可溶性ウロキナーゼ型プラスミノーゲン活性化因子受容体は心不全患者における運動耐容能を示しており有害心イベントを予測する

Soluble urokinase-type plasminogen activator receptor represents exercise tolerance and predicts adverse cardiac events in patients with heart failure

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Soluble urokinase type plasminogen activator receptor represents exercise tolerance and predicts adverse cardiac events in patients with heart failure

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Abstract

Background: Soluble urokinase type plasminogen activator receptor (suPAR) is a membrane binding protein that is released into the blood stream by immune activation. Recent reports suggest that circulating suPAR levels are associated with adverse cardiovascular outcomes. Exercise tolerance is an independent predictor of prognosis in patients with heart failure (HF); however, the relationship between serum suPAR level and exercise tolerance is unclear.

Methods and results: We prospectively enrolled 94 patients who were hospitalized for worsening of HF. All patients underwent a symptom-limited cardiopulmonary exercise test to evaluate exercise tolerance. The median value of serum suPAR was 4848 pg/ml. During follow-up, 44 patients (47%) were admitted for all-cause mortality and re-hospitalization for HF. Median serum suPAR was significantly higher in the patients with cardiac events than in the patients with non-event group. Patients were divided into 2 groups according to circulating suPAR levels. Kaplan-Meier analysis demonstrated that adverse cardiac events were significantly higher in the high suPAR group (log-rank p=0.023). Multivariate analysis revealed that suPAR was independently correlated with the parameters of exercise tolerance such as anaerobic threshold (p=0.007) and peak oxygen uptake (p=0.005).

Conclusion: suPAR levels predicted adverse cardiac events and independently correlated with the parameters of exercise tolerance. suPAR could be a useful surrogate biomarker of exercise tolerance in patients with HF.

Key Words: Soluble urokinase type plasminogen activator receptor; Exercise tolerance; Heart failure

Introduction

Soluble urokinase type plasminogen activator receptor (suPAR) is glycosylphosphatidylinositol-anchored glycoprotein that is expressed mainly in inflammatory cells [1, 2]. SuPAR is proteolytically cleavaged and released into the blood stream in response to various inflammatory stimuli [3, 4]. It has been suggested that activated neutrophils release suPAR, and it is supposed that this release from neutrophils contributes to recruitment of monocytes to sites of active inflammation [5, 6]. Because a part of suPAR is released into the circulation by proteolytic shedding, its circulating levels are expected to be a potential biomarker for low-grade inflammation [7], and suPAR may play an important role in various disease. For instance, previous reports suggested that circulating suPAR levels are independently associated with severity of chronic kidney disease[8], and suPAR levels are specifically elevated in patients with focal segmental glomerulosclerosis (FSGS), and have an important role in the pathogenesis of FSGS[9]. In the cardiovascular field, previous studies have reported that circulating suPAR levels are associated with an increased incidence of atherosclerosis and coronary artery disease [10, 11]. Moreover, Koller et al. recently reported that suPAR is a strong and independent predictor of mortality in patients with heart failure (HF) [12]. These data indicate that circulating suPAR could be used as a biomarker to reflect low-grade chronic inflammatory status and various diseases.

HF is a common syndrome that develops from various comorbidities, and it is anticipated that the number of patients will increase with the growth of an aging society [13, 14]. It has been demonstrated that exercise capacity is an independent prognostic indicator for patients with HF [15, 16]. To date, however, there are few biomarkers that reflect exercise tolerance in these patients. It was reported that circulating suPAR levels

affected acute or chronic exercise [17, 18]. In addition, a recent study showed that suPAR levels even reflect lifestyles, including physical activity, in a cardiovascularly healthy cohort [19]. However, another study reported that long-term physical activity per se does not appear to have an effect on circulating suPAR levels [20].

Given that serum suPAR levels reflect the prognosis of HF, we hypothesize that it could be used as a surrogate biomarker of the exercise tolerance. In this study, we measured serum suPAR levels in patients with HF and determined its significance in the assessment of exercise capacity, disease severity, and prognosis.

