

# Serum cholinesterase as a prognostic biomarker for acute heart failure

Masayuki Shiba<sup>1</sup>, Takao Kato <sup>1\*</sup>, Takeshi Morimoto<sup>2</sup>, Hidenori Yaku<sup>3</sup>, Yasutaka Inuzuka<sup>4</sup>, Yodo Tamaki<sup>5</sup>, Neiko Ozasa<sup>1</sup>, Yuta Seko<sup>1</sup>, Erika Yamamoto<sup>1</sup>, Yusuke Yoshikawa<sup>1</sup>, Takeshi Kitai <sup>6</sup>, Yugo Yamashita<sup>1</sup>, Moritake Iguchi<sup>7</sup>, Kazuya Nagao<sup>8</sup>, Yuichi Kawase<sup>9</sup>, Takashi Morinaga<sup>10</sup>, Mamoru Toyofuku<sup>11</sup>, Yutaka Furukawa <sup>6</sup>, Kenji Ando<sup>10</sup>, Kazushige Kadota<sup>9</sup>, Yukihiro Sato<sup>12</sup>, Koichiro Kuwahara<sup>13</sup>, and Takeshi Kimura<sup>1</sup>

<sup>1</sup>Department of Cardiovascular Medicine, Kyoto University Graduate School of Medicine, 54 Shogoin Kawahara-cho, Sakyo-ku, Kyoto 606-8507, Japan; <sup>2</sup>Department of Clinical Epidemiology, Hyogo College of Medicine, Nishinomiya, Japan; <sup>3</sup>Department of Cardiology, Mitsubishi Kyoto Hospital, Kyoto, Japan; <sup>4</sup>Cardiovascular Medicine, Shiga General Hospital, Moriyama, Japan; <sup>5</sup>Division of Cardiology, Tenri Hospital, Tenri, Japan; <sup>6</sup>Department of Cardiovascular Medicine, Kobe City Medical Center General Hospital, Kobe, Japan; <sup>7</sup>Department of Cardiology, National Hospital Organization Kyoto Medical Center, Kyoto, Japan; <sup>8</sup>Department of Cardiology, Osaka Red Cross Hospital, Osaka, Japan; <sup>9</sup>Department of Cardiology, Kurashiki Central Hospital, Kurashiki, Japan; <sup>10</sup>Department of Cardiology, Kokura Memorial Hospital, Kokura, Japan; <sup>11</sup>Department of Cardiology, Japanese Red Cross Wakayama Medical Center, Wakayama, Japan; <sup>12</sup>Department of Cardiology, Hyogo Prefectural Amagasaki General Medical Center, Amagasaki, Japan; and <sup>13</sup>Department of Cardiovascular Medicine, Shinshu University Graduate School of Medicine, Nagano, Japan

Received 29 October 2020; revised 16 December 2020; editorial decision 26 December 2020; accepted 29 December 2020; online publish-ahead-of-print 18 January 2021

## Aims

The association between serum cholinesterase and prognosis in acute heart failure (AHF) remains to be elucidated. We investigated the serum cholinesterase level at discharge from hospitalization for AHF and its association with clinical outcomes in patients with AHF.

## Methods and results

Among 4056 patients enrolled in the Kyoto Congestive Heart Failure multicentre registry, we analysed 2228 patients with available serum cholinesterase data. The study population was classified into three groups according to serum cholinesterase level at discharge: low tertile (<180 U/L,  $N = 733$ ), middle tertile ( $\geq 180$  U/L and <240 U/L,  $N = 746$ ), and high tertile ( $\geq 240$  U/L,  $N = 749$ ). Patients in the low tertile had higher tricuspid pressure gradient, greater inferior vena cava diameter, and higher brain natriuretic peptide (BNP) levels than those in the high tertile. The cumulative 1-year incidence of the primary outcome measure (a composite endpoint of all-cause death and hospitalization for HF) was higher in the low and middle tertiles than in the high tertile [46.5% (low tertile) and 31.4% (middle tertile) vs. 22.1% (high tertile),  $P < 0.0001$ ]. After adjustment for 26 variables, the excess risk of the low tertile relative to the high tertile for the primary outcome measure remained significant (hazard ratio 1.37, 95% confidence interval 1.10–1.70,  $P = 0.006$ ). Restricted cubic spline models below the median of cholinesterase demonstrated incrementally higher hazards at low cholinesterase levels.

## Conclusions

Low serum cholinesterase levels are associated with congestive findings on echocardiography, higher BNP, and higher risks for a composite of all-cause death and HF hospitalization in patients with AHF.

## Keywords

Heart failure • Prognosis • Liver • Cholinesterase

\* Corresponding author. Tel: +81 75 751 3190, Fax: +81 75 751 3203, Email: [tkato75@kuhp.kyoto-u.ac.jp](mailto:tkato75@kuhp.kyoto-u.ac.jp)

© The Author(s) 2021. Published by Oxford University Press on behalf of the European Society of Cardiology.

This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc/4.0/>), which permits non-commercial re-use, distribution, and reproduction in any medium, provided the original work is properly cited. For commercial re-use, please contact [journals.permissions@oup.com](mailto:journals.permissions@oup.com)

## Introduction

The existence of mutual interactions between heart failure (HF) and liver diseases or malnutrition has recently been reported.<sup>1–3</sup> Liver damage in HF is caused by venous congestion with tricuspid regurgitation (TR), with concomitant malnutrition due to intestinal congestion.<sup>1–4</sup> Many studies have shown that hypoalbuminaemia contributes to unfavourable outcomes in patients with HF.<sup>5,6</sup> However, serum albumin levels are influenced by factors other than liver function, such as ultrafiltration from the kidney and intestinal loss.<sup>7–9</sup> Even under steady conditions, serum albumin passes through the glomerular and intestinal capillaries.<sup>7,8</sup> With respect to the liver function, cholinesterase is suggested to be a more specific marker than albumin.<sup>9</sup> Large-scale data on the association between serum cholinesterase and HF, which may be helpful in further understanding the relationship between liver function and HF, are scarce.

In the present study, we investigated the serum cholinesterase level at discharge from hospitalization for acute HF (AHF) and its association with clinical outcomes after discharge from hospital in patients with AHF.

