Peer Review File

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Reviewer A

The authors present an interesting manuscript that describes the epidemiology of ECMO related infections in a sizable cohort of Chinese patient and describe the novel finding that dynamic decline in lymphocytes is significantly associated with ECMO-related infections. This is interesting, as it may provide a novel way of monitoring these patients.

Major comments:

To question 1: In the introduction, in line 60-61 it is stated that it is important to identify risk factors of ECMO-related infections. There are many studies that have attempted to do so, which should (in brief) be mentioned here before moving on to the very specific topic of dynamic decline in lymphocytes, especially as the manuscript also goes into detail about some other risk factors.

Author reply:

We sincerely appreciate the valuable comments. We added the text "More attention should pay to immune system although duration of ECMO and age were widely perceived as contributing to infection." To Introduction section.

To question 2: In the methods it is missing who collected the data. This is important, as ECMO related infections are subject to high interrater variability. If there may be interrater agreement in this for the current study as well, it may be important to include in the discussion (line 246).

Author reply:

Thanks for your professional comments. As your suggestion, we added the text "The data were collected by Songqiao Liu. ECMO-related infection was diagnosed by two intensivists (TH and CW)." in the **Methods** section.

To question 3: In the discussion, the second and third paragraph (lines 249-273)

elaborate extensively on topics that are not the main focus of the manuscript. This could be shortened significantly, if not removed.

Author reply:

Thank you for your professional comments. We removed the second and third paragraph in the Discussion section.

To question 4: The paragraph of the discussion from line 323 to 325 feels like stating the obvious. I would rather see more elaboration on the implication of the novel insights from this study for the monitoring of ECMO patients.

Author reply:

Thanks for your comments. We added the text "Gram-negative bacteria have become dominant species of nosocomial infection due to the extensive use of glycopeptides and vancomycin(31). Consistent with this, gram-negative bacteria were involved in ECMO-related infection up to 73.9% in our study. Acinetobacter baumannii and Klebsiella pneumoniae have increased dramatically which counting for 54.1% in VAP and 57.1% in BSI respectively. Clinical managements for patients supported by ECMO should be improved even more." In the **Discussion** section.

To question 5: In figure 1, it says that 210 patients that were supported by VA-ECMO were included. However, later there were also patients that were supported by VV-ECMO (Table 1). Should it not say 210 VA- or VV-ECMO supported in figure 1?

Author reply:

We feel great thanks for your professional review work on our manuscript. We apologize for my mistake. A total of 174 adult patients that received ECMO including VA-ECMO and VV-ECMO were included in this study. We have corrected and reuploaded **Figure 1**.

Minor comments:

To question 6: Line 17: should probably say: "... adult patients that received ECMO and underwent ..."

Author reply:

Thank you for your kindly advices. The original text "A total of 174 adults patients received ECMO underwent 1670 ECMO-days were included in this study." in the **Abstract** section is revised as "A total of 174 adult patients that received ECMO and underwent 1670 ECMO-days were included in this study.".

To question 7: Line 25: 95% CI is defined here, but in lines 21-22 I assume also 95% CI's are given. Then, it should be defined there.

Author reply:

Thanks for your professional suggestions. The original text "Infected patients had longer durations of mechanical ventilation (20.2(12.6,30.7) vs 9.0(5.8,14.7) days, *P*<0.001), ECMO support (11.6(8.1,17.3) vs 7.6(5.6,9.7) days, *P*<0.001) and hospital stays (28.2(20.7) vs 22.0(15.6) days, *P*<0.001)." in the **Abstract** section is revised as "Infected patients had longer durations of mechanical ventilation (20.2(Interquartile range (IQR)12.6,30.7) vs 9.0(IQR 5.8,14.7) days, *P*<0.001), ECMO support (11.6(IQR 8.1,17.3) vs 7.6(IQR 5.6,9.7) days, *P*<0.001) and hospital stays (28.2(20.7) vs 22.0(15.6) days, *P*<0.001).".

To question 8: Line 38/39: should probably say: "... cohort of patients receiving ECMO was analyzed for incidence, microbial etiology and risk factors of EMO-related infection."

