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ORIGINAL RESEARCH ARTICLE

Elevated plasma phenylalanine predicts mortality in critical patients with heart failure

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Abstract

Aims Previous studies found a relationship between elevated phenylalanine levels and poor cardiovascular outcomes. Potential strategies are available to manipulate phenylalanine metabolism. This study investigated whether increased phenylalanine predicted mortality in critical patients with either acute heart failure (HF) or acute on chronic HF, and its correlation with inflammation and immune cytokines.

Methods and results This study recruited 152 subjects, including 115 patients with HF admitted for critical conditions and 37 normal controls. We measured left ventricular ejection fraction (LVEF), plasma concentrations of phenylalanine, C-reactive protein, albumin, pre-albumin, transferrin, and pro-inflammatory and immune cytokines. Acute Physiology and Chronic Health Evaluation (APACHE II), Sequential Organ Failure Assessment (SOFA), and maximal vasoactive–inotropic scores (VIS_{max}) were calculated. Patients were followed up until death or a maximum of 1 year. The primary endpoint was all-cause death. Of the 115 patients, 37 (32.2%) were admitted owing to acute HF, and 78 (67.8%) were admitted owing to acute on chronic HF; 64 (55.7%) had ST elevation/non-ST elevation myocardial infarction. An LVEF measured during the hospitalization of $<$40%, 40–50%, and $\geq$50% was noted in 51 (44.3%), 15 (13.1%), and 49 (42.6%) patients, respectively. During 1 year follow-up, 51 (44.3%) patients died. Death was associated with higher APACHE II, SOFA, and VIS_{max} scores; higher levels of C-reactive protein and phenylalanine; higher incidence of atrial fibrillation and use of inotropic agents; lower cholesterol, albumin, pre-albumin, and transferrin levels; and significant changes in pro-inflammatory and immune cytokines. Phenylalanine levels demonstrated an area under the receiver operating characteristic curve of 0.80 for mortality, with an optimal cut-off value set at 112 μM. Phenylalanine $\geq$ 112 μM was associated with a higher mortality rate than was phenylalanine $<$ 112 μM (80.5% vs. 24.3%, $P < 0.001$) [hazard ratio = 5.07 (2.83–9.05), $P < 0.001$]. The Kaplan–Meier curves revealed that phenylalanine $\geq$ 112 μM was associated with a lower accumulative survival rate (log rank = 36.9, $P < 0.001$). Higher phenylalanine levels were correlated with higher APACHE II and SOFA scores, higher C-reactive protein levels and incidence of using inotropic agents, and changes in cytokines suggestive of immunosuppression, but lower levels of pre-albumin and transferrin. Further multivariable analysis showed that phenylalanine $\geq$ 112 μM predicted death over 1 year independently of age, APACHE II and SOFA scores, atrial fibrillation, C-reactive protein, cholesterol, pre-albumin, transferrin, and interleukin-8 and interleukin-10.

Conclusions Elevated phenylalanine levels predicted mortality in critical patients, phenotypically predominantly presenting with HF, independently of traditional prognostic factors and cytokines associated with inflammation and immunity.

Keywords Phenylalanine; Prognosis; Heart failure; Biomarkers

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Introduction

The mortality rate is high among patients in critical condition admitted to the intensive care unit with heart failure (HF). Risk stratification, currently estimated by general risk scores such as the Acute Physiology and Chronic Health Evaluation (APACHE II) and Sequential Organ Failure Assessment (SOFA) scores,[1,2] could be better. Multifaceted assessment of critical care patients remains an unmet need.

On the basis of advanced high-throughput bioinformatics, we and others recently demonstrated that blood phenylalanine concentrations provide both diagnostic and prognostic value in patients with HF.[3–8] Moreover, the SABRE study and the British Women's Health and Heart Study, which have statistically powerful community cohorts, showed that higher phenylalanine levels were associated with increased cardiovascular risk.[9] Furthermore, a study based on the PROSPER and FINRISK cohorts demonstrated that elevated phenylalanine levels predicted HF-related hospitalization in community cohorts at cardiovascular risk.[10] All evidence suggests a relationship between phenylalanine levels and poor cardiovascular outcomes. In these studies, the definition of HF included HF with reduced and preserved ejection fraction (HFrEF and HFpEF, respectively).

The metabolism of phenylalanine relates to the enzyme phenylalanine hydroxylase, which is functionally attenuated by inflammation.[11,12] The association between high phenylalanine levels and increased mortality rates in patients with sepsis also warrants further exploring inflammatory and immune profiles.[13,14] Furthermore, potential strategies are currently available to manipulate the function of phenylalanine hydroxylase.[15] Accordingly, this study was designed to investigate (i) whether phenylalanine levels predicted poor outcomes in critical patients with either acute HF or acute on chronic HF, including both HFrEF or HFpEF, because a substantial portion of patients with acute decompensated HF had HFpEF; (ii) the correlation of phenylalanine levels to inflammatory and immune cytokines; and (iii) whether the prognostic value of phenylalanine was independent of traditional risk stratification parameters and inflammation and immune profiles.

