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RESEARCH ARTICLE

Prognostic significance of endogenous erythropoietin in long-term outcome of patients with acute decompensated heart failure

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Aims Although previous reports suggest that an elevated endogenous erythropoietin (EPO) level is associated with worse clinical outcomes in chronic heart failure (HF) patients, the prognostic implication of EPO in patients with acute decompensated HF (ADHF) and underlying mechanisms of the high EPO level in severe HF patients who have a poor prognosis remain unclear.

Methods and results We examined 539 consecutive ADHF patients with EPO measurement on admission from our registry. During a median follow-up period of 329 days, a higher EPO level on admission was independently associated with worse clinical outcomes [hazard ratio (HR) 1.25, 95% confidence interval (CI) 1.06–1.48, $P = 0.008$], and haemoglobin level was the strongest determinant of EPO level ($P < 0.001$), whereas estimated glomerular filtration rate (eGFR) was not significant in multivariate regression analysis. In the anaemic subgroup of 318 patients, a higher EPO level than expected on the basis of their haemoglobin level was related to increased adverse events (HR 1.63, 95% CI 1.05–2.49, $P = 0.028$). Moreover, estimated plasma volume excess rate was positively associated with EPO level ($P = 0.003$), and anaemic patients with a higher than expected EPO level tended to have a higher estimated plasma volume excess rate and plasma lactate level, and lower systemic oxygen saturation level with the preservation of the reticulocyte production index than those with a lower than expected EPO level.

Conclusion A high EPO level predicts long-term worse clinical outcomes in ADHF patients, independent of anaemia and impaired renal function. Anaemia and hypoxia due to severe congestion may synergistically contribute to a high EPO level in high-risk HF patients.

Keywords Acute heart failure • Anaemia • Biomarker • Erythropoietin • Prognosis

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Introduction

Despite dramatic advances in diagnosis and treatment, the prognosis of heart failure (HF) remains poor. Indeed, the Framingham Heart Study indicated that HF is still highly fatal, and in subjects who were diagnosed with HF in the 1990s, more than half of them died within 5 years, and the mortality had declined by only one-third from that in the 1950s.[1] Failure to improve the mortality of HF patients has prompted investigators to seek novel mechanisms related to prognostic determinants of HF.

Multiple co-morbid conditions are frequently observed in patients with HF. Among them, anaemia and renal insufficiency were reported to be strong prognostic determinants of HF patients.[2,3] Furthermore, the interaction between these conditions has been suggested to form a vicious cycle, termed the 'cardio-renal–anaemia syndrome', where HF may cause progressive renal dysfunction and both may lead to anaemia, which in turn can worsen HF and renal insufficiency.[4]

Endogenous erythropoietin (EPO) is mostly produced in the kidney by stimulation such as hypoxia, which is the fundamental physiological stimulus for EPO production through a rapid increase in the number of renal EPO-producing cells.[5] Impairment of EPO production is a major cause of anaemia in HF patients with renal insufficiency; therefore, EPO has been expected to be reduced in the cardio-renal–anaemia syndrome. In contrast, several previous studies revealed that an elevated EPO level was frequently observed and was associated with impaired survival, independent of anaemia and renal function, in chronic HF patients.[6–10] Of them, Belonje *et al.* reported that the EPO measurement was useful for future risk stratification in a large cohort of chronic HF patients, and that a higher than expected EPO level based on the prediction by haemoglobin level in the anaemic subgroup could predict worse clinical outcomes.[10] These results indicate that EPO plays an important role in identifying chronic HF patients at risk for future adverse events.

However, the prognostic implication of EPO in patients with acute decompensated HF (ADHF) and the underlying mechanisms of the high EPO level in patients with severe HF who have a poor prognosis remain unclear, despite several mechanisms having been hypothesized.[9–12]

Thus, the aim of the present study was to investigate whether the high EPO level in the decompensated phase of HF was associated with worse clinical outcomes, and to explore the pathophysiological mechanisms of a high EPO level in high-risk patients in a relatively large ADHF cohort.

Methods

Study design

Data from the NaDEF (National cerebral and cardiovascular center acute DEcompensated heart Failure) registry, which were obtained between January 2013 and March 2015, were retrospectively analysed. The NaDEF registry is a single-centre, observational, ongoing, prospective cohort that includes all patients requiring hospitalization to our institution from January 2013 for the first time with a diagnosis of ADHF by at least two experienced cardiologists according to the Framingham ADHF criteria,[13] and follow-up was performed at 3, 6, 12, and 24 months after discharge by direct contact with patients or patients' physicians at the hospital or outpatient clinic, telephone interview of patients, or, if deceased, of family members, and by mail, by dedicated co-ordinators and investigators. In this study, because patient information was anonymized and de-identified prior to analyses, written informed consent was not obtained from each patient. However, we publicized the study by posting a summary of the protocol (with an easily understood description) on the website of the National Cerebral and Cardiovascular Center; the notice clearly informed patients of their right to refuse enrolment. These procedures for informed consent and enrolment were in accordance with the detailed regulations regarding informed consent described in the guidelines, and this study, including the procedure for enrolment, has been approved by the Institutional Review Board of the National Cerebral and Cardiovascular Center (M22-025), and registered under the Japanese UMIN Clinical Trials Registration (UMIN000017024).

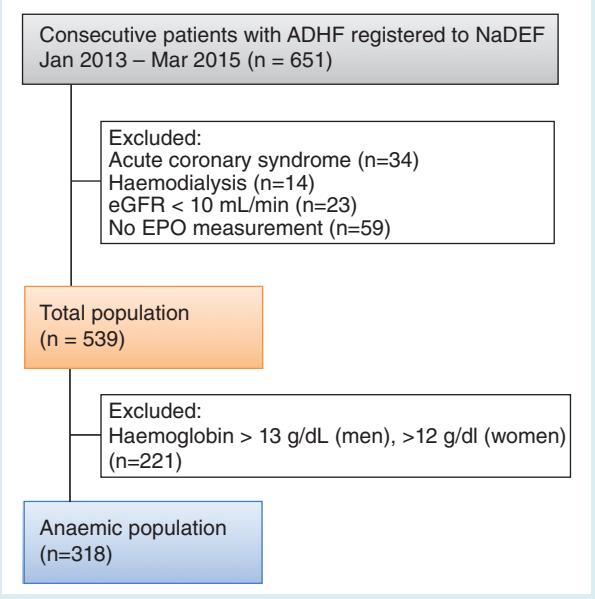

Figure 1 Study population. ADHF, acute decompensated heart failure; eGFR, estimated glomerular filtration rate; EPO, erythropoietin.

