 (46.15)	0.875
Post-chemotherapy	10 (52.63)	9 (47.37)	0.983
COPD secondary to smoking	13 (72.22)	5 (27.78)	0.078
Thyroid	6 (46.15)	7 (53.85)	0.641
Chronic kidney disease	42 (60.87)	27 (39.13)	0.085

Medications *N (%)*			
Beta blockers	106 (51.96)	98 (48.04)	0.361
ACE inhibitors	86 (51.50)	81 (48.50)	0.597
Calcium antagonists	9 (42.86)	12 (57.14)	0.356
Angiotensin receptor antagonists	12 (40.00)	18 (60.00)	0.141
Mineralocorticoid receptor antagonists	75 (51.49)	71 (48.63)	0.647
Diuretics	95 (52.49)	86 (47.51)	0.951
Proton pump inhibitors	16 (40.00)	24 (60.00)	0.080
Antiplatelet agents	19 (65.52)	10 (34.48)	0.127
Iron supplements	5 (45.45)	6 (54.55)	0.635
Vitamin K antagonists	15 (50.00)	15 (50.00)	0.776
Antiarrhythmic agents	15 (53.57)	13 (46.43)	0.894
Vasodilators	28 (50.91)	27 (49.09)	0.796
Biochemical parameters			
Mean corpuscular volume (fL) *Mean (SD)*	91.55(11.83)	87.49 (9.75)	**0.008**
Haemoglobin level (g/DL) *Mean (SD)*	13.75 (1.96)	12.75 (2.06)	**<0.001**
Mean cell haemoglobin level (g/DL) *Median(IQR)*	29.9 (28.6 – 32.4)	29.1 (27.1 – 30.6)	**<0.001**
Mean cell haemoglobin concentration *Mean (SD)*	32.09 (1.44)	31.48 (1.94)	**0.010**
Pro BNP (ng/L) *Median(IQR)*	1724.5(294 – 6255)	2509.5(166 – 5390)	1.000
CRP (mg/L) *Median(IQR)*	10 (10 – 33)	10 (10 – 25)	0.677
Serum sodium (mmol/L) *Mean (SD)*	139.62 (3.55)	140.91 (2.68)	**0.004**
HIV Positive *N (%)*	14 (53.85)	12 (46.15)	0.875
if positive CD4 count *Median(IQR)*	300(251 – 452)	303(287 – 412)	0.913
Gender			
Male	63 (71.59)	25 (28.41)	
Female	46 (38.33)	74 (61.67)	**<0.001**
NYHA Class			
I	36 (72.00)	14 (28.00)	**0.016**
II	51 (48.11)	55 (51.89)	0.4769
III	21 (41.18)	30 (58.82)	0.1035
IV	1 (100.00)	0 (0.00)	**0.004**
ECG findings			
Arrhythmia on ECG *N (%)*	15 (75.00)	5 (25.00)	**0.033**
Heart rate (beats per minute) *Mean (SD)*	83.71 (18.03)	83.50 (16.73)	0.931
QRS duration (milliseconds) *Median(IQR)*	94 (80 – 106)	92 (80 – 108)	0.783
Bundle branch block *N (%)*	15 (55.56)	12 (44.44)	0.725
PR interval (milliseconds) *Mean (SD)*	169.49 (32.19)	165.75 (38.61)	0.470
Left ventricular ejection fraction *Mean (SD)*	31.46 (10.83)	31.33 (10.17)	0.930

**Normally distributed numeric variables were described using the mean and standard deviation. The Student's T-test for equality of means was used to compare means of normally distributed variables. Non-normally distributed variables were described using the median and interquartile range. The two-sample Wilcoxon rank-sum (Mann-Whitney) test was used to compare medians for non-normal data. For categorical variables, Pearson's chi-squared test was used to compare proportions.

If n<5 in any of the cells, the Fischer's exact test for comparison of proportions was used. P values <0.05 were considered statistically significant and are shown in bold font.

