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Usefulness of Relative Hypochromia in Risk Stratification for Non-Anemic Patients with Chronic Heart Failure

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Abstract

The purpose of this study is to investigate the impact of a reduction of hemoglobin (Hb) content in the erythrocytes as estimated by mean corpuscular hemoglobin concentration (MCHC) on long-term clinical outcomes in non-anemic patients with heart failure (HF). We prospectively enrolled 1,579 subjects with HF undergoing coronary angiography enrolled in the GeneBank study with 5-year follow-up of all-cause mortality. Levels of Hb and MCHC were assessed at enrollment and after 6 months of follow up. Anemia was defined as Hb levels <13 g/dL in males, and <12 g/dL in females. In our non-anemic cohort (n=785, 49.7%), mean Hb and median MCHC were 13.8±1.1 g/dL and 34.3 (interquartile range 33.6-35) g/dL, respectively. Non-anemic HF patients with lower MCHC had higher mortality risk (Quartiles 1 vs 4, Hazard ratio = 2.1, 95% confidence interval 1.4-3.3, p=0.001). In a subset of non-anemic patients with persistent normal Hb on follow-up (n=206), the mean time between baseline and follow up MCHC levels was 169.3±41.6 days. In comparison to patients with levels of MCHC above the first quartile (>33.6 g/dL) on baseline and follow up, patients with persistently low MCHC (<33.6 g/dL) had a significantly increased mortality risk (log rank <0.001). All models remained significant even after adjusting for

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traditional cardiac risk factors, left ventricular ejection fraction, baseline Hb levels and mean corpuscular volume. In conclusion, relative hypochromia is an independent predictor of increased mortality risk in patients with HF, even in the setting of normal Hb levels.

Keywords

Heart failure; relative hypochromia; anemia; outcomes; oxidative stress

Anemia has been reported to affect up to 20-30% of patients with heart failure (HF), and has consistently been associated with increased morbidity and mortality.¹⁻⁵ Multiple mechanisms are involved in the development of anemia in the setting of HF, including nutritional deficiencies (such as malabsorption, impaired metabolism), acute or chronic blood loss (such as gastrointestinal bleeding),³ intrinsic renal disease leading to insufficient erythropoietin production,³ hemodilution from volume expansion,⁶ increased levels of circulating inflammatory cytokines (as in anemia of chronic inflammation),^{7,8} medication use or hematinic abnormalities.⁴ The presence of anemia has become increasingly recognized as an important factor in the development and progression of HF.⁵ As one of the most common causes of anemia, iron deficiency is associated with impaired functional capacity, worse cardiac function, and adverse cardiac outcome.^{4,9,10} However, the definition of iron deficiency is largely restricted to quantifying levels of circulating iron and its binding proteins. Mean corpuscular hemoglobin concentration (MCHC), the index of hemoglobin (Hb) in blood cells, reported in complete blood cell count, reflects amount of iron incorporated into circulating erythrocytes.¹¹⁻¹³ We have previously reported the prognostic implications of relative hypochromia (defined as low MCHC in the setting of normal Hb) in a small observational series of ambulatory patients with significant left ventricular impairment.¹⁴ Herein, we investigate the effect of hypochromia on a larger and broader patient population of stable HF patients especially regarding the impact of changes in MCHC levels over time on long-term survival. We also investigate the association between relative hypochromia and severity of HF assessed by biochemical, physiological, and functional measures, as well as the association between relative hypochromia and various inflammatory and oxidative stress markers in the setting of HF.

Methods

The Cleveland Clinic GeneBank study is a large, prospective cohort study from 2001 to 2007 with clinical and longitudinal outcomes data composed of consenting subjects undergoing elective diagnostic cardiac catheterization procedure. All participants gave written informed consent approved by the Cleveland Clinic Institutional Review Board. History of HF was detected by: a) Directly asking patient by research personnel, b) reviewing medical records for confirmation (all patients were seen by cardiologist at Cleveland Clinic before the left heart catheterization), and c) ICD codes and adjudication by research personnel. This analysis included 1,579 HF subjects with New York Heart Association functional class II-IV without evidence of myocardial infarction (cardiac troponin I <0.03 ng/mL) with hematologic data available for analysis. Five-year survival was ascertained for all subjects following enrollment via a combination of prospective

patient contact, electronic medical record review, and confirmed by the Social Security Death Index.