Methods

Patient enrolment

From April 2017 to September 2018, patients were consecutively enrolled at the cardiac intensive care unit on the basis of these inclusion criteria: (i) they were transferred to the intensive care unit owing to acute HF or acute on chronic HF; (ii) they had reduced left ventricular ejection fraction (LVEF $<$ 40%, HFrEF), mid-range (LVEF 40–49%, HFmrEF), or preserved LVEF (LVEF $\geq$ 50%, HFpEF) on the basis of the 2016 European Society of Cardiology guidelines for the diagnosis and treatment of acute and chronic HF[16]; (iii) they had N-terminal-pro B-type natriuretic peptide (NT-proBNP) $>$ 900 pg/mL[17,18]; (iv) they were at critical status with an APACHE II score $>$15; (v) they needed to stay in the intensive care unit $>$ 48 h; and (vi) they were older than 20 years. The exclusion criteria included the following: (i) patients with co-morbid disorders other than the main cause for admission that might compromise their survival within 3 months, such as terminal stage cancer; or (ii) patients who died before the baseline blood collection for measuring phenylalanine. Acute on chronic HF presentation was defined by a prior diagnosis of HF on admission, chronic HFrEF, HFmrEF, or HFpEF. Prior diagnosis of HF was determined by reviewing medical records from our institution as well as any referring institution. Acute HF was characterized as a new diagnosis of HF on admission, including a variety of acute HF-associated aetiologies and acute myocardial infarction with acute pulmonary oedema.

All patients provided informed consent. As normal controls (n = 37), we also enrolled participants who had normal LVEFs and did not have any systemic disease. The study was designed and carried out in accordance with the principles of the Declaration of Helsinki and with the approval from the Ethics Review Board of Chang Gung Memorial Hospital.

Scoring systems

Disease severity was evaluated by calculating SOFA,[1] APACHE II,[2] and maximal vasoactive–inotropic scores (VIS_{max})[19] on the first day of admission to the intensive care unit.

Blood sampling and examination

Fasting blood samples were collected in ethylenediaminetetraacetic acid-containing tubes in the early morning, the day after obtaining informed consent. We analysed plasma phenylalanine by ultra-performance liquid chromatography (UPLC) workflow. NT-proBNP was measured using Elecsys proBNP sandwich immunoassay on an Elecsys 2010 (Roche Diagnostics, Basel, Switzerland). Measurement of other parameters, including estimated glomerular filtration rate, C-reactive protein, haemoglobin, and albumin, was performed in the central laboratory.

Echocardiography

Two-dimensional echocardiographic images (GE Vivid E9) with patients in the left lateral decubitus position were obtained, conducted according to the guidelines suggested by

the American Society of Echocardiography.[20] We calculated the LVEF using the Simpson method. Patients with HFpEF or HFmrEF had to fit one of these criteria: (i) left atrial volume index $>$ 34 mL/m^2 or a left ventricular mass index $\geq$ 115 g/m^2 for men and $\geq$95 g/m^2 for women; and/or (ii) an E/e′ $\geq$ 13 and a mean e′ septal and lateral wall $<$ 9 cm/s.[16] For a diagnosis of chronic HF, an echocardiogram performed in the 6 months before enrolment was acceptable. However, all patients had echocardiograms during hospitalization, either before enrolment or within 2 days of enrolment, and these were used for the final analysis.

Phenylalanine measurement

Plasma concentration of phenylalanine was quantified by UPLC. Plasma samples (100 μL) were precipitated with 10% sulfosalicylic acid. After protein precipitation and centrifugation, derivatization was initiated by AQC in acetonitrile. Amino acids were then analysed using the ACQUITY UPLC System, consisting of a binary solvent manager, a sample manager, and a tunable UV detector. We used EmpowerTM 2 Software to control the system and collect data. Separations were performed on a 2.1 × 100 mm ACQUITY BEH C18 column was at a flow rate of 0.70 mL/min. The average intra-assay coefficient of variation was 2.6% for phenylalanine. The total coefficient of variation was 2.7% for phenylalanine. The detection limit was 3.3 μM. The linear range was 25–500 μM.

Cytokine measurement

Milliplex MAP Human high-sensitivity T cell Magnetic Bead Panel (MILLIPLEX HSTCMAG-28SK kit, Millipore Corporation, Billerica, MA, USA) was used to quantify the plasma cytokine levels. Antibody beads, controls, wash buffer, serum matrix, and standards were prepared following the manufacturer's instructions. After all procedures, the plate was analysed using the MAGPIX with xPONENT software. Measured cytokines included granulocyte-macrophage colony-stimulating factor (GM-CSF), interferon (IFN), IFN-inducible T cell alpha chemoattractant (ITAC), macrophage inflammatory protein (MIP), tumour necrosis factor (TNF), fractalkine, and a variety of interleukins (ILs).

Follow-up program

Follow-up data were prospectively obtained from hospital records, personal communication with the patients' physicians, telephone interviews with patients, and patients' regular visits to staff physician outpatient clinics. Patients were followed up until death or a maximum of 1 year. The primary endpoint was death from all causes.

Statistical analyses

Results are expressed as the mean ± SD for variables with normal distribution, as the median [inter-quartile range (IQR)] for variables with skewed distribution, and as the number (percentage) for categorical variables. We compared data using the Mann–Whitney *U* test, Kruskal–Wallis *H* test, and χ^2, when appropriate. We estimated receiver operating characteristic (ROC) curve and used Youden's index to identify the cut-off value of variables. Area under the curve (AUC) of ROCs was presented. A univariate Cox proportional hazards model was used to determine the variables' predictive value on mortality. By Cox multivariable analysis, we adjusted for covariates to better identify strong independent predictors of mortality using a forward selection model. Variables with a *P* value $<$ 0.05 in a univariate analysis were included in the multivariable analysis. Hazard ratios (HRs) and 95% confidence intervals (CIs) were calculated. To compare time-dependent outcomes, we performed Kaplan–Meier analyses with a log-rank test. All statistical analyses were two-sided and performed using SPSS software (Version 22.0, SPSS, Chicago, IL, USA). A *P* value of $<$0.05 was considered significant.