Methods

Study population

We enrolled 94 consecutive patients who were hospitalized for worsening HF at Osaka City University Hospital between July 2013 and March 2015. To diagnose HF, all patients had to meet the diagnosis with two major criteria or one major criterion in conjunction with two minor Framingham criteria [21]. The patients who met the following conditions were excluded; (1) experienced acute coronary syndrome within the preceding 30 days; (2) underwent open heart surgery within the preceding 3 months; (3) underwent percutaneous coronary angioplasty or cardiac resynchronization therapy during cardiac rehabilitation; (4) had symptomatic valvular heart disease; (5) had end-stage renal failure (estimated glomerular filtration rate (eGFR) < 15 ml/min/1.73 m^2); (6) had chronic inflammatory disease; (7) could not perform cardiopulmonary exercise testing (CPX) owing to musculoskeletal problems or paralysis; and (8) was unwilling to provide informed consent.

Follow-up

The endpoints of this study were a composite of all cause mortality and unplanned admission to hospital for worsening HF. Patients were followed up for a median of 796 days (interquartile range (IQR) 427-1076 days).

Clinical characteristics, laboratory, and echocardiogram data were collected from the patients' medical records at the time of enrollment. An echocardiographic examination was performed by at least two experienced technicians in a blinded manner, and the ejection fraction (EF) was determined by the modified Simpson's method.

The study protocol conformed to the principles of the Declaration of Helsinki

and was conducted after approval of the ethics committee of our institution (approval number 2569) and, written informed consent was obtained from each patient.

Cardiopulmonary exercise test

All patients underwent a symptom-limited CPX before discharge. The CPX was performed using a cycle ergometer (Strength Ergo 8; Fukuda Denshi, Tokyo, Japan) with a ramp protocol. The ramp protocol is characterized by a gradual increase in work rate, which is evenly distributed within each minute of the exercise phase [22]. In this study, the ramp protocol started at 0 or 10 W for a 4-min warm-up and was followed by an incrementally increasing work rate of 10 W every minute (this increased the work rate by 1 W every 6 s) up to the patient's limit. The electrocardiogram was monitored continuously and blood pressure was recorded every minute before, during, and after exercise (ML-9000; Fukuda Denshi, Tokyo, Japan). Expired gas analysis was performed with the breath-by-breath method using an expired gas analyzer (Cpex-1; Inter Reha, Tokyo, Japan). The following parameters were measured and calculated: peak oxygen uptake (peak VO_2), minute ventilation (VE), and carbon dioxide production (VCO_2). Peak VO_2 was defined as the highest VO_2 value achieved at peak exercise. The peak respiratory exchange ratio (carbon dioxide production/VO_2) was used to determine maximal effort. The anaerobic threshold (AT) was determined using the V-slope method. The slope of the relationship between VE and VCO_2 (VE/VCO_2 slope) as a marker of ventilator inefficiency was obtained. The oxygen uptake efficiency slope (OUES), as an indicator of cardiopulmonary functional reserve, was obtained from the relationship between VO_2 and the logarithmic change in VE [23].

Laboratory analysis

Blood samples were obtained by venipuncture at the time of hospital admission. Standard laboratory measurements were determined from fresh samples. Circulating levels of serum creatinine, high-sensitivity C-reactive protein (hs-CRP), high-sensitivity troponin T (hs-TnT), and B-type natriuretic peptide (BNP) were analyzed using certified methods in the Department of Clinical Laboratory, Osaka City University. eGFR was calculated using the modified IDMS-MDRD study equation [24]: eGFR (ml/min/1.73 m^2) = 194 × (serum creatinine)$^{-1.094}$ × (age)$^{-0.287}$ × (0.739, when female).

Blood samples for analysis of suPAR were collected before CPX. After centrifugation at 4 °C and 3000 rpm for 15 min, serum samples were stored at -80 °C until analysis. Serum concentrations of suPAR were determined from thawed samples, using commercially available enzyme-linked immunosorbent assay (ELISA) kits (R&D System, Minneapolis, MN, USA).

Statistical analysis

Continuous variables were presented as mean ± standard deviation (SD) or median with inter-quartile range (IQR) for non-normally distributed variables. Categorical variables were presented as number (percent). Receiver operating characteristic (ROC) curves were performed to detect the optimal cutoff point of suPAR to estimate the event free rate. To compare each parameter between the low and high suPAR groups, we used an unpaired t test, or the Wilcoxon-Mann-Whitney test or Fisher's exact test for categorical variables.

The Spearman correlation coefficient between suPAR and each continuous variable was calculated. The Kaplan-Meier curves were constructed for the times to

events, which was death or to hospitalization for worsening HF, and the log-rank test was used for initial comparison. Multivariate linear regression analysis was used to adjust for clinical covariates, such as age, hemoglobin, and eGFR. There were reports showing that age, eGFR are strongly related to serum suPAR level [12, 25], we analyzed with these factors and AT (model 1) or peak VO_2 (model 2). Because AT and peak VO_2 have an internal correlation[26], these parameters were needed to be analyzed separately.

