Circulating Thrombospondin-2 Reflects Disease Severity and Predicts Outcome of Heart Failure With Reduced Ejection Fraction

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Background: Thrombospondin-2 (TSP-2) is a matricellular protein found in human serum. Deletion of TSP-2 causes age-dependent dilated cardiomyopathy. We hypothesized that TSP-2 is a useful biomarker in patients with heart failure with reduced ejection fraction (HFrEF).

Methods and Results: Serum TSP-2 was measured in 101 patients with HFrEF, and mortality and cardiovascular events were followed. Serum TSP-2 in the HFrEF group was significantly higher than in the non-HF group (n=17). Mean NYHA functional class was significantly higher in the high TSP-2 group (>median) than the low TSP-2 group (2.26 vs. 1.76, P=0.004). Circulating TSP-2 level was significantly associated with that of B-type natriuretic peptide (BNP; r=0.40, P<0.0001) on multivariate linear regression analysis. On Kaplan-Meier curve analysis the high TSP-2 group had a lower event-free rate than the low TSP-2 group (log-rank test, P=0.03). Multivariate Cox hazard analysis identified hemoglobin (hazard ratio [HR], 0.66; 95% confidence interval [CI]: 0.53–0.82, P<0.0001), and TSP-2 (ln[TSP-2]; HR, 3.34; 95% CI: 1.03–10.85, P=0.045) as independent predictors of adverse outcome. The area under the curve for 1-year events increased when TSP-2 was added to Framingham risk score (FRS; alone, 0.60) or BNP (alone, 0.69; FRS+TSP-2, 0.75; BNP+TSP-2, 0.76).

Conclusions: TSP-2 is a potentially useful biomarker for assessment of disease severity and prognosis in HFrEF. (Circ J 2014; 78: 903–910)

Key Words: Biomarker; Heart failure; Matricellular protein; Prognosis

Thrombospondins (TSPs) are glycoproteins known to play an essential role in cell-matrix interactions. Thrombospondin-2 (TSP-2) is a TSP expressed in extracellular matrix, developing blood vessels, and the basal epidermal keratinocyte layer. Its expression increases during tissue remodeling associated with wound healing, foreign body reactions, carcinogenesis, ischemia and inflammation. The expression of TSP-2 is low in the myocardium, but increases in response to pathological stress stimuli. Previous experimental studies have shown that genetic ablation of TSP-2 exacerbated the frequency of cardiac rupture after pressure overload, apoptosis of the myocardium in aging heart, and lethality in doxorubicin-induced cardiomyopathy.
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20 HFrEF was defined as left ventricular ejection fraction (LVEF) $\leq 50\%$, in addition to the presence of HF. The non-HF group consisted of 17 non-HF patients who also underwent cardiac catheterization for suspected angina pectoris and who were confirmed as normal on coronary angiography. The exclusion criteria were acute coronary syndrome; malignancy; or serum creatinine $>2.0$ mg/dl. Hypertension, diabetes mellitus (DM), and dyslipidemia were defined as follows: hypertension: anti-hypertensive medication, or blood pressure $>140/90$ mmHg; DM: hypoglycemia medication, or hemoglobin A1c $\geq 6.5\%$, casual plasma glucose $\geq 200$ mg/dl, fasting plasma glucose $\geq 126$ mg/dl, and/or plasma glucose $2$ h after $75$-g glucose load $\geq 200$ mg/dl; dyslipidemia: medication for dyslipidemia, or high-density lipoprotein cholesterol $<40$ mg/dl, low-density lipoprotein cholesterol $\geq 140$ mg/dl, triglycerides $\geq 150$ mg/dl.

The study was conducted after approval of the and viral myocarditis. These data suggest that TSP-2 functions as a cardioprotective factor. In contrast to animal models, there is little or no information on the clinical importance of circulating TSP-2 concentration in patients with cardiovascular disease. In this study, we measured serum TSP-2 level in patients with HFrEF and determined its significance in the assessment of disease severity and prediction of cardiovascular-related mortality.

