



Endotoxin Activity in Patients With Extracorporeal Membrane Oxygenation Life Support: An Observational Pilot Study

Chen-Tse Lee¹, Chih-Hsien Wang^{2*†}, Wing-Sum Chan³, Yun-Yi Tsai¹, Tzu-Jung Wei¹, Chien-Heng Lai², Ming-Jiuh Wang¹, Yih-Shang Chen² and Yu-Chang Yeh^{1*†}

¹ Department of Anesthesiology, National Taiwan University Hospital, College of Medicine, National Taiwan University, Taipei, Taiwan, ² Department of Surgery, National Taiwan University Hospital, College of Medicine, National Taiwan University, Taipei, Taiwan, ³ Department of Anesthesiology, Far Eastern Memorial Hospital, New Taipei, Taiwan

OPEN ACCESS

Edited by:

Xiaotong Hou,
Capital Medical University, China

Reviewed by:

Laura Borgstedt,
Technical University of
Munich, Germany
Koichiro Shinozaki,
Feinstein Institute for Medical
Research, United States

*Correspondence:

Chih-Hsien Wang
wchemail@ntu.edu.tw
Yu-Chang Yeh
tonyyeh@ntuh.edu.tw

[†]These authors have contributed
equally to this work

Specialty section:

This article was submitted to
Intensive Care Medicine and
Anesthesiology,
a section of the journal
Frontiers in Medicine

Received: 08 September 2021

Accepted: 31 October 2021

Published: 29 November 2021

Citation:

Lee C-T, Wang C-H, Chan W-S,
Tsai Y-Y, Wei T-J, Lai C-H, Wang M-J,
Chen Y-S and Yeh Y-C (2021)
Endotoxin Activity in Patients With
Extracorporeal Membrane
Oxygenation Life Support: An
Observational Pilot Study.
Front. Med. 8:772413.
doi: 10.3389/fmed.2021.772413

Background: Extracorporeal membrane oxygenation (ECMO) life support has become an integral part of intensive care. The endotoxin activity assay (EAA) is a useful test to measure endotoxemia severity in whole blood. To date, no information is available regarding the EAA levels and their effect on clinical outcomes in critically ill patients with ECMO support.

Methods: This prospective observational pilot study enrolled adult critically ill patients with ECMO support from August 2019 to December 2020. The EAA levels were measured within 24 h (T1), and at 25–48 (T2), 49–72 (T3), and 73–96 h (T4) after ECMO initiation. This study primarily aimed to investigate the incidence of high EAA levels (≥ 0.6) at each time point. Subsequent exploratory analyses were conducted to compare the EAA levels of venoarterial ECMO (VA-ECMO) patients between 30-day survivors and non-survivors. *Post-hoc* analysis was performed to compare the clinical outcomes of VA-ECMO patients with elevated EAA levels at T3 (vs. T1) and those without elevated EAA levels.

Results: A total of 39 VA-ECMO patients and 15 venovenous ECMO (VV-ECMO) patients were enrolled. At T1, the incidence of high EAA level (≥ 0.6) was 42% in VV-ECMO patients and 9% in VA-ECMO patients ($P = 0.02$). At T2, the incidence of high EAA level was 40% in VV-ECMO patients and 5% in VA-ECMO patients ($P = 0.005$). In VA-ECMO patients, EAA levels at T3 were significantly higher in 30-day non-survivors than in survivors (median [interquartile range]: 0.49 [0.37–0.93] vs. 0.31 [0.19–0.51], median difference 0.16 [95% confidence interval [CI], 0.02–0.31]; $P = 0.024$). Moreover, VA-ECMO patients with elevated EAA levels at T3 (vs. T1) had lower 30-day survival than patients without elevated EAA levels (39 vs. 83%, $P = 0.026$) and fewer ECMO free days by day 30 (median: 3 vs. 23 days, median difference 12 days [95% CI, 0–22]; $P = 0.028$).

Conclusions: A certain proportion of patients experienced high EAA levels (≥ 0.6) after VV-ECMO or VA-ECMO initiation. VA-ECMO patients with an elevated EAA level at 49–72 h were associated with poor clinical outcomes.

