Evaluation of Endothelial Biomarkers on the Prognosis of Patients on Extracorporeal Membrane Oxygenation

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Abstract

Background: Extracorporeal membrane oxygenation (ECMO) is often used in critical patients with severe myocardial failure. However, acute kidney injury (AKI) commonly occurs in patients on ECMO and usually brings about poor outcome. Recent studies suggest that renal vascular endothelial cell injury participates in the extent and maintenance of AKI. This study aimed to determine whether the endothelial biomarkers could serve as prognostic factors for the outcome of patients on ECMO.

Methods: This prospective study enrolled total 23 critically ill patients on veno-arterial ECMO in the intensive care units of a tertiary care hospital between March 2014 and February 2015. Serum samples were tested for thrombomodulin, angiopoietin (Ang)-1, Ang-2, and vascular endothelial growth factor (VEGF). Demographic, clinical, and laboratory data were also collected.

Results: The overall mortality rate was 56.5%. The combination of Ang-2 at the time of ECMO support (day 0) and VEGF at day 2 had modest prognostic ability of discriminating mortality (area under receiver operating characteristic curve [AUROC], 0.854; 95% confidence interval: 0.645-0.965).

Conclusions: In this study, we found that the combination of Ang-2 at day 0 and VEGF at day 2 was a modest model for mortality discrimination in this group of patients.

Introduction

Extracorporeal membrane oxygenation (ECMO) is often used in critical patients with severe myocardial failure (e.g., cardiogenic shock or myocarditis). It provides these patients with temporary circulatory support and has been utilized as a bridging therapy for further treatment. However, acute kidney injury (AKI) commonly occurs in patients on ECMO and usually brings poor outcome. The mortality increases as AKI stage progresses.1-4 Therefore, it is important to detect AKI as early as possible and give proper treatment soon to prevent AKI progression and further complications.

Previous studies showed that several intensive care unit (ICU) scoring systems and AKI classification systems have good ability in outcome prediction for patients on ECMO.5-9 However, these systems mostly used serum creatinine (SCr) and/or urine amount as renal function evaluation, which is not very sensitive in predicting early AKI. SCr increases slowly after the onset of AKI, and the timing of the intervention for AKI may be missed or delayed in critical patients until SCr level increases.

Recent studies suggested that renal vascular endothelial cell injury participates in the extent and maintenance of AKI. Early changes in peritubular capillary blood flow during reperfusion are related to loss of endothelial cell function, and a series of vascular physiologic alterations are associated with the progression of AKI.10 Thrombomodulin (TM) is a transmembranous glycoprotein found on the vascular endothelium.11 It is a marker of endothelial injury12 and related to the outcome of AKI.13,14

Angiopoietin (Ang)-1, Ang-2, and vascular endothelial growth factor (VEGF) are proteins associated with angiogenesis. Ang-1 has an anti-inflammatory effect with limiting endothelium activation, while Ang-2 triggers an inflammatory response by activating the endothelium. Besides, Ang-1 downregulates VEGF expression and reduces thrombin-induced permeability.15-17 In recent studies, lower Ang-1 concentration and high Ang-2 concentration are associated with increased risk of AKI.18-21 VEGF expresses increasingly in AKI and is important for maintenance of renal vasculature during AKI.22-23

Although endothelial activation and injury are involved in AKI, these has been no associated study on patients on ECMO. Compared to SCr, the serum biomarkers are more likely to be measured for early AKI detection, because they fluctuate during endothelial injury in the early phase of AKI. Therefore, this study aimed to determine whether the serum biomarkers of endothelial injury and activation could serve as prognostic factors for the outcome of patients on ECMO.