Results

Baseline characteristics and laboratory data

All patients (*n* = 115) had a high NT-proBNP level [5922 pg/mL (IQR 1917–11 698 pg/mL)]. The baseline characteristics, laboratory data, and medications are shown in *Table 1* and *Table S1*. An LVEF measured during the hospitalization of $<$40%, 40–50%, and $\geq$50% was noted in 51 (44.3%), 15 (13.1%), and 49 (42.6%) patients, respectively. Of the 115 patients, 64 (55.7%) had ST elevation/non-ST elevation myocardial infarction; 37 (32.2%) were admitted owing to acute HF, and 78 (67.8%) were admitted owing to acute on chronic HF. Seventeen (14.8%) had infection-induced acute decompensation of chronic HF. All patients were at functional classification IV. Their average age was 71.7 years, and the average APACHE II and SOFA scores were 25.8 and 8.32, respectively.

Factors associated with mortality

During the 1 year follow-up period, 51 (44.3%) patients died. In 31 (60.8%) patients, death occurred owing to infection, 6 (11.8%) died of HF, and 14 (27.5%) owing to critical cardiovascular conditions. Mortality was associated with higher APACHE II, SOFA, and VIS_{max} scores; higher incidence of atrial fibrillation; and use of inotropic agents (*Table 1*).

Table 1 Demographic and laboratory data

	All $n = 115$	Survival $n = 64$	Death $n = 51$	*P* value
Age (years)	71.7 ± 13.3	69.6 ± 13.7	74.3 ± 12.3	0.061
Male (%)	73 (63.4)	38 (59.3)	35 (68.6)	0.335
APACHE II score	25.8 ± 7.02	23.6 ± 5.23	28.5 ± 8.01	<0.001
SOFA score	8.32 ± 3.76	6.91 ± 3.29	10.1 ± 3.58	<0.001
VIS_{max} score	1.3 (0–16.4)	1.1 (0–11.9)	4.8 (0–30.5)	0.009
LVEF (%)	46.3 ± 17.9	47.2 ± 17.5	45.3 ± 18.6	0.566
NYHA Fc IV (%)	115 (100)	64 (100)	51 (100)	1.00
Body mass index (kg/m^2)	25.0 ± 5.18	25.2 ± 4.95	24.7 ± 5.49	0.621
Co-morbidity				
Diabetes mellitus (%)	60 (52.1)	35 (54.6)	25 (49.0)	0.577
Hypertension (%)	83 (72.1)	46 (71.8)	37 (72.5)	1.00
Coronary artery disease (%)	77 (67.0)	40 (62.5)	37 (72.5)	0.431
Atrial fibrillation (%)	23 (20)	7 (10.9)	16 (31.3)	0.009
COPD (%)	8 (6.9)	7 (10.9)	1 (1.9)	0.075
Chronic kidney disease (%)	25 (21.7)	17 (26.5)	8 (15.6)	0.179
Ventilator use (%)	99 (86.1)	54 (84.3)	45 (88.2)	0.599
Inotropic agent use (%)	53 (46.0)	19 (29.6)	34 (66.7)	<0.001
Days in ICU (day)	13.9 ± 10.6	13.2 ± 10.5	14.9 ± 10.8	0.367
Laboratory data				
WBC (1000/μL)	13.5 ± 6.04	12.9 ± 5.79	14.1 ± 6.34	0.308
Haemoglobin (g/dL)	10.9 ± 2.85	11.2 ± 2.78	10.5 ± 2.92	0.245
CRP (mg/L)	17.0 (53.6–106)	33.6 (6.9–58.1)	83.0 (44.9–157)	<0.001
Cholesterol (mg/dL)	131 ± 47.8	143.4 ± 47.9	116 ± 43.5	0.002
Triglyceride (mg/dL)	109 (84–152)	110 (82–152)	109 (88–156)	0.632
Albumin (g/dL)	3.20 ± 0.55	3.30 ± 0.48	3.08 ± 0.61	0.044
eGFR ($mL/min/1.73\ m^2$)	38.6 ± 32.3	43.8 ± 34.3	32.0 ± 28.5	0.053
ALT (U/L)	29 (18–57)	29.5 (21.3–54.5)	26 (16–72)	0.630
Serum sodium (mEq/L)	141 ± 6.78	139 ± 5.74	142 ± 7.72	0.053
Pre-albumin (mg/dL)	14.4 ± 6.77	16.7 ± 7.33	11.5 ± 4.65	<0.001
Transferrin (mg/dL)	152 ± 48.6	164 ± 51.7	136 ± 39.9	0.002
NT-proBNP (ng/mL)	5920 (1917–11 698)	5900 (1874–10 680)	5960 (1917–12 680)	0.536
Phenylalanine (μM)	98.5 ± 50.6	80.1 ± 26.0	122 ± 63.3	<0.001

APACHE II, Acute Physiology and Chronic Health Evaluation; ALT, alanine aminotransferase; COPD, chronic obstructive pulmonary disease; chronic kidney disease, estimated glomerular filtration rate (eGFR) < 60 $mL/min/1.73\ m^2$; CRP, C-reactive protein; ICU, intensive care unit; LVEF, left ventricular ejection fraction; NT-proBNP, N-terminal-pro B-type natriuretic peptide; NYHA Fc, New York Heart Association functional classification; SOFA, Sequential Organ Failure Assessment; VIS_{max}, maximal vasoactive–inotropic score in 24 h after enrolment; WBC, white blood cell count.
Data are expressed as the mean ± SD for variables with normal distribution, median [inter-quartile range (IQR)] for variables with skewed distribution, and as number (percentage) for categorical variables.

