ORIGINAL ARTICLE

Fetuin-A and sodium concentrations are independently associated with all-cause mortality in patients awaiting heart transplantation

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KEY WORDS

coronary sinus blood, heart failure, fetuin-A, prognosis, sodium

ABSTRACT

INTRODUCTION End-stage heart failure (HF) is a clinical condition with complex pathophysiology and poor prognosis.

OBJECTIVES This study aimed to identify factors associated with mortality during a 1.5-year follow-up in patients with end-stage HF.

PATIENTS AND METHODS We prospectively analyzed 72 patients hospitalized with end-stage HF. During right heart catheterization, 10 ml of coronary sinus (CS) blood was collected. The endpoint was all-cause mortality during a 1.5-year follow-up. We used a multivariable logistic regression model to find factors associated with all-cause mortality. We created 2 separate models for CS fetuin and peripheral blood (PB) fetuin.

RESULTS The median (interquartile range) age of the patients was 58 (50–61.50) years. During the follow-up, 43.1% of the patients died. Lower levels of fetuin-A in the CS (OR, 1.103; 95% CI, 1.045–1.164; $P < 0.001$, per 10-unit decrease in fetuin concentration) and PB samples (OR, 1.098; 95% CI, 1.046–1.153; $P < 0.001$, per 10-unit decrease in fetuin concentration), along with lower plasma sodium levels (OR, 1.563; 95% CI, 1.134–2.156; $P = 0.006$ in the first model and OR, 1.639; 95% CI, 1.209–2.227; $P = 0.002$ in the second model; per 1-unit decrease in sodium concentration) were independently associated with death during the follow-up period. The area under the receiver operating characteristics curve (AUC) indicated a good prognostic power of CS and PB fetuin-A levels (AUC, 0.917 and AUC, 0.850, respectively) and an acceptable prognostic power of sodium concentration (AUC, 0.788).

CONCLUSIONS Lower levels of CS and PB fetuin-A, as well as lower sodium levels, are associated with an increased risk of death in patients with end-stage HF.

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INTRODUCTION End-stage heart failure (HF) is a clinical condition with a complex pathophysiology and poor prognosis. A variety of pathophysiological processes involved in the development and progression of the disease—such as fibrosis, inflammation, myocardial injury, and remodeling—can be monitored with biomarkers.[1,2] Over the past few years, the role of biomarkers in HF has increased significantly.[3] Cardiac biomarkers are used as a minimally invasive way to assess the condition of patients with HF. They facilitate the initial diagnosis and prognostic stratification and they play an important role in the identification of a patient's possible response to a therapeutic intervention.[2-5] However, with the exception of natriuretic peptides and troponins, the reliability

WHAT'S NEW?

In this single-center study, we found that lower coronary sinus (CS) and peripheral blood (PB) fetuin-A levels as well as PB sodium concentrations were associated with an increased risk of death from end-stage heart failure (HF). CS fetuin-A level had an excellent prognostic power, allowing for the successful prediction of survival versus nonsurvival outcomes among HF patients. Moreover, the prognostic utility of CS fetuin-A level was superior to that of PB fetuin level, while CS amino-terminal pro–B type natriuretic peptide (NT-proBNP) concentration was comparable to peripheral venous NT-proBNP concentration in this respect. Furthermore, PB fetuin-A level, with its good prognostic power, may have an adequate clinical utility in outpatients with HF. However, during protocol-consistent right heart catheterization, the collection of blood from the CS is relatively simple and safe and may provide more reliable data for assessing the risk of death in advanced HF patients.

and clinical utility of novel, emerging biomarkers in clinical practice have not been fully investigated.[2] Moreover, most of the biomarkers in HF have been assessed only in the peripheral venous blood and it is therefore unknown to what extent they reflect the processes resulting from heart damage. It seems that the levels of biomarkers measured only from peripheral circulation may not reflect their intracardiac levels.[6] Previous single studies have shown that coronary sinus biomarkers may have a better prognostic power than those from peripheral venous blood samples in predicting outcomes among some patients with HF and may provide better insight into the pathophysiology of HF.[6,7] Furthermore, coronary sinus sampling may offer enhanced sensitivity and specificity by allowing an assessment of the local intracardiac milieu and avoiding the dilution effect.[7,8] In addition, the collection of blood from the coronary sinus is relatively simple and safe during standard procedures, such as right catheterization, performed in experienced cardiology centers. However, there are no data examining the effect of biomarkers obtained from the coronary sinus on the mortality of patients with end-stage HF who are registered on a heart transplant (HT) waiting list.

It is a well-established fact that some new biomarkers could be of particular interest in HF management. In recent years, several studies have focused on the role of fetuin-A in the pathophysiology of cardiovascular diseases.[9-12] Fetuin-A (α_2-Heremans-Schmid glycoprotein) is a multifunctional glycoprotein which is viewed as an important component of various normal and pathological processes, including macrophage deactivation, insulin resistance, protease activity control, recovery from acute inflammation, and bone metabolism regulation.[9,10] Considering the properties of fetuin-A and its potential role in the pathophysiology of cardiovascular diseases, it may also become a useful marker for evaluating patients with end-stage HF.

Therefore, in this study, we sought to analyze selected factors associated with an increased risk of death in patients with end-stage HF during a 1.5-year follow-up. Furthermore, we examined the differences in the coronary sinus and peripheral venous blood levels of 2 biomarkers: the established N-terminal pro–B-type natriuretic peptide (NT-proBNP) and a new HF biomarker, fetuin-A.