Materials And Methods

Study population and data collection

The local Institutional Review Board of Chang Gung Memorial Hospital approved the study protocol (Institutional Review Board No. 103-1569C). The study was performed in the ICUs of a tertiary care hospital in Taiwan between March 2014 and February 2015. Patients who met the inclusion criteria were invited to participate in the study on the first day of ECMO support. Written informed consent was obtained from the next-of-kin of the patients before their participation. The following patients were excluded: pediatric patients younger than 18 years old, patients with end stage renal disease undergoing regular renal replacement therapy, and patients whose next-of-kin declined study enrollment. Besides, patients with veno-venous (V-V) ECMO support were also excluded due to different pathophysiologic changes between veno-arterial (V-A) and V-V ECMO. For patients with repeated ECMO support during hospitalization, we only collected the data on the first ECMO support. Finally, total 23 patients were enrolled.

The following data were prospectively collected: demographic data, indications for ECMO support, and outcomes. Physiological calculations utilized the worst physiological values on the day of ECMO support. The primary study outcome was in-hospital mortality. Follow-up at 6 months after hospital discharge was performed via chart records or telephone interviews if necessary.

Sampling and quantifying serum biomarkers

Ten milliliters of blood were collected from each patient with routine blood tests performed at the time of ECMO support (day 0), the morning of the first post-ECMO day (day 1), and the morning of the second post-ECMO day (day 2). The blood samples were centrifuged at 1000 g for 5 minutes, and the supernatants were stored at -80°C. Serum biomarkers (Ang-1, Ang-2, VEGF, and TM) were quantified by an enzyme-linked immunosorbent assay (R&D system, Minneapolis, MN, USA) according to manufacturer instructions.

Acute kidney injury definition

SCr and urine amount on the day of ECMO support were used for AKI definition. AKI status was determined by changes in the SCr and urine amount according to the Kidney Disease Improving Global Outcomes (KDIGO) criteria.24 The lowest SCr level within 7 days before ECMO implementation was used as baseline SCr. AKI was defined as an increase of the SCr level exceeding 0.3 mg/dl within 48 hours, or an increase in SCr to 1.5 times the baseline value within 7 days, or a urine output less than 0.5 ml/kg/hr for 6 hours. Severe AKI was defined as KDIGO stages 2 or 3.

Clinical management

The ECMO device (Medtronic, Inc., Anaheim, CA) was composed of a centrifugal pump and a hollow-fiber microporous membrane oxygenator with an integrated heater. All ECMO circuits had a heparin-bound Carmeda bioactive surface. A silicone oxygenator (Medtronics, Minneapolis, MN, USA) was incorporated into the ECMO circuit. A 17–19 Fr percutaneous arterial (outflow) cannula and a 19–21 Fr percutaneous venous (inflow) cannula (DLP; Medtronic Inc., Minneapolis, MN) were chosen according to patients' body size. Percutaneous access through the common femoral vein (inflow) and the common femoral artery (outflow) was preferred for V-A ECMO. If cyanosis was noted on the cannulated limb, an 8 Fr distal perfusion catheter would be implanted into the ipsilateral superficial femoral artery.

Statistical analysis

There was no sufficient power to test normality of continuous variables due to the small sample size of this study. Therefore, all statistical tests were done using nonparametric statistics. Descriptive statistics of continuous variables were expressed as median with interquartile range. Data between the survivors and non-survivors were compared using Mann-Whitney U test for continuous variables or Fisher's exact test for categorical variables. The performance of discriminating mortality by those biomarkers at day 0, day 1, and day 2 of ECMO support was assessed using receiver operating characteristic (ROC) curve analysis. Finally, those significant biomarkers in the univariate analysis were introduced into the multivariable logistic regression model with adjustment of age and sex. All statistical tests were two-tailed, and a value of $P < 0.05$ was considered statistically significant. No adjustment for multiple testing (multiplicity) was made in this study. Statistics analysis was conducted using SPSS 22 software (IBM SPSS, Armonk, NY: IBM Corp).

