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Original article

# Metabolic profile provides prognostic value better than galectin-3 in patients with heart failure

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ABSTRACT

*Background:* Metabolic profiles have been shown to provide prognostic information in patients with heart failure (HF). Galectin-3 (Gal-3), indicating cardiac fibrosis, is a documented biomarker of prognosis in HF. It is unknown whether metabolic profiles provide prognostic value better than Gal-3.
*Methods and results:* This study analyzed 212 hospitalized HF patients, measuring metabolic score (composed by butyrylcarnitine, dimethylarginine/arginine ratio, spermidine, and total essential amino acids) and Gal-3. Endpoints were composite events (death/HF-related re-hospitalization). The median of metabolic scores and Gal-3 levels were 3.1 (1.3–5.2) and 17.8 ng/mL (4.7–100 ng/mL), respectively. Patients with higher metabolic scores had worse functional classes, higher atrial fibrillation incidences, levels of Gal-3 and B-type natriuretic peptide (BNP), but lower albumin levels and glomerular filtration rate. Correlations of metabolic score to Gal-3 and BNP were significant, but weak ($r = 0.34$ and 0.41, respectively, both $p < 0.001$). During a follow-up period of $4.2 \pm 1.4$ years, there were 91 (42.9%) composite events. In univariate analysis, significant predictors of composite events were age, functional class, atrial fibrillation, levels of hemoglobin, log (Gal-3), log (BNP) and metabolic score. In multivariable analysis, adjusted for above variables, metabolic score remained a strong predictor of combined endpoints (hazard ratio = 2.596, 95% confidence interval = 1.649–4.087, $p < 0.001$). C-statistics for the prediction of composite events significantly increased when metabolic score was incorporated into the model with established risk factors, BNP and Gal-3 [0.76 (0.70–0.83) vs. 0.66 (0.58–0.74), $p = 0.032$].
*Conclusions:* Metabolic profile provides prognostic value for HF patients better than Gal-3.


## Introduction

Heart failure (HF) represents the end stage of various cardiovascular diseases, affecting approximately 26 million people worldwide [1]. Although substantial advances have been made, short- and long-term HF-related re-hospitalization and mortality remain high and consume tremendous healthcare resources [2]. The limited effectiveness of current treatment strategies indicates a need for novel assessment tools for phenotyping, risk stratification, and novel interventions.

B-type natriuretic peptide (BNP) and amino-terminal pro-BNP have been recognized as biomarkers for diagnosis and prognosis of HF [3,4]. However, galectin-3 (Gal-3), a peptide indicating cardiac fibrosis, has emerged as a biomarker with powerful prognostic value in HF patients [5–7]. Gal-3 is higher in the serum of patients with acute decompensated HF, and has been shown to provide additional prognostic value over natriuretic peptides for predicting short-term mortality.

Accumulated evidence suggests that HF is associated with metabolic dysfunction [8,9]. Advances in metabolite analysis and bio-informatics techniques have enabled efficient metabolite profiling for a few systemic diseases [10–13]. Previously, a metabolic profile has been developed to estimate metabolic

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abnormalities and provide robust prognostic information for patients with HF [14]. The prognostic metabolic profile consists of four components: butyrylcarnitine, dimethylarginine (DMA)/arginine ratio, spermidine, and total essential amino acids, representing different facets of metabolic disturbances. They are associated with anomalous lipid and energy metabolism, endothelial dysfunction, pathological remodeling, and nutritional status, which also involve systemic inflammation and fibrosis. Gal-3 can be viewed as a regulatory molecule of inflammation and tissue fibrogenesis [15]. This study would like to unravel whether the prognostic value of metabolic profiling is not only independent of but also better than Gal-3.

## Methods

### *Patients and study design*

From January 2009 to June 2010, patients hospitalized due to acute or decompensated chronic HF, and aged 20–85 years, were consecutively enrolled. Exclusion criteria included: (i) the presence of systemic diseases such as hypothyroidism, decompensated liver cirrhosis, and systemic lupus erythematosus; (ii) the presence of disorders other than HF that might compromise survival within 6 months; (iii) patients being bed-ridden for >3 months and/or unable to stand alone; (iv) patients with a serum creatinine of >2.5 mg/dL; and (v) 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.

