International Journal of Infectious Diseases 85 (2019) 143–149

Contents lists available at ScienceDirect

International Journal of Infectious Diseases

journal homepage: www.elsevier.com/locate/ijid

Phenylalanine- and leucine-defined metabolic types identify high mortality risk in patients with severe infection

Shie-Shian Huang[a,b], Jui-Ying Lin[c], Wei-Siang Chen[b,d,e], Ming-Hui Liu[b,e], Chi-Wen Cheng[b,e], Mei-Ling Cheng[f,g,h], Chao-Hung Wang[b,e,*]

[a] Division of Infectious Diseases, Department of Internal Medicine, Chang Gung Memorial Hospital, Keelung, Taiwan
[b] Chang Gung University College of Medicine, Taoyuan, Taiwan
[c] Nutrition Department, Chang Gung Memorial Hospital, Keelung, Taiwan
[d] Intensive Care Unit, Division of Cardiology, Department of Internal Medicine, Chang Gung Memorial Hospital, Keelung, Taiwan
[e] Heart Failure Research Center, Division of Cardiology, Department of Internal Medicine, Chang Gung Memorial Hospital, Keelung, Taiwan
[f] Metabolomics Core Laboratory, Healthy Aging Research Center, Chang Gung University, Taoyuan, Taiwan
[g] Department and Graduate Institute of Biomedical Sciences, College of Medicine, Chang Gung University, Taoyuan, Taiwan
[h] Clinical Metabolomics Core Laboratory, Linkou Chang Gung Memorial Hospital, Taoyuan, Taiwan

ARTICLE INFO

Article history:
Received 17 April 2019
Received in revised form 23 May 2019
Accepted 25 May 2019
Corresponding Editor: Eskild Petersen, Aarhus, Denmark

Keywords:
Amino acids
Metabolic profile
Infection
Prognosis
Biomarkers

ABSTRACT

Objective: To investigate the prognostic value of phenylalanine and leucine in patients with severe infection.
Methods: Ninety-three patients with infection who had a quick Sequential Organ Failure Assessment (qSOFA) score ≥2 were enrolled. Plasma phenylalanine, leucine, albumin, C-reactive protein, pre-albumin, and transferrin were measured and the SOFA score at enrollment was calculated after hospitalization.
Results: During the 3-month follow-up, 30 (32.3%) patients died. Death was associated with higher SOFA scores, a higher incidence of bacteremia and admission to the intensive care unit, higher C-reactive protein and phenylalanine levels, worse kidney function, and lower pre-albumin and transferrin levels. Patients were categorized into three groups: high-risk type 1 (phenylalanine ≥84 μM), high-risk type 2 (phenylalanine <84 μM and leucine <93 μM), and low-risk (other). Compared to the low-risk type patients, high-risk type 1 and 2 patients had higher mortality rates (hazard ratio 10.1 (95% CI 2.33–43.5) and hazard ratio 5.56 (95% CI 1.22–25.4), respectively). Type 1 patients had higher SOFA scores, a higher incidence of admission to the intensive care unit, and higher C-reactive protein and leucine levels. Type 2 patients had lower albumin and hemoglobin levels. Multivariable analysis showed that both high-risk types were independent predictors of death.
Conclusions: Phenylalanine- and leucine-defined risk classifications provide metabolic information with prognostic value for patients with severe infection.

Introduction

Although infection is heterogeneous among hospitalized patients, it is generally associated with increased morbidity and mortality rates (Fleischmann et al., 2016). Patients with infection who have deteriorated consciousness, tachycardia, or tachypnea are high-risk populations and have a high mortality rate (Iwashyna et al., 2010). Among patients suspected of having infection, the quick Sequential Organ Failure Assessment (qSOFA) score is a tool that can rapidly screen for patients with more severe infection (Singer et al., 2016). However, there is an unmet need for biomarkers that can identify subpopulations at extremely high risk of mortality among patients with high qSOFA scores. The aim of this study was to provide such biomarkers. Currently, risk stratification is estimated by general risk scores, such as the Sequential Organ Failure Assessment (SOFA) score (Seymour et al., 2016), C-reactive protein (CRP) (Garnacho-Montero et al., 2014), and nutritional indexes including albumin, pre-albumin, and transferrin (Levitt and Levitt, 2016; Ning et al., 2016; Szatanik et al., 2011). However, one assessment tool cannot be applied to all

* Corresponding author at: Heart Failure Research Center, Division of Cardiology, Department of Internal Medicine, Chang Gung Memorial Hospital, 222 Mai Chin Road, Keelung, Taiwan.
E-mail address: bearty@adm.cgmh.org.tw (C.-H. Wang).

https://doi.org/10.1016/j.ijid.2019.05.030

patients because different patient populations have distinct characteristics, highlighting the need for a multifaceted assessment of critical patients.

