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CIRRHOSIS AND LIVER FAILURE

Cardiac and proinflammatory markers predict prognosis in cirrhosis

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Keywords
chronic liver disease – cirrhotic cardiomyopathy – inflammation – portal hypertension

Abbreviations
ANP, atrial natriuretic peptide; BNP, brain natriuretic peptide; BP, blood pressure; CBV, central blood volume; CCT, central circulation time; CO, cardiac output; HBF, hepatic blood flow; HR, heart rate; hs-CRP, high-sensitive CRP; hs-TnT, high-sensitivity troponin T; HVPG, hepatic venous pressure gradient; ICG, indocyanine green; IL-6, interleukin-6; IL-8, interleukin-8; IP-10, interferon-gamma induced protein 10; IQR, inter-quartile range; LBP, lipopolysaccahride binding protein; LLQ, lower level of quantification; MAP, mean arterial pressure; MELD, model of end-stage liver disease; proANP, prohormone of ANP; proBNP, prohormone of BNP; RAP, right atrial pressure; suPAR, soluble urokinase-type plasminogen activator receptor; SVR, systemic vascular resistance; SV, stroke volume; VEGF, vascular endothelial growth factor.

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Abstract

Background & Aims: Inflammation and cardiac dysfunction plays an important role in the development of complications leading to increased mortality in patients with cirrhosis. Novel cardiac markers such as prohormone of ANP (proANP), copeptin and high-sensitivity troponin T (hs-TnT) and proinflammatory markers including soluble urokinase-type plasminogen activator receptor (suPAR) and high-sensitive C-reactive protein (hs-CRP) are related to these complications. We aimed to investigate if cardiac and proinflammatory markers are related to severity of liver disease, cardiac and haemodynamic changes, and long-term survival. *Methods:* One hundred and ninety-three stable cirrhotic patients (Child class: A = 46; B = 97; C = 50) had a full haemodynamic investigation performed with measurement of splanchnic and systemic haemodynamics and measurement of circulating levels of proBNP, proANP, copeptin, hs-TnT, LBP, IL 6, IL 8, IP 10, VEGF, hs-CRP and suPAR. *Results:* Soluble urokinase-type plasminogen activator receptor soluble urokinase-type plasminogen activator receptor, hs-CRP, and hs-TnT were significantly different throughout the Child classes ($P < 0.01$; $P < 0.01$; $P < 0.02$). All markers except copeptin correlated with indicators of disease severity in cirrhosis; ProANP and suPAR correlated with hepatic venous pressure gradient ($r = 0.24$ and $r = 0.34$; $P < 0.001$) and systemic vascular resistance ($r = -0.24$ and $r = -0.33$; $P < 0.001$). Cardiac (proANP, hs-TnT; $P < 0.01$) and proinflammatory (hs-CRP, suPAR; $P < 0.05$) markers were associated with mortality in a univariate Cox analysis, however, the strongest predictors of mortality in a multivariate Cox analysis were hs-TnT, ascites and hepatic venous pressure gradient (reg.coeff.: 0.34, $P < 0.001$; 0.16, $P < 0.001$; 0.06, $P = 0.04$). *Conclusion:* Markers of cardiac dysfunction and inflammation are significantly associated with disease severity, degree of portal hypertension and survival in cirrhosis. In particular, hs-TnT and suPAR seem to contain prognostic information.

Patients with advanced cirrhosis have a generalized circulatory dysfunction characterized by portal hypertension and a hyperdynamic circulation causing development of severe complications, such as ascites, esophageal varices, renal impairment and hepatic encephalopathy (1–3). Appearance of these complications is

associated with increased mortality (2). It has been hypothesized that inflammation and cardiac dysfunction may augment these complications and thereby deteriorate the prognosis (4–6).

Cardiac dysfunction has been shown to precede the development of renal failure and the poor outcome in advanced cirrhosis (2, 7). Circulating concentrations of natriuretic peptides brain natriuretic peptide (BNP) and prohormone of BNP (proBNP) that reflect cardiac dysfunction have been related to the degree of circulatory dysfunction and disease severity in cirrhosis (8, 9). Other novel cardiovascular markers such as prohormone of ANP (proANP), high-sensitivity troponin T (hs-TnT) and copeptin (provasopressin) are related to a poor outcome in patients with decompensated congestive heart failure.

In addition, inflammatory activation has been shown to aggravate circulatory failure, to impair the immune system thereby facilitating infections, and ultimately to worsen the prognosis (10). Elevated plasma levels of markers of immune activity such as interleukin-6 (IL-6), interleukin-8 (IL-8) and lipopolysaccahride binding protein (LBP) indicate an activated state of inflammation. Novel proinflammatory markers such as interferon-gamma induced protein 10 (IP10), soluble urokinase-type plasminogen activator receptor (suPAR), vascular endothelial growth factor (VEGF) and high-sensitive C-reactive protein (hs-CRP) have also been found increased in patients with cirrhosis reflecting low-grade inflammation, therefore these markers may contain prognostic information (11–13).

Established models such as Child-Turcotte score and model of end-stage liver disease (MELD) does not take the circulatory and inflammatory condition into consideration (6). Addition of these aspects may therefore improve the risk assessment.

The aim of the present explorative study was to investigate whether novel proinflammatory and cardiac markers are related to severity of liver disease, cardiac function, haemodynamic changes and long-term survival in stable cirrhotic patients.

Methods

Demographics

In the period 2002–2007, 250 consecutive patients with cirrhosis were referred from the outpatient clinic, Hvidovre Hospital, for liver vein catheterization to determine the presence of portal hypertension. The study comprised of the 193 patients (63 female and 130 male) in whom an informed consent for study enrollment was obtained. The diagnosis of cirrhosis was either verified by biopsy or based on established clinical, biochemical and ultrasonographic criteria. Median age was 55 years (interquartile range, IQR 50;62). 166 patients had a history of alcohol abuse (that is, a consumption exceeding 50 g/day for more than 5 years). They had all abstained from alcohol for at least 1 week before the haemodynamic investigations and had no signs of withdrawal symptoms at the time of the study. Twenty patients had non-alcoholic cirrhosis, classified either as post-hepatitic, cryptogenic, autoimmune, or because of rare conditions (haemocromatosis, Budd Chiari, alfa-one-antitrypsine deficiency). One patient had combined aetiology. The proportion of alcoholic liver disease reflects the prevalence in the Danish population of patients with cirrhosis (14). According to the modified Child-Turcotte classification, 46 patients were in class A, 97 in class B and 50 in class C. Ninety patients had ascites. Ninety-five patients were receiving diuretics and had been put on a sodium-restricted diet, 36 patients received beta-blocker treatment. Diuretics and beta-blockers were withdrawn 24 h prior to the investigation. None of the patients had hepatic encephalopathy above grade I, alcoholic hepatitis, received antibiotics or had experienced gastrointestinal bleeding within the last week. In addition, all patients were without clinical signs of either infection or cardiac decompensation and their ECGs were without evidence of ischaemia at the time of inclusion. Survival analyses were performed with December 31st 2011 as the cut-off date, giving a mean follow-up of 4.3 years. Causes of death were obtained from the electronic patient files. Patients transplanted during follow-up were censored from the analyses at the time of transplantation.