### *Blood sampling and assays*

The length of stay in our study was 8.8 ± 6.7 days (median, 7 days, inter-quartile range, 5–10 days), similar to that reported in the Taiwan Society of Cardiology-HF with reduced ejection fraction (TSOC-HFrEF) registry (median, 8 days, inter-quartile range, 5–15 days) [16]. Blood samples were collected when patients were stabilized to the state before discharge. The measurement of BNP and other parameters, including estimated glomerular filtration rate (eGFR), hemoglobin, sodium, lipid profile, albumin, and C-reactive protein, were immediately conducted in the central core laboratory. The serum was stored at −80 °C for later measurement of Gal-3 and metabolic profile.

#### *BNP assay*

BNP was measured with the Triage BNP Test (Biosite, San Diego, CA, USA), which was a fluorescence immunoassay for quantitative determination of plasma BNP. Precision, analytical sensitivity, and stability characteristics of the assay were previously described [4].

#### *Gal-3 assay*

For Gal-3 measurement, an enzyme-linked fluorescent assay (bioMérieux ref. 411191, Marcy-l'Étoile, France) on a mini-VIDAS® analyzer (bioMérieux) was used. The total coefficient of variation for the assay was <7%, the linear range was 3.3–100.0 ng/mL, and the limit of detection was 2.4 ng/mL.

### *Metabolic profiling*

Liquid chromatographic separation for processed plasma was achieved on a 100 mm × 2.1 mm Acquity 1.7-μm C8 column (Waters Corp., Milford, MA, USA) using an ACQUITY TM UPLC system. The eluent was introduced into the TOF MS system (SYNAPT G1 high-definition mass spectrometer, Waters Corp.) and operated in an ESI-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) to discover potential metabolites. For quantification, plasma samples were mixed with isotopically labeled internal standards. Amino acids and biogenic amines were analyzed by LC–MS/MS. Other metabolites were analyzed by flow injection analysis coupled with tandem mass spectrometric analysis. The analysis was performed in positive and negative electrospray ionization mode. Identification and quantification were standardized by spiking in of isotopically labeled standards. LC-MS analysis was performed with Waters Zevo TQ coupled to an UPLC (Waters Corp.). Metabolic score was calculated based on the methods published previously [12], including four components: butyrylcarnitine, DMA/arginine ratio, spermidine, and total essential amino acids.

### *Follow-up program*

The starting time of follow-up was the day of discharge. Follow-up data were prospectively obtained every month from hospital records, personal communication with the patients' physicians, telephone interviews, and patients' regular visits to staff physician outpatient clinics. "Re-hospitalization" was defined as HF-related re-hospitalizations. A committee of 3 cardiologists adjudicated all hospitalizations without knowledge of patients' clinical variables to determine whether the events are related to worsening HF. "Death" was also chosen as an endpoint. Deaths include sudden death (unexpected death, witnessed or not), worsening HF-related death (decompensated HF or treatment-resistant HF), and other cardiovascular origin [acute myocardial infarction (directly related, whether due to mechanic, hemodynamic, or arrhythmic complications); stroke]. Because of the interrelationship of HF with other comorbidities, deaths due to comorbidities such as infection, multi-organ failure, etc. were also included. However, deaths due to cancer, surgery, suicide, or traffic accident (not related to heart) were censored. Death and a composite event of HF-related re-hospitalization and death were selected as endpoints in this prognostic study.

### *Statistical analyses*

Results are expressed as the mean ± SD for continuous variables and as the number (percentage) for categorical variables. The information on New York Heart Association (NYHA) functional class and medications was recorded on the day of blood sample collection before discharge. Data were compared by two-sample *t*-tests, ANOVA (post hoc analysis by Tukey's test), and Chi-square, when appropriate. Correlations between variables were analyzed by Pearson correlation. Follow-up data were collected as scheduled or at the last available visit. Cox proportional hazards models were used to determine independent predictors of the first defined events (death, or HF-related re-hospitalization) after controlling for covariates (those variables with a *p*-value of <0.1 in the univariable analysis). Hazard ratios (HRs) and 95% CIs were also calculated. C-statistics summarize the diagnostic discrimination. C-statistics between models were compared using the Mann–Whitney *U* test for equality concordance [17]. All statistical analyses were 2-sided and performed using SPSS software (version 15.0, SPSS, Chicago, IL, USA). A *p*-value of <0.05 was considered significant.

## Results

### *Baseline characteristics*

During the study, 255 patients were hospitalized and met the inclusion criteria. A total of 43 patients were excluded. Of these,

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