Original Article

A Low Arginine/Ornithine Ratio is Associated with Long-Term Cardiovascular Mortality

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Aims: The long-term prognostic value of the bioavailability of ʟ-arginine, an important source of nitric oxide for the maintenance of vascular endothelial function, has not been investigated fully. We therefore investigated the relationship between amino acid profile and long-term prognosis in patients with a history of standby coronary angiography.

Methods: We measured the serum concentrations of ʟ-arginine, ʟ-citrulline, and ʟ-ornithine by high-speed liquid chromatography. We examined the relationship between the ʟ-arginine/ʟ-ornithine ratio and the incidence of all-cause death, cardiovascular death, and major adverse cardiovascular events (MACEs) in 262 patients (202 men and 60 women, age 65 ± 13 years) who underwent coronary angiography over a period of ≤ 10 years.

Results: During the observation period of 5.5 ± 3.2 years, 31 (12%) patients died, including 20 (8%) of cardiovascular death, while 32 (12%) had MACEs. Cox regression analysis revealed that ʟ-arginine/ʟ-ornithine ratio was associated with an increased risk for all-cause death (unadjusted hazard ratio, 95% confidence interval) (0.940, 0.888–0.995) and cardiovascular death (0.895, 0.821–0.965) ($p < 0.05$ for all). In a model adjusted for age, sex, hypertension, hyperlipidemia, diabetes, current smoking, renal function, and $\log_{10}$-transformed brain natriuretic peptide level, cardiovascular death (0.911, 0.839–0.990, $p = 0.028$) retained an association with a low ʟ-arginine/ ʟ-ornithine ratio. When the patients were grouped according to an ʟ-arginine/ʟ-ornithine ratio of 1.16, the lower ʟ-arginine/ʟ-ornithine ratio group had significantly higher incidence of all-cause death, cardiovascular death, and MACEs.

Conclusion: A low ʟ-arginine/ʟ-ornithine ratio may be associated with increased 10-year cardiac mortality.

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1. Introduction

Arteriosclerosis increases with age, partly due to lifestyle-related diseases such as hypertension, diabetes, renal dysfunction, and physical dysfunction, and the progression is associated with further cardiovascular events[1-5]. Atherosclerosis is caused by endothelial dysfunction due to vascular stiffening and hypercoagulability[6-11]. Nitric oxide (NO) plays an important role in the maintenance of vascular endothelial function by alleviating vasoconstriction, vascular smooth muscle cell proliferation, leukocyte adhesion, and platelet aggregation. The sole source of nitrogen for NO is ʟ-arginine, which produces NO via the activity of endogenous NO synthase (NOS). Therefore, endothelial dysfunction due to decreased

NO bioavailability is associated with decreased L-arginine bioavailability[12]. L-arginine is obtained from the diet and from the endogenous synthesis and degradation of proteins, and serum L-arginine concentrations are generally maintained at levels sufficient for NO production[13, 14].

However, despite normal serum L-arginine concentrations, patients with hypertension, hyperlipidemia, and diabetes mellitus may experience reduced L-arginine bioavailability and associated endothelial dysfunction. This phenomenon has been termed the "arginine paradox"[15]. The global L-arginine bioavailability ratio (GABR)[16], defined as the ratio of L-arginine levels to the sum of its major metabolite levels (L-arginine / [L-citrulline + L-ornithine]), is an important indicator in a variety of clinical situations, including stable coronary artery disease (CAD)[17-24]. Patients with cardiogenic shock due to acute myocardial infarction (AMI) have been reported to have a lower GABR compared with patients with stable CAD[25]. We also previously reported that the GABR is lower in patients with acute coronary syndrome (ACS)[26-28]. Thus, many cross-sectional studies have examined amino acid profiles; however, long-term mortality according to the amino acid profile has not been fully investigated.

2. Aim

In this study, we investigated the relationship between the amino acid profile and 10-year mortality in patients with a history of standby coronary angiography.

3. Methods

3.1. Study Patients

We recruited patients who underwent elective cardiac catheterization for suspected or previously diagnosed CAD at the National Defense Medical College (Tokorozawa, Japan) from November 2012 to November 2013 and who had serum amino acid measurements. This study was approved by the Ethics Committee of the National Defense Medical College (no. 1084) and was conducted in accordance with the Declaration of Helsinki. The study protocol was registered on the website of University Hospital Medical Information Network (UMIN) Center in Japan (URL: https://center6.umin.ac.jp/cgi-open-bin/ctr_e/ctr_view.cgi?recptno=R000010380, UMIN000009635). We obtained written informed consent from each patient. The exclusion criteria were age < 20 years, ongoing treatment for malignancy, and acute emergent status including acute coronary

syndrome and acute heart failure. All patients included in the study were fasting.

3.2. Primary and Secondary Endpoints

The patients were followed prospectively until June 2021. The condition of each patient was assessed from our hospital records by at least two of the authors. The primary endpoints were all-cause death and cardiovascular death. The secondary endpoint was major adverse cardiovascular events (MACEs). We defined MACEs as death from cardiovascular causes, non-fatal myocardial infarction, and non-fatal cerebral stroke.

### 3.3. Coronary Angiography and Amino Acid Measurements

Coronary angiography was performed using a 4Fr catheter system. We obtained angiograms from four standard projections for each of the right and left coronary arteries. Obstructive stenosis was defined as a narrowing of more than 75% of the lumen; we defined CAD as coronary stenosis in at least one major coronary artery or its branches or a clinical history of myocardial infarction, percutaneous coronary intervention, or coronary artery bypass graft surgery. We collected blood samples in plain tubes from the guide sheath during coronary angiography prior to heparin administration, and we refrigerated the samples immediately. We obtained serum by centrifugation at 3,000 rpm for 10 min at 4℃ and stored the samples at −80℃ until needed for amino acid analysis. Serum L-arginine, L-citrulline, and L-ornithine levels were measured by high-speed liquid chromatography using a Shimadzu RF-20A system (Shimadzu Corporation, Kyoto, Japan) with a Symmetry C18 column (3.9×150 mm, particle size 5 µm; Waters Corp., Milford, MA). The detection method was based on fluorescence derivatization using the AccQ-Fluor™ reagent (Waters Corp.), as previously described[29, 30].