Study population

From the 651 patients enrolled in the NaDEF registry, those with acute coronary syndrome or without measurement of EPO were excluded from this study. To minimize errors influencing the level of endogenous EPO, patients with estimated glomerular filtration rate (eGFR) <10 mL/min or on maintenance haemodialysis on admission were also excluded. Finally, 539 patients were examined (*Figure 1*).

Renal function and anaemia

The eGFR was determined using the abbreviated Modification of Diet in Renal Disease equation: $eGFR = 186.3 \times (\text{creatinine}/88.4)^{-1.154} \times (\text{age})^{-0.203}$ ($\times 0.742$ if female).[14] Anaemia was defined according to the World Health Organization criteria as a haemoglobin level <13.0 g/dL in men and <12.0 g/dL in women.

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Table 1 Baseline characteristics of the total population

Variable	All patients	EPO quartile				*P*-value
		1	2	3	4	
Number	539	134	135	135	135	–
EPO range, U/L	–	<23.0	23.0–38.3	38.4–78.9	≥79.0	–
Age, years	75.8 ± 11.8	72.0 ± 13.1	76.8 ± 10.6	76.6 ± 12.2	77.7 ± 10.2*	<0.001
Male sex, *n* (%)	320 (59)	82 (61)	84 (62)	74 (55)	80 (59)	0.61
NYHA III or IV, *n* (%)	442 (89)	109 (89)	112 (89)	106 (86)	116 (90)	0.54
Past history						
AF, *n* (%)	278 (52)	58 (43)	67 (50)	69 (51)	84 (62)	0.018
Hypertension, *n* (%)	378 (70)	89 (66)	101 (75)	95 (70)	93 (69)	0.43
Diabetes mellitus, *n* (%)	190 (35)	46 (35)	48 (36)	45 (33)	51 (38)	0.87
Systolic BP, mmHg	139.3 ± 32.4	140.2 ± 34.0	143.8 ± 30.8	139.9 ± 31.9	133.2 ± 32.1	0.057
Heart rate, b.p.m.	92.4 ± 28.6	95.6 ± 29.4	88.0 ± 26.5	94.2 ± 29.8	92.0 ± 28.3	0.148
SpO_2, %	94.1 ± 5.1	94.5 ± 4.1	93.8 ± 4.7	94.7 ± 5.0	93.6 ± 6.3	0.20
LVEF, %	38.2 ± 17.1	37.5 ± 17.9	38.0 ± 16.4	39.0 ± 16.1	38.5 ± 18.2	0.92
Estimated PV excess rate, %	9.3 ± 11.8	5.3 ± 11.1	7.9 ± 10.6	10.3 ± 11.2	13.8 ± 12.9	<0.001
Aetiology						
ICM, *n* (%)	121 (23)	26 (20)	33 (24)	30 (22)	32 (24)	0.30
NICM, *n* (%)	187 (35)	53 (40)	42 (31)	46 (34)	46 (34)	
Valvular disease, *n* (%)	134 (25)	28 (21)	36 (27)	34 (25)	36 (27)	
Other, *n* (%)	93 (17)	24 (18)	24 (18)	25 (19)	20 (15)	
Oral medications at admission						
ACE inhibitors/ARBs, *n* (%)	280 (52)	63 (47)	77 (57)	68 (50)	72 (53)	0.40
Beta-blockers, *n* (%)	284 (53)	59 (44)	82 (61)	64 (47)	79 (59)	0.012
Diuretics, *n* (%)	302 (56)	63 (47)	75 (56)	75 (56)	89 (66)	0.020
Spironolactone, *n* (%)	110 (20)	33 (25)	22 (16)	22 (16)	33 (24)	0.12

Continuous variables are presented as mean ± SD.
Categorical variables are presented as number of patients (%).
BP, blood pressure; EPO, erythropoietin; ICM, ischaemic cardiomyopathy; NICM, non-ischaemic cardiomyopathy; PV, plasma volume; SpO_2, oxygen saturation by pulse oximetry.
*$P < 0.001$ vs. quartile 1.

Blood sampling and erythropoietin measurement

Venous blood samples for routine laboratory measurements and EPO were measured on admission. Blood samples were collected in pyrogen-free tubes containing EDTA (Becton Dickinson, San Jose, CA, USA) and were immediately centrifuged at 2000 *g* for 30 min at 4 °C. Platelet-poor serum was separated and stored at −80 °C until analysis. Serum EPO level was measured with a Recombigen EPO Kit (LSI Medience Corp., Tokyo, Japan). This assay consists of an anti-EPO rabbit capture antibody, iodine-labelled recombinant human EPO, and secondary antibody (serum anti-rabbit IgG). The amount of serum EPO was quantified by radioimmunoassay. The analytical parameters for the assay were intra-assay coefficient of variation (CV), 2.6–4.0%; interassay CV, 4.7–5.3%; limit of detection, 4.0 mIU/mL; and linearity, 4.0–200 mIU/mL.

Erythropoietin responsiveness

We used a similar approach to those used in previous studies to determine the response of endogenous EPO to the haemoglobin level in anaemic patients.[10,15,16] To avoid interference with EPO production or EPO activity due to renal function and HF, a sample of 35 anaemic patients in our outpatient clinic cohort (different from our ADHF cohort) without renal disease or HF (previous history of HF, symptoms or signs of HF, LVEF <50%, or moderate to severe valvular heart disease) was obtained to construct a regression equation as a reference. The equation, 9.626 − (0.503 × haemoglobin) = log EPO, was used to calculate the predicted EPO value for a given haemoglobin level. The observed log EPO level in anaemic patients in the present study was divided by the predicted log EPO level to obtain the observed/predicted (O/P) ratio. The mean O/P ratio for the reference subjects was 1.001 [95% confidence interval (CI), 0.938–1.064]. An O/P ratio <0.938 indicates a lower than expected EPO production. An O/P ratio >1.064 implies a higher than expected EPO production. Moreover, the erythropoietic response was also determined by calculating the reticulocyte production index (RPI), a standard measure of reticulocyte production that corrects for both the degree of anaemia and the early release of reticulocytes from bone marrow in anaemic patients.[17]

RPI was calculated using the following equation:

RPI = reticulocytes (%) × [observed haematocrit/normal haematocrit (45 for men, 40 for women)] × (1/maturation factor defined by sex and observed haematocrit).