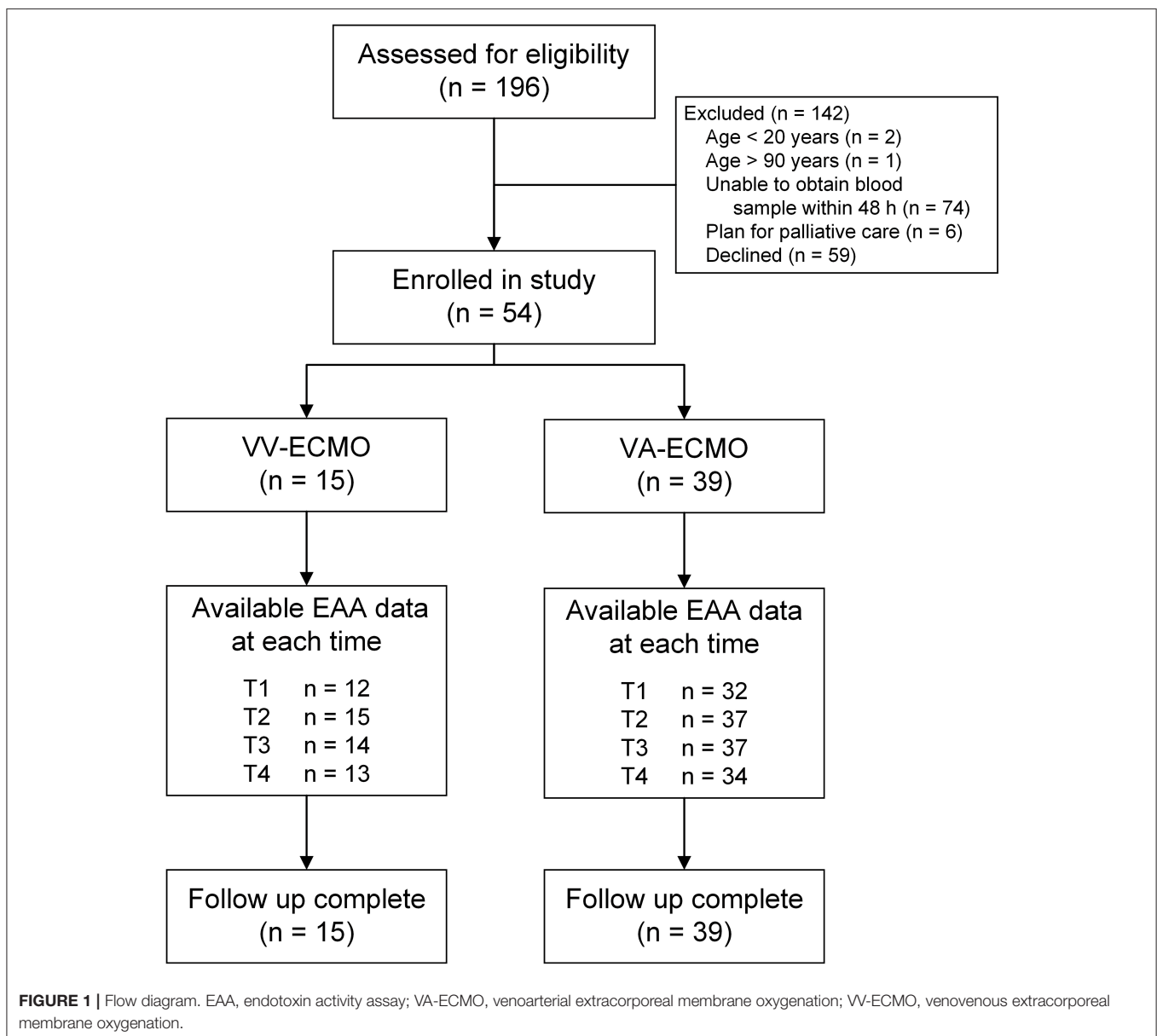
Keywords: critical care, endotoxin, extracorporeal membrane oxygenation (ECMO), survival, infection

INTRODUCTION

Extracorporeal membrane oxygenation (ECMO) life support has become an integral part of intensive care (1), but contingent infection increases morbidity and mortality in patients receiving ECMO (2, 3). Venovenous ECMO (VV-ECMO) is primarily indicated for patients with acute respiratory distress syndrome (ARDS), which is frequently associated with pneumonia and sepsis. Venoarterial ECMO (VA-ECMO) is primarily used for supporting patients with refractory cardiogenic shock. According to our clinical experience, fever and sepsis after VA-ECMO are common. In addition to some common nosocomial infections, severe hypoperfusion can disrupt the intestinal barrier and result in bacteremia and endotoxemia (4, 5).

High endotoxin levels are associated with poor survival outcomes in critically ill patients (6). A novel immunoassay

using neutrophil-dependent chemiluminescence, the endotoxin activity assay (EAA), was developed to measure endotoxin activity in whole blood (7). Previous research demonstrated that an EAA level ≥ 0.6 was strongly associated with the development of septic shock and mortality in critically ill patients (8). However, the incidence and effects of high endotoxin levels on clinical outcomes in critically ill patients on ECMO support remain unclear. The primary goal of this pilot study was to investigate that how many critically ill patients with VV-ECMO or VA-ECMO support would have an EAA level higher than 0.6. In addition, for these patients with VV-ECMO or VA-ECMO support, the secondary goals of this study were to investigate whether survivors and non-survivors were different in EAA levels, and to investigate whether patients with or without elevated EAA levels had different clinical outcomes within 30 days after ECMO initiation.



MATERIALS AND METHODS

Patients

This prospective observational pilot study was approved by the Research Ethics Committee of National Taiwan University Hospital (approval number 201811061RINC), and was registered at clinicaltrials.gov (NCT03978728). This study was conducted between August 2019 and December 2020 and was reported in accordance with the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines (9). Adult critically ill patients who underwent VV-ECMO or VA-ECMO were evaluated for eligibility within 24 h of ECMO initiation. Patients were excluded if they aged younger than 20 years or elder than 90 years, had do-not-resuscitate orders, underwent extracorporeal endotoxin adsorption treatment during the study period, had no blood sample obtained within 48 h of ECMO initiation, or did not speak Taiwanese natively. Informed consent was obtained from the legally authorized representatives of all participants before enrollment. A total of 196 critically ill patients receiving ECMO support were assessed for the eligibility of this study.

ECMO Components

The implementation and principal components of ECMO for all enrolled patients were as described in our previous study (10). The VA-ECMO was placed in the femoral vein (21–23 French) and artery (15–19 French), and VV-ECMO was achieved using femoral inflow (21–23 French) and jugular outflow (15–19 French). To avoid malperfusion of the distal limb, an antegrade distal perfusion catheter was used when the mean pressure of the superficial femoral artery was <50 mmHg (11). All enrolled patients received standard ECMO management and intensive care unit (ICU) care. Heparin was continuously administered to maintain an activated clotting time of 160–180 s if no active bleeding or other complications were observed. Antibiotic prophylaxis with vancomycin and ceftazidime were administered shortly after ECMO to patients without antibiotic use; the antibiotics were subsequently downgraded in accordance with institutional protocols.

