Amino acid profiling to predict prognosis in patients with heart failure: an expert review

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Abstract

Heart failure is a complex disease with a poor prognosis. A number of widely used prognostic tools have limitations, so efforts to identify novel predictive markers and measures are important. As a metabolomics tool, amino acid profiling has shown promise in predicting heart failure prognosis; however, the evidence has not yet been sufficiently evaluated. We describe the utilization of amino acids in the healthy heart and in heart failure before reviewing the literature on amino acid profiling for prognostic prediction. We expertly interpret the findings and provide suggestions for future research to advance the understanding of the prognostic potential of amino acid profiling in heart failure. Our analysis revealed correlations between amino acid biomarkers and traditional prognostic factors, the additional prognostic value of amino acid biomarkers over traditional prognostic factors, and the successful use of amino acid biomarkers to distinguish heart failure aetiology. Although certain amino acid biomarkers have demonstrated additional prognostic value over traditional measures, such as New York Heart Association functional class, these measures are deeply rooted in clinical practice; thus, amino acid biomarkers may be best placed as additional prognostic tools to improve current risk stratification rather than as surrogate tools. Once the metabolic profiles of different heart failure aetiologies have been clearly delineated, the amino acid biomarkers with the most value in prognostic prediction should be determined. Amino acid profiling could be useful to evaluate the pathophysiology and metabolic status of different heart failure cohorts, distinguish heart failure aetiologies, and improve risk stratification and prognostic prediction.

Introduction

Heart failure is a global public health concern. Worldwide, the number of patients with heart failure increased from 33.5 million in 1990 to 64.3 million in 2017.[1] In addition to its increasing prevalence, heart failure is associated with poor survival. In 2019, a large study of 55 959 patients with heart failure showed a survival rate of 75.9% at 1 year, 45.5% at 5 years, 24.5% at 10 years, and 12.7% at 15 years.[2] As well as being associated with a poor prognosis, heart failure is complicated by the large number of possible aetiologies, including myocardial infarction, myocarditis, and cardiomyopathy. Other cardiovascular-related insults, such as hypertension, diabetes mellitus, atrial fibrillation, valvular disease, and coronary artery disease, can also lead to heart failure.

These variable aetiologies mean that there is substantial heterogeneity in the clinical progression and prognosis of patients with heart failure.[3] As such, efforts to improve the clinical management of patients with heart failure, including diagnosis, prognostic prediction, and treatment, are continuously evolving.

There are a large number of widely used prognostic prediction factors for heart failure, such as New York Heart Association functional class,[4] prior heart failure hospitalization,[5] brain natriuretic peptide concentration,[6] resting cardiac output,[7] and cardiac power index,[8] among others.[9,10] Comprehensive risk-scoring tools for heart failure are also widely used, including Get With the Guidelines-Heart Failure,[11–13] the Emergency Heart Failure Mortality Risk Grade,[14] the Multiple Estimation of Risk based on the Emergency department

Spanish Score in patients with Acute Heart Failure score,[15] the Heart Failure Survival Score,[16] and the AHEAD score[17,18] for acute heart failure; and the Seattle Heart Failure Model[19,20] and the Meta-Analysis Global Group in Chronic Heart Failure risk score[21,22] for chronic heart failure. Other indices are used to assess the nutritional status of patients with heart failure[23–25] and are calculated based on body weight, albumin concentration, cholesterol concentration, and lymphocyte count. In addition, the Global Leadership Initiative on Malnutrition, which is a new framework of nutritional indicators, can also be used in patients with heart failure due to the presence of systemic inflammation.[26] However, some of these prognostic methods have demonstrated important limitations; for example, New York Heart Association functional class has high inter-operator variability.[27] Moreover, according to a published study, prior heart failure hospitalization does not predict 180 day mortality.[5] In addition, general risk stratification tools, such as the Acute Physiology and Chronic Health Evaluation II and the Sequential Organ Failure Assessment,[28,29] which can be used to assess the severity of disease and prognosis of patients admitted to the intensive care unit, including patients with acute heart failure, have high inter-operator variability. In one analysis, only 48% of Sequential Organ Failure Assessment scores were fully in agreement with gold-standard assessment by two expert examiners due to inconsistency among clinicians.[30] Thus, despite the availability of validated tools and measurement indices, prognostic accuracy for patients with heart failure remains poor, and there is still scope for improvement.