Iron deficiency was defined as a ferritin level <100 μg /L or between 100 and 299 μg/L, if transferrin saturation (TSAT) was <20%, based on the major previous clinical trial.[18]

Plasma volume estimation

To assess the influence of congestion and dilutional anaemia on EPO production on admission (decompensated phase) in anaemic ADHF

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Table 2 Laboratory data and initial treatments of the total population

Variable	All patients	EPO quartile				*P*-value
		1	2	3	4	
Number	539	134	135	135	135	–
EPO range, U/L	–	<23.0	23.0–38.3	38.4–78.9	≥79.0	–
Laboratory data						
EPO, U/L	38 (23–79)	19 (16–21)	30 (26–34)	51 (43–63)	151 (103–277)*	<0.001
Log EPO, U/L	3.88 ± 0.99	2.89 ± 0.19	3.39 ± 0.15*	3.96 ± 0.22*	5.27 ± 0.79*	<0.001
Haemoglobin, g/dL	12.0 ± 2.1	12.9 ± 2.0	12.6 ± 1.7	11.7 ± 1.9*	10.9 ± 2.1*	<0.001
Platelets, $\times 10^4/\mu L$	16.6 ± 5.8	17.7 ± 6.1	16.0 ± 5.2	16.6 ± 5.8	16.1 ± 5.9	0.077
Fe, μg/dL	50 (33–70)	52 (34–80)	53 (35–74)	50 (36–68)	45 (28–63)	0.22
Ferritin, μg/L	109 (58–225)	140 (76–262)	117 (65–222)	97 (59–206)	90 (38–213)	0.31
TSAT, %	18.4 (12.3–26.2)	19.6 (13.4–28.9)	19.0 (12.8–29.5)	18.7 (13.5–25.8)	16.8 (9.1–24.5)	0.18
Iron deficiency, *n* (%)	320 (59)	71 (53)	81 (60)	84 (62)	84 (62)	0.35
eGFR, mL/min/1.73 m^2	47.1 (30.9–60.1)	52.6 (34.9–67.6)	49.5 (36.0–60.7)	45.6 (33.7–54.0)	41.0 (26.2–54.0)*	<0.001
Sodium, mEq/L	139.6 ± 4.4	139.2 ± 4.1	140.0 ± 3.9	139.5 ± 4.8	139.5 ± 4.6	0.53
BNP, pg/mL	594 (308–1077)	479 (182–804)	541 (276–976)	606 (332–1233)	743 (429–1366)*	<0.001
hs TnT, ng/mL	0.04 (0.02–0.07)	0.03 (0.02–0.05)	0.04 (0.02–0.07)	0.04 (0.02–0.07)	0.05 (0.03–0.07)	0.50
CRP, mg/dL	0.42 (0.14–1.22)	0.36 (0.12–1.19)	0.28 (0.10–0.88)	0.44 (0.13–1.23)	0.70 (0.25–1.88)	0.48
Renin activity, ng/mL/h	1.7 (0.6–6.6)	2.2 (0.6–8.3)	1.6 (0.6–4.7)	1.5 (0.5–4.3)	1.8 (0.6–9.6)	0.003
Aldosterone, ng/dL	9.5 (5.8–24.2)	10.4 (6.8–20.1)	7.9 (5.6–18.3)	10.5 (6.1–21.7)	9.2 (4.8–43.5)	0.001
Lactate, mg/dL	13.5 (10.0–18.0)	14.0 (10.1–18.7)	14.0 (10.4–18.0)	13.5 (10.3–17.1)	12.9 (9.0–20.9)	0.75
Urinary albumin, mg/g creatinine	59 (23–175)	51 (15–151)	54 (23–117)	65 (22–216)	79 (35–260)	0.24
Urine NAG, U/L	8.4 (4.7–14.7)	9.2 (4.8–15.4)	6.9 (4.6–11.5)	9.3 (5.7–16.4)	8.4 (3.7–13.7)	0.083
Urine β2-MG, μg/L	293 (97–1353)	317 (102–1433)	304 (129–1360)	275 (82–1215)	272 (77–1630)	0.63
Intravenous treatments						
Diuretics, *n* (%)	398 (74)	100 (75)	91 (68)	100 (75)	107 (79)	0.20
Vasodilators, *n* (%)	319 (60)	77 (58)	78 (59)	83 (61)	81 (60)	0.94
Inotropes, *n* (%)	79 (15)	23 (17)	12 (9)	17 (13)	27 (20)	0.050

Continuous variables are presented as mean ± SD if normally distributed, and median (interquartile range) if not normally distributed.
Categorical variables are presented as number of patients (%).
CRP, C-reactive protein; eGFR, estimated glomerular filtration rate; EPO, erythropoietin; Fe, serum iron; hs TnT, high-sensitive troponin T; MG, microglobulin; NAG, *N*-acetyl-β-D-glucosaminidase; TSAT, transferrin saturation.
*$P < 0.001$ vs. quartile 1.

patients, we estimated plasma volume (PV) excess on admission. Total blood volume was estimated from their ideal weight as previously reported (ideal body weight method).[19] Briefly, from the deviation in real body weight from ideal body weight, which was normalized for height, the ratio of total blood volume to body weight was determined for each patient. Then, estimated PV was calculated using the following equation:

Estimated PV = body weight × (designated total blood volume/body weight ratio) × (1 − haematocrit).

The estimated PV excess rate, defined as the ratio of (admission − pre-discharge) estimated PV to the estimated PV pre-discharge, was then calculated in each patient. This method was validated by a study that analysed relatively large healthy volunteers who underwent scintigraphy with radiolabelled albumin,[19] and was used in ADHF patients.[20]

Study endpoints

The study endpoint was all-cause death and worsening HF, which was defined as worsening of symptoms and signs of HF requiring intensification of intravenous therapy or initiation of mechanical support during hospitalization, as used in major AHF clinical trials,[21,22] or readmission due to HF after discharge.

