Peer Review File

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Reviewer A

Minor changes.

1. In the sentence "Compared with the survivors, non-survivors had severer disease upon admission based on neurological status and imaging features and a shorter disease course, and were more likely to receive conservative treatment," the word "severer" is grammatically correct, but it's less commonly used. It might be better to rephrase it as "more severe."
2. Recommend changing analgosedation to the word "analgo-sedation" is more commonly written with a hyphen.
3. In the sentence "Long-term MV is associated with poor functional outcomes in patients with aSAH (7)", it would be better to replace "is" with "has been". So, the corrected sentence would read "Long-term MV has been associated with poor functional outcomes in patients with aSAH (7)".
4. Line 159: "sedative and analgesia" - It should be "sedatives and analgesia" or "sedative and analgesics" to match the plural noun.
5. Line 280: "that reported in a previous multi-state population-based study (6)" - The citation should be placed outside the parentheses.

Overall The study acknowledged its limitations, including the retrospective design, single-center nature, and sample size, which affect the ability to establish causal relationships and generalize the findings to all aSAH patients. Prospective validations and external validations are needed to further confirm these associations. Additionally, the study focused on conventional laboratory parameters and recommended exploring unconventional indicators and state-of-the-art laboratory tests for outcome prediction in aSAH patients requiring mechanical ventilation.

Overall, the study provided valuable insights into the prognostic value of initial laboratory tests, particularly glucose and ionized calcium levels, in mechanically ventilated patients with aSAH. These findings contribute to better clinical decision-making and highlight the importance of optimizing glycemic control and calcium management in the critical care of aSAH patients.

Reply: We thank the reviewers for their enthusiasm, helpful comments, and inspiring suggestions. We have carefully considered all of the minor changes and corrected them. Our responses and other changes we have made to the manuscript are detailed below, along with page and line references for convenience. The revised parts related to your comments are marked by track changes. As a result of these changes, we think this manuscript is much improved, and we hope that it is now suitable for publication in **J Thorac Dis**.

Changes in the text: Comment 1 (*Pg 3 Ln 89*); Comment 2 (*Pg 4 Ln 109*); Comment 3 (*Pg 4 Ln 117*); Comment 4 (*Pg 7 Ln 201*); Comment 5 (*Pg 10 Ln 323*);

Reviewer B

Comment 1: The text is too extensive to be published in this form, although carefully written.
Reply 1: We apologize for any confusion since we might not elaborate well the study design. After revisiting the data and the logic of this manuscript, we found there were at least two points that needed to be clarified here.

The first one is the selection of variables. Prior studies concerning stroke usually focus on imaging parameters and clinical presentations, but with less attention on laboratory tests. Our previous study showed that initial laboratory blood test provided a useful tool for COVID-19 management in identifying patients with critical condition and stratifying risk levels of death (1). We believe that laboratory tests performed on blood samples reflect individual physiological and biochemical states as well as body response to stress induced by both infection and stroke. Considering these, we included a series of laboratory test items reflecting multi-organ function, such as the coagulation system, the liver, the kidney, and the cardiac system. Interestingly, we found initial glucose and ionized calcium levels are associated with the disease outcome of aSAH patients requiring MV. After adjustment by other variables in the categories of clinical data, radiographic data, and treatment, both of them were revealed as independent risk factors for mortality. Given above, we believe that a broad inclusion of laboratory blood tests may better identify meaningful and reliable risk factors for mortality of this critical ill population.

Although initial logistic analysis indicate WFNS score was associated with mortality of these patients. However, after adjustment, it was not considered as an independent risk factor for mortality in this population. These data seemed to not support prior studies indicating the role of medical imaging and clinical factors in predicting disease outcomes of aSAH (2). However, this discrepancy might be owing to the different object of study since here we focused on patients with aSAH requiring MV. This population usually exhibited severe symptoms and diffuse bleeding in the CT scans (Table 1). Thus, imaging and clinical factors reflecting the severity of the etiology may not well discriminate those with unfavorable outcomes. Instead, laboratory tests as an objective indicator, may better reflect individual response to the stress induced by brain injury (i.e., reflecting the interaction between the internal environment and the etiolgoy). In this revision, we have added these discussion. ***Please check Pg 13 Ln 415-425.***

It is worth mentioning that laboratory test has a broad connotation, including routine laboratory blood test, genetic test, immunological test, etc. In this study we only examined the first cluster. Thus, in the revision, we corrected the expression of “laboratory tests” as “routine laboratory blood tests” in the title as well as throughout the main text.

