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# Prognostic Value of Elevated Serum Ceruloplasmin Levels in Patients With Heart Failure

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## ABSTRACT

**Background:** Ceruloplasmin (Cp) is a copper binding acute phase protein that is increased in inflammatory states and deficient in Wilson's disease. Recent studies demonstrate that increased levels of Cp are associated with increased risk of developing heart failure. Our objective was to test the hypothesis that serum Cp provides incremental and independent prediction of survival in stable patients with heart failure. **Methods and Results:** We measured serum Cp levels in 890 patients with stable heart failure undergoing elective cardiac evaluation that included coronary angiography. We examined the role of Cp levels in predicting survival over 5 years of follow up. Mean Cp level was  $266 \pm 6.9$  mg/dL and demonstrated relatively weak correlation with B type natriuretic peptide (BNP;  $r = 0.187$ ;  $P < .001$ ). Increased Cp levels were associated with increased 5 year all cause mortality (quartile [Q] 4 vs Q1 hazard ratio [HR] 1.9, 95% confidence interval [CI] 1.4-2.8;  $P < .001$ ). When controlled for coronary disease traditional risk factors, creatinine clearance, dialysis, body mass index, medications, history of myocardial infarction, BNP, left ventricular ejection fraction (LVEF), heart rate, QRS duration, left bundle branch blockage, and implantable cardioverter defibrillator placement, higher Cp remained an independent predictor of increased mortality (Q4 vs Q1 HR 1.7, 95% CI 1.1-2.6;  $P < .05$ ). Model quality was improved with addition of Cp to the aforementioned covariables (net reclassification improvement of 9.3%;  $P < .001$ ). **Conclusions:** Ceruloplasmin is an independent predictor of all cause mortality in patients with heart failure. Measurement of Cp may help to identify patients at heightened mortality risk. (*J Cardiac Fail* 2014;20:946-952)

Ceruloplasmin (Cp) is a 151-kDa glycoprotein predominantly synthesized in the liver.<sup>2</sup> Ceruloplasmin transports > 95% of the body's copper, an essential cofactor for cytochromes and enzymes. Over the past decades, Cp became increasingly recognized as a multifunctional metalloprotein as well as for maintaining copper homeostasis. Furthermore, Cp can directly mobilize iron into the serum and provides the major molecular link between copper and iron metabolism.<sup>3</sup> However, recent studies have demonstrated

that Cp may act as an oxidase to nitric oxide,<sup>4</sup> and may adversely affect endothelial function.<sup>5</sup> Emerging literature also supports an important role of Cp in the development of neurodegenerative diseases<sup>6</sup> and in atrial fibrillation,<sup>7</sup> suggesting a potential pathogenic role of Cp.

The potential mechanistic link between Cp and cardiovascular disease has been debated,<sup>8</sup> although the focus has been primarily on its contribution to the development of atherosclerosis and lipid oxidation rather than its

protective capacity.<sup>2,9</sup> High levels of Cp were observed to be an independent risk factor for coronary artery disease (CAD),<sup>10</sup> and our group has further demonstrated that elevated Cp is an independent predictor of major adverse cardiovascular events.<sup>8</sup> Several recent reports have indicated that Cp levels are elevated in patients with heart failure (HF), in both acute and chronic states and regardless of etiology.<sup>11,12</sup> Furthermore, serum Cp levels appeared to be inversely correlated with left ventricular (LV) ejection fraction (LVEF) but directly correlated with symptom severity, especially in the nonischemic group of patients.<sup>11</sup> The objective of the present study was to examine the prognostic value of Cp in a large well characterized cohort of patients with a history of HF, particularly in its clinical utility in the context of standard cardiorenal biomarkers such as B-type natriuretic peptide (BNP).

