CLINICAL STUDY

Association of Redox State of Human Serum Albumin with Severity in Patients with Heart Failure
A Cross-Sectional Study
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Summary
Oxidative stress plays a crucial role in the progression of heart failure (HF). We surveyed the fraction of human mercaptalbumin [f(HMA)], an indicator of the redox state of human serum albumin (HSA), in patients with HF and examined whether f(HMA) is associated with the severity of HF.

We enrolled consecutive elderly patients hospitalized for acute HF or exacerbation of HF. The redox state of HSA was measured by the high-performance liquid chromatography with postcolumn bromocresol green method using serum samples collected close to discharge. First, the distribution of f(HMA) in HF was compared to that in community-dwelling elderly individuals (n = 125; median age, 80 years) as a control group analyzed in a previous study. Overall, 133 patients (median age, 81 years; 75 men) were included. Patients with HF showed a lower level of f(HMA) than those of the control group (55.0% [IQR 47.7-61.3] versus 66.3% [IQR 62.8-70.0], P < 0.001). Multiple regression analysis showed a negative correlation between f(HMA) and log-transformed B-type natriuretic peptide (standardized beta = −0.19).

Patients with HF showed lower f(HMA) than those in the control group. Additionally, f(HMA) was related to HF independently with log-transformed B-type natriuretic peptide in the multivariate regression analysis, suggesting that f(HMA) is a biomarker that reflects the redox state in HF patients.

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Key words: Oxidative stress, Fraction of human mercaptalbumin, Case-control

The prevalence of patients with heart failure (HF) worldwide is currently approaching 30 million.1 The economic burden of HF has become a global problem.2,3 HF is not completely curable and patients with HF undergo repeated exacerbations; therefore, preventing rehospitalization is an important clinical goal.4 From the perspective of disease management, there is a general agreement that oxidative stress evaluation is meaningful for patients with HF. Oxidative stress plays a key role in the progression and severity of HF.5-7 It occurs in patients with HF because of various mechanisms such as increased neurohumoral factors (activation of the sympathetic nervous system and renin-angiotensin-aldosterone system), overexpression of proinflammatory cytokines, bacterial translocation, and hypoxia.8 Increased oxidative stress is caused by an imbalance between reactive oxygen species and antioxidant defenses.9,10 The importance of oxidative stress is growing with respect to the pathophysiological mechanism of cardiac remodeling responsible for the development and progression of HF.11 Due to difficulties in assessing the level of oxidative stress directly in clinical settings, surrogate biomarkers (e.g., malondialdehyde, myeloperoxidase, serum uric acid, reactive oxygen metabolites, plasma-oxidized low-density lipoproteins, and xanthine oxidase) have been used, and they have been reported to reflect the symptomatic status and severity of HF.8,11 However, none has reached the stage of being recommended for routine clinical use in clinical guidelines for HF management.2,3

Human serum albumin (HSA) is a significant and predominant antioxidant in plasma, and the redox state of HSA has recently been reported as a novel biomarker of systemic oxidative stress.12 Based on the redox state, HSA is divided into two main forms: human nonmercaptalbumin (HNA, oxidized form) and human mercaptalbumin (HMA, reduced form).13 Therefore, the fraction of HMA in HSA (f[HMA]) has been considered as a biomarker that reflects the redox state in the human body.14 Several studies have reported the association between the redox state of HSA and the severity or progression of chronic disease in clinical settings (e.g., kidney and hepatic diseases).5,15,16 Due to the strong association between HF and...
oxidative stress, we hypothesized that there is an association between the severity of HF and f(HMA) in patients with HF. However, few investigations have reported the level of f(HMA) in patients with HF. The goals of this study were 1) to survey the f(HMA) in patients with HF and 2) examine the validity of f(HMA) as a marker reflecting the severity of HF.