Liver vein catheterization

All patients underwent a haemodynamic investigation in the morning after an overnight fast and after at least one hour of resting in supine position. The routine biochemical blood samples were determined within one week prior to the haemodynamic investigation. Catheterization of hepatic veins and femoral artery were performed as described elsewhere (15, 16). The mean arterial pressure (MAP) was determined by electronic integration of the pressure signal. Right atrial pressure (RAP) was determined as the mean pressure over a period of 15 s. The hepatic venous pressure gradient (HVPG) was determined as wedged (WHVP) minus free hepatic venous pressure (FHVP). Hepatic blood flow (HBF) was determined by the indocyanine green (ICG) constant infusion technique. ICG clearance was measured as the infusion rate divided by the arterial plasma concentration of ICG. The post-sinusoidal resistance was determined as HVPG/HBF (15). Cardiac output (CO) was measured by the indicator dilution technique after a bolus injection of ^{125}I-labelled human serum albumin into the right atrium, followed by arterial sampling. The systemic vascular resistance (SVR) expressed in dynes·s/cm^5 was assessed as $80 \times$ (MAP-RAP/CO) pressures expressed in mmHg and CO in liters per minute. Heart rate (HR) was determined by electrocardiography. The central circulation time (CCT) was determined independently of CO by a

quantitative injection of ^{99m}Tc-labelled human serum albumin as previously described (16). The central blood volume (CBV) was assessed as CO determined by ^{125}I-labelled human serum albumin multiplied by CCT determined by ^{99m}Tc-labelled human serum albumin (15). Thus, the variables for the determination of the CBV were independently assessed. The plasma volume (PV) was calculated as the injected amount of ^{125}I-labelled serum albumin divided by the plasma concentration of radioactivity 10 min after injection. The total blood volume (BV) was determined from PV and haematocrit with correction for plasma trapping as PV/(1 − 0.89 × haematocrit). The non-central blood volume (non-CBV) was calculated as the difference between BV and the CBV. Arterial compliance was calculated as stroke volume (SV) relative to pulse pressure, where SV = CO/HR and pulse pressure = systolic BP − diastolic BP. Blood samples for analyses of proinflammatory and cardiovascular markers were obtained from a cubital vein during the catheterization.

Proinflammatory markers

Plasma from one 9 ml EDTA-coated tube was separated by centrifugation and stored at −80°C until measurements were performed. All proinflammatory markers were measured in duplicates. A few samples were only measured once because of lack of sample volume. Plasma levels of the cytokines IL-6, IL-8, VEGF and IP-10 were determined by the coloured bead multiplex technology (Luminex Corporation, Austin, TX, USA). hs-CRP and suPAR were measured by enzyme-linked immunosorbent assays (ELISA) according to the manufacturer's instructions (DRG Instruments GmbH, Marburg, Germany; Quantikine, R & D Systems, MN, USA; suPARnostic, Virogates, Denmark). Measurements were fitted with linear regression analysis in Graph pad prisme programme version 5.0. When the sample concentration was above the effective linear range according to the manufacturer′s instruction the sample was run in a higher dilution. All proinflammatory marker concentrations are presented after correcting for dilution and after the background was subtracted, based on standard curves. Only measurements with CV ≤20% and ≥50 beads counted/well were included in the analysis. If the concentration of one duplicative measurement was < lower level of quantification (LLQ), and the other ≥, the concentration of the measurement < LLQ was set to the LLQ for the said biomarker in the assay. Interassay imprecision was ≤6% for suPAR, ≤10% for hs-CRP and IP10 and ≤15% for VEGF, LBP, IL-6 and IL-8, respectively.

Cardiovascular markers

High-sensitivity troponin T and proBNP were measured on an automated Modular E platform (Roche, Mannheim, Germany). LLQ was 13 ng/L and 5.9 pmol/L, respectively. Intra-assay imprecisions of hs-TnT were <7.8% for concentrations <6.5 ng/L and <1.4% for concentrations >23.4 ng/L. Intra-assay imprecisions of proBNP were <1.9 % for concentrations <7.55 pmol/L and <1.3% for concentrations >1669 pmol/L. High-sensitivity copeptin and mid-regional proANP were measured on a Kryptor Plus platform (ThermoFisher, Waltham, MA, USA). LLQ was 1.9 pmol/L and 4.5 pmol/L, respectively. Inter-assay imprecisions for copeptin were <17% for concentrations between 3–4 pmol/L and <5 % for concentrations >15 pmol/L. Interassay imprecisions for proANP concentrations between 20–1000 pmol/L were <6.5%.We have previously reported on assay performance and characteristics [for hs-TnT and proBNP (17, 18); for copeptin and proANP (19, 20)].

Ethics

Patients participated after giving their informed consent in accordance with the Helsinki II Declaration and the study was approved by the local Ethics Committee for Medical Research in Copenhagen and Danish Data Protection Agency (J-No.2008-41-2020).

Statistical analysis

Data were summarized as median and IQR. One-way analysis of variance (ANOVA) was used to compare groups. In cases of non-normality, the non-parametric Kruskal–Wallis ANOVA was applied. Correlations were estimated using Spearman's rank correlation coefficient. Univariate Cox regression analysis was performed to evaluate the association between each new biomarker and long-term survival. Afterwards a multiple Cox regression analysis was performed to evaluate the association of the new biomarkers *and* well-known clinical, biochemical and haemodynamic variables with long-term survival using the backward elimination method. The Kaplan-Meier plot was used to illustrate survival with the patients divided into three strata of approximately equal size using round cut-off values of the variable in question and probability curves were compared by the log-rank test. The two-tailed significance level of type 1 error was fixed at 0.05.