Results

Between March 2014 and February 2015, 23 patients on ECMO support at the ICU were enrolled. The average age was 57 years and 19 (82.6%) were male. The in-hospital mortality rate was 56.5% (13/23). Table 1 presents the patients' demographic data and clinical characteristics. No significant difference was found for diabetes mellitus, coronary artery disease, intraaortic balloon pumping use, ECMO indication, and biochemistry data between survivors and non-survivors. Table 2 shows the concentration changes of biomarkers at day 0, day 1, and day 2 of ECMO support. TM and Ang-1 concentrations

showed no significant difference between survivors and non-survivors during the first two days. Noticeably, decreased Ang-2 level at day 0 (median: 15.7 vs. 24.4 ng/mL, $P = 0.035$) and tremendously increased VEGF level at day 2 (median: 119.9 vs. 24.2 pg/mL, $P = 0.005$) were observed in the survivors as compared to non-survivors (Figure 1). Figure 2 depicts the ROC curves of the four biomarkers in discriminating mortality at day 0, day 1, and day 2 of ECMO support. We found that the combined predicted probability of Ang-2 at day 0 and VEGF at day 2 had modest prognostic ability of discriminating mortality (area under the ROC curve, 0.854; 95% confidence interval [CI], 0.645-0.965; as shown in Figure 2D). As shown in Table 3, the multivariable logistic regression model identified that decreased VEGF level at day 2 was associated with higher risks of in-hospital mortality (Odds ratio, 0.97; 95% CI, 0.93–0.999; $P = 0.044$).

Discussion

This study is the first one investigating the relationship between endothelial biomarkers and mortality in patients on ECMO. In this study, we noticed an increased Ang-2 level in non-survivors compared with survivors. Besides, we also observed that the combination of Ang-2 at day 0 and VEGF at day 2 showed a modest performance on mortality discrimination in patients on ECMO.

The initiation of ECMO brings an immediate and complex inflammatory reaction in patients, as seen in systemic inflammatory response syndrome. The inflammatory reaction then results in the widespread activation of the endothelium and induces pro-inflammatory cytokines secretion.25 Moreover, active diseases that require ECMO support may be associated with endothelial inflammation, such as cardiotomy surgery and acute myocardial infarction. AKI following ECMO support is also related to endothelial injury.10 Therefore, endothelial injury is an important issue in patients on ECMO.

Previous studies showed that Ang-2 level was associated with mortality in critically-ill patients.18, 26-29 Ang-2, a competitive antagonist of Ang-1, reacted with Tie2 receptor to keep vascular stabilization. Upon inflammatory stimulus, Ang-2 was released from the Weibel-Palade bodies, causing capillary leakage and facilitating leukocyte migration.30 In patients on ECMO, Ang-2 increased in response to early endothelial activation. Although it didn't reveal a close relationship with AKI in our study, it still provided a potential marker for mortality prediction in patients on ECMO.

VEGF is considered as an endothelial survival factor that prevents microvascular apoptotic cell loss in vitro.31 Both low and high VEGF concentrations have been reported in critically-ill patients27, 32-33, and the significance of which is not fully understood. In our study, the VEGF concentration in the survivor group continued to increase over the first 72 hours and was higher than the non-survivor group, which was similar to previous studies.32 VEGF modulates the effect of Ang-2 in a context-dependent fashion: Ang-2 promotes basal lamina remodeling and endothelial cell proliferation at high VEGF concentration, but causes endothelial cell death and vessel regression if VEGF is inhibited.34 In our study, we observed that survivors had significantly higher 72-hour VEGF concentration compared to non-survivors. Higher VEGF concentration may modulate the Ang-2 effect and help endothelial cell proliferation and

neovascularization, but the detailed relationship with mortality needs further studies to evaluate and confirm.