Complete blood cell count was performed on blood samples collected at the day of enrollment using ADVIA 120 hematology analyzer (Siemens, New York, NY). Anemia was defined as Hb <13 g/dL for males and <12 g/dL for females.¹⁵ Indices of anemia included Hb, hematocrit, MCHC (ratio of Hb to hematocrit) and mean corpuscular volume (MCV). The normal range of MCHC is 33 to 36 g/dL.¹⁶ Non-anemic patients were grouped into quartiles based on MCHC levels.

Follow-up levels of Hb and MCHC levels were obtained from reviewing the electronic medical records when these tests were measured for clinical purposes. To study change of MCHC levels over time, a fixed cutoff of 180 ± 90 days was used, and level closest to day 180 was selected. Only non-anemic patients on baseline and follow up were analyzed to assess effect of MCHC change over time on patient's outcome. To study the effect of MCHC change overtime on outcomes, patients were grouped using cutoff of Quartile 1 for MCHC, thus 4 groups were identified: persistent hypochromia, resolving hypochromia, new hypochromia and no hypochromia (Figure 1).

HF severity was assessed based on plasma B-type natriuretic peptide levels (BNP; using Abbott Architect ci8200, Abbott Park, IL) and Duke Activity Score Index (DASI),^{9,17} on day of enrollment. Left ventricular ejection fraction (LVEF) was collected for all patients. High sensitivity C-reactive protein (hsCRP), ceruloplasmin, and serum arylesterase activity were all measured at the time of enrollment in a randomly selected sample of 790 patients. Ceruloplasmin and hsCRP were measured by Abbott Architect ci8200 (Abbott Laboratories, Abbott Park IL).^{18,19} Serum arylesterase activity level was determined using a modification of a spectrophotometry-based assay as previously described.²⁰

The Student's *t* test, Wilcoxon-Rank sum test or Kruskal-Wallis test for continuous variable and Chi square test for categorical variables were used to examine the difference between the groups. Kaplan Meier analysis and Cox proportional hazards regression were used for time-to-event analysis to determine hazard ratio and 95% confidence intervals (95% CI) for 5-year survival. Levels of MCHC were adjusted for traditional cardiac risk factors in a multivariate model, including age, gender, diabetes mellitus, systolic blood pressure, hyperlipidemia, creatinine clearance, smoking, coronary artery disease, LVEF, body mass index and medications (angiotensin converting enzyme inhibitors/angiotensin receptor blockers, beta-blockers, and aspirin). Analyses were repeated after adjusting for Hb, MCV and DASI. All statistical analyses were performed using R 2.15.1(Vienna, Austria).

Results

Table 1 shows the baseline characteristics of 1,579 patients with stable HF enrolled in this study. Mean Hb and MCHC were 12.6 ± 1.6 g/dL and 33.9 ± 1.2 g/dL, respectively (all normally distributed). About half of the patients [$n=785$ (49.7%)] were non-anemic. Among this non-anemic group, the median MCHC was 34.3 g/dL (interquartile range of 33.6-35 g/dL). Patients with low levels of MCHC were more likely to be females, have lower

creatinine clearance and have less prevalence of smoking and coronary artery disease (Table 1).

Anemic patients with HF showed increased risk of 5-year mortality (Hazard ratio 2.2, 95% CI 1.8-2.7, $p<0.001$). Overall, patients with lower MCHC had increased mortality risk (Quartiles 1 vs 4, Hazard ratio 2.5, 95% CI 2.0-3.3, $p<0.001$). Among patients with normal Hb levels, patients with decreased MCHC levels have increased mortality risk in a graded pattern (Figure 2, Table 2). All results remained significant even after multivariate adjustments with traditional coronary risk factors, as well as known prognostic cardio-renal, hematologic variables and DASI (Table 2). Furthermore, patients with low MCHC levels at baseline had significantly increased HF severity assessed by BNP levels (neurohormonal upregulation) and DASI (functional capacity) but not with LVEF (Table 3).

