Reviewer's report

Title: Vagus nerve stimulation improves coagulopathy in hemorrhagic shock: a thromboelastometric animal model study.

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In this manuscript the authors evaluate the effects of vagal nerve stimulation (VNS) on coagulopathy in a model of hemorrhagic shock (HS). The authors found that VNS prevented the HS-induced changes in circulating IL-1B and IL-10 levels. The also found that VNS improved coagulopathy after HS as measured by TEG. The manuscript is generally well written; however, the data set as currently presented is limited and does not provide any mechanistic insights into the mechanism by which VNS attenuates coagulopathy in this model of HS.

Specific Comments:

1. This study utilized a model of hemorrhagic shock only (no trauma) with resuscitation using Lactated ringers. Why was the shed blood not used as part of the resuscitation?

2. The experimental protocol is confusing as currently written in the methods section. Shock was induced for 15 minutes followed by resuscitation for 45 minutes. The authors describe VNS lasting for 35 minutes post-injury. Is this after, or during, the resuscitation phase? The protocol also states that blood is collected at 1 hours post HS. A diagram may help better explain the sequence of events in this model.

3. Why did the authors choose to stimulate the vagus nerve for 30 seconds at a time using 7 different stimulations over 35 minutes. This is not a common protocol for performing VNS in the literature. This frequency and duration of VNS is also not clinically applicable using either an electrical or pharmacologic approach to VNS.

4. Was their any difference in the shed blood volume between experimental groups?

5. Were the TEG results compared to classical measures of coagulopathy (PT, PTT, platelets, bleeding time)?

6. As the authors acknowledge, the addition of a trauma to this model would increase clinical relevance. It must be done.

7. What is the rationale for measuring changes in TEG and circulating cytokines at only 1 hour post injury? Were any other time points tested.

8. There is a decrease in IL-1B levels in the VNS group when comparing baseline to post-shock. This is an interesting finding; can the authors provide any explanation for this finding? How does it relates to the EG results? A mechanistic
explanation is needed. The down regulation of pro inflammatory cytokines after VNS has been known for more than a decade. How does it relate to improvements on coagulation? Is this a spleen dependent or independent mechanism? Is this a direct effect on cells involved in the coagulation process? if so, which cells?

9. There appears to be very minimal coagulopathy in the HS group (group 2) as only clot firmness is different from baseline. This injury in this current model may not be sufficient to induce significant coagulopathy and limits the findings presented here.

10. TEG data demonstrates very small, but significant changes in maximum clot firmness and alpha angle. Are these small changes clinically relevant?

11. A representative TEG tracing from each group should be added to Figure 3.

12. The authors discuss the potential role of syndecan-1 in mediating the inflammatory response to injury and the relation to IL-1B and IL-10. Can the authors provide any data regarding changes in syndecan-1 in this model. Further exploration for the mechanism mediating potential changes in coagulation would improve enthusiasm for this series of experiments.

Minor Comments:

1. The title of figure 3c is misspelled. Should read “alpha” angle.

2. The authors mention the “anti-inflammatory cholinergic pathway”. This is usually termed the “Cholinergic anti-inflammatory pathway”

**Level of interest:** An article of importance in its field

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.