

# The Role of Endothelial Dysfunction in Development of Acute Kidney Injury in Patients With Acute Decompensated Heart Failure

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## Research

**Keywords:** Cardiorenal syndrome, heart failure, endothelial dysfunction, kidney injury

**Posted Date:** October 19th, 2020

**DOI:** <https://doi.org/10.21203/rs.3.rs-92710/v1>

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# Abstract

**Objective:** Cardiorenal syndrome type I (CRS I) is defined as the development of acute kidney injury (AKI) following acute decompensated heart failure (ADHF). Note that the clinical significance of endothelial markers in ADHF-associated AKI has yet to be clarified. This study investigated the biological processes linking ADHF and AKI to determine whether plasma markers of endothelial injury and activation could serve as predictors for AKI in patients with ADHF.

**Methods:** The study prospectively recruited 125 consecutive patients admitted to a coronary critical unit due to ADHF. Patients with and without AKI were compared in terms of plasma levels of soluble thrombomodulin (sTM), angiotensin (Ang)-1 and 2, and baseline characteristics.

**Results:** Among the study population, 14 (11.2%) patients developed CRS within 7 days after admission. The hemoglobin levels ( $11.4 \pm 2.1$  vs.  $13.3 \pm 2.2$  g/dL,  $p = 0.003$ ) and baseline eGFR ( $65.7 \pm 34.5$  vs.  $85.5 \pm 35.0$  mL/minute/1.73m<sup>2</sup>,  $p = 0.048$ ) of patients with CRS were lower than those of patients without CRS. Patients with CRS also presented elevated plasma levels of BNP ( $1,797.2 \pm 1,649.1$  vs.  $687.8 \pm 976.6$  pg/mL,  $p = 0.008$ ), Ang-2 ( $7,524.7 \pm 8,485.5$  vs.  $3,325.3 \pm 4,740.9$  pg/mL,  $p = 0.006$ ), and sTM ( $7,763.8 \pm 3,803.7$  vs.  $4,661.3 \pm 1,896.8$  ng/mL,  $p < 0.001$ ) compared to patients without CRS. Areas under the ROC curves (AUROC) revealed that Ang-2 and TM plasma levels had significantly discriminative powers pertaining to the development of CRS (0.704, 95% CI 0.55-0.859,  $p = 0.013$ ; and 0.789, 95% CI 0.675-0.903,  $p < 0.001$ , respectively).

**Conclusion:** These biomarkers suggest a novel avenue for kidney injury in the context of ADHF and indicate that baseline biomarker profiles could potential be used to identify individuals at risk of developing AKI.

## Background

The intersection of cardiac and renal dysfunction has important therapeutic and prognostic implications. The association between heart failure and renal insufficiency has been demonstrated in previous studies [1, 2]. Cardiorenal syndromes are disorders of the heart and kidneys, whereby dysfunction in one organ may lead to dysfunction in the other organ. Cardiorenal syndrome type I (CRS I) is defined as the development of acute kidney injury (AKI) following acute decompensated heart failure (ADHF) [3]. In one study, the incidence of CRS I in cases of patients hospitalized for acute heart failure ranged from 12% to 37% [4]. CRS I is associated with increased cardiovascular death, duration of hospitalization, and incidence of re-hospitalization [5].

The complex pathophysiology of CRS type 1 has yet to be fully elucidated. Recent evidence suggests that endothelial activation and subsequent vascular barrier injury is a critical pathogenic mechanism in CRS [6]. Endothelial injury induced by shear stress, angiotensin II, aldosterone, or inflammatory cytokines decreases glomerular filtration rates and hinders diuretic efficacy, resulting in fluid retention, decreased cardiac output, venous congestion, and decreased renal perfusion [7]. Several molecules, such as

thrombomodulin (TM) and angiopoietins (Ang), are implicated in mediating endothelial activation and injury under various stimuli [8, 9]. TM, a membrane-bound glycoprotein predominantly expressed in endothelial cells, neutralizes the clotting activity of thrombin and activates the anti-coagulant and anti-inflammatory properties of protein C [10]. A circulating soluble form of TM (sTM) is released into the plasma through proteolytic degradation. The fact that sTM is released from the surfaces of endothelial cells only after injury makes it a recognizable marker of endothelial injury [11]. One previous study reported elevated sTM levels in patients with acute heart failure but not in those with chronic heart failure [12]. Researchers have also reported that sTM plasma levels are associated with kidney injury following sepsis [8] and acute myocardial infarction [13].

Ang-1 and Ang-2 bind to the same site of the endothelial cell-specific Tie-2 receptor [14]. Ang-1 and Ang-2 are essential for vascular development, maturation, and inflammation. After binding to the Tie-2 receptor, Ang-1 triggers the activation of Tie-2. By contrast, Ang-2 exerts an antagonistic response in Tie-2. Ang-1 is protective in its anti-inflammatory effects and stabilization of the endothelium. By contrast, Ang-2 promotes inflammatory response by activating the endothelial cells and enhancing vascular leakage [15]. The importance of angiopoietins in endothelial activation and injury to the vascular barrier has prompted extensive research into their role as biomarkers in critical illness [16]. Plasma Ang-2 levels are significantly higher in cases of acute heart failure than in healthy controls and are a predictor of poor outcomes [17]. Plasma Ang-2 levels are also an independent predictor for mortality in patients undergoing dialysis in intensive care units [18].

Biomarkers of endothelial injury and dysfunction have been associated with poor outcomes in cases of cardiovascular disease; however, the clinical significance of endothelial markers in heart failure associated AKI has not been clarified. This study investigated the underlying biological processes linking ADHF and AKI with the aim of determining whether the plasma markers of endothelial injury and activation could serve as predictors for AKI in patients with ADHF.