Results

Clinical, haemodynamic and biochemical data of the patients

Clinical, biochemical and haemodynamic variables included in the study are shown in Table 1. Patients showed signs of portal hypertension and a hyperdynamic circulation with an increased median HVPG of 16 mmHg, a decreased median SVR of 1058 dyn·s/cm^5, a decreased median CCT of 11.3 s and a resting median CO high within normal range 6.9 L/min. Circulating

Table 1. Clinical, biochemical and haemodynamic characteristics of 193 patients with cirrhosis

		n
Clinical		
Age (years)	55 (50; 62)	193
Gender (male/female)	130/63	193
Aetiology (alcohol/viral hepatitis/other*)	167/5/15	187
Ascites (+/−)	90/96	186
Esophageal varices (+/−)	121/57	178
Child class (A/B/C)	46/97/50	193
MELD score	11 (8, 14)	175
Biochemical		
S-creatinine (μmol/L; <120)	74 (62; 89)	183
S-sodium (mmol/L; 136–146)	139 (136; 141)	184
S-alanine aminotransferase (U/L; 10–40)	37 (27; 53)	178
S-bilirubin (μmol/L; 2–17)	19 (11; 32)	178
S-albumin (μmol/L; 540–800)	486 (416; 555)	179
P-coagulation factors 2,7,10 (units; 0.70–1.30)	0.56 (0.46; 0.70)	189
Splanchnic hemodynamics		
HVPG (mmHg; <5)	16.0 (11.8; 19,3)	193
HBF (L/min; 0.5–2.3)	1.1 (0.84; 1.58)	157
ICG clearance (ml/min; 300–700)	188 (126; 342)	169
Galactose elimination capacity (mmol/min; F > 1.4, M > 1.7)	1.5 (1.23; 1.73)	169
Systemic hemodynamics		
Arterial compliance (ml/mmHg)	1.34 (1.01; 1.70)	190
SVR (dyn s/cm^5; 1600–2300)	1058 (800; 1343)	190
MAP (mmHg)	93 (84; 103)	193
HR (/min)	75 (65; 84)	193
SV (ml)	94 (74; 109)	190
CO (L/min)	6.9 (5.6; 8.3)	190
RAP (mmHg; <5)	5.0 (4.0; 7.0)	192
CBV (L)	1.3 (1.1; 1.6)	189
Plasma volume (L)	3.8 (3.2; 4.4)	191
CCT (s; 14–28)	11.3 (9.8; 13.7)	174

*Autoimmune, Budd–Chiari, Haemocromatosis, NASH, Alfa-1-antrypsin deficiency.

Data are listed as number or median (inter-quartile range). CBV, central blood volume; CCT, central circulation time; CO, cardiac output; HBF, hepatic blood flow; HR, heart rate; HVPG, hepatic venous pressure gradient; ICG, indocyanine green, MAP, mean arterial pressure; MELD, model of end-stage liver disease; *n*, number of patients with cirrhosis; RAP, right atrial pressure; SV, stroke volume; SVR, systemic vascular resistance.

concentrations of cardiac and proinflammatory markers are shown in Table 2 according to the Child classification and presence of ascites. The circulatory levels of suPAR and hs-CRP were significantly higher in Child class C patients compared with class A and B patients ($P < 0.01$). Plasma hs-TnT was significantly higher in Child class C compared with class A patients ($P < 0.02$). A tendency towards increasing median levels of proANP with increasing Child classes was seen, but the difference did not reach statistical significance. Patients with ascites had significantly higher levels of proBNP, proANP, copeptin, suPAR and IL-8 ($P < 0.03$).

A low CO (below the median 6.9 L/min) was associated with significantly higher proBNP and copeptin levels, 14.9 vs. 11.4 pmol/L ($P < 0.03$) and 8.9 vs. 5.7 pmol/L ($P < 0.01$) respectively. Whereas, suPAR levels were significantly higher in patients with a high CO (above 6.9 L/min), 11.1 vs. 8.1 ng/ml ($P < 0.01$). Beta-blocker treatment was associated with significantly lower suPAR levels 7.8 vs. 11.4 ng/ml ($P < 0.01$). The distribution of all other markers did not show any significant differences according to neither the CO level nor treatment with beta-blockers.

Cardiac and proinflammatory markers and bivariate correlations

Bivariate correlation analyses were performed for all markers. Significant correlations are shown in Table 3. Circulating levels of suPAR were significantly related to the Child-Turcotte classification ($r = 0.50$, $P < 0.0001$), HVPG ($r = 0.34$, $P < 0.0001$), and inversely related to indicators of the hyperdynamic circulation including SVR ($r = -0.33$, $P < 0.0001$) and MAP ($r = -0.29$, $P < 0.0001$). In addition, circulating levels of proANP were significantly related to the Child-Turcotte classification ($r = 0.32$, $P < 0.0001$) but showed weaker correlations to HVPG, SVR and MAP ($r = 0.24$, $r = -0.24$, $r = -0.28$, $P < 0.001$). ProANP was related to renal function (serum creatinine, $r = 0.24$, $P < 0.001$). Similarly hs-TnT levels were significantly related to Child classification ($r = 0.25$, $P < 0.001$) and renal function (serum creatinine, $r = 0.31$, $P < 0.0001$), although the first correlation was weaker. Furthermore, circulating levels of both proANP, suPAR, and hs-TnT were associated with disease severity by significant relations to the MELD score ($r = 0.42$, $r = 0.54$, $P < 0.0001$ and $r = 0.26$, $P = 0.001$). ProANP and hs-TnT, but not suPAR were significantly related to age ($r = 0.22$, $P = 0.002$, $r = 0.20$, $P = 0.01$ and $r = 0.09$, $P = 0.23$ respectively). Among the cardiac and proinflammatory markers several significant correlations were seen. SuPAR was related to both hs-TnT and proANP ($r = 0.28$, $P < 0.0001$ and $r = 0.34$, $P = 0.0002$ respectively). ProANP and hs-TnT related to each other ($r = 0.45$, $P < 0.0001$). All three markers were related to proBNP ($r = 0.53$, $r = 0.32$, $r = 0.27$, $P < 0.001$, proANP, hs-TnT and suPAR respectively).