The second one is the inclusion of subjects. Although this study aimed at aSAH patients requiring MV, ventilation was not initiated at the same time. Some patients required mechanical ventilation at admission and while others required it later in the hospital course. We admit this is a major limitation, and in our future studies we will investigate the prognostic value of initial laboratory blood test in aSAH patients requiring early-ventilation in the neurosurgery ICU. ***Please check Pg 14 Ln 445-451.***

Comment 2: Hyperglycemia is an issue common in critically ill patients, even in the absence of preexisting DM, and is associated with higher morbidity and mortality. That should be taken

into account.

Reply 2: The Reviewer raised an excellent point. Hyperglycemia is frequent during critical illness and is perceived by the clinician as part of the systemic metabolic response to stress. Of all patients with "stress hyperglycemia" only one third are known to have diabetes mellitus (3). Hyperglycemia is significantly associated with increased mortality in critically ill individuals owing to its many detrimental effects, including reduced immune function, increased inflammation and coagulation, and modulation of the endothelium (4). Thus, current clinical evidence support ongoing consideration of hyperglycemia as a therapeutic target to improve outcomes in hospitalized patients (5). We have discussed this issue in the revised manuscript. ***Please check Pg 12 Ln 362-369.***

Comment 3: Also, predisposing factors of hypocalcemia like magnesium depletion or blood transfusions should also be discussed. I assumed they did not exist.

Reply 3: We thank the Reviewer for raising this point. Both magnesium depletion (6) and blood transfusions (7) have been revealed as predisposing factors of hypocalcemia. Interestingly, both hypocalcemia and hypomagnesemia were associated with the severity and prognosis of both SAH and ICH (8, 9). However, in this study, there is a lack of the data concerning magnesium levels upon admission or blood transfusions before admission. Thus, we failed to determine whether these factors were associated with hypocalcemia in this specific critically ill patients.

Comment 4: Were the findings compared with those of other critical care populations?

Reply 4: We thank the Reviewer for raising this point and the opportunity to clarify our findings. The main conclusion of this manuscript is that initial glucose and ionized calcium levels, have the potential to predict survival in patients with aSAH requiring MV. Here, we will review previous literature concerning the role of these indicators in other types of critically ill patients in the field of neurosurgery, including spontaneous intracerebral hemorrhage, traumatic brain injury.

Hypocalcemia has been revealed to correlate with the extent of bleeding in patients with ICH (10, 11). A low calcium level may be associated with a subtle coagulopathy predisposing to increased bleeding and might therefore be a promising therapeutic target for acute ICH treatment trials (12). Hyperglycemia on admission, induced by either diabetes or stress, is a common finding after ICH and serves as a sensitive predictor of the risk of pulmonary infection and all-cause death after ICH (13, 14). These evidence support guideline recommendations for good glycemic control in patients with intracerebral hemorrhage (15).

Early studies unanimously indicated that early hyperglycemia was associated with higher mortality in traumatic brain injury (16). However, it was believed that stress-induced hyperglycemia predicts for mortality, while hyperglycemia related to diabetes is of less importance compared with SIH in terms of mortality in the acute trauma and TBI patient (17). Further mechanistic studies showed that blood glucose was associated with brain tissue acidosis in patients with major head injury (18). As is known, ionized calcium (Ca^{2+}) is an essential cofactor in the coagulation cascade and platelet aggregation, and hypocalcemia may contribute to the progression of intracranial bleeding. On the other hand, Ca^{2+} is an important mediator of cell damage after TBI and cellular hypocalcemia may have a neuroprotective

effect after brain injury (19). Considering the dual character of Ca^{2+} in the pathophysiology of TBI, current evidence indicated that admission hypocalcemia were positively (19), negatively (20) or even not (21, 22) correlated with the neurological outcome of patients with traumatic brain injury

All studies mentioned above suggest hyperglycemia and hypocalcemia is associated with adverse outcomes in critical ill neurologic patients. At a more macroscopic level, hyperglycemia (23-25) and hypocalcemia (26-28) also correlate with a broader spectrum of critically ill patients in the intensive care unit, including cardiac, infectious, and septic patients. In this revision, we summarized above findings in the discussion. ***Please check Pg 10 Ln 322-326.***

Reviewer C

Comment 1: First of all, the authors should not describe the purpose of this study as "laboratory tests predict mortality" or similar terms because the authors only examined the prognostic role of laboratory tests, not their predictive accuracy.