Death was also associated with higher C-reactive protein and phenylalanine levels and lower cholesterol, albumin, pre-albumin and transferrin levels but was not associated with NT-proBNP levels.

Association of phenylalanine and cytokines to mortality

The association between phenylalanine levels and mortality was analysed. The ROC curve demonstrated an AUC of 0.80, with an optimal cut-off value for phenylalanine set at 112 μM (*Figure 1A*). The mortality rate in patients with phenylalanine ≥ 112 μM was significantly higher than that in patients with phenylalanine < 112 μM (80.5% vs. 24.3%, $P < 0.001$) (HR = 5.07, 95% CI = 2.83–9.05, $P < 0.001$) (*Figure 1B*). In *Figure 1C*, the Kaplan–Meier curve revealed that a phenylalanine ≥ 112 μM was associated with a lower accumulative survival rate (log rank = 36.9, $P < 0.001$). For patients with LVEF < 40% and LVEF ≥ 40%, the AUCs were 0.83 and 0.78, respectively. The analysis was performed again after excluding patients with worsening HF induced by infection; it showed that the AUC was 0.81. As for pro-inflammatory and immune cytokines, mortality correlated to higher levels of IL-6, IL-8, IL-10, TNFα, MIP1β, MIP3α, and ITAC but lower levels of IL-23 (*Table 2*).

Characteristics of patients with high phenylalanine

Compared with patients with phenylalanine < 112 μM, patients with phenylalanine ≥ 112 μM had higher APACHE II

Figure 1 Prognostic value of phenylalanine and leucine. (A) The prognostic value of phenylalanine (Phe) is shown by the receiver operating characteristic curve. (B) The mortality rates in patients with Phe ≥ 112 μM vs. Phe < 112 μM. (C) The Kaplan–Meier curves for patients with Phe ≥ 112 μM vs. Phe < 112 μM.