Cardiac and proinflammatory markers and survival

At the end of the follow-up period 132 patients had died and 1 patient had received a liver transplantation. Table 4 summarises the causes of death. Cirrhosis and related complications were responsible for 69 (53%) of the cases.The patient, who was transplanted, died because of kidney failure after an arterial thrombosis, another patient died in multiorgan failure after a third degree burn and a third patient of a large saddle embolus. 25 patients died outside hospital, hence the causes

Table 2. Plasma levels of cardiac and proinflammatory markers in patients with cirrhosis

	Child-class					
	A (n = 46)	B (n = 97)	C (n = 50)	Ascites (n = 90)	No ascites (n = 96)	Normal range
Cardiac						
ProBNP (pmol/L)	12.0 (5.9; 22.0)	13.0 (6.2; 19.5)	15.3 (9.0; 32.4)	14.5 (8.2; 33.0)#	11.3 (5.9; 19.7)	<6
ProANP (pmol/L)	513.0 (366.0; 666.0)	660.0 (495.0; 882.0)	753.0 (570.0; 1059.0)	702.0 (514.5; 949.5)#	591.0 (456.0; 807.0)	None
Copeptin (pmol/L)	4.7 (3.2; 11.9)	7.1 (3.9; 12.6)	7.5 (4.4; 16.8)	8.1 (4.7; 16.0)#	5.5 (3.4; 11.5)	None
hs-TnT (ng/L)	3.0 (3.0; 4.7)	3.0 (3.0; 8.1)	5.3 (3.0; 15.8)*	5.2 (3.0; 9.9)	3.0 (3.0; 6.2)	<14
Proinflammatory						
IL 6 (pg/ml)	7.3 (2.1; 56.2)	7.6 (2.7; 27.9)	10.4 (2.7; 22.0)	9.4 (2.7; 21.2)	8.0 (2.1; 41.1)	1–2
IL 8 (pg/ml)	34.7 (14.0; 74.4)	58.2 (28.2; 105.9)	93.6 (44.0; 178.0)	70.1 (32.2; 144.6)#	47.9 (16.0; 93.4)	0.6–7.5
LBP (mikrog/ml)	49.5 (42.4; 55.7)	45.9 (33.8; 52.9)	42.4 (31.6; 58.8)	46.3 (36.1; 56.5)	47.6 (33.5; 54.1)	5–15
SuPAR (ng/ml)	5.5 (4.7; 9.0)	9.9 (7.6; 12.0)*	13.6 (9.1; 17.1)**	10.8 (7.7; 15.5)#	8.3 (5.1; 11.3)	0.1–4.0
hs-CRP (µg/ml)	2.5 (1.4; 4.7)	3.5 (1.7; 7.7)	8.9 (3.5; 29.7)**	4.3 (2.0; 11.2)	3.3 (1.4; 6.9)	≤1
IP 10 (pg/ml)	752.1 (392.0; 1027.3)	929.4 (568.4; 1473.7)	1219.2 (722.3; 1609.3)	1064.0 (574.0; 1480.9)	885.8 (531.0; 1431.4)	30–50
VEGF (pg/ml)	270.7 (99.5; 733.2)	205.2 (81.0; 534.5)	358.6 (97.4; 569.0)	231.2 (81.5; 531.3)	228.4 (86.0; 592.9)	10–147

Significant differences: * from class A ($P < 0.02$), ** from class A and B ($P < 0.01$), # from patients without ascites ($P < 0.03$).
Data are listed as median (inter-quartile range). hs-CRP, high-sensitive CRP; hs-TnT, high-sensitivity troponin T; IL 6, interleukin-6; IL-8, interleukin-8; IP 10, interferon-gamma induced protein 10; LBP, lipopolysaccharide binding protein; ProANP, prohormone of ANP; ProBNP, prohormone of BNP; SuPAR, soluble urokinase-type plasminogen activator receptor; VEGF, vascular endothelial growth factor.

of death was not registered, and in three patients follow-up was not possible because of inability to locate their patient records both electronically and in paper form. In univariate proportional hazard Cox regression analyses, cardiac markers were significantly associated with increased mortality: ProBNP ($P < 0.04$), proANP ($P < 0.01$) and hs-TnT ($P < 0.001$). Similarly, a significant association was established to the proinflammatory markers hs-CRP ($P < 0.05$) and suPAR ($P < 0.01$). However, the prognostic significance of hs-CRP was not maintained when performing Kaplan-Meier analyses of 1-year and overall survival. Results are shown in Table 5, Figs 1 and 2 with patients at risk summarized in Appendix Tables 1 and 2. Furthermore, the risk of dying within 1 year predicted by the MELD score is increased by a factor 1.6 if hs-TnT is 4–8 ng/L and by a factor of 2.7 if hs-TnT is more than 8 ng/L, as illustrated in Fig. 3. Subsequently, the cardiac and proinflammatory markers *and* well-known clinical, biochemical and haemodynamic variables including Child and MELD scores were included in a multivariate proportional hazard Cox regression analysis in a backward elimination strategy to evaluate their association with long-term survival. Ascites, HVPG, and hs-TnT ($P < 0.001$) remained as the strongest markers independently associated with long-term survival. Results are shown in Table 6. Excluding the five patients with cardiac failure from the Cox analyses and Kaplan–Meier plots did not result in significant changes of the models.

Discussion

The main result of this explorative cross-sectional study is that markers of cardiac dysfunction and inflammation are associated with disease severity and long-term survival in patients with cirrhosis. In particular, hs-TnT proved significance as a new useful prognostic marker. Secondly, the markers were related to indicators of portal hypertension, hyperdynamic circulation, renal function, and MELD and Child scores.