## Methods

### Patient population

The Kyoto Congestive Heart Failure (KCHF) registry is a physician-initiated, prospective, observational, multicentre cohort study that enrolled consecutive patients who were hospitalized for AHF for the first time between 1 October 2014 and 31 March 2016. These patients were admitted to 19 secondary and tertiary hospitals, including rural and urban and large and small institutions, throughout Japan. The overall design of the KCHF study and the patient enrolment process have been previously described in detail.<sup>10,11</sup> We enrolled consecutive patients with AHF, as defined by the modified Framingham criteria, who were admitted to the participating centres and underwent HF-specific treatment with intravenous drugs within 24 h of hospital presentation. A test for cholinesterase was not mandatory to the enrolment but was ordered as part of routine clinical practice. This study was a *post hoc* analysis for an impact of serum cholinesterase on outcomes in patients in whom the biomarker was assessed; thus, this was a retrospective analysis of a prospective cohort study.

### Ethics

The investigation conformed to the principles outlined in the Declaration of Helsinki. The study protocol was approved by the ethical committees of Kyoto University Hospital (local identifier: E2311) and each participating hospital. A waiver of written informed consent from each patient was granted by the institutional review boards of Kyoto University and each participating centre, as the study met the conditions of the Japanese ethical guidelines for an epidemiological study.<sup>10,11</sup>

### Outcomes

One-year clinical follow-up data with an allowance of 1 month were collected in October 2017. The attending physicians or research assistants at each participating hospital collected data on clinical events after the index of hospitalization from hospital charts or by contacting patients, their relatives, or their referring physicians (with consent).

The primary outcome measure in this study was a composite of all-cause death and hospitalization for HF. The secondary outcome

measures were the individual components of the primary composite outcome measure (i.e., all-cause death and hospitalization for HF). The definitions of causes of death are described in [Supplementary material online, Methods](#).

### Statistical analysis

The baseline patient characteristics and clinical outcome measures were compared among three groups based on serum cholinesterase level at discharge. Continuous variables are expressed as means and standard deviations or medians with interquartile ranges. Continuous variables were compared using one-way analysis of variance or the Kruskal–Wallis test, according to their distributions. Categorical variables are expressed as counts and percentages. Categorical variables were compared using the  $\chi^2$  test. The cumulative incidences of the primary outcome measure and all-cause death were estimated using Kaplan–Meier analysis, and the among-group differences were assessed using the log-rank test. The cumulative incidences of HF hospitalization were estimated using the Gray method, accounting for a competing risk of all-cause death.<sup>12</sup> The time zero for follow-up was set on the day of discharge from the index hospitalization.

We set the high tertile as the reference, and estimated the adjusted risk of the low tertile vs. the high tertile and that of the middle tertile vs. the high tertile for the primary outcome measure and all-cause death using multivariable Cox proportional hazard models. To account for a competing risk of all-cause death, the hazard ratio (HR) of HF hospitalization was described using the Fine-Gray subdistribution hazard model.<sup>13</sup> Two multivariable models were constructed. Model 1 included 26 clinically relevant risk-adjusting variables, as follows: age, sex, body mass index (BMI), a variable related to the aetiology of AHF (acute coronary syndrome), echocardiographic measurements [left ventricular ejection fraction (LVEF), left ventricular end-diastolic diameter (LVEDD), and moderate/severe TR], variables related to medical history (previous HF hospitalization, atrial fibrillation or flutter, hypertension, diabetes, previous stroke, current smoking, and chronic lung disease), variables related to vital signs (systolic blood pressure and heart rate), variables related to laboratory tests [natriuretic peptide, estimated glomerular filtration rate (eGFR), albumin, sodium, haemoglobin, total bilirubin, gamma-glutamyl transpeptidase ( $\gamma$ -GTP), aspartate transaminase (AST)], and medications at discharge [angiotensin-converting enzyme inhibitor (ACE-I) or angiotensin receptor blocker (ARB), and  $\beta$ -blocker]. Most of the 26 adjusting variables was preliminarily designed. We included BMI, haemoglobin and albumin as nutritional status, bilirubin,  $\gamma$ -GTP, and AST as liver enzyme, natriuretic peptide, LVEF and LVEDD as factors strongly associated with severity of HF, and TR severity as a surrogate of right-sided HF into the multivariable models. We also included brain natriuretic peptide (BNP) levels or N-terminal pro brain natriuretic peptide (NT-proBNP) levels as a binary variable in the adjusted model (Model 1) because we measured either the BNP or NT-proBNP level. BNP >704 pg/mL and NT-proBNP >4936 pg/mL were based on the median, and NT-proBNP values were adopted if no BNP values were available. To appreciate the effect of cholinesterase on outcomes over its full range, we constructed a restricted cubic spline (RCS) regression model with three knots, unadjusted or adjusted using the same variables used in Model 1. The reference was the median value of cholinesterase at discharge (208 U/L). As a sensitivity analysis, we included BNP as a continuous variable in the adjusted model (Model 2) in patients with available data. We also included cholinesterase levels as a continuous variable instead of tertiles in Model 1. We expressed HRs per serum cholinesterase decrements of 10 U/L. The results are presented as HRs and their 95% confidence intervals (CIs). Missing data were handled without imputation and 1852 patients were finally included into the fully adjusted model. Detailed methods of