Statistical analyses

Results are presented as mean ± SD when normally distributed, and as median and interquartile range (IQR) when non-normally distributed. Comparisons of differences between groups were made by analysis of variance (ANOVA) with Bonferroni's post-hoc testing for continuous variable, and by χ^2 test or Fisher's exact test for dichotomous variables, when appropriate. For the total population, multivariate linear regression analysis was performed based on the variables achieving $P < 0.10$ on univariate linear regression analysis to explore the strongest independent determinant of log EPO level. For the total population, Kaplan–Meier survival plots were constructed by dividing the EPO level at baseline into quartiles to study the influence of EPO level on all-cause mortality and worsening HF. For the anaemic population, Kaplan–Meier plots were constructed for the different groups of O/P ratio of EPO levels on admission, and log-rank testing was performed. The association between parameters and the composite of all-cause mortality and worsening HF was assessed by Cox proportional hazards regression. Univariate factors that had a value of $P < 0.10$ were identified. Finally, these factors were entered into the multivariate model to assess the impact of EPO level on admission on the composite of all-cause mortality and worsening HF. Moreover, stepwise selection with a *P*-value of 0.10 for backward elimination was used to select

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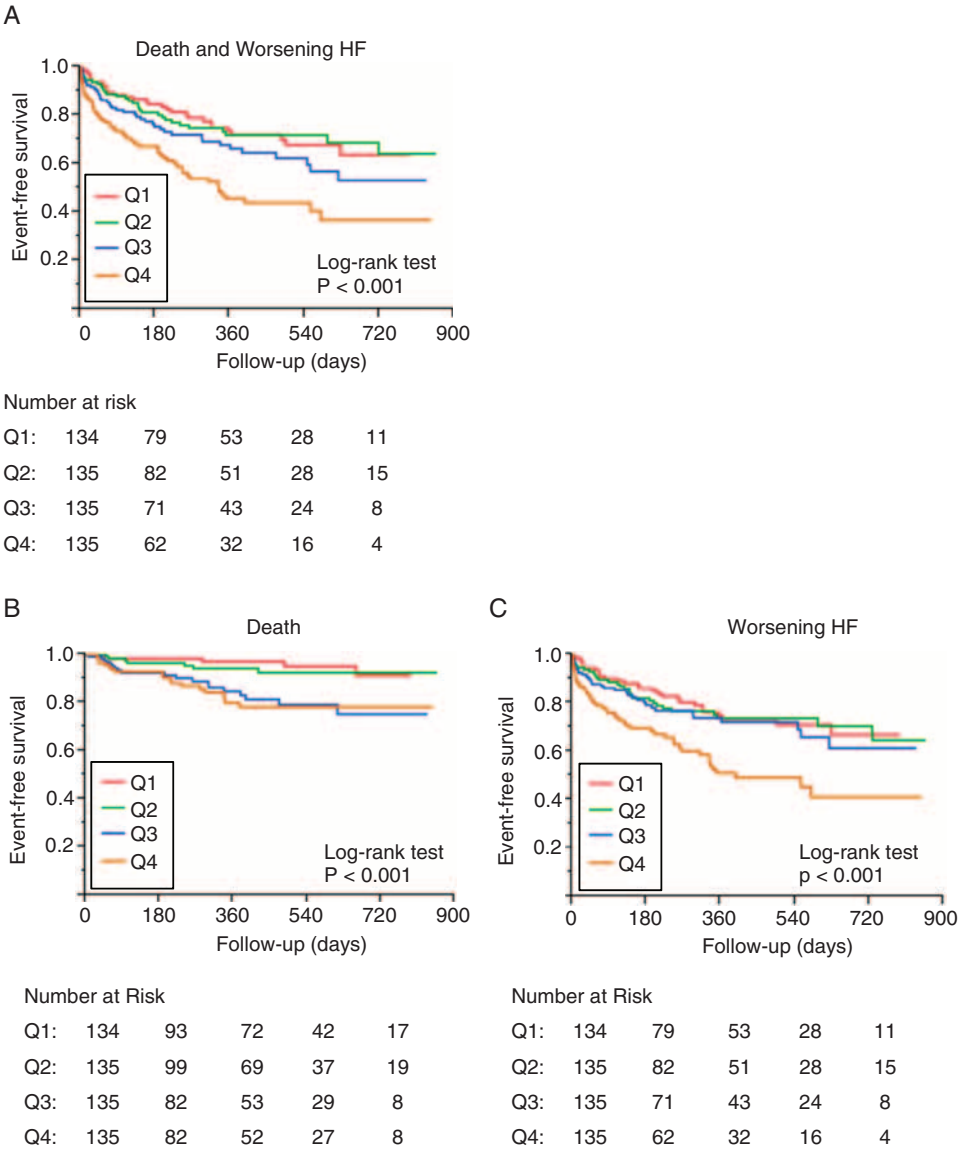

Figure 2 Kaplan–Meier analyses of clinical outcomes categorized by erythropoietin (EPO) level. (*A*) Composite of all-cause death and worsening heart failure. (B) All-cause death. (C) Worsening heart failure. Q, quartile.

the best predictive model in the total population. Adding to the main effect, the interaction between log EPO and other covariates was also examined in the total population. All tests were two tailed, and a value of $P < 0.05$ was considered statistically significant. All analyses were performed with SPSS® for Windows version 21.0 (IBM, Corp., Armonk, NY, USA) and STATA® 13 (Stata Corp, College Station, TX, USA).

Results

Patient characteristics

The clinical characteristics of the total 539 patients are shown in *Tables 1* and *2*. Although patients with higher EPO quartiles were older and had lower systolic blood pressure, haemoglobin, and eGFR levels, and higher plasma BNP level compared with those with lower EPO quartiles, there were no significant differences in sex, NYHA functional class, LVEF, aetiology of HF, oxygen saturation level by pulse oximetry (SpO_2), estimated PV excess rate, platelet count, iron status, urinary albumin level, renal tubular injury markers, plasma lactate level, and intravenous treatment among the quartiles. The median EPO level on admission was 38 U/L.