Data Collection

Demographic data including age, sex, height, body weight, indications for ECMO support, Survival after VA-ECMO (SAVE) score, Respiratory ECMO Survival Prediction (RESP) score, and Acute Physiology and Chronic Health Evaluation (APACHE) II score were recorded after enrollment. Blood samples were obtained at four time points after ECMO initiation: within 24 h (T1) and at 25–48 (T2), 49–72 (T3), and 73–96 h (T4). White blood cell (WBC) counts; lactate, procalcitonin, diamine oxidase (DAO), and cystatin C levels; and EAA levels were examined. The inotropic score (IE) was calculated at the four time points as $100 \times \text{epinephrine dose } (\mu\text{g/kg/min}) + 100 \times \text{norepinephrine dose } (\mu\text{g/kg/min}) + \text{dopamine dose } (\mu\text{g/kg/min}) + \text{dobutamine dose } (\mu\text{g/kg/min})$ (12). The duration of ECMO support and survival status at 30 days were recorded.

Measurements of EAA Levels and Blood Inflammatory Biomarkers

The blood samples were collected from the peripheral arterial line. An EAA measurement was performed within 3 h after blood sample collection using the EAA Kit (Spectral Medical Inc., Toronto, Canada) according to the manufacturer's instructions. An EAA level ≥ 0.6 was considered high. Biomarkers were measured using commercially available enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturer's instructions. Procalcitonin was measured using the Human Procalcitonin SimpleStep ELISA Kit (Abcam, Cambridge, UK). DAO was measured using the IDK DAO ELISA Kit (Immundiagnostik AG, Bensheim, Germany). Finally, cystatin C was measured using the Cystatin C (CST3) (Human) ELISA Kit (Biovision, Inc., CA, USA).

The primary goal was to obtain the EAA levels, particularly the incidence of high EAA levels (≥ 0.6), at each time point in patients with VV-ECMO or VA-ECMO. Exploratory

TABLE 1 | Characteristics and clinical outcomes of patients with VV-ECMO or VA-ECMO.

	VV-ECMO (n = 15)	VA-ECMO (n = 39)
Age (year)	64 (51–71)	63 (52–69)
Height (cm)	164 (159–168)	168 (157–174)
Weight (kg)	62.9 (57.0–75.2)	67.4 (56.3–82.3)
Sex (female/male)	6/9	7/32
APACHE II score	31 (26–40)	29 (24–36)
SAVE score	-	-8 (-10 to -2)
RESP score	0 (-3 to 4)	-
RRT, n (%)	4 (27%)	22 (56%)
ECMO indication		
E-CPR	-	16
Heart failure	-	17
Postcardiotomy	-	4
Septic shock	-	2
ARDS	15	-
EAA level		
T1 median (IQR)	0.44 (0.31–0.71)	0.33 (0.22–0.50)
T2 median (IQR)	0.50 (0.36–0.66)	0.38 (0.28–0.52)
T3 median (IQR)	0.50 (0.33–0.78)	0.40 (0.23–0.57)
T4 median (IQR)	0.49 (0.37–0.59)	0.44 (0.29–0.56)
ECMO free days ^a	0 (0–17)	14 (0–24)
ICU free days ^b	0	0 (0–2)
30-day survival, n (%)	11 (73%)	21 (54%)

Data are presented as medians (interquartile ranges, IQRs), numbers, and percentages as appropriate. Four study time points: within 24 h (T1) and 25–48 (T2), 49–72 (T3), and 73–96 h (T4) after ECMO initiation. APACHE II, Acute Physiology and Chronic Health Evaluation II; ARDS, acute respiratory distress syndrome; EAA, endotoxin activity assay; ECMO, extracorporeal membrane oxygenation; E-CPR, extracorporeal cardiopulmonary resuscitation; ICU, intensive care unit; IQR, interquartile range; RRT, renal replacement therapy; SAVE, Survival after Venoarterial ECMO; RESP, Respiratory ECMO Survival Prediction; VA-ECMO, venoarterial ECMO; VV-ECMO, venovenous ECMO.

^aECMO free days were defined as days free from ECMO support, between ECMO initiation and day 30.

^bICU free days were defined as days bot in the ICU, between ECMO initiation and day 30.

analyses were conducted to compare EAA levels between 30-day survivors and non-survivors. *Post-hoc* exploratory analysis was performed to compare the clinical outcomes between VA-ECMO patients with and without an elevated EAA level at T3 (vs. T1).