All data were analyzed using R software package (version 3.2.1; R Foundation for Statistical Computing, Vienna, Austria). A value of $P < 0.05$ was considered statistically significant.

Results

The clinical characteristics of all study participants are presented in Table 1. The subjects consisted of patients with ischemic and non-ischemic HF (17% and 83%, respectively). The mean age of the patients was 68 years, and mean EF was 38.4%.

In this population, the median serum suPAR level was 4848.5 pg/ml (IQR: 3261.2 to 6589.0 pg/ml). Median serum suPAR was significantly higher in the patients with cardiac events (5530.5 pg/ml) than in the patients with non-event group (4590.0 pg/ml, P=0.047; Figure 1). Based on the ROC curve, the optimal cutoff value of the suPAR level to detect mortality and rehospitalization was determined to be 4170 pg/ml with 79% sensitivity and 49% specificity (area under the curve 0.64). Using the value, all patients were divided into 2 groups (Table 1): high suPAR group (median, 6170.0 pg/ml) and low suPAR group (median, 2953.6 pg/ml). The high suPAR group was older than the low suPAR group. In addition, the high suPAR patients more likely suffered from renal dysfunction. On echocardiography, EF was not significantly different between the 2 groups. There was no significance difference in cardiovascular medication between the high and low groups.

During follow-up, 44 of the 94 patients died or needed to be re-hospitalized because of worsening HF (cardiac death, n=3, all cause death, n=8, rehospitalization for HF, n=36). As shown in Figure 2, Kaplan-Meier analysis revealed that the high suPAR group had a significantly higher probability of death or re-hospitalization (log-rank test, p=0.023).

CPX parameters were compared between the high and low suPAR groups (Table 2). The values of AT and peak VO_2 were significantly higher in the low suPAR group compared with the high suPAR group (Figure 3). Minimum VE/VCO_2 was lower and OUES was higher in the low suPAR group than in the high group. Other parameters such

as the VE/VCO_2 slope were not different between the 2 groups.

We evaluated the correlation between serum suPAR levels and each continuous variable, including AT and peak VO_2 (Table 3). Spearman correlation coefficient analysis showed a statistically significant positive correlation between serum suPAR levels and age, and a negative correlation between suPAR and hemoglobin, eGFR, AT, and VO_2. As shown in Table 4, multivariate analysis revealed that suPAR was independently correlated with exercise tolerance parameters such as peak VO_2 ($p=0.007$) and AT ($p=0.005$).

Discussion

In the present study, we showed an association between serum suPAR levels and exercise capacity in patients with HF. The present study reported the following findings: (1) the prevalence of adverse cardiac events was significantly higher in the high suPAR group, (2) serum suPAR levels were inversely correlated with the exercise tolerance parameters such as AT and peak VO_2. Together, these findings indicate that circulating suPAR is a potentially useful surrogate marker of exercise tolerance in patients with HF, and could thus predict the prognosis in these patients.

Exercise tolerance is an established independent prognostic factor for patients with HF [15, 16]. It has been shown that some cardio-protective biomarkers such as brain derived neurotrophic factor (BDNF) [27] correlate with aerobic exercise performance in patients with HF. On the other hand, experimental rodent HF models showed that pro-inflammatory cytokines such as tumor necrosis factor α (TNF-α) and interleukin-6 (IL-6) caused skeletal muscle exhaustion and accelerate sarcopenia, which ultimately reduced exercise tolerance [28]. These inflammatory cytokines are regarded as a surrogate biomarkers of exercise tolerance in patients with HF [29-31]. Because suPAR has been reported as an inflammatory biomarker [7], we hypothesized that the serum suPAR level reflects exercise capacity in patients with HF and demonstrated that its level independently correlated with the exercise tolerance parameters.