3.4. Patient Classification

To obtain Kaplan–Meier curves based on survival time analysis, we classified the patients into four groups using the quartile range of the L-arginine/L-ornithine ratio: from Q1 to Q4 for the lowest to the highest quartile range, respectively.

3.5. Coronary Risk Factors

We assessed coronary risk factors using the following definitions. We defined hypertension as blood pressure greater than 140/90 mmHg or a prior diagnosis of hypertension with blood pressure-lowering medication. We defined diabetes mellitus as
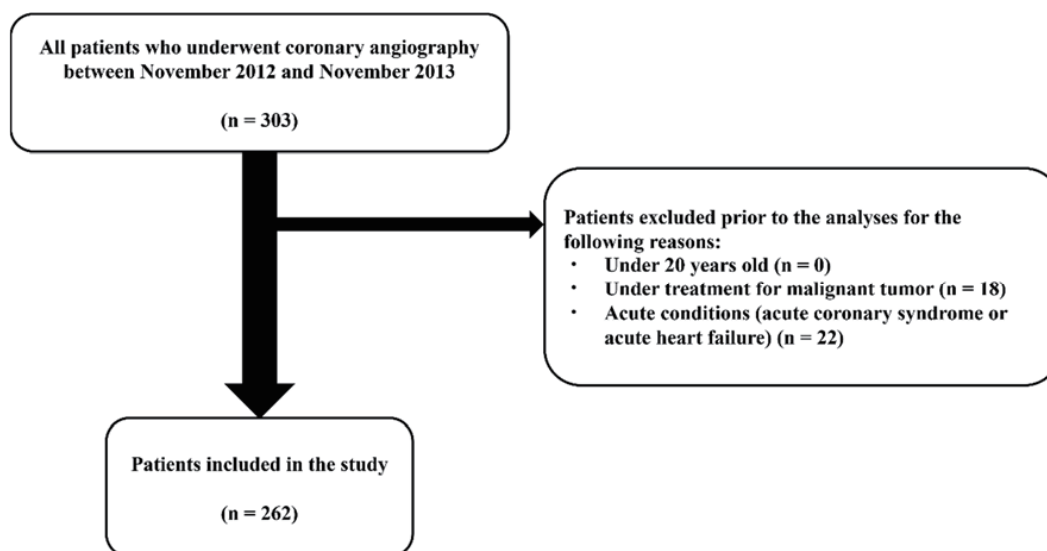
Fig. 1. Flowchart of patient inclusion

fasting blood glucose >126 mg/dL or the use of insulin or oral hypoglycemic agents. We defined hyperlipidemia as total cholesterol >220 mg/dL, low-density lipoprotein cholesterol >140 mg/dL, or a prior diagnosis of hyperlipidemia with lipid-lowering medication. We calculated the estimated glomerular filtration rate (eGFR) using the Modification of Diet in Renal Disease equation modified for the Japanese population [31, 32].

3.6. Statistical Analysis

Summary data are presented as mean ± standard deviation and 95% confidence interval (CI) for parametric variables and median (interquartile range) for nonparametric variables. We performed cross-table analysis using the chi-square test or Fisher's exact test, as appropriate. The comparison of measured variables by four groups was performed using analysis of variance (ANOVA). For nonparametric variables, we used the Kruskal–Wallis test. Residual analysis was performed using Bonferroni's post-hoc test after ANOVA. In Cox proportional hazards model analysis, we added age and sex as explanatory variables in Model 1. We added hypertension, hyperlipidemia, diabetes mellitus, current smoking, CAD, eGFR, and log10-transformed brain natriuretic peptide level (Log10BNP) to Model 1 for Model 2 because these factors are known to influence vascular endothelial function [33-35]. We further added diabetes mellitus, prescription of statin, calcium channel blocker and nitrates, eGFR, and Log10BNP to Model 1 for Model 3 because statins and calcium channel blockers have been reported to improve vascular endothelial

function [36, 37]. We used the Kaplan–Meier method with the log-rank test. We performed most statistical analyses using JMP version 15.0 (SAS Institute, Inc., Cary, NC) or SPSS version 22.0 (SPSS Japan, Tokyo, Japan), while cubic spline analysis was performed using R version 4.1.0 (R Core Team 2020). For all analyses, we considered $p < 0.05$ to be statistically significant. We also considered a two-sided p-value < 0.05 to be statistically significant.

4. Results

4.1. Clinical Characteristics of the Patients

We included data from 262 patients (202 men, 60 women; mean age 65 ± 13 years) who underwent elective cardiac catheterization and serum amino acid measurements in the analysis **(Fig. 1)**. Of these patients, 129 (49%) had CAD **(Table 1)**. The observation period was 5.5 ± 3.2 years. The rank of quartiles of L-arginine/L-ornithine increased with age, CAD, and previous coronary artery bypass surgery. There were differences in eGFR and Log10BNP among the quartile groups.

4.2. Association between the Endpoints and Amino Acid Profile

The number of patients with all-cause death, cardiovascular death, or MACEs during the observation period is shown in **Table 2**. Thirty-one patients died during the observation period, of which cardiovascular death occurred in 20 patients. MACEs were counted in 32 patients. The lowest ranked group had a higher frequency of death from heart failure and

Table 1. Patient characteristics and laboratory measurements classified by quartiles of the L-arginine/L-ornithine ratio (Q1-Q4)