**Table 1** Patient characteristics

| Variable                                                 | High tertile<br>(N = 749) | Middle tertile<br>(N = 746) | Low tertile<br>(N = 733) | P-value | N of patients<br>analysed |
|----------------------------------------------------------|---------------------------|-----------------------------|--------------------------|---------|---------------------------|
| Clinical characteristics                                 |                           |                             |                          |         |                           |
| Age (years) <sup>a,b</sup>                               | 72.5 ± 13.7               | 79.8 ± 9.8                  | 81.1 ± 10.1              | <0.0001 | 2228                      |
| Women <sup>a,b</sup>                                     | 337 (45%)                 | 348 (47%)                   | 321 (44%)                | 0.54    | 2228                      |
| BMI (kg/m <sup>2</sup> ) <sup>a,b</sup>                  | 24.4 ± 4.9                | 22.5 ± 4.0                  | 21.7 ± 4.2               | <0.0001 | 2121                      |
| Aetiology                                                |                           |                             |                          |         |                           |
| Acute coronary syndrome <sup>a,b</sup>                   | 48 (6.4%)                 | 33 (4.4%)                   | 29 (4.0%)                | 0.07    | 2228                      |
| Medical history                                          |                           |                             |                          |         |                           |
| Previous heart failure hospitalization <sup>a,b</sup>    | 217 (29%)                 | 265 (36%)                   | 279 (38%)                | 0.0005  | 2228                      |
| Atrial fibrillation or flutter <sup>a,b</sup>            | 279 (37%)                 | 334 (45%)                   | 353 (48%)                | <0.0001 | 2228                      |
| Hypertension <sup>a,b</sup>                              | 547 (73%)                 | 575 (77%)                   | 526 (72%)                | 0.05    | 2228                      |
| Diabetes <sup>a,b</sup>                                  | 315 (42%)                 | 259 (35%)                   | 252 (34%)                | 0.003   | 2228                      |
| Dyslipidaemia                                            | 363 (48%)                 | 305 (41%)                   | 235 (32%)                | <0.0001 | 2228                      |
| Previous ischaemic stroke <sup>a,b</sup>                 | 116 (15%)                 | 124 (17%)                   | 122 (17%)                | 0.79    | 2228                      |
| Current smoking <sup>a,b</sup>                           | 126 (17%)                 | 76 (10%)                    | 66 (9.2%)                | <0.0001 | 2228                      |
| Chronic lung disease <sup>a,b</sup>                      | 81 (11%)                  | 109 (15%)                   | 102 (14%)                | 0.06    | 2228                      |
| Vital signs at admission                                 |                           |                             |                          |         |                           |
| Systolic BP (mmHg) <sup>a,b</sup>                        | 152.5 ± 35.3              | 148.2 ± 34.9                | 144.0 ± 32.8             | <0.0001 | 2219                      |
| Diastolic BP (mmHg)                                      | 89.1 ± 25.2               | 83.8 ± 24.3                 | 81.6 ± 22.2              | <0.0001 | 2214                      |
| HR (b.p.m.) <sup>a,b</sup>                               | 100.5 ± 29.5              | 94.0 ± 27.4                 | 93.1 ± 25.5              | <0.0001 | 2218                      |
| Laboratory tests                                         |                           |                             |                          |         |                           |
| BNP > 704 pg/mL or NT-proBNP > 4936 pg/mL <sup>a,c</sup> | 306 (41%)                 | 373 (51%)                   | 431 (59%)                | <0.0001 | 2213                      |
| BNP (pg/mL) <sup>b</sup>                                 | 544 (306–1000)            | 714 (429–1255)              | 827 (462–1509)           | <0.0001 | 2038                      |
| NT-proBNP (pg/mL)                                        | 4731 (2740–7766)          | 4518 (2225–9786)            | 6229 (3047–19 762)       | 0.10    | 175                       |
| eGFR (mL/min/1.73 m <sup>2</sup> ) <sup>a,b</sup>        | 52.6 ± 21.8               | 46.5 ± 23.1                 | 41.8 ± 23.4              | <0.0001 | 2226                      |
| Total bilirubin (mg/dL) <sup>a,b</sup>                   | 0.84 ± 0.51               | 0.87 ± 0.54                 | 0.96 ± 0.77              | 0.03    | 2201                      |
| γ-GTP (IU/L) <sup>a,b</sup>                              | 63.7 ± 72.7               | 58.2 ± 61.7                 | 60.2 ± 82.1              | 0.002   | 2096                      |
| AST (IU/L) <sup>a,b</sup>                                | 46.2 ± 70.8               | 61.6 ± 205.3                | 60.7 ± 243.1             | 0.50    | 2224                      |
| Albumin (g/dL) <sup>a,b</sup>                            | 3.6 ± 0.46                | 3.5 ± 0.45                  | 3.3 ± 0.49               | <0.0001 | 2197                      |
| Sodium (mEq/L) <sup>a,b</sup>                            | 139.8 ± 3.6               | 139.1 ± 4.3                 | 138.4 ± 4.9              | <0.0001 | 2222                      |
| Haemoglobin (g/dL) <sup>a,b</sup>                        | 12.5 ± 2.3                | 11.4 ± 2.2                  | 10.7 ± 2.2               | <0.0001 | 2227                      |
| Cholinesterase (U/L)                                     | 297.4 ± 52.4              | 207.9 ± 17.0                | 141.9 ± 29.8             | <0.0001 | 2228                      |
| Medications at discharge                                 |                           |                             |                          |         |                           |
| ACE-I or ARB <sup>a,b</sup>                              | 498 (67%)                 | 440 (59%)                   | 329 (45%)                | <0.0001 | 2228                      |
| β-blocker <sup>a,b</sup>                                 | 555 (74%)                 | 489 (66%)                   | 439 (60%)                | <0.0001 | 2228                      |
| Mineralocorticoid receptor antagonist                    | 387 (52%)                 | 330 (44%)                   | 311 (42%)                | 0.0008  | 2228                      |
| Loop diuretic                                            | 603 (81%)                 | 612 (82%)                   | 618 (84%)                | 0.15    | 2228                      |

Categorical variables were presented as number (%), and continuous variables were presented as mean ± standard deviation or median (interquartile range).

ACE-I, angiotensin-converting enzyme inhibitor; ARB, angiotensin-receptor blocker; AST, aspartate transaminase; BMI, body mass index; BNP, brain natriuretic peptide; BP, blood pressure; eGFR, estimated glomerular filtration rate; HR, heart rate; NT-proBNP, N-terminal pro brain natriuretic peptide; γ-GTP, gamma-glutamyl transpeptidase.

<sup>a</sup>Risk adjusting variables selected for Model 1.

<sup>b</sup>Risk adjusting variables selected for Model 2.

<sup>c</sup>Median value of BNP and NT-proBNP was 704 pg/mL and 4936 pg/mL, respectively.

prediction ability of cholinesterase and analysis based on serum cholinesterase at admission were included in the [Supplementary material online, Methods](#).