## Methods

### Study Population

The Cleveland Clinic Genebank study is a large, prospective cohort study from 2001 to 2006 comprising stable 9,880 subjects undergoing elective diagnostic cardiac catheterization procedure. Each of the research subjects,  $\geq 18$  years old, gave written informed consent that was approved by the Cleveland Clinic Institutional Review Board. 1,909 patients had a history of HF detected by 3 methods: a) directly asking patient by research personnel regarding past medical problems, including self reporting history of HF; b) reviewing medical records for confirmation, especially outpatient visits to cardiology department (all patients were seen by cardiologist at Cleveland Clinic before the left heart catheterization); and c) International Classification of Diseases codes and adjudication by research personnel. This analysis included 890 subjects with a history of HF without evidence of recent acute coronary syndrome (cardiac troponin I  $< 0.03$  ng/mL) and with plasma samples available for Cp measurements. An estimate of creatinine clearance was calculated with the use of the Cockcroft Gault equation. The presence of clinically relevant CAD was confirmed by luminal stenosis of  $\geq 50\%$  in any major coronary arteries. Adjudicated 5 year all cause survival after enrollment was ascertained for all subjects by prospective patient contact as well as chart review and Social Security Death Index data ascertainment up to 2011.

### Sample Storing and Ceruloplasmin Assay

About 70 mL fasting arterial blood was drawn for each patient on the day of enrollment. Blood was spun and serum samples placed in small 5 mL tubes. About 7–10 small tubes were stored at  $80^{\circ}\text{C}$  for each patient. Quantitative determination of Cp mass was performed with the use of an immunoturbidimetric assay (Architect ci8200; Abbott Laboratories, Abbott Park, Illinois) as previously described.<sup>8</sup> All samples had either not previously thawed or thawed only once before the analysis, which is within the specifications of this FDA approved assay as intended. This assay provides highly sensitive measurement of Cp levels with an intra assay coefficient of variation of 3.7%, interassay precision of up to 4%, and a reference range of 20–60 mg/dL. Serum BNP levels were measured with the use of an immunoassay on the same platform.

## Statistical Analyses and Patient Grouping

The Student *t* test or Wilcoxon rank sum test for continuous variables and chi square test for categorical variables were used to examine the difference between assigned groups. We also studied Cp in combination with BNP. In this combination model, receiver operator characteristic (ROC) curve analyses and 5 fold cross validation were used to determine the optimal Cp cutoffs. For a given cutoff, we used a Cox model to estimate the risk of 5 year mortality. The 5 fold cross validation divides the data into 5 approximately equal size portions. A Cox model is trained on 4 parts of the data and then estimates the risk of 5 year mortality in the 5th part. This is repeated for each of the 5 parts. We calculated the area under the ROC curve (AUC) with the estimated risk. The optimal cutoff is chosen to maximize AUC values. Patients were grouped based on BNP levels into those with low BNP ( $< 100$  pg/mL), borderline “gray zone” BNP (100–400 pg/mL), and high BNP ( $> 400$  pg/mL), which had previously been reported to be associated with increased risk of mortality in HF patients.<sup>13</sup> Kaplan Meier analysis and Cox proportional hazards regression were used for time to event analysis to determine hazard ratios (HRs) and 95% confidence intervals (CIs) for 5 year survival. Levels of Cp were then adjusted for traditional coronary heart disease risk factors in a multivariable model, including age, sex, systolic blood pressure, body mass index (BMI), low density lipoprotein cholesterol, high density lipoprotein cholesterol, smoking, diabetes mellitus, creatinine clearance, dialysis use, and medications (angiotensin converting enzyme [ACE] inhibitors, beta blockers, statins, nitrate, and aspirin). Analyses were repeated after adjusting for history of myocardial infarction (MI), logarithm transformed BNP, LVEF, electrocardiographic data (heart rate, QRS duration, and left bundle branch block [LBBB]), and presence of implantable cardioverter defibrillator (ICD). Net reclassification index for evaluating the improvement in prediction performance gained by adding Cp to all of the aforementioned risk factors was calculated according to the Pencina method.<sup>14</sup> All statistical analyses were performed with the use of SAS version 9.2 (SAS Institute, Cary, North Carolina) and R 2.15.1 (Vienna, Austria).

## Results

### Study Population

Baseline characteristics of the study population are shown in Table 1. The mean and median serum Cp levels were 26.6 mg/dL and 25.6 mg/dL, respectively (interquartile range [IQR] 21.5–30.2 mg/dL). Patients with elevated Cp levels were more likely to be female and with a history of diabetes mellitus, but they were also less likely to have history of coronary artery disease or history of MI.

### Correlation With Cardiac and Inflammatory Indexes

There was no statistically significant correlation between Cp levels and LVEF ( $r = 0.05$ ;  $P = .174$ ). Also, there was no statistically significant association between Cp and extent of underlying CAD (number of vessels affected). However, there was a weak correlation between Cp and plasma BNP levels ( $r = 0.187$ ;  $P < .001$ ).