## **Materials And Methods**

### ***Study population***

This study was conducted from November 2012 to September 2013 in the 14-bed coronary care unit (CCU) of a tertiary medical center (Chang Gung Memorial Hospital). The study prospectively recruited 125 consecutive patients admitted to the CCU due to ADHF. Exclusion criteria included age of less than 18 years old, or having undergone organ transplant prior to admission. Plasma specimens were obtained from all patients within 24h of CCU admission, and all patients were followed until hospital discharge or death. The Institutional Review Board of Chang Gung Memorial Hospital approved the protocols for recruitment and sample collection, which was performed with informed consent of the participants.

### ***Data collection***

Demographic data were collected on the first day of admission to the CCU, including body mass index, blood pressure, heart rate, smoking status, and Killip class as well as previous diagnosis of hypertension, hyperlipidemia, diabetes, and coronary artery disease. The left ventricular ejection fraction (LVEF) was acquired using the modified Simpson's method via echocardiography upon admission. Prior history of major adverse cardiac events (MACE) was also collected, including recurrent myocardial infarction (MI), hospital admission for heart failure, unplanned repeat revascularization, malignant dysrhythmia, stroke, or pulmonary embolism [16].

The estimated glomerular filtration rate (eGFR) was calculated using the Modification of Diet in Renal Disease formula [19]. High-sensitive C-reactive protein (hsCRP), hemoglobin (Hb), platelet, and white blood cell (WBC) counts were measured upon admission.

### ***Outcome assessment***

The occurrence of acute CRS 1 was the dependent variable of this study. AKI events were defined as any of the following criteria occurring within 7 days after admission: an increase in serum creatinine level of  $\geq 0.3$  mg/ dL within a period of 48 h or an increase in serum creatinine level of  $\geq 1.5$  times from baseline within a period of 7 days. Note that both of these criteria were suggested by the practical clinical Kidney Disease Improving Global Outcomes (KDIGO) guidelines for AKI [20].

### **Enzyme-linked immunosorbent assay (ELISA)**

Plasma was collected from blood taken upon admission. The concentrations of sTM, von Willebrand factor (vWF), Ang-1, Ang-2, and Tie-2 were determined by ELISA in accordance with the manufacturers' instructions (Sekisui Diagnostics, MA). The measured values were compared between patients with and without AKI.

### **Statistical analysis**

All data were expressed as mean  $\pm$  SD or percentage. The Student's t-test was used to compare the means of continuous variables and normally distributed data; otherwise, the Mann-Whitney test was used. Categorical variables were tested using the Chi-square test or Fisher exact test. Multivariate analysis was not performed due to the low incidence of CRS events in the study cohort. Receiver operating characteristic (ROC) curves for day 1 plasma levels of Ang-2 and sTM were plotted to predict the development of AKI and the respective areas under the curves were calculated. A probability value ( $p$ )  $< 0.05$  using a 2-sided test was considered statistically significant. All analyses were performed using SPSS software package, version 20.0 (SPSS, Inc, Chicago, IL).

## **Results**

For this study, 125 consecutive patients were recruited. Among them, 14 (11.2%) patients developed CRS within 7 days after admission to the CCU. The demographic characteristics, biomarkers, and clinical presentations are listed in Table 1. Compared with patients without CRS, those with CRS were older (71.9

$\pm 7.5$  vs.  $62.9 \pm 14.5$  years old;  $p=0.024$ ), had a lower incidence of coronary heart disease (35.7% vs. 71.2%,  $p = 0.013$ ), were less likely to use ACEI/ARB (57.1% vs. 84.7%,  $p = 0.022$ ), were more likely to have a history of cerebrovascular disease (28.6% vs. 8.1%,  $p=0.040$ ), and were more likely to use loop diuretics (78.6% vs. 36.9%,  $p = 0.004$ ). CRS patients presented lower hemoglobin levels ( $11.4 \pm 2.1$  vs.  $13.3 \pm 2.2$  g/dL,  $p = 0.003$ ) and baseline eGFR levels ( $65.7 \pm 34.5$  vs.  $85.5 \pm 35.0$  mL/minute/1.73m<sup>2</sup>,  $p = 0.048$ ) compared to patients without CRS. CRS patients presented higher BNP plasma levels ( $1797.2 \pm 1649.1$  vs.  $687.8 \pm 976.6$  pg/mL,  $p = 0.008$ ), Ang-2 ( $7524.7 \pm 8485.5$  vs.  $3325.3 \pm 4740.9$  pg/mL,  $p = 0.006$ ), and sTM plasma levels ( $7763.8 \pm 3803.7$  vs.  $4661.3 \pm 1896.8$  ng/mL,  $p < 0.001$ ) compared to patients without CRS. No significant differences were observed between the two groups in terms of gender, comorbidities, smoking status, body mass index (BMI), blood pressure, heart rate, LVEF, leukocyte count, hsCRP, VEGF, vWF, Tie-2, or Ang-1. Multivariate analysis was not performed due to the low incidence of CRS events in the study cohort.