Author reply:

Thanks for your suggestions. The original text "In the present study, a large cohort of patients receiving ECMO for incidence, microbial etiology and risk factors of ECMO-related infection were analyzed." in the **Strengths and limitations of this study** section is revised as "In the present study, a large cohort of patients receiving ECMO was analyzed for incidence, microbial etiology and risk factors of ECMO-related infection.".

To question 9: Line 41: ECMO instead of ECNO

Author reply:

Thanks for your careful checks. The original text "ECNO-related infection was the

independent risk factor of 90-day mortality." in the **Strengths and limitations of this study** section is revised as "ECMO-related infection was the independent risk factor of 90-day mortality.".

To question 10: Line 62: should probably say: "Lymphocytes are the cornerstone component of the adaptive immune system ..."

Author reply:

Thanks for your advice. The original text "Lymphocytes were the cornerstone component of adaptive immune system which distinguishes self from foreign and provides specialized immune defense." in the **Introduction** section is revised as "Lymphocytes are the cornerstone component of adaptive immune system which distinguishes self from foreign and provides specialized immune defense."

To question 11: Line 63/64: what is meant by can be hindered?

Author reply:

Thanks for your comments. The original text "Lymphocytes were the cornerstone component of adaptive immune system which distinguishes self from foreign and provides specialized immune defense can be hindered." in the **Introduction** section is revised as "Lymphocytes were the cornerstone component of adaptive immune system which distinguishes self from foreign and provides specialized immune defense.".

To question 12: Line 66: less is grammatically incorrect, should say e.g. little

Author reply:

Thanks for your suggestion. The original text "There is relatively less research on the correlation between lymphocyte and infectious complication in patients on ECMO." in the **Introduction** section is revised as "There is relatively little research on the correlation between lymphocyte and infectious complication in patients on ECMO.".

To question 13: Line 68: should include a source

Author reply:

Thank you for your key advice and we added the reference "Aging of immune system:

Immune signature from peripheral blood lymphocyte subsets in 1068 healthy adults".

To question 14: Line 77-78: says approved twice

Author reply:

We would like to thank you for your careful reading. The original text "The study was conducted in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of Zhongda Hospital Affiliated to Southeast University approved this study (2022ZDSYLL177-P01)." in the **Methods** section is revised as "The study was conducted in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of Zhongda Hospital Affiliated to Southeast University (2022ZDSYLL177-P01)."

To question 15: Line 91: NIs are not specified, also not in the abbreviations

Author reply:

Thank you for the suggestion. The original text "Exclusion criteria included: 1) age under 18 years old; 2) ECMO support duration less than 48 hours; 3) occurrence of NIs before ECMO support." in the **Methods** section is revised as "Exclusion criteria included: 1) age under 18 years old; 2) ECMO support duration less than 48 hours; 3) occurrence of nosocomial infections before ECMO support." And the original text "The association between the risk of NIs and ECMO duration has been reported in many studies." in the **Discussion** section is revised as "The association between the risk of nosocomial infections and ECMO duration has been reported in many studies.".

To question 16: Line 117: main diagnosis instead of diagnose

Author reply:

We would like to thank you for your careful reading. The original text "The following baseline patient data and ECMO-related variables were collected: demographics (i.e., gender, age, body mass index(BMI)), main diagnose at admission, reason for ECMO initiation, type of ECMO support, comorbidities(i.e., hypertension, diabetes mellitus), immunocompromised status(26), broad-spectrum antibiotics use within 48 hours before ECMO initiation, illness severity at admission stratified according to the Sepsis-related

Organ Failure Assessment (SOFA)(27) and Acute Physiology and Chronic Health Evaluation II (APACHE II) score(28), renal replacement therapy (RRT) during ECMO support, and duration of ECMO and mechanical ventilation (MV) use." in the **Methods** section is revised as " The following baseline patient data and ECMO-related variables were collected: demographics (i.e., gender, age, body mass index(BMI)), main diagnosis at admission, reason for ECMO initiation, type of ECMO support, comorbidities(i.e., hypertension, diabetes mellitus), immunocompromised status(26), broad-spectrum antibiotics use within 48 hours before ECMO initiation, illness severity at admission stratified according to the Sepsis-related Organ Failure Assessment (SOFA)(27) and Acute Physiology and Chronic Health Evaluation II (APACHE II) score(28), renal replacement therapy (RRT) during ECMO support, and duration of ECMO and mechanical ventilation (MV) use."