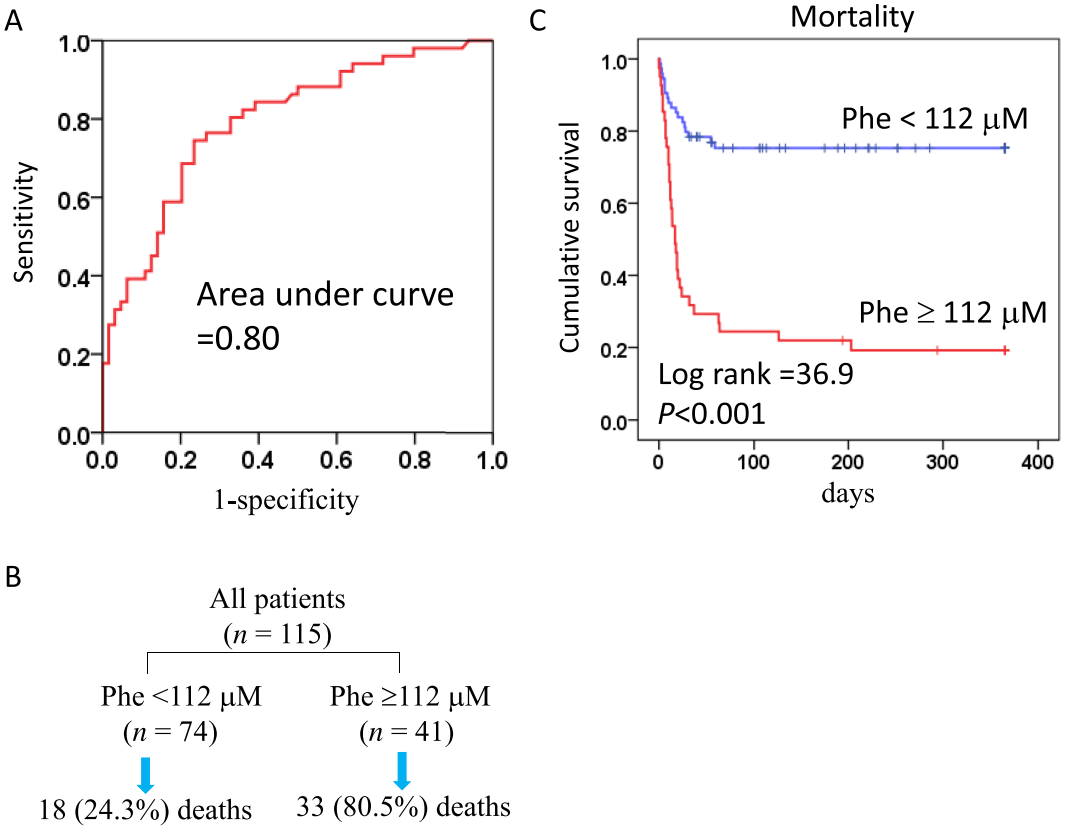

Table 2 Cytokines in patients of survival or death

pg/mL	All $n = 115$	Survival $n = 64$	Death $n = 51$	*P* value
IL-1β	0.31 [0.21–0.68]	0.37 [0.18–0.73]	0.26 [0.21–0.63]	0.800
IL-2	0.53 [0.25–1.04]	0.54 [0.28–0.99]	0.50 [0.25–1.08]	0.848
IL-4	12.2 [6.09–37.3]	18.0 [5.65–45.3]	9.45 [6.09–32.8]	0.190
IL-5	1.97 [1.02–3.57]	2.07 [1.22–3.69]	1.81 [0.96–3.49]	0.181
IL-6	11.1 [3.60–28.9]	6.99 [3.39–17.8]	13.9 [5.62–38.2]	0.005
IL-7	3.41 [1.99–4.91]	3.58 [2.00–5.49]	3.17 [1.99–4.83]	0.642
IL-8	19.3 [9.55–40.9]	12.6 [7.53–20.9]	35.0 [20.4–89.9]	<0.001
IL-10	9.93 [5.54–14.5]	7.73 [4.42–12.6]	13.0 [7.94–24.8]	<0.001
IL-12-p70	1.02 [0.55–1.55]	1.07 [0.55–1.61]	0.91 [0.51–1.45]	0.327
IL-13	0.86 [0.33–1.78]	0.86 [0.40–1.92]	0.86 [0.33–1.78]	0.589
IL-17A	3.06 [2.04–4.83]	3.40 [2.16–5.63]	1.86 [2.95–4.02]	0.111
IL-21	1.18 [0.54–2.48]	1.23 [0.65–2.81]	1.17 [0.54–2.27]	0.230
IL-23	110 [54.9–233]	134 [61.7–260]	72.3 [42.2–163]	0.011
IFN-γ	2.74 [1.58–5.33]	2.87 [1.76–5.47]	2.67 [1.35–4.80]	0.485
TNFα	7.07 [5.09–11.3]	6.15 [4.35–8.73]	9.30 [5.91–15.0]	0.001
MIP1α	11.7 [8.93–15.9]	11.3 [8.00–15.8]	12.8 [9.02–17.5]	0.365
MIP1β	8.79 [5.87–12.6]	7.78 [5.35–11.3]	10.4 [6.33–16.2]	0.011
MIP3α	18.2 [8.58–47.9]	11.8 [7.67–33.7]	27.0 [14.1–86.0]	<0.001
ITAC	13.5 [8.86–27.1]	12.3 [8.58–18.9]	16.5 [9.27–32.3]	0.028
GM-CSF	6.37 [3.82–12.4]	7.00 [3.95–13.7]	5.61 [3.82–10.5]	0.337
Fractalkine	65.2 [40.2–113]	75.8 [40.7–115]	54.4 [37.8–113]	0.219

GM-CSF, granulocyte-macrophage colony-stimulating factor; IL, interleukin; IFN, interferon; ITAC, interferon-inducible T cell alpha chemoattractant; MIP, macrophage inflammatory protein; TNF, tumour necrosis factor.
Data are expressed as the median [inter-quartile range (IQR)] because most of the variables are skewed distribution.

Table 3 Comparisons of demographic and laboratory data in patients with different levels of phenylalanine

	Phe < 112 μM n = 74	Phe ≥ 112 μM n = 41	P value
Age (years)	70.6 ± 13.5	73.6 ± 12.6	0.253
Male (%)	45 (60.8)	28 (68.2)	0.545
APACHE II score	18.5 ± 6.16	21.1 ± 5.32	0.029
SOFA score	7.62 ± 3.66	9.59 ± 3.64	0.007
VIS_{max} score	0 (0–12.8)	1.04 (0–22.5)	0.561
LVEF (%)	48.0 ± 18.5	43.2 ± 16.4	0.161
NYHA Fc IV	74 (100)	41 (100)	1.00
Body mass index (kg/m^2)	25.3 ± 5.33	24.3 ± 4.88	0.289
Co-morbidity			
Diabetes mellitus (%)	41 (55.4)	19 (46.3)	0.436
Hypertension (%)	52 (70.2)	31 (75.6)	0.665
Coronary artery disease (%)	49 (66.2)	28 (68.3)	1.00
Atrial fibrillation (%)	11 (14.8)	12 (29.2)	0.088
COPD (%)	6 (8.1)	2 (4.8)	0.710
Chronic kidney disease (%)	15 (20.2)	10 (24.3)	0.642
Ventilator use (%)	60 (81.0)	39 (95.1)	0.048
Inotropic agent use (%)	30 (40.5)	23 (56.0)	0.122
Days in ICU (day)	13.1 ± 10.7	15.4 ± 10.3	0.275
Laboratory data			
WBC (1000/μL)	13.1 ± 5.68	14.0 ± 6.65	0.419
Haemoglobin (g/dL)	10.8 ± 2.42	10.8 ± 3.51	0.937
CRP (mg/L)	36.5 (9.36–61.3)	89.3 (51.0–154)	<0.001
Cholesterol (mg/dL)	137 ± 50.9	119 ± 39.6	0.040
Triglyceride (mg/dL)	108 (82.0–149)	110 (85.0–168)	0.524
Albumin (g/dL)	3.20 ± 0.53	3.18 ± 0.58	0.841
eGFR (mL/min/1.73 m^2)	42.6 ± 35.8	31.2 ± 23.1	0.041
ALT (U/L)	18.0 (28.5–53.5)	18.0 (37.0–139)	0.308
Serum sodium (mEq/L)	139 ± 6.10	142 ± 7.72	0.086
Pre-albumin (mg/dL)	15.7 ± 7.19	12.0 ± 5.21	0.002
Transferrin (mg/dL)	154 ± 49.5	146 ± 47.0	0.371
NT-proBNP (ng/mL)	5760 (1959–10 591)	8360 (1819–15 420)	0.538