**Table 1.** Baseline Characteristics

| Demographics Characteristics                                                   | Total (n = 890) | Q1 (n = 225)      | Q2 (n = 222)      | Q3 (n = 221)     | Q4 (n = 222)    | P Value |
|--------------------------------------------------------------------------------|-----------------|-------------------|-------------------|------------------|-----------------|---------|
| Range (mg/dL)                                                                  | 12–54.3         | <21.5             | 21.5–25.6         | 25.6–30.1        | >30.1           |         |
| Age, y, mean (SD)                                                              | 66.6 (10)       | 65.7 (10)         | 67.1 (10)         | 67.5 (10)        | 66 (10)         | .232    |
| Male, n (%)                                                                    | 543 (61)        | 194 (86)          | 148 (67)          | 116 (52)         | 85 (38)         | <.001   |
| BMI, kg/m <sup>2</sup> , mean (SD)                                             | 30 (7)          | 29 (6)            | 29 (6)            | 30 (7)           | 31 (8)          | .107    |
| Diabetes mellitus, n (%)                                                       | 269 (30)        | 40 (18)           | 61 (28)           | 74 (33)          | 94 (43)         | <.001   |
| Hypertension, n (%)                                                            | 670 (76)        | 175 (79)          | 167 (76)          | 161 (74)         | 167 (77)        | .572    |
| Hyperlipidemia, n (%)                                                          | 700 (81)        | 194 (87)          | 175 (80)          | 165 (78)         | 166 (77)        | .041    |
| CAD, n (%)                                                                     | 684 (77)        | 190 (85)          | 165 (74)          | 167 (76)         | 162 (75)        | .022    |
| MI, n (%)                                                                      | 493 (59)        | 146 (69)          | 117 (56)          | 115 (56)         | 115 (55)        | .008    |
| CABG, n (%)                                                                    | 384 (43)        | 122 (54)          | 91 (41)           | 90 (41)          | 81 (36)         | .001    |
| Cigarette smoking, n (%)                                                       | 620 (70)        | 163 (72)          | 157 (71)          | 155 (70)         | 145 (65)        | .381    |
| Baseline medications, n (%)                                                    |                 |                   |                   |                  |                 |         |
| ACE inhibitor                                                                  | 601 (68)        | 167 (74)          | 154 (69)          | 144 (65)         | 136 (61)        | .024    |
| Beta blocker                                                                   | 592 (67)        | 168 (75)          | 137 (62)          | 151 (68)         | 136 (61)        | .007    |
| Statin                                                                         | 534 (60)        | 152 (68)          | 140 (63)          | 123 (56)         | 119 (54)        | .008    |
| Aspirin                                                                        | 566 (64)        | 154 (68)          | 142 (64)          | 135 (61)         | 135 (61)        | .303    |
| Nitrate                                                                        | 347 (39)        | 97 (43)           | 83 (37)           | 90 (41)          | 77 (35)         | .278    |
| BNP, pg/mL, median (IQR)                                                       | 300 (114–678)   | 233 (87–497)      | 255 (102–563)     | 371 (162–880)    | 381 (151–930)   | <.001   |
| LVEF, %, median (IQR)                                                          | 35 (25–50)      | 40 (30–50)        | 35 (25–50)        | 35 (25–55)       | 38 (25–50)      | .904    |
| Creatinine clearance, mL min <sup>-1</sup> 1.73 m <sup>-2</sup> , median (IQR) | 83 (60–110)     | 88.1 (69.4–117.2) | 81.7 (57.2–113.1) | 78.4 (55.4–98.5) | 80 (54.7–111.2) | <.001   |
| Dialysis use, n (%)                                                            | 19 (2)          | 8 (4)             | 6 (3)             | 3 (1)            | 2 (1)           | .013    |
| Electrocardiography data                                                       |                 |                   |                   |                  |                 |         |
| Heart rate, beats/min, median (IQR)                                            | 71 (62–83)      | 69 (57–81)        | 72 (63–82)        | 71 (63–81)       | 75 (66–86)      | <.001   |
| QRS duration, ms, median (IQR)                                                 | 108 (94–138)    | 108 (96–135)      | 108 (94–138)      | 108 (94–142)     | 106 (92–142)    | .908    |
| LBBB, n (%)                                                                    | 101 (12)        | 23 (10)           | 29 (13)           | 25 (12)          | 24 (11)         | .794    |

Q, quartile; BMI, body mass index; CAD, coronary artery disease; MI, myocardial infarction; CABG, coronary artery bypass graft surgery; ACE, angiotensin converting enzyme; BNP, B type natriuretic peptide; IQR, interquartile range; LBBB, left bundle branch block.