There were some controversial results showing the relationship between circulating suPAR levels and exercise ability [17, 18, 32]. In fact, long-term physical activity per se does not appear to affect circulating suPAR levels [20]. The reason was thought to be that the methods of evaluating exercise capacity were different. Most previous studies investigating circulating suPAR level and exercise tolerance employed acute exercise as

the mode of exercise, so it was difficult to evaluate exercise capacity quantitatively. In contrast, in the present study, we evaluated exercise capacity in all patients using a precise CPX test, and demonstrated a significant negative correlation between suPAR and AT and, peak VO_2. The reason for no correlation existing between suPAR and the VE/VO_2 slope may be explained as the CPX measurement including not only cardiopulmonary function, but also respiratory muscle, skeletal muscle, and sympathetic function. Therefore, the specific value of the ventilation perfusion mismatch was not unambiguously linked.

Expression of suPAR is strongly activated during inflammation, immune responses, tissue injury, and wound healing [33]. Additionally, suPAR is thought to be involved in pathophysiological pathways that are associated with atherosclerosis in a different manner from inflammatory processes [33]. We speculate that these mechanisms might be the cause of suPAR reflecting not only chronic inflammation, but also exercise tolerance at the same time. However, the reason why suPAR levels are elevated in patients with heart failure remains unclear. Recently, Fujisawa et al. reported that serum suPAR levels were associated with diastolic dysfunction independent of confounding factors in patients with heart failure with preserved EF [34]. Because suPAR is involved in many pathways, further investigations are required to determine the mechanism by which suPAR affects development of heart failure.

There were several limitations in the present study. First, this was a single center study and included only a small number of cases. Second, our cohort included several etiologies, such as ischemic heart disease, dilated cardiomyopathy, and secondary cardiomyopathy and arrhythmia. Although the precise cellular source of suPAR could not be determined in this study, our data suggest that suPAR could be used as a surrogate marker of exercise tolerance in patients with HF. Further studies are needed to assess the

value of suPAR in a large number of patients.

In conclusion, suPAR level predicted adverse cardiac events and independently correlated with exercise tolerance parameters. SuPAR could be a useful surrogate biomarker of the exercise tolerance in patients with HF.

Conflict of interest: The authors declare that they have no conflict of interest.

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Figure Legends

Figure 1. Serum soluble urokinase plasminogen activator receptor (suPAR) level in patients with clinical events (mortality or re-hospitalization of worsening HF) (n=44) and patients with no clinical event (n=50).

Figure 2. Kaplan-Meier analysis for the probability of death and rehospitalization because of heart failure in patients with high (n=55) and low suPAR (n=39). Primary events were significantly higher in the high suPAR group than in the low group (log-rank p=0.023).

Figure3. Comparison in AT and peak VO_2 between high and low suPAR group.

Table 1. Baseline characteristics of the patients				
	Total n=94	Low suPAR group (≤4170 pg/ml) n=39	High suPAR group (> 4170 pg/ml) n=55	P
Age (years)	68.0±14.5	63.0±15.2	71.5±13.0	**0.003**
Male (%)	60 (63.8)	28 (73.7)	32 (56.1)	0.18
Current Smoking (%)	11 (11.7)	5 (10.5)	6 (12.3)	0.97
BMI (kg/m²)	22.2±4.1	21.7	21.9	0.843
SBP (mmHg)	108.9±19.1	110.5±19.0	107.8±19.2	0.49
Heart rate (bpm)	75.1±13.6	74.1±13.4	75.8±13.9	0.56
Comorbidity				
HT (%)	42 (44.7)	18 (47.3)	24 (42.1)	0.98
DM (%)	28 (29.8)	9 (23.7)	19 (33.3)	0.33
DLP (%)	42 (44.7)	15 (39.4)	27 (49.1)	0.42
Af (%)	36 (38.3)	10 (25.6)	26 (47.3)	**0.032**
Ischemic HF (%)	16 (17.0)	6 (15.8)	10 (17.5)	0.94
Baseline use of medications				
ACE inhibitor or ARB (%)	59 (62.8)	29 (74.4)	30 (54.5)	0.055
β-blocker (%)	75 (79.8)	32 (82.1)	43 (78.2)	0.80
Aldosterone inhibitor (%)	44 (46.8)	21 (53.8)	23 (41.8)	0.30
Statin (%)	38 (40.4)	20 (51.3)	18 (32.7)	0.60
Baseline use of devices				
Pacemaker (%)	8 (8.5)	0	8 (14.5)	0.059
ICD (%)	4 (4.3)	0	4 (7.2)	0.14
CRT (%)	3 (3.2)	1 (2.5)	2 (3.6)	1.00
Echocardiogram				
LVEDD (mm)	55.2±9.8	57.7±10.9	53.4±8.5	**0.037**
LVESD (mm)	43.0±13.1	46.4±14.4	40.6±11.5	**0.032**
IVS (mm)	8.9±2.0	8.5±2.3	9.2±1.7	0.12
LAD (mm)	45.8±7.8	43.9±6.4	47.1± 8.5	0.052
LVEF (%)	38.4±14.8	35.9±15.7	40.1±13.9	0.14
DCT (msec)	177.6±62.5	182.1±66.9	174.2±59.4	0.57
E/e' ratio	20.5±10.1	19.3±11.8	21.5±8.7	0.35
TRPG (mmHg)	31.2±13.7	31.6±18.7	31.0±9.3	0.85
Laboratory data				
Hemoglobin (g/dl)	12.5±2.3	13.6±2.2	11.7±2.0	**<0.001**
Serum Sodium (mEq/l)	139.5±2.9	140 (137-141)	140 (138-142)	0.34
eGFR (ml/min/1.73 m^2)	50.620.8	58.6±12.8	44.8±23.5	**0.001**
BNP (pg/ml)	452.1 (197.7-827.0)	464.1 (208.7-806.0)	450.5 (198.8-817.1)	0.78
hs-CRP (g/dl)	0.23 (0.11-0.51)	0.13 (0.10-0.36)	0.29 (0.155-0.52)	0.068
hs-Troponin T (ng/ml)	0.017 (0.010-0.042)	0.015 (0.010-0.029)	0.024 (0.010-0.048)	0.074