PATIENTS AND METHODS

Study population and data collection This is a prospective study involving 93 patients with end-stage HF who were hospitalized in the cardiology department for right heart catheterization and who were registered on the HT waiting list between 2015 and 2016. Patients who underwent HT or mechanical circulatory support implantation during the 1.5-year follow-up (n = 21) were excluded from the study.

At the time of enrollment in the study, the baseline evaluation included a medical history, the New York Heart Association (NYHA) classification, a panel of laboratory tests on peripheral blood samples, an ergospirometric exercise test, spirometry, echocardiography, and right heart catheterization. Peripheral venous and coronary sinus blood samples were collected from the HF patients at the time of right heart catheterization.

Right heart catheterization was performed with a Swan-Ganz catheter (Edwards Lifesciences, Irvine, California, United States) inserted transcutaneously through the right internal jugular vein and advanced into the pulmonary artery. During the right heart catheterization, blood was drawn from the coronary sinus. Using the vascular sheath, a guidewire was inserted into the right atrium, followed by a JR diagnostic catheter. In the left oblique projection (30 ° to 45 °), after being rotated along the posterior atrial wall, the catheter was placed immediately above the septal leaflet of the tricuspid valve and then inserted into the coronary sinus. The correct position of the catheter was checked by fluoroscopy by administering 5 to 10 ml of contrast. After rinsing with saline and waiting for 3 minutes, a 10-ml sample of coronary sinus blood was taken and, simultaneously, peripheral venous blood was drawn from one of the upper extremity veins. The samples were immediately centrifuged and the aliquoted plasma and serum were stored in microcentrifuge tubes at –80 °C until assayed. All analyses were performed on the first freeze / thaw cycle.

The endpoint of the study was defined as all-cause mortality during a 1.5-year follow-up after inclusion in the study. Follow-up data were obtained during follow-up appointments and from telephone interviews with patients or their families at the end of the 1.5-year follow-up. No patients were lost to follow-up.

The Medical University of Silesia's local Institutional Review Board approved the study protocol and all patients gave informed consent before being included in the study (no., KNW/0022/KB1/88/15).

Laboratory measurements Complete blood count and hematological parameters of the patients were analyzed using automated blood cell counters (Sysmex XS1000i and XE2100, Sysmex Corporation, Kobe, Japan). The intra- and interassay coefficients of variation in the blood samples were 5% and 4.5%, respectively. Hepatic and renal function parameters, as well as cholesterol and albumin plasma concentrations, were measured with a COBAS Integra 800 analyzer (Roche Instrument Center AG, Rotkreuz, Switzerland). The plasma concentration of fibrinogen was measured using an STA Compact analyzer (Roche). A highly sensitive latex-based immunoassay was used to detect plasma C-reactive protein (CRP) with a Cobas Integra 70 analyzer (Roche Diagnostics, Ltd). CRP levels were determined with a typical detection limit of 0.0175 mg/dl. The plasma concentration of NT-proBNP was measured with a commercially available kit from Roche Diagnostics (Mannheim, Germany) on an Elecsys 2010 analyzer with an analytical sensitivity of less than 5 pg/ml (the upper limits were 100 pg/ml in men and 150 pg/ml in women, as suggested by the manufacturer). Human fetuin-A was measured by a sandwich enzyme-linked immunosorbent assay (ELISA) with a commercially available Human Fetuin-A ELISA kit (SunRedBio Technology Co., Ltd., Shanghai, China). This ELISA kit was designed, developed, and produced for the quantitative measurement of human fetuin-A in serum samples. The concentration of fetuin-A was expressed as mg/l. The inter- and intra-assay coefficients of variation were less than 12% and less than 10%, respectively. The minimum detectable concentration for the fetuin-A assay was 7.115 mg/l. The ELISA test was performed using a BioTek Elx50 reader (BioTek Instruments Inc., Tecan Group, Switzerland).

Statistical analysis The statistical analysis was performed using SAS software, version 9.4 (SAS Institute Inc, Cary, North Carolina, United States). Descriptive statistics were expressed as mean (SD) or median with interquartile ranges for continuous variables and as frequency and percentages for categorical variables, as appropriate. Differences between the study groups were assessed using the *t* test, the Mann-Whitney test, or the χ^2 test.

The multivariable logistic regression model was applied to study the predictive factors of the 1.5-year follow-up. The covariates were determined according to univariable results ($P \leq 0.3$) and clinical relevance. We created 2 separate models for coronary sinus fetuin and peripheral blood fetuin. Due to the relatively small sample of patients, we were limited in the number of variables that could potentially be included in the multivariable model; therefore, we proposed 2 scenarios with different sets of explanatory variables. The correlation between the explanatory variables was checked and multicollinearity was evaluated by means of the tolerance and variance inflation factor. The results are presented as odds ratios with 95% CIs. Receiver operator characteristic (ROC) curves were plotted and the Youden index was used to determine the cutoff for the parameters that were significant in the multivariable analysis and for NT-proBNP. The prognostic strength of biomarkers for predicting 1.5-year mortality was evaluated by calculating for each the area under the ROC curve (AUC), the sensitivity, the specificity, the negative predictive value, the positive predictive value, the negative likelihood ratio, the positive likelihood ratio, and the accuracy. The ROC curves were quantitatively compared using the DeLong test, while the differences between AUC values were tested using the method of Hanley and McNeil. An AUC of more than 0.7 was considered clinically relevant.[13] Kaplan–Meier curves with the log-rank test were performed to compare mortality rates in patients dichotomized according to the cutoff values from the ROC curves for biomarkers. A *P* value of less than 0.05 was considered statistically significant.