In the study population, 99 patients had baseline eGFR  $> 60$  mL/min/1.73m<sup>2</sup>, while 26 patients had eGFR  $< 60$  mL/min/1.73m<sup>2</sup>. The incidence of AKI among patients with eGFR  $< 60$  mL/min/1.73m<sup>2</sup> (5/26, 19.2%) was similar to that among patients with eGFR  $> 60$  mL/min/1.73m<sup>2</sup> (9/99, 9.1%; odds ratio, 1.13; 95% C.I. 0.92-1.37;  $p=0.166$ ). Among patients with eGFR  $< 60$  mL/min/1.73m<sup>2</sup>, we observed higher Ang-2 plasma levels ( $11573 \pm 9441$  pg/mL,  $n=5$  vs.  $4652 \pm 7415$  pg/mL,  $n=21$ ;  $p = 0.021$ ) and sTM plasma levels ( $11641 \pm 3795$  pg/mL,  $n=5$  vs.  $7032 \pm 2147$  ng/mL,  $n=21$ ;  $p = 0.008$ ) in patients with AKI than in patients without AKI (Figure 1). Among patients with eGFR  $> 60$  mL/min/1.73m<sup>2</sup>, we also observed elevated Ang-2 plasma levels ( $5883 \pm 7425$  pg/mL,  $n=9$  vs.  $2951 \pm 3825$  pg/mL,  $n= 90$ ;  $p = 0.05$ ) and sTM plasma levels ( $6165 \pm 2874$  pg/mL,  $n=9$  vs.  $4102 \pm 1323$  pg/mL,  $n=90$ ;  $p = 0.0002$ ) in AKI patients compared with those without AKI. This indicates that baseline eGFR levels were not associated with the difference between sTM and Ang-2 levels in patients with and without CRS.

Table 2 demonstrates the ROC curves of Ang-2 and sTM to predict development of CRS. Areas under the ROC curves (AUROC) revealed that Ang-2 and TM plasma levels had modest discriminative powers pertaining to the development of CRS (0.704, 95% CI 0.55-0.859,  $p = 0.013$ ; and 0.789, 95% CI 0.675-0.903,  $p < 0.001$ , respectively). The cutoff values for Ang-2 and sTM in predicting AKI were 2479 pg/mL and 4855.2g/mL, respectively.

Figure 2 shows the incidence of AKI stratified by the quartiles of Ang-2 and sTM plasma levels. The incidences of AKI were markedly higher in the 4<sup>th</sup> quartile group Ang-of2 (8/32, 25%;  $p=0.032$ ) and 4<sup>th</sup> quartile group of sTM (8/30, 26.7%;  $p=0.001$ ) than in the other three quartiles. By contrast, the incidence of AKI was significantly lower in the Ang-2 1<sup>st</sup> quartile group (1/31, 3.2%) and sTM 1<sup>st</sup> quartile group (0/32, 0%) than in the other three quartiles.

We also examined the correlation between Ang-2, sTM, Hb concentration, WBC count, hsCRP, and BNP levels. Table 3 lists the correlations between serum mediators and these variables. sTM levels were correlated with the concentrations of Hb ( $r=-0.516$ ;  $p<0.001$ ) and BNP ( $r=0.434$ ;  $p=0.001$ ). Ang-2 levels

were positively correlated with hsCRP ( $r=0.314$ ;  $p=0.005$ ) and BNP levels ( $r=0.696$ ;  $p<0.001$ ) but negatively correlated with Hb ( $r=-0.313$ ;  $p<0.001$ ) and LVEF ( $r=-0.405$ ;  $p<0.001$ ) levels.

sTM plasma levels were higher in patients with MACE than in patients without MACE ( $5892.1 \pm 3495.9$  vs.  $4754.7 \pm 1896.8$  pg/mL,  $p=0.026$ ) (Figure 3A). Ang-2 plasma levels were similar in patients with and without MACE ( $2902.7 \pm 3028.2$  vs.  $4061 \pm 5919$  pg/mL,  $p=0.321$ ). Ang-2 plasma levels were significantly higher in patients with LVEF  $< 45\%$  ( $7178 \pm 8416$  pg/ml) than in patients with LVEF  $> 45\%$  ( $2361 \pm 2295$  pg/ml,  $p<0.0001$ ) (Figure 3B) as well as significantly higher in patients with Killip class 2 to 4 than in patients with Killip class 1 ( $4314 \pm 5028$  vs.  $2334 \pm 3887$  pg/ml,  $p = 0.0001$ ) (Figure 3C).

## Discussion

The study revealed that BNP, Ang-2, and sTM plasma levels were higher and Hb levels were lower in ADHF patients with AKI than in those without AKI. ROC curves revealed that the day-1 Ang-2 and sTM levels had modest discriminative power in predicting the development of AKI. The difference between patients with and without CRS in terms of Ang-2 and sTM levels was unaffected by baseline renal function, as indicated by eGFR. The incidence of AKI was markedly higher in the 4<sup>th</sup> quartiles of Ang-2 and sTM than in the other three quartiles. sTM levels were higher in patients with MACE than in those without MACE. Ang-2 levels were higher in patients with impaired LV function.

Our results revealed higher sTM plasma levels in ADHF patients with AKI. In previous studies, sTM levels have been identified as a specific marker of endothelial injury [11]. Our data suggest that endothelial injury may play a crucial role in mediating kidney injury in cases of ADHF. Endothelial injury can be triggered by altered hemodynamics, hypoxia, or inflammatory response in cases of heart failure [6]. The injured endothelial cells subsequently undergo procoagulatory processes, which may be responsible for the formation of microthrombotic foci leading to organ microcirculation failures or complete organ failure [21]. Elevated sTM levels have been reported in patients with AKI induced by sepsis [8] or acute myocardial infarction [13]. However, elevated sTM levels have never been reported in patients with CRS type I. Elevated sTM levels are related to several vascular related diseases, including coronary disease, stroke, and peripheral occlusive arterial disease [22]. MACE has been associated with poor prognosis in patients with AMI [16] or diabetes [23]. Endothelial injury is reportedly involved in MACE development [24]. In the current study, we observed higher sTM levels in patients with MACE than in those without MACE, due perhaps to more severe vascular atherosclerosis or endothelial injury. The Ang-Tie2 receptor system is an important regulator of vascular barrier function. Ang-2 is released from endothelial cells in response to a variety of stimuli, such as inflammatory cytokines, activated leukocytes, and hypoxemia [15]. Ang-2 inhibits the binding of Ang-1 to the Tie2 receptor, thereby impairing endothelial integrity and promoting vascular leakage [14]. Ang-2 also has proinflammatory properties, promotes endothelial cell apoptosis, and increases adhesion of neutrophils [15]. Elevated Ang-2 levels are strongly associated with poor outcomes in patients with cardiogenic shock [17] or sepsis [25] and have also been linked to kidney injury induced by sepsis [26] or liver cirrhosis [27]. Our data indicate that Ang-2 could serve as a clinical predictor of the development of AKI in patients with ADHF. We also observed a link between elevated Ang-

