



Metabolic Disturbances Identified in Plasma Are Associated With Outcomes in Patients With Heart Failure

Diagnostic and Prognostic Value of Metabolomics

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ABSTRACT

BACKGROUND Identification of novel biomarkers is needed to improve the diagnosis and prognosis of heart failure (HF). Metabolic disturbance is remarkable in patients with HF.

OBJECTIVES This study sought to assess the diagnostic and prognostic values of metabolomics in HF.

METHODS Mass spectrometry-based profiling of plasma metabolites was performed in 515 participants; the discovery phase study enrolled 51 normal control subjects and 183 HF patients, and the validation study enrolled 63 control subjects and 218 patients with stage C HF. Another independent group of 32 patients with stage C HF who recovered to New York Heart Association functional class I at 6 and 12 months was profiled as the "recovery" group.

RESULTS A panel of metabolites, including histidine, phenylalanine, spermidine, and phosphatidylcholine C34:4, has a diagnostic value similar to B-type natriuretic peptide (BNP). In the recovery group, the values of this panel significantly improved at 6 and 12 months. To evaluate the prognostic values, events were defined as the combined endpoints of death or HF-related re-hospitalization. A metabolite panel, which consisted of the asymmetric methylarginine/arginine ratio, butyrylcarnitine, spermidine, and the total amount of essential amino acids, provided significant prognostic values ($p < 0.0001$) independent of BNP and traditional risk factors. The prognostic value of the metabolite panel was better than that of BNP (area under the curve of 0.85 vs. 0.74 for BNP) and Kaplan-Meier curves (log rank: 17.5 vs. 9.95). These findings were corroborated in the validation study.

CONCLUSIONS Metabolomics demonstrate powerful diagnostic value in estimating HF-related metabolic disturbance. The profile of metabolites provides better prognostic value versus conventional biomarkers. (J Am Coll Cardiol 2015;65:1509-20) © 2015 by the American College of Cardiology Foundation.

A complex clinical syndrome, heart failure (HF) represents the end stage of various cardiac diseases. In the past few decades, substantial advances have been made in understanding the underlying pathophysiology and hemodynamics of HF,

leading to the development of novel pharmaceuticals and interventional therapies. Nevertheless, short- and long-term, HF-related hospitalization and mortality remain high, requiring substantial amounts of health care resources (1). The limited

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ABBREVIATIONS AND ACRONYMS

ACC = American College of Cardiology

AHA = American Heart Association

AUC = areas under the curve

BNP = B-type natriuretic peptide

HF = heart failure

NO = nitric oxide

OPLS-DA = orthogonal-projection-to-latent-structure-discriminant-analysis

VIP = variable importance in the projection

effectiveness of current treatments for late-stage HF necessitates novel interventional measures to curb maladaptive molecular processes and avoid the progression of HF to advanced stages.

A variety of HF biomarkers have been identified, with B-type natriuretic peptide (BNP) and the N-terminal fragment of the proprotein, emerging as clinically useful markers for the diagnosis and prognosis of HF (2,3). Natriuretic peptides also have prognostic value for individuals at moderate risk of cardiovascular disease without overt symptoms (4). Unfortunately, these biomarkers do not provide additional information on molecular targets for therapeutic interven-

tions. In addition, application of a single biomarker may be insufficient for evaluating patients with HF; a combination of multiple molecules may be better for such an evaluation.

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The etiology of a substantial proportion of HF patients remains unexplained according to the current knowledge of cardiovascular risk factors. Regardless of the heterogeneous etiologies, HF development is causally related to the heart's inability to meet the metabolic demands of the body. The accompanying changes in global metabolism—sometimes referred to as a “metabolic storm”—suggest a clinical application of an HF-specific metabolome for diagnostic and prognostic purposes. The current staging of HF is on the basis of the consensus developed by the American College of Cardiology (ACC) and the American Heart Association (AHA), instead of pathogenic mechanisms (5). Taking advantage of the high throughput and the potential of developing multiple biomarkers, metabolomics is a platform for identifying metabolic signatures in patients with pre-HF to those with advanced HF, independent of traditional risk factors. A thorough understanding of HF-associated metabolic perturbation, together with advances in nutrigenomic research, might permit the development of personalized preventive measures.

Previously, on the basis of analysis of urine samples from a limited number of patients, Kang et al. (6) showed that concentrations of 1-methylnicotinamide, 2-oxoglutarate, and pyruvate were lower in patients with ischemic HF, but that concentrations of acetate, ketone bodies, cytosine, methylmalonate, and phenylacetylglycine were all higher. Another study identified pseudouridine and 2-oxoglutarate as 2 biomarkers of HF (7). In the present study, we recruited more HF patients and examined the clinical

applicability and significance of plasma metabolomic analysis in the diagnosis and prognosis of HF. We also sought to determine whether metabolomic analysis provides sensitive evaluation of HF at different stages and in disease regression after therapeutic interventions.

METHODS

PATIENTS AND STUDY DESIGN. For the discovery phase study, patients at HF stages B and C were enrolled from January 2011 to December 2012. Patients at stage A HF and normal control subjects were then enrolled from October 2011 to December 2012. HF stages A, B, and C were classified according to the ACC/AHA HF classification system (5). Stage C patients were those hospitalized due to acute or decompensated chronic HF and ages 20 to 85 years. Exclusion criteria included the following: 1) the presence of systemic disease, such as hypothyroidism, decompensated liver cirrhosis, and systemic lupus erythematosus; 2) the presence of disorders other than HF that might compromise 6-month survival; 3) patients who were bedridden for >3 months and/or unable to stand alone; 4) patients with a serum creatinine of >3 mg/dl; and 5) patients with severe coronary artery disease without complete revascularization therapy. Informed consent was obtained from all patients. The study was designed and carried out in accordance with the principles of the Declaration of Helsinki and with approval from the Ethics Review Board of Chang Gung Memorial Hospital.

The validation phase study set was composed of another independent population that included 63 normal control subjects and 218 patients at stage C HF who were recruited from July 2011 to May 2013. (Online Figure 1 depicts the study flow).

UNTARGETED METABOLIC ANALYSIS. Liquid chromatographic separation for processed plasma was achieved on a 100 × 2.1 mm Acquity 1.7-μm C8 column (Waters Corp., Milford, Massachusetts) using an ACQUITY™ UPLC system (Waters Corp.). The eluent was analyzed via high-definition, time-of-flight mass spectrometry (MS) (SYNAPT G1, Waters Corp.) operating in electrospray ionization-positive ion mode. Raw mass spectrometric data were processed using MassLynx V4.1 and MarkerLynx software (Waters Corp.). The multivariate data matrix was analyzed by SIMCA-P software (version 13.0, Umetrics AB, Umea, Sweden).