RESULTS The final study group consisted of 72 patients with end-stage HF awaiting HT. All participants were classified as NYHA functional classes III and IV (80.6% and 19.4%, respectively) and as profiles 4 to 6 according to the Interagency Registry for Mechanically Assisted Circulatory Support classification. The baseline characteristics of the study population are shown in **TABLE 1**. During the 1.5-year follow-up, 31 patients (43.1%) died.

The results of the univariable and multivariable analysis are shown in **TABLE 2**. In both models of the multivariable logistic regression analysis, fetuin-A and sodium concentrations were independently associated with an increased risk of death during the 1.5-year follow-up.

The ROC curves and Kaplan–Meier survival curves for coronary sinus and peripheral fetuin--A levels and peripheral sodium levels are shown in **FIGURE 1A–1F**. The areas under the curves of coronary sinus fetuin-A (AUC, 0.9174), peripheral fetuin A (AUC, 0.8497), and sodium concentrations (AUC, 0.7876) had good sensitivities and specificities, which allows for the prediction of mortality during a 1.5-year follow-up. The difference between the calculated AUCs for coronary sinus fetuin-A and sodium concentrations was 0.1298 ($P = 0.04$). In turn, the difference between the calculated AUCs for peripheral fetuin-A and sodium concentrations was 0.0622 ($P = 0.4$).

According to the Kaplan–Meier curves (**FIGURE 1B**), patients with lower coronary sinus fetuin-A levels (≤632.36 mg/l) had a worse 1.5-year survival rate than those with higher coronary sinus fetuin-A levels (>632.36 mg/l) (20.6% vs 89.5%; log-rank $P < 0.001$). Similarly, lower peripheral fetuin-A levels (≤584.30 mg/l) were associated with a significantly worse 1.5-year survival rate than higher peripheral fetuin-A levels (>584.30 mg/l) (28.6% vs 96.7%; log-rank $P < 0.001$) (**FIGURE 1D**). Patients with lower sodium concentrations (≤137 mmol/l) had a worse

TABLE 1 Baseline characteristics of the study population (continued on the next page)

Parameter	General population (n = 72)	Survival (n = 41)	Nonsurvival (n = 31)	*P* value
Baseline data				
Age, y	58 (50–61.5)	58 (53–61)	57 (47–63)	0.83
Male gender, n (%)	66 (91.7)	36 (87.8)	30 (96.8)	0.17
Ischemic etiology of HF, n (%)	44 (61.1)	27 (65.9)	17 (54.8)	0.56
BMI, kg/m^2	27.45 (24.42–30.61)	27.40 (23.85–30.48)	29.05 (26.01–30.67)	0.43
HR, bpm	71.04 (8.11)	71.51 (7.99)	70.42 (8.35)	0.58
Comorbidities				
Hypertension, n (%)	43 (59.7)	28 (68.3)	15 (48.4)	0.09
Type 2 diabetes, n (%)	33 (45.8)	17 (41.5)	16 (51.6)	0.39
Persistent AF, n (%)	31 (43.1)	16 (39)	15 (48.4)	0.43
Reversible PH, n (%)	31 (43.1)	13 (31.7)	18 (58.1)	0.03
Laboratory parameters				
WBC count, × 10^9/l	6.84 (5.77–8.16)	6.81 (5.69–8.27)	7.13 (5.79–7.84)	0.82
Hemoglobin, mmol/l	8.65 (1)	8.64 (0.98)	8.66 (1.05)	0.92
Creatinine, µmol/l	115.5 (94.5–142)	99 (90–123)	138 (113–163)	<0.001
Platelet count, ×10^9/l	181.40 (49.78)	189.85 (50.42)	170.23 (47.43)	0.10
Total bilirubin, µmol/l	22.30 (15.35–35.15)	21.40 (14.20–36.30)	23.10 (16.00–35.00)	0.64
Albumin, g/l	43.35 (4.37)	43.90 (4.21)	42.65 (4.55)	0.23
Uric acid, µmol/l	487.02 (157.51)	481.78 (158.39)	493.95 (158.68)	0.75
Urea, µmol/l	8.9 (6.5–12.8)	7.6 (5.6–10)	12.1 (8.4–17.7)	0.002
Sodium, mmol/l	137. 78 (3.39)	139.22 (2.76)	135.87 (3.23)	<0.001
Fibrinogen, mg/dl	386 (309–481)	341 (284–422)	405 (373–498)	<0.001
AST, U/l	28 (21.5–35)	28 (22–38)	28 (21–33)	0.25
ALT, U/l	24 (18–35)	26 (21–42)	20 (17–27)	0.01
ALP, U/l	81 (64–131)	71 (55–109)	85 (67–135)	0.047
GGTP, U/l	87.06 (53.42)	67.39 (36.49)	113.06 (61.25)	<0.001
Cholesterol, mmol/l	3.87 (3.22–4.89)	3.98 (3.25–4.98)	3.56 (2.73–4.56)	0.23
LDL cholesterol, mmol/l	1.99 (1.53–3.05)	1.99 (1.53–3.02)	1.99 (1.57–3.13)	0.94
hs-CRP, mg/l	2.46 (1.44–6.05)	1.66 (1.02–4.75)	4.39 (2.17–8.75)	0.003
HbA_{1c}, %	6.07 (0.75)	6 (0.74)	6.17 (0.78)	0.35
Peripheral venous NT-proBNP, pg/ml	3777 (2008.5–5719.5)	3082 (1587–6337)	4114 (3252–5640)	0.09
Coronary sinus NT-proBNP, pg/ml	4356 (2472–6512)	3243 (1699–6856)	4790 (3785–6502)	0.08
Coronary sinus fetuin, mg/l	672.92 (478.28–990.66)	976.99 (691.80–1178.65)	476.95 (400.78–625.42)	<0.001
Peripheral blood fetuin, mg/l	610.98 (259.42)	749.64 (259.77)	427.58 (90.93)	<0.001
Hemodynamic parameters				
sPAP, mm Hg	39 (29–49)	39 (30–53)	39 (27–49)	0.47
mPAP, mm Hg	26.32 (8.67)	26.39 (9.72)	26.23 (7.21)	0.94
CI, l/min/m^2	1.80 (0.14)	1.80 (0.13)	1.79 (0.16)	0.78
TPG, mm Hg	8 (7–9.5)	8 (7–9)	8 (7–11)	0.8
PVR, Wood units	2.06 (1.81–2.35)	2.05 (1.81–2.31)	2.19 (1.82–2.56)	0.43
Spirometry				
FEV_1, %	77.85 (13.58)	77.39 (15.08)	78.45 (11.51)	0.75
FVC, %	83 (74–91)	83 (74–88)	84 (74–93)	0.47
FEV_1 to FVC ratio, %	97.5 (92–102)	98 (91–103)	97 (92–101)	0.6
Echocardiographic parameters				
LA, mm	54.33 (7.47)	51.51 (7.34)	58.06 (5.91)	<0.001
RVEDd, mm	32 (29.5–34)	31 (28–34)	33 (30–35)	0.06
TAPSE, mm	14.04 (3.19)	15.02 (2.77)	12.74 (3.29)	0.002
LVEDd, mm	75.19 (9.64)	72.95 (9.19)	78.16 (9.56)	0.02
LVEF, %	16 (15–18)	17 (15–18)	16 (15–18)	0.49