Statistical analyses were performed by three physicians (M.S., T.K., and Y.Y.) and one statistician (T.M.) using JMP Pro software (version 14; SAS Corp., Cary, NC, USA), R version 3.6.3 (R Foundation for Statistical Computing, Vienna, Austria), and EZR.<sup>14</sup> A two-tailed *P*-value of <0.05 was considered statistically significant in all analyses, except in the case of multiple comparisons.

## Results

### Clinical characteristics

Among 4056 patients enrolled in the KCHF registry, 2228 patients who were discharged alive and had cholinesterase data at discharge ([Supplementary material online, Figure S1](#)) were included in the current study population. We excluded 271 patients who died during the index hospitalization, 57 patients who were lost to follow-up

**Table 2** Echocardiographic parameters

| Variable                                                     | High tertile<br>(N = 749) | Middle tertile<br>(N = 746) | Low tertile<br>(N = 733) | P-value | N of patients<br>analysed |
|--------------------------------------------------------------|---------------------------|-----------------------------|--------------------------|---------|---------------------------|
| Left ventricular end-diastolic dimension (mm) <sup>a,b</sup> | 53.0 ± 9.7                | 50.5 ± 9.2                  | 49.9 ± 8.8               | <0.0001 | 2107                      |
| Left ventricular end-systolic dimension (mm)                 | 40.5 ± 12.4               | 37.9 ± 11.4                 | 37.3 ± 10.9              | <0.0001 | 2076                      |
| LVEF (%) <sup>a,b</sup>                                      | 45.3 ± 16.7               | 47.5 ± 16.2                 | 47.1 ± 16.4              | 0.03    | 2172                      |
| LVEF <40%                                                    | 294 (39%)                 | 261 (35%)                   | 243 (33%)                | 0.04    | 2226                      |
| Left atrial diameter (mm)                                    | 44.8 ± 8.2                | 44.6 ± 8.3                  | 46.0 ± 10.1              | 0.14    | 2043                      |
| Moderate/severe mitral regurgitation                         | 239 (32%)                 | 264 (35%)                   | 286 (39%)                | 0.02    | 2228                      |
| Moderate/severe tricuspid regurgitation <sup>a,b</sup>       | 145 (19%)                 | 185 (25%)                   | 263 (36%)                | <0.0001 | 2228                      |
| Tricuspid regurgitant pressure gradient (mmHg)               | 29.8 ± 13.4               | 30.1 ± 14.1                 | 31.7 ± 15.9              | 0.04    | 1828                      |
| Inferior vena cava (mm)                                      | 15.4 ± 4.6                | 15.6 ± 4.7                  | 17.0 ± 5.3               | <0.0001 | 2057                      |

LVEF, left ventricular ejection fraction.

<sup>a</sup>Risk adjusting variables selected for Model 1.

<sup>b</sup>Risk adjusting variables selected for Model 2.

after discharge, 46 patients with liver cirrhosis determined from history taking or judged by each attending physician, and 1454 patients with no available serum cholinesterase data at discharge (Supplementary material online, Table S1). The study population was classified into three groups according to serum cholinesterase level at discharge: low tertile (<180 U/L, N = 733), middle tertile (≥180 U/L and <240 U/L, N = 746), and high tertile (≥240 U/L, N = 749) (Supplementary material online, Figure S2).

The baseline characteristics were significantly different across the three groups (Table 1). Patients in the low and middle tertiles of cholinesterase were older, had lower BMI, were less likely to have dyslipidaemia and diabetes, and more often had a history of HF and atrial arrhythmia than those in the high tertile (Table 1). Patients in the low and middle tertiles had lower serum albumin, haemoglobin, and eGFR, and had higher serum BNP levels than those in the high tertile (Table 1). With respect to medications at discharge, patients in the low and middle tertiles were less likely to take ACE-I or ARB, β-blocker, mineralocorticoid receptor antagonist, and tolvaptan than those in the high tertile (Table 1).

## Echocardiographic measurements

Significant differences were found in all echocardiographic measurements except for the left atrial diameter. Patients in the low tertile of cholinesterase had higher LVEF, higher TR pressure gradient, and greater inferior vena cava (IVC) diameter than those in the high tertile (Table 2).

## Clinical outcomes

The median follow-up duration after discharge was 475 (interquartile range 364–629) days, with a 96.0% follow-up rate at 1 year. The cumulative 1-year incidence of the primary outcome measure (a composite endpoint of all-cause death and hospitalization for HF) was significantly higher in the low and middle tertiles than in the high tertile of cholinesterase (46.5% in the low tertile and 31.4% in the middle tertile vs. 22.1% in the high tertile,  $P < 0.0001$ ) (Figure 1A). After adjustment for confounders, the excess risk of the low tertile relative to the high tertile remained significant for the primary outcome measure (HR 1.37, 95% CI 1.10–1.70;  $P = 0.006$ ), whereas the excess

risk of the middle tertile relative to the high tertile was no longer significant for the primary outcome measure (HR 1.06, 95% CI 0.86–1.31;  $P = 0.59$ ) (Supplementary material online, Table S2). For the secondary outcome measures, the cumulative 1-year incidence of all-cause death was significantly higher in the low and middle tertiles than in the high tertile (28.8%, 13.9%, and 7.3%, respectively;  $P < 0.0001$ ) (Figure 1B). After adjustment for confounders, the excess risk of the low tertile relative to the high tertile of cholinesterase remained significant for all-cause death (HR 1.87, 95% CI 1.35–2.57;  $P < 0.0001$ ) (Supplementary material online, Table S2). The cumulative 1-year incidence of hospitalization for HF was also significantly higher in the low and middle tertiles than in the high tertile (27.7%, 21.9%, and 17.3%, respectively;  $P < 0.0001$ ) (Figure 1C). However, the excess adjusted risk of the low tertile relative to the high tertile was no longer significant for hospitalization for HF (HR 1.12, 95% CI 0.85–1.47;  $P = 0.42$ ) (Supplementary material online, Table S2).