Given the limitations of these predictive measures, efforts to identify novel biomarkers that could be incorporated into existing risk stratification tools to improve prognostic prediction and inform patient outcomes could hold the key to future progress. For example, researchers have shown that the discriminatory ability of the Get With the Guidelines-Heart Failure tool was improved by addition of brain natriuretic peptide concentration.[12]

Metabolomics is a relatively new and rapidly expanding research tool that can be used to measure metabolites, such as amino acids, in biological samples, such as plasma, saliva, urine, and tissues. As a metabolomics tool, amino acid profiling using high-resolution mass spectroscopy or nuclear magnetic resonance spectroscopy is useful to study disease pathogenesis,[31] identify novel biomarkers of prognosis, and identify possible therapeutic targets.[32] It has been utilized to study a number of diseases and conditions, such as diabetes mellitus,[33,34] coronary artery disease,[35] neurodegenerative diseases,[36] cancer,[37] and functional limitation in the elderly.[38] Moreover, amino acid profiling is receiving increasing research interest in cardiovascular diseases.[39] However, until recently, the usefulness of amino acid profiling in heart failure has received less attention. Amino acids are not routinely measured during blood tests in patients with heart failure, and none of the existing heart failure risk scores include

amino acid biomarkers. However, when considering the pathophysiology of heart failure, it appears that amino acids might be useful as prognostic biomarkers because heart failure is accompanied by chronic inflammation, which promotes catabolism, resulting in sarcopenia and cachexia. In recent years, a number of studies on amino acid profiling in heart failure have been published, showing evidence that amino acid profiling has the potential to become a novel prognostic tool. The evidence on amino acid profiling in heart failure has been reviewed in the context of diagnosis[40] and treatment,[40,41] but its use in predicting heart failure prognosis has not yet been evaluated in detail.

In this study, we aim to review the usefulness of amino acid profiling to predict the prognosis of patients with heart failure, particularly as a tool that can be incorporated into existing heart failure risk scores to predict prognosis. We will introduce the role of amino acids in the heart and describe their utilization in both the healthy heart and in heart failure. Next, we will review the literature on amino acid profiling for prognostic prediction in heart failure and provide an expert interpretation of the findings. Finally, we will suggest directions for future research to advance the understanding of the prognostic potential of amino acid profiling in heart failure.

Importance of amino acids in the failing heart

Under physiological conditions, approximately 70–90% of cardiac adenosine triphosphate is produced by fatty acid oxidation to fuel cardiac contraction. The remaining energy is produced by glucose and lactate oxidation, and to a lesser degree, ketone body and amino acid metabolism.[42,43] In the failing heart, the metabolism of these substrates changes dramatically, from primary reliance on fatty acids for energy production to primary reliance on glucose metabolism.[44,45] Thus, amino acids are not readily utilized as substrates to generate energy in the form of adenosine triphosphate; instead, they are primarily used for protein synthesis and turnover.[46–48] It follows that in heart failure, the increase in anabolic activity increases the utilization of amino acids.[49]

Branched-chain amino acids, including leucine, isoleucine, and valine, are associated with protein metabolism in the heart and skeletal muscles[50] and may function as regulators of cell signaling[51] while aromatic amino acids, including phenylalanine and tyrosine, are associated with hepatic metabolism.[52] In fact, a wide range of amino acids are produced from protein metabolism and tissue degradation, and these amino acids can be measured to inform the state of metabolism in various diseases, including heart failure (*Figure 1*). Adding to the complexity of amino acid metabolism, there are complex interactions between the heart and other organs, such as the kidneys,[53] blood vessels,[54] liver,[55]