	Total (*n*=280)	Q1 (*n*=65)	Q2 (*n*=66)	Q3 (*n*=65)	Q4 (*n*=66)	*p*-value
L-arginine/L-ornithine		<1.16	1.16 - 1.65	1.66 - 2.16	2.16<	
Age (years)*	65 ± 13	68 ± 11	67 ± 12	62 ± 14	64 ± 14	0.022
Sex (male/female), *n* (%)	202/60 (23/77)	15/50 (23/77)	14/52 (21/79)	11/64 (17/83)	20/46 (30/70)	0.324
BMI (kg/m²)	23.5 ± 4.0	23.7 ± 3.3	23.1 ± 3.5	23.4 ± 3.7	23.8 ± 5.2	0.766
Hypertension, *n* (%)	165 (63)	47 (72)	45 (68)	39 (60)	34 (52)	0.067
Hyperlipidemia, *n* (%)	136 (52)	41 (63)	34 (52)	33 (51)	28 (42)	0.129
Diabetes mellitus, *n* (%)	88 (34)	23 (35)	26 (39)	19 (29)	20 (30)	0.580
Current smoking, *n* (%)	75 (29)	24 (37)	15 (23)	20 (31)	16 (24)	0.253
Previous conditions						
CAD, *n* (%)*, †	128 (49)	39 (60)	39 (59)	26 (40)	24 (36)	0.007
OMI, *n* (%)	47 (18)	12 (18)	17 (26)	9 (14)	9 (14)	0.229
CABG, *n* (%)‡	16 (6)	8 (12)	5 (8)	3 (5)	0	0.027
PCI, *n* (%)	64 (24)	17 (26)	18 (27)	15 (23)	14 (21)	0.843
HCM, *n* (%)	27 (10)	5 (8)	8 (12)	9 (14)	5 (8)	0.546
DCM, *n* (%)	20 (8)	3 (5)	4 (6)	5 (8)	8 (12)	0.398
Atrial fibrillation, *n* (%)	29 (11)	9 (14)	8 (12)	7 (11)	5 (8)	0.704
Medications						
Bata-blocker, *n* (%)	99 (38)	28 (43)	23 (35)	26 (40)	22 (33)	0.635
ACE inhibitor, *n* (%)	24 (9)	7 (11)	7 (11)	6 (9)	4 (6)	0.770
ARB, *n* (%)	121 (46)	32 (49)	29 (44)	25 (38)	35 (53)	0.365
Calcium channel blocker, *n* (%)	94 (36)	25 (38)	28 (42)	19 (29)	22 (33)	0.415
Nitrates, *n* (%)	18 (7)	6 (9)	8 (12)	2 (3)	2 (3)	0.094
Furosemide, *n* (%)	41 (16)	12 (18)	9 (14)	9 (14)	11 (17)	0.849
Spironolactone, *n* (%)	27 (10)	9 (14)	3 (5)	9 (14)	6 (9)	0.237
Statin, *n* (%)	126 (48)	37 (57)	31 (47)	34 (52)	24 (36)	0.104
Insulin, *n* (%)	21 (8)	5 (8)	7 (11)	4 (6)	5 (8)	0.818
Warfarin, *n* (%)	36 (14)	14 (22)	10 (15)	7 (11)	5 (8)	0.109
Hb (g/dL)	13.4 ± 1.8	13.4 ± 1.8	13.1 ± 1.8	13.7 ± 1.8	13.6 ± 1.7	0.314
AST (IU/L)	26 ± 10	25 ± 9	27 ± 10	27 ± 13	27 ± 10	0.768
ALT (IU/L)	25 ± 17	23 ± 15	23 ± 14	27 ± 18	26 ± 19	0.383
LDL cholesterol (mg/dL)	104 ± 30	106 ± 26	102 ± 29	102 ± 37	104 ± 29	0.865
TG (mg/dL)	114 (84. 172)	127 (79. 185)	107 (85. 171)	112 (90. 162)	114 (77. 164)	0.212
HDL cholesterol (mg/dL)	54 ± 17	51 ± 12	54 ± 16	56 ± 17	57 ± 21	0.179
HbA1c (%)	5.9 ± 1.0	5.7 ± 0.8	6.0 ± 1.1	6.0 ± 1.2	5.9 ± 0.9	0.401
Cr (mg/dL)§	1.1 ± 1.2	1.1 ± 1.1	1.5 ± 1.8	0.9 ± 0.3	0.9 ± 0.7	0.020
CRP (mg/dL)	0.30 (0.30. 0.30)	0.30 (0.30. 0.50)	0.30 (0.30. 0.30)	0.30 (0.30. 0.30)	0.30 (0.30. 0.30)	0.930
eGFR (mL/min)‡, §	66 ± 22	63 ± 24	59 ± 23	69 ± 18	73 ± 23	0.001
Log₁₀BNP§	1.76 ± 0.66	1.76 ± 0.68	1.96 ± 0.71	1.70 ± 0.63	1.63 ± 0.59	0.030
Dd (mm)	50 ± 8	48 ± 9	51 ± 8	51 ± 8	49 ± 8	0.186
Ds (mm)	33 ± 10	32 ± 9	34 ± 11	35 ± 11	32 ± 9	0.331
EF (%)	61 ± 15	62 ± 14	60 ± 17	59 ± 16	62 ± 14	0.538
DT (ms)	218 ± 83	230 ± 111	200 ± 73	215 ± 75	227 ± 65	0.189
E/E'	14.5 ± 8.0	13.8 ± 6.4	16.2 ± 9.3	14.7 ± 10.7	13.3 ± 4.2	0.285
LAD (mm)	40 ± 9	39 ± 11	40 ± 10	41 ± 8	39 ± 8	0.531
L-arginine (µmol/L)‖	82.8 ± 24.1	76.1 ± 24.5	87.0 ± 24.0	82.2 ± 22.1	85.9 ± 24.9	0.041
L-ornithine (µmol/L)§, ¶, #	57.1 ± 29.2	90.3 ± 30.8	62.2 ± 17.5	43.5 ± 12.0	32.8 ± 11.0	<0.001
L-citrulline (µmol/L)	132.0 ± 75.7	142.5 ± 76.0	140.6 ± 95.7	119.1 ± 55.6	125.9 ± 68.6	0.220
L-arginine/L-ornithine§, ¶, #	1.73 ± 0.76	0.87 ± 0.19	1.41 ± 0.15	1.90 ± 0.15	2.72 ± 0.61	<0.001
L-arginine/L-citrulline	0.80 ± 0.48	0.66 ± 0.33	0.83 ± 0.57	0.86 ± 0.51	0.83 ± 0.47	0.061
GABR§, ¶	0.50 ± 0.21	0.36 ± 0.12	0.48 ± 0.18	0.56 ± 0.21	0.61 ± 0.23	<0.001