Cardiac markers

This study is among the first to investigate the combined effect of proANP and hs-TnT on survival in patients with cirrhosis. Natriuretic peptides are secreted from the cardiac atria and ventricles in response to myocardial injury and hypertrophy. In patients with cardiomyopathy regardless of aetiology increased plasma concentrations of BNP and ANP are found and have been associated with poorer survival (8, 21). Recent studies have suggested a better prediction of proANP than ANP because of the following: proANP is co-secreted with ANP, undergoes less enzymatic

Table 3. Significant Spearman correlations between proANP, hs-TnT and suPAR and clinical, biochemical and hemodynamic variables

	Coefficient	*P*-value
ProANP		
Child class	0.32	<0.0001
HVPG	0.24	0.0008
MELD	0.42	<0.0001
S-Creatinine	0.24	0.0009
S-Bilirubin	0.26	<0.001
Coagulation factors 2, 7 and 10	−0.22	<0.01
SVR	−0.24	0.0009
MAP	−0.28	<0.0001
RAP	0.20	0.005
Age	0.22	0.002
ProBNP	0.53	<0.0001
hs-TnT	0.45	<0.0001
hs-TnT		
Child class	0.21	0.006
MELD	0.26	0.001
S-Creatinine	0.31	<0.0001
S-Albumin	−0.20	0.01
HR	0.21	0.004
Age	0.20	0.01
ProANP	0.45	<0.0001
ProBNP	0.32	<0.0001
SuPAR		
Child class	0.50	<0.0001
HVPG	0.34	<0.0001
MELD	0.54	<0.0001
S-Bilirubin	0.48	<0.0001
S-Albumin	−0.45	<0.0001
Coagulation factors 2, 7 and 10	−0.36	<0.0001
SVR	−0.33	<0.0001
MAP	−0.29	<0.0001
ProANP	0.34	<0.0001
ProBNP	0.27	0.0002
hs-TnT	0.28	0.0002

HR, heart rate; HVPG, hepatic venous pressure gradient; hs-TnT, high-sensitivity troponin T; MAP, mean arterial pressure; MELD, model of end-stage liver disease; ProANP, prohormone of ANP; ProBNP, prohormone of BNP; RAP, right atrial pressure; SuPAR, soluble urokinase-type plasminogen activator receptor; SVR, systemic vascular resistance.

Table 4. Causes of death in the patients

	n
Cirrhosis and related complications	69
Liver failure	12
HRS	11
Coma hepaticum	8
Variceal bleeding	9
SBP	2
HCC	5
Combination of the above*	22
Infections (sepsis/pneumonia/other)	11 (5/5/1)
Cancer (lung/gastrointestinal/other)	11 (4/3/4)
Cardiac failure	5
Bowl perforations	4
Intracerebral hemorrhage	4
Other	3
Unknown	25

*Primarily combinations of SBP and either HRS, coma or liver failure.
Data are listed as number of dead patients (*n*). HRS, hepatorenal syndrome; SBP**,** Spontaneous Bacterial Peritonitis; HCC, hepatocellular carcinoma.

Table 5. Cardiac and proinflammatory markers significantly associated with long-term survival at univariate proportional hazard Cox regression analysis

	Regression coefficient, *b*	SE, *b*	HR	*P*-value
ProBNP	0.21	0.10	1.24	0.037
ProANP	0.47	0.16	1.59	0.003
hs-TnT	0.39	0.10	1.48	0.0001
hs-CRP	0.17	0.08	1.18	0.048
SuPAR	0.43	0.16	1.53	0.007

Data were ln-transformed before analysis to approximate linearity. HR, hazard ratio, hs-CRP, high-sensitive CRP; hs-TnT, high-sensitivity troponin T; ProANP, prohormone of ANP; ProBNP, prohormone of BNP; SuPAR, soluble urokinase-type plasminogen activator receptor.

degradation, and less receptor binding than ANP; hence giving higher and more stable circulating plasma levels (22). Furthermore, proANP has been shown to be associated with progression of heart failure and increased mortality (22, 23). Several studies have investigated the role of natriuretic peptides in cirrhosis. Circulating concentrations of BNP and proBNP have been found elevated in both patients with compensated and decompensated cirrhosis, and seem to relate to the severity of cirrhosis, cardiac dysfunction and survival (8). ANP has been found increased in decompensated cirrhosis (24). However, proANP has not, to our knowledge, been evaluated with regard to haemodynamic and prognosis in cirrhosis. TnT is a filament-associated protein in the cardiac muscle and elevated plasma levels are seen in patients with myocardial ischaemia. Furthermore, elevated TnT occur in non-ischaemic conditions such as heart failure, left ventricular hypertrophy, chronic kidney disease and diabetes (25). Troponin I has previously been shown to be increased in cirrhotic patients and these patients showed signs of subclinical myocardial injury with a lower stroke volume and left ventricular mass index (26). A new troponin assay, hs-TnT, has been proposed as a better predictor than the established troponin assays with respect to development of heart failure and survival (27). In general, hs-TnT is associated with age, left ventricular hypertrophy, left ventricular systolic dysfunction, chronic kidney disease and all-cause mortality (28). hs-TnT has not previously been investigated in cirrhosis.

Our findings were that hs-TnT concentrations in class C patients were significantly higher than in class A patients, and a significant correlation was observed between hs-TnT and the MELD score indicating a