Figure 1 Schematic illustrating the relationship between heart failure and amino acid plasma levels. Heart failure is a complex syndrome characterized by its aetiology (e.g. coronary heart disease, valvular heart disease, and dilated cardiomyopathy), stage of progression, patient background (e.g. sex and age), and comorbidities (e.g. hypertension, atrial fibrillation, and chronic kidney disease). Heart failure leads to a state of systemic inflammation, hormonal dysregulation, and multi-organ dysfunction that in turn causes a systemic imbalance between a catabolic and anabolic state favouring global catabolism. As a result, atrophy of multiple tissues as well as insulin and growth hormone resistance lead to sarcopenia, cachexia, protein degradation, and release of amino acid from tissues into the systemic circulation. This is further exacerbated by reduced kidney and hepatic clearance of amino acids due to tissue hypoperfusion. Given the central phenomenon of tissue breakdown in heart failure and subsequent changes to systemic amino acid levels, metabolomics and amino acid profiling hold great potential as diagnostic, prognostic, and therapeutic tools in heart failure. Specifically, the plasma leucine/phenylalanine ratio, Fischer's ratio (ratio of branched-chain amino acid to aromatic amino acid levels), and levels of individual amino acids have been shown to have prognostic value in heart failure.
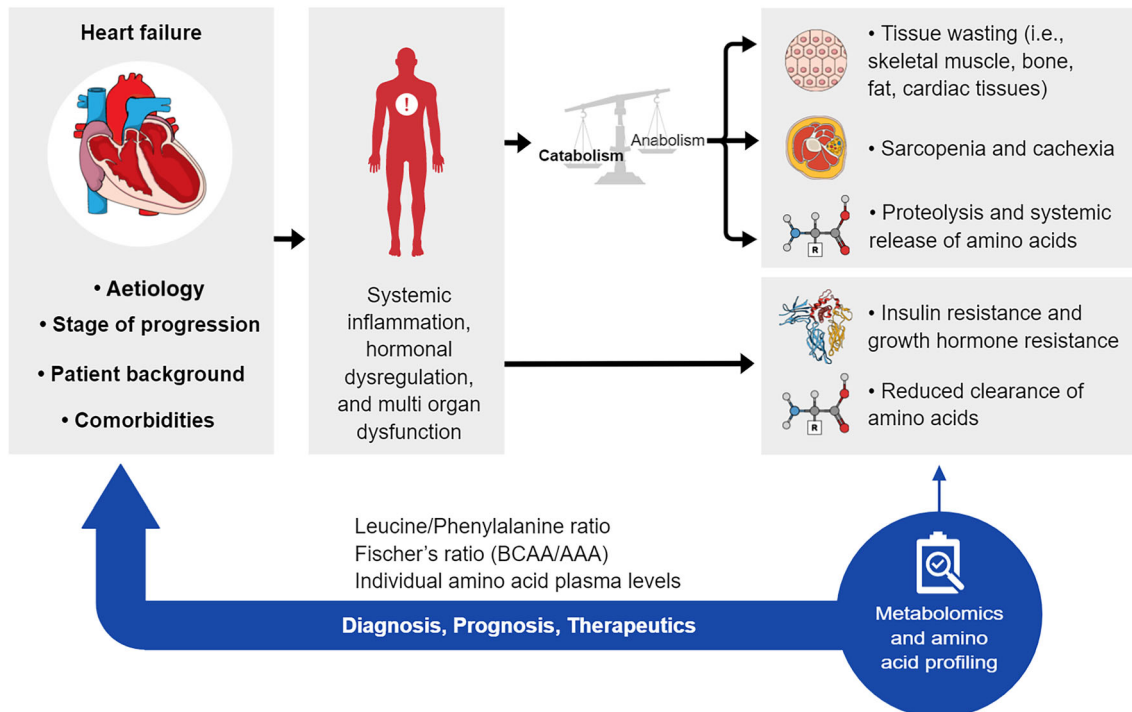
and skeletal muscle.[56,57] For example, patients with heart failure are in a hypercatabolic state that causes amino acids to be released from muscles.[58] Moreover, a previous study observed a change in plasma branched-chain amino acid levels in myocardial infarction, which was thought to be affected by enhanced branched-chain amino acid metabolism in skeletal muscle.[59] Thus, changes in amino acid concentrations may reflect interactions between the heart and other organs.

Prior studies have demonstrated that an increase in plasma branched-chain amino acids is a risk factor for cardiac events in patients with ischaemic heart disease, which is one of the most common causes of heart failure.[60,61] Furthermore, several reports have described changes in both branched-chain amino acid metabolism[50,62] and aromatic amino acid metabolism[63] as well as in the ratio of branched-chain amino acids to aromatic amino acids (Fischer's ratio), in heart failure. Building on these observations that show changes in amino acid concentrations in heart failure, recent studies have suggested that these amino acid biomarkers could be useful to predict heart failure prognosis. The evidence on the use of

amino acid profiling in predicting heart failure prognosis will be reviewed herein.

Amino acid profiling to predict heart failure prognosis

Correlations between amino acid biomarkers and traditional prognostic factors in heart failure

Given the interactions between the heart and other organs, systemic factors, including oxidative stress, nitric oxide, and inflammation, are closely correlated with cardiac function and prognosis in heart failure.[64,65] Recent studies have demonstrated correlations between systemic factors/cardiac function and different amino acid biomarkers in patients with heart failure (*Table 1*; reference values provided in *Supporting information*, *Table S1*), suggesting that amino acid biomarkers could also be useful predictors of prognosis.