Significant *p*-values are indicated in bold. *$p<0.05$ Q1,2 vs. Q3; †$p<0.05$ Q1,2 vs. Q4; ‡$p<0.05$ Q1 vs. Q4; §$p<0.05$ Q2 vs. Q3,4; ‖$p<0.05$ Q1 vs. Q2,4; ¶ $p<0.01$ Q1 vs. Q2,3,4; #$p<0.01$ Q3 vs. Q4.
ACE, angiotensin-converting enzyme; ALT, alanine aminotransferase; ARB, angiotensin receptor blocker; AST, aspartate aminotransferase; BMI, body mass index; BNP, brain natriuretic peptide; BP, blood pressure; BUN, blood urea nitrogen; CABG, coronary artery bypass graft; CAD, coronary artery disease; Cr, creatinine; CRP, c-reactive protein; DCM, dilated cardiomyopathy; Dd, left ventricular end-diastolic dimension; Ds, left ventricular end-systolic dimension; DT, deceleration time; EF, ejection fraction; eGFR, estimated glomerular filtration rate; GABR, global L-arginine bioavailability ratio; Hb, hemoglobin; HbA1c, hemoglobin A1c; HCM, hypertrophic cardiomyopathy; HDL, high-density lipoprotein; Ht, hematocrit; LAD, left atrial dimension; LDL, low-density lipoprotein; OMI, old myocardial infarction; PCI, percutaneous coronary intervention; TG, triglyceride.

Table 2. All events that occurred in the observation period

	Total	1st Quartile	2nd Quartile	3rd Quartile	4th Quartile
L-arginine/L-ornithine		<1.16	1.16 - 1.65	1.66 - 2.16	2.16<
All patients, *n*	262	65	66	65	66
All-cause death, n (%)	31 (12)	15 (23)	5 (8)	7 (11)	4 (6)
Cancer, *n* (%)	6 (2)	1 (2)	1 (2)	2 (3)	2 (3)
Others, *n* (%)	5 (2)	2 (3)	0	3 (5)	0
Cardiovascular death, *n* (%)	20 (8)	12 (18)	4 (6)	2 (3)	2 (3)
Heart failure, *n* (%)	7 (3)	3 (5)	3 (5)	1 (2)	0
Stroke, *n* (%)	4 (2)	2 (3)	0	1 (2)	1 (2)
Aortic dissection, *n* (%)	2 (1)	1 (2)	0	0	1 (2)
Sudden death, *n* (%)	6 (2)	5 (8)	1 (2)	0	0
Infective endocarditis, *n* (%)	0	1 (2)	0	0	0
MACEs, *n* (%)	32 (12)	14 (22)	6 (9)	5 (8)	7 (11)
Non-fatal AMI, *n* (%)	4 (2)	0	1 (2)	1 (2)	2 (3)
Non-fatal stroke, *n* (%)	8 (3)	2 (3)	0	2 (3)	4 (6)
Survivor of VF, *n* (%)	0	0	1 (2)	0	0

There was a patient in Q4 who experienced both non-fatal AMI and non-fatal stroke. AMI, acute myocardial infarction; MACEs, major adverse cardiovascular events.

sudden death.

4.3. Survival Curves

The Kaplan–Meier curves of the quartile groups are shown in **Fig. 2**. Increased death from all causes and from cardiovascular events were associated with lower L-arginine/L-ornithine ratios. Therefore, dividing the patients into two groups at an L-arginine/L-ornithine ratio of 1.16, which is the highest boundary of the lowest quartile group and suggested to be the most effective cut-off point in the ROC curve, showed that the lower group had a higher incidence of all-cause death, cardiovascular death, and MACEs **(Fig. 3)**.

4.4. Cox Regression Analysis for the Primary and Secondary Endpoints

The results of the Cox hazard analysis for the L-arginine/L-ornithine ratio for the primary and secondary endpoints are shown in **Table 3**. In the univariate Cox analysis, the hazard ratios (HR) (95% CI) were 0.940 (0.888-0.995) for all-cause death and 0.890 (0.821-0.965) for cardiovascular death ($p < 0.05$ for all). This ratio also tended to be associated with the incidence of MACEs but was not significant ($p = 0.093$). In the multivariate Cox analysis of Models 1, 2, and 3, the risk of cardiovascular death decreased with increasing L-arginine/L-ornithine ratio.

In the quartile group, the lowest-ranked group had a significantly higher risk of cardiovascular death compared with the highest-ranked group. In the binary classification, a cutoff point of 1.16 for the L-arginine/L-ornithine ratio was useful for assessing risk for all endpoints. The risk of an L-arginine/L-ornithine ratio less than 1.16 was 2.7 times higher for all-cause death, 4.9 times higher for cardiovascular death, and 2.5 times higher for MACEs compared with the others.

### 4.5. Cubic Spline Analysis for the Primary and Secondary Endpoints

The results of the cubic spline analysis for the primary and secondary endpoints of the L-arginine/L-ornithine ratio are shown in **Fig. 4**. In the univariate analysis, HR tended to decrease with increasing L-arginine/L-ornithine ratio for all-cause death, cardiovascular death, and MACEs. In particular, the HR for cardiovascular death decreased with increasing L-arginine/L-ornithine ratio in the multivariate analysis in Model 2.

5. Discussion

We found that a low L-arginine/L-ornithine ratio was associated with an increased risk of cardiovascular death after statistical adjustment for the following clinical and laboratory factors: hypertension, hyperlipidemia, diabetes mellitus, current smoking, CAD, eGFR, and $Log_{10}BNP$. These results suggest that the L-arginine/L-ornithine ratio may be an independent biomarker for assessing long-term mortality from cardiovascular death.