STATISTICAL ANALYSES. Results are expressed as the mean ± SD for continuous variables and as the number (percent) for categorical variables. Data were compared by 2-sample or paired Student's *t* tests,

analysis of variance, and chi-square test, when appropriate. Metabolomics analysis was performed using several software programs. Data import and pre-processing steps for targeted MS data were done using TargetLynx (Waters Corp.). The integrated MetIDQ software (Biocrates, Innsbruck, Austria) was applied to streamline data analysis by automated calculation of metabolite concentrations. To maximize identification of differences in metabolic profiles between groups, the orthogonal-projection-to-latent-structure-discriminant-analysis (OPLS-DA) model was applied using the SIMCA-P software (version 13.0, Umetrics AB). The variable importance in the projection (VIP) value of each variable in the model was calculated to indicate its contribution to the classification. A higher VIP value represented a stronger contribution to discrimination among groups. VIP values >1.0 were considered significantly different. Logistic regression analysis was used for diagnosis of HF. Odds ratios and 95% confidence intervals were calculated.

Follow-up data were collected as scheduled or at the last available visit. Cox proportional hazard models were used to determine independent predictors of the first defined events (death or HF-related re-hospitalization) after controlling for covariates. Hazard ratios and 95% confidence intervals were also calculated. All statistical analyses were 2-sided and performed using SPSS software (version 15.0, SPSS, Chicago, Illinois). A p value of <0.05 was considered significant.

Detailed methods are provided in the [Online Appendix](#).

RESULTS

A total of 515 participants included 114 normal control subjects and 401 patients. The discovery phase included 234 participants, including 51 normal control subjects and 183 HF patients at stages A ($n = 43$), B ($n = 67$), and C ($n = 73$) ([Online Figure 1](#)). Baseline characteristics and laboratory data are shown in [Table 1](#). A significant trend of changes was noted in most of the variables for the normal control subjects to patients at HF stages A, B, and C. Compared with the normal control subjects, stage C patients had remarkably higher BNP levels and a wider QRS complex, but they also had lower levels of total cholesterol, low- and high-density lipoprotein cholesterol, sodium, hemoglobin, albumin, and in the estimated glomerular filtration rate. Although there were no significant differences in age among the patient groups, HF patients were older than the normal control subjects. In addition, the percent of men was

higher in the patient groups. Coronary artery disease was the major HF etiology for study patients. The validation phase study included 63 normal control subjects and 218 stage C HF patients; the related demographic characteristic data are shown in [Online Table 1](#).

Initially, for the untargeted metabolomics analysis, 119 plasma samples, from 51 normal control subjects and 68 stage C HF patients from the 234 discovery phase participants, were examined ([Online Figure 1](#)). In the metabolomic profiling, 897 positive-mode features were identified and applied for SIMCA-P analysis. The OPLS-DA score plot and loading plot showed remarkable separation between the control subjects and stage C HF patients ([Online Figure 2](#)). The results of untargeted analysis revealed amino acids and phospholipids to be important discriminators between the normal control subjects and stage C HF patients. Therefore, stable isotope dilution-multiple reaction monitoring MS was performed in all 234 participants to quantify amino acids, biogenic amines, acylcarnitines, and phospholipids in both the discovery phase and validation groups.

To test whether metabolites could discriminate patients from control subjects, plasma from 51 normal control subjects and from 73 stage C HF patients in the discovery phase study were subject to targeted metabolite analysis, and datasets were analyzed by the OPLS-DA model. The OPLS-DA score plots demonstrated considerable separation between the normal control subjects and the stage C HF patients in the first principal component ([Figure 1A](#); x-axis, called $t[1]$), which probably reflected the HF-related pathological variations between the subjects. The metabolites responsible for the discrimination between these 2 groups (those with $VIP >1.5$) are listed in [Table 2](#) and [Online Table 2](#). This model (i.e., the “training set”) was then used to estimate HF status in the following analyses. The calculated value of this model is called $tPS[1]$.

[Figure 1B](#) is the score plot of data corresponding to the age-matched control subjects ($n = 51$) and stage C HF patients ($n = 50$) in the discovery phase study, whose demographic characteristic data are shown in [Online Table 3](#). The distribution of the data in the score plots was similar to that shown in [Figure 1A](#), suggesting that discrimination of the 2 groups was independent of age. On the basis of this training set, patients at stage A ($n = 43$) and stage B ($n = 67$) were calculated by $tPS[1]$ ([Figures 1C and 1D](#), respectively). The score plots of stage B patients showed that the data spanned the regions over the stage C HF patients and the normal control subjects, suggesting the heterogeneity of stage B as defined by the AHA/ACC criteria.