There are some limitations in our study. First, our study was performed in a tertiary care center with a small sample size. Although it was a prospective study, many next-of-kin of the patients declined to join the study at the time of ECMO support due to the critical condition of the patients. Large-scale studies at multiple centers should be performed to confirm these findings. Second, although we excluded patients on V-V ECMO support and only collected patients on V-A ECMO support, the diversity of the diseases indicated for ECMO support may still affect the results, and further subgroup investigations are needed to explore the relationship between specific diseases and endothelial biomarkers. Third, we did not compare the differences in the endothelial biomarkers levels with a control group because we could not find a group of patients with the same disease severity but without ECMO support.

In summary, we presented the relationship between endothelial biomarkers change and mortality in patients on V-A ECMO. The combination of Ang-2 at day 0 and VEGF at day 2 was a modest model for mortality discrimination in this group of patients. However, further larger studies are warranted due to the small sample size at a single tertiary-care medical center in this study.

Declarations

Ethics approval and consent to participate:

Written informed consent was obtained from the next-of-kin of the patients before their participation. The study was approved by the local Institutional Review Board of Chang Gung Memorial Hospital approved the study protocol (Institutional Review Board No. 103-1569C).

Consent for publication:

Not applicable.

Availability of data and material:

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

Competing interests:

The authors declare that they have no competing interests.

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No funding was received.

Authors' contributions:

TYT contributed to collecting data and manuscript drafting. KHT and CHC revised the manuscript and conducted the statistical analysis. FCT and YYN helped with acquisition and interpretation of data. PCF conducted the statistical analysis. YCT, JTF, and CWY contributed to provide intellectual content of the work and involved in editing the manuscript. YCC contributed to the conception, design, and interpretation of data. All authors critically revised the manuscript. All authors have seen and approved the final draft of the manuscript.

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Table 1. Patients' Demographic Data And Clinical Characteristics

Variable	All Patients (n = 23)	Non-Survivors (n = 10)	Survivors (n = 13)	P value
Age (years)	57 (19)	55 (7)	58 (20)	0.250
Male sex, n (%)	19 (82.6)	7 (70)	12 (92.3)	0.281
Diabetes mellitus, n (%)	4 (17.4)	1 (10)	3 (23.1)	0.604
Coronary artery disease, n (%)	15 (65.2)	5 (50)	10 (76.9)	0.221
IABP, n (%)	18 (78.3)	8 (80)	10 (76.9)	1.000
Indication for ECMO, n (%)				0.119
Postcardiotomy	12 (52.2)	5 (50)	7 (53.8)	
Myocarditis	1 (4.3)	1 (10)	0 (0)	
Acute myocardial infarction	6 (26.1)	1 (10)	5 (38.5)	
Heart transplantation	1 (4.3)	1 (10)	0 (0)	
Profound shock with desaturation	2 (8.7)	2 (20)	0 (0)	
VT with cardiogenic shock	1 (4.3)	0 (0)	1 (7.7)	
Biochemistry data on ECMO 1^{st} day				
MAP (mmHg)	58 (19)	55 (21)	59 (14)	0.306
UO (L/day)	1.6 (1.9)	1.7 (2.4)	1.6 (1.4)	0.852
SCr (mg/dL)	1.4 (0.8)	1.3 (0.5)	1.5 (0.9)	0.321
WBC count (cu/mm) x 1000	16.0 (17.8)	16.9 (12.3)	15.7 (17.8)	0.756
Hemoglobin (g/dL)	9.2 (1.5)	9.1 (1.1)	9.4 (2.0)	0.710
Platelets (x10^9/L)	9.7 (8.6)	9.0 (7.9)	10.2 (10.9)	0.535
Sodium (mEq/L)	143 (18)	147 (20)	143 (11)	0.456
Potassium (mEq/L)	3.2 (1.8)	3.2 (2.0)	3.6 (1.5)	0.926
Albumin (g/L)	2.7 (0.7)	2.8 (0.2)	2.7 (1.1)	1.000
Lactate (mmol/L)	79.4 (48.9)	83.2 (75.2)	75.3 (15.2)	0.710
APACHE II score	23 (10)	26 (10)	23 (8)	0.153
Acute kidney injury, n (%)	18 (78.3)	8 (80.0)	10 (76.9)	1.000

Continuous data were presented median (interquartile);

IABP, intraaortic balloon pumping; ECMO, extracorporeal membrane oxygenation; VT, ventricular tachycardia; MAP, mean arterial pressure; UO, urine output; SCr, serum creatinine; WBC, white blood cell; APACHE II, acute physiology and chronic health evaluation II.