Recently, based on advanced bioinformatics platforms, we and others have demonstrated that patient plasma-based metabolomics provide information about metabolic disturbances and have prognostic value for critically ill patients (Alexander et al., 2011; Cheng et al., 2015; Hunter et al., 2016; Tenori et al., 2013; Wang et al., 2017, 2016; Wurtz et al., 2015). We subsequently simplified the metabolomics assessment to an amino acid-based profile. Although phenylalanine and leucine are essential amino acids and the building blocks of the body, it was found that the concentrations of these two amino acids could be used to classify patients into different risk types proven to be strongly related to disease worsening or death (Wang et al., 2018a,b). One high-risk type is characterized by high phenylalanine levels, which are associated with severe metabolic disturbance. The other high-risk type is associated with extremely low leucine concentrations, indicating malnutrition. Different high-risk metabolic types represent populations in different metabolic states.

Building on our previous findings, it was hypothesized that the prognostic value of this phenylalanine- and leucine-defined metabolic profile could be demonstrated in a population hospitalized due to infection with high qSOFA scores. The prognostic value of this profile was compared with traditional assessment tools. In addition, the characteristics of patients categorized into the different risk types were explored to shed light on the mechanisms that explain how metabolic risk types correlate with mortality.

Methods

Patient enrollment

Patients at the hospital were consecutively enrolled from April 2017 to December 2018, based on these inclusion criteria: (1) hospitalized due to suspected infection; and (2) qSOFA score ≥2. Exclusion criteria were: (1) infection with HIV; or (2) the presence of disorders other than the main cause of admission that might compromise survival within the next 3 months. Informed consent was obtained from patients or their family if the patient was unconscious. The study was designed and conducted in accordance with the principles of the Declaration of Helsinki and with approval from the Ethics Review Board of Chang Gung Memorial Hospital.

Scoring systems

Disease severity was evaluated by calculating the qSOFA score on the first day of hospital admission. The qSOFA score ranges from 0 to 3 points, with 1 point each for systolic hypotension (≤100 mmHg), high respiratory rate (≥22 breaths per min), and altered mentation (Glasgow coma scale score <15). Each patient's SOFA score was also calculated (Vincent et al., 1996).

Blood sampling and examination

Blood samples were collected, after overnight fasting for 8 h, in ethylenediaminetetraacetic acid (EDTA)-containing tubes early in the morning the day after the patient or their family gave informed consent. Plasma was analyzed by ultra-performance liquid chromatography (UPLC) workflow. Other parameters were measured at the central core laboratory, including estimated glomerular filtration rate (eGFR), hemoglobin, CRP, white blood cell count, pre-albumin, transferrin, and albumin.

Follow-up program

Patients received guideline-based medical treatment for their diagnosed diseases. Admission to the intensive care unit (ICU) was

Table 1
Demographic and laboratory data of all patients at baseline.[a]

	All *n* = 93	Survived *n* = 63	Died *n* = 30	*p*-Value
Age (years)	72.1 ± 13.6	70.7 ± 13.1	75.1 ± 14.3	0.144
Male (%)	58 (62.4)	37 (58.7)	21 (70.0)	0.294
SOFA score	6.25 ± 4.07	5.11 ± 3.56	8.63 ± 4.11	<0.001
Admission to ICU (%)	37 (39.8)	19 (30.2)	18 (60)	0.006
Co-morbidity				
Diabetes mellitus (%)	41 (44.1)	26 (41.3)	15 (50.0)	0.428
Hypertension (%)	70 (75.3)	45 (71.4)	25 (83.3)	0.214
COPD (%)	12 (12.9)	10 (15.9)	2 (6.7)	0.216
Stroke (%)	28 (30.1)	20 (31.7)	8 (26.7)	0.618
Laboratory data				
Albumin (g/dl)	2.90 ± 0.60	2.93 ± 0.61	2.85 ± 0.58	0.551
White blood cells (1000/μl)	12.5 ± 6.25	11.9 ± 6.00	13.7 ± 6.69	0.197
Hemoglobin (g/dl)	10.8 ± 2.50	10.8 ± 2.26	10.9 ± 2.99	0.879
C-reactive protein (mg/l)	60.7 (21.3, 121)	48.6 (7.17, 105)	94.4 (46.6, 160)	0.003
eGFR (ml/min/1.73 m^2)	77.7 ± 61.3	90.4 ± 62.1	51.2 ± 51.1	0.003
Pre-albumin (mg/dl)	13.1 ± 6.50	14.2 ± 7.01	10.8 ± 4.55	0.016
Transferrin (mg/dl)	144 ± 40.9	150 ± 43.7	131 ± 31.1	0.034
Cholesterol (mg/dl)	125 ± 42.6	129 ± 43.8	117 ± 39.4	0.187
Triglyceride (mg/dl)	96.5 (77, 119)	97 (76.5, 128)	95.5 (76.5, 116)	0.934
ALT (U/l)	30 (16.5, 54.5)	27 (15, 51)	38.5 (18, 68.8)	0.091
Body mass index (kg/m^2)	22.1 ± 5.70	21.8 ± 5.70	22.7 ± 5.74	0.480
Bacteremia (%)	14 (15.1)	5 (7.9)	9 (30)	0.011
Amino acid profile	118 ± 118	123.7 ± 141.2	105 ± 40.3	0.481
Leucine (μM)	104 ± 38.5	101 ± 33.9	108 ± 47.2	0.439
Phenylalanine (μM)	84.8 ± 41.7	72.1 ± 20.8	112 ± 59.2	<0.001

SOFA, sequential organ failure assessment; ICU, intensive care unit; COPD, chronic obstructive pulmonary disease; eGFR, estimated glomerular filtration rate; ALT, alanine aminotransferase.