Erythropoietin level and clinical outcome

During a median follow-up period of 329 days (IQR 110–541 days), adverse events (composite of death and worsening HF,

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Table 3 Cox proportional hazards model for composite of death and worsening heart failure in the total population

Variable	Univariate		Multivariate (significant covariates in univariate analyses)		Multivariate (stepwise selection)	
	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
Age, 5 years	1.12 (1.04–1.21)	0.003	1.05 (0.96–1.16)	0.25	Not selected	–
Systolic BP, 10 mmHg	0.94 (0.89–0.99)	0.018	0.98 (0.92–1.04)	0.44	Not selected	–
BNP, 100 pg/mL	1.03 (1.01–1.04)	0.002	1.02 (1.00–1.04)	0.048	1.02 (1.00–1.04)	0.066
eGFR, 10 mL/min/1.73 m^2	0.81 (0.74–0.89)	<0.001	0.85 (0.76–0.94)	0.002	0.82 (0.74–0.90)	<0.001
Haemoglobin, 1 g/dL	0.88 (0.81–0.95)	0.001	0.98 (0.89–1.09)	0.74	Not selected	–
Log EPO, 1 U/L	1.43 (1.24 –1.64)	<0.001	1.21 (1.02 –1.45)	0.032	1.25 (1.06–1.48)	0.008
SpO_2, 1 %	0.98 (0.95–1.01)	0.157	Not selected	–	Not selected	–
Sodium, 1 mEq/L	0.96 (0.92–0.99)	0.029	0.97 (0.93–1.01)	0.147	Not selected	–
Male sex	1.19 (0.85–1.68)	0.31	Not selected	–	Not selected	–
Lactate, 1 mg/dL	1.00 (0.98–1.02)	0.68	Not selected	–	Not selected	–
Urinary albumin, 100 mg/g creatinine	1.00 (0.97–1.03)	0.80	Not selected	–	Not selected	–
Platelets, $10^4/\mu L$	0.97 (0.95–1.01)	0.17	Not selected	–	Not selected	–
Estimated PV excess rate, 1%	1.00 (0.98–1.01)	0.89	Not selected	–	Not selected	–

BP, blood pressure; CI, confidence interval; eGFR, estimated glomerular filtration rate; EPO, erythropoietin; HR, hazard ratio; PV, plasma volume; SpO_2, oxygen saturation by pulse oximetry.

death, and worsening HF) occurred in 166 (30.8%), 50 (9.3%), and 142 (26.3%) study patients, respectively. Kaplan–Meier analyses revealed that a higher EPO level on admission was significantly associated with worse clinical outcomes including the composite of death and worsening HF, death, and worsening HF (log-rank; $P < 0.001$, $P < 0.001$, and $P < 0.001$, respectively; *Figure 2*). In the multivariate Cox proportional hazards model, a higher log EPO level and lower eGFR were significantly associated with the composite of death and worsening HF among variables including higher age and plasma BNP level, and lower systolic BP, haemoglobin, and serum sodium levels (*Table 3*). Furthermore, in adjusted Cox models, the risk of the composite of death and worsening HF in the highest quartile was almost 1.7-fold higher than that in the lowest quartile of log EPO levels [hazard ratio (HR) 1.68, 95% CI 1.03–2.73; *P* for trend = 0.011]. Although a significant interaction between log EPO and age was identified ($P = 0.004$), there were no interactions between log EPO and other variables on outcomes. The risk was stratified by the mean age of 75 years (HR 1.49, 95% CI 1.10–2.02, $P = 0.004$, <75 years; HR 1.05, 95% CI 0.83–1.32, $P = 0.68$, ≥75 years).

Erythropoietin responsiveness in the anaemic subgroup

In multivariate linear regression analysis, haemoglobin level was the strongest determinant of log EPO level among eGFR, plasma BNP level, systolic BP, estimated PV excess rate, and iron status (Fe, ferritin, TSAT) (*Table 4*). Then the subanalyses in the 'anaemic' population were performed to explore the pathophysiological mechanisms of the high EPO level. The median EPO level in the anaemic subgroup of 318 patients was 49 U/L. The subgroup was divided into three groups based on the O/P ratio. As shown in *Tables 5* and *6*, anaemic patients with a higher EPO level than expected had higher haemoglobin, serum sodium, and plasma BNP levels compared with those with a lower EPO level than expected. Moreover, anaemic patients with a higher EPO level than expected tended to have a higher estimated PV excess rate and plasma lactate level, and a lower SpO_2 level on admission, which might reflect severe congestion on admission compared with those with a lower EPO level than expected (*Tables 5* and *6*). Regarding EPO responsiveness, anaemic patients with a higher EPO level than expected had a significantly higher RPI, which could reflect the preserved reticulocyte-producing potential of bone marrow in

Table 4 Linear regression analyses of log erythropoietin level in the total population

Variable	Univariate		Multivariate	
	β coefficient	P-value	β coefficient	P-value
Age	0.14	<0.001	−0.004	0.93
BNP	0.23	<0.001	0.23	<0.001
eGFR	−0.23	<0.001	−0.07	0.11
Haemoglobin	−0.40	<0.001	−0.35	<0.001
SpO_2	−0.03	0.52	Not selected	–
Estimated PV excess rate	0.29	<0.001	0.13	0.004
Systolic BP	−0.13	0.003	−0.02	0.68
Platelets	−0.09	0.041	−0.03	0.49
Urinary albumin	0.04	0.41	Not selected	–
Fe	−0.07	0.067	0.25	0.004
Ferritin	0.12	0.006	0.13	0.002
TSAT	−0.08	0.062	−0.27	0.003

BP, blood pressure; eGFR, estimated glomerular filtration rate; Fe, serum iron; PV, plasma volume; SpO_2, oxygen saturation by pulse oximetry; TSAT, transferrin saturation.