Table 6: Prevalence of anaemia in chronic heart failure patients

	N (%)
Anaemia (Hb <13g/dl in males or Hb<12 in females)	55 (26.44)
Iron deficiency (Ferritin level ≤ 100 micrograms/L)	99 (47.60)
Iron deficiency anaemia (Hb <13g/dl in males or Hb<12 in females AND Ferritin level ≤ 100 micrograms/L)	32 (15.38)
Microcytic anaemia (MCV < 78.9 AND Hb <13g/dl in males or Hb<12 in females)	14 (6.73)
Macrocytic anaemia (MCV > 98.5 AND Hb <13g/dl in males or Hb<12 in females)	8 (3.85)
Pallor	15 (8.11)

Table 7: Logistic regression models of clinical factors predicting absolute iron deficiency

Clinical Feature	**Univariable model**		**Multivariable model**	
Clinical findings	**OR (95% CI)**	**P value**	**OR (95% CI)**	**P value**
Gender				
Male	Reference		Reference	
Female	4.05 (2.24 – 7.32)	**<0.001**	2.97 (1.47 – 6.01)	**0.002**
NYHA Class				
I	Reference		Reference	
II	2.77 (1.34 – 5.73)		2.18 (0.89 – 5.33)	0.086
III & IV	3.51 (1.53 – 8.02)	**0.006**	2.33 (0.73 - 7.41)	0.151
Minnesota score	1.01 (1.00 – 1.02)	**0.012**	1.00 (0.99 – 1.02)	0.484
Symptoms typical of heart failure				
Orthopnoea	2.51 (1.16 – 5.39)	**0.019**	1.41 (0.54 – 3.68)	0.480
Paroxysmal nocturnal dyspnoea	1.76 (1.01 – 3.06)	**0.045**	1.27 (0.59 – 2.73)	0.538
Ankle swelling	1.57 (0.82 – 3.01)	0.173		
Specific signs of heart failure				
Hepato-jugular reflux	2.11 (1.15 – 3.89)	**0.016**	1.80 (0.84 – 3.84)	0.129
Laterally placed apical impulse	1.89 (1.09 – 3.29)	**0.024**	1.57 (0.80 – 3.05)	0.186
Aetiology of heart failure				
Ischaemic heart disease	0.50 (0.24 – 1.02)	0.057	1.15 (0.42 – 3.10)	0.784
Viral (including HIV)	0.55 (0.22 – 1.36)	0.197		
Peripartum cardiomyopathy	4.01 (1.41 – 11.40)	**0.009**	3.55 (1.07 – 11.73)	**0.038**
Medical conditions				

COPD secondary to smoking	0.39 (0.13 – 1.14)	0.087	0.52 (0.13 – 1.99)	0.337
Medications				
Angiotensin receptor antagonists	1.80 (0.82 – 3.94)	0.145		
Proton pump inhibitors	1.86 (0.92 – 3.75)	0.083	1.40 (0.62 – 3.16)	0.421
Antiplatelet agents	0.53 (0.23 – 1.21)	0.132		
Biochemical parameters				
CRP (mg/L)	1.00 (0.99 – 1.00)	0.494		
Serum sodium (mmol/L)	1.14 (1.04 – 1.25)	**0.005**	1.11 (0.99 – 1.24)	0.054
eGFR (mL/min/1.73 m2)	0.99 (0.99 – 1.00)	0.995		
ECG findings				
Arrhythmia on ECG	0.33 (0.12 – 0.95)	**0.041**	0.42 (0.13 – 1.42)	0.164

Variables (Signs and symptoms) with p value<0.2 for T-tests/Rank-sum/Chi-squared/Fischer's exact (Tables 3 and 4 above) were considered for further exploration in logistic models. Variables with p<0.1 were included in the multivariable logistic model. P values<0.05 were considered statistically significant and are shown in bold font.