TABLE 1 Patient Characteristics in the Discovery Phase Study

	Control Subjects (n = 51)	Stage A (n = 43)	Stage B (n = 67)	Stage C (n = 73)	p Value for Trend
Age, yrs	55.2 ± 4.4	60.1 ± 10.8	59.9 ± 12.8	64.1 ± 12.8	<0.001
Male	19 (37.3)	35 (81.4)	57 (85.1)	41 (56.2)	0.073
LVEF	72.3 ± 8.0	70.1 ± 8.9	50.5 ± 14.1	37.2 ± 15.6	<0.001
Blood pressure, mm Hg					
Systolic	125.7 ± 15.7	124.3 ± 16.8	123.4 ± 19.9	124.7 ± 19.7	0.742
Diastolic	75.6 ± 12.1	76.7 ± 11.8	77.1 ± 12.8	72.0 ± 12.5	0.156
Heart rate, beats/min	72.3 ± 11.3	76.7 ± 11.3	73.6 ± 9.2	79.5 ± 14.1	0.006
Comorbidity					
Diabetes mellitus	0 (0)	18 (41.9)	22 (32.8)	38 (52.1)	<0.001
Chronic kidney disease	0 (0)	11 (25.6)	16 (23.9)	27 (37)	<0.001
Hypertension	0 (0)	31 (72.1)	48 (71.6)	54 (74.0)	<0.001
Atrial fibrillation	0 (0)	3 (7.0)	7 (10.4)	21 (28.8)	<0.001
COPD	0 (0)	3 (7.0)	2 (3.0)	12 (16.4)	0.002
Ischemic	0 (0)	31 (72.1)	59 (88.1)	44 (60.3)	<0.001
Body mass index, kg/m ²	24.4 ± 3.3	26.1 ± 4.2	25.8 ± 4.3	24.9 ± 4.6	0.601
Medication					
ACEI or ARB	0 (0)	15 (34.9)	48 (71.6)	62 (84.9)	<0.001
Beta-blocker	0 (0)	18 (41.9)	53 (79.1)	51 (69.9)	<0.001
Digoxin	0 (0)	0 (0)	5 (7.5)	21 (28.8)	<0.001
Diuretic agent	0 (0)	4 (9.3)	15 (22.4)	49 (67.1)	<0.001
Statins	0 (0)	21 (48.8)	40 (59.7)	27 (37.0)	<0.001
Fenofibrate	0 (0)	4 (9.3)	1 (1.5)	0 (0)	0.325
Statins/fenofibrate	0 (0)	23 (53.5)	41 (61.2)	27 (37.0)	<0.001
OHA/insulin	0 (0)	15 (34.9)	21 (31.3)	29 (39.7)	<0.001
Laboratory data					
BNP, pg/ml	9.2 ± 8.8	43.0 ± 69.4	209.2 ± 359.7	851.6 ± 793.8	<0.001
Log (BNP)	0.88 ± 0.23	1.34 ± 0.49	1.94 ± 0.60	2.71 ± 0.52	<0.001
Cholesterol, mg/dl	214.7 ± 35.5	175.1 ± 43.0	182.3 ± 41.9	169.2 ± 58.6	<0.001
Triglyceride, mg/dl	99.2 ± 55.9	186.3 ± 110.3	138.4 ± 100.6	120.2 ± 68.6	0.762
LDL-C, mg/dl	139.2 ± 30.1	104.3 ± 37.8	112.9 ± 39.8	108.1 ± 52.1	0.001
HDL-C, mg/dl	55.3 ± 12.9	39.5 ± 11.3	42.2 ± 13.3	38.6 ± 17.8	<0.001
Serum sodium, mEq/l	140.4 ± 1.3	138.5 ± 3.0	139.3 ± 2.2	138.6 ± 4.1	0.006
Hemoglobin, g/dl	13.9 ± 1.2	13.9 ± 1.6	13.8 ± 1.9	12.6 ± 2.1	<0.001
Total bilirubin, mg/dl	0.8 ± 0.3	0.8 ± 0.3	0.8 ± 0.4	1.1 ± 0.6	0.001
Albumin, g/dl	4.4 ± 0.2	4.1 ± 0.3	3.9 ± 0.3	3.5 ± 0.6	<0.001
Hemoglobin A1c, %	5.7 ± 0.3	6.6 ± 1.4	6.5 ± 1.4	6.7 ± 1.5	<0.001
eGFR, ml/min/1.73 m ² *	99.1 ± 19.2	78.6 ± 24.6	79.7 ± 32.2	65.6 ± 27.9	<0.001
QRS complex, ms	89.6 ± 8.6	94.3 ± 15.6	96.9 ± 19.4	106.3 ± 24.9	<0.001

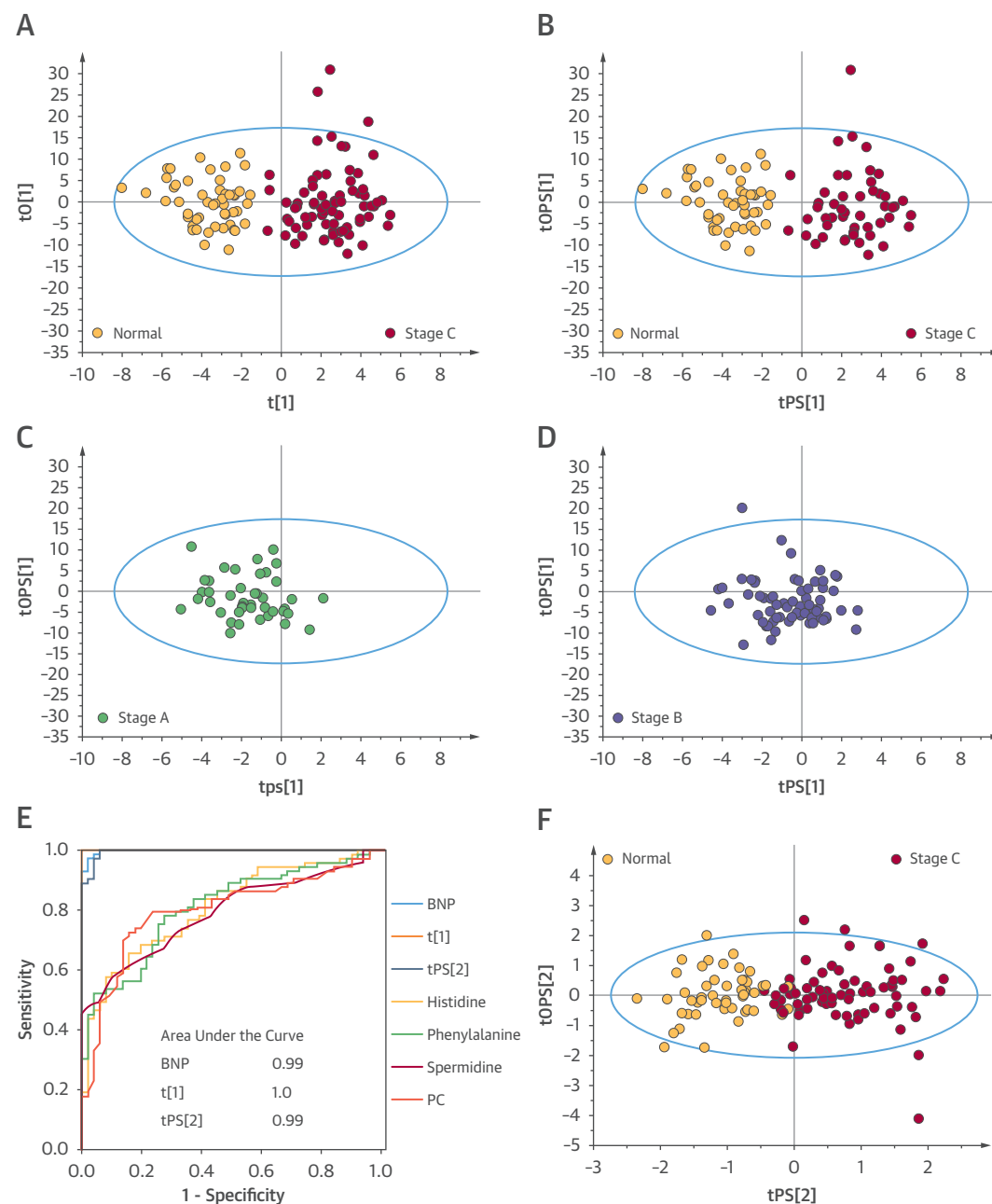
Values are mean ± SD or n (%). *Chronic kidney disease = eGFR <60 ml/min/1.73 m².

ACEI = angiotensin-converting enzyme inhibitor; ARB = angiotensin receptor blocker; BNP = B-type natriuretic peptide; COPD = chronic obstructive pulmonary disease; eGFR = estimated glomerular filtration rate; HDL-C = high-density lipoprotein cholesterol; LDL-C = low-density lipoprotein cholesterol; LVEF = left ventricular ejection fraction; OHA = oral hypoglycemic agents.

