Peer Review File

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Reviewer's Comments

I would like to congratulate the authors for such an interesting report. It is a great effort to understand the complex physiopathology underlying the trauma induced coagulopathy using the MAR ratio, a marker of a coagulopathic response to injury. Hemodynamic parameters have not influenced results in authors´previous work, however I would still include shock index in their SHOCK subgroup. It is described as an accurate predictor for need of transfusion and mortality in severe trauma patients. In addition, the index is more sensitive than both heart rate and systolic blood pressure alone as it seems to occur with MAR. I feel very interesting that despite reporting normal values in regards to MA and R time, the authors have found important differences in cytokine expression and inflammatory coordination. Unfortunately, such differences didn't have an impact on morbidity (except from need for transfusion), nor mortality as ICU or hospital length of stay and mortality don't show differences between groups. I have missed a more thorough explanation about this fact in the discussion section.

Reply: Thank you for these comments. In response to these comments, as well as those regarding mortality, the reviewer is correct that this study did not show an association between the ratio and mortality outcomes. We attribute this to the fact that this study is focusing on the inflammation/coagulation interaction and was not powered to detect mortality. In previous work by our group, we did demonstrate a strong relationship between mortality and low ratios (reference 10).

At the same time, some of the importance of this ratio is the fact that both CRITICAL and NORMAL groups appear very similar but there are very different, and dysfunctional, processes happening at the molecular level. We did add some additional text in the conclusions (lines 455-63) to try and expand upon this concept.

Specific comments

Abstract:

Line 51: MAR ratio meaning should be explained

Reply: We have added two sentences to the Abstract Background (lines 50-53) to orient the reader regarding the MAR ratio.

Background: Line 95: Reference for genotypic contribution should be given.

Reply: We have added references from JBA MacLeod and Christie et al to support the discussion of genotypes of coagulopathy.

Methods:
Line 137: Exclusion criteria. Do pre-existing clotting disorders include those on antiplatelet and/or anticoagulant therapy? This should be clarified.
Reply: Line 140 references pre-existing clotting disorders, which includes use of antiplatelet and anticoagulant therapies. However, the reviewer is correct in noting it is not clear. We have added the antiplatelet/anticoagulant disqualification to the text for clarity.

Line 146: Explanation for CAT+ that is developed in lines 247-248 should be given here.
Reply: We have corrected the text so that the explanation for CAT is given in the Methods section, at the time of first reference.

Lines 189-190: Shock group might include shock index in addition to base deficit.
Reply: The Reviewer is correct that we could consider using shock index in the propensity matched subgroup analysis. We chose not to include both variables, due to concerns of collinearity and further restriction of cohort size. As base deficit was the basis of the original analysis, we kept this as the sole reflection of physiologic status at admission in this subgroup analysis. However, shock index is an important variable and as the reviewer notes, a well-supported reflection of the burden of shock at admission. We have included shock index information in the tables and text as recommended but opted to keep base deficit as the variable reflecting physiologic status at admission in the subgroup analysis.

Results:
Line 228: As previously mentioned, shock index might be included. Shock index >0.9 has been reported as an early predictor of hemorrhagic shock, mortality and need for transfusion in the trauma population1-4
1. Cannon CM, Braxton CC, Kling-Smith M, et al. Utility of the shock index in predicting mortality in traumatically injured patients. J Trauma 2009;67:1426–30.
2. McNab A, Burns B, Bhullar I, et al. An analysis of shock index as a correlate for outcomes in trauma by age group. Surgery 2013;154:384–7.
3. Singh A, Ali S, Agarwal A, et al. Correlation of shock index and modified shock index with the outcome of adult trauma patients: a prospective study of 9860 patients. N Am J Med Sci 2014;6:450– 2.
4. Montoya KF, Charry JD, Calle-Toro JS, et al. Shock index as a mortality predictor in patients with acute polytrauma. Journal of Acute Disease 2015;4:202–4).

Reply: Thank you for the references and suggestions. We added Shock index, as well as admission base deficit, to table 1 and page 10 (RESULTS). We also added a brief section to the conclusions (lines 375-386) commenting on these admission physiologic factors.

Conclusions:

Line 307: Sub-cohorts matched for injury severity (INJURY) and for injury severity and magnitude of shock (SHOCK) might be biased by size (15 vs 15 and 11 vs 11 respectively) and this could influence the results.

