γ-Butyrobetaine Is A Proatherogenic Intermediate in Gut Microbial Metabolism of L-Carnitine to TMAO

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Usefulness of Relative Hypochromia in Risk Stratification for Nonanemic Patients With Chronic Heart Failure

Muhammad Hammadah, MD, Marie-Luise Brennan, MD, PhD, Yuping Wu, PhD, Stanley L. Hazen, MD, PhD, and W.H. Wilson Tang, MD

Anemia has been reported to affect up to 20% to 30% of patients with heart failure (HF) and has consistently been associated with increased morbidity and mortality.\textsuperscript{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),\textsuperscript{3} intrinsic renal disease leading to insufficient erythropoietin production,\textsuperscript{7} hemodilution from volume expansion,\textsuperscript{6} increased levels of circulating inflammatory cytokines (as in anemia of chronic inflammation),\textsuperscript{7, 8} and medication use or hematocrit abnormalities.\textsuperscript{4} The presence of anemia has become increasingly recognized as an important factor in the development and progression of HF.\textsuperscript{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.\textsuperscript{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.\textsuperscript{11, 12} 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.\textsuperscript{13} Here, we investigate the effect of hypochromia on a larger and broader patient population of stable patients with heart failure 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 and 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 who underwent elective diagnostic cardiac catheterization procedure. All participants gave written informed
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 indexes (Hb, MCV, and MCHC), suggesting this subgroup is a good representative of the total study population (Supplemental Table 1). In this longitudinal cohort, mean baseline Hb and MCHC were 12.6 ± 1.6 and 33.9 ± 1.2 g/dl, respectively. Mean follow-up Hb and MCHC were 12.8 ± 2.0 and 33.0 ± 1.3 g/dl, respectively. About half of the patients were nonanemic (n = 271, 49.7%) at baseline, and most were nonanemic on follow-up (n = 311, 56.3%). In the nonanemic group at baseline, and most were nonanemic on follow-up
patients with low MCHC did not have increased risk of developing anemia (less than vs more than quartile 1, odds ratio 1.8, 95% CI 0.96 to 3.2, p = 0.067).

Effect of MCHC change over time 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,” less than quartile 1) were compared with those with high MCHC (“normochromia,” quartile 1 or more). Most of the patients with low MCHC on baseline have also low MCHC on follow-up “persistent hypochromia,” whereas about half of the patients with high MCHC at baseline continued to have high MCHC levels “no hypochromia” (Figure 1). In comparison with patients with no hypochromia, patients with persistent hypochromia had significant increased mortality risk (log rank <0.001, Figure 3).

We also examined the relation between MCHC levels in nonanemic patients with heart failure and markers of inflammation and systemic oxidative stress (Table 3). Of the 790 patients with available biomarkers levels, 398 patients were nonanemic. In nonanemic patients with HF with low MCHC levels, we observed graded and significantly high-level hsCRP and ceruloplasmin, consistent with heightened systemic inflammation. Serum levels of arylesterase activity, a cardioprotective antioxidant 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 and 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 nonanemic patients with HF, a subgroup 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 antioxidative 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.

Because 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 which has a high specificity (up to 96%) in detecting iron deficiency.7,21 Iron is involved in multiple physiological functions including oxygen transport (Hb), oxygen storage (myoglobin), oxidative metabolism (mitochondrial oxidative enzymes),7,22 and antioxidant activity (ferritin, myoglobin) and also is involved in the synthesis and degradation of lipids, carbohydrates, and nucleotides.7,22 Traditionally, iron deficiency is only considered important in the 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 patients with heart failure.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 Nonanemic iron-deficient patients had a twofold greater risk for death than anemic iron-replete subjects.13 Furthermore, treatment with iron compounds in nonanemic 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 event rates (only 2%). Gaber et al24 found that correction of iron deficiency improves functional class and walking distance in nonanemic iron-deficient patients with systolic HF. They also found significant improvement of diastolic and systolic functions 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 nonanemic 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) because of low iron intake, gastrointestinal blood loss, or iron malabsorption.25