[a] Data are expressed as the mean ± standard deviation for variables with a normal distribution, median (interquartile range) for variables with a skewed distribution, and as the number (percentage) for categorical variables.

defined as a patient transferred to the ICU within 48 h after hospitalization due to critical condition with the need for mechanical ventilation or aggressive intensive care. All patients were followed until death or for a maximum of 3 months. The primary endpoint was all-cause death.

UPLC-based amino acid measurement

Plasma samples (100 μl) were precipitated with 10% sulfosalicylic acid. After protein precipitation and centrifugation, derivatization was initiated by AQC in acetonitrile. The amino acids were then analyzed using the Acquity UPLC System, consisting of a binary solvent manager, a sample manager, and a tunable UV detector. The system was controlled and data collected using Empower 2 software. Separations were performed using a 2.1 × 100 mm Acquity BEH C18 column at a flow rate of 0.70 ml/min. The average intra-assay coefficient of variation was 4.5% for leucine and 4.6% for phenylalanine. The total coefficient of variation was 4.1% for leucine and 3.7% for phenylalanine. The detection limit was 0.9 μM for leucine and 3.3 μM for phenylalanine. The linear range was 25–500 μM for these two amino acids.

Statistical analysis

The results are expressed as the mean ± standard deviation for variables with a normal distribution, as the median (interquartile range) for variables with a skewed distribution, and as the number (percentage) for categorical variables. Data were compared by *t*-test, Mann–Whitney *U*-test, Kruskal–Wallis *H*-test, and Chi-square test (multiple comparison with Bonferroni-adjusted *p*-values) as appropriate. For phenylalanine and leucine levels, the level at baseline was used in the analysis. Receiver operating characteristic (ROC) curves were estimated, and Youden's index was used to identify the cut-off value of variables. The area under the curve (AUC) of the ROCs was determined. The univariate Cox proportional hazards model was used to determine the predictive value of variables on mortality. Cox multivariable analysis was performed to adjust the prognostic value of predictors by CRP, eGFR, bacteremia, and admission to the ICU, or to identify strong independent predictors of mortality by using a forward selection model. Variables with a *p*-value of <0.05 in the univariate analysis were included in the multivariable analysis. Hazard ratios (HRs) and 95% confidence intervals (CIs) were calculated. To compare the time-dependent outcomes, Kaplan–Meier analyses were performed with a log-rank test. All statistical analyses were two-sided and performed using IBM SPSS Statistics version 22.0 (IBM Corp., Armonk, NY, USA). A *p*-value of <0.05 was considered significant.

Results

Baseline characteristics and laboratory data

This study enrolled 93 patients (Table 1). The patients were hospitalized for the following conditions: 19 (20.4%) for urinary tract infections, 43 (46.2%) for pneumonia, six (6.5%) for wound infections, two (2.2%) for gastrointestinal infections, 17 (18.3%) for combined urinary tract infection and pneumonia, and six (6.5%) for other reasons. Bacteremia was found in 14 (15.1%) patients. A total of 37 (39.8%) patients were transferred to the ICU after hospitalization. The average age of the patients was 72.1 years and their average SOFA score was 6.25. Generally, these patients had low levels of albumin, hemoglobin, pre-albumin, transferrin, cholesterol, and triglycerides, but increased white blood cell counts and CRP levels.

Factors associated with mortality

During the 3-month follow-up period, 30 (32.3%) patients died (Table 1). Mortality was associated with higher SOFA scores and admission to the ICU. Mortality was also associated with a higher incidence of bacteremia, higher CRP and phenylalanine levels, but lower pre-albumin and transferrin levels.

Phenylalanine- and leucine-based profiles

Phenylalanine, an amino acid, was strongly associated with mortality. In predicting mortality, phenylalanine at baseline had an AUC of 0.712. The optimal cut-off value for phenylalanine was 84 μM, with sensitivity, specificity, and accuracy of 60%, 71.4%, and 67.7%, respectively. The mortality rate for patients with phenylalanine ≥84 μM was significantly higher than the rate for those with phenylalanine <84 μM (HR 3.18, 95% CI 1.53–6.61, $p = 0.002$).

Next, the prognostic value of leucine was estimated in patients with phenylalanine <84 μM. Leucine levels significantly predicted mortality in this subgroup. In predicting mortality, leucine at baseline had an AUC of 0.746. The optimal cut-off value of leucine was 93 μM, with sensitivity, specificity, and accuracy of 91.7%, 57.8%, and 63.2%, respectively. The mortality rate among patients with leucine <93 μM was significantly higher than the rate among those with leucine ≥93 μM (HR 5.77, 95% CI 1.26–26.4, $p = 0.024$).