**Methods**

**Subjects**

The study subjects were 101 consecutive patients with HFrEF scheduled to undergo coronary angiography for stable or suspected coronary artery disease or a diagnostic workup for HF at Kumamoto University Hospital between March 2009 and April 2012. Eligible patients were aged $\geq 20$ years with HF, defined as American College of Cardiology/American Heart Association Stage B or C. HFrEF was defined as left ventricular ejection fraction (LVEF) $\leq 50\%$, in addition to the presence of HF. The non-HF group consisted of 17 non-HF patients who also underwent cardiac catheterization for suspected angina pectoris and who were confirmed as normal on coronary angiography. The exclusion criteria were acute coronary syndrome; malignancy; or serum creatinine $>2.0$ mg/dl. Hypertension, diabetes mellitus (DM), and dyslipidemia were defined as follows: hypertension: anti-hypertensive medication, or blood pressure $>140/90$ mmHg; DM: hypoglycemia medication, or hemoglobin A1c $\geq 6.5\%$, casual plasma glucose $\geq 200$ mg/dl, fasting plasma glucose $\geq 126$ mg/dl, and/or plasma glucose $2$ h after $75$-g glucose load $\geq 200$ mg/dl; dyslipidemia: medication for dyslipidemia, or high-density lipoprotein cholesterol $<40$ mg/dl, low-density lipoprotein cholesterol $\geq 140$ mg/dl, triglycerides $\geq 150$ mg/dl. The study was conducted after approval of the

**Table 1. Subject Clinical Characteristics**

|                        | Non-HF (n=17) | HFrEF (n=101) | P-value |
|------------------------|---------------|---------------|---------|
| Age (years)            | 64.6±7.6      | 66.6±11.8     | 0.49    |
| Female                 | 8 (47)        | 27 (27)       | 0.15    |
| BMI (kg/m²)            | 23.8±3.5      | 23.5±3.4      | 0.73    |
| Mean NYHA class        | NA            | 2.0±0.83      | NA      |
| Ischemic etiology      | NA            | 52 (51)       | NA      |
| Atrial fibrillation    | 0 (0)         | 26 (26)       | 0.022   |
| Hypertension           | 13 (77)       | 71 (70)       | 0.78    |
| Diabetes mellitus      | 5 (29)        | 41 (41)       | 0.43    |
| Dyslipidemia           | 10 (59)       | 64 (63)       | 0.79    |
| Current smoking        | 0 (0)         | 11 (11)       | 0.36    |
| BNP (pg/ml)            | 17.4 (10.0–32.9) | 188.3 (68.0–471.0) | <0.0001 |
| hsTnT (ng/ml)          | 0.005 (0.004–0.007) | 0.014 (0.007–0.023) | <0.0001 |
| hsCRP (mg/L)           | 0.030 (0.020–0.050) | 0.050 (0.030–0.022) | 0.009   |
| TSP-2 (ng/ml)          | 14.84 (11.84–15.97) | 17.79 (13.30–22.95) | 0.017   |
| Hemoglobin (g/dl)      | 13.1±1.2      | 13.3±2.1      | 0.64    |
| eGFR (mL·min⁻¹·1.73m⁻²) | 68.4±16.3   | 69.0±16.0     | 0.058   |
| LVEF                   | 65.8±3.7      | 40.1±3.7      | <0.0001 |
| E/e’ (%)               | 12.0±4.3      | 16.6±8.5      | 0.041   |
| PCWP (mmHg)            | NA            | 12.8±6.8      | NA      |

Data given as mean±SD, median (IQR) or n (%). †Cardiac amyloidosis (n=1), cardiac sarcoidosis (n=1), tachycardia-induced cardiomyopathy (n=2), diabetic cardiomyopathy (n=1), LV non-compaction (n=2), congenital heart disease (n=1), unclassified cardiomyopathy (n=7).