Table 1 Summary of recent studies examining the prognostic value of amino acids, ratios, and metabolites associated with heart failure

Publication	Study design	Population	No. of patients	Biomarkers examined	Key results
Hakuno et al., 2015	Case–control study	Stable HF of NYHA class >II, BNP > 40 pg/mL, LVEF <45% vs. asymptomatic population with normal BNP	HF: n = 38; control: n = 33	41 AAs	Multivariate correlations: • Fischer's ratio with BNP • Monoethanolamine with LVEF • Methionine with LVEDVi • Tyrosine with IVC diameter • 1-methylhistidine with ratio of early diastolic velocity of the mitral inflow to mitral annulus • Histidine with BNP
Du et al., 2018	Observational study	STEMI with acute HF	n = 138	26 AAs	• BCAA levels were independent predictors of adverse cardiovascular events • Prognostic value of BCAA was better than NT-proBNP, but the combination of BCAA levels and NT-proBNP demonstrated stronger predictive value • Addition of BCAAs and NT-proBNP to the Global Registry of Acute Coronary Events led to a net reclassification improvement
Wang et al., 2018	Observational study	Acute/decompensated HF	Acute/decompensated HF: n = 599; control: n = 94	Leucine, phenylalanine	• Leucine ≥145 μM and phenylalanine ≥88.9 μM associated with age, lower BP, lower eGFR, and higher BNP • Leucine <81.2 μM associated with age, lower BP, lower eGFR, and higher BNP, lower BMI, lower cholesterol, lower haemoglobin, and lower albumin • Both predicted the composite endpoint (death/HF re-hospitalization) after adjusting for age, sex, BNP, DM, CKD, and hypertension
Wang et al., 2019	Observational study	HF of NYHA Class ≤III; prior hospitalization for acute/decompensated HF and discharged >1 month prior to enrolment; aged 20–85 years	n = 890	Histidine, ornithine, phenylalanine	• HOP score ≥8.8 predicted 1-year composite event (HF re-hospitalization and all-cause death) independent of age, albumin, and BNP in HFrEF and HFpEF, and independent of age, BMI, and BNP in HFmrEF
Chen et al., 2020	Observational study	HF with reduced LVEF <40% (HFrEF), 40–49% (HFmrEF), or ≥50% (HFpEF); NT-proBNP >900 pg/mL; critical status (APACHE II > 15); ICU stay >48 h; aged >20 years	HF: n = 115; control: n = 37	Phenylalanine	• Phenylalanine ≥112 μM predicted mortality over 1 year, independent of age, APACHE II and SOFA scores, AF, CRP, cholesterol, pre-albumin, IL-8, and IL-10 • In multivariate analysis, phenylalanine ≥112 μM correlated with higher APACHE II and SOFA scores, inflammation, higher IL-8 and IL-10, higher CRP, and lower pre-albumin and transferrin
Hiraiwa et al., 2020	Retrospective cohort study	Worsening HF	n = 157	Fischer's ratio	• Low FR independently predicted cardiac events

(Continues)

Table 1 (continued)

Publication	Study design	Population	No. of patients	Biomarkers examined	Key results
Kimura et al., 2020	Observational study	NIDCM (reduced LVEF <50%) and LV dilation, in the absence of CAD and VHD	n = 39	BCAA/total AA ratio	• In univariate analysis, BCAA ratio/total AA ratio was associated with an increased risk of cardiac events (composite of cardiac death, hospitalization for worsening HF, and lethal arrhythmia)
Kouzu et al., 2021	Retrospective observational study	Patients admitted to hospital for HF diagnosis and management	n = 301	3-methylhistidine, beta-alanine, valine, hydroxyproline, tryptophan	• Multivariate analysis showed that a high 3-methylhistidine concentration and low beta-alanine and valine concentrations were independently associated with adverse events (all-cause death, unscheduled readmission for worsening HF, or lethal arrhythmia) • Inclusion of both high 3-methylhistidine and low beta-alanine or low valine into the adjusted model with NT-proBNP improve the accuracy of adverse event prediction after discharge • 3-methylhistidine associated with muscle mass and nutritional status
Liu et al., 2021	Case–control study	HF	HF: n = 96; control: n = 97	23 AAs	• Of 23 AAs and 26 carnitines screened, histidine, arginine, citrulline, glutamine, valine, hydroxyhexadecenyl-carnitine, acylcarnitine C22, hydroxytetradecanoyl-carnitine, and carnitine were associated with HF occurrence • Arginine, glutamine, and hydroxytetradecanoyl-carnitine distinguished DCM from CHD • Aspartic acid and hydroxytetradecanoyl-carnitine helped to classify CHD and VHD

