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Targeted Metabolomic Profiling of Plasma and Survival in Heart Failure Patients

David E. Lanfear, MD, MS,[a,b] Joseph J. Gibbs, MD,[a] Jia Li, PhD,[c] Ruicong She, MS,[c] Christopher Petucci, PhD,[d] Jeffrey A. Culver, MS,[d] W.H. Wilson Tang,[e] Yigal M. Pinto,[f] L. Keoki Williams,[b] Hani N. Sabbah,[a] Stephen J. Gardell, PhD[d]

ABSTRACT

OBJECTIVES This study sought to derive and validate plasma metabolite associations with survival in heart failure (HF) patients.

BACKGROUND Profiling of plasma metabolites to predict the course of HF appears promising, but validation and incremental value of these profiles are less established.

METHODS Patients (n = 1,032) who met Framingham HF criteria with a history of reduced ejection fraction were randomly divided into derivation and validation cohorts (n = 516 each). Amino acids, organic acids, and acylcarnitines were quantified using mass spectrometry in fasting plasma samples. We derived a prognostic metabolite profile (PMP) in the derivation cohort using Lasso-penalized Cox regression. Validity was assessed by 10-fold cross validation in the derivation cohort and by standard testing in the validation cohort. The PMP was analyzed as both a continuous variable (PMPscore) and dichotomized at the median (PMPcat), in univariate and multivariate models adjusted for clinical risk score and N-terminal pro-B-type natriuretic peptide.

RESULTS Overall, 48% of patients were African American, 35% were women, and the average age was 69 years. After a median follow-up of 34 months, there were 256 deaths (127 and 129 in derivation and validation cohorts, respectively). Optimized modeling defined the 13 metabolite PMPs, which was cross validated as both the PMPscore (hazard ratio [HR]: 3.27; $p < 2 \times 10^{-16}$) and PMPcat (HR: 3.04; $p = 2.93 \times 10^{-8}$). The validation cohort showed similar results (PMPscore HR: 3.9; $p < 2 \times 10^{-16}$ and PMPcat HR: 3.99; $p = 3.47 \times 10^{-9}$). In adjusted models, PMP remained associated with mortality in the cross-validated derivation cohort (PMPscore HR: 1.63; p = 0.0029; PMPcat HR: 1.47; p = 0.081) and the validation cohort (PMPscore HR: 1.54; p = 0.037; PMPcat HR: 1.69; p = 0.043).

CONCLUSIONS Plasma metabolite profiles varied across HF subgroups and were associated with survival incremental to conventional predictors. Additional investigation is warranted to define mechanisms and clinical applications. (J Am Coll Cardiol HF 2017;5:823-32) © 2017 by the American College of Cardiology Foundation.

Despite the cumulative success of neurohormonal interventions, heart failure (HF) remains an enormous health problem with a substantial residual disease burden that exhibits a wide range in the course of the disease and response to treatment (1,2). Powerful risk prediction models of survival have been produced using clinical risk scores and natriuretic peptides (3,4), but crucial knowledge gaps still exist regarding variability in the course of disease, the additional biological axes at play, and

From the [a]Heart and Vascular Institute, Henry Ford Hospital, Detroit, Michigan; [b]Center for Health Policy and Health Services Research, Henry Ford Hospital, Detroit, Michigan; [c]Department of Public Health Sciences, Henry Ford Hospital, Detroit, Michigan; [d]Sanford Burnham Prebys Medical Discovery Institute, Orlando, Florida; [e]Department of Cardiovascular Medicine, Cleveland Clinic Prevention Research Laboratory, Cleveland Clinic, Cleveland, Ohio; and the [f]Department of Cardiology, University of Amsterdam, Amsterdam, the Netherlands. This research was supported by the National Heart, Lung, and Blood Institute (NHLBI) (Lanfear R01HL103871, R01HL132154). Dr. Williams is supported by the NHLBI (R01HL118267), the National Institute of Allergy and Infectious Diseases (R01AI079139), and the National Institute of Diabetes and Digestive and Kidney Diseases (R01DK064695). Dr. Sabbah's work is supported by the NHLBI (PO1HL074237, R01HL132154). Drs. Gardell and Petucci are supported by the NIH Common Fund (Southeast Center for Integrated Metabolomics U24 DK097209). Dr. Pinto owns stock in ACS Biomarker. Dr. Williams has been a consultant for Merck & Co. All other authors have reported that they have no relationships relevant to the contents of this paper to disclose.