ACEI, angiotensin-converting enzyme inhibitor; AT1, angiotensin type 1; BMI, body mass index; BNP, B-type natriuretic peptide; eGFR, estimated glomerular filtration rate; HF, heart failure; hsCRP, high-sensitivity C-reactive protein; hsTnT, high-sensitivity troponin T; LVEF, left ventricular ejection fraction; NA, not available; NYHA, New York Heart Association; PCWP, pulmonary capillary wedge pressure; TSP, thrombospondin.
ethics committee and written informed consent was obtained from each patient.

Follow-up
After blood sampling, patients were followed in the outpatient clinic for a median of 820 days (interquartile range [IQR], 591–1,060 days). The endpoint of this study was a composite of all-cause mortality, non-fatal myocardial infarction (MI), stroke, and cardiovascular-related admission to the hospital (eg, HF, arrhythmia, including ventricular fibrillation, ventricular tachycardia, atrial fibrillation, and advanced atrioventricular block). Mortality and cardiovascular events were identified by searching the medical records and confirmed via direct contact with the patients/relatives, and caring physicians.

Procedure
Blood samples were withdrawn from resting participants at supine position to assess serum TSP-2 using an enzyme-linked immunosorbent assay (ELISA, R&D Systems, Minneapolis, MN). Plasma BNP levels were measured using the M102 Shionogi BNP kit (Shionogi, Osaka, Japan). Serum high-sensitivity cardiac troponin T (hsTnT) was measured using the Elecsys 2010 Troponin T hs kit (Roche Diagnostics, Indianapolis, IN, USA). The serum and plasma samples were kept frozen at −80°C until performance of the lab assays. The intra-assay and inter-assay variability of the TSP-2 were 5.8% and 2.9%, respectively. Echocardiography findings obtained from all study participants using Aplio XG (Toshiba, Tokyo, Japan) or Vivid 7 (GE-Vingmed Ultrasound, Horten, Norway) ultrasound systems were evaluated by 2 independent investigators who were blinded to all clinical data. LVEF was calculated using the modified Simpson method. Early (E) and late atrial (A) transmitral peak flow velocities were measured from mitral inflow velocities. Early diastolic mitral annular (e’) velocity was determined after pulsed wave tissue Doppler imaging and E/e’ ratio was calculated.

Statistical Analysis
Continuous variables are expressed as mean±SD, but TSP-2, BNP, high-sensitivity C-reactive protein (hsCRP), and hsTnT had a skewed distribution and are therefore expressed as median (IQR) and were log transformed before Pearson’s correlation analysis, multivariate linear regression analysis, and Cox regression analysis. Categorical variables are expressed as number (percentage). Continuous and categorical variables were compared using Student’s t-test or Mann-Whitney U-test and Fisher’s exact test, respectively. The correlation between circulating TSP-2 and BNP was evaluated using Pearson’s correlation. Multivariate linear regression analysis was used to adjust for clinical covariates, such as age, sex, hypertension, DM, and estimated glomerular filtration rate (eGFR). The Kaplan-Meier method, log-rank test, simple and multiple Cox regression analysis were used to assess prognostic association. We also used multivariate Cox hazard analysis with the forced inclusion models of the following parameters: model 1, age and TSP-2; model 2, age, DM, and TSP-2; model 3: age, BNP, and TSP-2. To assess whether the addition of TSP-2 improves the prognostic value of Framingham risk score (FRS)24 or BNP, we generated receiver operating characteristics (ROC) curves and compared their area under the curve (AUC; ie, c-statistic). We performed DeLong’s test to compare AUC for the combination of TSP-2 and FRS, or BNP with FRS, or BNP alone. Two-tailed P<0.05 indicated statistical significance. All data were analyzed using SPSS v17.0J for Windows (SPSS Japan, Tokyo, Japan), and EZR,25 with a graphical user interface for R (R Foundation for Statistical Computing version 2.13.0).