### Association of Serum Ceruloplasmin Levels With Survival

A total of 261 patients (29%) died by the 5-year follow-up. The average time to event was 1,535 days. Table 2 illustrates the Cox proportional Hazard analysis of increased levels of Cp with 5-year all-cause mortality outcomes. Compared with patients with the lowest Cp levels (quartile 1), patients with increased Cp levels had higher risk of 5-year all-cause mortality (quartile 4 vs quartile 1 unadjusted HR 1.94,

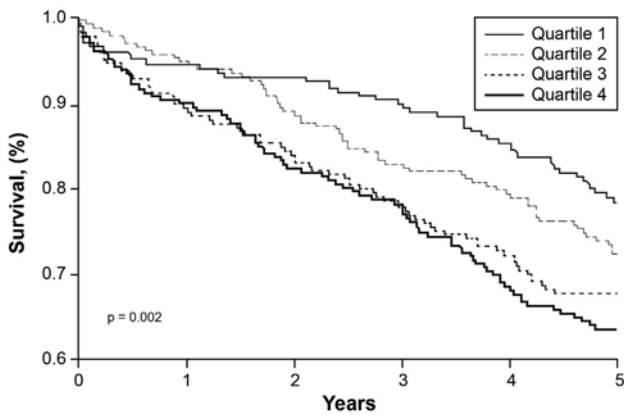
95% CI 1.36–2.77;  $P < .001$ ; Table 2; Fig. 1). After adjusting for coronary heart disease traditional risk factors, medications, creatinine clearance, dialysis use, BMI, history of MI, BNP, and LVEF, higher Cp remained a significant predictor of increased 5-year mortality (adjusted HR 1.71, 95% CI 1.15–2.55;  $P < .01$ ). Analysis was also repeated after adjustment for heart rate, QRS duration, LBBB, and ICD placement. In this model, Cp remained an independent predictor of worse 5-year outcome (Table 2). We also

**Table 2.** Hazard Ratio for 5 Year Mortality by Ceruloplasmin (Cp) Quartiles (Qs)

|                      | Serum Ceruloplasmin Level (Range) |                  |                    |                     |
|----------------------|-----------------------------------|------------------|--------------------|---------------------|
|                      | Q1                                | Q2               | Q3                 | Q4                  |
| Range (mg/dL)        | <21.5                             | 21.5–25.6        | 25.6–30.2          | ≥30.2               |
| Unadjusted HR        | 1                                 | 1.34 (0.92–1.95) | 1.71 (1.18–2.46)** | 1.94 (1.36–2.77)*** |
| Adjusted HR, model 1 | 1                                 | 1.19 (0.81–1.74) | 1.64 (1.12–2.41)*  | 1.93 (1.31–2.85)*** |
| Adjusted HR, model 2 | 1                                 | 1.22 (0.83–1.78) | 1.62 (1.11–2.37)*  | 1.9 (1.29–2.81)***  |
| Adjusted HR, model 3 | 1                                 | 1.3 (0.87–1.94)  | 1.55 (1.04–2.3)*   | 1.71 (1.15–2.55)**  |
| Adjusted HR, model 4 | 1                                 | 1.36 (0.91–2.05) | 1.57 (1.04–2.37)*  | 1.68 (1.11–2.53)*   |
| Adjusted HR, model 5 | 1                                 | 1.34 (0.88–2.03) | 1.45 (0.95–2.22)   | 1.68 (1.1–2.55)*    |
| Event rate           | 47/221                            | 61/223           | 72/223             | 81/223              |

Model 1: Adjusted for coronary heart disease traditional risk factors: age, sex, systolic blood pressure, diabetes, high density lipoprotein, low density lipoprotein, body mass index, smoking, creatinine clearance and dialysis use; model 2: model 1 plus medications (angiotensin converting enzyme inhibitors, beta blockers, statins, nitrate, and aspirin); model 3: model 2 plus myocardial infarction, log transformed B type natriuretic peptide and left ventricular ejection fraction; model 4: model 3 plus ventricular rate, QRS duration, and left bundle branch block; model 5: model 4 plus implantable cardioverter defibrillator placement.