Statistical Analysis

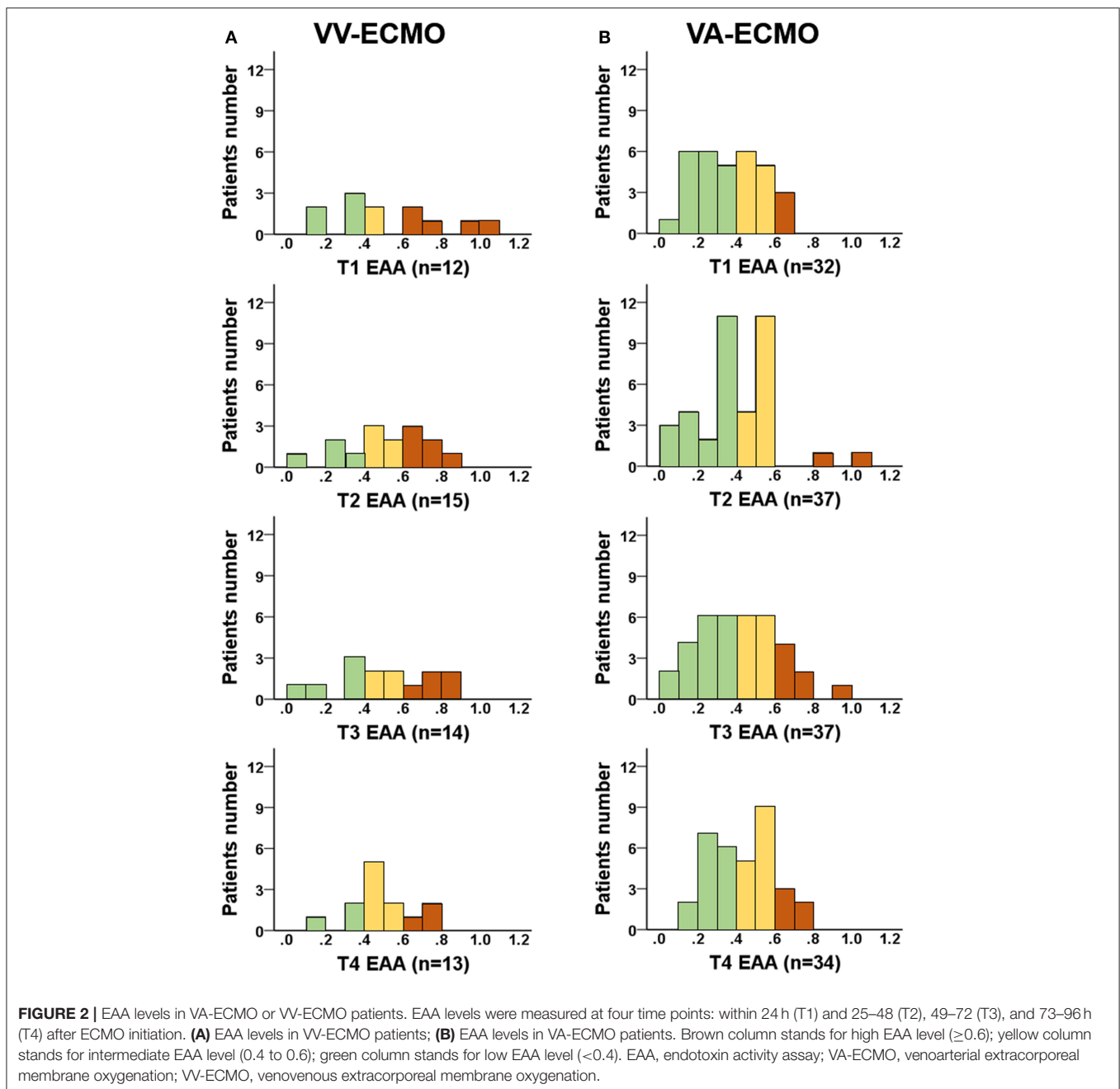
Data were analyzed in SPSS Version 27.0 (IBM, Armonk, NY, USA). Continuous variables are reported as medians (interquartile ranges) and were compared using the Mann-Whitney *U* test; median differences (95% confidence intervals [CIs]) between groups were calculated using the

Hodges–Lehmann estimator. Categorical variables are reported as numbers (percentages) and were compared using chi-square tests or Fisher's exact tests as appropriate. The Kaplan–Meier survival curve was employed to visualize the survival differences between groups, which were tested using log-rank tests. A $P < 0.05$ indicated a significant difference.

RESULTS

Patient Characteristics and EAA Levels

After eligibility assessment and informed consent, 39 VA-ECMO patients and 15 VV-ECMO patients were enrolled (Figure 1).



The patient characteristics, indications for ECMO, and EAA levels are summarized in **Table 1**. The 30-day survival rates were 54 and 73% in VA-ECMO and VV-ECMO patients, respectively. The EAA levels in VV-ECMO patients and VA-ECMO patients are presented in **Figure 2**. At T1, the incidence of high EAA levels (≥ 0.6) was 42% in VV-ECMO patients and 9% in VA-ECMO patients ($P = 0.02$). At T2, the incidence of high EAA levels was 40% in VV-ECMO patients and 5% in VA-ECMO patients ($P = 0.005$).

Clinical and Laboratory Data in 30-Day Survivors and Non-survivors

The EAA levels and clinical and laboratory data for VA-ECMO patients surviving or not surviving at 30 days are summarized in **Table 2**. Significantly higher APACHE II scores and SAVE scores at VA-ECMO initiation were found in 30-day non-survivors. In VA-ECMO patients, EAA levels at T3 were higher in 30-day non-survivors than in survivors (median [interquartile range]: 0.49 [0.37–0.93] vs. 0.31 [0.19–0.51], median difference 0.16 [95% CI, 0.02–0.31]; $P = 0.024$). Biomarker levels are presented in **Table 3**. Procalcitonin levels at T3 were higher in 30-day non-survivors than in survivors.

Post hoc Analysis: Clinical and Laboratory Data in VA-ECMO Patients With or Without Elevated EAA Levels at T3

The EAA levels and clinical and laboratory data of VA-ECMO patients with or without elevated EAA levels at T3 (vs. T1) are summarized in **Table 4**. In VA-ECMO patients, patients with an elevated EAA level at T3 (vs. T1) had lower 30-day survival (39 vs. 83%, $P = 0.026$) and fewer ECMO free days by day 30 (median [interquartile range]: 3 [0–21] vs. 23 [15–25] days, median difference 12 days [95% CI, 0–22]; $P = 0.028$) than did those without an elevated EAA level. Biomarker levels are presented in **Table 5**. The DAO levels at T1 and the WBC counts at T4 were higher in VA-ECMO patients with an elevated EAA level than in those without. The 30-day survival curves for VA-ECMO patients with or without elevated EAA levels at T3 are presented in **Figure 3**. The EAA levels in VA-ECMO patients with a proven infection within 96 h are presented in **Table 6**.

DISCUSSION

This study discovered that 42% of VV-ECMO patients and 9% of VA-ECMO patients had high EAA levels (≥ 0.6) at the initiation of ECMO support. In VA-ECMO patients, EAA levels at T3 were higher in 30-day non-survivors than in survivors. VA-ECMO patients with elevated EAA levels at T3 (vs. T1) had lower 30-day survival than those without elevated EAA levels had.

Our study demonstrated that incidence of high EAA levels (≥ 0.6) was higher in VV-ECMO patients than in VA-ECMO patients; this finding is compatible with a report that patients with VV-ECMO have a higher risk of infection than do those with VA-ECMO (13). It has been reported that 63% of ventilator-associated pneumonia were due to gram-negative bacteria (GNB) (14), and the GNB sepsis directly result in

TABLE 2 | Data of 30-day survivors and non-survivors with VA-ECMO.