Defining risk by phenylalanine and leucine levels

Based on the findings reported above, patients with phenylalanine ≥84 μM were classified as 'high-risk type 1' and patients with phenylalanine <84 μM and leucine <93 μM were classified as

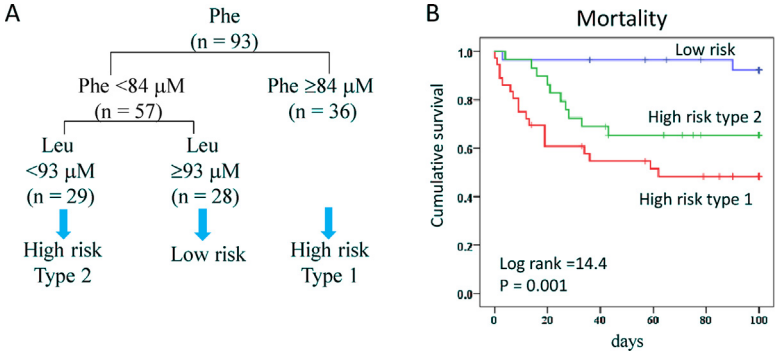

Figure 1. Prognostic value of phenylalanine and leucine. (A) The three types of mortality risk defined by levels of phenylalanine (Phe) and leucine (Leu). (B) Kaplan–Meier curves for the three different risk types.

'high-risk type 2'; all other patients were considered 'low risk' (Figure 1A). Compared to low-risk patients, high-risk type 1 patients had higher SOFA scores and incidence of admission to the ICU, as well as higher CRP and leucine levels (Table 2). High-risk type 2 patients had lower albumin and hemoglobin levels. The mortality rate was 50% in high-risk type 1 patients, 34.5% in high-risk type 2 patients, and 7.1% in low-risk patients. Kaplan–Meier curves demonstrated that, compared to patients at low risk of mortality, patients classified as high-risk types 1 and 2 had significantly worse survival (HR 10.1, 95% CI 2.33–43.5, $p = 0.002$, and HR 5.56, 95% CI 1.22–25.4, $p = 0.027$, respectively) (Figure 1B).

Cox univariate and multivariable analysis of mortality

Cox univariate analysis was performed to identify factors associated with mortality, which showed that high-risk types 1 and 2, SOFA score, CRP, pre-albumin, transferrin, eGFR, bacteremia, and admission to the ICU were significantly associated with death (Table 3). However, only high-risk types 1 and 2 and pre-albumin remained significant after adjusting for CRP, eGFR, bacteremia, and admission to the ICU. Furthermore, multivariable analysis by including high-risk types 1 and 2 and pre-albumin, showed that only high-risk types 1 and 2 were significant predictors of death.

Discussion

Metabolism-based assessment is a novel approach for stratifying patients at high mortality risk. This study showed that the short-term mortality rate among patients hospitalized due to severe infection defined by a high qSOFA score was as high as 32.3%. Amino acid-based profiles including phenylalanine and leucine further identified patients at high risk of mortality, in addition to traditional risk factors such as the SOFA score and levels of CRP, pre-albumin, and transferrin. Amino acid profiling defined two high-risk types, namely high-risk types 1 and 2, which were associated with remarkably high short-term mortality rates when compared to low-risk patients. These two high-risk types represent distinct pathophysiological characteristics. Furthermore, the prognostic value of the amino acid-based profile was independent of traditional risk factors.

Traditional risk assessment tools

Risk assessment is essential for patients with severe infection (Singer et al., 2016). However, since the population of patients admitted for infections is heterogeneous, various scoring systems and nutritional indexes have been developed to predict outcomes. These include the SOFA score (Vincent et al., 1996), albumin, pre-albumin (Arabi et al., 2017; Lavrentieva et al., 2014; Lee et al., 2018; Park et al., 2018, 2017), and transferrin (Lasocki et al., 2018; Shander et al., 2014). The SOFA score provides continuous assessment; however, it does not translate into therapeutic recommendations. As regards nutritional indexes, the severity of hypoalbuminemia does not further discriminate high-risk patients in the overall hypoalbuminemic population. Although pre-albumin and transferrin have been found to be prognostic, their turnover rate, half-life, and potential interference by inflammation and anemia substantially limit their universal use (Ferrie and Tsang, 2017; Geisler et al., 2007; Guerra et al., 2009).