SuPAR (pg/ml)	4848.5 (3261.2-6589.0)	2953.6 (2398.4-3609.6)	6170.0 (5220.2-7324.0)	**<0.001**

Values are presented mean ±standard deviation, median (inter-quartile range), or n (%)
BMI; body mass index, SBP; systolic blood pressure, HT; hypertension, DM; diabetes mellitus, DLP; dyslipidemia, Af; atrial fibrillation, HF; heart failure LVEF; left ventricular ejection fraction, ACE; angiotensin convert enzyme, ARB; angiotensin type II receptor blocker, ICD; implantable cardioverter-defibrillator, CRT; cardiac resynchronization therapy, LVEDD; left ventricular end-diastolic diameter, LVESD; left ventricular end-systolic diameter, IVS; interventricular septum, LAD; left atrium diameter, DCT; deceleration time, TRPG; tricuspid regurgitation pressure gradient, BNP; B-type natriuretic peptide, hs-CRP; high-sensitivity C-reactive protein, eGFR; estimated glomerular filtration

Table 2. Cardiopulmonary exercise test data				
	Total n=94	Low suPAR group (≤ 4170 pg/ml) n=39	High suPAR group (> 4170 pg/ml) n=55	P
Peak work rate (watts)	56.7 (40-70)	63.0 (45.5-75.0)	51.0 (39.0-62.0)	**0.024**
Anaerobic threshold (mg/kg/min)	12.8 (11.5-14.9)	14.2 (11.9- 15.4)	12.4 (11.1-14.2)	**0.009**
peakVO_2 (mg/kg/min)	17.1 (14.9-20.6)	19.3 (16.1-21.3)	15.7 (13.4-18.7)	**0.001**
Peak O_2 pulse (ml/beats)	8.54 (7.10-10.23)	8.99 (8.07-10.98)	8.15 (6.59-9.42)	0.133
VE/VCO_2 slope	32.4 (28.0-36.5)	31.6 (27.1-34.4)	33.6 (28.7-38.4)	0.110
Minimum VE/VCO_2	26.4 (24.0-30.5)	25.4 (22.6-27.1)	27.9 (25.0-31.4)	**0.038**
ΔHR/ΔWR (beats/min/watt)	0.51 (0.33-0.64)	0.52(0.31-0.70)	0.46(0.36-0.62)	0.798
OUES	1352 (1053-1763)	1134.5 (1479.0-1950.5)	939.3 (475.0-1192.5)	**0.011**

Values are presented as median (inter-quartile range).
VO_2; oxygen uptake, VE; ventilatory equivalent, VCO_2; carbon dioxide output, HR; heart rate, WR; work rate, OUES; oxygen uptake efficiency slope