VA-ECMO	30-day survivors (n = 21)	30-day non-survivors (n = 18)	P values
Age (year)	65 (55–71)	62 (49–66)	0.367
Sex (female/male)	2/19	5/13	
APACHE II score	27 (24–32)	36 (27–39)	0.020
SAVE score	−5 (−9–−1)	−10 (−15–−7)	0.003
ECMO free days ^a	22 (17–26)	1 (0–13)	0.001
ICU free days ^b	0 (0–15)	0	0.009
T1 (within 24 h)			
EAA	0.38 (0.20–0.51)	0.29 (0.20–0.46)	0.319
WBC (k/ μ L)	12.2 (9.5–17.4)	12.1 (9.7–15.9)	0.988
Inotropic score	4.1 (0–20.7)	12.9 (1.9–19.4)	0.483
Lactate (mmol/L)	10.1 (3.4–13.5)	9.5 (7.7–13.1)	0.478
T2 (25–48 h)			
EAA	0.38 (0.20–0.52)	0.38 (0.33–0.52)	0.660
WBC (k/ μ L)	12.1 (10.6–15.3)	12.8 (8.8–15.6)	0.885
Inotropic score	4.6 (0.7–11.5)	9.2 (3.3–18.8)	0.147
Lactate (mmol/L)	2.9 (1.7–4.5)	3.1 (2.0–4.4)	0.641
T3 (49–72 h)			
EAA	0.31 (0.19–0.51)	0.49 (0.37–0.63)	0.024
WBC (k/ μ L)	12.3 (9.0–15.2)	10.4 (7.9–12.2)	0.128
Inotropic score	2.3 (0.0–8.1)	7.5 (1.1–13.9)	0.220
Lactate (mmol/L)	1.9 (1.4–3.0)	2.5 (1.8–5.3)	0.056
T4 (73–96 h)			
EAA	0.41 (0.29–0.52)	0.49 (0.29–0.61)	0.391
WBC (k/ μ L)	10.1 (8.8–13.5)	11.3 (7.9–11.9)	0.977
Inotropic score	0.9 (0–7.9)	3.2 (0–15.4)	0.427
Lactate (mmol/L)	1.8 (1.2–2.5)	2.5 (1.4–4.0)	0.126

Data are presented as medians (interquartile ranges), numbers, and percentages as appropriate. Study time points (T1–T4) represent the time after ECMO initiation. APACHE II, Acute Physiology and Chronic Health Evaluation II; EAA, endotoxin activity assay; ECMO, extracorporeal membrane oxygenation; ICU, intensive care unit; SAVE, Survival after Venoarterial ECMO; VA-ECMO, venoarterial ECMO; WBC, white blood cell.

^aECMO free days were defined as days free from ECMO support from ECMO initiation until day 30.

^bICU free days were defined as days not in the ICU, between ECMO initiation and day 30.

high endotoxin activity. However, in patients with non-GNB sepsis, severe hypoperfusion or hypoxemia could damage the intestinal barrier and results in the translocation of intestinal resident bacteria (mainly GNB) and endotoxins (5). Therefore, the translocation could secondarily result in high endotoxin toxicity in these patients with non-GNB sepsis. Moreover, current surviving sepsis guidelines suggest using VV-ECMO for patients with sepsis-induced severe ARDS when conventional mechanical ventilation fails (15). We suggest that more concerns should be raised for high endotoxin activity in VV-ECMO patients, and further investigations of the impact of high endotoxin activity on clinical outcomes are needed.

Elevated EAA levels at T3 were associated with lower 30-day survival and fewer ECMO-free days in VA-ECMO patients of this study. Several factors may contribute to subsequent elevated endotoxin activity in the whole blood of

TABLE 3 | Biomarker levels in 30-day survivors and non-survivors with VA-ECMO.

VA-ECMO	30-day survivors	30-day non-survivors	P values
T1 (within 24 h)			
Procalcitonin	4 (1–17)	8 (4–15)	0.442
DAO	14 (8–33)	18 (8–76)	0.419
Cystatin C	0.7 (0.4–1.0)	1.0 (0.3–1.3)	0.338
T2 (25–48 h)			
Procalcitonin	4 (1–13)	10 (4–15)	0.165
DAO	9 (5–26)	13 (5–23)	0.705
Cystatin C	0.6 (0.5–1.0)	1.0 (0.4–1.2)	0.338
T3 (49–72 h)			
Procalcitonin	3 (1–5)	8 (3–12)	0.022
DAO	5 (4–11)	6 (4–13)	0.752
Cystatin C	0.7 (0.4–1.0)	0.9 (0.5–1.2)	0.209
T4 (73–96 h)			
Procalcitonin	2 (0–7)	4 (2–8)	0.202
DAO	6 (4–9)	6 (3–12)	0.667
Cystatin C	0.6 (0.4–1.1)	1.0 (0.6–1.1)	0.131

Data are presented as medians (interquartile ranges), numbers, and percentages as appropriate. Study time points (T1–T4) represent the time after ECMO initiation. DAO, diamine oxidase; VA-ECMO, venoarterial ECMO.

TABLE 4 | Data of VA-ECMO patients with or without elevated EAA levels at T3.