Metabolism-based assessment is a novel approach for identifying patients at the highest risk of mortality (Alexander et al., 2011; Cheng et al., 2015; Hunter et al., 2016; Tenori et al., 2013; Wang et al., 2018a,b, 2017, 2016; Wurtz et al., 2015). Metabolism was estimated after overnight fasting (the early fasting state). The post-absorptive state is not appropriate for amino acid study, since secreted insulin promotes the uptake of branched-chain amino acids by muscle. However, in the early fasting state, amino acids reach homeostasis, not used for gluconeogenesis that happens

Table 2
Comparisons of demographic and laboratory data in patients of different risk types.[a]

	Low risk $n = 28$	Type 1 high risk $n = 36$	Type 2 high risk $n = 29$	p-Value
Age (years)	72.7 ± 14.8	73.5 ± 13.5	69.9 ± 12.6	0.552
Male (%)	17 (60.7)	27 (75)	14 (48.3)	0.085
SOFA score	4.07 ± 2.55	8.25 ± 4.47[c]	5.86 ± 3.64	<0.001
Admission to ICU	6 (21.4)	23 (63.9)[c]	8 (27.6)	0.001
Co-morbidity				
Diabetes mellitus (%)	14 (50)	15 (41.7)	12 (41.4)	0.752
Hypertension (%)	23 (82.1)	27 (75)	20 (69)	0.514
COPD (%)	4 (14.3)	6 (16.7)	2 (6.9)	0.489
Stroke (%)	10 (35.7)	9 (25)	9 (31)	0.645
Laboratory data				
Albumin (g/dl)	2.99 ± 0.42	3.04 ± 0.61	2.63 ± 0.66[b]	0.013
White blood cell (1000/μl)	10.7 ± 4.17	13.3 ± 7.05	13.2 ± 6.70	0.188
Hemoglobin (g/dl)	11.3 ± 1.56	11.4 ± 3.14	9.75 ± 2.02[b]	0.017
C-reactive protein (mg/l)	43.4 (5.89, 101)	98.1 (55.7, 156)[d]	45.7 (6.97, 83.1)	0.004
eGFR (ml/min/1.73 m^2)	93.2 ± 46.6	60.7 ± 68.7	83.9 ± 60.8	0.087
Pre-albumin (mg/dl)	14.7 ± 5.94	11.9 ± 6.63	13.1 ± 6.74	0.230
Transferrin (mg/dl)	158 ± 43.7	136 ± 41.3	139 ± 34.7	0.065
Cholesterol (mg/dl)	126 ± 35.2	125 ± 47.2	125 ± 44.7	0.999
Triglyceride (mg/dl)	92 (80.3, 127)	104 (83, 114)	90 (72, 121)	0.577
ALT (U/l)	21 (13.3, 54)	36 (19, 70)	30 (16.5, 48)	0.091
Body mass index (kg/m^2)	22.0 ± 5.72	22.4 ± 5.22	21.7 ± 6.38	0.875
Bacteremia (%)	3 (10.7)	6 (16.7)	5 (17.2)	0.743
Amino acid profile				
Leucine (μM)	110 ± 19.1	129 ± 39.3[b]	65.9 ± 16.1[c]	<0.001
Phenylalanine (μM)	64.5 ± 9.30	123 ± 44.2[c]	57.3 ± 11.5	<0.001

SOFA, sequential organ failure assessment; ICU, intensive care unit; COPD, chronic obstructive pulmonary disease; eGFR, estimated glomerular filtration rate; ALT, alanine aminotransferase; ANOVA, analysis of variance.

[a] Data are expressed as the mean ± standard deviation for variables with a normal distribution, as the median (interquartile range) for variables with a skewed distribution, and as the number (percentage) for categorical variables.

[b] $p < 0.05$.

[c] XXX, compared to 'low-risk' by one-way ANOVA and Chi-square test (multiple comparison with Bonferroni adjusted p-value).

Table 3
Cox univariate and multivariable analysis for predictors of mortality in patients with severe infection.

	Univariate		Multivariable[a]		Multivariable[b]	
	HR (95% CI)	*p*-Value	HR (95% CI)	*p*-Value	HR (95% CI)	*p*-Value
Phenylalanine (μM)	1.02 (1.01–1.03)	<0.001	1.02 (1.01–1.03)	<0.001	1.02 (1.01–1.02)	<0.001
Risk types						
High-risk type 1	10.2 (2.36–44.2)	0.002	5.65 (1.20–26.7)	0.029	8.81 (2.01–38.5)	0.004
High-risk type 2	5.60 (1.23–25.6)	0.026	4.92 (1.07–22.6)	0.040	4.88 (1.06–22.5)	0.042
Low-risk (reference)						
SOFA score	1.17 (1.08–1.26)	<0.001	1.01 (0.98–1.24)	0.12		
Pre-albumin (mg/dl)	0.91 (0.85–0.98)	0.015	0.91 (0.84–0.99)	0.021	0.94 (0.88–1.01)	0.060
Transferrin (mg/dl)	0.99 (0.98–1.00)	0.046	0.99 (0.98–1.01)	0.325		
CRP (log)	3.19 (1.49–6.84)	0.003				
eGFR (ml/min/1.73 m^2)	0.99 (0.98–0.99)	0.005				
Bacteremia	3.07 (1.39–6.72)	0.005				
Admission to ICU	2.60 (1.25–5.41)	0.011				

HR, hazard ratio; CI, confidence interval; SOFA, sequential organ failure assessment; CRP, C-reactive protein; eGFR, estimated glomerular filtration rate; ICU, intensive care unit.
[a] Adjusted by CRP, estimated glomerular filtration rate (eGFR), bacteremia, and admission to ICU.
[b] Multivariable analysis by including pre-albumin and high-risk types 1 and 2.

after prolonged starvation (Berg et al., 2002). This study demonstrated that phenylalanine- and leucine-defined metabolic types have prognostic value and potentially offer feedback that could help guide medical and nutritional interventions.