Methods

Study participants and protocol: This cross-sectional study was conducted as a secondary analysis of our ongoing cohort study undertaken in the Graduate School of Medicine of Nagoya University, Japan. The main cohort study enrolled consecutive elderly patients hospitalized for acute HF or exacerbation of HF at the Nagoya Daiichi Red Cross Hospital in Japan. In this study, we enrolled patients admitted between July 2019 and July 2020. Patients were excluded if they refused to participate, underwent cardiac surgery, or died during the hospital stay. Patients readmitted to the hospital during the study period were enrolled at the time of their first hospitalization. Informed consent was obtained from each patient or patient’s family. The demographic data, etiologies of HF, comorbidities, laboratory assessments, echocardiographic findings, and medications at discharge were collected from the medical records of each patient.

The healthy controls were not recruited in this study. To compare serum f(HMA) between healthy adults and patients with HF, the dataset of f(HMA) in our previous study, which reported f(HMA) level in 125 community-dwelling older women, was used in this study.

The study protocol was approved by the Ethics Review Committee of Nagoya University Graduate School of Medicine (approval number: 2019-0026) and the Japan Red Cross Aichi Medical Center Nagoya Daiichi Hospital (approval number: 2019-059).

Measurement of the redox state of HSA and other biomarkers: Blood samples were collected close to hospital discharge. From each patient, 500 μL was drawn from the serum obtained by centrifugation and stored at −80°C until analysis. To measure the redox state of HSA, high-performance liquid chromatography with postcolumn −80°C until analysis. To measure the redox state of HSA, from the serum obtained by centrifugation and stored at −80°C until analysis. To measure the redox state of HSA, from the serum obtained by centrifugation and stored at −80°C until analysis. To measure the redox state of HSA, from the serum obtained by centrifugation and stored at −80°C until analysis.

The plasma level of B-type natriuretic peptide (BNP) shows the basic characteristics of the study participants. Table I presents the demographic data, etiologies of HF, comorbidities, laboratory assessments, echocardiographic findings, and medications at discharge were collected from the medical records of each patient.

Each variable was tested for normality with postcolumn −80°C until analysis. To measure the redox state of HSA, from the serum obtained by centrifugation and stored at −80°C until analysis. To measure the redox state of HSA, from the serum obtained by centrifugation and stored at −80°C until analysis. To measure the redox state of HSA, from the serum obtained by centrifugation and stored at −80°C until analysis. To measure the redox state of HSA, from the serum obtained by centrifugation and stored at −80°C until analysis.

The prevalence of NYHA classes I, II, III, and IV was 13 (9.8%), 45 (33.8%), 63 (47.4%), and 12 (9.0%), respectively. The f(HMA) was estimated using the following equation: f(HMA) = HMA area / (HMA area + HNA area). Quality control samples were analyzed in each assay series, and the coefficient of variation was 4.5% for f(HMA).

The plasma level of B-type natriuretic peptide (BNP) was measured at the Medical Laboratory of Japan Red Cross Aichi Medical Center Nagoya Daiichi Hospital using a commercially available kit BNP-JP (Abbott Japan, Tokyo, Japan).

Statistical analysis: Each variable was tested for normality using the Shapiro-Wilk test. Continuous variables were presented as mean ± standard deviation in the case of a normal distribution or as median and interquartile range in the case of a non-normal distribution. In this study, participants with missing f(HMA) data were excluded from the main analysis.

First, the validity of f(HMA) was tested using the known-groups method, which compares scale scores across groups known to differ in the health construct. The levels of f(HMA) of groups differing in known health conditions were compared using the Mann-Whitney test. We hypothesized that patients with HF would present a lower level of f(HMA) (more oxidative stress) than community-dwelling older adults based on the conceptualization of disease-specific oxidative stress. For comparison, only women were selected from the enrolled patients to match the control population. The Student’s T-test, Mann-Whitney’s U-test, or chi-square test, as appropriate, was used to compare patient characteristics of the two groups. In addition, to understand the differences in f(HMA) according to sex, we compared men and women among the patients with HF.

Second, the relationship between the severity of HF and f(HMA) was examined. We compared the group means of f(HMA) between the New York Heart Association (NYHA) functional class and American College of Cardiology Foundation (ACCF)/American Heart Association (AHA) stage. Kruskal-Wallis test was used to compare the overall difference between groups of the median f (HMA), and Steel-Dwass multiple comparison test was used as a post hoc test.