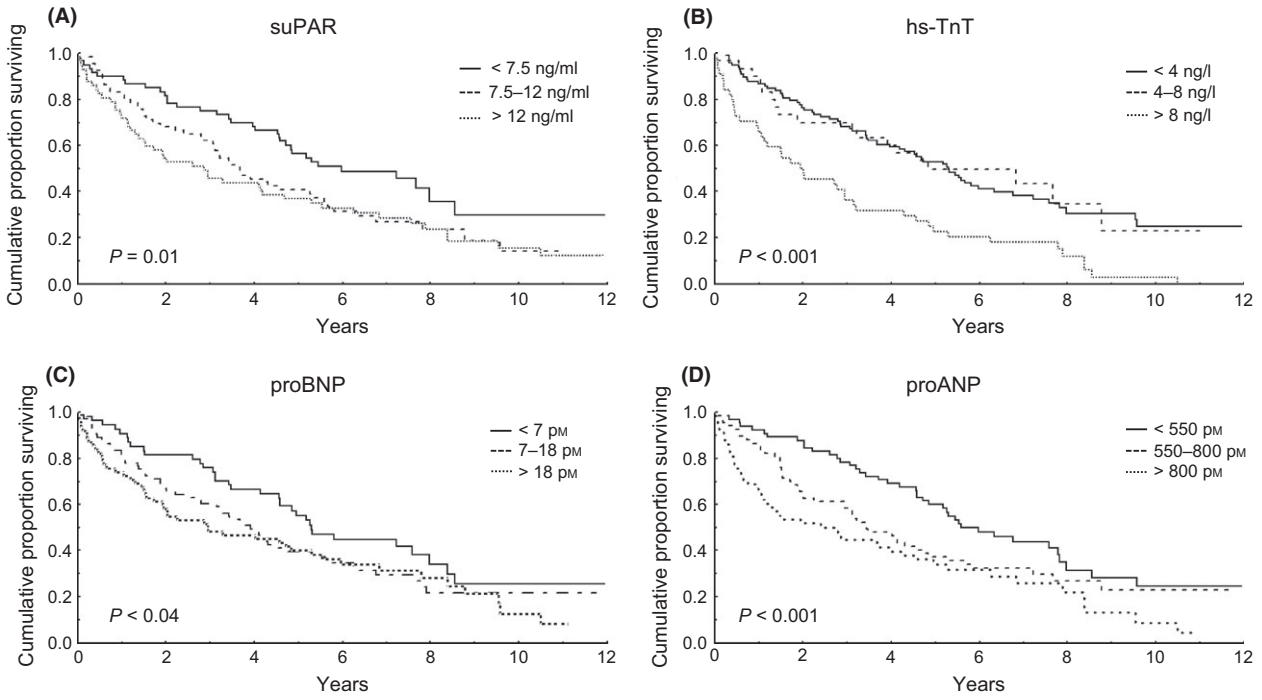

Fig. 1. Kaplan-Meier plots of long-term survival in relation proinflammatory and cardiac markers. The panels show survival in relation circulating levels of soluble urokinase-type plasminogen activator receptor (suPAR) (panel A), high-sensitivity troponin T (hs-TnT) (panel B), pro-brain natriuretic peptide (proBNP) (panel C) and pro-atrial natriuretic peptide (proANP) (panel D) with patients divided into three strata of approximately equal size using round cut-off values of the variable in question and the probability curves were compared by the log-rank test.

relation with the severity of liver disease. Furthermore, median proANP levels tended to increase throughout the Child classes, although the difference did not reach statistical significance. ProANP also related to disease severity with significant correlations to both increasing Child class and increasing MELD score. This is in keeping with earlier observations that cardiac dysfunction is more frequent and pronounced in patients with more advanced stages of the disease (24). Interestingly, proANP and hs-TnT did not significantly relate to haemodynamic markers of cardiac function (CO, CI, SV) in this study. Increasing levels of proANP, proBNP and hs-TnT were all associated with a poor long-term survival in the Kaplan-Meier plot and log rank analyses and in the univariate analysis, however only hs-TnT proved independent prognostic significance. These associations were constant even after excluding the five patients with cardiac failure from the analyses. Interestingly, copeptin levels were significantly higher in patients with a low CO. This biomarker has previously been associated with a poor outcome in patients with decompensated congestive heart failure. We and others have previously shown a relation between reduced cardiac output and development of refractory ascites and renal failure in cirrhosis, and this study indicates that copeptin may have a potential as a marker of development of cardiac dysfunction in these patients (7, 29). Even though, proBNP in our study seems a less strong prognostic marker, than proANP and hs-TnT, the significant correlations between proANP, hs-TnT and suPAR on one hand and proBNP on the other do suggest that proBNP still contain prognostic information. A recent study reported troponin I to correlate closely with BNP in patients with non-ischaemic heart failure indicating an association with left ventricular myocardial strain (30). Also a strong correlation between proBNP and proANP has been documented in patients with chronic heart failure indicating a combined atrial and ventricular pathophysiology (22). The observed increases in plasma concentrations of the cardiac biomarkers and their relation to disease severity and prognosis in these patients could merely be thought to represent alcoholic heart muscle disease, which can be difficult to separate from other types of cardiac dysfunction in cirrhosis including cirrhotic cardiomyopathy, because of the high prevalence of alcoholic aetiology in the study population. However, recent studies have substantiated that cardiac dysfunction is present in cirrhotic patients irrespective of aetiology indicating that cardiomyopathy relates to the cirrhotic process *per se* (9, 31, 32). The correlation between proANP and hs-TnT and elevated serum creatinine could be interpreted as an association between cardiac dysfunction and renal impairment as observed by Krag *et al.* (7). However, hs-TnT is known to be associated with