L-arginine is the only source of nitrogen for NO production and is supplied by the body either via the
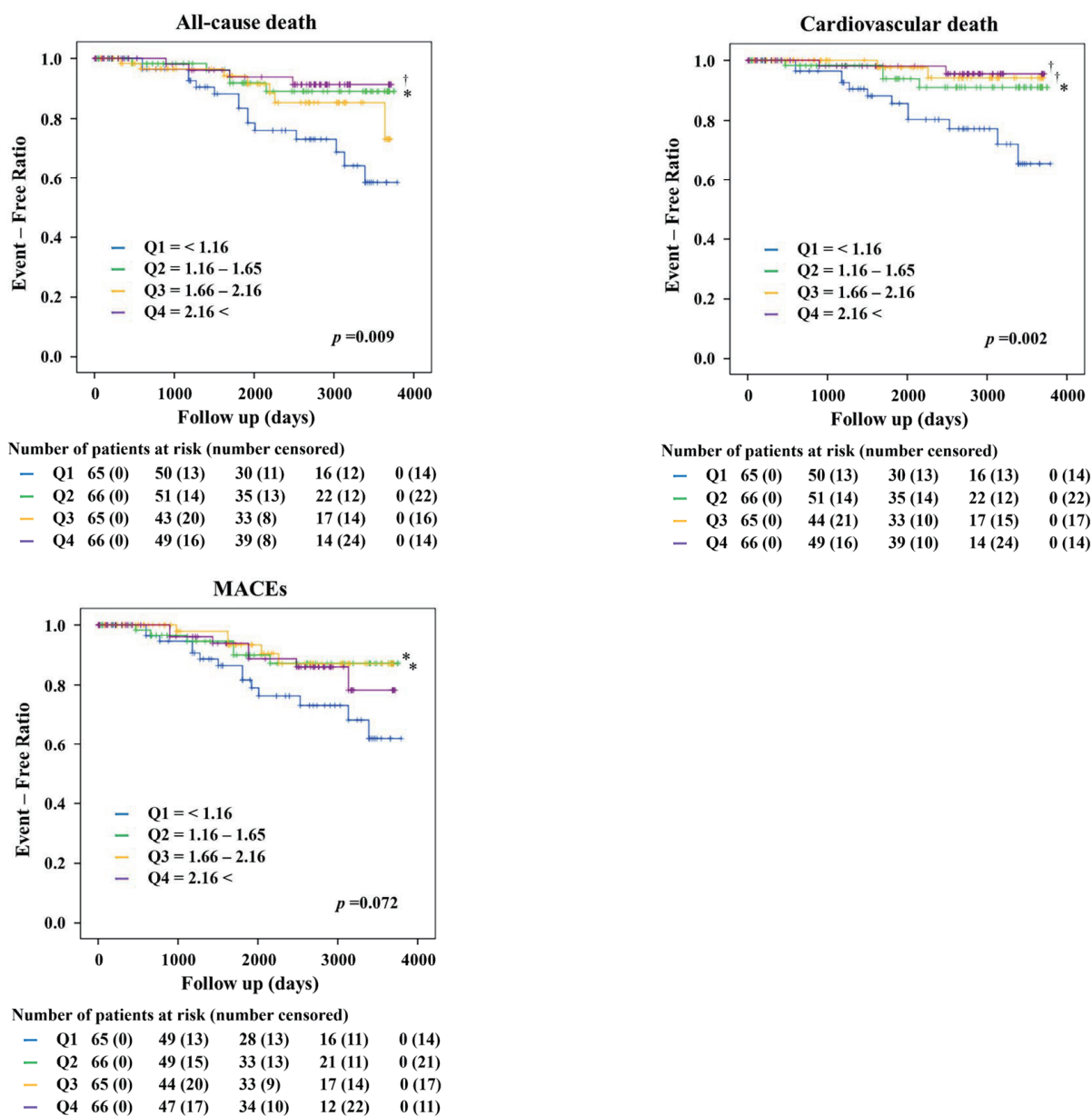
Fig. 2. Kaplan–Meier curves by quartile (Q1–Q4) showing that lower L-arginine/L-ornithine ratios are associated with increased mortality from all-cause death and cardiovascular death

The *p*-values in the graphs indicates overall difference among the quartile groups. *$p < 0.05$, †$p < 0.01$ vs. Q1. MACEs, major adverse cardiovascular events.

diet or the ornithine circuit. Serum L-arginine concentrations are generally maintained at a level sufficient for NO production[13, 14]. However, in acute severe conditions (e.g., sepsis), L-arginine concentrations in plasma and intracellular muscle are significantly reduced compared with healthy individuals[38, 39]. There is no change in plasma L-arginine production in patients with sepsis[40], suggesting that the decrease in L-arginine concentration in these patients is due to altered arginine metabolism or increased transport

across the cell membrane. The arginine transport system is modulated by bacterial endotoxins and inflammatory cytokines, which may upregulate cationic amino acid transporter-2 (CAT-2) and downregulate cationic amino acid transporter-1 (CAT-1)[41, 42]. As a result, the transport of arginine to inducible NOS-2 is increased, while its transport to NOS-3 (endothelial NOS) is decreased.