TABLE 1 Baseline characteristics of the study population (continued from the previous page)

Parameter	General population (n = 72)	Survival (n = 41)	Nonsurvival (n = 31)	*P* value
Cardiac medications, n (%)				
B-Blockers	72 (100)	41 (100)	31 (100)	1.0
ACEI/ARB	70 (97.2)	40 (97.6)	30 (96.8)	0.84
Loop diuretics	69 (95.8)	38 (92.7)	31 (100)	0.12
MRA	72 (100)	41 (100)	31 (100)	1.0
Digoxin	28 (38.9)	16 (39)	12 (38.7)	0.98
Ivabradine	13 (18.1)	11 (26.8)	2 (6.5)	0.03
Statin	60 (83.3)	34 (82.9)	26 (83.9)	0.92
Coumarin derivatives	44 (61.1)	27 (65.9)	17 (54.8)	0.34
Acetylsalicylic acid	26 (36.1)	15 (36.6)	11 (35.5)	0.92
Sildenafil	30 (41.7)	12 (29.3)	18 (58.1)	0.01
ICD	47 (65.3)	31 (75.6)	16 (51.6)	0.03
CRT-D	25 (34.7)	10 (24.4)	15 (48.4)	
Other				
Vo_2 max, ml/kg/min	10.8 (9.7–12)	11.3 (10.3–12.1)	10.2 (8.9–11.5)	0.004

Data are presented as median (interquartile range) or mean (SD) unless otherwise indicated.

Abbreviations: ACEI, angiotensin-converting enzyme inhibitor; ALP, alkaline phosphatase; ALT, alanine aminotransferase; ARB, angiotensin II receptor blocker; AST, aspartate aminotransferase; BMI, body mass index; CI, cardiac index; CRT-D, cardiac resynchronization therapy-defibrillator; AF, atrial fibrillation; FEV_1, forced expiratory volume in 1 second; FVC, forced vital capacity; GGTP, γ-glutamyl transpeptidase; HbA_{1c}, glycated hemoglobin; HF, heart failure; HR, heart rate; hs-CRP, high-sensitivity C-reactive protein; ICD, implantable cardioverter-defibrillator; LA, left atrium; LDL, low-density lipoprotein; LVEDd, left ventricular end-diastolic dimension; LVEF, left ventricular ejection fraction; mPAP, mean pulmonary artery pressure; MRA, mineralocorticoid receptor antagonists; NT-proBNP, N-terminal pro–B-type natriuretic peptide; PH, pulmonary hypertension; PVR, pulmonary vascular resistance; RVEDd, right ventricular end-diastolic dimension; sPAP, systolic pulmonary artery pressure; TAPSE, tricuspid annular plane systolic excursion; TPG, transpulmonary gradient; Vo_2 max, maximal oxygen uptake; WBC, white blood cell

TABLE 2 Univariable and multivariable analysis of factors associated with worse prognosis