Results
Table 1 lists the clinical characteristics of all study participants. Median serum TSP-2 was significantly higher in the HFpEF group (17.79 ng/ml) than in the non-HF group (14.84 ng/ml, P=0.017; Figure 1). The subjects consisted of patients with ischemic and non-ischemic HF (51%; ischemic etiology, 49%; non-ischemic etiology; Table 1). There was no significant difference in serum TSP-2 level between these groups (data not shown). Using the aforementioned median, HFpEF patients were divided into 2 groups: the high TSP-2 group (median, 22.95 ng/ml) and the low TSP-2 group (median, 13.28 ng/ml; Table 2). The proportions of patients with a history of hypertension, DM, and atrial fibrillation were significantly higher in the high TSP-2 group than the other group. High TSP-2 patients also tended to more likely suffer from anemia and renal dysfunction, and presented with more severe symptoms than those with low TSP-2 (mean New York Heart Association [NYHA] functional class: 2.26 vs. 1.76, P=0.004). Moreover, circulating levels of traditional biomarkers, for example BNP, hsCRP, and hsTnT, were significantly higher in the high than low TSP-2 group. On echocardiography, E/e’ was significantly higher in the high TSP-2 group than in the low TSP-2 group, but LVEF was not significantly different between the 2 groups. Pulmonary capillary wedge pressure was significantly higher in the high TSP-2 group than in the low TSP-2 group. Loop diuretics were prescribed more frequently to patients in the high TSP-2 group. Table 3 lists the result of linear regression analysis. On univariate analysis, hypertension, DM, BNP, and eGFR were associated with TSP-2. Multivariate analysis showed that BNP correlated significantly with TSP-2 independent of age, sex, hypertension, DM, and eGFR (Table 3).

Data for 101 patients with HFpEF were available for analysis of mortality and cardiovascular events. During follow-up, 26
(25.7%) of the 101 patients died or were hospitalized for cardiovascular disease. The following events were registered: death (n=2), non-fatal MI (n=0), stroke (n=0), hospitalization for HF decompensation (n=20), and hospitalization for arrhythmia (n=4). On Kaplan-Meier analysis the high TSP-2 group had a significantly higher probability of death or cardiovascular events (log-rank test, P=0.03; Figure 2A). Furthermore, the combination of high TSP-2 and BNP (cut-off: 188.3 pg/ml [median]) identified patients with a significantly higher probability of adverse cardiovascular events (log-rank test, P=0.038; Figure 2B).

Univariate Cox proportional hazards analysis showed that high TSP-2, advanced age, the presence of DM, low hemoglobin, low eGFR, and high BNP correlated significantly with future events (Table 4). Furthermore, stepwise multivariate Cox proportional hazard analysis identified TSP-2 (ln[TSP-2]; hazard ratio [HR], 3.34; 95% confidence interval [CI]: 1.03–10.85; P=0.045), and hemoglobin (HR, 0.66; 95% CI: 0.53–0.82; P<0.0001) as independent predictors of risk of death and cardiovascular events (Table 4). In addition, forced entry multivariate Cox hazard analysis was done to adjust for the factors suspected to increase the probability of adverse cardiovascular events. In the 3 forced entry models, TSP-2 still significantly predicted future cardiovascular events (Table 4).

On ROC analysis (excluding those subjects with <1-year follow-up, n=100), TSP-2 was a significant marker of increased 1-year event risk, with AUC=0.71 (95% CI: 0.58–0.84; P=0.005), which was greater than that of FRS (AUC, 0.60; 95% CI: 0.45–0.76; P=0.17), and BNP (AUC, 0.69; 95% CI: 0.54–0.84; P=0.01). The combination with TSP-2 improved the AUC of FRS (AUC for the combination, 0.75; 95% CI: 0.63–0.88; P=0.001), and of BNP (AUC for the combination, 0.76; 95% CI: 0.63–0.88; P=0.001; Figure 3), although these differences did not reach statistical significance (FRS vs. FRS+TSP-2, BNP vs. BNP+TSP-2).