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Table 5 Baseline characteristics of anaemic patients

Variable	All anaemic patients ($n = 318$)	Lower EPO level than expected ($n = 149$)	EPO level as expected ($n = 71$)	Higher EPO level than expected ($n = 98$)	*P*-value
Age, years	79.2 ± 9.7	80.1 ± 8.9	80.1 ± 10.0	77.2 ± 10.5	0.056
Male sex, *n* (%)	186 (58)	78 (52)	43 (61)	65 (66)	0.084
NYHA III or IV, *n* (%)	261 (82)	126 (85)	56 (79)	79 (81)	0.53
Past history					
AF, *n* (%)	170 (53)	77 (52)	40 (56)	53 (54)	0.80
Hypertension, *n* (%)	230 (72)	109 (73)	51 (72)	70 (71)	0.95
Diabetes mellitus, n (%)	122 (38)	64 (43)	24 (34)	34 (35)	0.30
Systolic BP, mmHg	138.4 ± 30.1	139.9 ± 28.9	141.0 ± 32.8	134.1 ± 29.9	0.24
Heart rate, b.p.m.	87.7 ± 26.8	84.8 ± 25.8	87.4 ± 24.5	92.2 ± 29.6	0.107
SpO_2, %	94.3 ± 5.3	94.6 ± 4.8	94.9 ± 4.1	93.5 ± 6.5	0.161
LVEF, %	41.3 ± 16.9	43.6 ± 16.9	40.7 ± 16.9	38.4 ± 16.8	0.089
Estimated PV excess rate, %	11.6 ± 11.2	10.6 ± 10.8	12.5 ± 11.0	12.4 ± 12.0	0.37
Aetiology					
ICM, *n* (%)	78 (25)	34 (23)	24 (34)	20 (34)	0.107
NICM, *n* (%)	86 (27)	36 (24)	15 (21)	35 (21)	
Valvular disease, *n* (%)	96 (30)	51 (34)	22 (31)	23 (31)	
Other, *n* (%)	58 (18)	28 (19)	10 (14)	20 (14)	
Oral medications at admission					
ACE inhibitor/ARBs, *n* (%)	173 (54)	85 (57)	35 (49)	53 (54)	0.56
Beta-blockers, *n* (%)	165 (52)	75 (50)	35 (49)	55 (56)	0.59
Diuretics, *n* (%)	195 (61)	94 (63)	32 (45)	69 (70)	0.003
Spironolactone, *n* (%)	65 (20)	29 (19)	15 (21)	21 (21)	0.92

Continuous variables are presented as mean ± SD.
Categorical variables are presented as number of patients (%).
BP, blood pressure; EPO, erythropoietin; ICM, ischaemic cardiomyopathy; NICM, non-ischaemic cardiomyopathy; PV, plasma volume; SpO_2, oxygen saturation by pulse oximetry.
*$P < 0.01$ vs. lower EPO level than expected.

response to circulating EPO, compared with those with a lower EPO level than expected (*Tables 5* and *6*). However, age, sex, systolic blood pressure, eGFR, C-reactive protein, plasma renin activity, aldosterone levels, platelet count, iron status, urinary albumin level, and renal tubular injury markers were comparable among the groups. In the anaemic population, during a median follow-up period of 330 days (IQR 119–554 days), Kaplan–Meier analyses demonstrated that a higher EPO level than expected was associated with worse clinical outcomes, especially with worsening HF (*Figure 3*). Furthermore, multivariate Cox proportional hazard modelling revealed that a higher than expected EPO level was independently associated with worse clinical outcomes (composite of death and worsening HF) among other confounders including plasma BNP and haemoglobin levels, eGFR, and systolic blood pressure (*Table 7*).

Discussion

The present findings indicate that a high circulating EPO level on admission was significantly associated with future adverse events in patients with ADHF. We also found that a higher than expected EPO level was independently associated with worse clinical outcomes in the anaemic ADHF subgroup. Importantly, anaemic ADHF patients with a higher than expected EPO level had higher bone marrow reticulocyte production compared with those with a lower than expected EPO level, indicating that blunted reticulocyte responsiveness to EPO of bone marrow might not contribute to a high systemic EPO level. Furthermore, patients with a higher than expected EPO level tended to have a higher estimated PV excess rate and plasma lactate level, with a lower SpO_2 level compared with those with a lower than expected EPO level, suggesting that the high EPO level might be partly caused by severe congestion and hypoxia during the decompensated phase.

Previous studies have demonstrated that an elevated EPO level was an independent determinant of worse clinical outcomes in chronic HF and the compensated phase of hospitalized HF.[6–8,10,15] In the present study, we confirmed these findings in a relatively larger cohort of ADHF patients in whom EPO was measured on admission. In addition, our ADHF cohort had a higher prevalence of anaemic patients and worse clinical outcomes compared with the previous cohort of compensated hospitalized patients with HF which included acute and chronic HF (59.0% vs. 22.3%).[10] Interestingly, 31% of anaemic ADHF patients had a higher than expected EPO level, given their haemoglobin value, and this rate was also higher in the ADHF cohort than in compensated HF patients (only 9% of anaemic patients).[10] Therefore, our cohort was considered to be appropriate for exploring the

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Table 6 Laboratory data and initial treatments of anaemic patients