AA, amino acid; AF, atrial fibrillation; BCAA, branched-chain amino acid; BMI, body mass index; BNP, brain natriuretic peptide; BP, blood pressure; CKD, chronic kidney disease; CRP, C-reactive protein; DM, diabetes mellitus; eGFR, estimated glomerular filtration rate; FR, Fischer's ratio; HF, heart failure; HFmrEF, heart failure with mid-range ejection fraction; HFpEF, heart failure with preserved ejection fraction; HFrEF, heart failure with reduced ejection fraction; ICU, intensive care unit; IL-8, interleukin-8; IL-10, interleukin-10; IVC, inferior vena cava; LVEDVi, left ventricular end-diastolic volume index; LVEF, left ventricular ejection fraction; NIDCM, non-ischaemic dilated cardiomyopathy; NT-proBNP, N-terminal pro-brain natriuretic peptide; NYHA, New York Heart Association; and STEMI, ST-segment elevation myocardial infarction.

Hakuno et al.[39] analysed the plasma concentrations of 41 amino acids in 38 patients with stable heart failure (New York Heart Association functional Class ≥2). The authors found that Fischer's ratio and five amino acids, namely, monoethanolamine, methionine, tyrosine, 1-methylhistidine, and histidine, correlated significantly with brain natriuretic peptide concentration, left ventricular ejection fraction, left ventricular end-diastolic volume index, inferior vena cava diameter, ratio of early diastolic velocity of the mitral inflow to mitral annulus, and brain natriuretic peptide concentration, respectively. In another study, Hiraiwa et al.[63] showed that patients with worsening heart failure with a low Fischer's ratio had more heart failure hospitalizations and more cardiac events than patients with a high Fischer's ratio. Moreover, Hiraiwa et al.[63] observed a negative correlation between Fischer's ratio and the concentrations of aspartate transaminase and alkaline phosphatase as indicators of liver function, as well as significant positive correlations between Fischer's ratio and cholinesterase concentration, albumin concentration, and geriatric nutritional risk index, which are all traditional prognostic predictors in heart failure. The correlation between Fischer's ratio and geriatric nutritional risk index could suggest that a change in Fischer's ratio in heart failure reflects a change in nutritional status.[63]

Chen et al.[66] examined the correlation of phenylalanine with inflammation and immune cytokines in 115 patients with New York Heart Association functional class IV heart failure on the basis that phenylalanine metabolism is functionally attenuated by inflammation.[67,68] The data showed that an increased phenylalanine concentration in the plasma predicted mortality in patients with New York Heart Association functional Class IV heart failure and correlated with higher Acute Physiology and Chronic Health Evaluation II and Sequential Organ Failure Assessment scores, inflammation, changes in cytokine concentrations, and malnutrition. Kimura et al.[69] evaluated plasma branched-chain amino acids in patients with non-ischaemic dilated cardiomyopathy as one cause of heart failure. The authors showed that the branched-chain amino acid/total amino acid ratio was positively correlated with body mass index, total cholesterol, and left ventricular ejection fraction, and negatively correlated with total bilirubin and brain natriuretic peptide concentration. Moreover, patients with a low branched-chain amino acid/total amino acid ratio had a poorer prognosis than those with a higher ratio, and a low ratio was a predictor of composite cardiac events in patients with non-ischaemic dilated cardiomyopathy.

The above evidence demonstrates the presence of correlations between traditional prognostic markers of heart failure and certain amino acids, suggesting that amino acids may also have prognostic value. Although a number of amino acids have demonstrated correlations with traditional prognostic factors, their relative predictive value has not yet been determined, and the amino acid biomarkers with the greatest prognostic potential in heart failure are yet to be determined. Thus, given that this field is in its infancy, identifying which amino acids (either individually or in combination) produce the most accurate prognostic score will be important in the future.