Table 3. Spearman correlation coefficient analysis between serum suPAR levels and each continuous variable		
	Spearman r	P
Age (years)	0.226	0.028
eGFR (ml/min/1.73m^2)	-0.422	< 0.001
BNP (pg/ml)	0.053	0.614
hs-CRP (mg/dl)	0.198	0.055
BMI (kg/m^2)	-0.023	0.824
Hemoglobin (g/dl)	-0.373	< 0.001
LVEF (%)	0.089	0.392
AT (mg/kg/min)	-0.348	< 0.001
Peak VO_2 (mg/kg/min)	-0.367	< 0.001

eGFR; estimated glomerular filtration, BNP; B-type natriuretic peptide, hs-CRP; high-sensitivity C-reactive protein, BMI; body mass index LVEF; left ventricular ejection fraction, AT; anaerobic threshold

Table 4. Factors associated with ln (suPAR)				
Factors	Multivariate analysis model 1		Multivariate analysis model 2	
	B (95% CI)	P	B (95% CI)	P
Age (years)	-0.002 (-0.005 to 0.001)	0.161	-0.003 (-0.006 to 0.001)	0.099
eGFR (ml/min/1.73 m^2)	-0.003 (-0.005 to -0.001)	0.002	-0.021 (-0.005 to -0.001)	0.003
Hb (g/dl)	-0.021 (-0.039 to -0.002)	0.025	-0.021 (-0.039 to 0.002)	0.026
AT(mg/kg/min)	-0.020 (-0.035 to -0.006)	**0.007**	Not selected	
Peak VO_2 (mg/kg/min)	Not selected		-0.014 (-0.024 to -0.004)	**0.005**

CI; confidence interval.
Hb; Hemoglobin
Other abbreviations as in Table 1.