ALT, alanine aminotransferase; chronic kidney disease, estimated glomerular filtration rate (eGFR) < 60 mL/min/1.73 m^2; COPD, chronic obstructive pulmonary disease; CRP, C-reactive protein; ICU, intensive care unit; LVEF, left ventricular ejection fraction; NT-proBNP, N-terminal-pro B-type natriuretic peptide; NYHA Fc, New York Heart Association functional classification; VIS_{max}, maximal vasoactive–inotropic score in 24 h after enrolment.
Data are expressed as the mean ± SD for variables with normal distribution, median [inter-quartile range (IQR)] for variables with skewed distribution, and number (percentage) for categorical variables.

and SOFA scores, higher incidence of using inotropic agents, and higher C-reactive protein but lower pre-albumin and transferrin levels (*Table 3*). We examined the differences in cytokines between normal controls and patients with different phenylalanine levels. The demographic characteristics of the normal controls are shown in *Table S2*. The phenylalanine levels in the normal controls were 67.8 ± 13.1 μM (95% CI = 63.4–72.2 μM). Compared with normal controls, patients admitted to the intensive care unit had significantly higher levels of IL-6, IL-8, IL-10, TNFα, MIP1β, MIP3α, and ITAC but lower levels of IL-1β, IL-2, IL-4, IL-5, IL-7, IL-12-p70, IL-17A, IL-21, IL-23, IFN-γ, GM-CSF, and fractalkine (*Table 4*). Because normal controls were younger than our patients, patients were age and sex matched to the normal controls (*Table S3*). The comparison in cytokines between normal controls and matched patients reconfirmed these findings (*Table S4*). Furthermore, compared with patients with phenylalanine < 112 μM, patients with phenylalanine ≥ 112 μM had significantly higher levels of IL-8, IL-10, TNFα, MIP1β, MIP3α, and ITAC (*Table 4*).

Cox univariate and multivariable analyses for mortality

The value of cytokines in predicting mortality was estimated by Cox univariate and multivariable analyses (*Table S5*). The univariate analysis showed that higher IL-6, IL-8, IL-10, TNFα, MIP1β, MIP3α, and ITAC but lower IL-23 were associated with a higher mortality rate. Multivariable analysis revealed that only IL-8 and IL-10 were independent predictors of mortality. Then we analysed all significant prognostic parameters in the univariate analysis, including clinical and laboratory variables, phenylalanine, and cytokines, together (*Table 5*). The multivariable analysis with a forward selection model demonstrated that only phenylalanine ≥ 112 μM, VIS_{max}, IL-8, and

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Table 4 Comparisons of cytokines in normal controls and patients with different levels of phenylalanine (Phe)

Variable	Normal (n = 37)	Phe < 112 μM (n = 74)	Phe ≥ 112 μM (n = 41)	P value[a]
IL-1β	0.91 [0.57–1.49]	0.30 [0.20–0.70]**	0.36 [0.21–0.66]**	<0.001
IL-2	0.94 [0.68–1.17]	0.49 [0.22–0.93]**	0.61 [0.35–1.13]*	<0.001
IL-4	42.3 [27.5–67.5]	13.73 [5.35–41.86]**	9.88 [7.03–36.7]**	<0.001
IL-5	3.83 [2.94–5.08]	2.01 [1.01–3.55]**	1.88 [1.02–3.64]**	<0.001
IL-6	0.87 [0.60–1.31]	9.18 [3.47–25.71]**	12.6 [5.30–36.5]**	<0.001
IL-7	5.94 [4.39–8.06]	3.71 [2.02–5.09]**	3.13 [1.99–4.75]**	<0.001
IL-8	3.06 [2.68–4.02]	14.08 [7.98–26.63]**	36.2 [21.9–58.9]**,‡	<0.001
IL-10	7.47 [5.11–11.05]	7.84 [4.67–13.50]	13.1 [9.84–23.9]**,‡	<0.001
IL-12-p70	1.92 [1.61–2.69]	1.05 [0.54–1.59]**	0.93 [0.54–1.52]**	<0.001
IL-13	1.13 [0.67–2.23]	0.68 [0.33–1.70]	1.00 [0.40–2.31]	<0.062
IL-17A	4.94 [4.15–6.78]	3.11 [2.07–4.56]**	3.06 [2.01–4.85]**	<0.001
IL-21	2.54 [1.74–3.60]	1.18 [0.54–2.55]**	1.29 [0.57–2.42]**	<0.001
IL-23	259 [166–380]	118.83 [54.23–224.03]**	91.7 [52.1–243]**	<0.001
IFN-γ	5.66 [4.63–7.29]	2.42 [1.51–5.02]**	3.34 [1.82–5.98]**	<0.001
TNFα	3.35 [2.75–3.82]	6.65 [4.62–10.76]**	8.12 [5.64–14.10]**,†	<0.001
MIP1α	13.4 [10.8–16.0]	11.05 [7.87–16.078]	13.1 [10.4–16.7]	0.092
MIP1β	4.95 [3.52–6.44]	7.92 [5.31–11.36]**	11.0 [6.39–20.3]**‡	<0.001
MIP3α	7.49 [5.23–9.75]	13.26 [7.91–34.10]**	32.6 [14.4–84.1]**‡	<0.001
ITAC	7.74 [6.28–9.67]	12.46 [8.35–18.93]**	17.0 [10.61–34.38]**,†	<0.001
GM-CSF	16.9 [12.3–23.8]	6.54 [3.83–12.84]**	5.79 [3.82–12.03]**	<0.001
Fractalkine	104 [70.9–126]	64.38 [39.23–118.50]*	65.3 [42.0–105]*	0.01