A total of 552 (35%) subjects had follow-up Hb levels that met our inclusion criteria. Mean time between baseline and follow up samples was 169 ± 42 days. No significant differences were found between selected patients with follow-up level and those without, in regard to HF severity (LVEF, DASI and BNP), adverse outcomes (5-year mortality) and hematologic indices (Hb, MCV and MCHC), suggesting this subgroup is a good representative of the total study population (Supplemental Table 1s, online only). In this longitudinal cohort, mean baseline Hb and MCHC were 12.6 ± 1.6 g/dL and 33.9 ± 1.2 g/dL, respectively. Mean follow up Hb and MCHC were 12.8 ± 2.0 g/dL and 33.0 ± 1.3 g/dL, respectively. About half of the patients were non-anemic ($n=271$, 49.1%) at baseline, and most were non-anemic on follow up ($n=311$, 56.3%). In the non-anemic group at baseline, patients with low MCHC did not have increased risk of developing anemia (below vs above Quartile 1, odds ratio 1.8, 95% CI 0.96-3.2, $p=0.067$).

Effect of MCHC change overtime on long term survival was studied in patients with normal Hb both at baseline and at follow up ($n=206$). Patients with low MCHC ("relative hypochromia", $<$ Quartile 1) were compared to those with high MCHC ("normochromia", Quartile 1). Most of the patients with low MCHC on baseline have also low MCHC on follow up "persistent hypochromia", while about half of the patients with high MCHC at baseline continued to have high MCHC levels "no hypochromia" (Figure 1). In comparison to patients with no hypochromia, patients with persistent hypochromia had significant increased mortality risk (log rank <0.001 , Figure 3).

We also examined the relationship between MCHC levels in non-anemic HF patients and markers of inflammation and systemic oxidative stress (Table 3). Out of 790 patients with available biomarkers levels, 398 patients were non-anemic. In non-anemic HF patients with low MCHC levels, we observed graded and significantly higher levels hsCRP and ceruloplasmin, consistent with heightened systemic inflammation. Serum levels of arylesterase activity, a cardioprotective anti-oxidant activity catalyzed by high density lipoprotein-associated paraoxonase-1,²⁰ were significantly lower in patients with low MCHC – an indication of heightened oxidative stress (Table 3).

Discussion

The primary finding of our study is the association between the presence as well as persistence of relative hypochromia (as defined by low MCHC levels, a readily available parameter in complete blood count analysis) and increased risk of 5-year all-cause mortality. This was especially notable in non-anemic patients with HF, a sub-group for whom no clinical consensus yet exists regarding approach, monitoring and treatment. Furthermore, we observed significant impairment in functional activity in patients with relative hypochromia and heightened inflammatory and reduced anti-oxidative processes. Interestingly, low MCHC levels on baseline were not associated with increased risk of developing anemia on follow up. These findings highlight the importance of recognizing and exploring the mechanistic underpinnings of relative hypochromia in the setting of chronic HF, above and beyond its contribution to anemia.

Since MCHC is a direct assessment of the amount of Hb incorporated into the erythrocytes, MCHC may serve as a reliable and readily accessible indicator of erythrocyte iron load,^{11-13,21} that has a high specificity (up to 96%) in detecting iron deficiency.²¹ Iron is involved in multiple physiologic functions including oxygen transport (Hb), oxygen storage (myoglobin), oxidative metabolism (mitochondrial oxidative enzymes),^{7,22} antioxidant activity (ferritin, myoglobin), and also is involved in the synthesis and degradation of lipids, carbohydrates and nucleotides.^{4,7,22} Traditionally, iron deficiency is only considered important in presence of anemia. Our current findings argue that strict cut-points for assessing iron deficiency may miss out on identifying patients with relative impairment in iron metabolism/utilization.