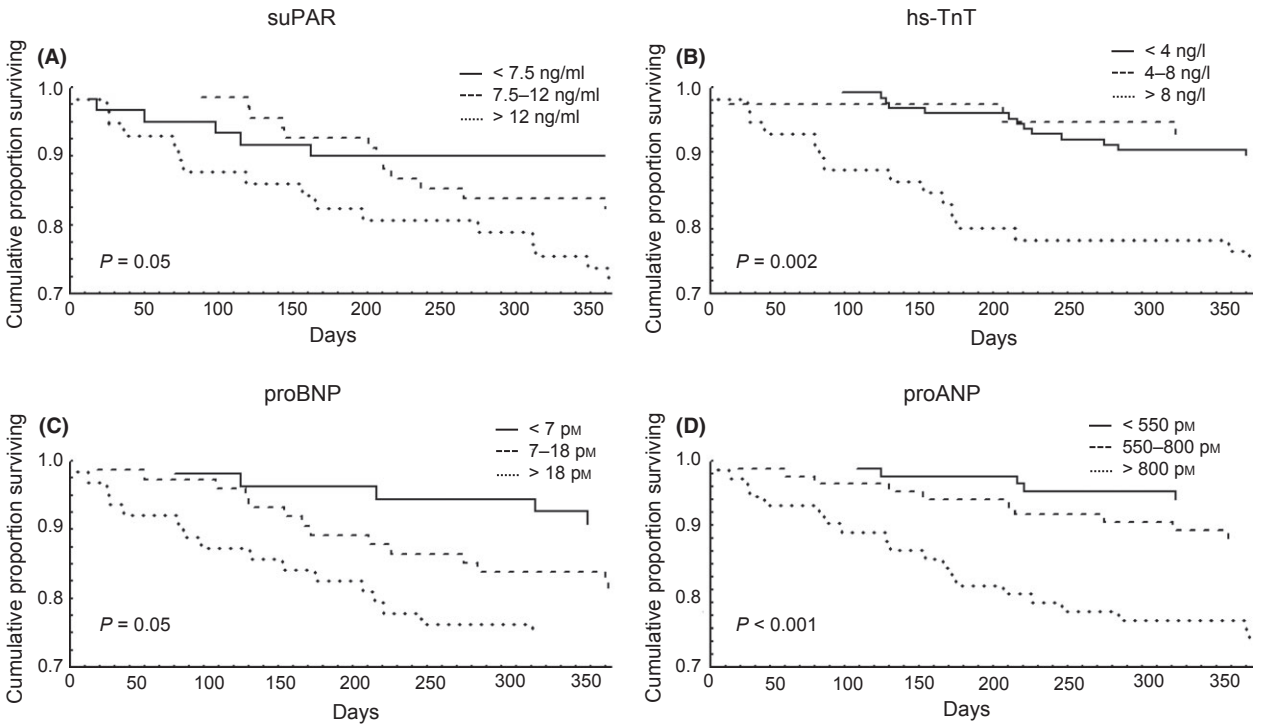

Fig. 2. Kaplan–Meier plots of 1-year survival in relation proinflammatory and cardiac markers. The panels show survival in relation circulating levels of soluble urokinase-type plasminogen activator receptor (suPAR) (panel A), high-sensitivity troponin T (hs-TnT) (panel B), pro-brain natriuretic peptide (proBNP) (panel C) and pro-atrial natriuretic peptide (proANP) (panel D) with patients divided into three strata of approximately equal size using round cut-off values of the variable in question and probability curves were compared by the log-rank test.

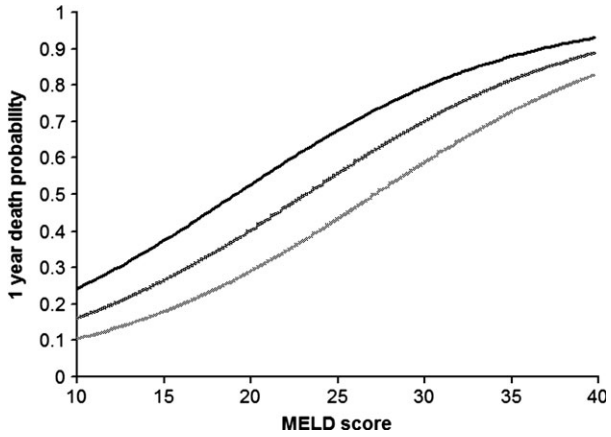

Fig. 3. Estimated 1 year probability of dying as a function of :(i) MELD score alone (lower curve) (ii) MELD score and hs-TnT of 4–8 ng/L (middle curve) and 3) MELD score and hs-TnT >8 ng/L (upper curve). The estimation was done as described in ref.(35) using the following logistic regression equation: Logit = −3.385 + 0.125 × MELD-score + 0.499 × hs-TnT score (0 for values <4 ng/L, 1 for values of 4–8 ng/L and 2 for values >8 ng/L).

chronic kidney disease. The mechanism remains unclear but could be a combination of cardiac abnormalities and impaired renal excretion of the marker (33).

Table 6. Variables independently associated with long-term survival at multivariate proportional hazard Cox regression analysis*

	Regression coefficient, *b*	SE, *b*	HR	*P*-value
Ascites	0.16	0.08	1.17	0.04
HVPG	0.06	0.02	1.06	<0.001
hs-TnT	0.34	0.10	1.41	<0.001

*Multivariate analysis included well-known clinical, biochemical and haemodynamic variables in patients with cirrhosis (ascites, MELD, Child score, bilirubin, albumin, creatinine, INR, sodium, HVPG, RAP, CO, SVR, MAP, GEC, ICG,CTT,CBV) plus all cardiac and proinflammatory markers.

hs-TnT was ln-transformed before analysis to approximate linearity. HR, hazard ratio; HVPG, hepatic venous pressure gradient; hs-TnT, high-sensitivity troponin T.

Inflammatory markers

Bacterial translocation and low grade inflammation (LGI) with increased levels of cytokines seem to play an important role in the course of cirrhosis by contributing to complications such as hepatorenal syndrome, variceal bleeding, hepatic encephalopathy and increasing susceptibility to infections; all accounting for significant mortality (4, 5). SuPAR is a highly sensitive marker of inflammatory processes because of the following: Excre-

tion from activated leucocytes and macrophages in response to inflammation, exertion of chemotactic properties and involvement in numerous immunological functions (34). Hence, suPAR is believed to be one of the most potential markers of LGI. In the general population elevated levels of suPAR have been associated with age, cancer, cardiovascular disease, diabetes, and increased all-cause mortality (34). However, knowledge concerning its role as prognostic marker in cirrhosis is limited.

In our study, we found increasing levels of suPAR in cirrhotic patients with the highest levels in patients with decompensated cirrhosis. Furthermore, we established significant correlations between suPAR and markers of deteriorated liver function including the MELD score. Similar findings were done by Zimmerman *et al.* (13). Moreover suPAR was also related to increased HVPG, decreased SVR and increased CO, indicating an association to the hyperdynamic circulation. To our knowledge, this is the first study to report a connection between suPAR and the splanchnic and systemic haemodynamic changes in cirrhosis. In addition, we demonstrated that suPAR was closely related to all markers of cardiac dysfunction, and therefore suggests a link between LGI and the cardiac changes seen in cirrhosis. Surprisingly, we found no significant relation between suPAR and age in our study. Zimmerman *et al.* have suggested the prognostic use of suPAR as an independent marker of poor outcome in cirrhosis. In a univariate Cox regression analysis, we found suPAR to be independently related to mortality. However, after performing a multivariate Cox regression analysis this relation did not remain significant, indicating that other variables, such as hs-TnT contain a higher level of prognostic information. Prior studies have documented that suPAR correlates with increasing hs-CRP (34). Recent results from our group suggest hs-CRP as a strong prognostic proinflammatory indicator in cirrhosis (12). Interestingly, in the current study, hs-CRP was inferior to suPAR both in terms of predicting a poor outcome and by weaker correlations to haemodynamic variables.

Limitations

The study population primarily consists of patients with alcoholic cirrhosis wherefore it may be difficult to extrapolate the results to populations with a different combination of aetiologies. Moreover, the lack of an age-matched control group does not enable us to adjust for the well-established relation between age and plasma levels of pro-ANP, hs-TnT and suPAR. However, the distribution of alcoholic aetiology and age was the same throughout the Child classes, therefore this cannot explain the increasing plasma levels seen in this study. Finally, a control group in the present study would not have affected the essential results of our study. Beta-blockers were only withdrawn 24 h prior to the investigations because of safety reasons, thus in the 36 patients treated with beta-blockers, there could be a potential beta-blocker interference left, which could affect our results. However, only suPAR levels were significantly different in these patients, whereas all other biomarkers were unaffected of treatment with a beta-blocker. We cannot exclude, that some of the analysed markers reflect identical aspects of inflammatory and circulatory dysfunction in cirrhosis, and thereby may be subject to co-variation, However, the multivariate analyses largely work to eliminate presence of co-variation.