To question 17: Line 129: should probably say "episodes per 1000 ECMO-days ..."

Author reply:

Thank you for your kindly advices. The original text "The episodes of 1000 ECMO-days was used as the measurement for the incidence rate of ECMO-related infection." in the **Methods** section is revised as "The episodes per 1000 ECMO-days was used as the measurement for the incidence rate of ECMO-related infection."

To question 18: Line 146: should probably say "were performed" instead of "was"

Author reply:

Thank you for the suggestion. The original text "The Kaplan-Meier estimate of the cumulative probability of survival in different groups of ECMO patients was performed, and the log-rank test was used." in the **Methods** section is revised as "The Kaplan-Meier estimate of the cumulative probability of survival in different groups of ECMO patients were performed, and the log-rank test was used."

To question 19: Line 155: should probably say "... flowchart is summarized ..."

Author reply:

Thank you for your advice. The original text "The study flowchart summarized in **Fig**

1." is the **Results** section is revised as "The study flowchart is summarized in **Fig 1**."

To question 20: Line 170: should probably say "G- bacteria were the most common cause ..."

Author reply:

Thank you for the suggestion. The original text "G^- bacteria was the most common causes of infection, especially Acinetobacter baumannii and Klebsiella pneumoniae counting for 54.1% in VAP and 57.1% in BSI." in the **Results** section was revised as "G^- bacteria was the most common cause of infection, especially Acinetobacter baumannii and Klebsiella pneumoniae counting for 54.1% in VAP and 57.1% in BSI.".

To question 21: Line 239: ECMO instead of ECNO

Author reply:

Thank you for your kindly advices. The original text "ECNO-related infection was the independent risk factor of 90-day mortality." in the **DISCUSSION** section was revised as "ECMO-related infection was the independent risk factor of 90-day mortality.".

To question 22: Line 299: should probably say "... and ECMO duration has been"

Author reply:

Thank you for the suggestion. The original text "The association between the risk of NIs and ECMO duration had been reported in many studies." in the **DISCUSSION** section was revised as "The association between the risk of nosocomial infections and ECMO duration has been reported in many studies."

Reviewer B

It is an interesting well conducted and written investigation addressing a hot topic in critical care medicine. Only minor issues.

To question 1: No comorbidities except diabetes resulted to be related to ECMO infections. Was smoking habit investigated?

Author reply:

We feel great thanks for your professional review work on our manuscript. Smoking habit was not investigated.

To question 2: Invasive procedure such as coronary angiography may be related to infections. Was this datum specifically addressed in VA ECMO?

Author reply:

We sincerely appreciated the valuable comments. Patients who receiving VA-ECMO or VV-ECMO support were all included in our study.

To question 3: Was the site of cannulation (in VV ECMO) related to infections?

Author reply:

Thank you for your question. The cannulation sites of ECMO in our center are all peripheral. All patients are received femoro-femoral cannulation or femoro-intrajugular cannulation.

To question 4: How many patients developed endocarditis?

Author reply: Thanks for your question. Two patients developed endocarditis. The original text "Of 174 patients included, 24(13.8%) developed VAPs, 21(12.1%) developed BSIs and 1(0.6%) developed UTI during their ECMO course, corresponding to 14.4 episodes/1000 ECMO days, 6.0 episodes/1000 ECMO days, 12.6 episodes/1000 ECMO days, respectively." in the **Results** section was revised as "Of 174 patients included, 24(13.8%) developed VAPs, 21(12.1%) developed BSIs with two of them were endocarditis and 1(0.6%) developed UTI during their ECMO course, corresponding to 14.4 episodes/1000 ECMO days, 6.0 episodes/1000 ECMO days, 12.6 episodes/1000 ECMO days, respectively.".