Finally, simple linear regression was performed using the redox state of albumin as a dependent variable. Clinical variables (age, sex, and body mass index), pathological variables (left ventricle ejection fraction, NYHA class, and BNP), and biological variables (HSA, hemoglobin, estimated glomerular filtration rate (eGFR), total bilirubin, serum sodium, and uric acid) were used as independent variables. Forward-backward stepwise regression was performed, and a P value cutoff of 0.20 was used for variable inclusion and removal. The collinearity between variables was checked using the variance inflation factor (VIF). All data were analyzed using JMP Pro 15.1.0 (SAS Institute, Cary, NC, USA), and a P value < 0.05 was regarded as statistically significant.

Results

Study participants: A total of 320 consecutive patients were admitted due to HF at Japan Red Cross Aichi Medical Center Nagoya Daiichi Hospital during the study period. Some patients were not recruited for some reasons (Figure 1). A total of 162 patients provided informed consent (participation rate, 77.9%). Of these 162 patients, we excluded 29 who did not provide serum samples or information regarding pathological parameters. Finally, 133 patients with HF were included in the analysis. Table I shows the basic characteristics of the study participants. The median age was 81 years (interquartile range, 73-86 years), and 56.4% of the patients were men. The median left ventricle ejection fraction was 50.0% (35.5%-59.0%). The prevalence of NYHA classes I, II, III, and IV was 13 (9.8%), 45 (33.8%), 63 (47.4%), and 12 (9.0%), respec-
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Figure 1. Flowchart of inclusion and exclusion criteria for participants.

Admitted because of heart failure (N = 320)

Excluded (n = 158)
- Refused to participate (n = 26)
- No family permission (n = 18)
- Cardiac surgery (n = 14)
- Hospital death (n = 23)
- Omission (n = 72)
- Others (n = 5)

Enrolled in this study (n = 162)

Missing data for f(HMA) (n = 29)
- No serum sample (n = 19)
- Other (n = 10)

Participants analyzed in this study (n = 133)

- f(HMA) in patients with HF: The median value of f(HMA) in patients with HF was 55.0% (interquartile range, 47.0-61.3). The results from the Shapiro-Wilk test showed that f(HMA) was non-normally distributed (Figure 2). There was no statistically significant gender difference in f(HMA) among patients with HF. In a female-only comparison, the f(HMA) levels of patients with HF were significantly lower than those of the control group (median f(HMA) in HF versus control: 54.7% (interquartile range, 47.0-61.2) versus 66.3% (interquartile range, 62.8-70.0), P < 0.001) (Table II). No significant differences regarding age were observed between the two groups. BMI, serum albumin, and eGFR in HF group were lower than in the control group.

Table III shows the results of the linear regression analysis of the association between selected variables and f(HMA). Variables with clearly skewed distributions were log-transformed before being included in the linear regression analysis. Univariate analysis revealed that log-transformed BNP was significantly correlated with f(HMA) (r = −0.24, P = 0.0186) (Figure 3). Correlations were also observed with other variables, such as age, ACCF/AHA stage, NYHA functional class at discharge, log-transformed eGFR, serum sodium, and serum uric acid. Moreover, according to stepwise multivariate analysis, log-transformed BNP (standardized beta = −0.19, P = 0.0232), age (standardized beta = −0.18, P = 0.0443), log-transformed eGFR (standardized beta = 0.35, P = 0.0003), and serum sodium (standardized beta = 0.26, P = 0.0024) were independently associated with f(HMA) (Table III). VIFs for each variable in multivariate analysis were less than 5, suggesting no multicollinearity. The Clinical Scenario classification showed no significant association (median f(HMA) in CS 1 versus 2: 54.5% (interquartile range, 47.9-61.4) versus 55.7% (interquartile range, 47.0-61.9), P = 0.7854).