In conclusion, the current study shows that markers of inflammation and cardiac dysfunction are significantly associated with the haemodynamic derangement and long-term survival in cirrhosis. In particular hs-TnT and suPAR seem to contain prognostic information pointing to the importance of inflammatory and cardiac disturbances for the course of the disease. Therefore, future longitudinal studies are warranted to assess the temporal relationship between the development of complications to cirrhosis and cardiac and immune dysfunction.

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Appendix

Table A1. Number of patients entering and dying in each yearly interval of the observation period for overall survival and hs-CRP, hs-TnT, suPAR, proBNP and proANP

	Interval	Year 0–1	Year 1–2	Year 2–3	Year 3–4	Year 4–5	Year 5–6	Year 6–7	Year 7–8	Year 8–9	Year 9–10	Year 10–11	Year 11–12	Year 12–13
hs-CRP														
<0.2 μg/ml	Number entering	44	38	31	29	23	16	12	6	4	1	1		
	Number dying	6	7	2	6	5	3	1	0	2	0	0		
0.2–0.7 μg/ml	Number entering	51	39	34	29	25	23	16	14	6	5	4		
	Number dying	12	5	5	4	1	3	0	1	0	1	1		
>0.7 μg/ml	Number entering	43	30	25	24	21	14	12	7	3	2	1		
	Number dying	13	5	1	3	6	1	3	2	1	1	0		
hs-TnT														
<4 ng/L	Number entering	98	85	75	67	58	47	33	24	13	12	8	3	1
	Number dying	13	10	8	9	6	10	2	4	0	2	0	0	0
4–8 ng/L	Number entering	30	27	21	21	18	12	9	7	4	2	2	1	
	Number dying	3	6	0	3	3	0	1	1	1	0	0	0	
>8 ng/L	Number entering	44	29	22	16	14	10	9	8	4	1	1		
	Number dying	15	7	6	2	4	1	1	2	3	0	1		
suPAR														
<7.5 ng/ml	Number entering	60	54	49	45	41	31	22	17	6	5	4	2	1
	Number dying	6	5	4	4	7	4	0	3	1	0	0	0	0
7.5–12 ng/ml	Number entering	66	54	45	41	30	22	16	10	7	4	3		
	Number dying	12	9	4	11	3	5	2	1	1	1	0		
>12 ng/ml	Number entering	57	41	31	26	25	20	16	13	9	7	5	2	
	Number dying	16	10	5	1	4	2	2	2	2	1	1	0	
proBNP														
<7 pmol/L	Number entering	54	49	44	41	36	27	20	15	8	6	5	3	1
	Number dying	5	5	3	5	6	5	0	3	2	0	0	0	0
7–18 pmol/L	Number entering	73	59	49	44	35	26	21	15	7	5	5	1	
	Number dying	14	10	5	9	6	3	3	3	0	0	0	0	
>18 pmol/L	Number entering	62	46	36	30	29	22	15	12	8	6	3	1	
	Number dying	16	10	6	1	4	3	1	1	2	2	1	0	

Table A1. (continued)

	Interval	Year 0–1	Year 1–2	Year 2–3	Year 3–4	Year 4–5	Year 5–6	Year 6–7	Year 7–8	Year 8–9	Year 9–10	Year 10–11	Year 11–12	Year 12–13
proANP														
<7 pmol/L	Number entering	65	60	57	51	45	37	25	19	9	8	7	3	1
	Number dying	5	3	6	6	6	7	2	4	1	1	0	0	0
7–18 pmol/L	Number entering	67	57	43	39	32	23	20	14	9	6	4	2	
	Number dying	10	14	4	7	7	3	0	2	1	0	0	0	
>18 pmol/L	Number entering	58	38	30	26	23	15	11	9	5	3	2		
	Number dying	20	8	4	3	3	1	2	1	2	1	1		

hs-CRP, high-sensitive CRP; hs-TnT, high-sensitivity troponin T; proANP, prohormone of ANP; proBNP, prohormone of BNP; suPAR, soluble urokinase-type plasminogen activator receptor.

Table A2. Number of patients entering and dying in each 3 monthly interval in the first year of the observation period for hs-CRP, hs-TnT, suPAR, proBNP and proANP

	Interval	Months 0–3	Months 4–6	Months 7–9	Months 10–12
hs-CRP					
<0.2 μg/ml	Number Entering	44	40	38	38
	Number Dying	4	2	0	0
0.2–0.7 μg/ml	Number Entering	51	48	43	40
	Number Dying	3	5	3	1
>0.7 μg/ml	Number Entering	43	39	37	34
	Number Dying	4	2	3	4
hs-TnT					
<4 ng/L	Number Entering	98	97	93	87
	Number Dying	1	4	6	2
4–8 ng/L	Number Entering	30	29	29	28
	Number Dying	1	0	1	1
>8 ng/L	Number Entering	44	37	32	31
	Number Dying	7	5	1	2
suPAR					
<7.5 ng/ml	Number Entering	60	56	54	54
	Number Dying	4	2	0	0
7.5–12 ng/ml	Number Entering	66	65	61	55
	Number Dying	1	4	6	1
>12 ng/mL	Number Entering	57	50	47	46
	Number Dying	7	3	1	5
proBNP					
<7 pmol/L	Number Entering	54	53	52	51
	Number Dying	1	1	1	2
7–18 pmol/L	Number Entering	73	71	65	62
	Number Dying	2	6	3	3
>18 pmol/L	Number Entering	62	54	51	47
	Number Dying	8	3	4	1
proANP					
<7 pmol/L	Number Entering	65	65	63	61
	Number Dying	0	2	2	1
7–18 pmol/L	Number Entering	67	64	62	59
	Number Dying	3	2	3	2
>18 pmol/L	Number Entering	58	50	44	41
	Number Dying	8	6	3	3

hs-CRP, high-sensitive CRP; hs-TnT, high-sensitivity troponin T; proANP, prohormone of ANP; proBNP, prohormone of BNP; suPAR, soluble urokinase-type plasminogen activator receptor.