Table 2. Patients' Endothelial Biomarkers In The First 3 Days

Biomarker	All Patients ($n = 23$)	Non-Survivors ($n = 10$)	Survivors ($n = 13$)	P value
Thrombomodulin (ng/mL)				
Day 0	5.9 (2.4)	6.3 (1.9)	5.6 (1.8)	0.420
Day 1	6.3 (1.9)	6.0 (1.9)	6.7 (1.6)	0.535
Day 2	7.5 (2.7)	6.8 (3.0)	7.5 (2.2)	0.215
Angiopoietin-1 (ng/mL)				
Day 0	29.0 (16.1)	30.8 (13.0)	22.1 (18.7)	0.203
Day 1	24.9 (17.1)	24.2 (8.8)	26.2 (15.8)	0.107
Day 2	20.7 (13.8)	20.1 (6.6)	22.2 (9.5)	0.172
Angiopoietin-2 (ng/mL)				
Day 0	19.2 (24.8)	24.4 (58.2)	15.7 (23.2)	0.035
Day 1	24.7 (35.3)	25.6 (29.0)	17.7 (26.6)	0.137
Day 2	22.7 (15.4)	23.7 (14.7)	20.3 (9.2)	0.577
VEGF (pg/mL)				
Day 0	8.5 (13.7)	15.3 (32.4)	7.9 (2.1)	0.071
Day 1	33.0 (65.0)	24.2 (37.6)	35.6 (58.1)	0.438
Day 2	62.1 (119.2)	24.2 (33.9)	119.9 (105.8)	0.005

Data were presented median (interquartile);

ECMO, extracorporeal membrane oxygenation; VEGF, vascular endothelial growth factor.

Table 3. Multivariable Logistic Regression Analysis For Predictive Markers Of Mortality

Variable	Odds ratio (95% CI)	P value
Age (years)	0.96 (0.87–1.05)	0.358
Male sex	0.77 (0.04–16.14)	0.866
Angiopoietin-2 at day 0	1.00 (0.99–1.02)	0.619
VEGF at day 2	0.97 (0.93–0.999)	0.044

CI, confidence interval; VEGF, vascular endothelial growth factor.