2 plasma levels and poor cardiac function (as assessed in terms of Killip class and echocardiography results). This observation is in line with a previous report indicating a link between elevated Ang-2 plasma levels and a parallel functional decline in HF patients, as denoted by NYHA class [28]. Thus, the activation of endothelial cells (as indicated by Ang-2 plasma levels) may play a key role in impaired kidney function in patients with heart failure.

In accordance with previous studies, the current study determined that decreased hemoglobin levels and increased BNP levels are associated with CRS. BNP is mainly secreted from the cardiomyocytes in response to cardiac stretch and ischemia and plays an important role in cardiorenal protection [29]. One recent prospective study on patients with acute heart failure reported significantly higher BNP levels in patients with renal dysfunction than in patients with normal renal function [30]. Emerging evidence suggests that decreased shear stress secondary to pump failure is a potential mechanism for endothelial dysfunction in cases of heart failure [31]. Note that shear stress depends not only on blood flow but also on blood viscosity. Decreased blood viscosity due to a reduction in hemoglobin concentration can result in decreased shear stress and subsequent ischemia-induced vasodilation [32]. We therefore postulate that anemia (common in cases of CRS) is a contributor to decreased shear stress, leading to degraded endothelial function and contributing to degraded cardiac and renal function [33]. This study also identified correlations among BNP, hemoglobin, and the markers of endothelial injury, as indicated by Ang-2 and sTM plasma levels. These data provide evidence for a link between endothelial injuries induced by heart failure and subsequent kidney injury.

The results in this study demonstrate that Ang-2 and sTM plasma levels early in the course of heart failure may be predictive of AKI development. Note that we are in no way suggesting the use of Ang-2 or sTM plasma levels as sole biomarkers for the prediction of clinical outcomes or treatment decisions. We are only suggesting that in combination with other biomarkers and clinical markers, Ang-2 or sTM plasma levels are associated with CRS development, which could have implications for pathogenesis and risk stratification. Researchers are increasingly recognizing the clinical and biological heterogeneity of CRS. The pathophysiology of CRS represents the confluence of several hemodynamic, neuro-hormonal, inflammatory, and endothelial perturbations [34]. Incorporating novel biomarkers in combination with clinical predictors could greatly enhance the efficacy of diagnostic algorithms in predicting CRS and the differential responses to treatment [34]. These data may enhance our understanding of dysregulated coagulation and endothelial dysfunction as a pathogenic mechanism in CRS. In addition, the impact of endothelial dysfunction in AKI development may be an important target for future research in heart failure patients.

This is the first study to report on the potential role of endothelial biomarkers for the prediction of AKI in ADHF patients. Note however that this study has a number of limitations that should be considered in interpreting the results. This observational study included only a small number of patients recruited from a single medical facility, which prevented the use of multivariate analysis. It is highly likely that baseline characteristics, such as illness severity, are linked to endothelial dysregulation as well as the risk of developing AKI. Furthermore, a causal association between the endothelial dysfunction and AKI

development cannot be established based solely on these results. It will be necessary in the future to conduct larger scale studies with adjustments for potential confounding factors. Further studies should also be conducted to evaluate the efficacy of monitoring plasma sTM and Ang-2 levels for the prevention of AKI in patients with ADHF.

## Conclusion

In conclusion, this study revealed elevated sTM and Ang-2 plasma levels in ADHF patients with AKI. If confirmed in large-scale prospective studies, TM and Ang-2 could potentially be used as biomarkers in predicting the development of AKI in patients with heart failure. These biomarkers suggest a novel avenue for kidney injury in the context of ADHF and indicate that baseline biomarker profiles could potentially be used to identify individuals at risk of developing AKI.

## Declarations

### **Ethics approval and consent to participate:**

The Institutional Review Board of Chang Gung Memorial Hospital approved the protocols for recruitment and sample collection, which was performed with informed consent of the participants.

### **Consent for publication:**

Not applicable

### **Availability of data and materials:**

The datasets analyzed during the current study available from the corresponding author on reasonable request.

### **Competing interests:**

The authors declare that they have no competing interests

### **Funding:**

This research was supported by Chang Gung Medical Research Projects (CIRPG3H0051) and Minister of Science and Technology, Taiwan (MOST 108-2314-B-182A-130).