Amino acid biomarkers demonstrate additional value over traditional prognostic factors in heart failure

In addition to examining the correlations between amino acid biomarkers and traditional prognostic factors of heart failure, some recent studies have also examined whether amino acid profiling has additional value over traditional prognostic factors.

In their multivariate analysis, Chen et al.[66] showed that phenylalanine predicted mortality over 1 year in critically ill patients with heart failure, independent of several traditional measures, such as Acute Physiology and Chronic Health Evaluation II and Sequential Organ Failure Assessment scores, C-reactive protein, cholesterol, and atrial fibrillation. Although the correlation was only relevant for patients with acute/decompensated heart failure in whom the Acute Physiology and Chronic Health Evaluation II and Sequential Organ Failure Assessment scores were used to predict disease severity and prognosis while in the intensive care unit, the observation that phenylalanine predicted 1 year mortality independent of the general Acute Physiology and Chronic Health Evaluation II and Sequential Organ Failure Assessment scores suggests that it could provide more information on longer term risk in selected patient populations. In another study, Wang et al.[70] demonstrated that a leucine concentration of ≥145 μM and phenylalanine concentration of ≥88.9 μM, as well as a leucine concentration of <81.2 μM, were significant predictors of prognosis in patients with acute/decompensated heart failure, even after adjusting for other risk factors, such as age, sex, brain natriuretic peptide, diabetes mellitus, chronic kidney disease, and hypertension. These observations suggest that leucine and/or phenylalanine may provide additional prognostic information over traditional risk factors in patients with acute/decompensated heart failure; thus, the usefulness of these amino acid biomarkers in other types of heart failure should also be evaluated.

In their 2019 study, Wang et al.[71] measured histidine, ornithine, and phenylalanine in 890 patients with heart failure, 387 of whom underwent cardiopulmonary exercise testing to measure metabolic equivalents. In this study, metabolic equivalents were used to define functional class. The authors showed that the concordance between metabolic equivalents functional class and New York Heart Association functional class was only 47%. In fact, the histidine, ornithine, and phenylalanine score was more effective than New York Heart

Association functional class for distinguishing metabolic equivalents functional Class II and III patients from metabolic equivalents functional Class I patients. Moreover, a histidine, ornithine, and phenylalanine score of ≥8.8 was a powerful predictor of a poor prognosis (death/heart failure re-hospitalization), independent of other risk factors, including brain natriuretic peptide, albumin, and age, in both heart failure with reduced ejection fraction and heart failure with preserved ejection fraction, and brain natriuretic peptide, body mass index, and age in heart failure with mid-range ejection fraction. New York Heart Association functional class is a subjective classification based on clinical presentation and varies by clinician[72]; however, it is still very commonly used in clinical practice. Although it cannot be simply compared with the New York Heart Association functional classification, as they differ in terms of the information they provide, the histidine, ornithine, and phenylalanine score based on cardiopulmonary exercise testing could provide an accurate and objective means to classify patients with heart failure based on exercise-induced metabolism, which could reflect skeletal muscle metabolism and hepatic reserve. Thus, prognostic scores based on exercise-induced amino acid metabolism may be rare, but they are worthy of further exploration.

In another study, Du *et al.*[60] measured 26 amino acids using mass spectrometry in patients with acute heart failure. In a multivariate analysis, branched-chain amino acid concentrations were independent predictors of cardiovascular events in patients with acute heart failure, and the prognostic value of these markers was better than that of N-terminal pro-brain natriuretic peptide concentration. Furthermore, the authors showed that the combination of branched-chain amino acids and N-terminal pro-brain natriuretic peptide had stronger prognostic value than N-terminal pro-brain natriuretic peptide alone, further supporting the hypothesis that amino acid biomarkers could provide additional prognostic value in heart failure.

Hiraiwa *et al.*[63] suggested that the ability of a low Fischer's ratio to predict cardiac events in heart failure was independent of liver function tests and geriatric nutritional risk index. The observation that Fischer's ratio was correlated with measurements of liver function and the geriatric nutritional risk index, but could still independently determine the prognosis of patients with heart failure, suggests that some aspects of liver function and nutritional status may not be expressed in patients with heart failure. Moreover, it could suggest that amino acids have important effects on the heart that are separate from the liver and skeletal muscle and that influence prognosis; however, the exact role of Fischer's ratio in determining heart failure prognosis remains to be determined.