Figures

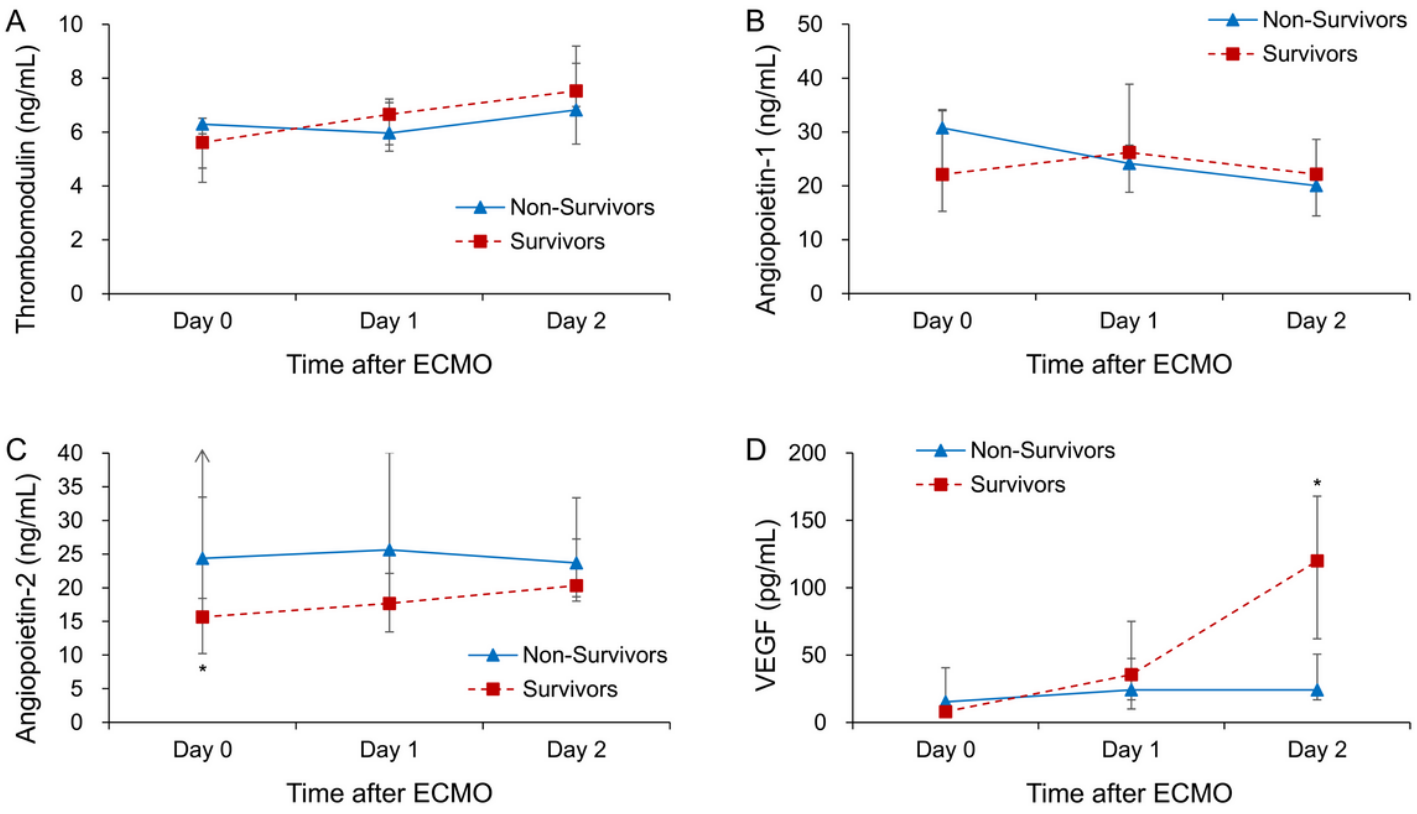

Figure 1

Median values (lower limit of bar represents 25th percentile and upper limit of bar represents 75th percentile) of endothelial biomarkers in the non-survivors and survivors. * indicates $P < 0.05$ between non-survivors and survivors. VEGF, vascular endothelial growth factor.