Appendix 1 – Protocol and extended Literature Review

Introduction

Iron deficiency (ID) is the most prevalent nutritional deficiency globally and has been identified to cause adverse consequences in chronic heart failure (CHF) [1]. It is currently emerging as a prognosticator and therapeutic target in this population group [1-3]. Much of the currently available data regarding ID and CHF is from high-income countries, and there is a paucity of data from poorly resourced settings such as South Africa. [4]

Background and significance of the problem

Chronic heart failure is a common and growing problem in the world today, while ID in the general population affects between 1-1.5 billion people globally and 400 million of these will complicate with anaemia [1,3]. Estimates from high - income countries indicate that the prevalence of CHF is 1-2% in the general population [1,4,7]. CHF remains a significant cause of mortality and morbidity with significant effects on quality of life measures [1,2,3,6,7]. Advances in the medical management of CHF have improved the natural history of the disease and patient outcomes [3,5,8]. However, despite these advances, activities of daily living remain difficult for this group of patients, as there are significant functional limitations due to symptoms of dyspnea and fatigue [1,9,10]. Unfortunately, treatments that improve symptoms and the quality of life are currently limited, and newer therapies are needed.

Hence, treatment of iron deficiency is emerging as an important therapeutic target in chronic heart failure. Traditionally iron deficiency has been considered usually with the presence of concurrent anaemia, but recent studies suggest a role for treatment regardless of the anaemia status of the patient [1,2,6,7,9, 11]. Iron deficiency irrespective of anaemia is associated with worsened symptoms and predicts a poorer prognosis in this population group [1,6,]. Iron deficiency without anaemia as a therapeutic target alone has been largely ignored until recently, when new evidence suggested that the replacement of intravenous iron, in ID without anaemia, may be of value in improving symptoms in the chronic heart failure patient [7,9,10].

The physiological role of iron

Iron is a micronutrient essential for normal cellular functioning. The homeostasis of iron is tightly regulated and controlled by the body to prevent toxic levels. It is a metabolically active nutrient with multiple physiologic oxidative states including Feryl 4+, ferric 3+, and ferrous 2+ [1,8]. These physiological states allow iron to have biochemical interactions with oxygen, nitrogen and sulfur atoms [1]. Hence, iron is an essential co-factor for various enzymatic complexes including metalloenzymes that act as catalysts for redox reactions within the cell. Six of the enzymes utilised in the generation of adenosine triphosphate are haem-containing cytochrome C proteins and another six are iron sulphur proteins [1,2,8]. Therefore, iron plays a key role in energy production in the mitochondrial electron chain, which is responsible for cellular energy generation. Due to its role as an enzymatic co-factor for mitochondrial energetic reactions, cells with a high-energy demand such as the cardiac myocyte may be more susceptible to the effects of iron deficiency [2].

Iron is also an important component of transport proteins required for biological roles, which includes haemoglobin and myoglobin. The iron in the body plays a role in oxygen transport as a constituent of haem in haemoglobin. Optimal haemotopoiesis thus requires iron, and the majority is endocytosed by reticulocytes to synthesise haemoglobin [1,2,8]. Iron is also essential for the cellular energetic processes and metabolism in extra haematopoietic tissues.

Iron Homeostasis

There are a series of complex processes involved in iron haemostasis in the body. These processes can be divided into absorption, recycling and storage which are in turn regulated by various transport proteins. The average iron content of the human body is 3-4 grams [8]. Most of this exists predominantly complexed as haemoglobin and other iron - containing proteins (myoglobin) as well as plasma transferrin-bound [8]. The remainder of iron exits as ferritin or haemosiderin. All of these biologic iron forms are primarily controlled by the molecule hepcidin which is produced by the liver

[1,8].

Furthermore, iron can be conceptualised into two pools of iron reservoirs in the body, namely that which is utilised and that which is stored [1]. These two interact by way of circulating iron bound to transferrin which is a source of soluble iron and delivers iron to target cells.

The average iron intake is approximately 10 - 20 mg per day. Iron absorption occurs via specific absorption transport systems in the duodenal enterocytes. Inorganic (non-haem) iron absorption occurs via the apical surface of the duodenal brush border cells using the divalent metal transporter 1 (DMT1) [1,8]. Enzymes (ferrireductases) found on the membrane surface are responsible for the reduction of ferric to ferrous iron. Haem iron absorption occurs via a haem carrier protein and haem-oxygenase 1 reduces the iron prior to entering the cytosol. The absorbed iron binds to plasma transferrin and is distributed throughout the body to its target cells especially the bone marrow [1,8]. Iron-loaded transferrin binds to the transferrin receptor 1 on the surface of most mammalian body cells and undergoes endocytosis. Iron then enters the cytoplasm via DMT1 in the endosomal membrane [1,2,8].