### Table 2. Subject Characteristics vs. TSP-2 Level

| TSP-2 Level | Low TSP-2 (<17.79 ng/ml) (n=50) | High TSP-2 (≥17.79 ng/ml) (n=51) | P-value |
|-------------|---------------------------------|---------------------------------|---------|
| Age (years) | 65.7±13.3                       | 67.5±10.1                       | 0.43    |
| Female      | 10 (20)                         | 17 (33)                         | 0.20    |
| BMI (kg/m²) | 23.3±3.4                        | 23.6±3.5                        | 0.54    |
| Mean NYHA class | 1.76±0.72              | 2.26±0.87                       | 0.004   |
| Ischemic etiology   | 29 (58)                      | 23 (45)                         | 0.23    |
| Atrial fibrillation | 8 (16)                      | 18 (35)                         | 0.040   |
| Hypertension       | 29 (58)                       | 42 (82)                         | 0.009   |
| Diabetes mellitus  | 12 (24)                       | 29 (57)                         | 0.001   |
| Dyslipidemia       | 31 (62)                        | 33 (65)                         | 0.84    |
| Current smoking   | 6 (12)                         | 5 (10)                          | 0.76    |
| BNP (pg/ml)       | 105.5 (46.7–199.4)             | 324.8 (179.9–641.3)             | <0.0001 |
| hsTnT (ng/ml)     | 0.008 (0.005–0.014)            | 0.020 (0.011–0.031)             | <0.0001 |
| hsCRP (mg/L)      | 0.035 (0.020–0.089)            | 0.12 (0.040–0.52)               | 0.0006  |
| TSP-2 (ng/ml)     | 13.28 (11.73–15.29)            | 22.95 (19.71–26.77)             | <0.0001 |
| Hemoglobin (g/dl) | 13.9±1.7                       | 12.8±2.3                        | 0.006   |
| eGFR (ml · min⁻¹ · 1.73m⁻²) | 67.0±14.4               | 54.9±15.3                       | 0.0001  |
| LVEF           | 40.6±8.3                       | 39.6±8.4                        | 0.44    |
| E/A            | 1.04±0.72                      | 1.51±1.16                       | 0.060   |
| DCT            | 195.1±62.0                     | 189.4±80.5                      | 0.38    |
| E/e' (%)       | 14.8±7.7                       | 18.5±8.9                        | 0.018   |
| PCWP (mmHg)     | 10.0±4.4                       | 15.4±7.6                        | <0.0001 |

Data given as mean±SD, median (IQR) or n (%). †Cardiac amyloidosis (n=1), cardiac sarcoidosis (n=1), tachycardia-induced cardiomyopathy (n=2), diabetic cardiomyopathy (n=1), LV non-compaction (n=2), congenital heart disease (n=1), unclassified cardiomyopathy (n=7).

DCT, deceleration time. Other abbreviations as in Table 1.
In that study, the authors found that high TSP-2 was associated with risk of cardiovascular mortality in older men screened for abdominal aortic aneurysm. Their data suggested the clinical utility of measuring circulating TSP-2 in humans for risk stratification of adverse cardiovascular events, but the median circulating TSP-2 level in their subjects (66.77 ng/ml) was markedly higher than that reported in other clinical studies (patients with pre-eclampsia, 13.2 ng/ml; patients with ischemic stroke, 31.2 ng/ml), suggesting that their study covered only particular candidates. In contrast, median circulating TSP-2 in the present study was similar to those reported in previous studies.27,28 These data indicate that TSP-2 could be used as a screening biomarker in general patients.

Traditionally, risk stratification of cardiovascular disease is done using the FRS.24 In HF patients, BNP was reported to be useful and is currently the most commonly used prognostic biomarker.