Parameter	Univariable data		Multivariable data	
	OR (95% CI)	*P* value	OR (95% CI)	*P* value
Model 1				
Coronary sinus fetuin[a], mg/l	1.104 (1.052–1.157)	<0.001	1.103 (1.045–1.164)	<0.001
Fibrinogen[b], mg/dl	1.008 (1.003–1.014)	0.002	–	–
CRP[b], mg/l	1.182 (1.029–1.359)	0.02	–	–
Creatinine[b], µmol/l	1.044 (1.022–1.066)	<0.001	–	–
Sodium[c], mmol/l	1.462 (1.195–1.789)	<0.001	1.563 (1.134–2.156)	0.006
Urea[b], µmol/l	1.163 (1.048–1.291)	0.004	–	–
Vo_2 max[c], ml/kg/min	1.616 (1.163–2.247)	0.004	–	–
Model 2				
Peripheral blood fetuin[a], mg/l	1.093 (1.045–1.142)	<0.001	1.098 (1.046–1.153)	<0.001
ALP[b], U/l	1.009 (0.999–1.019)	0.09	–	–
GGTP[b], U/l	1.021 (1.008–1.033)	0.001	–	–
NT-proBNP[d], pg/ml	1.008 (0.990–1.025)	0.39	–	–
Sodium[c], mmol/l	1.462 (1.195–1.789)	<0.001	1.639 (1.209–2.227)	0.002
LVEDD[b], mm	1.062 (1.007–1.121)	0.03	–	–
TAPSE[c], mm	1.289 (1.083–1.531)	0.004	–	–
Reversible PH	2.982 (1.130–7.869)	0.03	–	–

a per 10 units decrease

b per 1 unit increase

c per 1 unit decrease

d per 100 units decrease

Abbreviations: OR, odds ratio; others, see TABLE 1

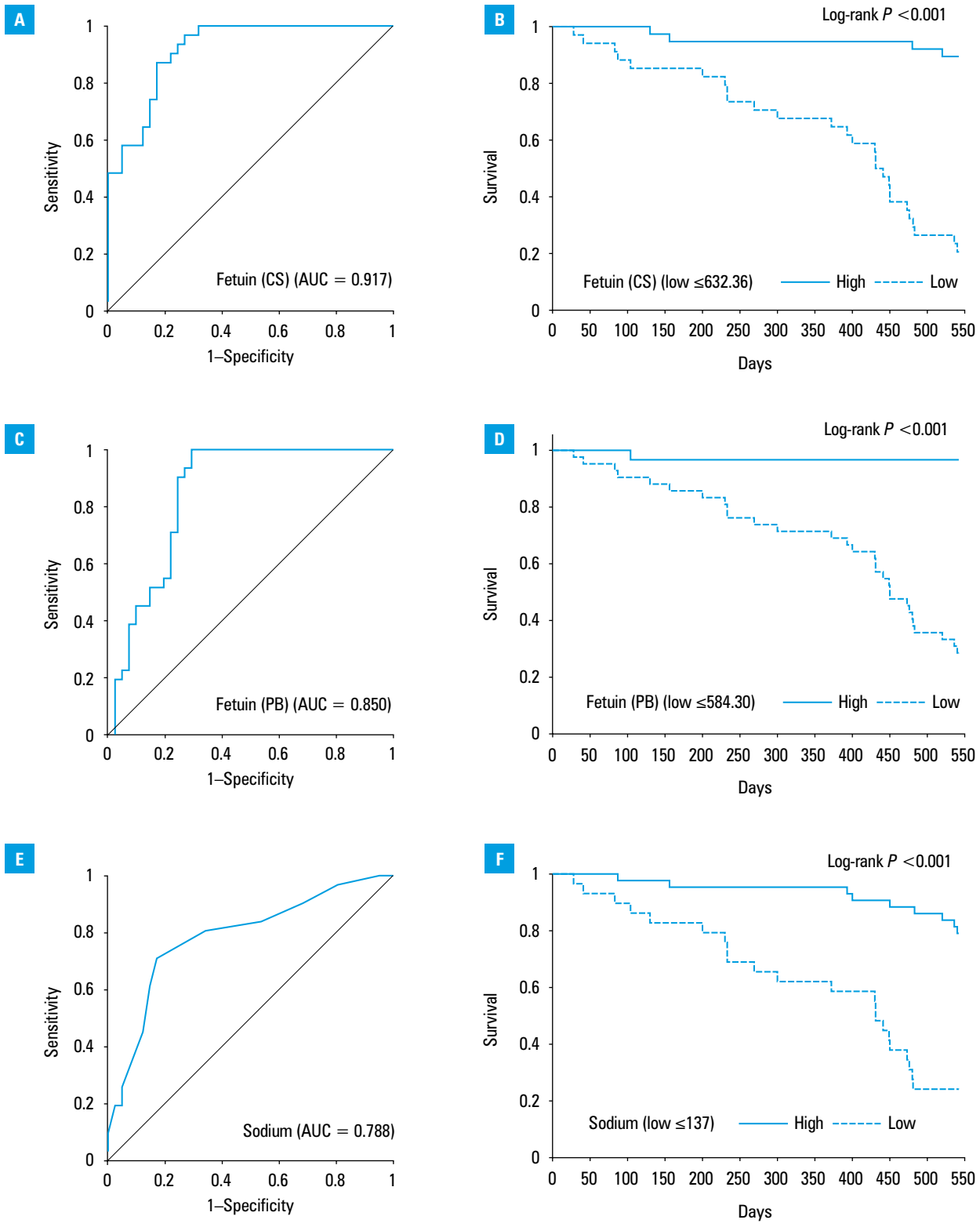

FIGURE 1 Receiver operating characteristic curves (left) and Kaplan–Meier survival curves (right) for: **A**, **B** – coronary sinus fetuin-A; **C**, **D** – peripheral fetuin-A; and **E**, **F** – sodium concentrations
Abbreviations: AUC, area under the curve; CS, coronary sinus; PB, peripheral blood

survival rate than the group with higher sodium concentrations (>137 mmol/l) (24.1% vs 79.1%; log-rank P <0.001) (FIGURE 1F).