f(HMA) and severity of HF: Figure 4 compares f(HMA) or BNP in the two HF severity categories (NYHA functional class and ACCF/AHA stage). A trend for f(HMA) to decrease with increasing severity of the NYHA class is shown in Figure 4A. The Kruskal-Wallis test showed a significant difference in the value of f(HMA) between the groups (P = 0.012), and for a post hoc test, the Steel-Dwass multiple comparison test showed a significant difference between class I and IV (P = 0.04). Similarly, in the ACCF/AHA stage, f(HMA) decreased with increasing severity (Figure 4B). The median and interquartile range for f(HMA) was 48.6% [39.7-53.5] in stage D patients compared with 55.2% [47.9-61.3] in stage C patients. Patients in stage D showed lower levels of f(HMA) than those in stage C (P = 0.02). Figure 4C shows no differences in BNP levels between the NYHA classes at discharge. The median and interquartile range for BNP was 306.4 pg/mL [82.3-678.4] in NYHA class I patients, 288.1 pg/mL [152.2-464.8] in class II, 202 pg/mL [114.3-340.2] in class III, and 422 pg/mL [154.4-809.1] in class IV. Kruskal-Wallis test showed no significant difference in the value of f(HMA) between the groups (P = 0.485). To confirm the reproducibility, we performed an additional statistical test (trend test) using Jonckheere-Terpstra test and the results were similar, with no association between BNP and NYHA class. Similarly in the ACCF/AHA stage, there was no difference in BNP between stage C and stage D (Figure 4D). The median and interquartile range for BNP was 211.0 pg/mL [136.3-444.3] in stage C patients and 366.1 pg/mL [178.4-462.1] in stage D patients (P = 0.4853).

Discussion

To the best of our knowledge, this is the first study to investigate the redox state of HSA in a large number of patients with HF. We found that the f(HMA) was significantly lower in patients with HF than those in the healthy...
control group, implying that patients with HF are highly exposed to oxidative stress. Moreover, patients with HF with higher severity showed significantly lower f(HMA). Multivariate analyses showed that log-transformed BNP was independently associated with f(HMA) after adjusting for other confounders. These results support our hypothesis that f(HMA) is a biomarker that reflects the pathogenesis and severity of HF.

A comparison between HF and population data showed that patients with HF presented significantly lower levels of f(HMA) than those in the control group. Although the BNP level was not measured in the control group, they were a relatively healthy population. Thus, this comparison may reflect the pathophysiology of HF to some extent. A small-sample size study showed that the f(HMA) of patients with HF (n = 15) was lower than that of the control patients (n = 10). Increased oxidative stress is known to aggravate endothelial damage and cardiac dysfunction. Regarding its free radical scavenging properties, HSA exerts its antioxidant effects primarily through the presence of HMA. The decrease in HMA is a result of the oxidative stress level exceeding the background and representing the oxidative stress level and its scavenging ability. There are several established and emerging biomarkers in HF: biomarkers associated with myocyte stretch, ANP, BNP, and N-terminal fragment pro-BNP; myocardial injury biomarkers, troponin T and troponin I; oxidative stress, myeloperoxidase, midregional proadrenomedullin, oxidized low-density lipoprotein, urinary biopyrrins, and plasma malondialdehyde. f(HMA) is