Once intracellular, iron can then be used for metabolic functions or stored within the cells in nontoxic cytosolic ferritin shells. Ferroportin is responsible for cellular iron export [1,8]. Cellular iron is tightly regulated by iron regulatory proteins and chaperones namely iron regulatory protein 1 (IRP1) and iron regulatory protein 2 (IRP2) to avoid toxicity and free radical generation [1]. Much of intracellular iron exists within erythrocytes and reticulocytes as haemoglobin.

Hepcidin is the major protein governing the control of iron homeostasis, and it controls iron absorption via ferroportin [1,2,7,8]. Ferroportin is the only molecular channel by which iron can leave the cell. It is expressed mainly in cells critical for iron handling which includes the duodenal enterocytes, the splenic pulp macrophages which are responsible for the iron recycling of old red blood cells as well as the hepatocytes which are responsible for the storage of iron [1,8]. Red blood cells contain the most considerable amount of iron, and the element is recycled

efficiently via phagocytosis to ensure the availability of iron. Typically, there are minimal losses of iron via skin or mucosal surfaces as well as menstruation with women. Currently, there are no known specific mechanisms within the human body for iron excretion [8].

The hepcidin and ferroportin transporters are essential regulators in the critical balance between erythropoiesis and iron absorption. The significant determinants governing their control are body iron stores, erythropoietic activity and inflammation [1,8]. Hepcidin upregulation occurs when the body has sufficient iron stores and prevents iron absorption in the gut whereas, it is suppressed in iron deficiency to allow the converse to happen [1,8]. Chronic inflammation stimulates hepcidin production via the cytokines interleukin 6 and 1beta which causes iron sequestration into macrophages and iron -restricted erythropoiesis leading to anaemia of inflammation which includes CHF [1,2,8]

Chronic Heart Failure - Heart failure with reduced ejection fraction

As defined by the European Society of Cardiology (ESC) 2016 guidelines for the diagnosis and treatment of acute and chronic heart failure, “Heart failure is a clinical syndrome characterized by typical symptoms”. “Signs (e.g. elevated jugular venous pressure, pulmonary crackles and peripheral oedema) may accompany these symptoms, caused by a structural and functional cardiac abnormality, resulting in a reduced cardiac output and elevated intracardiac pressures at rest or during stress.” [5] Heart Failure is further sub-classified by the echocardiographic findings of the left ventricular ejection fraction, with heart failure with reduced ejection fraction (HFrEF) defined as less than 40% [5].

CHF is a growing and concerning epidemic today with the prevalence being 1-2% of the adult population in the developed world rising to 10% in the age group of subjects over 70 [7]. Heart disease is both a current and emerging concern in South Africa and Sub-Saharan Africa (SSA) as a whole. It is predicted to affect 1.3 million people per annum within the next 20 years in Africa [12]. There is a dual epidemiological burden to be faced due to the prevalence of both communicable

diseases such as HIV and rheumatic fever as well as non - communicable diseases of lifestyle such as hypertension, emerging as important causes of heart failure in SSA [12,13]. Research has indicated that most cases of heart failure in SSA are due to non - ischaemic causes such as hypertensive heart disease and idiopathic dilated cardiomyopathy [12,13].

Iron deficiency and chronic heart failure

The prevalence of iron deficiency in the chronic heart failure population is postulated to be between 30 - 50% dependent on the definitions of iron deficiency used in the study and the study population [1,3,4]. Iron deficiency has been demonstrated to have associations with worsened symptoms and prognosis in this population group independent of the anaemia status [1,3,4,9,10,11]. It is a comorbidity amenable to medical treatment, however, has been ignored until recently [9-11].