### **Authors' contributions:**

Conceptualization: CHC, SML

Data collection: CHC, SML, ACCH

Funding acquisition: SML

Methodology: PHC, YCC

Supervision: TYL

Writing – original draft: SML

Writing – review & editing: CHC, SML

## Acknowledgements

Not applicable

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## Tables

**Table 1 Baseline characteristics of patients with acute decompensated heart failure**

	Entire cohort (n=125)	CRS (n=14)	No CRS (n= 111)	P value
<b>Demography</b>				
Age, year	63.9±14.2	71.9±7.5	62.9±14.5	.024
BMI	24.6±3.8	23.2±4.2	24.8±3.7	.132
Male gender	92(73.6)	8(57.1%)	84(75.7%)	.195
Current smoker	56(44.8%)	5(35.7%)	51(75.7%)	.574
Previous heart failure	39(31.2%)	4(28.6%)	35(31.5%)	1.00
Coronary heart disease	83(66.4%)	5(35.7%)	79(71.2%)	.013
Myocardial infarction	22(17.6%)	3(21.4%)	19(17.1%)	.712
Hypertension	80(64.0%)	12(85.7%)	68(61.3%)	.084
Diabetes	52(41.6%)	6(42.9%)	46(41.4%)	1.00
Chronic atrial fibrillation	14(11.2%)	2(14.3%)	12(10.8%)	.657
Cerebrovascular disease	13(10.4%)	4(28.6%)	9(8.1%)	.040
MACE	28(22.2%)	6(42.8%)	22(19.8%)	.083
<b>Clinical presentation</b>				
NYHA class III/IV	45(36.0%)	5(35.7%)	40(36.0%)	1.00
Systolic BP, mmHg	128.6±25.6	132.8±26.3	128.1±25.5	.519
Diastolic BP, mmHg	72.9±14.5	70.7±11.9	73.1±14.8	.556
Heart rate, beat/min	80.5±15.8	76.8±11.9	80.9±16.2	.352
LVEF, %	52.8±15.4	45.5±19.6	53.8±14.7	.059
<b>Medications</b>				
Beta blocker	107(85.6%)	12(87.5%)	95(85.6%)	1.00
ACEI/ARB	102(81.6%)	8(57.1%)	94(84.7%)	.022
Spirolactone	24(19.2%)	2(14.3%)	22(19.8%)	1.00
Loop diuretics	52(41.6%)	11(78.6%)	41(36.9%)	.004
<b>Clinical outcomes</b>				
Length of stay, days	10.7±12.2	15.9±8.8	10.0±12.5	.089
In-hospital mortality	7(5.6%)	1(7.1%)	6(5.4%)	.574

<b>Laboratory parameters</b>				
Leukocyte count, per mL	10152.4±3695.4	11178.6±4812.3	10021.8±3534.9	.272
Hemoglobin, g/dL	13.1±2.3	11.4±2.1	13.3±2.2	.003
Sodium, mmol/L	139.0±3.1	139.9±3.6	138.9±3.0	.287
Potassium, mEq/L	3.9±0.5	4.1±0.9	3.9±0.4	.250
Creatinine, mg/dL	1.1±0.7	1.69±1.4	1.1±0.5	.010
eGFR, ml/minute/1.73m <sup>2</sup>	83.3±35.4	65.7±34.5	85.5±35.0	.048
hsCRP, mg/L	22.4±32.3	39.7±52.9	20.2±28.5	.087
BNP, pg/mL	835.7±1124.7	1797.2±1649.1	687.8±976.6	.008
Troponin-I, ng/mL	8.5±20.8	5.4±16.1	8.9±21.4	.576
<b>Biomarkers of endothelial injury</b>				
Thrombomodulin, pg/mL	5011.6±2385.3	7763.8±3803.7	4661.3±1896.8	<.0001
vWF, MU	683.7±249.4	569.5±212.4	698.2±250.8	.069
Tie-2, ng/mL	7.9±2.3	7.2±1.6	7.9±2.5	.334
Angiotensin-1, pg/mL	23122.2±12040.6	21919.1±12241.8	23275.5±12062.9	.691
Angiotensin-2, pg/mL	3799.4±5413.7	7914.8±8328.1	3275.7±4727.5	.002

Abbreviations:

CRS: Cardiorenal syndrome

BMI: body mass index

MACE: major adverse cardiac events

BP: blood pressure

LVEF: left ventricular ejection fraction

ACEI: angiotensin converting enzyme inhibitor

ARB: angiotensin II receptor antagonist

eGFR: estimated glomerular filtration rate

hsCRP: high-sensitive C-reactive protein

BNP: brain natriuretic peptide

vWF: von Willebrand factor

**Table 2. Acquisition of the areas under the receiver operating characteristic curves (AUROC) for day-1 plasma levels of angiotensin-2 and thrombomodulin in predicting the development of acute kidney injury within 48 hours after acute myocardial infarction**

	AUROC <sup>a</sup>	95% C.I. <sup>b</sup>	p value	Cutoff value	Sensitivity	Specificity
Angiotensin-2	0.704	0.550-0.859	0.013	2479.0pg/mL	0.714	0.627
Thrombomodulin	0.789	0.675-0.903	<0.001	4855.2pg/mL	0.786	0.691

<sup>a</sup> AUROC: areas under the receiver operating characteristic

<sup>b</sup> C. I.: confidence interval

**Table 3. Correlation between the levels of thrombomodulin angiotensin-2 and other markers**

	Hemoglobin	WBC count	hsCRP	LVEF	BNP
Thrombomodulin					
R	-0.516	-0.002	0.152	-0.059	0.434
P value	<0.001	0.978	0.182	0.517	0.001
Angiotensin-2					
R	-0.313	0.094	0.314	-0.405	0.696
P value	<0.001	0.301	0.005	<0.001	<0.001

Abbreviations:

WBC: white blood cells

hsCRP: high-sensitive C-reactive protein

LVEF: left ventricular ejection fraction

BNP: brain natriuretic peptide

# Figures

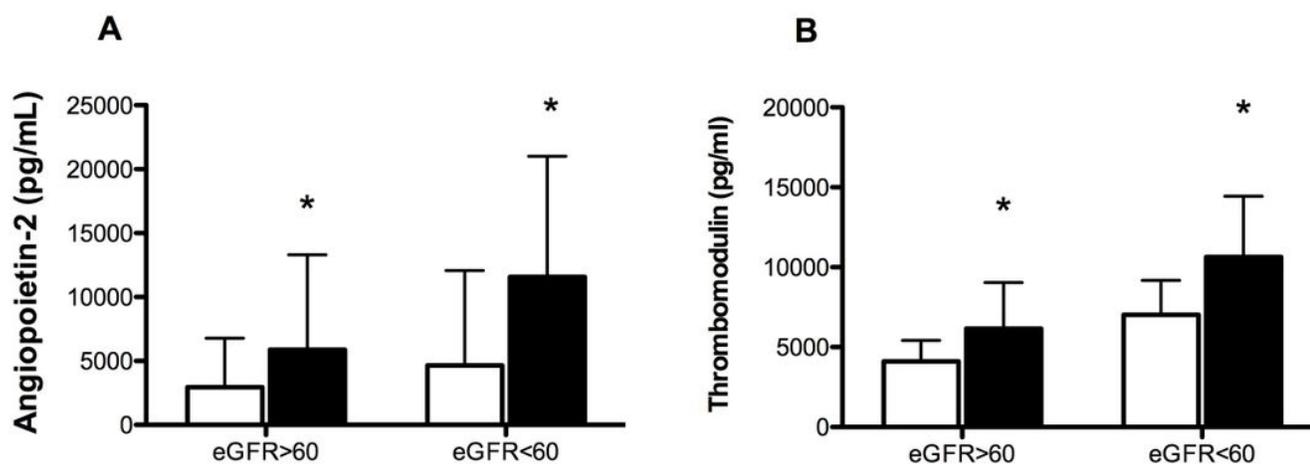
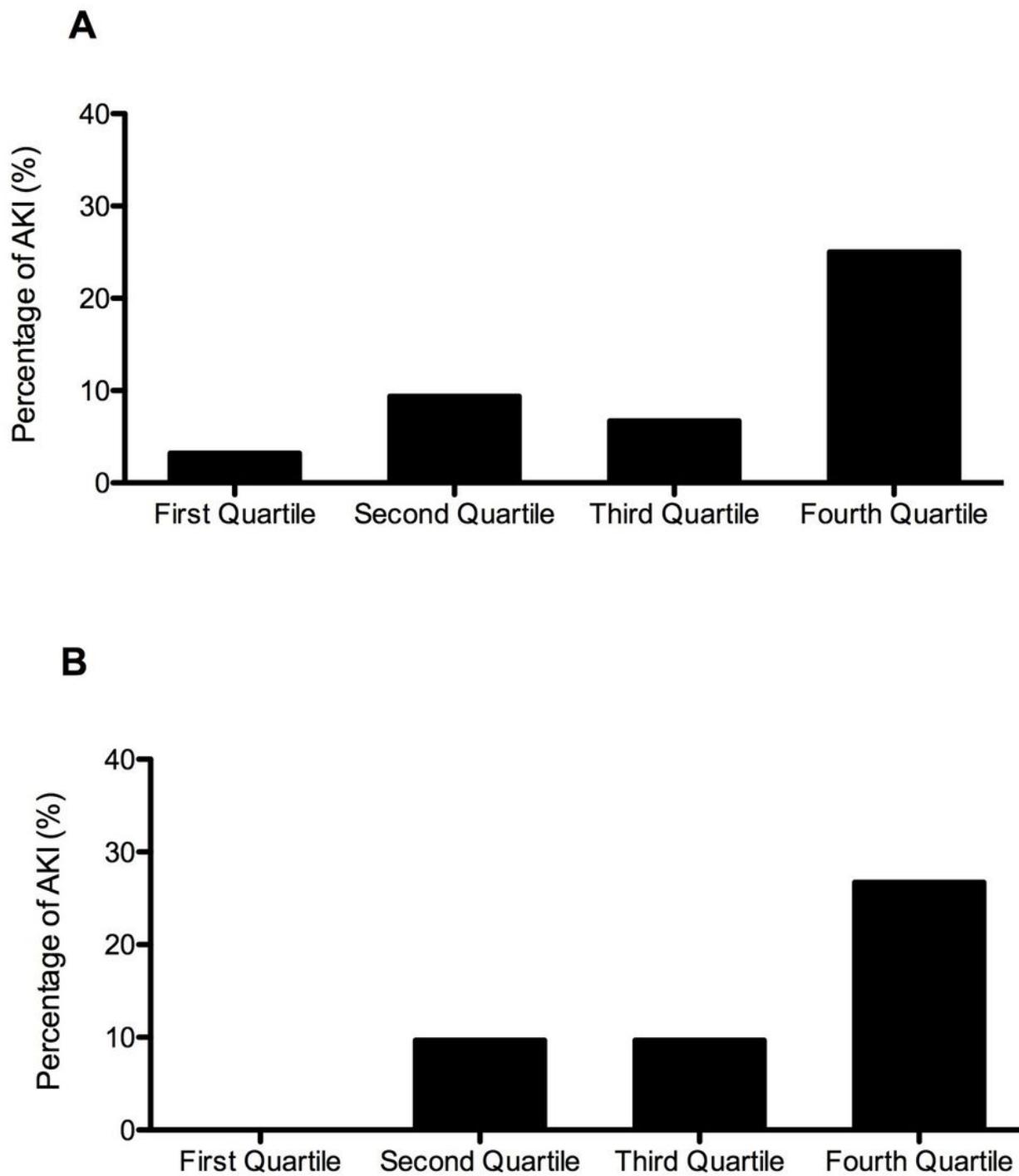


Figure 1

Concentrations of angiotensin-2 (A) and thrombomodulin (B) in patients with and without acute kidney injury. Open bars, patients without acute kidney injury (AKI); solid bars, patients with AKI. \*P < 0.05 compared with patients without AKI in the subgroup of estimated glomerular filtration rate (eGFR) < 60 ml/minute/1.73 m<sup>2</sup>. Data expressed as mean ± standard deviation.



**Figure 2**

Percentage of acute kidney injury (y-axis) among subjects with acute decompensated heart failure stratified by quartiles of plasma angiotensin-2 (A) and thrombomodulin (B).