GM-CSF, granulocyte-macrophage colony-stimulating factor; IL, interleukin; IFN, interferon; ITAC, interferon-inducible T cell alpha chemoattractant; MIP, macrophage inflammatory protein; TNF, tumour necrosis factor.
Data are presented as median [inter-quartile range].
*$P < 0.05$.
**$P < 0.01$, compared with 'normal'.
†$P < 0.05$.
‡$P < 0.01$, compared with 'Phe < 112 μM'.
[a]Indicates use of Kruskal–Wallis H test.

Table 5 Cox univariate and multivariable analyses of factors for predicting mortality

	Univariate		Multivariable[a]		Multivariable[b]	
	HR (95% CI)	P value	HR (95% CI)	P value	HR (95% CI)	P value
Phenylalanine ≥ 112 μM	5.06 (2.83–9.05)	<0.001	3.09 (1.66–5.76)	<0.001	2.57 (1.28–5.16)	<0.001
APACHE II score	1.08 (1.03–1.13)	0.002				
SOFA score	1.17 (1.09–1.26)	<0.001				
VIS_{max} score	1.04 (1.03–1.06)	<0.001	1.03 (1.01–1.05)	0.006	1.04 (1.02–1.07)	0.001
Atrial fibrillation	2.47 (1.36–4.47)	0.003				
CRP (log)	3.20 (1.82–5.63)	<0.001				
Cholesterol (mg/dL)	0.99 (0.98–0.99)	0.003				
Pre-albumin (mg/dL)	0.90 (0.85–0.95)	<0.001				
Transferrin (mg/dL)	0.99 (0.98–0.99)	0.004				
IL-8 (log) × 10^{-1}	1.24 (1.17–1.33)	<0.001	1.10 (1.01–1.19)	0.024	1.17 (1.08–1.28)	<0.001
IL-10 (log) × 10^{-1}	1.27 (1.16–1.39)	<0.001	1.13 (1.02–1.25)	0.026	1.20 (1.08–1.33)	0.001

APACHE II, Acute Physiology and Chronic Health Evaluation; CI, confidence interval; CRP, C-reactive protein; HR, hazard ratio; IL, interleukin; SOFA, Sequential Organ Failure Assessment; VIS_{max}, maximal vasoactive–inotropic scores in 24 h after enrolment.
[a]Multivariable analysis with forward selection model for all variables with $P < 0.05$ in the univariate analysis.
[b]Adjusted for age, APACHE II score, SOFA score, atrial fibrillation, CRP, cholesterol, pre-albumin, and transferrin.

IL-10 were independent predictors of mortality over 1 year. Further multivariable analysis revealed that phenylalanine ≥ 112 μM independently predicted mortality after adjusting for age, APACHE II score, SOFA score, atrial fibrillation, CRP, cholesterol, pre-albumin, and transferrin. After the analysis patients with decompensated HF induced by infection were excluded, phenylalanine ≥ 112 μM remained a significant predictor of mortality after adjusting for the same variables (HR = 2.88, 95% CI = 1.42–5.84, P = 0.003).

Discussion

Increased plasma concentrations of phenylalanine predicted mortality in critical patients, phenotypically predominantly presenting with HF. Higher phenylalanine levels were correlated with higher APACHE II and SOFA scores, substantial inflammation, changes in cytokines suggestive of immunodepression, and malnutrition. In addition, the prognostic value of phenylalanine was independent of traditional

risk scores and factors as well as pro-inflammatory and immune cytokines.

Traditional risk assessment tools

Recent comprehensive HF surveys reveal that the 1 year mortality rates after discharge from acute HF vary widely, from 10% to 32% in the REPORT-HF (International Registry to Assess Medical Practice with Longitudinal Observation for Treatment of HF)[21] to 40% in the GWTG-HF (Get With The Guidelines-Heart Failure) registry.[22] In the ARIC (Atherosclerosis Risk in Communities) study, the in-hospital mortality rate for patients with acute decompensated HF was 7%.[23] Our study consistently showed that the 1 year mortality rate was 44.3%, including both in-hospital and 1 year post discharge deaths. Risk assessment is mandatory for critical care. In this study, patients were admitted to the intensive care unit mainly for cardiovascular causes rather than infection. Natriuretic peptide levels were remarkably elevated in all patients and thus demonstrated no further prognostic value. Unexpectedly, LVEF was not a significant predictor of death within 1 year post discharge, probably because we included both HFrEF and HFpEF and because LVEF measured post coronary intervention or under use of inotropic agents had a confounding effect. A variety of scoring systems and nutritional indexes have been developed for predicting outcomes for patients with critical illness, such as APACHE II, SOFA, and VIS_{max} scores,[1,19,24] albumin, pre-albumin,[25–27] and transferrin.[28] The value of these parameters was repeatedly confirmed in our study. However, phenylalanine levels predicted mortality independently of all other parameters. Phenylalanine ≥ 112 μM, as a single variable, was associated with a 1 year mortality rate of 80.5%, remarkably higher than the 24.3% one-year mortality rate among patients with phenylalanine < 112 μM.