To discriminate between the patients at stage C HF and normal control subjects (diagnostic value), the receiver-operating characteristic curves were plotted for both BNP and t[1] (by taking all metabolites into the principal component analysis) (Figure 1E). The areas under the curves (AUCs) were 0.99 and 1.0, respectively. Notably, there were 4 important metabolites that significantly contributed to the diagnostic value for HF, namely, histidine, phenylalanine, spermidine, and phosphatidylcholine C34:4 (once known as “lecithin”) (Table 3). These 4 metabolites gave rise to an AUC of 0.99, which was better than

that of each metabolite alone (Figure 1E). On the basis of the training set composed of these 4 metabolites, the score plot (tPS[2]) distribution of the normal control subjects and the stage C HF patients are shown in Figure 1F. The odds ratio of the BNP and tPS[2] levels for identifying stage C HF (vs. normal control subjects) are presented in Table 3. The diagnostic value of tPS[2] was independent of diabetes mellitus and other comorbidities. The targeted quantitative analysis of the validation set revealed similar diagnostic levels for the 4-metabolite panel as observed in the discovery samples (Table 3, Online Table 4).

FIGURE 1 Diagnostic Values of Metabolomics for Heart Failure



The plasma samples from patients at different heart failure (HF) stages (stages A, B, and C) and normal subjects were collected to determine metabolite concentrations by liquid chromatography tandem mass spectrometry. **(A)** OPLS-DA score plots show the considerable separation between 51 normal control subjects and 73 stage C HF patients. This model (the "training set") was then used to calculate $tPS[1]$ of patients at different stages of HF. **(B)** The OPLS-DA score plots of age-matched stage C patients ($n = 50$) and normal control subjects ($n = 51$). **(C and D)** The OPLS-DA score plots of patients at stages A and B, respectively. The ellipse shown in the model represents the Hotelling T-square test with 95% confidence. **(E)** The ROC curves for the diagnosis of HF by B-type natriuretic peptide (BNP), $tPS[1]$, and $tPS[2]$. **(F)** The OPLS-DA score plots represent the $tPS[2]$ values calculated by combining 4 important metabolites. OPLS-DA = orthogonal-projection-to-latent-structure-discriminant-analysis; ROC = receiver-operating characteristic.

TABLE 2 Statistical Analysis of Targeted Metabolites* Between Control Subjects and Stage C Heart Failure Patients in the Discovery Phase Study

Metabolite, μM	VIP Score	Control Subjects (n = 51)	Stage C (n = 73)	p Value
Histidine	2.47	99.3 \pm 18.0	77.7 \pm 18.4	<0.001
Phenylalanine	2.29	57.4 \pm 8.9	76.9 \pm 22.6	<0.001
Ornithine/arginine	2.27	1.02 \pm 0.23	2.11 \pm 1.32	<0.001
Phosphatidylcholine diacyl C34:4	2.26	0.97 \pm 0.36	0.59 \pm 0.35	<0.001
Ornithine	2.13	56.9 \pm 18.7	84.0 \pm 32.5	<0.001
Phosphatidylcholine diacyl C36:2	2.12	226.4 \pm 58.4	172.9 \pm 52.4	<0.001
Octadecadienyl carnitine	1.99	0.053 \pm 0.034	0.105 \pm 0.070	<0.001
Phosphatidylcholine diacyl C36:1	1.98	44.8 \pm 11.1	35.3 \pm 10.2	<0.001
Log (glutamate)	1.95	1.28 \pm 1.03	1.68 \pm 1.12	<0.001
Phosphatidylcholine diacyl C36:0	1.89	4.98 \pm 1.16	3.85 \pm 1.43	<0.001
Spermidine	1.87	0.25 \pm 0.03	0.37 \pm 0.17	<0.001
Log (spermine)	1.84	-0.93 \pm 0.21	-0.63 \pm 0.39	<0.001
Phosphatidylcholine diacyl C40:5	1.83	9.32 \pm 3.25	6.97 \pm 2.60	<0.001
Phosphatidylcholine diacyl C36:6	1.83	0.91 \pm 0.46	0.56 \pm 0.39	<0.001
Phosphatidylcholine diacyl C33:3	1.80	0.23 \pm 0.06	0.17 \pm 0.07	<0.001
Citrulline/Ornithine	1.79	1.16 \pm 0.73	0.67 \pm 0.51	<0.001
Phosphatidylcholine diacyl C38:5	1.77	44.2 \pm 12.6	33.6 \pm 14.0	<0.001
Creatinine	1.72	103.9 \pm 41.6	185.3 \pm 135.5	<0.001
Phosphatidylcholine diacyl C36:3	1.69	94.0 \pm 21.5	78.1 \pm 21.1	<0.001
Sphingomyelin C20:2	1.68	1.12 \pm 0.29	0.87 \pm 0.35	<0.001
Phosphatidylcholine diacyl C38:3	1.68	31.1 \pm 8.5	24.9 \pm 8.2	<0.001
Phosphatidylcholine acyl-alkyl C38:6	1.65	7.10 \pm 2.28	5.52 \pm 2.14	<0.001
Putrescine/ornithine	1.64	0.008 \pm 0.005	0.005 \pm 0.003	0.001
Phosphatidylcholine acyl-alkyl C34:2	1.64	8.30 \pm 2.46	6.56 \pm 2.37	<0.001
Aromatic amino acids	1.62	163.9 \pm 24.6	193.3 \pm 49.8	<0.001
Hydroxybutyrylcarnitine	1.61	0.030 \pm 0.015	0.049 \pm 0.032	<0.001
Phosphatidylcholine diacyl C40:4	1.61	2.76 \pm 0.83	2.22 \pm 0.71	<0.001
Phosphatidylcholine acyl-alkyl C36:2	1.57	9.79 \pm 2.23	8.09 \pm 2.63	<0.001
Hexose	1.57	5,285 \pm 1,986	6,993 \pm 2,814	<0.001
Phosphatidylcholine acyl-alkyl C36:3	1.56	6.4 \pm 1.8	5.2 \pm 1.7	<0.001
Octadecenoylcarnitine	1.54	0.12 \pm 0.07	0.18 \pm 0.10	<0.001
Butyrylcarnitine	1.52	0.23 \pm 0.06	0.35 \pm 0.23	<0.001

Values are mean \pm SD. *Includes metabolites with a VIP score >1.5 .
VIP = variable importance in the projection.