Chronic iron deficiency causes reduced exercise capacity and ultrastructural alterations to cardiac myocytes [1,2]. The effects on exercise capacity including an exaggerated ventilatory response have been described, even without concurrent anaemia [1]. It is also an independent risk factor for mortality and transplantation in patients with chronic systolic heart failure [5,6,7] Signs and symptoms seen in ID may be due to the effects from anaemia (if present) as well as direct effects on skeletal muscle which need iron as a cofactor for mitochondrial enzymes. However, iron deficiency even without anaemia may be associated with impaired exercise capacity, increased fatigue, worsened cognitive symptoms, more severe disease and poorer outcomes [1,6]. Supplementation with intravenous iron has been shown to reverse these symptoms [1,2,9,10,11].

Dysfunction of the myocardium and skeletal muscles contribute to the pathophysiology of heart failure. This myocardial dysfunction is due to the increased energy demands as the myocardium's optimal function is dependent on an intact iron metabolism. Animal studies have been used to gather data regarding the myocardial consequences of ID [1,2]. In these studies, iron -deficient rats develop increased cardiac output due to sympathetic activation and subsequent left ventricular

hypertrophy and eventual left ventricular dilatation [1-3]. Myocardial cells undergo extracellular remodeling and mitochondrial dysfunction is also present [1]. Various triggers in heart failure lead to immune activation of T cells and monocytes with the release of inflammatory cytokines which may lead to abnormal energy generation within the cardiac myocytes and upregulation of pro - inflammatory molecules [1,2]. Expression of iron regulatory proteins and transferrin-1 receptors are also downregulated in the failing myocytes further pointing to mechanisms by which iron deficiency can worsen symptoms in CHF [1,2,3].

Inadequate oxygen supply and impaired oxygen usage by skeletal muscle is also seen in ID and CHF contributing to clinical status and symptomatology. Iron deficiency impairs the energy producing capacity of skeletal muscle by changing substrate preferences utilised by these cells and leading to deficiencies in oxidative capacity [1,3,5].

The causes of iron deficiency in patients with heart failure are multifactorial [1,2,3]. It may be linked to impaired absorption of iron in the duodenum because the gut may be oedematous [1,6]. There are also several medical drug interactions which may occur especially with the use of proton pump inhibitors [1,3,6]. Blood loss from the gastrointestinal tract may also occur secondary to anti-platelet agents which may be prescribed to patients with ischaemic cardiomyopathy [1-3,6]. Liver congestion may also contribute to the development of iron deficiency.

Recent studies have also shown that there is a suboptimal dietary intake of iron in NYHA class III - IV heart failure. Also, animal models have demonstrated disruption of transport systems in the duodenum but without increased hepcidin levels [1,3,7]. In these models, hypoxia-inducible factor 2 alpha was found to be a significant regulator of duodenal transport of iron, and this could indicate the dysregulation of physiological mechanisms to counteract depleted iron stores in the heart failure state [1].

Independent risk factors for iron deficiency highlighted in studies included female gender, advanced NYHA class, increased levels of serum plasma N terminal pro B type natriuretic peptide and increased serum C- reactive protein [1,5,7,9]. In patients

with HFrEF mortality was increased in patients with iron deficiency independent of the presence of anaemia [3,6,7].

Heart failure is found to be a pro-inflammatory state characterised by a generalised and upregulated immune response leading to the increased expression of inflammatory mediators in the damaged myocardium and peripheral tissues [1,2,5]. However, in the clinical setting, no association was found between the pro-inflammatory activation by interleukin 6 (IL -6) and increased hepcidin levels in CHF indicating that more research in this field is required [1,2]. Hypoxia upregulates the expression of hepcidin in the rat myocardium but interestingly did not increase hepatic expression of hepcidin [1]. This suggests that there may be other unrecognised factors contributing to iron deficiency in heart failure besides hepcidin. Therefore, the hypothesis from animal models is that there are pathophysiological changes that occur in the myocardium due to iron deficiency which could lead to worsening of HF symptoms [1].