The comparison of the prognostic values of fetuin-A and NT-proBNP levels from the peripheral and coronary sinus blood samples is presented in FIGURE 2A and 2B. The concentration of fetuin-A in the blood samples from the coronary sinus was higher by a mean (SD) of 138.52 (117.69) mg/l compared with the samples from the peripheral blood. Similarly, the concentration of NT-proBNP in the serum from the coronary sinus was higher by a median (IQR) of 401 (168–607.5) pg/ml compared with the samples from peripheral blood. The ROC curve analysis showed a higher prognostic value of coronary sinus fetuin-A levels than

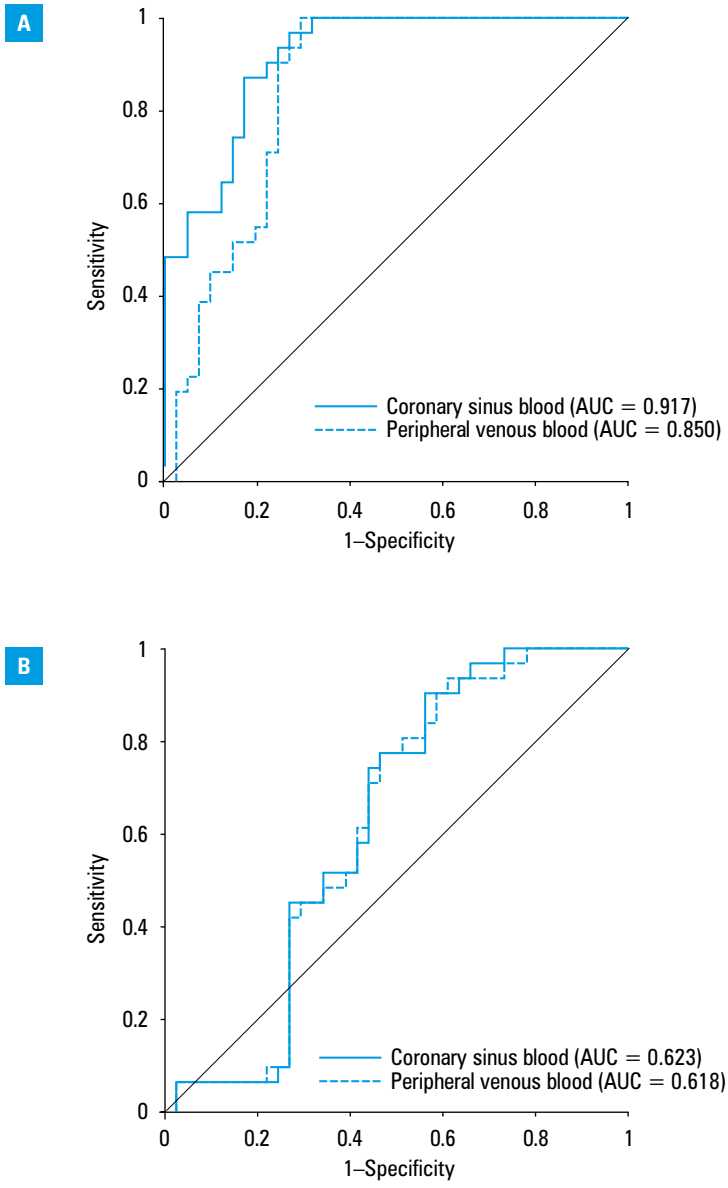

FIGURE 2 Comparison of the prognostic values of (**A**) fetuin-A and (**B**) N-terminal pro–B-type natriuretic peptide levels from peripheral and coronary sinus blood samples
Abbreviations: see **TABLE 1** and **FIGURE 1**

peripheral blood fetuin-A levels. Furthermore, the prognostic accuracy of NT-proBNP levels from peripheral and coronary sinus blood samples was comparable.

The difference between the calculated AUCs for coronary sinus fetuin-A and peripheral venous fetuin-A amounted to 0.068 (P = 0.02). However, the difference between AUCs for coronary sinus NT-proBNP and peripheral venous NT-proBNP was 0.005 (P = 0.5). A summary of the ROC curve analysis for selected biomarkers is presented in **TABLE 3**.

DISCUSSION To the best of our knowledge, this is the first study to demonstrate a strong, independent association between coronary sinus and peripheral blood fetuin-A levels and an increased risk of death in patients with end-stage HF. Coronary sinus fetuin-A level had an excellent prognostic power, as well as high sensitivity and specificity, which allows for survival and non-survival outcomes to be successfully predicted among patients on a HT waiting list. Furthermore, the prognostic utility of the coronary sinus fetuin-A level was significantly better than that of the peripheral venous blood fetuin-A level, as well as that of NT-proBNP and sodium concentrations. However, it should be emphasized that while the peripheral blood fetuin-A level had adequate prognostic power to assess the 1.5-year survival rate in the study group, its prognostic power was lower than that of coronary sinus fetuin-A level.