### Table 3. Factors Associated With Ln (TSP-2)

| Factors                        | Univariate analysis | Model 1 | Model 2 | Model 3 |
|--------------------------------|---------------------|---------|---------|---------|
|                                | r, B (95% CI)       | P-value | B (95% CI) | P-value | B (95% CI) | P-value | B (95% CI) | P-value |
| Age (years)                    | 0.077, 0.002        | 0.45    | -0.002, 0.59 | 0.59    | -0.005, 0.078 | 0.078    | -0.005, 0.11 | 0.11    |
|                                | (-0.004 to 0.008)   |         | (-0.008 to 0.004)   |         | (-0.011 to 0.001) |         | (-0.011 to 0.001) |         |
| Sex (female)                   | 0.062, 0.05         | 0.54    | 0.055, 0.47 | 0.47    | 0.019, 0.79 | 0.03    | 0.03, 0.69 | 0.69    |
|                                | (-0.11 to 0.21)     |         | (-0.095 to 0.21) |         | (-0.12 to 0.16) |         | (-0.12 to 0.18) |         |
| Hypertension (Yes)             | 0.30, 0.24         | 0.002   | -       |         | 0.076, 0.35 | 0.076   | 0.076, 0.35 | 0.076   |
|                                | (0.089 to 0.39)     |         |         |         | (-0.083 to 0.24) |         | (-0.083 to 0.24) |         |
| Diabetes mellitus (Yes)        | 0.20, 0.14         | 0.051   | -       |         | 0.003, 0.96 | 0.003   | 0.003, 0.96 | 0.003   |
|                                | (0.00 to 0.29)      |         |         |         | (-0.14 to 0.15) |         | (-0.14 to 0.15) |         |
| Hemoglobin (1-g/dl)            | 0.14, -0.024       | 0.1700  | -       |         | 0.013, 0.49 | 0.013  | 0.013, 0.49 | 0.013  |
|                                | (-0.058 to 0.01)    |         |         |         | (-0.025 to 0.052) |         | (-0.025 to 0.052) |         |
| ln (BNP)                       | 0.40, 0.11         | <0.0001 | 0.12, <0.0001 | 0.097   | 0.004, 0.073 | 0.012   | 0.012, 0.073 | 0.012   |
|                                | (0.061 to 0.16)     |         | (0.063 to 0.17) |         | (0.025 to 0.13) |         | (0.017 to 0.13) |         |
| eGFR (ml·min⁻¹·1.73m⁻²)        | 0.47, -0.011       | <0.0001 | -       |         | -0.009, <0.0001 | 0.009  | -0.009, <0.0001 | 0.009  |
|                                | (-0.015 to -0.007) |         |         |         | (-0.014 to -0.005) |         | (-0.014 to -0.004) |         |

CI, confidence interval. Other abbreviations as in Table 1.

**Figure 2.** Kaplan-Meier analysis for the probability of adverse cardiovascular events in patients with (A) high and low serum thrombospondin-2 (TSP-2), and (B) in subgroups of patients with high or low TSP-2 and B-type natriuretic peptide (BNP), using a cut-off of 17.79 ng/ml (median) for TSP-2 and 188.3 pg/ml (median) for BNP.

P=0.10; BNP vs. BNP+TSP-2, P=0.32; DeLong test).

**Discussion**

The main findings for HFrEF were: (1) serum TSP-2 level correlated with markers of disease severity, such as BNP; and (2) TSP-2 was an independent predictor of adverse cardiovascular outcome and was a stronger indicator of increased cardiovascular risk than FRS and plasma BNP. This indicates that circulating TSP-2 could be a useful biomarker for the assessment of disease severity and prediction of outcome for HFrEF.

Although TSP-2 has been shown in experimental studies to play an important role in cardiovascular pathophysiology, there is little information on the clinical significance of this glycoprotein in patients with HF. One recent study, however, showed that serum TSP-2 correlated with cardiovascular mortality.26 In that study, the authors found that high TSP-2 was associated with risk of cardiovascular mortality in older men screened for abdominal aortic aneurysm. Their data suggested the clinical utility of measuring circulating TSP-2 in humans for risk stratification of adverse cardiovascular events, but the median circulating TSP-2 level in their subjects (66.77 ng/ml) was markedly higher than that reported in other clinical studies (patients with pre-eclampsia, 13.2 ng/ml; patients with ischemic stroke, 31.2 ng/ml), suggesting that their study covered only particular candidates. In contrast, median circulating TSP-2 in the present study was similar to those reported in previous studies.27,28 These data indicate that TSP-2 could be used as a screening biomarker in general patients.