Diagnosis of absolute iron deficiency

The gold standard for the diagnosis of ID is bone marrow aspiration and assessment. However, the invasiveness of this test limits its clinical utility [1,4]. Therefore, biochemical markers are often used as surrogates to assess iron status. Absolute iron deficiency represents depleted iron stores in the body and its assessment is based on the level of circulating ferritin which acts as a reliable marker of iron stores. Serum ferritin and ferritin expression in storage tissues share a linear relationship. The serum ferritin used to diagnose absolute iron deficiency is <30 micrograms/l. However, in an inflammatory state such as chronic heart failure the expression of ferritin in its role as an acute phase reactant is stimulated, hence in this case a higher serum ferritin value of 100 mcg/L is used as a cut-off. [1,4,5].

Iron plays an important role in erythropoiesis hence red cell parameters are also considered in the diagnosis of ID however these may change late. These parameters include the haemoglobin level, mean corpuscular volume (MCV), mean corpuscular

haemoglobin concentration (MCHC). The diagnosis of restricted iron delivery to the red blood cell precursors uses these indices which leads to a decrease in the MCV and MCHC [1,8]. Hypochromic red blood cells with a low MCV and a low MCHC indicate ID but this is found in the advanced stage of the disease process. An increase in the percentage of these cells to greater than 25% is indicative of ID.

Absolute iron deficiency is a reflection of depletion of iron stores, but erythropoiesis may still be intact [1,6]. Anaemia is considered the end stage of ID leading to a decreased haemoglobin level less than 13g/dL in males and 12 g/dL in females. As iron metabolism is integral to erythropoiesis, subjects with ID are often found to have high levels of erythropoietin circulating, which also indicates iron - restricted erythropoiesis.

Iron deficiency and heart failure in Sub Saharan Africa

There is an abundance of data on ID from the developed world. However, there is a paucity of data from the developing world. Infectious causes and malnutrition may have a contributory role in SSA that is not a typical feature in developed countries [12-13]. The definitions of ID and anaemia may also need to be re-defined for developing countries as haemoglobin levels may be lower in the general population related to chronic infections, nutritional deficiencies and haemoglobinopathies. Therefore, there is a need for standardisation and further research in this field [4,12-13]. We found only one published study from Sub-Saharan Africa, the Tanzania heart failure study, which has documented the incidence of iron deficiency and anaemia in a heart failure population [12-13].

The diagnosis of iron deficiency in low - income settings is also problematic as there is a lack of access to laboratories with regards to the usage of biochemical markers. Cut-off values for serum ferritin levels still need to be standardised and explicitly validated for the African population. The diagnosis of ID may need to be established at higher ferritin levels as black Africans may have an increased level of circulating ferritin and therefore serum ferritin less than 150 micrograms /L may be diagnostic. However, this still needs to be confirmed [4]. The therapy available for ID in the

developed world includes intravenous iron. High cost, limited availability and access restrict the utilisation of this mode of therapy in the SSA [4, 12-13]. These specific issues highlight the need for further research into developing useful oral compounds to treat iron deficiency in the developing world.

Therapeutic implications for chronic heart failure

Currently, the treatment of iron deficiency in heart failure has generated much interest. Intravenous iron treatment has shown therapeutic benefits as opposed oral treatment due to the bioavailability of intravenous iron being superior to that of oral therapy [11].
The 2016 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure recommend investigating and elucidating the cause of iron deficiency in heart failure patients. It also recommends therapy for iron deficiency regardless of anaemia status [6]. Intravenous treatment is also well tolerated as opposed to oral therapy and more studies are currently underway investigating this hypothesis.

The therapeutic supplementation of iron in CHF populations improves exercise capacity, quality of life and heart failure symptoms [6,8, 9-11]. Importantly, to date, there are minimal adverse side effects related to intravenous iron replacement reported in the concluded clinical trials. There are also no documented adverse effects in terms of cardiovascular or infective consequences. A recent meta - analysis also showed benefit in reduced hospitalisations with iron supplementation but none have yet shown mortality benefits [6]. Further research is still required in the treatment of ID in CHF to determine if there are any mortality benefits [9-11,13].

The rationale for the study

As highlighted by this literature review, ID is an important, common and under recognised comorbidity in the CHF population. Therapy has been illustrated to be effective in improving symptoms, hospital admissions and clinical status. However, data on ID in CHF in SSA is lacking. This study aims to define the scope of this problem in our local clinic.