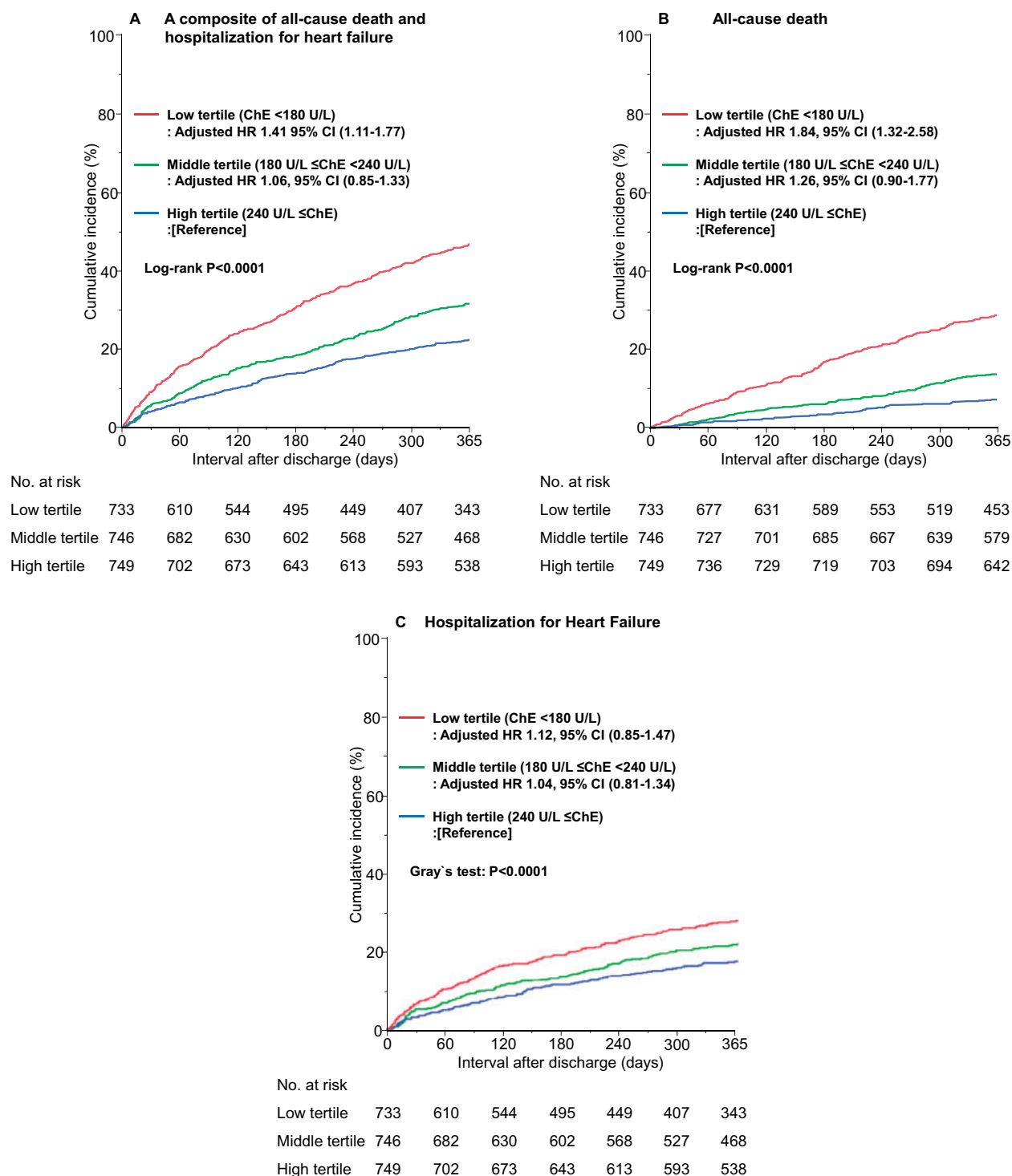
RCS models for the HR of cholinesterase demonstrated that the unadjusted risk for the primary outcome increased with lower cholinesterase below its median and the risk was low above its median (Figure 2A). After adjustment, the risk remained significant in patients with low cholinesterase levels below its median (Figure 2B).

## Sensitivity analyses

When we evaluated serum cholinesterase as a continuous variable, the adjusted risk per serum cholinesterase decrements of 10 U/L for the primary outcome measure and all-cause death was significant (HR 1.02, 95% CI 1.01–1.04;  $P = 0.0003$ , and HR 1.04, 95% CI 1.02–1.06;  $P < 0.0001$ , respectively; Supplementary material online, Table S3), which is consistent with the main analysis. When we included BNP level as a continuous variable into the adjusted model, the results were fully consistent with the main analysis (Supplementary material online, Table S2).

## Causes of death according to tertiles of serum cholinesterase at discharge

The cumulative 1-year incidence of cardiovascular death incrementally increased with decreasing cholinesterase levels (18.4% in the low tertile, 8.2% in the middle tertile, and 4.6% in the high tertile,



**Figure 1** Kaplan–Meier curves for (A) the primary outcome measure, (B) all-cause death, and (C) hospitalization for heart failure. ChE, cholinesterase; CI, confidence interval; HR, hazard ratio.

$P < 0.0001$ ) (Figure 3). The cumulative 1-year incidence of non-cardiovascular death also incrementally increased with decreasing cholinesterase levels (12.4% in the low tertile, 6.2% in the middle tertile, and 2.8% in the high tertile,  $P < 0.0001$ ).

