## PEER REVIEW HISTORY



## ARTICLE DETAILS

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| **TITLE (PROVISIONAL)** | The Ascorbic Acid, Corticosteroids, and Thiamine in Sepsis (ACTS) Protocol and Statistical Analysis Plan: a prospective, multi-center, double-blind, randomized, placebo controlled, clinical trial |
| **AUTHORS** | Moskowitz, Ari; Yankama, Tuyen; Andersen, Lars; David T, Huang; Donnino, Michael W.; Grossestreuer, Anne; Clinical Trial Investigators, ACTS |

## VERSION 1 – REVIEW

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| **REVIEWER** | Anthony Delaney<br>Intensive Care Unit, Royal North Shore Hospital, Sydney Australia |
| **REVIEW RETURNED** | 03-Oct-2019 |

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| **GENERAL COMMENTS** | Thank you for the opportunity to review this interesting study protocol. The authors present a study protocol for a double blind randomised clinical trial of vitamin C, hydrocortisone and thiamine for patients with septic shock. Some, even perhaps most, of these comments are moot in some respect, as the authors have already enrolled 75% of the target recruitment for the study. The word limit and format of this submission may also have limited the authors space to include all the details that they may have preferred to include.<br><br>There are a few issues that the authors might consider clarifying:-<br>• The choice of a placebo arm in this population, with a change in the SOFA score as the primary outcome requires a strong justification. Both the ADRENAL and APPROCHS trials showed that hydrocortisone is associated with a more rapid weaning of vasopressors and shorter duration of initial mechanical ventilation. These would be reflected in lower cardiovascular and respiratory SOFA scores. As such, with the current design and primary endpoint, the results of this trial can only lead to a very marginal increase in knowledge in this field. Can the authors provide a strong justification for not having a comparison group allocated to receive hydrocortisone?<br>• There are scant details provided as to the method used for allocation concealment? Will the randomisation be conducted via a web-based system, telephone system, envelopes?<br>• There are no details provided to describe how the blinding will be performed. The authors might consider providing some details to reassure readers that each of the three treatments and the concomitant placebos are identical.<br>• What volume of fluids are required to dilute all three treatments? Could this additional volume pose a risk of adverse effects for trial participants that need to be considered in the exclusion criteria (e.g. severe heart failure)? If each treatment is diluted in 100ml (300ml q6h), this would mean an additional 1.2 litres of fluid volume per day |

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| | solely for the purpose of delivering the trial medications.<br>• Fungal infection, active strongyloides and TB are at least relative contra-indications to the use of corticosteroids. Did the authors consider excluding patients with these infections from participating in the trial?<br>• The data collection and monitoring plan states that data will be monitored through the REDCap system. Will source data verification undertaken for a proportion of trial participants or for any data fields?<br>• With regards to the primary outcome measurement:-<br>o How will the authors deal with the SOFA scores at 72 hours of trial participants who have been discharged from the ICU and are more likely to have missing data?<br>o How will the authors deal with SOFA scores at 72 hours for trial participants that have died prior to 72 hours?<br>• The time from onset of shock to first dose of antimicrobial is a strong predictor for outcome in patients with septic shock. Did the authors consider presenting these data in the baseline characteristics table?<br>• It would be common for the baseline characteristics to include a global measure of severity of illness (APACHE II or III, SAPS). Can the authors provide a justification for not having this, particularly so when the results of this trial will be compared to the multitude of other similar studies.<br>• From the perspective of the statistical analysis plan, the authors have dealt with the problem of multiplicity in a reasonable fashion and have specified the subgroups that will be assessed in the first instance.<br>• The sample size calculation is somewhat optimistic, particularly with regards to the power available to assess mortality. With regards to the estimates for mortality, a baseline mortality of 40% seems a little high, especially in comparison to the ADRENAL study results. A 20% absolute risk reduction is beyond what has been found for any treatment in this field.<br>• The section on concomitant therapy is unusual. Why are trial participants allowed vitamin C/hydrocortisone/thiamine in the first 4 days? Patients who have an absolute indication for these medications are already excluded. This could be a big problem for the investigators if a large proportion of trial participants in the control group receive the intervention.<br><br>• As a minor point, the term “expired” in is used in places throughout the manuscript. Do the authors mean “died”?<br><br>The current enthusiasm for the use of this cocktail of medications in patients with sepsis certainly warrants critical review and more rigorous evidence and the investigative team are to be commended for improving the evidence base in this field. I wish them all the best with the trial. |