To the knowledge of the author this is the first study that seeks to define the prevalence of absolute iron deficiency in an outpatient setting in South Africa. This study can then be further extrapolated upon in order to possibly lead to further research on the therapeutic front.

Aim

This study seeks to define the prevalence of iron deficiency in a chronic heart failure (HFrEF) population at an urban tertiary hospital.

Objectives

Primary Objective

To define the prevalence of absolute iron deficiency in a chronic heart failure cohort (heart failure with reduced ejection fraction) at a tertiary hospital in Gauteng.

Absolute iron deficiency is defined as a ferritin level of 100 micrograms/L or less, as per the 2016 European Society of Cardiology guidelines for the diagnosis and treatment of acute and chronic heart failure.

Secondary objectives

1) Determine the prevalence of coexisting anaemia in this population
2) Define the symptoms associated with iron deficiency in this population with regards to the New York Heart Association classification and quality of life (using the Living with Heart Failure Minnesota questionnaire)
3) Describe clinical factors associated with iron deficiency in this population (including clinical findings such as heart rate, biochemical parameters and electrocardiogram findings in this population)

Methodology

This study will be a retrospective review of all outpatient clinical data at the chronic HFrEF clinic at the Charlotte Maxeke Johannesburg Academic hospital. We intend to review all the out-patient files, paying particular attention to the initial clinic visit. Information from the files reviewed will be recorded in a data collection sheet (see Appendix 1) focusing on 30 clinical variables. Each patient that attends the heart failure clinic undergoes a routine clinical examination, echocardiogram and electrocardiogram and baseline laboratory data recorded at the initial visit. Clinical data sheets are used to document all clinical information. Individual patient files are then used to store these data sheets, and the patient files are accessible via the clinic

Biochemistry results will also be reviewed from the National Health Laboratory Services database via the Trakcare viewer. The evaluation of patients attending the clinic also includes a six - minute walk test, quality of life evaluation using the Living with heart failure Minnesota questionnaire [14] and an electrocardiogram for each visit. Ferritin levels are also routinely investigated on the index presentation at the clinic. Therefore, all relevant clinical data collected will also be from the index presentation to the heart failure clinic.

Inclusion Criteria

All adult patients that are currently actively attending the clinic will be eligible to participate in the study. There are currently 525 patients at the clinic and all will be included in the study.

Exclusion Criteria

Patient clinic files with insufficient or missing data will not be eligible to participate in the study.

Funding

This study is a retrospective review of patient records. The principle investigator and the research team do not anticipate significant operational costs. As such, all costs will be accounted for by the primary investigator and hence no additional funding is required.

Statistical analyses

Data will be expressed as means and standard deviations where the data is normally distributed and as medians with quartiles when non-normally distributed. Data that is categorical will be expressed as numbers and percentages. Student's t-test and analysis of variance (ANOVA) with Fisher's test will be used to test for intergroup differences with the Chi - squared test being used to test for intergroup proportion differences. Univariate and multivariate logistic regression will also be used in order to determine clinical factors associated with iron deficiency. All tests will be two - sided with a p - value < 0.05 considered as statistically significant. Data collection will be done using Redcap software and all statistical analysis will be done with STATA version 14.

Ethics approval

This research proposal and an application for ethics approval will be forwarded to the WITS HREC in order to obtain ethics approval. Permission to access and utilise the data will be sought from Charlotte Maxeke Johannesburg Academic Hospital and the Division of Cardiology. Patient identification details will not be included on the data sheet hence anonymity is ensured and there is no possible risk or harm to the patient that may be incurred due to the study.