Reply 1: We thank the reviewers for their enthusiasm, helpful comments, and inspiring suggestions. We have carefully considered all of these points. Our responses and the changes we have made to the manuscript are detailed below, along with page and line references for convenience. The revised parts related to your comments are marked by track changes. As a result of these changes, we think this manuscript is much improved, and we hope that it is now suitable for publication in **J Thorac Dis**.

We thank the Reviewer for reminding us to be careful of the article rigor. We totally agree that the purpose of the study is to investigate the prognostic role of laboratory test in these patients, since no scoring system was made based on these indicators for predicting mortality of these patients. In the revision, we have corrected corresponding expressions as "the prognostic role" or "prognostic value" in the title as well as throughout the manuscript.

Comment 2: Second, in the abstract, the authors need to explain why they focused on the prognostic role of laboratory tests only and why these tests are associated with the prognosis in the background, please describe the follow up procedures of patients and briefly describe the main clinical factors and laboratory tests in the method, please briefly summarize the baseline clinical characteristics of the patient sample in the results, and have comments for the clinical implications of the findings in the conclusion.

Reply 2: We thank the Reviewer for his/her constructional suggestion. All the details you mentioned are important for the readers to briefly comprehend what the manuscript talks about in the abstract. However, the journal has requirement of a word limit number of the abstract (350 words). In the revised abstract, we tried to include all these information in a concise way. ***Please check Pg 3-4 Ln 69-100.***

Comment 3: Third, in the introduction of the main text, please further clarify "have not been thoroughly investigated" because this sentence is vague and not strict. Please have comments

on the clinical significance of the focus on laboratory tests and explain why these factors are potentially important, in comparison to medical imaging and clinical factors.

Reply 3: As is known, there are tremendous literature concerning the characteristics and outcomes of aSAH in the domain of neurology. In recent years, the management of aSAH patients requiring MV in the intensive care unit, which represents a specific critical ill population of patients with aSAH, has become an important research activity. As far as we known, there are only three studies focusing on this topic. Among them, two studies aimed at identifying risk factors for prolonged MV, while another one investigated risk factors of the complications (angiographic vasospasm and delayed cerebral ischemia) in aSAH patients requiring MV (29). However, the risk factors for survival of this specific population of critically ill patients have not been thoroughly investigated, and this is the aim of this study. In the revision, we have corrected the expression in the Introduction part. ***Please check Pg 4 Ln 121-123.***

Prior studies centering on the clinical characteristics of aSAH requiring MV mainly focus on clinical and imaging data (30, 31), with much less focus on laboratory tests. Laboratory tests performed on blood samples reflect individual physiological and biochemical states. In our previous studies, we found that laboratory screening provided a useful tool for COVID-19 management in identifying patients with critical condition and stratifying risk levels of death (1). Based on these findings, we believe routine laboratory test may have prognostic values in this specific population of critically ill patients.

Statistical analyses indicated that only initial laboratory indicators (ionized calcium and glucose), clinical data (WFNS score) and treatment modality (embolization and tracheotomy) were associated with mortality of these patients. However, after adjustment, WFNS was not considered as an independent risk factor for mortality in this population. These data seemed to not support prior studies indicating the role of medical imaging and clinical factors in predicting disease outcomes of aSAH. This discrepancy might be owing to the different object of study since here we focused on patients with aSAH requiring MV. According to our recent research progress, this critical ill population usually exhibited higher Hunt-Hess and mFisher scores (unpublished data), and medical imaging and clinical factors may fail to further discriminate those with unfavorable outcomes. However, laboratory tests as an objective indicator, better reflect individual response to the stress induced by brain injury. In this revision, we have added these discussions. ***Please check Pg 12 Ln 362-369.***

Comment 4: Fourth, in the methodology of the main text, please describe the sample size estimation and measurements of laboratory biomarkers. In statistics, it seems that the authors identified prognostic factors, not the independent prognostic roles of laboratory tests. The authors need to consider adjustment analysis.

Reply 4: This is a retrospective single-center cohort study (not a randomized controlled trial) of all patients admitted for aSAH requiring MV in the neurosurgical intensive care unit (ICU) from December 2019 to March 2022. Thus, the sample size estimation routinely used in prospective RCT was not adopted. In the multivariate logistic regression analysis, there were five variables included, with a total number of 75 survivors. Thus, the sample size of this study fulfilled the “Event Per Variable” principle (32).