Phenylalanine- and leucine-defined risk types

In a previous study, we demonstrated the prognostic value of the phenylalanine- and leucine-defined metabolic types in patients with heart failure (Cheng et al., 2015; Wang et al., 2018b). In this study, we tested the hypothesis that this amino acid-based profiling also provides prognostic value in patients suspected of having severe infection. It was observed that the two high-risk types were both associated with hypoalbuminemia. Additionally, type 1 was distinctly characterized by inflammation as indicated by high CRP and higher SOFA scores, with a higher incidence of admission to the ICU. Type 2 was characterized by extremely low albumin levels and low hemoglobin. In addition, more metabolism information related to these types can be obtained from our previous studies in heart failure patients (Cheng et al., 2015; Wang et al., 2018b). Patients with both high-risk types, compared to low-risk patients, were older, had lower blood pressure, and had worse kidney function and general functional capacity. Furthermore, high-risk type 1 patients had substantially more incompletely metabolized waste of fatty acids in their circulation, due to impaired mitochondrial β-oxidation for energy production, constituting a metabolic storm. High-risk type 2 patients had a lower body mass index and lower levels of cholesterol, hemoglobin, and albumin, suggesting a phenotype of severe malnutrition.

The pathophysiology and mechanisms that explain why these two high-risk types are associated with a higher mortality risk in cases of severe infection have not yet been established. Typical congenital phenylketonuria is characterized by an extremely high blood concentration of phenylalanine, which is associated with a genetic deficit of phenylalanine hydroxylase. However, hyperphenylalaninemia in adults involves a variety of other mechanisms related to disease background. We previously noted that the increase in blood phenylalanine concentration in high-risk type 1 patients indicated substantial tissue breakdown, which is probably related to insufficient tissue perfusion, increased insulin resistance, and dysfunctional energy production machinery associated with a critical status (Wang et al., 2018a,b). The body releases catecholamines in response to severe infection. In vitro studies have shown that epinephrine down-regulates the activity of phenylalanine hydroxylase in cultured hepatocytes, which could lead to the accumulation of phenylalanine (Miller and Shiman, 1976). Sepsis-induced inflammation and a pronounced increase in reactive oxygen species consume a significant portion of tetrahydrobiopterin (BH4), a co-factor of phenylalanine and tyrosine hydroxylases, and leave phenylalanine and tyrosine unmetabolized (Bendall et al., 2014; Nishijima et al., 2011). The perturbed phenylalanine metabolism can interfere with the production of catecholamine and cause unstable hemodynamics. Alternatively, sepsis-related activation of inducible nitric oxide synthase also potentially expends a substantial amount of BH4 for nitric oxide production. BH4 deficiency is associated with impaired microcirculation and subsequent mortality. Whether a phenylalanine level-guided BH4 intervention would be effective in lowering the mortality rate requires investigation.

Matthews et al. mimicked systemic stress by infusing epinephrine in young normal controls and found that it decreased the blood concentrations of all essential amino acids, including branched chain amino acids and phenylalanine (Matthews et al., 1990). Although the findings were inconclusive, speculative mechanisms included attenuated muscle protein breakdown. However, in actual critical patients, the findings on response to stress were different. A previous study of patients with sepsis showed that phenylalanine levels increased although other essential amino acids, including leucine, decreased (Matthews et al., 1990). However, the researchers did not elaborate the prognostic value of hyperphenylalaninemia (Su et al., 2015). Life-threatening situations may involve multiple organs and cause overwhelming stress and extraordinary inflammation, indicated by high cortisol levels. High cortisol levels can also cause muscle protein breakdown, leading to high phenylalanine levels (Christiansen et al., 2007). These pathophysiological mechanisms were present in the high-risk type 1 patients in the current study, who had remarkably high CRP levels. The exact mechanisms underlying the phenylalanine increase are multiple and overlapping, and warrant further elucidation.

The metabolic status in the high-risk type 2 group seems to be the consequence of catecholamine-induced general hypoaminoacidemia and severe malnutrition. However, based on the level of CRP, the pathophysiological stress in high-risk type 2 patients is likely not as great as that in high-risk type 1 patients. A previous study found that leucine levels could represent the amount of circulating essential amino acids, since the concentration of leucine was strongly correlated with the total amount of essential amino acids (Wang et al., 2018a,b). Extremely low levels of circulating amino acids can shut down a variety of protein and enzyme functions that are critical for defense against severe

infection. Intriguingly, although both high-risk types present as severe malnutrition based on albumin, pre-albumin, and transferrin levels, these high mortality risk types actually involve different pathways. Only with accurate assessment of these micromolecules can such patients be treated with appropriate strategies (Reid, 2006). The clinical applications of amino acid profiling definitely merit further investigations.