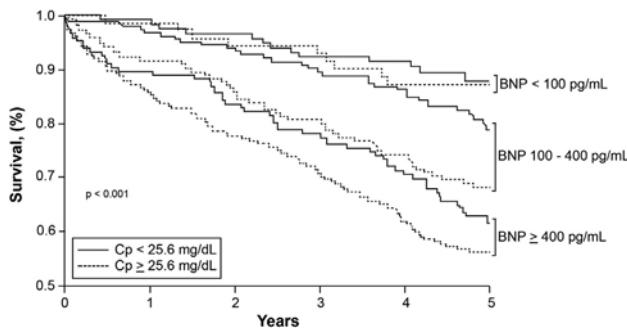
\* $P < .05$ ; \*\* $P < .01$ ; \*\*\* $P < .001$ .



**Fig. 1.** Kaplan Meier analysis of 5 year survival stratified according to ceruloplasmin quartiles.

stratified the analysis by history of MI ( $n = 493$ ). In both MI and non-MI patients, Cp was associated with increased 5-year mortality: Q4 vs Q1 HR 1.95 (95% CI 1.03–3.7;  $P = .041$ ) for the non-MI group and HR 1.98 (95% CI 1.3–3.1;  $P = .003$ ) for the MI group.

We further explored the prognostic value of Cp within specific BNP ranges. As illustrated in Figure 2, higher Cp levels were associated with poorer outcomes compared with lower Cp levels within each clinically defined BNP range. The optimal cutoff was 21.5 mg/dL based on ROC curve analysis. Higher Cp levels showing increased risk of 5-year mortality within the borderline BNP range (unadjusted HR 3.2, 95% CI 1.40–7.5;  $P = .006$ ) as well as in the high BNP range (unadjusted HR 5.8, 95% CI 2.6–13.1;  $P < .001$ ). After adjusting for the aforementioned risk factors, higher Cp still showed increased mortality risk when within the borderline BNP range (adjusted HR 3, 95% CI 1.1–8.5;  $P = .036$ ) or the high BNP range (adjusted HR 4.3, 95% CI 1.5–11.9;  $P = .006$ ; Fig. 2).



**Fig. 2.** Kaplan Meier analysis of 5 year survival in patients with heart failure based on ceruloplasmin (Cp) and B type natriuretic peptide (BNP) levels. Using Cp measurements in patients with different BNP levels (low,  $< 100$  pg/mL; borderline, 100–400 pg/mL; or high,  $> 400$  pg/mL) can help in identifying heightened risk HF patients.

## Association Between Ceruloplasmin Levels and Liver Congestion

Most of our patients had normal levels of liver enzymes: median (IQR) alanine transaminase (ALT) of 20 (16–26) U/mL and aspartate transaminase (AST) of 18 (16–26) U/mL. Slight positive correlation was found between Cp and ALT ( $R^2 = 0.004$ ;  $P = .048$ ). However, no significant correlation was found between Cp and AST ( $R^2 = 0.003$ ;  $P = .188$ ). Defining hepatic congestion as having  $2\times$  upper normal range of liver enzymes (AST reference range of 7–40 U/L; ALT reference range 0–45 U/L), only 8 patients had hepatic congestion. Levels of Cp were not significantly different between groups (medians of 25.9 mg/dL for hepatic congestion group vs 25.6 mg/dL for noncongestion group;  $P = .99$ ). Cp is a synthetic function of the liver that would be expected to be low in the setting of liver congestion, suggesting that our observation is independent from liver involvement with heart failure.

## Discrimination Testing

Both net reclassification improvement (NRI) and integrated discrimination improvement were used to quantify improvement in model performance.  $P$  values compare models with and without Cp. Both models were adjusted for traditional coronary heart disease risk factors, including age, sex, systolic blood pressure, diabetes, high-density lipoprotein, low-density lipoprotein, BMI, smoking, creatinine clearance, dialysis use, MI, log-transformed BNP, LVEF, heart rate, QRS duration, LBBB, and ICD placement. Cutoff values for NRI estimation used a ratio of 6:2:2 for low, medium, and high risk categories. The risk of mortality was estimated with the use of the Cox model. The net reclassification improvement of Cp for 5-year mortality was 9.33% ( $P < .001$ ). The relative integrated discrimination improvement for this model was 18% ( $P < .001$ ). This is responsible for correctly reclassifying 6.52% and 2.81% of events and nonevents, respectively (Table 3). The c-statistic of the model also improved from 0.687 to 0.701 with the addition of Cp but did not reach statistical significance ( $P = .18$ ).