Arterial and venous blood samples were obtained by venipuncture by the nurse upon arrival at the ICU. After blood draws, the samples were immediately delivered to the clinical lab and

analyzed immediately for each participant. Routine complete blood count (CBC) testing was performed on the UniCel DXH 800 analyzer. Coagulation tests were performed on the STAGO STA-R® Evolution multiparametric analyzer. Routine chemistry testing, including the basic and comprehensive metabolic panels, was performed on the Siemens ADVIA XPT analyzers and Centaur XP analyzers. Blood gas analysis was performed on the Instrumentation Laboratory GEM Premier 4000 analyzer. ***Please check Pg 6 Ln 173-182.***

In statistics, in order to identify independent factors of mortality in these patients, variables concerning clinical, radiographic, and laboratory data that were significant in univariate analysis were included in multivariate logistic regression analysis. Interestingly, both ionized calcium and serum glucose levels were found to be independent prognostic factors for mortality in these patients. ***Please check Pg 8 Ln 226-230 and Table 3.***

References

1. Bai Y, Wang E, Zhao S, Li J, Zhu Y, Zhang Y, et al. Implications of Laboratory Tests in Disease Grading and Death Risk Stratification of COVID-19: A Retrospective Study in Wuhan, China. Frontiers in medicine. 2021;8:629296.
2. Jaja BN, Cusimano MD, Etminan N, Hanggi D, Hasan D, Ilodigwe D, et al. Clinical prediction models for aneurysmal subarachnoid hemorrhage: a systematic review. Neurocritical care. 2013;18(1):143-53.
3. Miranda-Ruiz R, Castañón-González JA. [Hyperglycemia in critically ill patients: clinical implications for treatment]. Cirugia y cirujanos. 2004;72(6):517-24.
4. Knieriem M, Otto CM, Macintire D. Hyperglycemia in critically ill patients. Compendium (Yardley, PA). 2007;29(6):360-2, 4-72; quiz 72.
5. Becker CD, Sabang RL, Nogueira Cordeiro MF, Hassan IF, Goldberg MD, Scurlock CS. Hyperglycemia in Medically Critically Ill Patients: Risk Factors and Clinical Outcomes. The American journal of medicine. 2020;133(10):e568-e74.
6. Fong J, Khan A. Hypocalcemia: updates in diagnosis and management for primary care. Canadian family physician Medecin de famille canadien. 2012;58(2):158-62.
7. Sihler KC, Napolitano LM. Complications of massive transfusion. Chest. 2010;137(1):209-20.
8. Can A, Rudy RF, Castro VM, Dligach D, Finan S, Yu S, et al. Low Serum Calcium and Magnesium Levels and Rupture of Intracranial Aneurysms. Stroke. 2018;49(7):1747-50.
9. Can A, Du R. Calcium, magnesium, and subarachnoid hemorrhage. Aging. 2018;10(9):2212-3.
10. Morotti A, Charidimou A, Phuah CL, Jessel MJ, Schwab K, Ayres AM, et al. Association Between Serum Calcium Level and Extent of Bleeding in Patients With Intracerebral Hemorrhage. JAMA neurology. 2016;73(11):1285-90.
11. Sallinen H, Wu TY, Meretoja A, Putaala J, Tatlisumak T, Strbian D. Effect of baseline hypocalcaemia on volume of intracerebral haemorrhage in patients presenting within 72 hours from symptom onset. Journal of the neurological sciences. 2019;403:24-9.
12. Loggini A, El Ammar F, Mansour A, Kramer CL, Goldenberg FD, Lazaridis C. Association between electrolyte levels at presentation and hematoma expansion and outcome in spontaneous intracerebral hemorrhage: A systematic review. Journal of critical care. 2021;61:177-85.