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| **REVIEWER** | Tomoko Fujii<br>ANZIC-RC, Monash University, Australia<br><br>I am an investigator of the VITAMINS trial, which is similar to the ACTS trial. This might be a non-financial competing interest in relation to this paper. |
| **REVIEW RETURNED** | 24-Oct-2019 |

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| **GENERAL COMMENTS** | I appreciate the opportunity to review the manuscript of SAP for the ACTS trial. The ACTS trial will provide valuable information on this |

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| | topic, as such I read the manuscript with great interest. I would like to comment mainly on the consistency throughout the manuscript and the published information in the trial registration and the trial protocol published through the registration website, i.e. ClinicalTrials.gov.<br><br>1. As for the primary outcome, the protocol mentioned to the imputation method for the missing SOFA score for the patients who died before 72 hours. However, in the manuscript of SAP, the authors said multiple imputation with chained equations would be performed in case of missing data are >15%. This should be consistent.<br><br>2. The lists of secondary outcomes are variably presented across the registration site, study protocol, and the SAP. Furthermore, one of the key secondary outcomes in the SAP, Renal Failure, is reported differently between the text on page 8 and Table 2 in the SAP manuscript. I would suggest the authors make sure the list of secondary outcomes and the definitions including the observation period being consistent.<br><br>3. The authors assume different standard deviations in the two study groups to estimate the sample size. Would the authors be able to provide any rationale for this assumption and also the larger expected effect, i.e. delta SOFA -6 in treatment group, than that in the Marik's before-after study?<br><br>4. The investigators mentioned to the sample size adjustment after 100 patient enrolments in the protocol. As it appears the ACTS trial has already recruited >150 participants, the result of the sample size adjustment could be included in the SAP manuscript.<br><br>5. Minor comment. Page 25, line 1. The methods or procedures were not explained in the manuscript. Thus, 'as above' would not be appropriate.<br><br>6. Minor comment. Page 11, line18–19. The trial protocol allowed open-label hydrocortisone, but it appeared open-label thiamine and ascorbic acid are not allowed. This should be consistent with the trial protocol. |

## VERSION 1 – AUTHOR RESPONSE

**Reviewer 1 (R1):** Thank you for the opportunity to review this interesting study protocol. The authors present a study protocol for a double blind randomised clinical trial of vitamin C, hydrocortisone and thiamine for patients with septic shock. Some, even perhaps most, of these comments are moot in some respect, as the authors have already enrolled 75% of the target recruitment for the study. The word limit and format of this submission may also have limited the authors space to include all the details that they may have preferred to include.

**A:** Thank you for your time and thoughtful review.

**R1:** The choice of a placebo arm in this population, with a change in the SOFA score as the primary outcome requires a strong justification. Both the ADRENAL and APPROCHS trials showed that hydrocortisone is associated with a more rapid weaning of vasopressors and shorter duration of initial mechanical ventilation. These would be reflected in lower cardiovascular and respiratory SOFA scores. As such, with the current design and primary endpoint, the results of this trial can only lead to

a very marginal increase in knowledge in this field. Can the authors provide a strong justification for not having a comparison group allocated to receive hydrocortisone?

**A:** The reviewer makes an important point regarding the choice of a placebo as opposed to a hydrocortisone control arm. The original trial protocol was created and enrollment started prior to the publication of the ADRENAL and APPROCHS trials. At that time, there was high variability with respect to the routine use of corticosteroids in septic shock--such that mandating the use of corticosteroids would not be reflective of 'usual care' for the septic patient.