Traditionally, risk stratification of cardiovascular disease is done using the FRS.24 In HF patients, BNP was reported to be useful and is currently the most commonly used prognostic
TSP-2 has been reported to have anti-angiogenic properties.\textsuperscript{13,31,32} Furthermore, Kajihara et al. found high serum TSP-2 in patients with systemic sclerosis compared to control subjects, and that such patients tended to have dermal ulcers.\textsuperscript{33} Such anti-angiogenic properties might contribute to the high frequency of adverse cardiovascular outcome.

The present study has several limitations. First, a relatively small patient cohort was recruited at a single center. Further, extreme care should be taken to interpret the results of ROC analysis because of the aforementioned limitation and the relatively fewer cardiovascular events. Although the addition of TSP-2 improved the AUC (ie, c-statistic) of FRS, and of BNP, suggesting that measurement of circulating TSP-2 adds clinical value in risk stratification of patients with HFrEF, these differences did not reach statistical significance. Further multicenter studies of larger groups are needed to confirm the present results. In addition, we did not perform serial measurement of TSP-2 in all of the present patients, therefore we cannot identify TSP-2 as the marker guiding treatment of HF. Serial measurement of TSP-2 is needed to clarify this point. Second, although all sub-

\begin{table}
\centering
\caption{Cox Proportional Hazards Analysis for Future Adverse Events\textsuperscript{1}}
\begin{tabular}{lccc}
\hline
Factor & Univariate analysis & Multivariate analysis \\
& HR (95% CI) & P-value & HR (95% CI) & P-value \\
\hline
Age (years) & 1.04 (1.00–1.09) & 0.045 & Not selected & \\
Sex (female) & 0.80 (0.32–1.99) & 0.63 & 0.47 (0.19–1.21) & 0.12 \\
BMI (kg/m\textsuperscript{2}) & 0.91 (0.80–1.03) & 0.14 & Not selected & \\
NYHA functional class (3/4 vs. 1/2) & 1.57 (1.00–2.47) & 0.05 & Not selected & \\
Ischemic etiology (Yes) & 1.64 (0.74–3.61) & 0.22 & Not selected & \\
Hypertension (Yes) & 2.54 (0.87–7.37) & 0.087 & Not selected & \\
Diabetes mellitus (Yes) & 4.06 (1.76–9.38) & 0.001 & Not selected & \\
Dyslipidemia (Yes) & 1.13 (0.50–2.54) & 0.77 & Not selected & \\
Current smoking (Yes) & 0.76 (0.18–3.22) & 0.71 & Not selected & \\
Hemoglobin (1-g/dl) & 0.63 (0.51–0.79) & <0.0001 & 0.66 (0.53–0.82) & <0.0001 \\
ln(BNP) & 1.63 (1.16–2.30) & 0.005 & Not selected & \\
ln(TSP-2) & 4.42 (1.56–12.50) & 0.005 & 3.34 (1.03–10.85) & 0.045 \\
eGFR (<60 ml·min\textsuperscript{-1}·1.73 m\textsuperscript{-2}) & 0.97 (0.95–1.00) & 0.017 & Not selected & \\
LVEF (%) & 0.99 (0.94–1.03) & 0.51 & Not selected & \\
\hline
\end{tabular}
\textsuperscript{1}Whole subject group.
HR, hazard ratio. Other abbreviations as in Tables 1, 3.
\end{table}
Conclusions

Serum TSP-2 is a new biomarker for assessment of disease severity and prognosis in patients with HFrEF. Serum TSP-2 can increase the prognostic significance of traditional markers, such as BNP, leading to greater accuracy in risk stratification of patients with HFrEF.

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