TABLE 3 Univariable and Multivariable Analyses of the Associations of BNP and Metabolites With Heart Failure Diagnosis

	Discovery Phase			Validation		
	Odds Ratio	95% CI	p Value	Odds Ratio	95% CI	p Value
Univariable analysis						
Log (BNP) ($\times 10^{-2}$)	1.09	1.03-1.14	0.001	1.04	1.03-1.06	<0.0001
Histidine, μM	0.93	0.91-0.96	<0.0001	0.88	0.84-0.92	<0.0001
Phenylalanine, μM	1.11	1.06-1.16	<0.0001	1.20	1.12-1.26	<0.0001
PC aa C34:4, $\mu\text{M} \times 10^{-1}$	0.76	0.67-0.85	<0.0001	0.78	0.71-0.85	<0.0001
Spermidine, $\mu\text{M} \times 10^{-1}$	6.64	2.98-14.79	<0.0001	22.74	6.94-74.49	<0.0001
tPS[2] (4 metabolites: histidine, phenylalanine, PC aa C34:4, spermidine) ($\times 10^{-1}$)	2.72	1.44-5.14	<0.0001	2.71	2.09-3.50	<0.0001
Multivariable analysis*						
Log (BNP) ($\times 10^{-2}$)	1.16	1.01-1.34	0.048	1.08	1.03-1.12	<0.0001
tPS[2] (4 metabolites: histidine, phenylalanine, PC aa C34:4, spermidine) ($\times 10^{-1}$)	4.19	1.23-14.34	0.02	2.88	2.01-4.13	<0.0001

The range of tPS[2], values calculated by the combination of 4 metabolites, is from -2.5 to 2.65 (on the basis of logistic regression model). *Multivariable analysis adjusting for age, sex, diabetes mellitus, chronic kidney disease, and hypertension.
CI = confidence interval; PC aa = phosphatidylcholines diacyl; other abbreviations as in Table 1.

The metabolites that changed at different stages are shown in [Table 2](#) and [Online Table 2](#). These metabolites included amino acids, biogenic amines, and phospholipids. Compared with the control subjects, several plasma metabolites related to arginine metabolism, such as glutamine and citrulline, were lowered in stage C HF patients, but levels of glutamate, ornithine, spermine, and spermidine were elevated. Aromatic amino acids, such as tyrosine and phenylalanine, were elevated in stage C HF patients, although levels of several phosphatidylcholines were decreased, whereas taurine was increased. The metabolites with significant changes were mapped onto 7 biochemical pathways ([Central Illustration](#)).

To estimate the prognostic value of metabolomics and BNP, the following analyses focused on HF patients at stages B and C ($n = 140$). During a follow-up of 1.3 ± 0.8 years, there were 18 all-cause deaths and 29 HF-related re-hospitalizations. To identify any potential metabolic predictors of a composite of all-cause death and HF-related re-hospitalization, extensive analyses on the whole metabolite dataset were conducted. A combination of 4 metabolite components (dimethylarginine/arginine ratio, spermidine, butyrylcarnitine, and total essential amino acid amount) gave rise to an optimal prognostic value that was remarkably better than BNP. The tPS[3] was calculated from these 4 metabolite components and the AUCs of receiver-operating characteristics curves for tPS[3], tPS[1], and BNP levels were 85%, 78%, and 74%, respectively ([Figure 2A](#)). [Table 4](#) and [Online Table 5](#) show the Cox univariable and multivariable analyses for these parameters on the prognosis. The prognostic value of tPS[3] was still highly significant even after adjusting for BNP levels, age, left ventricular ejection fraction, diabetes mellitus, chronic kidney disease, and hypertension. The validation study reconfirmed these findings ([Table 4](#), [Online Table 5](#), [Figure 2D](#)).

The mean of tPS[3] (2.9, range 0.04 to 5.63) was set as the cutoff value for the prognostic prediction. In [Figure 2B](#), the Kaplan-Meier curves revealed that a tPS[3] of ≥ 2.9 at pre-discharge was associated with a higher composite of HF-related re-hospitalization and all-cause death (log rank = 17.5; $p < 0.0001$). In comparison, the Kaplan-Meier curves of patients categorized according to BNP (cutoff value set at 350 pg/ml) ([8](#)) are shown in [Figure 2C](#) (log rank = 9.9; $p = 0.002$). The targeted quantitative analysis for the 4 metabolite components of the validation phase set revealed better prognostic levels than BNP than that observed in the discovery samples ([Figures 2D to 2F](#)).

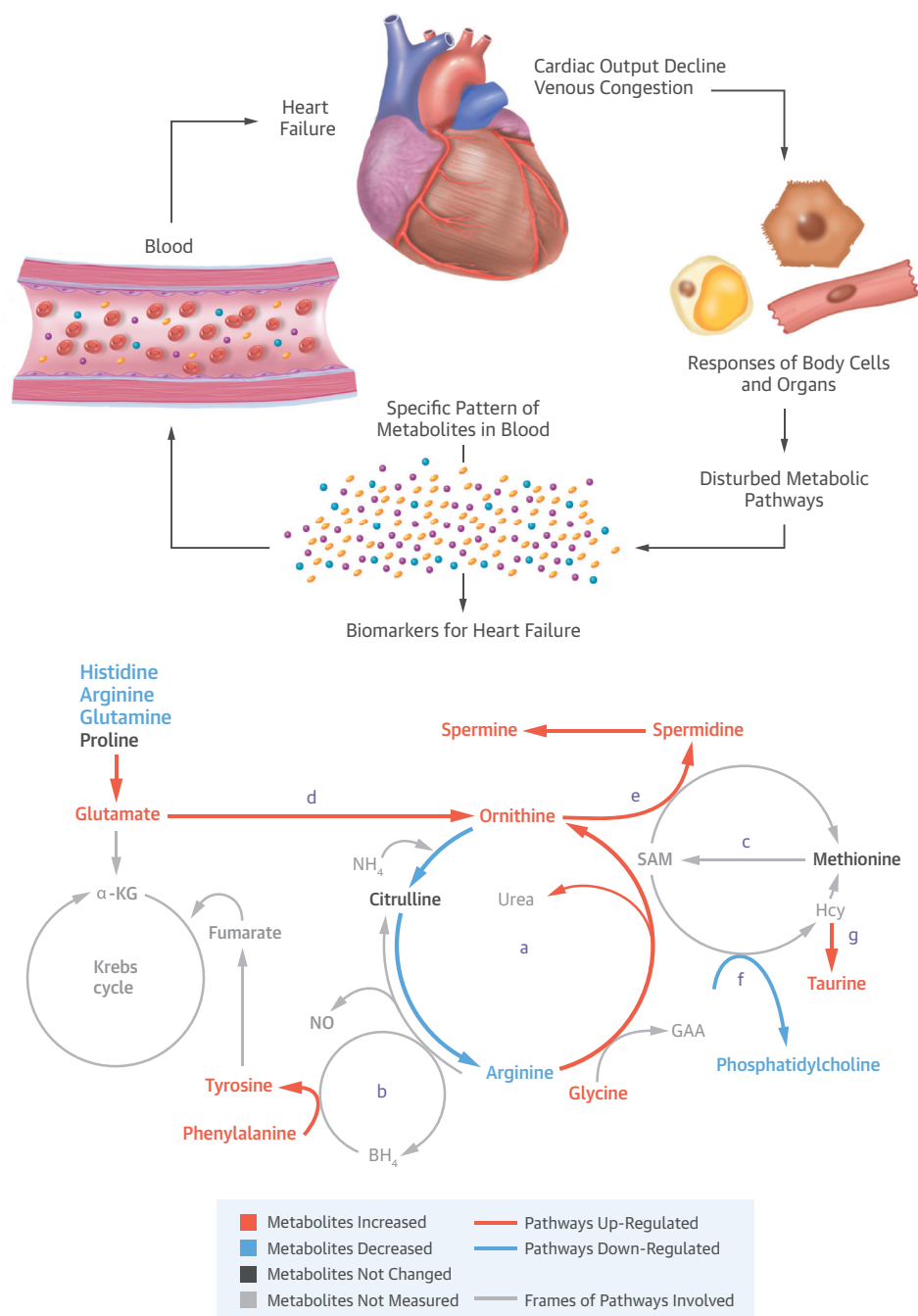
The correlation between tPS[2] and BNP levels was modest ($r = 0.53$; $p < 0.001$) ([Figure 3A](#)). To verify the diagnostic value of tPS[2], metabolomics analysis, together with BNP measurement, was also performed in 32 patients (22 men and 10 women; age 54 ± 11 years) who were recruited earlier than this study's enrollment period. These patients, named the "recovery" group, were initially hospitalized due to acute or decompensated chronic HF, but then they improved to New York Heart Association functional class I and survived for >1 year. Plasma was analyzed before discharge and again at 6 and 12 months post-discharge. The serial changes in tPS[2] and BNP values are shown in [Figure 3B](#). The tPS[2] values for the 32 patients at pre-discharge stage were significantly higher than those of the normal control subjects ($p < 0.001$). Compared with pre-discharge levels, both the values of tPS[2] and BNP remarkably decreased at 6 months and remained steady at 12 months after discharge ($p < 0.001$).