Figure 1

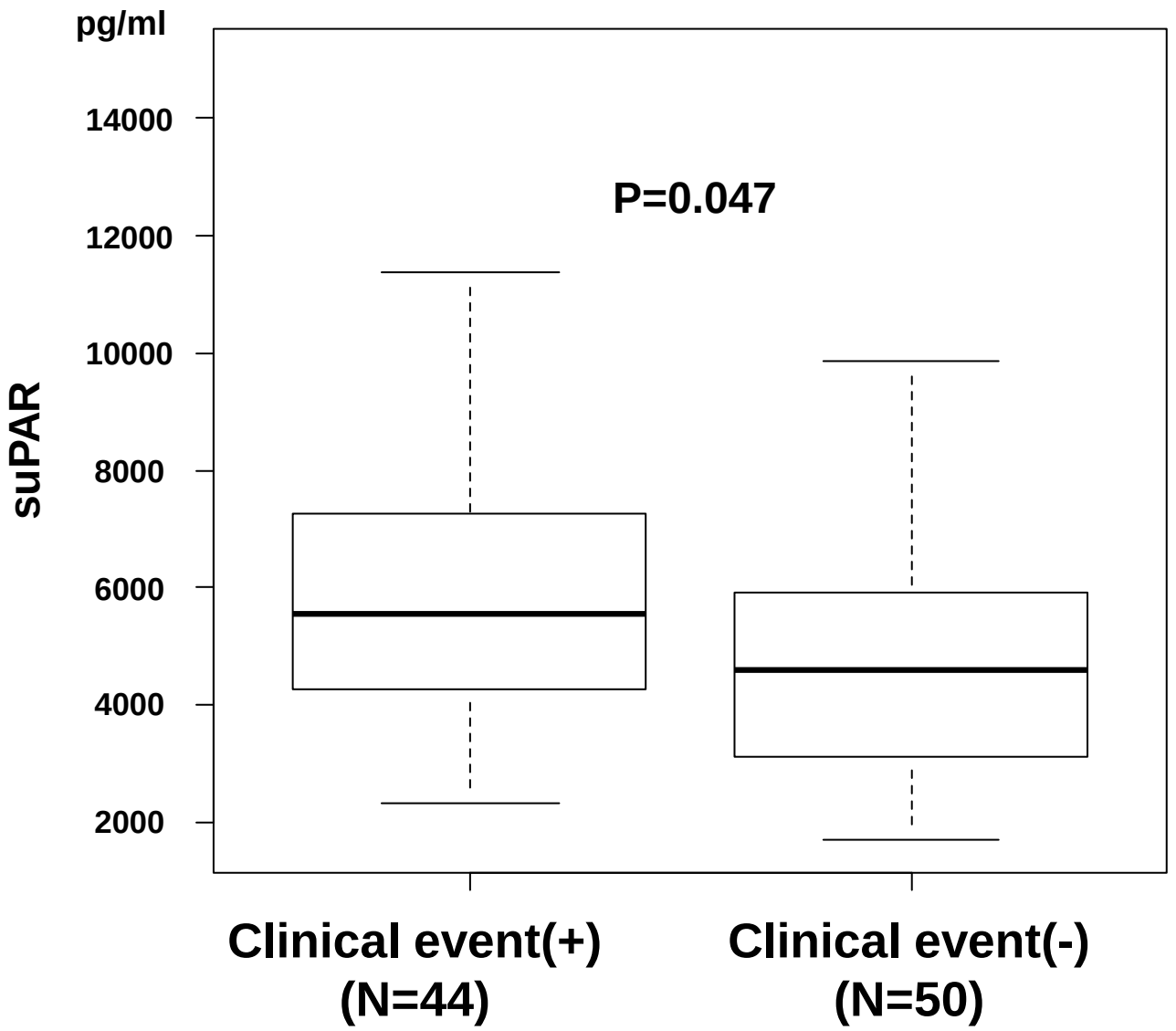

pg/ml
14000
12000
10000
8000
6000
4000
2000
suPAR
P=0.047
Clinical event(+)
(N=44)
Clinical event(-)
(N=50)