Fetuin-A is a multifunctional glycoprotein that is mainly secreted from the liver and adipose tissue and which acts systemically in blood and all extracellular fluids.[14] The protein is considered to be involved in various normal and pathological processes, including osteogenesis and bone resorption, the regulation of insulin activity and hepatocyte-growth-factor activity, the response to systemic inflammation, and the inhibition of unwanted mineralization.[9,14] Fetuin-A participates in the pathogenesis of HF by preventing fibrosis, increasing the cellular uptake of cationic inhibitors of proinflammatory cytokines, and participating in macrophage deactivation.[10,11,14,15] There is evidence that fetuin serum concentrations are inversely related to the levels of proinflammatory cytokines such as interleukin (IL) 1 and IL-6.[14-16] IL-1, which plays an important role in the pathogenesis of HF, has in turn been shown to inhibit fetuin-A transcript levels in cultured hepatocytes, which indirectly explains the low concentration of fetuin-A in patients with advanced HF.[17] Furthermore, fetuin deficiency has been associated with an increase in the expression of transforming growth factor β1 (TGF-β), collagen, and fibronectin, leading to fibrogenesis and excessive collagen deposition. These mechanisms are implicated in cardiac remodeling, leading to cardiac dysfunction. Thus, from the pathological point of view, fetuin-A can be associated with HF severity and may influence clinical outcomes in patients with HF.[14-19]

A study by Keçebaş et al[9] revealed that serum fetuin-A levels were markedly lower in patients with HF compared with healthy controls and that the high sensitivity and specificity of fetuin-A levels can be used to distinguish HF patients from healthy individuals. Furthermore, the authors observed lower fetuin-A concentrations among NYHA class III and IV patients than among those in lower functional capacity classes (NYHA I and II).[9] In accordance with our results, Keçebaş et al[9] reported that a worse prognosis was associated with lower serum fetuin concentrations. This finding could suggest that the anti-inflammatory activity of fetuin-A is downregulated in end-stage HF.

Another important property of fetuin-A—the inhibition of calcification—could potentially be related to the development and progression of

TABLE 3 A summary of receiver operating characteristic curves analysis for selected biomarkers

Parameter	AUC (95% CI)	*P* value	Cutoff	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)	LR+ (95% CI)	LR– (95% CI)	Accuracy
Coronary sinus fetuin, mg/l	0.9174 (0.8576–0.9772)	<0.001	≤632.36	0.87 (0.70–0.96)	0.83 (0.68–0.93)	0.79 (0.62–0.91)	0.89 (0.75–0.97)	5.00 (1.57–8.64)	0.156 (0.011–0.301)	0.85 (0.74–0.92)
Peripheral blood fetuin, mg/l	0.8497 (0.7585–0.9410)	<0.001	≤584.3	0.97 (0.83–0.99)	0.71 (0.54–0.84)	0.72 (0.56–0.85)	0.97 (0.83–0.99)	3.31 (1.71–4.91)	0.05 (–0.04 to 0.14)	0.82 (0.71–0.90)
Sodium, mmol/l	0.7876 (0.6781–0.8971)	<0.001	≤137	0.71 (0.52–0.86)	0.83 (0.68–0.93)	0.76 (0.56–0.90)	0.79 (0.64–0.90)	4.16 (1.18–8.13)	0.35 (0.15–0.55)	0.78 (0.66–0.87)
Peripheral NT-proBNP, pg/ml	0.6184 (0.4863–0.7505)	0.08	≥2017	0.94 (0.79–0.99)	0.33(0.24–0.56)	0.54 (0.40–0.67)	0.89 (0.65–0.99)	1.53 (1.13–1.94)	0.12 (–0.07 to 0.40)	0.63 (0.50–0.74)
Coronary sinus NT-proBNP, pg/ml	0.6231 (0.4921–0.7550)	0.07	≥2769	0.90 (0.74–0.98)	0.44 (0.28–0.60)	0.55 (0.40–0.69)	0.86 (0.64–0.97)	1.61 (1.33–2.09)	0.22 (–0.03 to 0.47)	0.64 (0.52–0.75)

Abbreviations: LR–, negative likelihood ratio; LR+, positive likelihood ratio; NPV, negative predictive value; PPV, positive predictive value; others, see **TABLE 1** and **FIGURE 1**

HF. Because of its high affinity to calcium phosphates, fetuin-A accumulates in the mineralized bone matrix, in atherosclerotic plaques, and in pathologically mineralized tissues.[18-19] However, the binding of minerals is not the only characteristic of fetuin-A, as it binds to many ligands, including TGF-β, thus acting as a soluble receptor-like antagonist of TGF-β actions.[20] A study by Merx et al[18] showed that in a fetuin-A-deficient mouse model, there was a spontaneous development of widespread myocardial calcifications, which are associated with a profound induction of pro-fibrotic TGF-β and downstream collagen, as well as fibronectin mRNA synthesis. Activation of these processes leads to cardiac fibrosis, systolic and diastolic dysfunction, impaired tolerance to ischemia, and catecholamine resistance, all of which are closely related to the pathophysiology of HF.[16,18] Furthermore, lower levels of fetuin-A negatively affected heart structure and function in a mouse model of myocardial ischemia.[18] A study by Feistritzer et al[21] demonstrated that adding fetuin-A to a model that included maximum cardiac troponin T, NT-proBNP, and CRP concentrations resulted in a larger AUC for the prediction of adverse left ventricle remodeling.