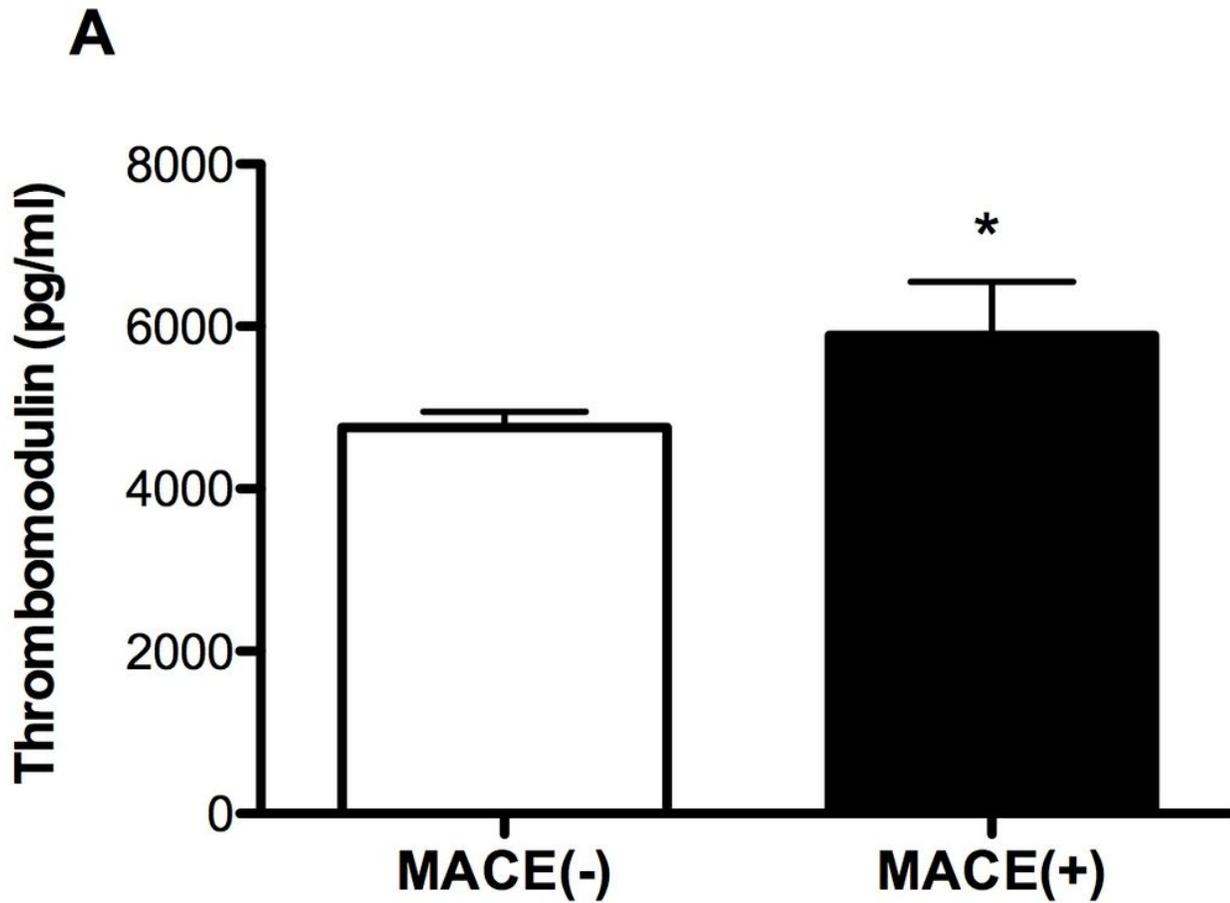


Figure 3

Plasma levels of thrombomodulin were significantly lower in patients without major adverse cardiac events (MACE) ( $4754.7 \pm 1896.8$  pg/ml) than in patients with multivessel disease ( $5892.1 \pm 3495.9$  pg/ml,  $p = 0.026$ ).