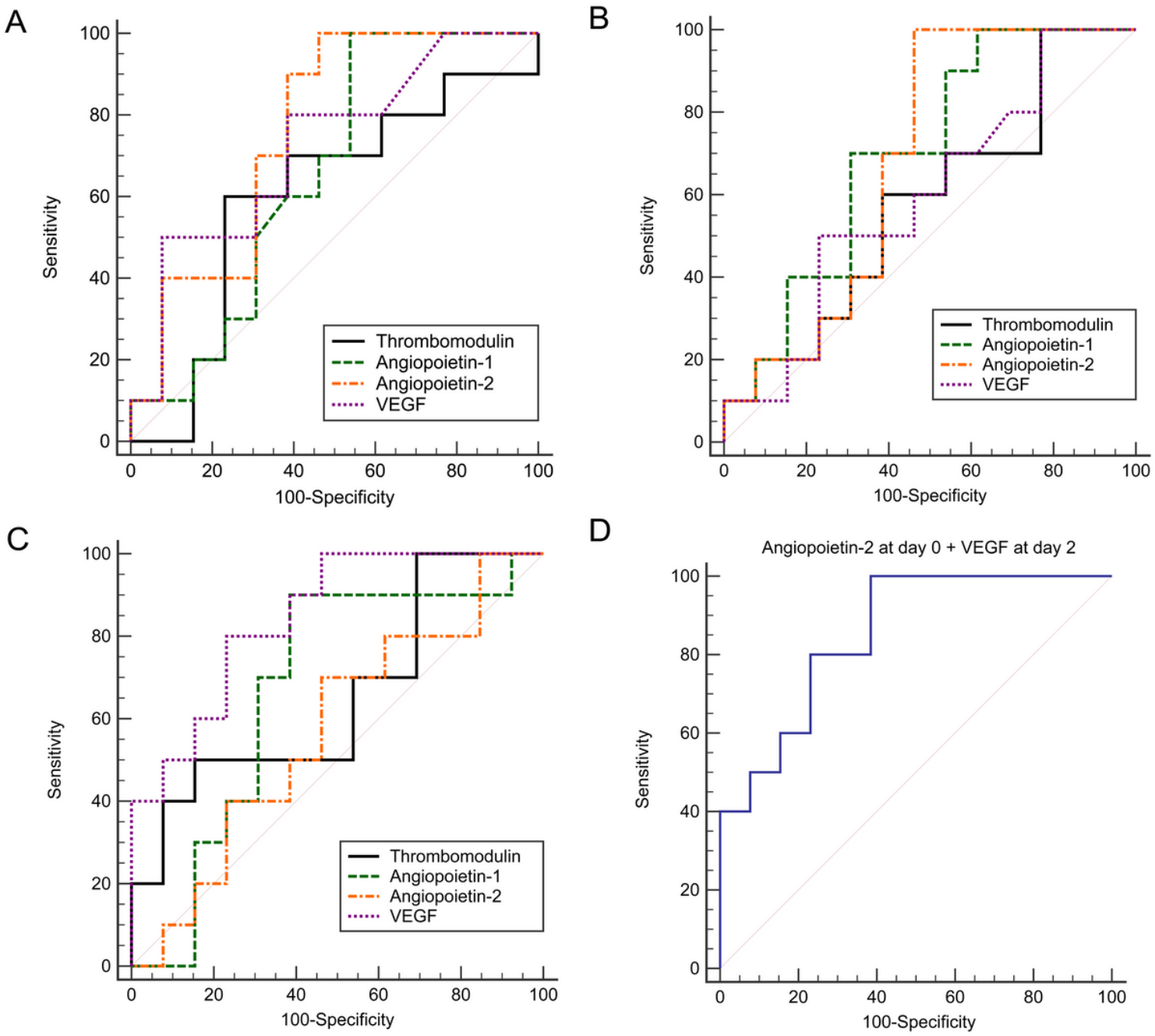

Figure 2

Receiver operating characteristic curves (ROC) of discriminating mortality for (A) at day 0, (B) at day 1, (C) at day 2, and (D) combination of angiopoietin-2 at day 0 and VEGF at day 2. The area under ROC of angiopoietin-2 at day 0 + VEGF at day 2 was 0.854 (95% confidence interval: 0.645 to 0.965).