DISCUSSION

In the present study, we demonstrated that metabolomics is a promising tool for the diagnosis and prognosis of HF. Plasma concentrations of metabolites, such as histidine, phenylalanine, ornithine, spermine, spermidine, phosphatidylcholines, and taurine, show significant differences among patients at different stages of HF. Combinations of metabolites are as good as the conventional biomarker BNP for diagnosis and better than BNP for prognosis. The value of estimating metabolic profiles in HF is far beyond diagnosis only, as demonstrated in the validation study. In contrast, the common changes in plasma metabolites identified in HF patients with different etiologies suggest that HF development may involve a universal metabolic disturbance related to HF-associated dysfunction in multiple organs ([9](#)). A number of pathways, namely, the glutamate-ornithine-proline pathway, polyamine synthesis, and phosphatidylcholines synthesis, may be specifically affected during HF progression ([Central Illustration](#)).

The key metabolites that show significant differences in abundance between stages C and A HF, between stage C HF and normal control subjects, and between the acute phase and 6 or 12 months later in the recovery group are histidine, phenylalanine, spermidine, and phosphatidylcholine C34:4. These metabolites are mapped to biochemical pathways ([Central Illustration](#)). Anomalous fluxes through these metabolic pathways may show the interplay between metabolism in multiple tissues and organs and

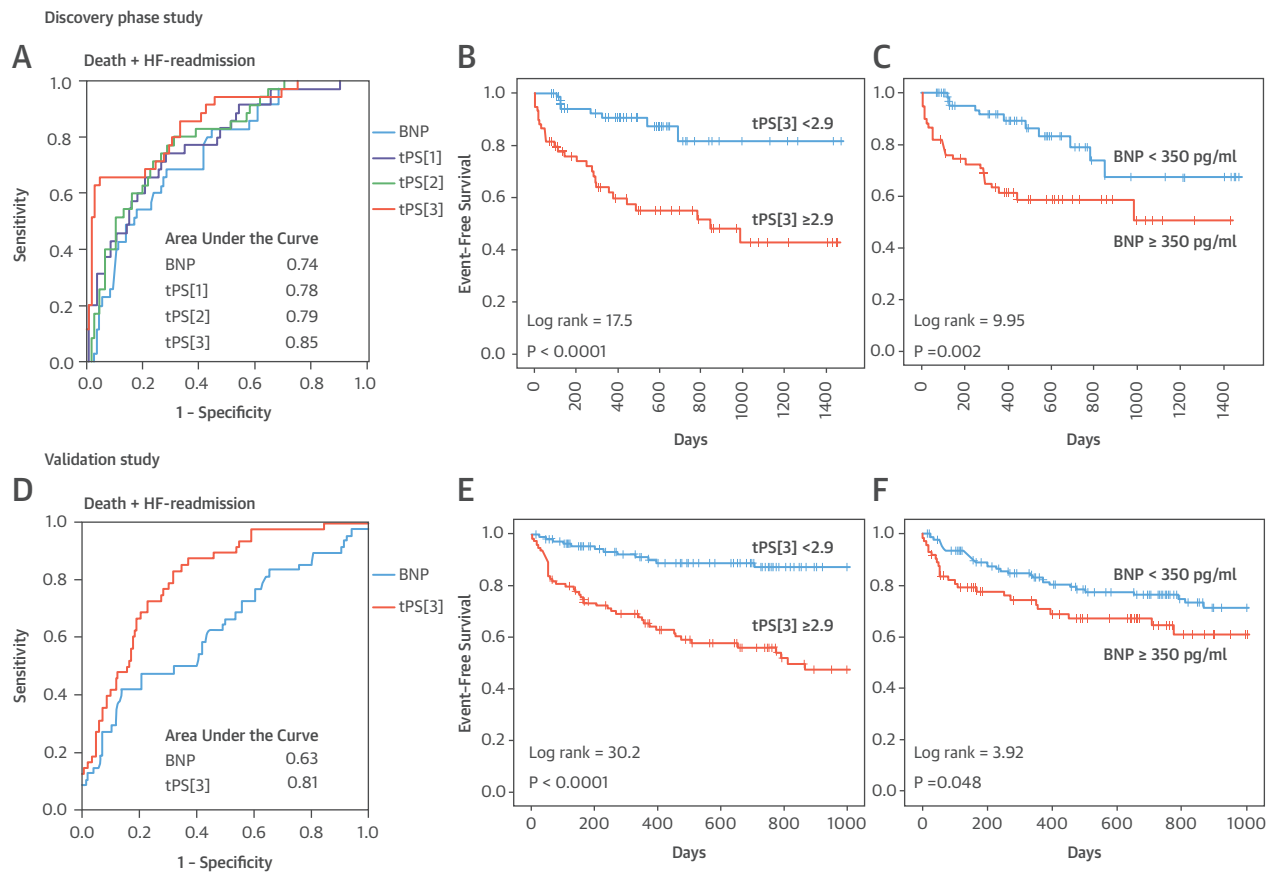
CENTRAL ILLUSTRATION Metabolic Pathways Implicated in HF Pathogenesis



Cheng, M-L. et al. J Am Coll Cardiol. 2015; 65(15):1509-20.