| Characteristic | Heart failure (n = 133) |
|---------------|-------------------------|
| Age (years)  | 81 (72.5–86) |
| Sex (male/female) | 75 (56.4) / 58 (43.6) |
| Body mass index (kg/m²) | 19.9 (17.6–22.6) |
| Left ventricle ejection fraction (%) | 50 (35.5–59) |
| < 40 / 40 ≤ / > 50 / 50 ≤ | 42 (31.6) / 17 (12.8) / 60 (45.1) |
| Mitral regurgitation: I/II/III/IV | 45 (35.1) / 55 (43.0) / 20 (15.6) / 3 (2.3) |
| Aortic stenosis | 3 (2.4) |
| Etiology | Ischemic 37 (27.8) |
| Myopathy | 17 (12.8) |
| Arrhythmia | 37 (27.8) |
| Valvular heart disease | 24 (18.0) |
| Hypertensive heart disease | 4 (3.0) |
| Other | 19 (14.3) |
| Clinical scenario: 1/2/3/4/5 | 76 (57.1) / 44 (33.1) / 8 (6.0) / 3 (2.3) / 2 (1.5) |
| ACCF/AHA stage C/D | 111 (89.5) / 13 (10.5) |
| NYHA functional class at discharge: I/II/III/IV | 13 (9.8) / 45 (33.8) / 63 (47.4) / 12 (9.0) |
| Comorbidities | Diabetes 37 (27.8) |
| Chronic renal disease | 37 (27.8) |
| Hemodialysis | 0 (0) |
| Liver disease | 6 (4.5) |
| Risk factor | Smoking 70 (52.6) |
| Alcohol | 36 (31.5) |
| Medication at discharge | Diuretics 97 (72.9) |
| ACEi/ARB | 65 (48.9) |
| β-blockers | 73 (57.5) |
| Ambulatory at discharge | 113 (85.0) |
| Biochemical data at discharge | f(HMA) (%) 55.0 (47.0–61.3) |
| Serum albumin (g/dL) | 3.4 (3.1–3.7) |
| Hemoglobin (g/dL) | 12.0 (10.8–13.7) |
| eGFR (mL/minute/1.73 m²) | 42.3 (30.2–53.7) |
| Brain natriuretic peptide (pg/mL) | 211.0 (137.1–432.5) |
| C-reactive protein (mg/dL) | 0.43 (0.16–1.10) |
| Serum uric acid (mg/dL) | 7.2 (2.2) |
| Total bilirubin (mg/dL) | 0.7 (0.5–0.9) |
| Serum sodium (mEq/L) | 139 (136–140) |

ARB indicates angiotensin II receptor blocker; ACEi, angiotensin-converting enzyme inhibitor; eGFR, estimated glomerular filtration; f(HMA), fraction of mercaptalbumin; ACCF, American College of Cardiology Foundation; AHA, American Heart Association; and NYHA, New York Heart Association. Continuous variables are presented as the mean ± standard deviation or median (interquartile range). Categorical variables are presented as n or n (%) .
classified as an oxidative stress-related biomarker, which is characterized by representing the oxidative status of albumin as well as its oxidative stress scavenging ability and is expected to have a longer half-life than other markers in the body. Thus, further study of the association of f(HMA) in HF may lead to applications in HF prevention, diagnosis, therapeutic efficacy, and prognosis prediction with or without previous oxidative stress markers.

As confounding factors, age, eGFR, and serum sodium have previously been reported, and the results were similar to those in this study (Table III). In patients with chronic kidney disease (CKD), HF is a leading cause of morbidity and mortality, and the population of patients with CKD and concurrent HF continues to grow. Decreased f(HMA) may be associated with decreased renal function. However, in the present study, a significant association between f(HMA) and log-transformed BNP, a specific biomarker of HF, was also found in the statistical analysis adjusted for eGFR. The decrease in f(HMA) seems to be the result of the effects of oxidative stress related to HF rather than renal failure. These results are consistent with previous studies showing an association between oxidative stress and severity of HF and, thus, the importance of assessing the quality of HSA for detecting individuals at risk of poor HF prognosis.

Previously, the concentration of HSA has been reported to be a prognostic factor in patients with HF. This is thought to be related to nutritional status and inflammation. In contrast, no association was found between the concentrations of HSA and f(HMA) in the present study. Previously, we reported similar results in elderly dwelling people, and that quality, but not quantity, of HSA, was a relevant factor in exercise tolerance, a robust prognostic factor. Thus, it seems that HSA quantity and quality reflect different parameters.

BNP is widely used at clinical sites in Japan for auxiliary diagnosis to support the treatment of HF. Maisel, et al. (2002) reported that BNP values increased significantly according to NYHA functional class in approximately 800 patients with HF. Conversely, in the present study, BNP levels did not change in an NYHA-dependent manner (Figure 4C). In previous studies, changes in BNP levels during hospitalization (delta BNP) predicted the prognosis of HF. We performed an additional analysis and found no association between delta BNP and f(HMA). These two variables seem to reflect different clinical features and further research is needed to investigate their relationship. As we have seen, f(HMA) reflects the severity of HF as assessed by the NYHA classification and ACCF/AHA stages, implying that f(HMA) is a biomarker of HF severity, even during the recovery period of HF. f(HMA) levels reflect the average redox state of the systemic body during a period that depends on the half-life of the protein. Given that HSA concentrations may reflect the average nutritional status over the past 2-3 weeks, and assuming that HNA production is irreversible, the half-life of HNA is also expected to be the same during these weeks. This long half-life of HSA or f(HMA) may be the most important feature as a