Iron deficiency has been studied in HF patients.^{4,22,23} Even in patients with normal Hb levels, patients with iron deficiency showed decreased functional capacity, LVEF and adverse outcomes.^{4,22,23} Non-anemic iron-deficient patients had a 2-fold greater risk for death than anemic iron-replete subjects.¹³ Furthermore, treatment with iron compounds in non-anemic patients with low iron load improved symptoms, functional capacity and quality of life but not survival.²³ The last could be related to short follow up period in this study (only 24 weeks) and low total events rate (only 2%). Gaber *et al.*, found that correction of iron deficiency improves functional class and walking distance in non-anemic iron deficient patients with systolic HF.²⁴ They also found significant improvement of diastolic and systolic function using echocardiogram after therapy despite lack of improvement of LVEF.²⁴ Ongoing clinical trials are addressing the role of iron supplementation in both anemic and non-anemic patients with HF.

Patients with HF are more susceptible to development of iron deficiency.²⁵ This could be related to gradual depletion of iron stores (absolute iron deficiency) due to low iron intake, gastrointestinal blood loss, or iron malabsorption.²⁵ In HF, there is also an activation of pro-inflammatory cytokines that block intestinal absorption of iron and divert iron from the circulation into the reticulo-endothelial system (functional iron deficiency).^{2,26} In both scenarios, there is decreased iron availability to targeted tissues and organs.⁴ Iron level in the blood or stores once it reaches a minimum threshold, is not as critical as how much iron that is getting into functioning erythrocytes.²⁷ Routine monitoring of blood iron levels in all

HF patients is costly. By using erythrocyte indices (such as MCHC in our study) as a surrogate marker of iron deficiency (both absolute and functional),^{10,14,21,27} high risk patients who might benefit from further iron studies/replacement therapy could be identified.

Interestingly, the association between MCHC and HF outcomes, severity and oxidative and inflammatory markers, was observed in a graded pattern. Thus even patients with low normal MCHC, showed adverse outcomes and increased oxidative stress. Both HF and iron deficiency are associated with increased oxidative stress.^{28,29} Since iron plays a central role in the formation and scavenging of reactive oxygen species,²⁹ low MCHC could be either a contributing factor to increased oxidative in HF, or a result of increased oxidative state due to HF, which impairs iron utilization. Taken together, the gradual decrease in MCHC may be more reflective of HF severity and iron metabolic disarrangement, more than arbitrary cut-offs of MCHC.

Limitations include lack of traditional measure of iron profiles (iron, transferrin and ferritin) to establish clinical evidence of iron deficiency; hence we can only establish the presence of relative hypochromia and not specifically iron deficiency. We don't have complete data about cause of death or other HF endpoints like hospitalization or 6-min walking test. Our study population is also a selected group of high-risk patients who all had elective coronary angiogram for clinical purposes. However, with the relatively large sample size, these findings corroborated the existing literature in HF and anemia and iron deficiency, while providing new evidence to support the prognostic role of relative hypochromia as an independent predictor of increase mortality in HF patients with normal Hb levels.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Change in MCHC levels in non-anemic patients over time

Abbreviations MCHC = mean corpuscular hemoglobin concentration

Figure 2. Kaplan-Meier plot for 5-year Mortality Stratified by Baseline MCHC levels in non-anemic HF patients (N=785). Log rank <0.001

Abbreviations MCHC = mean corpuscular hemoglobin concentration

Figure 3. Kaplan-Meier plot for 5-year mortality by Change in MCHC levels at Follow Up in non-anemic HF patients. Log rank <0.001

Abbreviations MCHC = mean corpuscular hemoglobin concentration

Table 1
Baseline Characteristics of total population and non-anemic population based on MCHC levels