In contrast, despite normal L-arginine concentrations in serum, endothelial dysfunction may
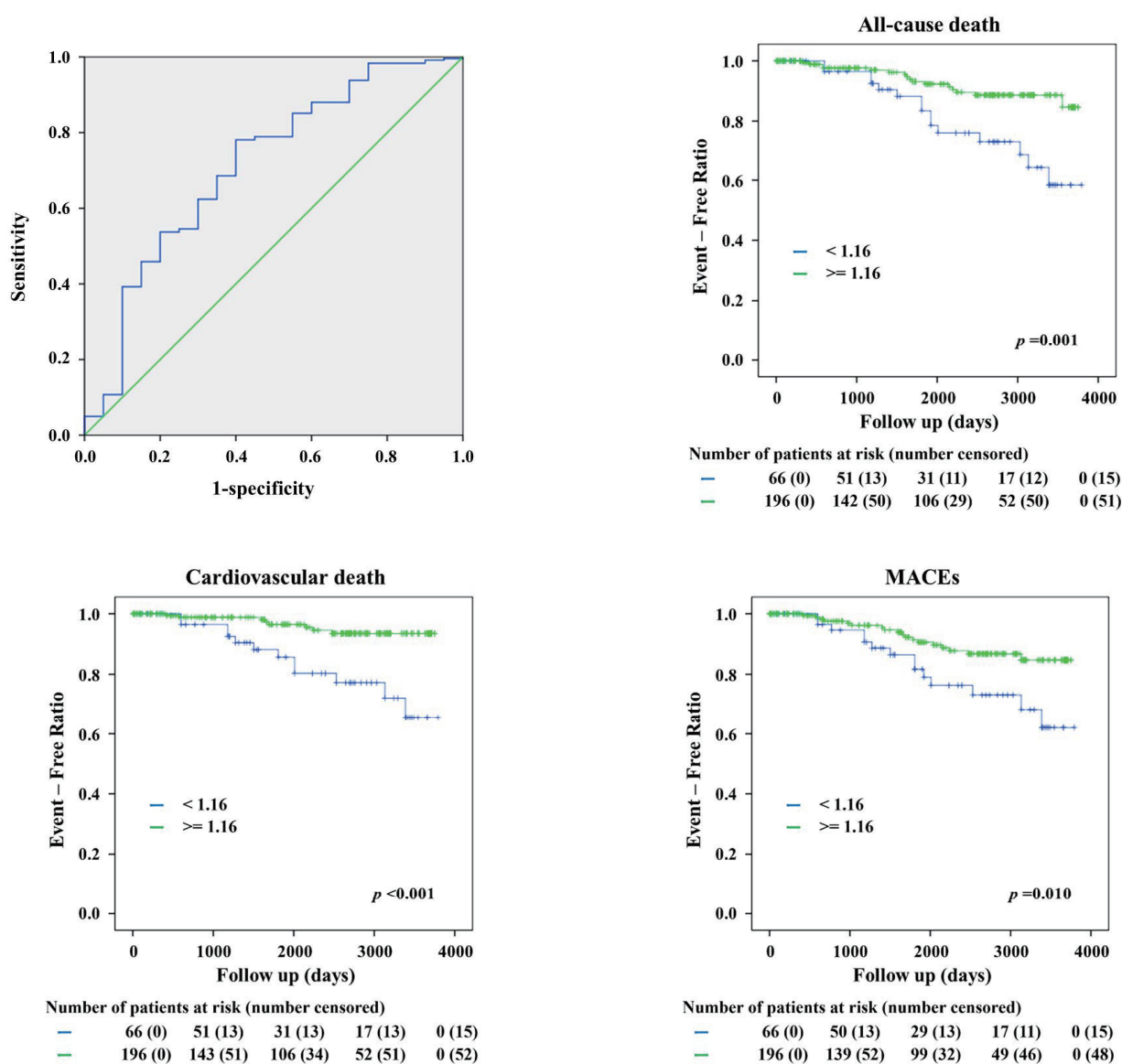
Fig. 3. Optimal L-arginine/L-ornithine ratio (1.16) obtained by receiver-operating characteristic curve; Kaplan–Meier curves classified according to the 1.16 ratio

For the endpoint of all-cause death, the optimal cut-off value for the L-arginine/L-ornithine ratio was 1.16 (area under the curve 0.657, sensitivity 0.450, specificity 0.854, *n* = 143, *p* = 0.025). This cut-off point was used in the subsequent analyses. Kaplan–Meier curves for two groups classified according to the L-arginine/ L-ornithine ratio of 1.16. The *p*-values in the graphs indicate the statistical significance of the differences among the groups. MACEs, major adverse cardiovascular events.

occur due to the chronically decreased bioavailability of L-arginine[15]. One possible cause is increased arginase activity[43-45]. L-arginine is converted to NO and L-citrulline by NOS and to L-ornithine and urea by arginase. Thus, L-arginine is a common substrate for NOS and arginase. Increased arginase activity in endothelial cells may lead to endothelial dysfunction by decreasing the concentration of L-arginine available for NO production. Another possibility may be the decreased bioavailability of arginine by metabolites of

L-arginine, given that L-ornithine and L-arginine share the same transporter (CAT-1), which may increase serum L-ornithine levels. When this occurs, L-ornithine competes with L-arginine for CAT-1, thus decreasing the bioavailability of L-arginine to endothelial cells[14]. This deficiency of L-arginine and excess of L-ornithine can have adverse effects such as structural problems in the vasculature, neuronal toxicity, and abnormal growth of tumor cells[46].

Because there is no net loss of L-arginine and its

Table 3. Unadjusted and adjusted risk for the study endpoints with the L-arginine/L-ornithine ratio