![Figure 2](histogram.png)

**Figure 2.** Histogram of f(HMA) in HF (gray) and Control (stripe white). For comparison of distributions, only women were selected. f(HMA) in patients with HF is skewed to the left compared to controls. f(HMA) indicates fraction of human mercaptalbumin; and HF, heart failure.

### Table II. Comparison between HF and Control

|                   | HF (n = 58) | Control (n = 125) | P value |
|-------------------|------------|-------------------|---------|
| Age (years)       | 81 (76.5–86) | 80 (78–83)       | 0.8725  |
| Body mass index (kg/m²) | 19.8 (16.7–22.6) | 21.8 (19.4–23.9) | 0.0004  |
| Comorbidities     |            |                   |         |
| Hypertension      | 29 (50.0)  | 89 (71.2)         | 0.0077  |
| Diabetes          | 16 (27.6)  | 16 (6.5)          | 0.0207  |
| Dyslipidemia      | 19 (33.3)  | 87 (69.6)         | <0.0001 |
| Smoking           | 14 (24.1)  | 1 (0.8)           | 0.0118  |
| Alcohol           | 8 (13.8)   | 8 (15.4)          | 0.1571  |
| Biochemical data at discharge |     |                   |         |
| f(HMA) (%)        | 54.7 (47.0–61.2) | 66.3 (62.8–70.0) | <0.0001 |
| Serum albumin (g/dL) | 3.4 (3.1–3.6)  | 4.3 (4.1–4.5)    | <0.0001 |
| eGFR (mL/minute/1.73 m²) | 40.2 (28.2–49.1) | 62.6 (52.9–74.0) | <0.0001 |
| Brain natriuretic peptide (pg/mL) | 221.1 (141.8–401.6) | N/A  | -       |

eGFR indicates estimated glomerular filtration; f(HMA), fraction of human mercaptalbumin; HF, heart failure; and N/A, not available. Only women were selected from heart failure to match controls. Continuous variables are presented as the mean ± standard deviation or median (interquartile range).
Table III. Results of Linear Regression Analysis of the Association between Selected Variables and f(HMA)

| Variable                                    | Univariate          | Multivariate         |
|---------------------------------------------|---------------------|----------------------|
|                                             | $\beta_s$ ($\beta_u$) (95% CI) | $P$ value | $\beta_s$ ($\beta_u$) (95% CI) | $P$ value |
| Age, per 1 year increment                   | $-0.26 (0.07)$ [-0.35, −0.08] | 0.0023 | $-0.18 (-0.13)$ [−0.26, −0.00] | 0.0443 |
| Sex (male)                                  | 0.04 (0.81) [-2.50, −4.12] | 0.6290 |                          |          |
| Body mass index, per 1 kg/m² increment      | 0.01 (0.02) [-0.39, −0.42] | 0.9361 |                          |          |
| Left ventricle ejection fraction, per 1% increment | $-0.11 (-0.07)$ [-0.18, 0.04] | 0.2193 |                          |          |
| ACCF/AHA (stage D)                          | $-0.21 (-1.35)$ [-5.81, −4.88] | 0.0208 | 0.11 (1.54) [-0.80, 3.87] | 0.1941 |
| NYHA functional class at discharge (III/IV) | $-0.26 (-5.06)$ [-8.26, −1.87] | 0.0021 |                          |          |
| Diabetes                                    | 0.06 (0.65) [-1.18, 2.48] | 0.4825 |                          |          |
| Chronic renal disease                       | 0.15 (1.59) [-0.24, 3.41] | 0.0882 |                          |          |
| Smoking                                     | $-0.16 (-2.18)$ [-4.50, 0.14] | 0.0649 |                          |          |
| Alcohol                                     | $-0.11 (-1.26)$ [-3.17, 0.65] | 0.1952 |                          |          |
| Serum albumin, per 1 mg/dL increment        | 0.00 (-0.04) [-3.18, 3.10] | 0.9807 |                          |          |
| Hemoglobin, per 1 mg/dL increment           | 0.06 (0.28) [-0.48, −1.03] | 0.4705 |                          |          |
| log (eGFR), per 1 unit increment            | 0.40 (8.15) [4.93, 11.36] | < 0.0001 | 0.35 (6.47) [3.07, 9.87] | 0.0003 |
| log (Total bilirubin), per 1 unit increment | 0.00 (-0.05) [-3.41, −3.31] | 0.9764 |                          |          |
| log (BNP), per 1 unit increment             | $-0.38 (-2.25)$ [-4.11, −0.38] | 0.0186 | $-0.19 (-1.84)$ [-3.43, −0.26] | 0.0232 |
| Serum sodium, per 1 mEq/L increment         | 0.28 (0.78) [0.33, 1.24] | 0.0009 | 0.26 (0.70) [0.26, 1.15] | 0.0024 |
| Log (C-reactive protein), per 1 unit increment | $-0.08 (-0.61)$ [-1.95, −0.74] | 0.3733 |                          |          |
| Serum uric acid, per 1 g/dL increment       | $-0.25 (-1.11)$ [-1.86, −0.35] | 0.0047 |                          |          |