Figure 2

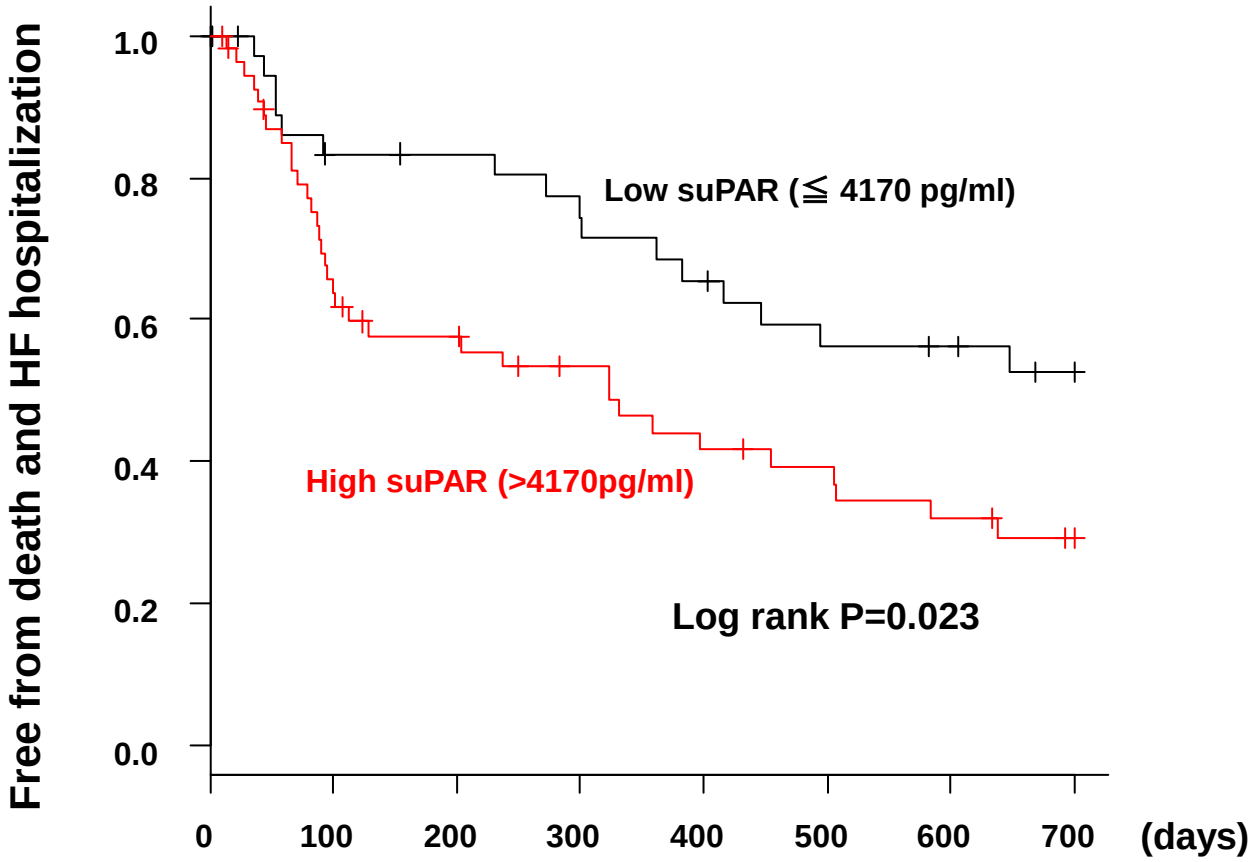
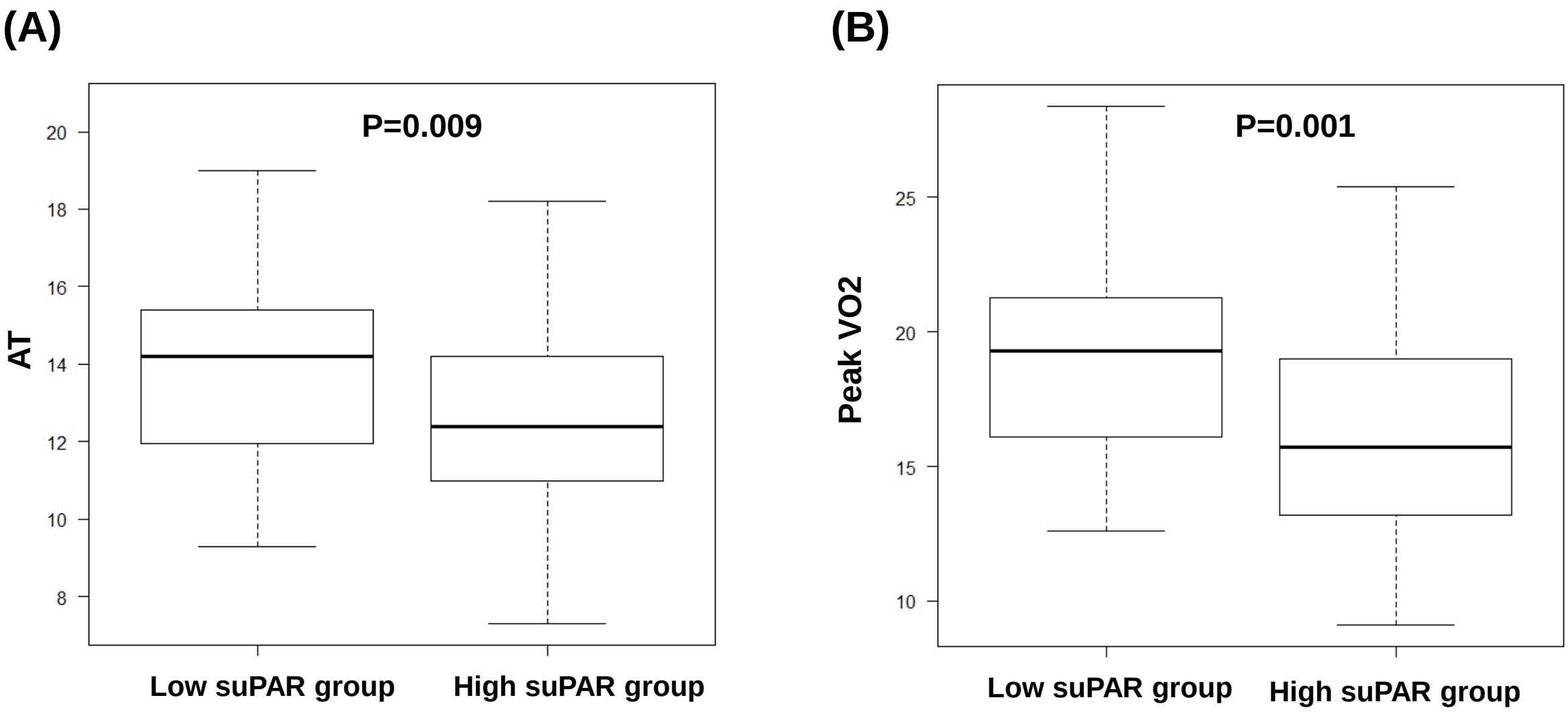

Free from death and HF hospitalization
1.0
0.8
0.6
0.4
0.2
0.0
0
100
200
300
400
500
600
700
(days)
Low suPAR (≦ 4170 pg/ml)
High suPAR (>4170pg/ml)
Log rank P=0.023

Figure 3


(A)
P=0.009
AT
20
18
16
14
12
10
8
Low suPAR group
High suPAR group
(B)
P=0.001
Peak VO2
25
20
15
10
Low suPAR group
High suPAR group