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ABBREVIATIONS AND ACRONYMS

6MWD = 6-min walking distance

AA = amino acid

AC = acylcarnitine

EF = ejection fraction

GC = gas chromatography

HF = heart failure

HFrEF = heart failure with reduced ejection fraction

MAGGIC = Meta-Analysis Global Group in Chronic Heart Failure

NT-proBNP = N-terminal pro-B-type natriuretic peptide

NYHA = New York Heart Association

OA = organic acid

PMP = prognostic metabolite profile

our limited ability to recognize pathophysiological subgroups that include the total HF population.

Our ability to biologically characterize patients has increased due to powerful technology platforms such as genomics and proteomics, which offer new hope of answering this challenge. A more recently maturing platform is metabolomics, which measures the levels of many small molecules of intermediary metabolism (i.e., metabolites) in tissues or fluids. Metabolomics is increasingly used to probe organ function and dysfunction, as well as to identify novel disease pathways. An early application of metabolomics profiling has been used in cardiovascular disease (5–7), with a focus on coronary syndromes (8–10). Similarly, there are growing data derived from the setting of HF, in which specific plasma metabolite levels are reported to be associated with incident disease or risk of death (11–15). Recently, a few systematic evaluations of the metabolites in HF have been published that further support the overall hypothesis that circulating metabolites may be deranged in the setting of HF, which may reflect the underlying disease state (16–18). Larger scale validation of the plasma metabolome regarding prognostic value, or as a way to stratify HF phenotypes, is still urgently needed.

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We profiled a pool of targeted metabolites in plasma obtained from an adequately sized group of patients with HF and HF with reduced ejection fraction (HFrEF) to describe phenotypic associations with the circulating metabolome, unveil plasma metabolites associated with mortality, and assess their risk association incremental to established predictors.

METHODS

PATIENTS. This study was conducted at Henry Ford Hospital and was approved by the study site institutional review board. All patients provided written informed consent at time of enrollment. The Henry Ford Heart Failure Pharmacogenomic registry has enrolled >1,700 HF patients of any type and collected blood and/or plasma samples and detailed phenotypic information. Patients enrolled in the registry are required to meet Framingham criteria for diagnosis of HF, including 2 major criteria (presence of paroxysmal nocturnal dyspnea, neck vein distention, rales, radiographic cardiomegaly, acute pulmonary edema, an S3 gallop, elevated central venous pressure, positive hepatojugular reflux, or sufficient weight loss with diuresis) or 1 major with 2 minor criteria (bilateral lower extremity edema, nocturnal cough, dyspnea with usual activity, hepatomegaly, pleural effusions, tachycardia, or decreased vital capacity). Registry participants are also required to have had an assessment of ejection fraction (EF) before enrollment, and patients on long-term dialysis were excluded.

For the present study, all registry patients with an EF <50% at the time of HF diagnosis were included (n = 1,070) and underwent metabolomic profiling of stored plasma (detailed in the following). Among these patients, we restricted analysis to those with information on N-terminal pro-B-type natriuretic peptide (NT-proBNP) levels and complete data for calculation of the MAGGIC (Meta-Analysis Global Group in Chronic Heart Failure) score. Thus, an analytical cohort of 1,032 patients were ultimately included in the study. This entire group was used for testing the association of metabolites with demographic and clinical characteristics. For the survival analyses, the total study group was divided randomly in a 1:1 ratio (n = 516 each) into a derivation cohort and a validation cohort.

MAGGIC scores were calculated as described in the original work, using 13 routinely available clinical factors, including age, sex, comorbid diabetes or chronic obstructive pulmonary disease, creatinine, duration of HF, tobacco abuse, current medications (angiotensin-converting enzymes inhibitors/angiotensin receptors blockers or beta-blockers), systolic blood pressure, EF, body mass index, and New York Heart Association (NYHA) functional class (3). Data used to populate the MAGGIC score were derived from direct abstraction of the medical record or from administrative data, including claims data. The duration of HF diagnosis was the time elapsed between first diagnostic code for HF in the system and date of study enrollment; this was categorized as more than or less than 18 months for MAGGIC score calculation. The NT-proBNP levels were measured on stored plasma samples, which were obtained at study enrollment and were frozen at −70°C until processing. These were quantified using an immunoelectrochemiluminescence assay on the Modular Analytics E 170 system (Roche Diagnostics, Basel, Switzerland). This assay has <0.001% cross reactivity with bioactive BNP.

METABOLOMIC STUDIES. Quantitative targeted metabolite profiling of individual amino acids (AAs), organic acids (OAs), and acylcarnitines (ACs) was performed at the Sanford Burnham Prebys Medical Discovery Institute using high pressure liquid chromatography/mass spectrometry or gas chromatography (GC)/mass