### Prediction ability of cholinesterase

For the prediction of the primary outcome measures, the area under the curve of cholinesterase was significantly greater than that of other liver enzymes [cholinesterase, 0.640 (95% CI 0.616–0.663); AST,

0.556 (95% CI 0.532–0.581); bilirubin, 0.540 (95% CI 0.515–0.565);  $\gamma$ -GTP, 0.538 (95% CI 0.512–0.563); albumin, 0.581 (95% CI 0.557–0.605) and cholinesterase vs. each liver enzyme,  $P < 0.0001$ , respectively]. The receiver operating characteristic (ROC) curves are presented in [Supplementary material online, Figure S3](#). When we compared the AHEAD (A: atrial fibrillation, H: haemoglobin, E: elderly, A: abnormal renal parameters, D: diabetes mellitus) score and the AHEAD score plus cholinesterase, a significant improvement in the prediction of all-cause death was noted [ROCs in [Supplementary material online, Figure S4](#), and also confirmed by net reclassification improvement (0.59, 95% CI 0.49–0.69,  $P < 0.0001$ ) and integrated discrimination improvement (0.07, 95% CI 0.06–0.08,  $P < 0.0001$ )].<sup>15</sup>

### Additional analysis based on serum cholinesterase at admission

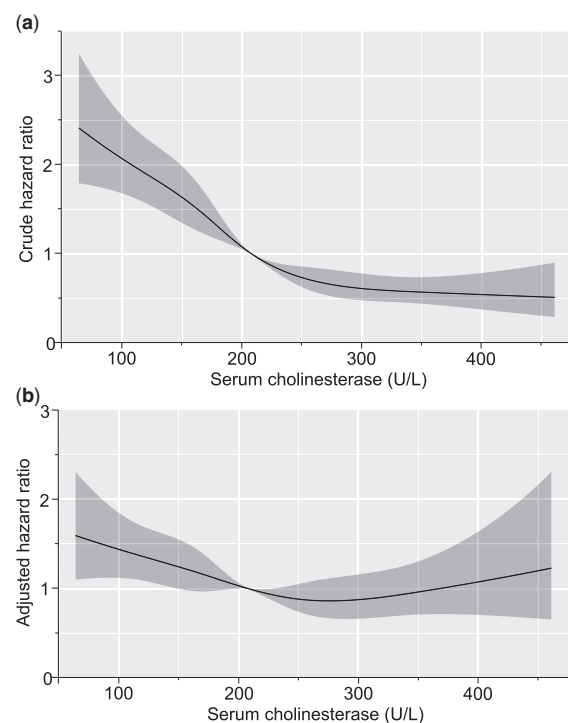
When stratified by the tertile of cholinesterase level at admission ([Supplementary material online, Table S4](#)), serum cholinesterase at admission was also significantly associated with in-hospital death ([Supplementary material online, Table S5](#)).

## Discussion

The main findings of the present study were as follows: (i) patients in the low tertile of cholinesterase at discharge from hospitalization for AHF were older and sicker than those in high tertile, (ii) low serum cholinesterase was associated with a higher adjusted risk for the composite of all-cause death and hospitalization for HF, and (iii) all-cause death was mostly contributed to the association between serum cholinesterase and the primary outcome measure.

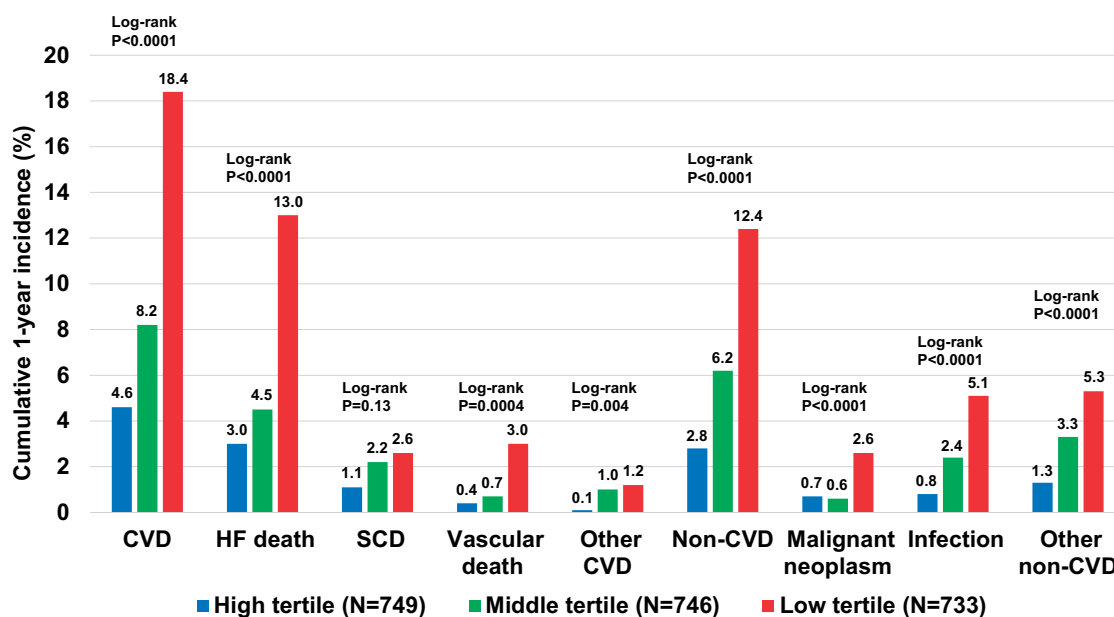
Liver function to synthesize proteins is critically important for the maintenance of the human body. In previous studies, deviation enzymes such as AST, alanine transaminase, and alkaline phosphatase were useful prognostic markers in HF patients<sup>1–3,5,6</sup>; however, these markers also reflect hepatocyte damage. Albumin is not only a specific marker of liver function but also a marker related to renal function with respect to albumin leakage from the kidney.<sup>7,9</sup> Serum cholinesterase is characterized by a large molecular weight (~340 kDa), contributing to its resistance to ultrafiltration from the kidney and intestinal excretion, as well as extravasation from the vascular bed.<sup>8,9,16,17</sup> It is a surrogate marker of hepatic function and nutrition, reflecting the ability of the liver to synthesize proteins.<sup>9</sup> In fact, we showed that cholinesterase was superior to other liver enzymes for the prediction of clinical outcomes in AHF. Only three small-scale studies have reported that serum cholinesterase is associated with clinical events in patients with HF.<sup>18–20</sup> In the present large-scale multicentre study, we elucidated the characteristics of patients with low cholinesterase levels and the association between serum cholinesterase levels and all-cause death, with adjustments for multiple confounding factors. This association was also observed in cardiovascular and non-cardiovascular deaths. These are the novel findings of the present study. In addition, our echocardiographic analyses provided new mechanistic insights into the link between low cholinesterase levels and outcomes.