Kouzu *et al.*[73] measured amino acids in 301 patients with stabilized heart failure. They showed that a high 3-methylhistidine concentration and low beta-alanine and valine concentrations were independently associated with ad-verse events, defined as all-cause death and unscheduled re-admission due to worsening heart failure, and event-free survival rates were lower. Elaborating on this, the authors showed that inclusion of both high 3-methylhistidine and low beta-alanine or low valine in the adjustment model with N-terminal pro-brain natriuretic peptide improved the accuracy of event prediction after discharge, suggesting that the evaluation of these amino acids provided additive value in prognostic prediction.

All of these studies demonstrate that some combinations of amino acid biomarkers and traditional prognostic factors have additional prognostic value over traditional factors alone. We also suggest that the combinations of leucine and N-terminal pro-brain natriuretic peptide, leucine/phenylalanine ratio and N-terminal pro-brain natriuretic peptide, and Fischer's ratio and N-terminal pro-brain natriuretic peptide are worthy of further investigation in terms of whether they provide additional prognostic value over each individual parameter alone.

Amino acid biomarkers to distinguish heart failure aetiology and pathophysiology

Previously, clinical trials evaluated patients with heart failure of different aetiologies collectively. However, a recent study by Liu *et al.*[74] emphasized the importance of distinguishing metabolic differences between patients with different heart failure aetiologies. In that study, the authors attempted to identify metabolic profile differences among coronary heart disease, dilated cardiomyopathy, and valvular heart disease. Their results showed that arginine, glutamine, and hydroxytetradecanoyl-carnitine could effectively distinguish heart failure caused by dilated cardiomyopathy from heart failure caused by coronary heart disease. Moreover, hydroxytetradecanoyl-carnitine and aspartic acid could distinguish heart failure caused by valvular heart disease from heart failure caused by coronary heart disease.

Notably, both the studies by Liu *et al.*[74] and Hakuno *et al.*[39] were case–control analyses in patients with heart failure; however, they demonstrated conflicting results in terms of the direction of change in amino acids. For example, Liu *et al.*[74] reported that the phenylalanine concentration increased in coronary heart disease and dilated cardiomyopathy, but decreased in valvular heart disease, whereas Hakuno *et al.*[39] demonstrated an increase in phenylalanine concentration in the general heart failure cohort. This disparity may be explained by heterogeneity among the patient populations evaluated. In the study by Liu *et al.*,[74] patients had either New York Heart Association III or IV heart failure, while in the study by Hakuno *et al.*,[39] patients with New York Heart Association II were also included. Differences in other patient characteristics, such as age, sex, comorbidities, treatment, and pathophysiology may also influence the balance between

catabolism and anabolism and may determine the direction of change in amino acids. Thus, it will also be important to determine the relative importance of amino acid biomarkers according to heart failure pathophysiology, such as in patients with acute/decompensated vs. chronic/stable disease, and to understand whether amino acid biomarkers demonstrate prognostic value in the short and/or long term in these different cohorts. However, to date, no studies have compared the amino acid profiles of patients with acute/decompensated vs. chronic/stable heart failure. In the future, it will be important to consider all such variables when examining changes in amino acids within the wider clinical population classified as having heart failure.

The study by Liu et al.[74] is particularly valuable as it illustrates the importance of examining the metabolic profiles of different heart failure aetiologies. Although the chronic inflammatory nature and cardiac insufficiency of heart failure are present irrespective of aetiology and stage, the metabolic profiles of patients with heart failure differ depending on the aetiology and stage of heart failure. Thus, examining the metabolic differences between cohorts could help to produce more personalized and precise prognostic prediction tools, which may have the potential to improve outcomes for patients.

Future perspectives

Although a number of studies on amino acid profiling in patients with heart failure have been published recently, this area of research is still in its infancy. This review has revealed a number of research avenues that would help to advance the knowledge of amino acid profiling in heart failure in the future.

First, amino acid tracing would be useful to identify which organs circulating amino acids originate from, and whether they act as signalling molecules between organs.[75] This could help researchers and clinicians to better understand disease pathogenesis and progression and, thus, to predict prognosis. Along this line, it would also be useful to identify cardiac-specific amino acids to examine metabolic changes in the heart that could influence cardiac function in heart failure.

Second, the amino acid biomarkers that best predict prognosis should be identified. Fischer's ratio appears to be particularly popular and has demonstrated correlations with BNP, which is commonly measured in patients with heart failure as a prognostic predictor.[76] However, the amino acids that would provide the most benefit in terms of prognostic prediction in heart failure are yet to be clarified.