VA-ECMO	Δ EAA T3–T1 < 0 (n = 12)	Δ EAA T3–T1 ≥ 0 (n = 18)	P-values
Age (year)	66 (58–71)	63 (48–68)	0.439
SAVE score	–5 (–10 to –1)	–10 (–11 to –4)	0.232
APACHE II score	30 (25–37)	28 (24–36)	0.692
ECMO indication			
E-CPR	4	6	
Heart failure	7	8	
Postcardiotomy	0	3	
Septic shock	1	1	
ECMO free days ^a	23 (15–25)	3 (0–21)	0.028
ICU free days ^b	0 (0–3)	0 (0–4)	0.602
30-day survival	10 (83%)	7 (39%)	0.026
T1 Number, n (%)			
EAA	0.42 (0.24–0.51)	0.28 (0.17–0.42)	0.146
WBC (k/μL)	12.9 (10.3–15.2)	11.9 (9.1–15.9)	0.602
Inotropic score	9.9 (3.9–30.6)	3.7 (0–15.8)	0.215
Lactate (mmol/L)	9.0 (3.4–13.2)	9.2 (7.0–12.5)	0.662
T2 Number, n (%)			
EAA	0.25 (0.12–0.47)	0.46 (0.35–0.52)	0.034
WBC (k/μL)	11.3 (9.3–13.0)	12.9 (11.3–16.9)	0.087
Inotropic score	4.8 (2.4–9.1)	7.6 (0.0–22.1)	0.647
Lactate (mmol/L)	2.5 (1.7–2.9)	3.3 (1.5–5.0)	0.245
T3 Number, n (%)			
EAA	0.20 (0.11–0.30)	0.51 (0.38–0.60)	0.001
WBC (k/μL)	9.0 (7.5–12.5)	11.4 (8.9–13.2)	0.146
Inotropic score	4.9 (1.7–8.5)	5.4 (0.0–15.9)	0.723
Lactate (mmol/L)	1.8 (1.4–2.5)	2.9 (1.4–5.0)	0.180
T4 Number, n (%)			
EAA	0.32 (0.21–0.51)	0.48 (0.30–0.52)	0.195
WBC (k/μL)	8.8 (6.8–10.5)	11.5 (9.4–12.8)	0.019
Inotropic score	1.6 (0–6.7)	1.6 (0.0–13.1)	1.000
Lactate (mmol/L)	1.8 (1.2–3.1)	2.1 (1.1–3.2)	0.645

Data are presented as medians (interquartile ranges), numbers, and percentages as appropriate. Four study time points: within 24 h (T1) and 25–48 (T2), 49–72 (T3), and 73–96 h (T4) after ECMO initiation. APACHE II, Acute Physiology and Chronic Health Evaluation II; EAA, Endotoxin Activity Assay; ECMO, extracorporeal membrane oxygenation; ICU, intensive care unit; SAVE, Survival after Venoarterial ECMO; VA-ECMO, venoarterial ECMO; WBC, white blood cell.

^aECMO free days were defined as days free from ECMO support from ECMO initiation until day 30.

^bICU free days were defined as days bot in the ICU, between ECMO initiation and day 30.

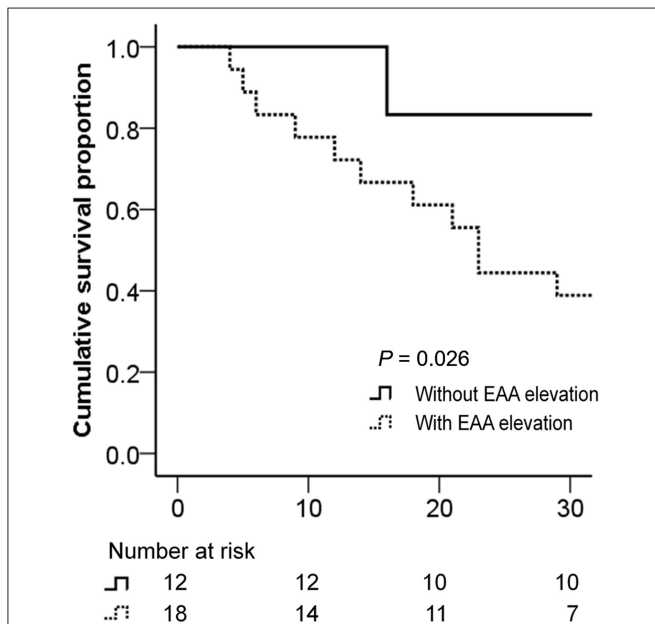


FIGURE 3 | Kaplan–Meier 30-day survival curve for VA-ECMO patients. Patients with or without elevated EAA level at T3 (vs. T1). Study time points: within 24 h (T1) and 49–72 (T3) after ECMO initiation. Result of statistical comparison using log-rank test. EAA, endotoxin activity assay; VA-ECMO, venoarterial extracorporeal membrane oxygenation.

VA-ECMO patients. First, ICU-related nosocomial infections (e.g., ventilator-associated pneumonia and bloodstream infection) are common prior to and during ECMO support (14, 16, 17). Among the 39 VA-ECMO patients in the present study, six presented with infections prior to ECMO initiation,

and nine developed infections within the first week after ECMO initiation. Most microorganisms reported within 96 h after VA-ECMO placement in this study were GNB. Second, microcirculatory dysfunction may disrupt the intestinal barrier and precipitate the translocation of resident intestinal bacteria and endotoxins (5, 18). Third, we found that DAO levels at T1 were higher in VA-ECMO patients with an elevated EAA level at T3 than in those without. This finding might be associated with intestinal barrier disruption. Moreover, several reports suggest that some *Klebsiella pneumoniae* infections originate from the gastrointestinal reservoir (19, 20), which is compatible

TABLE 5 | Biomarker levels in VA-ECMO patients with or without elevated EAA levels at T3.

VA-ECMO	Δ EAA T3-T1 < 0	Δ EAA T3-T1 \geq 0	P values
T1 (within 24 h)			
Procalcitonin	3 (1–16)	7 (3–16)	0.632
DAO	8 (7–21)	28 (13–76)	0.013*
Cystatin C	1 (0.6–1.2)	0.6 (0.3–1.0)	0.079
T2 (25–48 h)			
Procalcitonin	5 (2–13)	8 (2–19)	0.444
DAO	5 (4–17)	14 (6–23)	0.117
Cystatin C	1 (0.6–1.2)	0.5 (0.3–1.0)	0.080
T3 (49–72 h)			
Procalcitonin	2 (1–5)	8 (3–12)	0.065
DAO	5 (3–20)	6 (4–9)	0.851
Cystatin C	0.9 (0.5–1.3)	0.8 (0.4–1.0)	0.124
T4 (73–96 h)			
Procalcitonin	2 (1–4)	5 (2–10)	0.241
DAO	5 (4–14)	5 (4–8)	0.698
Cystatin C	0.9 (0.5–1.2)	0.7 (0.4–1.1)	0.100

Data are presented as medians (interquartile ranges), numbers, and percentages as appropriate. DAO; diamine oxidase; EAA, endotoxin activity assay; VA-ECMO, veno-arterial ECMO. *significant difference between patients with or without elevated EAA level from T1–T3. Study time points (T1–T4) represent the time after ECMO initiation.