$\beta_s$ indicates standardized $\beta$; $\beta_u$, unstandardized $\beta$; BNP, brain natriuretic peptide; CI, interval confidence; eGFR, estimated glomerular filtration; ACCF, American College of Cardiology Foundation; AHA, American Heart Association; and NYHA, New York Heart Association.

Figure 3. Correlation between log-transformed BNP and f(HMA). f(HMA) is negatively correlated with log-transformed BNP, which is statistically significant. Gray shadow shows 95% confidence intervals. f(HMA) indicates fraction of human mercaptalbumin; and BNP, brain natriuretic peptide.

**Limitations and perspectives:** We acknowledge that there are several limitations to this study. First, some patients were lost during the recruitment process. The lost patients were younger and had shorter hospital stays than the enrolled patients. Our participants may be older and more severely ill than the general population. Contrastingly, the number of ACCF/AHA stage D cases was too small to generalize the association between the severity of HF and f(HMA). Second, the timing of the measurement of f(HMA) and its change over time should be investigated in future studies. Finally, the present study could not examine cause-effect relationships because it was a cross-sectional study. Our ongoing prospective study will...
Figure 4. Comparison of f(HMA) and BNP by the severity of heart failure in box plots. NYHA class (A, C), ACCF/AHA (B, D) stage. Data were analyzed by Kruskal-Wallis test and as post hoc test, Steel-Dwass multiple comparison test was used. P value < 0.05 was considered to indicate a statistical difference. f(HMA) indicates fraction of human mercaptalbumin; NYHA, New York Heart Association; ACCF, American College of Cardiology Foundation; AHA, American Heart Association; and BNP, brain natriuretic peptide.

Conclusion

In conclusion, patients with HF showed lower f(HMA) than those in the control group and f(HMA) related to BNP independently in the multivariate regression analysis. The findings from the present study suggest that f(HMA) may be a biomarker reflecting the pathogenesis and severity of HF. Our ongoing prospective cohort study will verify the predictive validity of f(HMA).

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Disclosure

Conflicts of interest: The authors declare that there are no conflicts of interest.

IRB information: This study protocol was approved by the Ethics Review Committee of Nagoya University Graduate School of Medicine (approval number: 2019-0026) and Japan Red Cross Aichi Medical Center Nagoya Daiichi Hospital (approval number: 2019-059).

Author contributions: T.N. analyzed and interpreted the data and was a major contributor in drafting the manuscript. J.U. and T.N. measured the redox state of HSA. H.K. and S.S. explained the study and obtained informed consent from the patient. T.N. collected and assembled the dataset for this study. S.Y. and T.N. contributed to the conception and design of the study. S.Y. critically revised the draft and approved the final version for publication. All authors read and approved the final manuscript.

Data availability: The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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