Another interesting finding of the present study was the independent association found between decreased plasma sodium concentrations and worse survival rates in patients with end-stage HF. Sodium level had an acceptable prognostic power, sensitivity, and specificity for assessing the prognosis in patients on HT waiting lists. These findings are in agreement with our previous study, which demonstrated that lower plasma sodium concentrations at the time of being placed on a waiting list were associated with reduced 1-year survival rates in ambulatory patients with end-stage HF accepted for HT.[22] In that study, the prognostic power of sodium concentration (AUC, 0.778) in assessing the survival of patients on an HT waiting list was comparable to that obtained in the current study (AUC, 0.7876).[22]

Some other studies have also demonstrated that lower sodium concentrations are associated with an unfavorable prognosis in different populations of patients with HF.[23-25] Hyponatremia's incidence of 20% to 25% makes it one of the most common electrolyte abnormalities in HF patients and places it among the most important predictors of short- and long-term mortality.[23,25,26] The pathogenesis of hyponatremia in HF is multifactorial. It is believed that hyponatremia in HF is mainly due to low cardiac output, which impairs the kidneys' ability to excrete diluted urine.[23] In turn, reduced cardiac output results in increased secretion of arginine vasopressin (AVP), which entails an excessive activation of the sympathetic nervous system and the renin-angiotensin-aldosterone system, both observed in HF.[25,27] The exaggerated release of AVP primarily causes free water in the renal collecting ducts to be reabsorbed and the plasma

sodium concentration to be diluted, but it could also theoretically contribute to HF by aggravating systolic and diastolic wall stress.[26,28] Furthermore, sodium and water hemostasis might be directly affected by loop diuretics, which stimulate thirst and further secretion of AVP, thus promoting water retention and further predisposing the patient to hyponatremia.[29-31] A study by Kapłon-Cieślicka et al[23] reported that patients with hyponatremia are characterized by a higher NYHA class, lower systolic and diastolic pressures, higher creatinine concentrations, and lower hemoglobin levels. Sato et al[25] reported that hyponatremia in hospitalized HF patients was associated with a more critical condition, lower blood pressure, and higher brain natriuretic peptide levels. These findings indicate that patients with hyponatremia are in more advanced stages of HF, which contributes to a worse prognosis among this group.

We have also demonstrated that the prognostic accuracy of NT-proBNP in coronary sinus and peripheral blood samples was comparable and relatively modest. In clinical practice, NT-proBNP can help to distinguish cardiac from noncardiac causes of dyspnea, and is commonly used to rule out HF as well as to monitor the effectiveness of the treatment in decompensated HF.[32-34] However, the prognostic strength of NT-proBNP may be limited in a group of optimally treated patients with end-stage HF. NT-proBNP levels are affected by demographic variables such as age, gender, and ethnicity, as well as clinical characteristics such as hypertension, chronic pulmonary disease, atrial fibrillation, and renal insufficiency.[35,36] Thus, the presence of several confounding factors may significantly influence the prognostic value of NT-proBNP in predicting survival in patients with end-stage HF.[37] Plasma NT-proBNP level is considered a direct counter-regulatory response to myocardial stress and increased left ventricular filling pressure in HF.[37,38] After an optimal therapy with diuretics and angiotensin-converting enzyme inhibitors, significant reductions in plasma natriuretic peptide levels have been observed, which are responsible for a reduction in filling pressure and thus a reduction in the release of natriuretic peptides.[36] Moreover, it seems that in some patients with end-stage HF, the ability of their ventricles to synthesize and release natriuretic peptides may be exhausted as an expression of the end-stage disease. In such cases, lower levels of NT-proBNP are observed despite advanced HF, the consequence of which is an impaired ability of natriuretic peptides to provide risk stratification at this stage of the disease.[33] Given the multiple comorbidities accompanying advanced stages of HF that affect NT-proBNP levels and the impact of optimal neurohormonal suppression with maximal HF therapy on the release of NT-proBNP—as well as depletion of NT-proBNP release in some patients with end-stage HF—the role of this biomarker in assessing prognosis in clinically stable patients with advanced HF may be limited. Our previous study also confirmed the limited utility of NT-proBNP in predicting worse survival rates in clinically stable patients with end-stage HF.[37]

Limitations This study has several limitations that should be taken into consideration. Firstly, the study involved a relatively small number of patients in a single center. A further limitation is the lack of an independent validation cohort that would support the role of coronary sinus fetuin-A and sodium levels in the assessment of prognosis. It is likely that if an independent validation cohort had been used, the AUC for fetuin-A would have been lower. We created 2 models due to the small sample size and the relatively large number of relevant factors in univariable analysis; however, the adoption of various statistical models may reduce the value of the results obtained. Moreover, our study was limited to only those patients who survived 1.5 years from enrollment in the study or died during this period. It is also necessary to determine the role of fetuin among patients undergoing mechanical circulatory support device implantation and HT. Despite these limitations, this study provides pioneering evidence of the prognostic role of fetuin-A in advanced HF patients awaiting HT. Further in vivo and in vitro experiments will be needed to determine the mechanisms of increased fetuin-A production in HF. A multicenter trial with a large-scale study population is now warranted to further investigate the role and clinical significance of fetuin-A in HF.

Conclusions In this single-center, prospective study, we found that lower coronary sinus and peripheral blood fetuin-A levels and lower peripheral venous blood sodium concentrations were associated with an increased risk of death in patients with end-stage HF who were accepted for HT. Coronary sinus fetuin-A level has excellent prognostic power and high sensitivity and specificity, allowing for a successful identification of survival versus nonsurvival outcomes in patients on an HT waiting list; the prognostic power of sodium concentration was acceptable for assessing prognosis in the study group. Moreover, the prognostic utility of coronary sinus fetuin-A levels is superior to that of peripheral venous fetuin levels, while coronary sinus NT-proBNP concentration is comparable to peripheral venous NT-proBNP concentration in this respect. Furthermore, peripheral blood fetuin-A levels—with their good prognostic power, sensitivity, and specificity—may have adequate clinical utility in outpatients with HF. However, it should be emphasized that during protocol-consistent right heart catheterization in HT candidates, the collection of blood from the coronary sinus is relatively simple and safe and may provide more reliable data for assessing the prognosis in patients with advanced HF awaiting HT.

ARTICLE INFORMATION

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