Table 2
Association between MCHC levels and 5 year all cause mortality in non anemic HF patients

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

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

<table>
<thead>
<tr>
<th>MCHC levels</th>
<th>All</th>
<th>Quartile 1</th>
<th>Quartile 2</th>
<th>Quartile 3</th>
<th>Quartile 4</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>LVEF (%)</td>
<td>35 (25 55)</td>
<td>35 (20 55)</td>
<td>40 (30 55)</td>
<td>40 (30 55)</td>
<td>35 (25 50)</td>
<td>0.124</td>
</tr>
<tr>
<td>BNP (pg/ml)</td>
<td>204 (91 499)</td>
<td>398 (125 951)</td>
<td>266 (107 686)</td>
<td>173 (78 376)</td>
<td>140 (73 371)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>DASI Score</td>
<td>30 (18 43)</td>
<td>24 (13 38)</td>
<td>26 (17 43)</td>
<td>35 (19 50)</td>
<td>38 (23 50)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>hsCRP (mg/L)</td>
<td>3 (1 6)</td>
<td>4 (3 8)</td>
<td>3 (1 7)</td>
<td>3 (1.3 5.8)</td>
<td>2 (1.5)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Ceruloplasmin (mg/dL)</td>
<td>25 (21 29)</td>
<td>26.5 (23.6 30.9)</td>
<td>25.8 (21.8 30.7)</td>
<td>24.5 (20.5 28.3)</td>
<td>23 (20 26)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Arylesterase activity (mmoles/min/mL)</td>
<td>99 (81 117)</td>
<td>89 (75 113)</td>
<td>99 (86 114)</td>
<td>103 (83 120)</td>
<td>100 (81 117)</td>
<td>0.118</td>
</tr>
</tbody>
</table>

BNP B type natriuretic peptide; DASI Duke Activity Status Index score; hsCRP high sensitive C reactive protein; LVEF Left ventricular ejection fraction.
In HF, there is also an activation of proinflammatory cytokines that block intestinal absorption of iron and divert iron from the circulation into the reticuloendothelial system (functional iron deficiency). Routine monitoring of blood iron levels in all patients with heart failure is costly. Using erythrocyte indexes (such as MCHC in our study) as a surrogate marker of iron deficiency (both absolute and functional), high-risk patients who might benefit from further iron studies/replacement therapy could be identified.

Interestingly, the association among 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. Because iron plays a central role in the formation and scavenging of reactive oxygen species, low MCHC could be either a contributing factor to increased oxidative in HF or a result of increased oxidative state because of 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 cutoffs 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 do not have complete data about cause of death or other HF end points like hospitalization or 6-minute 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, whereas providing new evidence to support the prognostic role of relative hypochromia as an independent predictor of increased mortality in patients with heart failure with normal Hb levels.

Disclosures

Dr. Hazen and Dr. Brennan are named as co-inventors on active and pending patents held by the Cleveland Clinic relating to cardiovascular diagnostics. Dr. Hazen reports having been paid as a consultant or speaker for the following companies: Cleveland Heart Lab (Cleveland, OH), Esperion (Ann Arbor, MI), Lilly (Indianapolis, IN), Merck & Co., Inc. (Kenilworth, NJ), and Pfizer (Cambridge, MA), Inc. Dr. Hazen reports receiving research funds from Abbott (Chicago, IL), Cleveland Heart Lab, and Pfizer Inc. Dr. Hazen reports having the right to receive royalty payments for inventions or discoveries related to cardiovascular diagnostics or therapeutics from the following companies: Abbott Laboratories, Cleveland Heart Lab., Esperion, Frantz Biomarkers, and Siemens (Malvern, PA). Dr. Brennan is currently an employee of Epinomics. Dr. Hazen is also partially supported by a gift from the Leonard Krieger endowment. Drs. Tang, Hammadah, and Wu have no relations to disclose.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ajmcardio.2016.01.023.