TABLE 6 | EAA levels in VA-ECMO patients with a proven infection within 96 h.

No.	Type	Microorganisms	Day	EAA			
				T1	T2	T3	T4
4	Bacteremia	<i>Actinomyces odontolyticus</i>	0	0.38	0.35	0.59	0.49
10	Pneumonia	<i>Klebsiella pneumoniae</i>	1	0.47	0.57	0.47	0.52
14	Bacteremia	<i>Klebsiella pneumoniae</i>	0	-	0.57	0.76	0.61
26	Pneumonia	<i>Klebsiella pneumoniae</i>	0	0.17	0.38	0.52	0.56
31	Pneumonia	<i>Stenotrophomonas maltophilia</i>	3	0.49	0.35	0.61	0.5
37	Pneumonia	<i>Klebsiella pneumoniae</i>	0	0.26	0.52	0.9	0.59
44	Pneumonia	<i>Acinetobacter nosocomialis</i>	4	0.17	0.14	0.38	0.29
45	Pneumonia	<i>Klebsiella pneumoniae</i>	4	0.13	0.81	0.24	0.32
46	Pneumonia	<i>Klebsiella pneumoniae</i>	0	0.5	0.11	0.05	-
47	Pneumonia	<i>Elizabethkingia meningoseptica</i>	3	0.21	0.39	0.16	0.29
48	Pneumonia	<i>Pseudomonas aeruginosa</i>	4	0.4	0.35	0.4	-
51	Pneumonia	<i>Klebsiella pneumoniae</i>	0	-	0.36	0.28	0.32

Four study time points: within 24 h (T1) and 25–48 (T2), 49–72 (T3), and 73–96 h (T4) after ECMO initiation. EAA, endotoxin activity assay.

with the finding of this study that *Klebsiella pneumoniae* is the most common pathogen. Furthermore, extracorporeal endotoxin adsorption treatment has been reported to reduce EAA and inflammatory cytokine levels (21, 22). Further studies are warranted to investigate the clinical efficacy of extracorporeal

endotoxin adsorption treatment in VA-ECMO patients with elevated EAA levels.

This study has several limitations. First, with limited sample size, the results of exploratory analyses require further investigation in future studies. Second, the mechanism of increased endotoxin activity may vary among patients with different indications for VA-ECMO support. Further studies are warranted to investigate such variation. Third, an aggravated systemic inflammatory response resulting from the exposure of blood to extracorporeal circulation has been recognized as an independent cause of intestinal barrier dysfunction in porcine models (23, 24). Further studies are warranted to investigate the relationship between cytokine level and endotoxin activity.

CONCLUSIONS

A certain proportion of critically ill adult patients with ECMO have an EAA level \geq 0.6, and an elevated EAA levels at 48 h after VA-ECMO initiation was associated with lower 30-day survival. Further studies are warranted to investigate the risk factors for high endotoxin activity and the effects of high endotoxin activity on further clinical outcomes in larger patient groups. Additionally, new therapeutic strategies for high endotoxin activity are required to be investigated.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Research Ethics Committee of National Taiwan University Hospital. The legally authorized representatives of the patients provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

C-TL, C-HW, W-SC, and Y-CY: concept and design and drafting manuscript. C-HW, C-HL, and Y-CY: patient enrollment and data collection. T-JW, W-SC, M-JW, and Y-CY: interpretation of data. C-HW, M-JW, Y-SC, and Y-CY: critical revision of the manuscript. M-JW and Y-SC: study supervision. All authors contributed to the article and approved the submitted version.

FUNDING

This work was supported, in part, by a grant from the National Taiwan University Hospital (NTUH 109-S4468).

ACKNOWLEDGMENTS

The authors thank the Toray Industries for supporting the EAA Kits and device of endotoxin activity measurement.