Third, although certain amino acid biomarkers, such as histidine, ornithine, and phenylalanine, have shown benefit over traditional prognostic measures, such as New York Heart As-

sociation functional class, the traditional measures are deeply rooted in clinical practice. Thus, in future studies, it will be important to examine whether amino acid biomarkers are best placed as additional prognostic tools to improve current risk stratification rather than as surrogate tools. Notably, none of the existing heart failure risk scores include amino acid biomarkers. However, given that heart failure is accompanied by chronic inflammation, which promotes catabolism and subsequent sarcopenia and cachexia, amino acid biomarkers may have the potential to improve the prognostic power of existing risk tools.

Fourth, the clinical significance and interpretation of amino acid biomarkers may depend on the aetiology and pathophysiology of heart failure; thus, once the metabolic profiles of different types of heart failure and their associated physiological processes have been more clearly delineated, deciding which biomarkers should be assessed during both short-term and long-term prognostic prediction in the clinic will be important. A suitable balance should be achieved between ensuring the adequacy of amino acid measurements and minimizing the cost/labour of obtaining these measurements.

Finally, if amino acid profiling is implemented in the clinic, it will be important to consider the influence of diet and supplementation on plasma amino acid concentrations. Plasma amino acid concentrations increase with protein intake.[77] Schmidt et al.[78] showed that the concentrations of methionine, tryptophan, and tyrosine were higher in fish-eaters and vegetarians than meat-eaters and vegans. In fact, all 18 dietary amino acids differed by diet group, with amino acid concentrations being up to 47% lower in vegans than in meat-eaters. Moreover, Alcock et al.[79] demonstrated that dairy protein intake increases plasma leucine concentration. As such, when performing amino acid profiling, it would be important to consider this variability and to be able to distinguish with certainty whether changes in amino acid concentrations are associated with heart failure or whether they are due to other influencing factors, such as diet. One way to achieve this would be for patients to attend regular clinic visits to establish baseline concentrations for amino acids of interest considering their dietary intake, such that subsequent changes in serum amino acid concentrations can be better attributed to the progression of heart failure.

Conclusions

Amino acid profiling has received substantial interest in the areas of cardiovascular disease diagnosis, prognosis, and treatment, with numerous studies published on heart failure in recent years. Several studies have demonstrated correlations between certain amino acid biomarkers and traditional

prognostic factors in patients with heart failure. Moreover, some amino acid biomarkers have also demonstrated additional value over traditional prognostic factors for heart failure. However, many traditional factors are deeply rooted in clinical practice. Therefore, amino acid biomarkers may be best placed as additional prognostic tools to improve risk stratification rather than as surrogate tools. Amino acid profiling could be useful to distinguish heart failure aetiologies and to evaluate the pathophysiology and metabolic status of different heart failure cohorts, making it useful for more personalized prognostic prediction. In clinical practice, it will be important to determine which amino acids should be incorporated into existing prognostic models to improve current risk stratification and to ensure the use of amino acid profiling is justified in terms of cost and convenience.

Conflict of interest

Takahiro Okumura received research grants from Ono Pharmaceutical, Amgen Astellas BioPharma, Pfizer Japan, Alnylam Japan, and Alexion (not in connection with the submitted work) as well as honoraria from Ono Pharmaceutical, Otsuka Pharmaceutical, Novartis Pharma, and AstraZeneca. Toyoaki Murohara received lecture fees from Bayer Pharmaceutical, Daiichi-Sankyo, Dainippon Sumitomo Pharma, Kowa, MSD, Mitsubishi Tanabe Pharma, Nippon Boehringer Ingelheim, Novartis Pharma, Pfizer Japan, Sanofi-Aventis, and Takeda Pharmaceutical. Toyoaki Murohara also received an unrestricted research grant from the Department of Cardiology of Nagoya University Graduate School of Medicine, as well as honoraria from Astellas Pharma, Daiichi-Sankyo, Dainippon Sumitomo Pharma, Kowa, MSD, Mitsubishi Tanabe Pharma, Nippon Boehringer Ingelheim, Novartis Pharma, Otsuka Pharma, Pfizer Japan, Sanofi-Aventis, Takeda Pharmaceutical, and Teijin Pharma. All other authors declare that they have no relationships with industry relevant to the contents of this paper.

Supporting information

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

Table S1. Reference ranges for amino acids, ratios, and metabolites associated with heart failure in healthy patients.

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