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, patients on ECMO often have high mortality rate and poor prognosis. Recent studies suggest that endothelial activation with subsequent vascular barrier breakdown is a critical pathogenic mechanism in organ damage and related to the outcome in critical illness. 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, patients on ECMO still have high mortality rate and poor prognosis though the technique and post-operation care of ECMO supplement has progressed much in recent decades.1-4

Previous studies showed that several intensive care unit (ICU) scoring systems have good ability in outcome prediction for patients on ECMO.1,5,6 However, these scoring systems usually consist of many laboratory data and physiological measurements, and sometimes need complex calculation. Recently, several biomarkers are applied in predicting renal and neurologic outcomes in patients on ECMO, but no particular biomarker is associated with mortality in this patient group.

Recent studies suggest that endothelial activation with subsequent vascular barrier breakdown is a critical pathogenic mechanism in organ damage and related to the outcome in critical illness.9-12 Thrombomodulin (TM) is a transmembranous glycoprotein found on the vascular endothelium.13 It enhances thrombin-induced activation of protein C and has roles in inflammation, coagulation, and fibrinolysis.14 Soluble thrombomodulin levels are associated with mortality in patients with disseminated intravascular coagulation, sepsis, or acute respiratory distress syndrome.12,15,16 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.17-19 In recent studies, lower Ang-1 concentration and high
Ang-2 concentration are associated with increased mortality of patients with sepsis. However, the relationship between VEGF level and mortality is discordant in different studies.

Although endothelial activation and injury are involved in organ damage and associated with the prognosis in critical illness, there has been no associated study on patients on ECMO. 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. 66 patients were screened during the study period, but next-of-kin of 43 patients refused the study due to the critical condition of the patients. 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 30-day 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.

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 (Medtronic, 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. Non-survivors had higher vasopressor/inotrope dose and higher Sequential Organ Failure Assessment (SOFA) score than survivors at the day of ECMO supplement. 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. Moreover, active diseases that require ECMO support may be associated with endothelial inflammation, such as cardiectomy surgery and acute myocardial infarction. Non-pulsatile flow during aortic cross-clamping during cardiectomy is associated with diminished endothelial shear stress with reduction in endothelial nitrogen oxide production, while intra-aortic balloon pump support provides steady pulsatile flow, which induces a steady shear stress on the endothelial cells, reducing endothelial activation and inflammatory response. Acute kidney injury following ECMO support is also related to endothelial injury. 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. 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. In patients on ECMO, Ang-2 increased in response to early endothelial activation. Although it didn’t reveal a close relationship with acute kidney injury 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. Both low and high VEGF concentrations have been reported in critically-ill patients, 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. 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. 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.

**Abbreviations**

ECMO, Extracorporeal membrane oxygenation; Ang, angiopoietin; VEGF, vascular endothelial growth factor; AUROC, area under receiver operating characteristic curve; ICU, intensive care unit; TM, Thrombomodulin; V-V, veno-venous; V-A, veno-arterial; ROC, receiver operating characteristic; SOFA, sequential organ failure assessment; CI, confidence interval.

**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.

**Funding:**

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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Tables
**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   |
| Duration of ECMO support (days)         | 5 (5)                 | 7 (11)                 | 4 (1)              | 0.483   |
| Duration of ICU stay (days)             | 11 (10)               | 8 (11)                 | 17 (38)            | 0.020   |
| Mechanical ventilation (days)           | 8 (8)                 | 8 (11)                 | 8 (8)              | 0.454   |
| IABP, n (%)                             | 18 (78.3)             | 8 (80)                 | 10 (76.9)          | 1.000   |
| Myocardial failure during operation     | 10 (55.6)             | 6 (75)                 | 4 (40)             | 0.188   |
| Cardiogenic shock                       | 8 (44.4)              | 2 (25)                 | 6 (60)             | 0.188   |
| 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)            |         |
| Complication of ECMO, n (%)             |                      |                        |                    |         |
| Lower extremity ischemia                | 2 (8.7)               | 1 (10)                 | 1 (7.7)            | 1.000   |
| Stroke                                  | 1 (4.3)               | 1 (10)                 | 0 (0)              | 0.435   |
| Coma or brain hypoxia                   | 4 (17.4)              | 4 (40)                 | 0 (0)              | 0.024   |
| Significant bleeding                    | 8 (34.8)              | 4 (40)                 | 4 (30.8)           | 0.685   |
| Rethoractomy for bleeding               | 5 (21.7)              | 2 (20)                 | 3 (23.1)           | 1.000   |
| Vasopressor/inotrope on ECMO 1st day    |                      |                        |                    |         |
| Dopamine (μg/kg/min)                    | 0.0 (9.5)             | 0.0 (4.7)              | 0.0 (10.7)         | 0.538   |
| Norepinephrine (μg/kg/min)              | 0.1 (0.2)             | 0.1 (0.3)              | 0.0 (0.2)          | 0.324   |
| Dobutamine (μg/kg/min)                  | 0.0 (6.3)             | 5.0 (5.0)              | 0.0 (0.0)          | 0.032   |
|                         | 0.1 (0.4) | 0.4 (0.4) | 0.0 (0.2) | 0.027 |
|-------------------------|-----------|-----------|-----------|-------|
| **Biochemistry data on ECMO 1st day** |           |           |           |       |
| Epinephrine (μg/kg/min) |           |           |           |       |
| MAP (mmHg)              | 58 (19)   | 55 (21)   | 59 (14)   | 0.306 |
| Diuresis(ml/kg/hr)      | 0.9 (1.1) | 1.2 (1.0) | 0.9 (1.0) | 0.495 |
| 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 (x109/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 |
| PaO2/FiO2               | 384 (235) | 187 (411) | 395 (99)  | 0.193 |
| AaDO2                   | 237 (163) | 388 (382) | 235 (87)  | 0.172 |
| APACHE II score         | 23 (10)   | 26 (10)   | 23 (8)    | 0.153 |
| SOFA score              | 10 (5)    | 11 (5)    | 9 (2)     | 0.026 |
| Acute kidney injury, n (%)| 18 (78.3) | 8 (80)    | 10 (76.9) | 1.000 |
| KDIGO criteria (Stage 0/1/2/3) | 5/10/4/4 | 2/4/3/1 | 3/6/1/3 | 0.572 |
| Renal replacement therapy, n (%) | 10 (43.5) | 4 (40) | 6 (46.2) | 1.000 |

Continuous data were presented median (interquartile);

ECMO, extracorporeal membrane oxygenation; ICU, intensive care unit; IABP, intraaortic balloon pumping; VT, ventricular tachycardia; MAP, mean arterial pressure; SCr, serum creatinine; WBC, white blood cell; PaO2, partial pressure of oxygen; FiO2, fraction of inspired oxygen; AaDO2, alveolar-arterial oxygen tension difference; APACHE II, acute physiology and chronic health evaluation II; SOFA, sequential organ failure assessment; KDIGO, kidney disease improving global outcomes.

**Table 2.** Patients’ endothelial biomarkers in the first 3 days
### Biomarker Levels

| 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); 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).