Patients in the low tertile of cholinesterase showed a higher prevalence of TR and mitral regurgitation, with greater IVC diameter and higher BNP levels. These findings indicate the congestive status of



**Figure 2** Restricted cubic spline curves of the risk for the primary outcome measure. (A) Univariate model. (B) Multivariable model. The reference was the median value of cholinesterase at discharge (208 U/L).

patients in the low tertile.<sup>21</sup> TR and IVC diameter are associated with impairment of hepatic function.<sup>22</sup> In the present study, the low tertile of cholinesterase was associated with increased HF hospitalization and all-cause death. Therefore, the present study suggests that serum cholinesterase might be an indicator of liver dysfunction caused by congestive conditions. Seo et al.<sup>19</sup> reported that in 274 patients with HF with preserved ejection fraction, those with low cholinesterase levels showed elevated NT-proBNP and worse outcomes; however, they estimated the adjusted risks with limited numbers of confounders to avoid overfitting. In the present study, we included patients with HF with reduced ejection fraction and proposed a potential mechanism of low cholinesterase involving a congestive liver. Malnutrition is another factor related to low cholinesterase levels.<sup>23</sup> In the present study, BMI and haemoglobin decreased with decreasing cholinesterase levels. However, even after adjusting for factors related to nutritional status, such as BMI, serum albumin, and haemoglobin, the cholinesterase tertiles remained significantly associated with all-cause death. In previous reports,<sup>19,20</sup> cholinesterase level was reported to have an additional prognostic value on outcomes compared with simple nutritional indices. In addition, we showed the risk of low cholinesterase for in-hospital mortality as well as its superiority in predicting outcomes over other liver markers or the AHEAD score. Even after adjusting for 26 variables, low cholinesterase levels were significantly associated with worse outcomes in an incremental manner in patients with AHF. The linear correlation of low



**Figure 3** Causes of death according to the tertiles of serum cholinesterase at discharge. CVD, cardiovascular death; HF, heart failure; SCD, sudden cardiac death.

cholinesterase and the risk in the univariate model was hampered when we aggressively adjusted for congestive markers and other nutritional and liver markers, although the adjusted risk was significant with lower cholinesterase levels. Thus, our results suggest that cholinesterase may be a combined marker of a congestive liver and nutritional status, and may show the risk for adverse clinical outcomes of patients with AHF. Serum cholinesterase is easily and uniformly measured worldwide.<sup>9</sup> In the management of HF, evaluating serum cholinesterase levels may be a simple method to determine the risk for subsequent events, both cardiovascular and non-cardiovascular. If the value is low, particular attention should be paid to congestion and nutritional status, as well as future events. Further studies for different settings and different follow-up in patients with HF are needed to verify usefulness of evaluating cholinesterase along with other proposed scores such as the AHEAD score and the multiple estimation of risk based on the emergency department Spanish score in patients with acute heart failure (MESSI-AHF) score.<sup>15,24</sup>

## Limitations

Several limitations of the present study should be noted. First, data on serum cholinesterase at discharge were not available in a substantial proportion of patients in this registry. Selection bias might exist; data on cholinesterase was likely to be lacking depending on each centre (Supplementary material online, Figure S5) and those without cholinesterase had a higher prevalence of previous HF hospitalization, higher levels in albumin, a lower prevalence of atrial fibrillation/atrial flutter, hypertension, dyslipidaemia, and lower levels in eGFR and AST than those with (Supplementary material online, Table S1). Although clinical outcomes were comparable between the two

groups (Supplementary material online, Figure S6), it is possible that missing data of cholinesterase along with other missing values can alter the study results. Second, there was much uncertainty left at both sides of RCS curves (<150 U/L and 400 U/L<, Figure 2A and <150 U/L and 300 U/L<, Figure 2B, respectively). As in Supplementary material online, Figure S2, the distribution of cholinesterase was skewed. Therefore, there were too few events to draw solid conclusions at both sides. Third, there may be unadjusted confounding factors in the present study. We did not include lymphocyte count, tricuspid annular plane systolic excursion, pulmonary artery pressure, and loop diuretics doses into the fully adjusted model. Furthermore, we could not completely exclude patients with hyperthyroidism, nephrotic syndrome, fatty liver, chronic hepatitis, and other hepatic diseases, all of which are associated with cholinesterase levels.

## Conclusion

Low serum cholinesterase levels are associated with congestive findings on echocardiography, higher BNP levels, and higher risks for a composite of all-cause death and HF hospitalization in patients with AHF.

## Supplementary material

Supplementary material is available at *European Heart Journal: Acute Cardiovascular Care* online.

## Funding

This work was supported by the Japan Agency for Medical Research and Development [18059186 to T.K., T.K., and N.O.).