Increased phenylalanine levels and mortality

The association between increased phenylalanine levels and mortality has not been previously explored. Here, we provide a few indirect mechanisms. Higher phenylalanine levels were correlated with higher C-reactive protein levels and higher pro-inflammatory, innate, and adaptive T lymphocyte immune cytokines such as IL-8 and IL-10. Although the inflammation in our patients was raised, it was different from patients with sepsis, as shown by the low levels of IFN-γ compared with the normal controls. Generally, our patients, as compared with the normal controls, had a comparatively high level of inflammation (increased IL-6, IL-8, TNFα, MIP1β, and MIP3α) but suppressed immunity (increased IL-10 and decreased IL-4 and IL-12). Furthermore, these phenomena were even more remarkable in patients with higher phenylalanine levels. Although suppressed immunity was suggested only by the level of cytokines and needs further confirmation, it may be closely related to the increased mortality rate, because the leading cause of mortality in our study population was infection. The compromised immune system identified by increased phenylalanine may be a pivotal area for further research by immuno-phenotyping the patients and tackling their poor outcomes. On the other hand, it is worth noting that our previous study revealed that an elevated phenylalanine level was also associated with severe infection and mortality.[14] Therefore, it was necessary to clarify the confounding effect of infection on our findings in this study. After patients with infection-induced acute decompensation were excluded, our further analysis demonstrated that phenylalanine remained an independent predictor of mortality.

Congenital phenylketonuria involves a deficiency of phenylalanine hydroxylase leading to extremely high phenylalanine concentrations.[29] The moderately increased phenylalanine level in our patients might be associated with insufficient tetrahydrobiopterin (BH4), the co-factor for phenylalanine hydroxylase.[15] Inflammation-induced production of reactive oxygen species may consume a significant portion of BH4, which can be assumed to leave phenylalanine unmetabolized.[15,30] Moreover, in humans, it has been shown that pro-inflammatory cytokine-mediated inflammation paradoxically increases the production of neopterin at the expense of the production of BH4.[12,31] Insufficient bioavailability of BH4 also leads to dysfunction in multiple systems, including nitric oxide synthase, tryptophan metabolism, the catecholamine pathway, the neural system, and thyroid hormone production.[15,30–32] Moreover, recent reports also demonstrate that insufficient BH4 causes impaired T cell proliferation and function.[33] However, owing to its vulnerability to oxidation, it is difficult to correctly measure BH4 on a routine basis. Elevated phenylalanine is a surrogate for significant BH4 deficiency.

The prognostic value of phenylalanine was independent of inflammation and immune cytokines, suggesting that phenylalanine also predicted poor outcomes via other mechanisms. Previously, we found that patients with elevated phenylalanine levels had substantially more incompletely metabolized waste of fatty acids in the circulation owing to impaired mitochondrial β-oxidation, indicating dysfunctional energy production machinery.[8] The increase of blood phenylalanine concentration also represents substantial tissue breakdown, which is probably related to insufficient tissue perfusion and increased insulin resistance.[10] In addition, consistent with our previous reports,[8] these patients had low pre-albumin levels indicating severe malnutrition. All these factors potentially contribute to poor outcomes.

Although the cut-off value of phenylalanine is pivotal for clinical application, so far, no consensus has been reached. We hereby offer a cut-off at 112 μM in patients at critical status; however, cut-offs may differ in various populations and

for different outcomes of interest. For example, previously, we identified the cut-offs for predicting mortality at 84 μM in patients with severe infection[14] and at 88.9 μM among patients with not critically decompensated HF (for predicting composite events of all-cause death or HF-related re-hospitalization).[8] In the study by Delles *et al.*,[10] utilizing different measuring instruments (nuclear magnetic resonance), the phenylalanine concentration values for predicting HF-related hospitalization were 47.85 (43.30–52.40) vs. 45.10 (40.70–49.80) μM in the high-risk and low-risk community cohorts, respectively. The values were generally lower than our measurements done using UPLC. Future studies need to establish cut-offs on the basis of the same quantification platform.

Study limitations

BH4 deficiency was not directly measured in patients with increased phenylalanine levels. Further efforts need to quantify the amount of BH4 to provide direct evidence. With regard to immunity, although we measured the concentrations of cytokines that regulate or are secreted by T lymphocytes, enumerating innate and adaptive lymphocytes, as measured by flow cytometry, may strengthen the mechanisms associated with increased phenylalanine levels. Finally, the small sample size is definitely a limitation of our study. However, the findings of this study add further support to the notion that phenylalanine provides significant prognostic value, as we previously demonstrated in cohorts of HF and patients at high risk for sepsis.[3,7,8,14]

Conclusions

Increased plasma concentration of phenylalanine and disturbed phenylalanine metabolism predict poor outcomes in critical patients phenotypically predominantly presenting with HF, independently of traditional prognostic factors and cytokines associated with inflammation and T lymphocyte immunity. Our study provides additional support to the findings of recent large-scale cohort studies regarding the value of measuring phenylalanine.

Conflict of Interest

None.

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Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. Demographic and laboratory data in patients with different left ventricular ejection fractions.
Table S2. Demographic and laboratory data in patients and normal controls.
Table S3. Demographic and laboratory data of patients with sex and age matched to normal controls.
Table S4. Cytokines in patients with sex and age matched to normal controls.
Table S5. COX univariate and multivariable analysis of cytokines for predicting mortality.

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