(Top) The proposed scheme indicates that the decrease in cardiac output leads to disturbances of the metabolic pathways and the appearance of specific metabolites in the circulation of heart failure (HF) patients. These metabolites probably serve as biomarkers for HF. **(Bottom)** Disturbances occur in the following metabolic pathways: **(a)** urea cycle, **(b)** bipterin cycle, **(c)** MTA/methionine cycle, **(d)** ornithine-proline-glutamate, **(e)** polyamine synthesis, **(f)** methylation (phosphatidylcholine), and **(g)** transsulfuration (taurine) in HF patients. Metabolites marked in **salmon, blue, black, and gray** indicate metabolites, the abundance of which significantly increased, significantly decreased, remained unchanged, and were not measured, respectively. α -KG = alpha-ketoglutarate; BH_4 = tetrahydrobiopterin; GAA = guanidinoacetate; Hcy = homocysteine; MTA = 5-methylthioadenosine; NO = nitric oxide; PE = phosphatidylethanolamine; SAM = S-adenosylmethionine.

FIGURE 2 Prognostic Values of Metabolomics



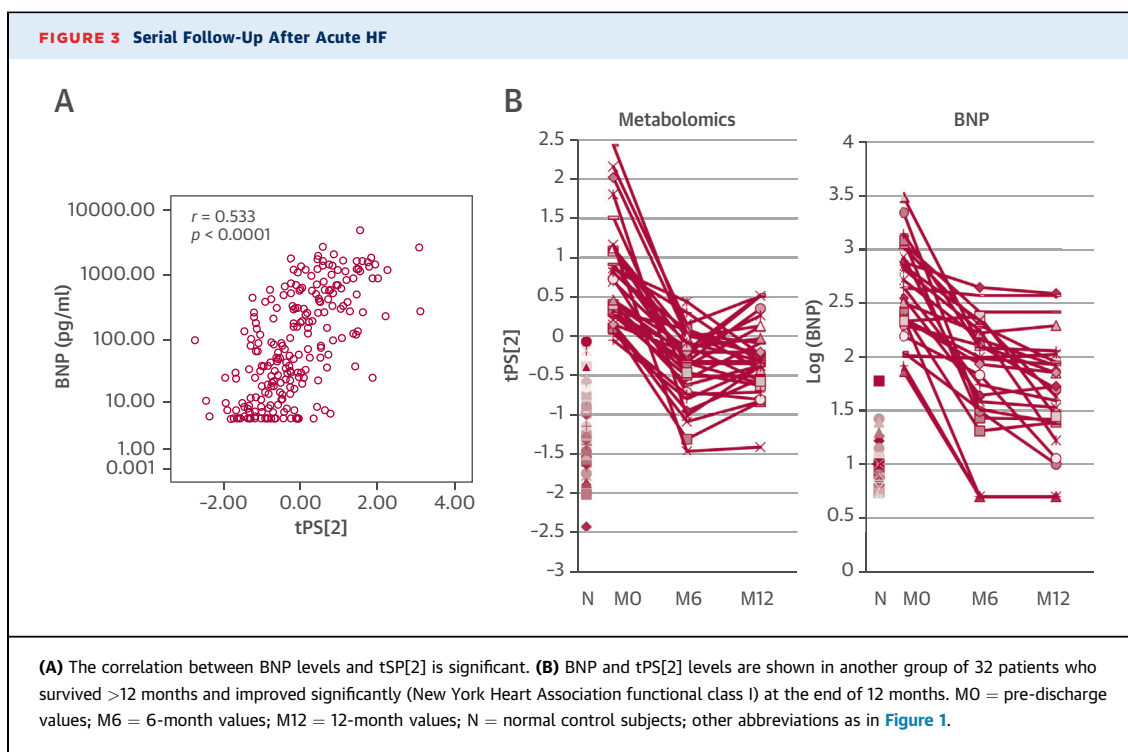
(A and D) The ROC curves for comparing the prognostic values of BNP, tPS[1], tPS[2], and tPS[3] in the discovery phase study; and BNP and tPS[3] in the validation studies. (B, C, E, and F) The Kaplan-Meier curves of tPS[3] and BNP for predicting a composite event of all-cause death and HF-related re-hospitalization in the discovery phase and the validation studies. Abbreviations as in Figure 1.

TABLE 4 Univariable Analysis on Prognostic Value in HF Patients

	Discovery Phase			Validation		
	HR	95% CI	p Value	HR	95% CI	p Value
Log (BNP) ($\times 10^{-1}$)	1.12	1.06-1.21	0.003	1.06	1.01-1.12	0.034
t[1] ($\times 10^{-1}$)	1.86	1.30-2.65	0.001			
tPS[2] ($\times 10^{-1}$)	1.98	1.43-2.74	<0.0001			
Total DMA/arginine ratio, μ M	1.85	1.49-2.29	<0.0001	1.42	1.05-1.94	0.02
Spermidine, μ M $\times 10^{-1}$	1.32	1.03-1.69	0.02	2.38	1.22-4.76	0.01
C4, μ M	1.36	1.08-1.71	0.01	6.23	2.51-15.49	<0.0001
Total essential amino acids (μ M)	0.66	0.45-0.97	0.03	0.93	0.91-0.94	0.001
tPS[3] (4 metabolites: total DMA/arginine ratio, spermidine, C4, total essential amino acids) ($\times 10^{-1}$)	2.78	2.04-3.78	<0.0001	3.08	2.19-4.33	<0.0001

The ranges of t[1] and tPS[2] are from -2.64 to 2.5 and from -2.5 to 2.65, respectively. The ranges of tPS[3]s are from 0.04 to 5.6 and from 1.2 to 6.3 for the discovery phase and validation studies, respectively (on the basis of regression models).

C4 = butyrylcarnitine; DMA = dimethylarginine; HR = hazard ratio; t[1] = values calculated by all metabolomics analysis; tPS[2] = values calculated by 4 metabolites for diagnosis; other abbreviations as in Tables 1 and 3.



the inadequate cardiac output in HF patients, together with the impact of systemic congestion. Plasma metabolomics represent the metabolic view of the so-called cardio-hepatic interaction, cardiorenal syndrome, and catabolic status in the muscular system. Keeping in line with the main findings, levels of histidine and phosphatidylcholine C34:4 increased, whereas levels of phenylalanine and spermidine decreased in patients whose medical condition improved.

