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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 interventions. 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 TM 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,