Study limitations

The pathophysiology behind the findings of elevated phenylalanine levels is currently speculative, such as the amount of BH4, protein breakdown, nitric oxide production, and the activity of phenylalanine and tyrosine hydroxylases. Future studies should be designed to quantify these associated items, which will also shed light on possible therapeutic targets. The small sample size is another limitation of this study. However, the results validate the prognostic value of amino acid profiling as found in our previous study, which included a large sample of patients with acute/decompensated heart failure.

Conclusions

Although the sample size was limited, this study demonstrates that phenylalanine- and leucine-defined metabolic types provide metabolic information and have prognostic value for assessing the mortality risk among patients hospitalized due to severe infection. Future studies should address whether interventions targeted at these biomarkers can improve survival.

Ethical approval

This study was approved by the Ethics Review Board of Chang Gung Memorial Hospital under numbers 201700160B0 and 201700157B0.

Declaration of interest

None.

Conflict of interest

None.

Acknowledgements

This study was supported in part by the Ministry of Science and Technology of Taiwan (MOST107-2314-B-182-071-MY2), Chang Gung Memorial Hospital (CMRPG2G0601, 2G0611, 2G0641, 2G0631, 2G0591, CORPG2J0021, 2J0011), and the Ministry of Education of Taiwan (EMRPD1G0251, EMRPD1H0401). The authors thank the Cardiology Section, Department of Internal Medicine, Chang Gung Memorial Hospital, Keeling, Taiwan for providing the patient samples. We also thank the Healthy Aging Research Center, Chang Gung University from the Featured Areas Research Center Program within the Framework of the Higher Education Sprout Project by the Ministry of Education in Taiwan.