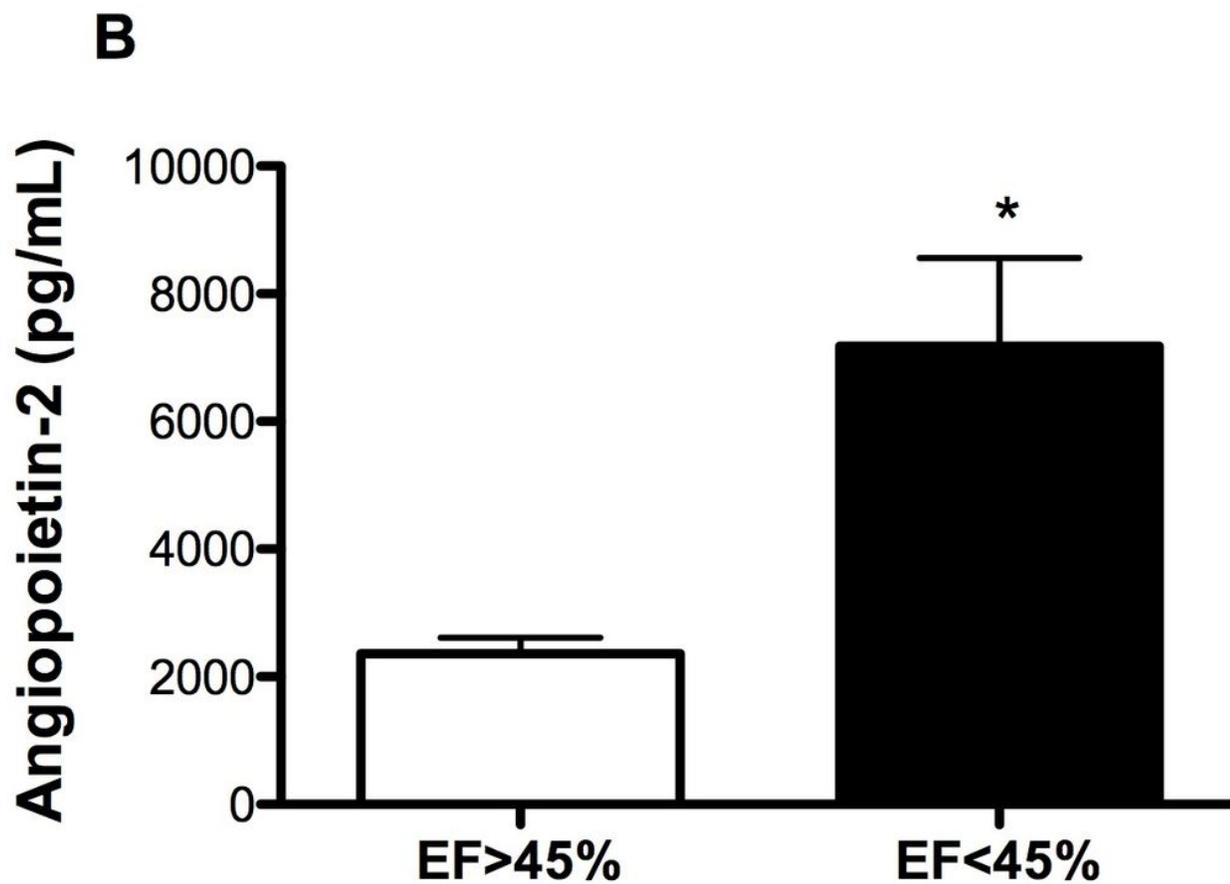


Figure 4

Plasma angiotensin-2 concentrations were significantly higher in patients with LVEF <45% (7178 ± 8416 pg/ml) than in patients with LVEF >45% (2361 ± 2295 pg/ml, p <0.0001).

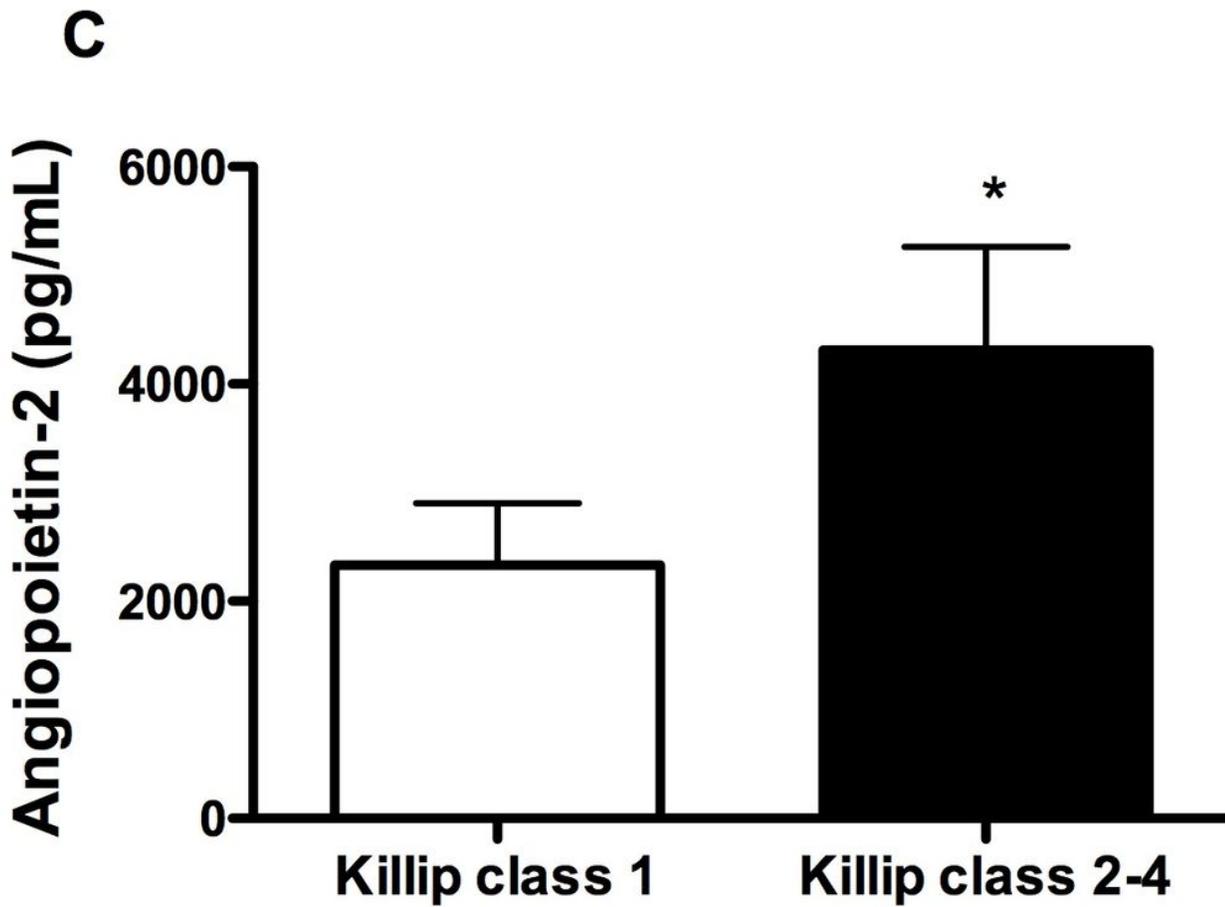


Figure 5

Plasma angiotensin-2 concentrations were significantly higher in patients with Killip class 2 to 4 ( $4314 \pm 5028$  pg/ml) than in those with Killip class 1 ( $2334 \pm 3887$  pg/ml,  $p = 0.001$ ).