REFERENCES

- Chen YS, Lin JW, Yu HY, Ko WJ, Jerng JS, Chang WT, et al. Cardiopulmonary resuscitation with assisted extracorporeal life-support versus conventional cardiopulmonary resuscitation in adults with in-hospital cardiac arrest: an observational study and propensity analysis. *Lancet*. (2008) 372:554–61. doi: 10.1016/S0140-6736(08)60958-7
- Zangrillo A, Landoni G, Biondi-Zoccai G, Greco M, Greco T, Frati G, et al. A meta-analysis of complications and mortality of extracorporeal membrane oxygenation. *Crit Care Resusc*. (2013) 15:172–8.
- Biffi S, Di Bella S, Scaravilli V, Peri AM, Grasselli G, Alagna L, et al. Infections during extracorporeal membrane oxygenation: epidemiology, risk factors, pathogenesis and prevention. *Int J Antimicrob Agents*. (2017) 50:9–16. doi: 10.1016/j.ijantimicag.2017.02.025
- van Deventer SJ, ten Cate JW, Tytgat GN. Intestinal endotoxemia. Clinical significance. *Gastroenterology*. (1988) 94:825–31. doi: 10.1016/0016-5085(88)90261-2
- Assimakopoulos SF, Triantos C, Thomopoulos K, Fligou F, Maroulis I, Marangos M, et al. Gut-origin sepsis in the critically ill patient: pathophysiology and treatment. *Infection*. (2018) 46:751–60. doi: 10.1007/s15010-018-1178-5
- Romaschin AD, Klein DJ, Marshall JC. Bench-to bedside review: Clinical experience with the endotoxin activity assay. *Crit Care*. (2012) 16:248. doi: 10.1186/cc11495
- Romaschin AD, Harris DM, Ribeiro MB, Paice J, Foster DM, Walker PM, et al. A rapid assay of endotoxin in whole blood using autologous neutrophil dependent chemiluminescence. *J Immunol Methods*. (1998) 212:169–85. doi: 10.1016/S0022-1759(98)00003-9
- Marshall JC, Foster D, Vincent JL, Cook DJ, Cohen J, Dellinger RP, et al. Diagnostic and prognostic implications of endotoxemia in critical illness: results of the MEDIC study. *J Infect Dis*. (2004) 190:527–34. doi: 10.1086/422254
- E. von Elm, Altman DG, Egger M, Pocock SJ, Gøtzsche PC, Vandenbroucke JP. The Strengthening of Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. *J Clin Epidemiol*. (2008) 61:344–9. doi: 10.1016/j.jclinepi.2007.11.008
- Yeh YC, Lee CT, Wang CH, Tu YK, Lai CH, Wang YC, et al. Investigation of microcirculation in patients with venoarterial extracorporeal membrane oxygenation life support. *Crit Care*. (2018) 22:200. doi: 10.1186/s13054-018-2081-2
- Huang SC, Yu HY, Ko WJ, Chen YS. Pressure criterion for placement of distal perfusion catheter to prevent limb ischemia during adult extracorporeal life support. *J Thorac Cardiovasc Surg*. (2004) 128:776–7. doi: 10.1016/j.jtcvs.2004.03.042
- Shore S, Nelson DP, Pearl JM, Manning PB, Wong H, Shanley TP, et al. Usefulness of corticosteroid therapy in decreasing epinephrine requirements in critically ill infants with congenital heart disease. *Am J Cardiol*. (2001) 88:591–4. doi: 10.1016/S0002-9149(01)01751-9
- Sun HY, Ko WJ, Tsai PR, Sun CC, Chang YY, Lee CW, et al. Infections occurring during extracorporeal membrane oxygenation use in adult patients. *J Thorac Cardiovasc Surg*. (2010) 140:1125–32.e2. doi: 10.1016/j.jtcvs.2010.07.017
- Grasselli G, Scaravilli V, Di Bella S, Biffi S, Bombino M, Patroniti N, et al. Nosocomial infections during extracorporeal membrane oxygenation: incidence, etiology, and impact on patients' outcome. *Crit Care Med*. (2017) 45:1726–33. doi: 10.1097/CCM.0000000000002652
- Evans L, Rhodes A, Alhazzani W, Antonelli M, Coopersmith CM, C. French, et al. Surviving sepsis campaign: international guidelines for management of sepsis and septic shock 2021. *Intensive Care Med*. (2021) 47:1181–247. doi: 10.1007/s00134-021-06506-y
- Abrams D, Grasselli G, Schmidt M, Mueller T, Brodie D. ECLS-associated infections in adults: what we know and what we don't yet know. *Intensive Care Med*. (2020) 46:182–91. doi: 10.1007/s00134-019-05847-z
- Schmidt M, Bréchet N, Hariri S, Guiguet M, Luyt CE, Makri R, et al. Nosocomial infections in adult cardiogenic shock patients supported by venoarterial extracorporeal membrane oxygenation. *Clin Infect Dis*. (2012) 55:1633–41. doi: 10.1093/cid/cis783
- Ohri SK, Bjarnason I, Pathi V, Somasundaram S, Bowles CT, Keogh BE, et al. Cardiopulmonary bypass impairs small intestinal transport and increases gut permeability. *Ann Thorac Surg*. (1993) 55:1080–6. doi: 10.1016/0003-4975(93)90011-6
- Gorrie CL, Mirceta M, Wick RR, Edwards DJ, Thomson NR, Strugnell RA, et al. Gastrointestinal carriage is a major reservoir of klebsiella pneumoniae infection in intensive care patients. *Clin Infect Dis*. (2017) 65:208–15. doi: 10.1093/cid/cix270
- Hsu CR, Pan YJ, Liu JY, Chen CT, Lin TL, Wang JT. Klebsiella pneumoniae translocates across the intestinal epithelium via Rho GTPase- and phosphatidylinositol 3-kinase/Akt-dependent cell invasion. *Infect Immun*. (2015) 83:769–79. doi: 10.1128/IAI.02345-14
- Mitaka C, Kusaoi M, Kawagoe I, Satoh D. Up-to-date information on polymyxin B-immobilized fiber column direct hemoperfusion for septic shock. *Acute Crit Care*. (2021) 36:85–91. doi: 10.4266/acc.2021.00150
- Rachoin JS, Foster D, Giese R, Weisberg LS, Klein DJ. Importance of endotoxin clearance in endotoxemic septic shock: an analysis from the evaluating use of polymyxinb hemoperfusion in a randomized controlled trial of adults treated for endotoxemic septic shock (EUPHRATES) Trial. *Crit Care Explor*. (2020) 2:e0083. doi: 10.1097/CCE.0000000000000083
- Kurundkar AR, Killingsworth CR, McIlwain RB, Timpa JG, Hartman YE, He D, et al. Extracorporeal membrane oxygenation causes loss of intestinal epithelial barrier in the newborn piglet. *Pediatr Res*. (2010) 68:128–33. doi: 10.1203/PDR.0b013e3181e4c9f8
- Jansen NJ, van Oeveren W, Gu YJ, van Vliet MH, Eijssman L, Wildevuur CR. Endotoxin release and tumor necrosis factor formation during cardiopulmonary bypass. *Ann Thorac Surg*. (1992) 54:744–7. doi: 10.1016/0003-4975(92)91021-Z

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Copyright © 2021 Lee, Wang, Chan, Tsai, Wei, Lai, Wang, Chen and Yeh. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.