		For All-cause death		For Cardiovascular death		For MACEs	
Events, *n* (%)		31 (12)		20 (8)		32 (13)	
Scale							
L-arginine/L-ornithine							
Unadjusted HR (95% CI), *p*		0.940 (0.888-0.995)	0.032	0.890 (0.821-0.965)	0.005	0.956 (0.906-1.008)	0.093
Adjusted HR (95% CI), *p*, Model 1		0.948 (0.895-1.005)	0.074	0.893 (0.824-0.968)	0.006	0.960 (0.910-1.012)	0.130
Adjusted HR (95% CI), *p*, Model 2		0.965 (0.909-1.024)	0.234	0.911 (0.839-0.990)	0.028	0.963 (0.911-1.017)	0.173
Adjusted HR (95% CI), *p*, Model 3		0.966 (0.912-1.024)	0.250	0.920 (0.849-0.998)	0.044	0.965 (0.914-1.019)	0.198
L-arginine/L-citrulline							
Unadjusted HR (95% CI), *p*		0.834 (0.724-0.961)	0.012	0.791 (0.651-0.962)	0.019	0.939 (0.846-1.042)	0.236
Adjusted HR (95% CI), *p*, Model 1		0.846 (0.726-0.986)	0.033	0.803 (0.650-0.991)	0.041	0.973 (0.870-1.087)	0.626
Adjusted HR (95% CI), *p*, Model 2		0.939 (0.815-1.083)	0.388	0.928 (0.769-1.12)	0.434	1.035 (0.933-1.149)	0.514
Adjusted HR (95% CI), *p*, Model 3		0.941 (0.816-1.086)	0.406	0.933 (0.775-1.124)	0.467	1.039 (0.935-1.154)	0.478
GABR							
Unadjusted HR (95% CI), *p*		0.704 (0.553-0.896)	0.004	0.598 (0.425-0.842)	0.003	0.850 (0.692-1.043)	0.119
Adjusted HR (95% CI), *p*, Model 1		0.723 (0.556-0.941)	0.016	0.597 (0.408-0.871)	0.008	0.897 (0.718-1.121)	0.341
Adjusted HR (95% CI), *p*, Model 2		0.888 (0.676-1.165)	0.390	0.784 (0.534-1.151)	0.214	1.036 (0.820-1.309)	0.768
Adjusted HR (95% CI), *p*, Model 3		0.894 (0.681-1.173)	0.418	0.813 (0.561-1.176)	0.271	1.043 (0.828-1.315)	0.719
Category							
L-arginine/L-ornithine (vs. Q4)							
Unadjusted HR (95% CI), *p*	Q3	1.854 (0.542-6.348)	0.325	1.087 (0.153-7.728)	0.934	0.716 (0.227-2.258)	0.569
	Q2	1.133 (0.303-4.243)	0.853	1.876 (0.342-10.296)	0.469	0.777 (0.260-2.321)	0.651
	Q1	4.048 (1.341-12.221)	0.013	6.517 (1.454-29.199)	0.014	2.026 (0.816-5.029)	0.128
	(trend)		0.016		0.007		0.089
Adjusted HR (95% CI), *p*, Model 1	Q3	2.297 (0.638-8.275)	0.203	1.766 (0.232-13.471)	0.583	0.946 (0.287-3.117)	0.927
	Q2	1.047 (0.275-3.991)	0.946	2.049 (0.365-11.485)	0.415	0.795 (0.261-2.417)	0.686
	Q1	3.760 (1.194-11.843)	0.024	7.685 (1.615-36.575)	0.010	2.091 (0.810-5.398)	0.127
	(trend)		0.030		0.010		0.141
Adjusted HR (95% CI), *p*, Model 2	Q3	2.346 (0.606-9.073)	0.217	1.822 (0.216-15.385)	0.581	0.934 (0.272-3.207)	0.914
	Q2	0.638 (0.154-2.651)	0.537	1.257 (0.201-7.865)	0.807	0.571 (0.179-1.82)	0.343
	Q1	3.016 (0.892-10.192)	0.076	6.320 (1.201-33.258)	0.030	1.996 (0.739-5.386)	0.173
	(trend)		0.027		0.025		0.091
Adjusted HR (95% CI), *p*, Model 3	Q3	2.193 (0.562-8.550)	0.258	1.523 (0.180-12.859)	0.699	0.802 (0.232-2.771)	0.727
	Q2	0.609 (0.142-2.604)	0.504	1.435 (0.214-9.642)	0.710	0.625 (0.190-2.056)	0.439
	Q1	2.650 (0.796-8.818)	0.112	4.989 (0.959-25.950)	0.056	1.756 (0.645-4.784)	0.271
	(trend)		0.058		0.080		0.091
Category							
L-arginine/L-ornithine <1.16							
Unadjusted HR (95% CI), *p*		3.084 (1.521-6.250)	0.002	4.862 (1.986-11.903)	0.001	2.443 (1.215-4.914)	0.012
Adjusted HR (95% CI), *p*, Model 1		2.735 (1.331-5.619)	0.006	4.797 (1.913-12.030)	0.001	2.313 (1.137-4.704)	0.021
Adjusted HR (95% CI), *p*, Model 2		2.666 (1.203-5.907)	0.016	4.912 (1.756-13.736)	0.002	2.527 (1.186-5.385)	0.016
Adjusted HR (95% CI), *p*, Model 3		2.387 (1.078-5.284)	0.032	3.803 (1.381-10.472)	0.010	2.227 (1.039-4.775)	0.040

Univariate and multivariate Cox proportional regression analysis of death from any cause, cardiac death, and MACEs. Model 1 adjusted for age and sex. Model 2 includes Model 1 plus hypertension, hyperlipidemia, diabetes mellitus, current smoking, CAD, eGFR, and Log10BNP. Model 3 includes Model 1 plus diabetes mellitus, prescription of statin, calcium channel blocker and nitrates, eGFR, and Log10BNP. Significant *p*-values are indicated in bold.