**Conflict of interest:** none declared.

## References

1. Sundaram V, Fang JC. Gastrointestinal and liver issues in heart failure. *Circulation* 2016;**133**:1696–1703.
2. Nikolaou M, Parissis J, Yilmaz MB, Seronde M-F, Kivikko M, Laribi S, Paugam-Burtz C, Cai D, Pohjanjous P, Laterre P-F, Deye N, Poder P, Cohen-Solal A, Mebazaa A. Liver function abnormalities, clinical profile, and outcome in acute decompensated heart failure. *Eur Heart J* 2013;**34**:742–749.
3. Samsky MD, Dunning A, DeVore AD, Schulte PJ, Starling RC, Wilson Tang WH, Armstrong PW, Ezekowitz JA, Butler J, McMurray JJ, Teerlink JR, Voors AA, Metra M, Mentz RJ, O'Connor CM, Patel CB, Hernandez AF. Liver function tests in patients with acute heart failure and associated outcomes: insights from ASCEND-HF. *Eur J Heart Fail* 2016;**18**:424–432.
4. Sze S, Pellicori P, Zhang J, Clark AL. Malnutrition, congestion and mortality in ambulatory patients with heart failure. *Heart* 2019;**105**:297–306.
5. Ancion A, Allepaerts S, Oury C, Gori A-S, Piérard LA, Lancellotti P. Serum albumin level and hospital mortality in acute non-ischemic heart failure. *ESC Heart Fail* 2017;**4**:138–145.
6. Liu M, Chan C-P, Yan BP, Zhang Q, Lam Y-Y, Li R-J, Sanderson JE, Coats AJS, Sun J-P, Yip GW-K, Yu C-M. Albumin levels predict survival in patients with heart failure and preserved ejection fraction. *Eur J Heart Fail* 2012;**14**:39–44.
7. 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**:229–255.
8. Levitt DG, Levitt MD. Protein losing enteropathy: comprehensive review of the mechanistic association with clinical and subclinical disease states. *Clin Exp Gastroenterol* 2017;**10**:147–168.
9. Santarpia L, Grandone I, Contaldo F, Pasanisi F. Butyrylcholinesterase as a prognostic marker: a review of the literature. *J Cachexia Sarcopenia Muscle* 2013;**4**:31–39.
10. Yamamoto E, Kato T, Ozasa N, Yaku H, Inuzuka Y, Tamaki Y, Kitai T, Morimoto T, Taniguchi R, Iguchi M, Kato M, Takahashi M, Jinnai T, Ikeda T, Nagao K, Kawai T, Komasa A, Nishikawa R, Kawase Y, Morinaga T, Kawashima T, Motohashi Y, Kawato M, Toyofuku M, Sato Y, Kuwahara K, Shioi T, Kimura T; on behalf of the KCHF study investigators. Kyoto Congestive Heart Failure (KCHF) study: rationale and design. *ESC Heart Fail* 2017;**4**:216–223.
11. Yaku H, Ozasa N, Morimoto T, Inuzuka Y, Tamaki Y, Yamamoto E, Yoshikawa Y, Kitai T, Taniguchi R, Iguchi M, Kato M, Takahashi M, Jinnai T, Ikeda T, Nagao K, Kawai T, Komasa A, Nishikawa R, Kawase Y, Morinaga T, Su K, Kawato M, Sasaki K, Toyofuku M, Furukawa Y, Nakagawa Y, Ando K, Kadota K, Shizuta S, Ono K, Sato Y, Kuwahara K, Kato T, Kimura T; on behalf of the KCHF Study Investigators. Demographics, management, and in-hospital outcome of hospitalized acute heart failure syndrome patients in contemporary real clinical practice in Japan—observations from the prospective, multicenter Kyoto Congestive Heart Failure (KCHF) registry. *Circ J* 2018;**82**:2811–2819.
12. Gray RJ. A class of K-sample tests for comparing the cumulative incidence of a competing risk. *Ann Stat* 1988;**16**:1141–1154.
13. Fine JP, Gray RJ. A proportional hazards model for the subdistribution of a competing risk. *J Am Stat Assoc* 1999;**94**:496–509.
14. Kanda Y. Investigation of the freely available easy-to-use software 'EZR' for medical statistics. *Bone Marrow Transplant* 2013;**48**:452–458.
15. Spinar J, Jarkovsky J, Spinarova L, Mebazaa A, Gayat E, Vitovec J, Linhart A, Widimsky P, Miklik R, Zeman K, Belohlavek J, Malek F, Felsoci M, Kettner J, Ostadal P, Cihalik C, Vackavik J, Taborsky M, Dusek L, Littnerova S, Parenica J. AHEAD score—long-term risk classification in acute heart failure. *Int J Cardiol* 2016;**202**:21–26.
16. Ono S, Egawa G, Kabashima K. Regulation of blood vascular permeability in the skin. *Inflamm Regen* 2017;**37**:11.
17. Tencer J, Torffvit O, Thysell H, Rippe B, Grubb A. Proteinuria selectivity index based upon alpha 2-macroglobulin or IgM is superior to the IgG based index in differentiating glomerular diseases. Technical note. *Kidney Int* 1998;**54**:2098–2105.
18. Sato T, Yamauchi H, Suzuki S, Yoshihisa A, Yamaki T, Sugimoto K, Kunii H, Nakazato K, Suzuki H, Saitoh S-I, Takeishi Y. Serum cholinesterase is an important prognostic factor in chronic heart failure. *Heart Vessels* 2015;**30**:204–210.
19. Seo M, Yamada T, Tamaki S, Hikoso S, Yasumura Y, Higuchi Y, Nakagawa Y, Uematsu M, Abe H, Fujii H, Mano T, Nakatani D, Fukunami M, Sakata Y. Prognostic significance of serum cholinesterase level in patients with acute decompensated heart failure with preserved ejection fraction: insights from the PURSUIT-HFpEF registry. *J Am Heart Assoc* 2020;**9**:e014100.
20. Seo M, Yamada T, Tamaki S, Morita T, Furukawa Y, Iwasaki Y, Kawasaki M, Kikuchi A, Kawai T, Abe M, Nakamura J, Yamamoto K, Kayama K, Kawahira M, Tanabe K, Kimura T, Ueda K, Sakamoto D, Sakata Y, Fukunami M. Prognostic significance of serum cholinesterase in patients with acute decompensated heart failure: a prospective comparative study with other nutritional indices. *Am J Clin Nutr* 2019;**110**:330–339.
21. Pellicori P, Carubelli V, Zhang J, Castiello T, Sherwi N, Clark AL, Cleland JGF. IVC diameter in patients with chronic heart failure: relationships and prognostic significance. *JACC Cardiovasc Imaging* 2013;**6**:16–28.
22. Chen Y, Seto W-K, Ho L-M, Fung J, Jim M-H, Yip G, Fan K, Zhen Z, Liu J-H, Yuen M-F, Lau C-P, Tse H-F, Yiu K-H. Relation of tricuspid regurgitation to liver stiffness measured by transient elastography in patients with left-sided cardiac valve disease. *Am J Cardiol* 2016;**117**:640–646.
23. Camarero Gonzalez E, Munoz Leira V, Iglesias Guerrero M, Fernández Alvarez JA, Cabezas-Cerrato J. [Protein-energy malnutrition: its effects on 4 metabolic parameters]. *Nutr Hosp* 1995;**10**:158–160.
24. Rossello X, Bueno H, Gil V, Jacob J, Javier Martín-Sánchez F, Llorens P, Herrero Puente P, Alquézar-Arbé A, Raposeiras-Roubín S, López-Díez MP, Pocock S, Miró Ò. MESSI-AHF risk score performance to predict multiple post-index event and post-discharge short-term outcomes. *Eur Heart J Acute Cardiovasc Care* 2020; Jul 17; doi: 10.1177/2048872620934318.