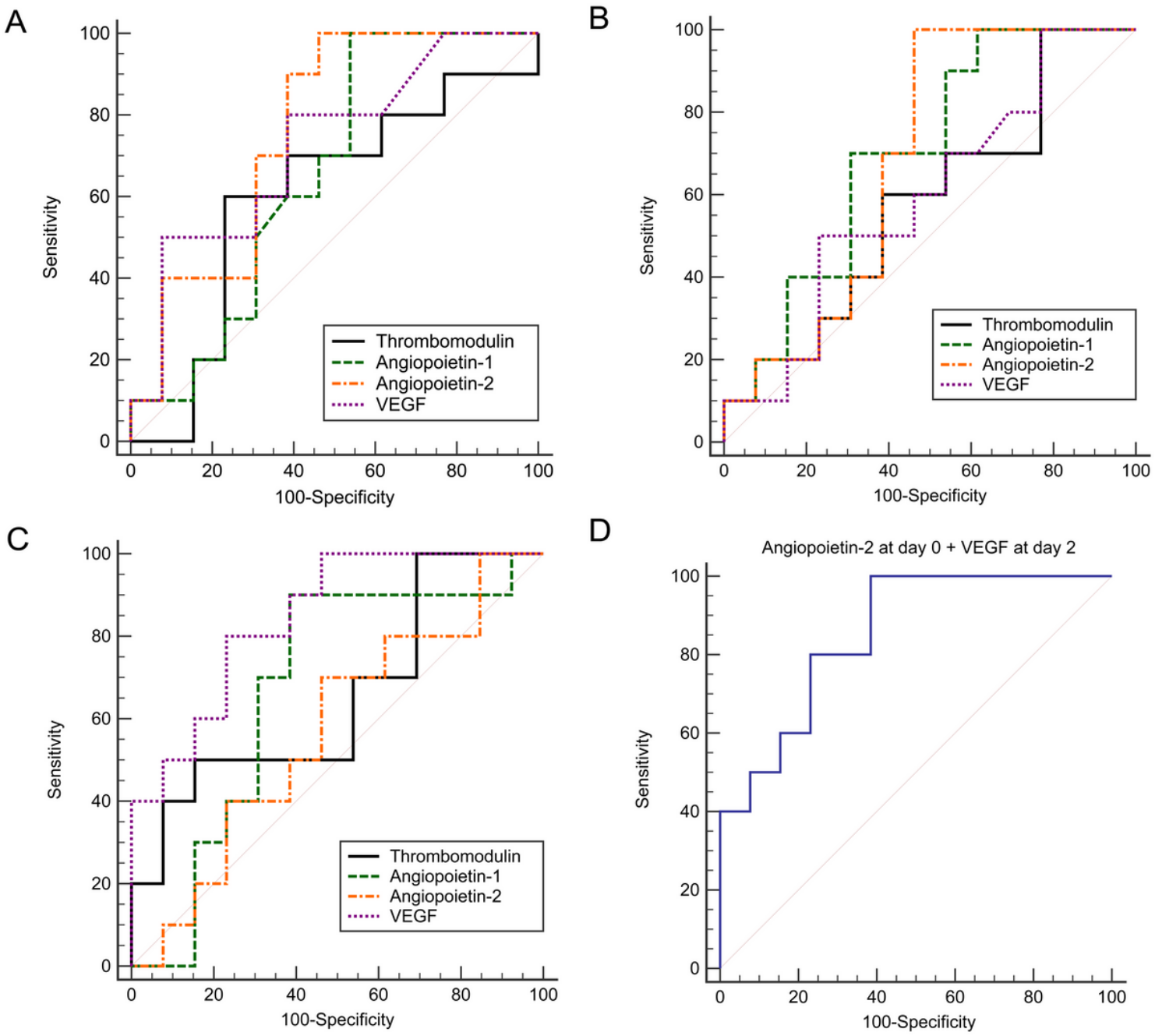
A
Sensitivity
100-Specificity
Thrombomodulin
Angiopoietin-1
Angiopoietin-2
VEGF
B
Sensitivity
100-Specificity
Thrombomodulin
Angiopoietin-1
Angiopoietin-2
VEGF
C
Sensitivity
100-Specificity
Thrombomodulin
Angiopoietin-1
Angiopoietin-2
VEGF
D
Angiopoietin-2 at day 0 + VEGF at day 2
Sensitivity
100-Specificity

Figure 3