Timeline

2018	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Literature review												
Preparing protocol												
Protocol assessment												
Ethics application												
Collecting data												
Data analysis												
Writing up - paper												

Appendix 2 - Data sheet

Variable	
Demographics	
Age	
Gender	
Clinical findings	
Resting heart rate (beats/minute)	
Blood pressure (mmHg)	
6 minute walk test	
Minnesota Score	
Body mass index	
Biochemical parameters	
Mean corpuscular volume (fL)	
Haemoglobin level (g/DL)	
Ferritin level (ug/L)	
Mean cell haemoglobin level (g/DL)	
Pro BNP (ng/L)	
CRP (mg/L)	
Serum sodium (mmol/L)	
eGFR (mL/min/1.73 m2)	
HIV status	
if positive CD4 count	
Specific factors related to heart failure	
Ejection fraction	
Aetiology of heart failure	
Diabetes mellitus	
Hypertension	
Ischaemic heart disease	

ECG findings	
Rhythm (Sinus or Atrial fibrillation)	
Heart Rate (beats per minute)	
QRS duration (miliseconds)	
Left bundle branch Block or Right bundle branch block present	
PR interval (miliseconds)	

MINNESOTA LIVING WITH HEART FAILURE® QUESTIONNAIRE

The following questions ask how much your heart failure (heart condition) affected your life during the past month (4 weeks). After each question, circle the 0, 1, 2, 3, 4 or 5 to show how much your life was affected. If a question does not apply to you, circle the 0 after that question.

Did your heart failure prevent you from living as you wanted during the past month (4 weeks) by -	**No**	**Very Little**				**Very Much**
1. causing swelling in your ankles or legs?	0	1	2	3	4	5
2. making you sit or lie down to rest during the day?	0	1	2	3	4	5
3. making your walking about or climbing stairs difficult?	0	1	2	3	4	5
4. making your working around the house or yard difficult?	0	1	2	3	4	5
5. making your going places away from home difficult?	0	1	2	3	4	5
6. making your sleeping well at night difficult?	0	1	2	3	4	5
7. making your relating to or doing things with your friends or family difficult?	0	1	2	3	4	5
8. making your working to earn a living difficult?	0	1	2	3	4	5
9. making your recreational pastimes, sports or hobbies difficult?	0	1	2	3	4	5
10. making your sexual activities difficult?	0	1	2	3	4	5
11. making you eat less of the foods you like?	0	1	2	3	4	5
12. making you short of breath?	0	1	2	3	4	5
13. making you tired, fatigued, or low on energy?	0	1	2	3	4	5
14. making you stay in a hospital?	0	1	2	3	4	5
15. costing you money for medical care?	0	1	2	3	4	5
16. giving you side effects from treatments?	0	1	2	3	4	5
17. making you feel you are a burden to your family or friends?	0	1	2	3	4	5
18. making you feel a loss of self-control in your life?	0	1	2	3	4	5
19. making you worry?	0	1	2	3	4	5
20. making it difficult for you to concentrate or remember things?	0	1	2	3	4	5
21. making you feel depressed?	0	1	2	3	4	5

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11/10/04

Appendix 3 [15]

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Appendix 4: Ethics Approval Certificate

UNIVERSITY OF THE WITWATERSRAND JOHANNESBURG

R14/49 Dr Karishma Singh

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M180841

NAME: (Principal Investigator)	Dr Karishma Singh
DEPARTMENT:	Internal Medicine Charlotte Maxeke Johannesburg Academic Hospital
PROJECT TITLE:	The prevelance of absolute iron defeciency in a chronic heart failure cohort (heart failure reduced ejection fraction) at a tertiary hospital in Gauteng
DATE CONSIDERED:	31/08/2018
DECISION:	Approved Unconditionally
CONDITIONS:	
SUPERVISOR:	Dr N Tsabedze
APPROVED BY:	Doctor CB Penny, Chairperson, HREC (Medical)
DATE OF APPROVAL:	03/09/2018

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office Secretary on the Third Floor, Faculty of Health Sciences, Phillip Tobias Building, 29 Princess of Wales Terrace, Parktown, 2193. University of the Witwatersrand. I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit the application to the Committee. **I agree to submit a yearly progress report**. The date for annual re-certification will be one year after the date of convened meeting where the study was initially reviewed. In this case, the study was initially reviewed in **August** and will therefore be due in the month of **August** each year. Unreported changes to the application may invalidate the clearance given by the HREC (Medical).

Principal Investigator Signature

16/09/2018
Date

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

Appendix 5: Turnitin report

Turnitin Originality Report

0501230r:Inline_Submission by Karishma Singh

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