References

Alexander D, Lombardi R, Rodriguez G, Mitchell MM, Marian AJ. Metabolomic distinction and insights into the pathogenesis of human primary dilated cardiomyopathy. Eur J Clin Invest 2011;41:527–38.
Arabi YM, Aldawood AS, Al-Dorzi HM, Tamim HM, Haddad SH, Jones G, et al. Permissive underfeeding or standard enteral feeding in high- and low-nutritional-risk critically ill adults. Post hoc analysis of the PermiT trial. Am J Respir Crit Care Med 2017;195:652–62.
Bendall JK, Douglas G, McNeill E, Channon KM, Crabtree MJ. Tetrahydrobiopterin in cardiovascular health and disease. Antioxid Redox Signal 2014;20:3040–77.
Berg JM, Tymoczko JL, Stryer L. Biochemistry. 5th ed. New York: WH Freeman; 2002.
Cheng ML, Wang CH, Shiao MS, Liu MH, Huang YY, Huang CY, et al. Metabolic disturbances identified in plasma are associated with outcomes in patients with heart failure: diagnostic and prognostic value of metabolomics. J Am Coll Cardiol 2015;65:1509–20.
Christiansen JJ, Djurhuus CB, Gravholt CH, Iversen P, Christiansen JS, Schmitz O, et al. Effects of cortisol on carbohydrate, lipid, and protein metabolism: studies of acute cortisol withdrawal in adrenocortical failure. J Clin Endocrinol Metab 2007;92:3553–9.
Geisler JP, Linnemeier GC, Thomas AJ, Manahan KJ. Nutritional assessment using prealbumin as an objective criterion to determine whom should not undergo primary radical cytoreductive surgery for ovarian cancer. Gynecol Oncol 2007;106:128–31.
Ferrie S, Tsang E. Monitoring nutrition in critical illness: what can we use?. Nutr Clin Pract 2017; 884533617706312.
Fleischmann C, Scherag A, Adhikari NK, Hartog CS, Tsaganos T, Schlattmann P, et al. Assessment of global incidence and mortality of hospital-treated sepsis. Current estimates and limitations. Am J Respir Crit Care Med 2016;193:259–72.
Garnacho-Montero J, Huici-Moreno MJ, Gutierrez-Pizarraya A, Lopez I, Marquez-Vacaro JA, Macher H, et al. Prognostic and diagnostic value of eosinopenia, C-reactive protein, procalcitonin, and circulating cell-free DNA in critically ill patients admitted with suspicion of sepsis. Crit Care 2014;18:R116.
Guerra LT, Rosa AR, Romani RF, Gurski RR, Schirmer CC, Kruel CD. Serum transferrin and serum prealbumin as markers of response to nutritional support in patients with esophageal cancer. Nutr Hosp 2009;24:241–2.
Hunter WG, Kelly JP, McGarrah 3rd RW, Kraus WE, Shah SH. Metabolic dysfunction in heart failure: diagnostic, prognostic, and pathophysiologic insights from metabolomic profiling. Curr Heart Fail Rep 2016;13:119–31.
Iwashyna TJ, Ely EW, Smith DM, Langa KM. Long-term cognitive impairment and functional disability among survivors of severe sepsis. JAMA 2010;304:1787–94.
Lasocki S, Lefebvre T, Mayeur C, Puy H, Mebazaa A, Gayat E. Iron deficiency diagnosed using hepcidin on critical care discharge is an independent risk factor for death and poor quality of life at one year: an observational prospective study on 1161 patients. Crit Care 2018;22:314.
Lavrentieva A, Kontakiotis T, Bitzani M. Enteral nutrition intolerance in critically ill septic burn patients. J Burn Care Res 2014;35:313–8.
Lee SH, Kim SJ, Choi YH, Lee JH, Chang JH, Ryu YJ. Clinical outcomes and prognostic factors in patients directly transferred to the intensive care unit from long-term care beds in institutions and hospitals: a retrospective clinical study. BMC Geriatr 2018;18:259.
Levitt DG, Levitt MD. Human serum albumin homeostasis: a new look at the roles of synthesis, catabolism, renal and gastrointestinal excretion, and the clinical value of serum albumin measurements. Int J Gen Med 2016;9:229–55.
Matthews DE, Pesola G, Campbell RG. Effect of epinephrine on amino acid and energy metabolism in humans. Am J Physiol 1990;258:E948–56.
Miller MR, Shiman R. Reversible inactivation of phenylalanine hydroxylase by catecholamines in cultured hepatoma cells. J Biol Chem 1976;251:3671–6.
Ning J, Shao X, Ma Y, Lv D. Valuable hematological indicators for the diagnosis and severity assessment of Chinese children with community-acquired pneumonia: Prealbumin. Medicine (Baltimore) 2016;95:e5452.
Nishijima Y, Sridhar A, Bonilla I, Velayutham M, Khan M, Terentyeva R, et al. Tetrahydrobiopterin depletion and NOS2 uncoupling contribute to heart failure-induced alterations in atrial electrophysiology. Cardiovasc Res 2011;91:71–9.
Park JE, Chung KS, Song JH, Kim SY, Kim EY, Jung JY, et al. The C-reactive protein/albumin ratio as a predictor of mortality in critically ill patients. J Clin Med 2018;7:.
Park YE, Park SJ, Park Y, Cheon JH, Kim TI, Kim WH. Impact and outcomes of nutritional support team intervention in patients with gastrointestinal disease in the intensive care unit. Medicine (Baltimore) 2017;96:e8776.
Reid C. Frequency of under- and overfeeding in mechanically ventilated ICU patients: causes and possible consequences. J Hum Nutr Diet 2006;19:13–22.
Seymour CW, Liu VX, Iwashyna TJ, Brunkhorst FM, Rea TD, Scherag A, et al. Assessment of clinical criteria for sepsis: for the Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). JAMA 2016;315:762–74.
Shander A, Goodnough LT, Javidroozi M, Auerbach M, Carson J, Ershler WB, et al. Iron deficiency anemia—bridging the knowledge and practice gap. Transfus Med Rev 2014;28:156–66.
Singer M, Deutschman CS, Seymour CW, Shankar-Hari M, Annane D, Bauer M, et al. The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). JAMA 2016;315:801–10.
Su L, Li H, Xie A, Liu D, Rao W, Lan L, et al. Dynamic changes in amino acid concentration profiles in patients with sepsis. PLoS One 2015;10:e0121933.
Szatanik M, Hong E, Ruckly C, Ledroit M, Giorgini D, Jopek K, et al. Experimental meningococcal sepsis in congenic transgenic mice expressing human transferrin. PLoS One 2011;6:e22210.
Tenori L, Hu X, Pantaleo P, Alterini B, Castelli G, Olivotto I, et al. Metabolomic fingerprint of heart failure in humans: a nuclear magnetic resonance spectroscopy analysis. Int J Cardiol 2013;168:e113–5.
Vincent JL, Moreno R, Takala J, Willatts S, De Mendonca A, Bruining H, et al. The SOFA (Sepsis-related Organ Failure Assessment) score to describe organ dysfunction/failure. On behalf of the Working Group on Sepsis-Related Problems of the European Society of Intensive Care Medicine. Intensive Care Med 1996;22:707–10.
Wang CH, Cheng ML, Liu MH. Amino acid-based metabolic panel provides robust prognostic value additive to B-natriuretic peptide and traditional risk factors in heart failure. Dis Markers 2018a;2018:3784589.

Wang CH, Cheng ML, Liu MH. Simplified plasma essential amino acid-based profiling provides metabolic information and prognostic value additive to traditional risk factors in heart failure. Amino Acids 2018b;50:1739–48.
Wang CH, Cheng ML, Liu MH, Kuo LT, Shiao MS. Metabolic profile provides prognostic value better than galectin-3 in patients with heart failure. J Cardiol 2017;70:92–8.
Wang CH, Cheng ML, Liu MH, Shiao MS, Hsu KH, Huang YY, et al. Increased p-cresyl sulfate level is independently associated with poor outcomes in patients with heart failure. Heart Vessels 2016;31:1100–8.
Wurtz P, Havulinna AS, Soininen P, Tynkkynen T, Prieto-Merino D, Tillin T, et al. Metabolite profiling and cardiovascular event risk: a prospective study of 3 population-based cohorts. Circulation 2015;131:774–85.