13. Chen S, Wan Y, Guo H, Shen J, Li M, Xia Y, et al. Diabetic and stress-induced hyperglycemia in spontaneous intracerebral hemorrhage: A multicenter prospective cohort (CHEERY) study. CNS neuroscience & therapeutics. 2023;29(4):979-87.
14. Chandrasekaran PN, Hanley DF, Hsu CY, Martin RH, Steiner T, Suarez JI, et al. Effect of Moderate and Severe Persistent Hyperglycemia on Outcomes in Patients With Intracerebral Hemorrhage. Stroke. 2022;53(4):1226-34.
15. Saxena A, Anderson CS, Wang X, Sato S, Arima H, Chan E, et al. Prognostic Significance of Hyperglycemia in Acute Intracerebral Hemorrhage: The INTERACT2 Study. Stroke. 2016;47(3):682-8.
16. Liu-DeRyke X, Collingridge DS, Orme J, Roller D, Zurasky J, Rhoney DH. Clinical impact of early hyperglycemia during acute phase of traumatic brain injury. Neurocritical care. 2009;11(2):151-7.
17. Bosarge PL, Shoultz TH, Griffin RL, Kerby JD. Stress-induced hyperglycemia is associated with higher mortality in severe traumatic brain injury. The journal of trauma and acute care surgery. 2015;79(2):289-94.
18. Diaz-Parejo P, Ståhl N, Xu W, Reinstrup P, Ungerstedt U, Nordström CH. Cerebral energy metabolism during transient hyperglycemia in patients with severe brain trauma. Intensive care medicine. 2003;29(4):544-50.
19. Badarni K, Harush N, Andrawus E, Bahouth H, Bar-Lavie Y, Raz A, et al. Association Between Admission Ionized Calcium Level and Neurological Outcome of Patients with Isolated Severe Traumatic Brain Injury: A Retrospective Cohort Study. Neurocritical care. 2023.
20. Ngatuvai M, Martinez B, Sauder M, Beeton G, Andrade R, Maka P, et al. Traumatic Brain Injury, Electrolyte Levels, and Associated Outcomes: A Systematic Review. The Journal of surgical research. 2023;289:106-15.
21. Hakim S, Al Jogol H, Parchani A, Peralta R, Rizoli S, Al-Thani H, et al. Initial Serum Levels of Magnesium and Calcium as Predictors of Mortality in Traumatic Brain Injury Patients: A Retrospective Study. Diagnostics (Basel, Switzerland). 2023;13(6).
22. Davis WT, Ng PC, Medellin KL, Cutright JE, Araña AA, Strilka RJ, et al. Association of Hypocalcemia With Mortality of Combat Casualties With Brain Injury and Polytrauma Transported by Critical Care Air Transport Teams. Military medicine. 2022.
23. Falciglia M, Freyberg RW, Almenoff PL, D'Alessio DA, Render ML. Hyperglycemia-related mortality in critically ill patients varies with admission diagnosis. Critical care medicine. 2009;37(12):3001-9.
24. van Vught LA, Wiewel MA, Klein Klouwenberg PM, Hoogendijk AJ, Scicluna BP, Ong DS, et al. Admission Hyperglycemia in Critically Ill Sepsis Patients: Association With Outcome and Host Response. Critical care medicine. 2016;44(7):1338-46.
25. Minasi A, Andreadi A, Maiorino A, Giudice L, De Taddeo S, D'Ippolito I, et al. Hypocalcemia is associated with adverse outcomes in patients hospitalized with COVID-19. Endocrine. 2023;79(3):577-86.
26. Kelly A, Levine MA. Hypocalcemia in the critically ill patient. Journal of intensive care medicine. 2013;28(3):166-77.
27. Dickerson RN, Morgan LG, Cauthen AD, Alexander KH, Croce MA, Minard G, et al. Treatment of acute hypocalcemia in critically ill multiple-trauma patients. JPEN Journal of

parenteral and enteral nutrition. 2005;29(6):436-41.
28. Lind L, Carlstedt F, Rastad J, Stiernström H, Stridsberg M, Ljunggren O, et al. Hypocalcemia and parathyroid hormone secretion in critically ill patients. Critical care medicine. 2000;28(1):93-9.
29. Zhao L, Cheng C, Peng L, Zuo W, Xiong D, Zhang L, et al. Alcohol Abuse Associated With Increased Risk of Angiographic Vasospasm and Delayed Cerebral Ischemia in Patients With Aneurysmal Subarachnoid Hemorrhage Requiring Mechanical Ventilation. Frontiers in cardiovascular medicine. 2022;9:825890.
30. Rass V, Ianosi BA, Lindlbauer M, Lindner A, Kofler M, Schiefecker AJ, et al. Factors Associated With Prolonged Mechanical Ventilation in Patients With Subarachnoid Hemorrhage-The RAISE Score. Crit Care Med. 2022;50(1):103-13.
31. Huang CH, Ni SY, Lu HY, Huang AP, Kuo LT. Predictors of Prolonged Mechanical Ventilation Among Patients with Aneurysmal Subarachnoid Hemorrhage After Microsurgical Clipping. Neurol Ther. 2022;11(2):697-709.
32. Peduzzi P, Concato J, Kemper E, Holford TR, Feinstein AR. A simulation study of the number of events per variable in logistic regression analysis. Journal of clinical epidemiology. 1996;49(12):1373-9.