MCHC levels, g/dL	Whole cohort	All Non-anemic	Non-Anemic population			
			Quartile1 <33.6	Quartile2 33.6-34.3	Quartile3 34.3-35	Quartile4 35
Number	1579	785	196	195	197	197
Age (years)	66±11	64±11	65±11	66±11	64±11	63±10
Male sex	65%	65%	50%	57%	71%	82%
Body mass index (kg/m ²)	30±7	31 ±7	32±9	31±8	31 ±7	30±6
Systolic blood pressure (mmHg)	131±22	131 ±2	134±23	131±21	131±21	128±19
Diabetes mellitus	39%	34%	38%	35%	29%	34%
Hyperlipidemia	83%	82%	81%	77%	83%	86%
Smoker	72%	73%	70%	74%	76%	72%
Creatinine clearance (mL/min)	83±4	94±43	87±45	91±40	98±43	98±44
Coronary artery disease	76%	70%	62%	70%	72%	77%
Myocardial infarction	58%	56%	50%	54%	56%	64%
Ejection fraction (%)	40 (25 – 55)	35 (25-55)	35 (20-55)	40 (30-55)	40 (30-55)	35 (25-50)
Brain natriuretic peptide (pg/mL)	310 (114-695)	204 (91-499)	398 (125-951)	266 (107-686)	173 (78-376)	140 (73-371)
Heart failure with preserved EF	37%	36%	35%	42%	38%	28%
Angiotensin converting enzyme inhibitors/angiotensin receptor blockers	68%	67%	66%	70%	61%	70%
Beta blockers	67%	66%	64%	63%	68%	68%
Aspirin	66%	66%	59%	66%	61%	76%
Hemoglobin (g/dL)	12.6±1.6	13.8±1.1	13.7±1.1	13.6±1.1	13.8±1	14.1±1.1
MCHC (g/dL)	33.9±1.2	34.2±1.1	32.8±0.8	34±0.2	34.6±0.2	35.5±0.4
MCV (fl)	88.8±5.7	89±5	89.3±5.8	89.5±5.2	89±4.4	88.3±4.3

Abbreviations: MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume.

* p value for the difference between MCHC quartiles in non-anemic population

Table 2
Association between MCHC levels and 5-Year All-Cause Mortality in non-anemic HF patients

MCHC levels (g/dL)	Quartile 1 <33.6	Quartile 2 33.6- 34.3	Quartile 3 34.3-35	Quartile 4 35
5-year Death	58/196=29.6%	50/195=25.6%	37/197=18.8%	32/197=16.2%
Unadjusted Hazard ratio	2.1 (1.4-3.3) **	1.7 (1.1-2.7) *	1.2 (0.8-2.0)	1
Adjusted Hazard ratio (Model 1)	1.9 (1.2-3.1) *	1.7 (1.0-2.7) *	1.3 (0.8-2.1)	1
Adjusted Hazard ratio (Model 2)	1.9 (1.1-3.0) *	1.7 (1.0-2.7) *	1.3 (0.8-2.1)	1
Adjusted Hazard ratio (Model 3)	1.7 (1.0-2.7) *	1.5 (0.9-2.4)	1.2 (0.7-1.9)	1

Model 1: Age, sex, diabetes, systolic blood pressure, smoking, hyperlipidemia, coronary artery disease, creatinine clearance, angiotensin converting enzyme inhibitor/angiotensin receptor blocker, beta blockers, aspirin use, body mass index and LVEF; Model 2: Model 1 + baseline hemoglobin and MCV; Model 3: Model 2 + DASI

p<0.01

*
p<0.05

Table 3
Distribution of Cardiac Severity, inflammatory and oxidative markers across MCHC Quartiles in Non-Anemic HF Patients*

MCHC levels	All	Quartile 1	Quartile 2	Quartile 3	Quartile 4	P value
LVEF (%)	35 (25-55)	35 (20-55)	40 (30-55)	40 (30-55)	35 (25-50)	0.124
BNP (pg/ml)	204 (91-499)	398 (125-951)	266 (107-686)	173 (78-376)	140 (73-371)	<0.001
DASI Score	30 (18-43)	24 (13-38)	26 (17-43)	35 (19-50)	38 (23-50)	<0.001
hsCRP (mg/L)	3 (1-6)	4 (3-8)	3 (1-7)	3 (1.3-5.8)	2 (1-5)	<0.001
Ceruloplasmin (mg/dL)	25 (21-29)	26.5 (23.6-30.9)	25.8 (21.8-30.7)	24.5 (20.5-28.3)	23 (20-26)	<0.001
Arylesterase activity (μmoles/min/mL)	99 (81-117)	89 (75-113)	99 (86-114)	103 (83-120)	100 (81-117)	0.118

LVEF: Left ventricular ejection fraction; BNP: B-type natriuretic peptide; DASI: Duke Activity Status Index score, hsCRP: high sensitive C reactive protein.