Variable	All anaemic patients ($n = 318$)	Lower EPO level than expected ($n = 149$)	EPO level as expected ($n = 71$)	Higher EPO level than expected ($n = 98$)	*P*-value
Laboratory data					
EPO, U/L	49 (29–108)	31 (21–44)	56 (37–98)	141 (77–308)*	<0.001
Log EPO, U/L	4.13 ± 1.04	3.49 ± 0.63	4.17 ± 0.71*	5.07 ± 1.02*	<0.001
O/P ratio	0.97 ± 0.23	0.79 ± 0.11	0.99 ± 0.04*	1.27 ± 0.19*	<0.001
Haemoglobin, g/dL	10.7 ± 1.4	10.3 ± 1.4	10.8 ± 1.3	11.2 ± 1.3*	<0.001
Platelets, $\times 10^4/\mu L$	16.4 ± 10.1	16.8 ± 13.4	16.7 ± 5.8	15.8 ± 6.1	0.72
Reticulocyte, %	1.6 (1.3–2.1)	1.5 (1.2–2.0)	1.6 (1.3–1.8)	1.7 (1.4–2.2)	0.093
RPI	0.9 (0.7–1.2)	0.8 (0.6–1.1)	0.9 (0.7–1.1)	1.1 (0.8–1.5)*	<0.001
Fe, μg/dL	47.6 ± 30.6	45.9 ± 29.3	45.9 ± 25.6	51.5 ± 35.6	0.33
Ferritin, μg /L	97 (48–224)	135 (58–267)	86 (45–151)	74 (39–163)	0.52
TSAT, %	18.3 ± 11.8	18.4 ± 10.7	16.8 ± 9.7	19.3 ± 14.4	0.41
Iron deficiency, *n* (%)	204 (65)	93 (62)	48 (68)	63 (64)	0.57
eGFR, mL/min/1.73 m^2	41.5 (27.7–54.7)	37.0 (25.7–53.7)	45.5 (34.5–55.6)	38.7 (27.7–56.0)	0.30
Sodium, mEq/L	139.4 ± 4.5	138.7 ± 5.4	139.9 ± 3.5	140.2 ± 3.6	0.019
BNP, pg/mL	600 (300–1066)	517 (262–930)	606 (326–935)	753 (335–1408)*	0.008
hs TnT, ng/mL	0.04 (0.03–0.07)	0.04 (0.03–0.07)	0.04 (0.02–0.06)	0.04 (0.03–0.07)	0.53
CRP, mg/dL	0.46 (0.14–1.62)	0.41 (0.10–1.71)	0.44 (0.16–1.80)	0.53 (0.21–1.50)	0.57
Renin activity, ng/mL/h	1.8 (0.7–6.4)	2.0 (0.8–6.5)	1.4 (0.4–3.4)	1.8 (0.5–9.6)	0.128
Aldosterone, ng/dL	8.5 (5.4–21.3)	8.4 (5.5–15.5)	8.0 (4.8–32.1)	9.5 (5.3–30.1)	0.166
Lactate, mg/dL	11.7 (9.0–17.0)	11.0 (8.7–15.8)	12.5 (8.8–15.1)	15.2 (9.0–19.0)	0.072
Urinary albumin, mg/g creatinine	69 (28–193)	80 (36–259)	70 (23–178)	49 (22–177)	0.191
Urine NAG, U/L	8.5 (4.8–14.0)	8.2 (4.8–13.9)	9.3 (5.3–13.7)	8.2 (4.4–14.7)	0.79
Urine β2-MG, μg/L	534 (140–2405)	649 (147–2650)	347 (127–2240)	346 (115–1630)	0.91
Intravenous treatments					
Diuretics, *n* (%)	235 (75)	112 (76)	45 (64)	78 (80)	0.074
Vasodilators, *n* (%)	192 (61)	86 (59)	39 (55)	67 (68)	0.153
Inotropes, *n* (%)	39 (13)	15 (10)	6 (8)	18 (19)	0.081

Continuous variables are presented as mean ± SD if normally distributed, and median (interquartile range) if not normally distributed.
Categorical variables are presented as number of patients (%).
CRP, C-reactive protein; eGFR, estimated glomerular filtration rate; EPO, erythropoietin; Fe, serum iron; hs TnT, high sensitive troponin T; MG, microglobulin; NAG, *N*-acetyl-β-D-glucosaminidase; O/P, observed/predicted; RPI, reticulocyte production index; TSAT, transferrin saturation.
*$P < 0.01$ vs. lower EPO level than expected.

pathophysiological mechanisms of a high EPO level independent of the stimulus of a lower haemoglobin level.

Several mechanisms have been hypothesized with respect to the circulating high EPO level in high-risk HF patients.[9–12] First, in HF, enhanced EPO production in the kidney may be a response to renal hypoxia due to reduced renal perfusion caused by decreased cardiac output, and not merely a response to a lower haemoglobin level. In our analyses, patients with a higher EPO level had lower blood pressure, impaired renal function, and more frequent requirement for inotropes than those with a lower EPO level; nevertheless, haemoglobin level rather than eGFR and iron status (Fe, ferritin, and TSAT) was the strongest independent determinant of EPO levels, and SpO_2 level tended to be decreased in anaemic patients with a higher than expected EPO level. Thus our results indicate that anaemia and hypoxia rather than renal perfusion might synergistically stimulate EPO production. Secondly, the renin–angiotensin–aldosterone system, which is often activated in HF, is known to enhance EPO production;[11] however, our study demonstrated that neither the systemic renin activity nor the aldosterone level was positively correlated with the EPO level. Thirdly, an altered metabolic state, causing the oxygen–haemoglobin dissociation curve to shift to the right, may influence EPO production, as previously suggested.[10] Fourthly, the active proinflammatory state in ADHF may cause an insufficient response of bone marrow to stimulation by EPO.[9,12] Contrary to this hypothesis, RPI was higher in our anaemic patients with a higher than expected EPO level compared with those with a lower than expected EPO level. Therefore, it might be difficult to explain a high EPO level by blunted bone marrow responsiveness to EPO stimulation. Fifthly, in our study, because the estimated PV excess rate was positively correlated with log EPO level in total patients, and anaemic patients with a higher than expected EPO level tended to show a higher estimated PV excess rate, severe congestion with hypoxia and a higher plasma lactate level in the acute decompensated phase might contribute to the elevated circulating EPO level. Taking all these findings together, anaemia and hypoxia due to severe congestive status, rather than decreased renal perfusion and bone marrow