**Table 3.** Reclassification With the Use of Ceruloplasmin (Cp) Levels

|                                       | Whole Cohort<br>( $n = 890$ ) |
|---------------------------------------|-------------------------------|
| Events correctly reclassified         | 6.52%                         |
| Nonevents correctly reclassified      | 2.81%                         |
| Net reclassification index            | 9.33%                         |
| Integrated discrimination improvement | 18%                           |

Baseline model included age, sex, systolic blood pressure, diabetes, high density lipoprotein, low density lipoprotein, body mass index, smoking, creatinine clearance, dialysis use, myocardial infarction, log transformed B type natriuretic peptide, left ventricular ejection fraction, resting heart rate, QRS duration, left bundle branch block, and implantable cardioverter defibrillator placement.

## Discussion

Considering that it is one of the most abundant circulating glycoproteins with a widely available biochemical measurement of its concentration, clinicians have associated low (rather than high) levels of Cp with Wilson disease a rare recessive autosomal hepatolenticular degeneration disease leading to pathologic deposition of copper in the liver, nervous system, and kidneys. At the other end of the spectrum, Cp is often considered as an acute-phase reactant that is elevated in the settings of acute inflammatory conditions and pregnancy<sup>1</sup> and has been widely measured in clinical practice. Our group and others have previously observed that Cp has been reported to be an independent risk factor for CAD,<sup>8,10,15-17</sup> and higher levels of Cp are associated with adverse outcomes.<sup>8</sup> However, despite early reports of elevated Cp levels and its catalytic activities in the setting of acute MI in the 1950s,<sup>18</sup> there have been limited investigations regarding the potential contribution of this relatively abundant circulating glycoprotein in the development and progression of myocardial dysfunction in humans. There are several key findings from our study. For the first time, particularly in a contemporary patient cohort, we observed that Cp provides prognostic value of 5-year mortality in patients with HF that was independent from traditional coronary heart disease risk factors, and the availability of Cp levels can reclassify risk for 5-year mortality by 9.33%. Second, the lack of strong relationship between Cp and measures of HF severity such as LVEF and BNP implies that Cp may provide distinct information of underlying pathophysiology. Taken together, the measurement of Cp (in combination with BNP) may help to identify patients at heightened long-term mortality risk. These findings, together with earlier reports suggesting the association between elevated Cp and increased likelihood of developing HF,<sup>19</sup> imply that the mechanistic underpinnings of the pathophysiologic role of Cp in myocardial dysfunction warrant further investigations.

The connection between Cp expression and development of HF in humans is based primarily on epidemiologic data. Data from the Atherosclerosis Risk in Communities study also confirmed the ability of Cp to predict future development of HF.<sup>19</sup> Only a few studies regarding association between Cp and HF have been done. Another study also found increased incidence of HF in patients with high Cp levels over 22 years of follow-up.<sup>20</sup> Meanwhile, in patients who were admitted with ST-segment elevated MI, increased Cp levels were shown to be associated with increased incidence of acute HF and decreased LVEF.<sup>21</sup>

Mechanism of cardiac involvement in Wilson disease is still not very well understood; however, copper deposition is the likely mechanism causing myocardial inflammation and interstitial fibrosis. Interestingly, defect in Wilson disease is related to impairment in hepatic copper transport protein, which impairs copper binding to apoceruloplasmin

to form Cp, resulting in low levels of serum Cp.<sup>22</sup> Cp levels do not seem to be related to amount of copper deposition in the liver or other tissues, given the fact that many Wilson patients who responded to medical treatment had persistently low levels of Cp levels. Furthermore, patients with aceruloplasminemia tend to have higher iron deposition in tissue rather than copper.<sup>23,24</sup>