Reply: The reviewer is correct that the small size of the subgroups is a limitation. We have added this concern to the discussion of limitations at the end of the manuscript. "Additionally, subgroup analysis resulted in small cohort sizes used in DyNa for both INJURY and SHOCK groups. Though the analysis controlled for important patient variable and did demonstrate significant difference in immunologic coordination, the small groups may have introduced an unintentional bias."

Line 317: Have the authors assessed a cutpoint for a "too high" MAR as a potential marker for an anti-fibrinolytic state?

Reply: The reviewer raises an interesting point. In the original MAR manuscript (10), we evaluated quartiles for the MAR ratio. Though we noted significantly higher mortality in patients with the lowest ratios (corresponding to CRITICAL in this manuscript), we did not detect a difference in the other three quartiles. We do not believe there is a direct relationship between different MAR ratios and different types of coagulopathy. Rather, our supposition is that CRITICAL patients correspond to a specific type of traumatic coagulopathy, which is largely driven by corresponding alterations in the inflammatory response. However, we are currently working on a multi-center study to validate the MAR ratio that we hope will further define these relationships. In some of our more recent work, we have utilized Youden's Index to identify a more specific cutpoint for CRITICAL and NORMAL patients.

Line 327: MAR ration should be MAR ratio

Reply: Thank you for identifying that error, we have corrected it.

Line 397: Study results do not support the idea that "a malfunction of the coagulation system that may initially be subtle, yet nevertheless ominous" as CRITICAL patients don´t show significant differences with NORMAL patients in terms of ICU/hospital length of stay nor mortality.

Reply: We believe the data support an association between a low ratio (CRITICAL) and traumatic coagulopathy. We have changed the referenced text to reflect this "*CRITICAL patients had the lowest MAR ratios, indicating a malfunction of the coagulation system that may initially be subtle, yet indicative of progressive traumatic coagulopathy*."

Lines 420-424: "Differences in initial immunologic response in TIC patients were affected by the magnitude of injury and hemorrhage, but these differences persisted in sub-cohorts of patients normalized for ISS and base deficit, indicating that patient-specific differences in response to injury may affect TIC." I think this is a very interesting statement that I would emphasize.
Reply: Thank you for making that point. We added additional comments to try and further emphasize this point. "In effect, injury merely initiates a complex interplay between clot formation and inflammation that is expressed as one phenotype of TIC. The MAR ratio is the earliest warning signal of one such pathologic phenotype."

Table 1 MAR values which define NORMAL and CRITICAL patients should be included Base deficit values should be added Shock index could be included
Reply: We have added both to Table 1.

Table 2 MAR values which define NORMAL and CRITICAL patients should be included Cytokines concentrations at the other times of measurement (at 8 hours, 24 hours, and daily to day seven following injury or TICU discharge) for CRITICAL and NORMAL subgroups could be added
Reply: We have added the reference values for NORMAL and CRITICAL patients. We have also added additional cytokine data at time points up to 48 hours in the figures and text of the RESULTS section.

Figure 1
I think it is intriguing why IL-4, and anti-inflammatory cytokine, is almost significantly lower in NORMAL patients compared to CRITICAL patients in INJURY subgroup at hour 0. In the same line, IL-8 is extremely higher in CRITICAL patients compared to NORMAL patients in SHOCK subgroup at hour 8. I would give any potential explanation about both facts.

Strong points:
- Great effort to understand the relationship between coagulation and inflammation in trauma induced coagulopathy

- The use of MAR ratio, a marker of a coagulopathic response to injury, that is created and already tested by the authors in a previous work.

Weak points:

- No impact on morbidity nor mortality except from need for transfusion. There are no differences between groups (MAR <14.2 vs MAR>14.2) on ICU or hospital length of stay nor mortality.

Reply: The reviewers are correct in this observation. However, this study was not powered to evaluate mortality. Our previous work on the MAR ratio does show a significant association between mortality and CRITICAL MAR ratios. We have added a paragraph at the end of the conclusions section discussing this limitation (466-475).

- Authors have not reported results on cytokine expression after admission time.

Reply: We have added cytokine data at time points up to 48 hours.

- There are no hemodynamic measurements

Reply: We have added Shock Index data as recommended by the Reviewer.