BNP, brain natriuretic peptide; CAD, coronary artery disease; CI, confidence interval; eGFR, estimated glomerular filtration rate; HR, hazard ratio; MACEs, major adverse cardiovascular events.
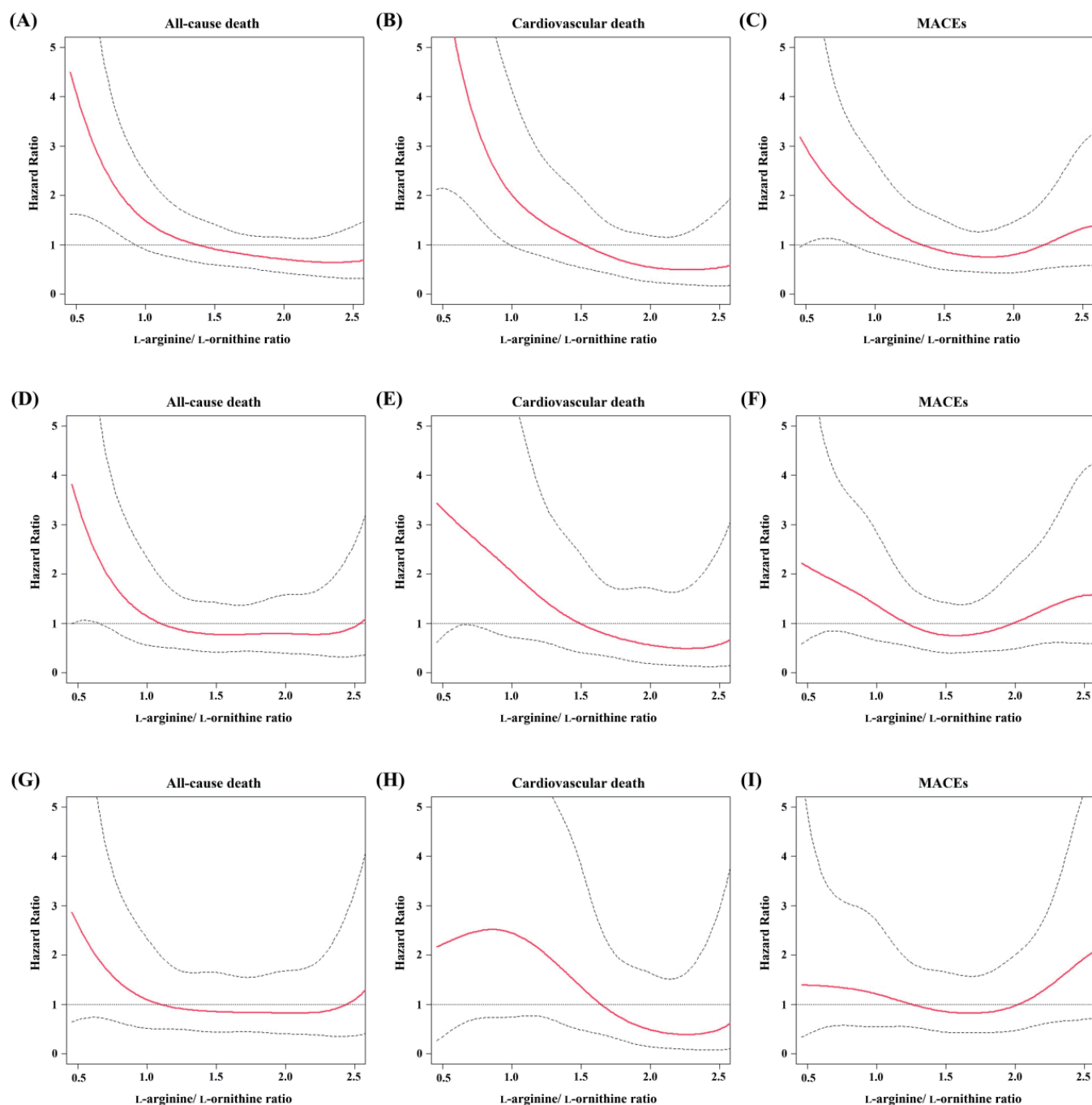
Fig. 4. Cubic spline analysis of the hazard ratio adjusted for the ʟ-arginine/ʟ-ornithine ratio

The spline curves were described to show the relationships of ʟ-arginine/ʟ-ornithine ratio with hazard ratios for all-cause death (A, D, G), cardiovascular death (B, E, H), and MACEs (C, F, I), respectively. The risks were analyzed in univariate (A-C), multivariate (D-F) adjusting for age, sex, hypertension, hyperlipidemia, diabetes mellitus, current smoking, CAD, eGFR, and Log10BNP, and multivariate (G-I) adjusting for age, sex, diabetes mellitus, prescription of statin, calcium channel blocker and nitrates, eGFR, and Log10BNP. BNP, brain natriuretic peptide; CAD, coronary artery disease; eGFR, estimated glomerular filtration rate; MACEs, major adverse cardiovascular events.

catabolic metabolites, ʟ-ornithine and ʟ-citrulline, during urea synthesis, a low ʟ-arginine/ʟ-ornithine ratio indicates decreased ʟ-arginine bioavailability with increased arginase activity. In previous cross-sectional studies, arteriosclerosis, CAD, and ACS were found to be strongly associated with the bioavailability of ʟ-arginine[26, 27, 47-49]. Decreased ʟ-arginine bioavailability is likewise associated with obesity, insulin resistance,

and metabolic syndrome[50]. Increased arginase activity causes a relative deficiency of ʟ-arginine[51, 52]. In addition, recent studies have reported that the inhibition of arginase activity and ʟ-arginine intake increases NO production[44, 53]. These results may highlight the importance of arginase in endothelial dysfunction.

However, few studies have investigated the effects of relative ʟ-arginine deficiency on long-term mortality. Molek *et al.* revealed that a decrease in ʟ-arginine concentration was reflective of the clinical impact and severity in AMI patients. A delay in the recovery of ʟ-arginine concentration at 6 months after onset was associated with death, myocardial infarction, or hospital admission for heart failure in the following 5 years[48]. Tang *et al.* reported that a lower GABR and higher ʟ-citrulline concentrations were associated with an increased risk of developing CAD and MACEs at 3 years[16]. However, to our knowledge, there are no other longer-term studies. Our results suggested that an ʟ-arginine/ʟ-ornithine ratio with a cut-off value of 1.16 was associated with the prognosis of cardiac death up to 10 years. However, the GABR was not associated with such a prognosis in the multivariate analysis including adjustment by eGFR (Table 3). This may be because serum levels of ʟ-citrulline are more dependent on renal function compared with those of ʟ-arginine and ʟ-ornithine. Another possible mechanism is that renal dysfunction prevents ʟ-citrulline from being metabolized to ʟ-arginine, resulting in an excess of ʟ-citrulline and a decrease in the GABR[54].

The present study had some limitations that should be considered. The first is the small number of patients. For example, regarding MACEs in this study, most of the events were cardiovascular deaths, whereas there were very few nonfatal AMI and stroke events. As a result, MACEs likely resembled the results for cardiovascular deaths. Therefore, further studies with a larger number of patients are needed. The second limitation is the effect of diurnal variation on amino acid profiles. Circadian rhythms of ʟ-arginine concentrations have been reported to be relatively high in the early morning and to decrease during the day[55]. In the present study, patients underwent cardiac catheterization at different times of the day, but we did not measure time-course changes of the amino acid profiles. The third limitation, which overlaps slightly with the second, is that the amino acid profile was measured only once. The possibility that amino acid profiles may have changed in individual patients during the 10-year prospective follow-up period cannot be ruled out.

6. Conclusion

A low ʟ-arginine/ʟ-ornithine ratio may be associated with increased long-term cardiovascular mortality. Activation of arginase in the urea cycle and decreased bioavailability of ʟ-arginine may also be associated with cardiovascular prognosis.

Conflict of Interest

The authors have no conflicts of interest directly relevant to the content of this article.

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