The underlying mechanisms for increased Cp expression in HF are not well understood, but the lack of tight association with standard cardiac indexes may suggest an underlying metabolic defect at play. Over the past decades, Cp has been shown to have multiple roles in copper transportation, coagulation, angiogenesis, defense against oxidant stress, and iron homeostasis.<sup>2,25</sup> In addition to transporting copper, Cp exhibits a copper-dependent ferroxidase activity, which is associated with possible oxidation of Fe<sup>2+</sup> (ferrous iron) into Fe<sup>3+</sup> (ferric iron), playing a possible fundamental mechanism of protection from iron-mediated free radical injury,<sup>4,26</sup> inhibiting lipid oxidation,<sup>27,28</sup> and blocking protein and DNA damage.<sup>2,25,29-32</sup> Despite these promising effects, most of these protective enzymatic activities have been demonstrated only in vitro, warranting further demonstration in humans particularly in disease states such as HF. It is important to point out that although measurement is widely available and inexpensive, clinically available assays, including the one used in the present study, represents only the concentration of Cp in the circulation rather than its activity. Interestingly, diminished Cp ferroxidase activities were observed in substantia nigra tissues of Parkinson disease patients versus control subjects,<sup>33</sup> as well as in blood samples of preeclampsia patients versus control subjects,<sup>34</sup> despite similar or increased Cp concentrations. This was also seen in a small HF group (n = 96), where patients with low Cp ferroxidase activities were found to have worse disease severity and outcomes.<sup>26,35</sup> Thus, there remains an intriguing possibility that the quality rather than the quantity of Cp may exert a stronger influence in the function of Cp, and the imbalance of pro- and antioxidant Cp enzymatic activities may be linked to heightened rather than diminished downstream nitrate stress.

In more advanced stages, HF promotes impaired tissue perfusion. It has been well established that the Cp gene exhibits multiple hypoxia-responsive elements, and up-regulation of Cp gene transcription was noticed during hypoxia.<sup>36,37</sup> Activation of Cp expression is mechanistically linked to hypoxia-inducible factor, whereby hypoxia-response element dependent gene regulation leads to transcriptional induction of the Cp gene promoter.<sup>37,38</sup> Recently, Cp has also been shown to have nitric oxide (NO) oxidase activity in vivo, converting NO, a potent short-lived vasodilator and antioxidant, to the less active reservoir form, nitrite.<sup>39</sup> It is possible that increased levels of Cp can decrease available plasma NO, thus enhancing reactive oxidant species formation and oxidative cell injury.<sup>4,5,39</sup> Interestingly, nitrite can be reduced back to

NO mainly during hypoxic situations.<sup>40</sup> Cp-knockout animals have lower nitrite reservoir, and they were found to have more hepatocellular infarction after ischemia and reperfusion than wild-type animals. Nitrate supplementation seems to reduce the injury.<sup>4</sup> Therefore, increased HF mortality with increased Cp levels could be related to decreased NO availability in the plasma, or a result of relative tissue hypoxia given the close gene location to hypoxia-response element.

### Study Limitations

Despite being the largest HF cohort reported with Cp levels and long-term outcomes, our study population represents a selected group of patients undergoing elective coronary angiography (~77% of our population has underlying CAD) with a relatively high proportion of patients with ischemic HF etiology. Despite finding significant association between Cp levels and all-cause mortality, we did not have complete data about cause of death, hospitalization, or consistent echocardiographic indices (such as LV hypertrophy or diastolic indices). We also did not have direct measures of Cp function (such as ferroxidase or NO oxidase activities). Despite these limitations, our intriguing findings should prompt further investigations into how Cp contributes to the pathophysiology of heart failure.

### Conclusion

Ceruloplasmin is an independent predictor of long-term all-cause mortality in patients with HF. Use of Cp in combination with BNP may help to identify patients at heightened mortality risk.

### Disclosures

Dr Hazen is named as coinventor on pending patents held by the Cleveland Clinic relating to cardiovascular diagnostics. Dr Hazen reports having been paid as a consultant or speaker for Cleveland Heart Lab, Esperion, Lilly, Liposcience, Merck & Co, and Pfizer. Dr Hazen reports receiving research funds from Abbott, Cleveland Heart Lab, Liposcience, Pfizer, and Takeda Pharmaceuticals. Dr Hazen reports having the right to receive royalty payments for inventions or discoveries related to cardiovascular diagnostics or therapeutics from Abbott Laboratories, Cleveland Heart Lab, Esperion, Frantz Biomarkers, Liposcience, and Siemens. All other authors have no relationships to disclose.

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