Application of tracer-based metabolic flux analysis on patients is associated with technical feasibility and ethical issues. Despite the lack of flux analysis, our metabolomics data still provided an excellent account of metabolic changes in patients.

Mitochondrial dysfunction and a shift from fatty acid to glucose utilization for energy production are the main metabolic changes seen in the failing heart. Histidine, which is capable of being converted to glutamate, enters the glutamate-ornithine-proline pathway or Krebs cycle as alpha-ketoglutarate, which supplies ornithine and energy for cardiac tissues. Glucose can be converted to phosphoribosyl pyrophosphate, which is essential to histidine biosynthesis. Over-consumption of glucose as an energy source may impair the cellular ability to replenish histidine deficiency, which is probably caused by inadequate intake. Uncoupling of nitric oxide

synthase may involve the pathogenesis of HF (10). Induction of nitric oxide synthase-2 and concurrent tetrahydrobiopterin depletion in the myocardium contribute to HF in animal models and human subjects (10,11). Consistent with such notions, accumulation of phenylalanine and tyrosine in HF patients during progression from stage A to C HF are indicative of tetrahydrobiopterin depletion. This occurs in parallel with a significant decrease in arginine levels, which may disturb nitric oxide (NO) production and lead to cardiac dysfunction (12). In contrast, the increased phenylalanine and tyrosine levels may also be associated with the accumulation of aromatic amino acids caused by increased muscular protein breakdown and impaired liver function.

Our study showed the stage-specific elevation of plasma polyamine metabolites, namely, spermidine and spermine. The polyamine and NO pathways are inter-regulated in several experimental models. NO inhibits ornithine decarboxylase and polyamines production (13,14). Ornithine, the upstream precursor to polyamines formation, can be produced from arginine by arginase or by arginine:glycine amidinotransferase. The decrease in arginine and the accumulation of ornithine in stage C HF patients may be associated with impaired NO synthesis and enhanced polyamine synthesis. The ornithine level

can be further increased as a result of a dysfunctional urea cycle in the liver. The involvement of polyamines in the pathogenesis of cardiac diseases is not unprecedented. Polyamines increase in cardiac tissue after ascending aortic stenosis and in various models of experimentally-induced cardiac hypertrophy (15,16). Moreover, spermidine has been shown to be detrimental to cardiomyocytes under hypoxic stress (17).

Reduction in plasma levels of most phosphatidylcholines in HF patients is intriguing. Previous studies reported alteration of lipid homeostasis, including changes in metabolism of phosphatidylcholines, in associated cardiomyopathies (18,19). Disturbances in the ratio of phosphatidylcholine to phosphatidylethanolamine and anomalies in membrane phospholipid homeostasis alter the membrane-associated protein complexes interactions that regulate myocardial metabolism and cell signaling (20). In contrast, because the liver plays a major role in phosphatidylcholine metabolism and lipoprotein secretion, anomalous phosphatidylcholine metabolism may be associated with abnormal hepatic functions in the failing heart (21). A congested liver is common in patients with congestive HF; it remains to be elucidated whether abnormal liver function plays an active role in HF pathogenesis or the liver merely responds passively to the remarkably increased metabolic input from nonhepatic tissues and organs.

Plasma metabolome is indicative of HF-associated global changes in metabolism rather than that of the heart alone, and likely involves interactions between various tissues and organs. As shown in our study, the limited correlation between BNP and tPS[2] suggests either novel HF-related metabolic pathways or general markers of poor status. Previous studies identified a few specific metabolic profiles of liver dysfunction, including elevated bile acids and decreased lysophosphatidylcholines and a decreased Fischer ratio, which is the molar ratio of plasma branched chain amino acids levels to aromatic amino acids levels (22).

Although some of these changes were also noted in our patients (Online Table 2), the main metabolic profile discovered in our study is distinct. Metabolic signatures are different between HF and non-HF cachexia. Cachexia related to chronic lung disease and cancer, for example, has been associated with increases in very low- and low-density lipoproteins (23), glutamine, aspartate, arginine, and asparagine, as well as decreases in amino adipate, beta-aminoisobutyrate, and 1-methylhistidine (24). Comparison of these reports and our findings suggest

that the metabolic profiles described in our study are specific to HF. However, the specificity of our profiles for both diagnostic and prognostic values needs to be assessed in patients with non-HF diseases or dyspnea. In contrast, the same AUCs of BNP and tPS[2] suggest that metabolomics is highly valuable for evaluating HF-related metabolic disturbances. It opens a new avenue for assessing patient response to nutritional interventions.

Our metabolomic findings demonstrate important clinical applications beyond diagnosis. Another metabolite set consisting of 4 metabolite components provides a better prognostic value than the conventional biomarker, BNP. This metabolic profile covers different aspects of pathogenesis. For instance, the total dimethylarginine/arginine ratio is indicative of endothelial dysfunction (25), spermidine is probably indicative of rescuing compensation or toxicity to cardiomyocytes, butyrylcarnitine is indicative of anomalous lipid and energy metabolism, and total essential amino acids is indicative of malnutrition. These findings raise the possibility of adjunctive nutritional therapy to improve the prognosis of patients with severe HF.

STUDY LIMITATIONS. Because systemic and myocardial metabolism can shift rapidly in response to HF-related pathophysiology and stress, the present study did not address the changes in patient metabolic profiles at different time points within a short period after hospital discharge. For better data interpretation and clinical applications in the future, the dynamics and stability of metabolomic profiles need to be established.

CONCLUSIONS

HF is associated with a variety of abnormalities in multiple metabolic pathways and subsequent occurrence of a complex “metabolic storm.” Apart from the understanding of pathogenesis, the profile of metabolites provides a more sensitive and better evaluation for HF staging than that defined by current classification schemes, paving the way for monitoring the outcome of therapeutic interventions.

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PERSPECTIVES

COMPETENCY IN MEDICAL KNOWLEDGE:

Plasma biomarkers that identify abnormalities of cardiac metabolism in patients with HF have prognostic value beyond that of natriuretic peptide levels or conventional clinical risk factors.

TRANSLATIONAL OUTLOOK:

Additional clinical studies are needed to examine the effects of nutritional and other interventions that target specific cardiac metabolic disturbances on clinical outcomes in patients with HF and their correlations with plasma levels of metabolic biomarkers.

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KEY WORDS B-type natriuretic peptide, diagnosis, liquid chromatography-mass spectrometry, prognosis

APPENDIX For supplemental text, figures, and tables, please see the online version of this article.