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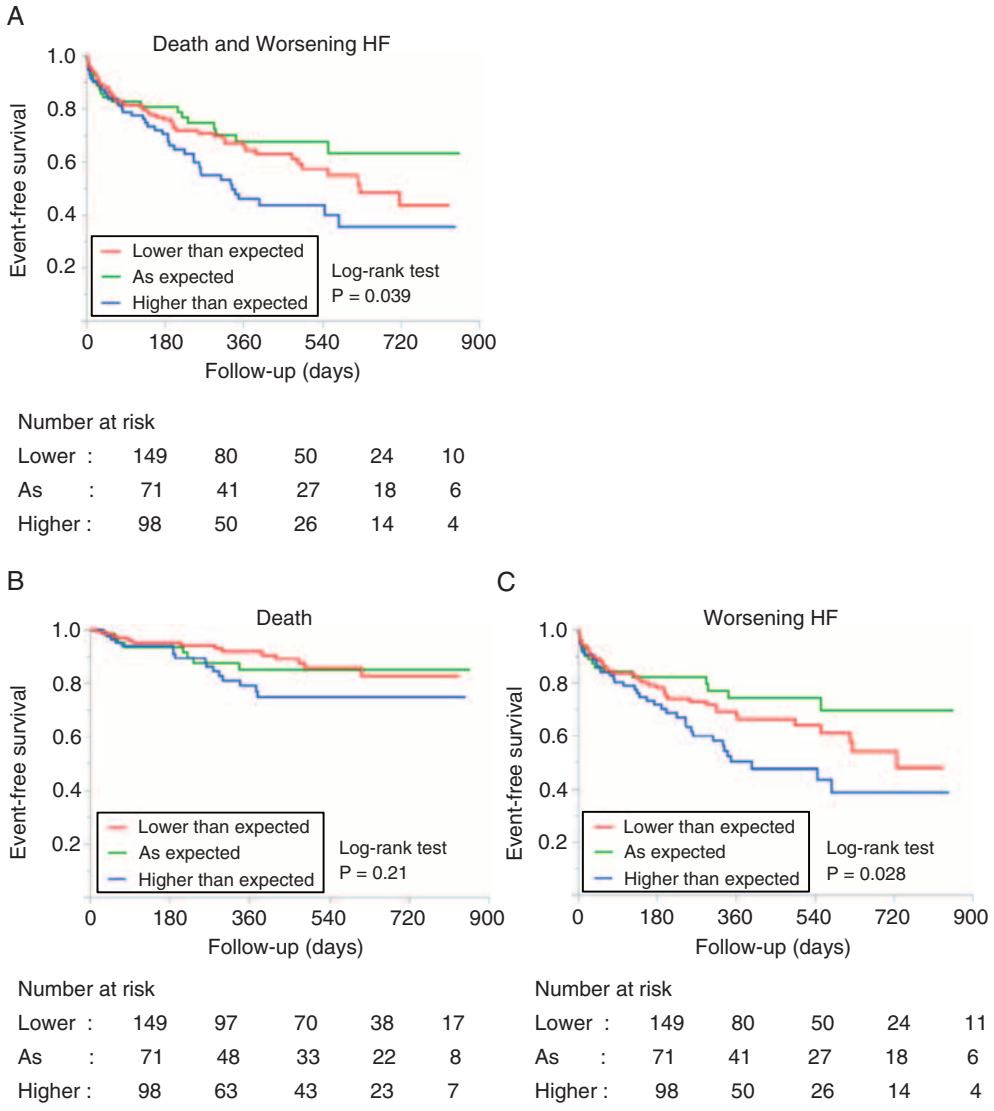

Figure 3 Kaplan–Meier analyses of clinical outcomes categorized by expected erythropoietin (EPO) level in the anaemic subgroup. (*A*) Composite of all-cause death and worsening heart failure. (*B*) All-cause death. (*C*) Worsening heart failure.

hyporesponsiveness to EPO stimulation, may cause circulating high EPO levels in the decompensated phase in high-risk ADHF patients.

Despite the fact that previous experimental studies have demonstrated that EPO ameliorates LV dysfunction by enhancing neovascularization and preventing apoptosis, major randomized clinical trials in HF patients with anaemia failed to demonstrate efficacy on clinical outcomes;[23–26] in contrast, concern about increased thrombotic complications was raised.[27,28] Indeed, high EPO levels have been reported to be associated with stroke in the general female population, possibly due to thrombogenicity and endothelial damage.[29] Nevertheless, in the present study, we found no significant association of markers of thrombogenicity (platelet count) and endothelial damage (urinary albumin level) with EPO level and clinical outcome. These results and our present findings suggest that circulating high EPO levels in high-risk HF patients may act as a surrogate marker rather than a therapeutic target.

Limitations

First of all, although the study population was relatively large compared with those of previous studies examining the clinical implication of EPO measurement, the present sample size, especially the anaemic subgroup, was still small, thereby limiting the ability to generalize the findings and the statistical power for detecting differences in negative data. Therefore, further prospective studies with a larger population are warranted. Secondly, several parameters other than RPI that can evaluate bone marrow responsiveness to EPO stimulation, such as reticulocyte haemoglobin content, immature reticulocyte fraction, and soluble

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Table 7 Cox proportional hazards model for composite of death and worsening heart failure in the anaemic population

	Univariate		Multivariate	
Variable	**HR (95% CI)**	***P*-value**	**HR (95% CI)**	***P*-value**
Higher than expected EPO level	1.59 (1.08–2.31)	0.021	1.63 (1.05–2.49)	0.028
Age, 5 years	1.02 (0.93–1.13)	0.64	Not selected	–
Systolic BP, 10 mmHg	0.89 (0.83–0.95)	<0.001	0.92 (0.85–0.98)	0.015
BNP, 100 pg/mL	1.04 (1.01–1.06)	0.005	1.03 (1.00–1.06)	0.020
eGFR, 10 mL/min/1.73 m^2	0.84 (0.75–0.93)	<0.001	0.91 (0.82–1.02)	0.107
Haemoglobin, 1 g/dL	0.88 (0.77–1.00)	0.042	0.85 (0.74–0.98)	0.025
SpO_2, 1%	0.98 (0.95–1.02)	0.32	Not selected	–
Sodium, 1 mEq/L	0.94 (0.91–0.98)	0.004	0.95 (0.92–0.99)	0.017
Urinary albumin, 100 mg/g creatinine	1.01 (0.99–1.03)	0.26	Not selected	–
Platelets, $10^4/\mu L$	0.97 (0.94–1.01)	0.18	Not selected	–
Male	1.12 (0.76–1.66)	0.57	Not selected	–
Lactate, 1 mg/dL	1.00 (0.95–1.03)	0.80	Not selected	–
Estimated PV excess rate, 1%	0.99 (0.97–1.00)	0.13	Not selected	–

BP, blood pressure; CI, confidence interval; eGFR, estimated glomerular filtration rate; EPO, erythropoietin; HR, hazard ratio; PV, plasma volume; SpO_2, oxygen saturation by pulse oximetry.

transferrin receptor, were not measured in our study. Finally, the true aetiology of anaemia was not confirmed in this observational study.

Conclusion

A high EPO level during the decompensated phase was independently related to worse clinical outcomes in ADHF patients. Anaemia and hypoxia due to severe congestion might cause a high EPO level, thereby strengthening its prognostic significance.

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