Additionally, while the use of corticosteroids was allowed at the discretion of the clinical team, just 14% of patients at the time of the most recent DSMB assessment (after 150 patients had been enrolled) received open label corticosteroids at any time during their hospitalization. This underscores the idea that a hydrocortisone control arm would not have matched the real-world care provided to most patients enrolled in this trial. We are aware that corticosteroid use varies substantially in septic shock, and that receipt of corticosteroids in septic shock likely leads to some improvement in the SOFA score. The results of the present study will need to be interpreted in that context.

We now note the above in the protocol.

**R1:** There are scant details provided as to the method used for allocation concealment? Will the randomisation be conducted via a web-based system, telephone system, envelopes?

**A:** Unique randomization lists were created for each site by an independent statistician. The list was then held by the research pharmacy at each site, which supplied treatment allocation to the research team at the time of randomization. This information has been added to the manuscript.

**R1:** There are no details provided to describe how the blinding will be performed. The authors might consider providing some details to reassure readers that each of the three treatments and the concomitant placebos are identical.

**A:** Additional information regarding blinding procedures has been added to the manuscript.

**R1:** What volume of fluids are required to dilute all three treatments? Could this additional volume pose a risk of adverse effects for trial participants that need to be considered in the exclusion criteria (e.g. severe heart failure)? If each treatment is diluted in 100ml (300ml q6h), this would mean an additional 1.2 litres of fluid volume per day solely for the purpose of delivering the trial medications.

**A:** To limit the amount of excess crystalloid given, the ascorbic acid and thiamine are mixed in 100ml of normal saline and hydrocortisone is given as an IV push. Thus, the total amount of excess volume administered is <500ml/day.

This information has been added to the manuscript.

**R1:** Fungal infection, active strongyloides and TB are at least relative contra-indications to the use of corticosteroids. Did the authors consider excluding patients with these infections from participating in the trial?

**A:** Patients were only included in the trial if agreeable to the clinical team caring for the patient. Given that there are a number of infectious processes which could theoretically be worsened by the administration of corticosteroids, we relied on the judgement of site investigators and clinical teams to appropriately balance the risks/benefits of study participation for individual patients.

**R1:** The data collection and monitoring plan states that data will be monitored through the REDCap system. Will source data verification undertaken for a proportion of trial participants or for any data fields?

**A:** In addition to close monitoring via the REDCap system as described, verification of primary data for the primary and key secondary outcomes was undertaken during in-person site visits by study monitors. Site visits were conducted after the fifth patient at each site was enrolled or earlier, at the discretion of the coordinating center. A close out site visit was also performed.

This information has been added to the manuscript.

**R1:** With regards to the primary outcome measurement:-
o How will the authors deal with the SOFA scores at 72 hours of trial participants who have been discharged from the ICU and are more likely to have missing data?
o How will the authors deal with SOFA scores at 72 hours for trial participants that have died prior to 72 hours?

**A:** Thank you for raising this important question, especially given the findings of the CITRUS-ALI trial. With regards to the first point, patients discharged from the ICU still undergo a blood draw at 72-hours for collection of SOFA score variables. Thus, SOFA missingness is generally constrained to those patients who die before 72 hours. At the time of this writing, ~9% of the cohort expired before 72 hours.

Missing SOFA variables will be indirectly imputed through the use of the mixed model described in the manuscript. However, if there is a large imbalance between groups in early death as seen in CITRUS-ALI, this may not be sufficient to guard against a Type 2 error. Thus, we have added an additional planned sensitivity analysis that includes a specific imputation strategy for SOFA scores that are missing due to early death.

**R1:** The time from onset of shock to first dose of antimicrobial is a strong predictor for outcome in patients with septic shock. Did the authors consider presenting these data in the baseline characteristics table?

**A:** While this would be interesting, we unfortunately do not capture this information in our CRF.

**R1:** It would be common for the baseline characteristics to include a global measure of severity of illness (APACHE II or III, SAPS). Can the authors provide a justification for not having this, particularly so when the results of this trial will be compared to the multitude of other similar studies.

**A:** Baseline SOFA score will be included as a measure of illness severity prior to study drug administration.

**R1:** From the perspective of the statistical analysis plan, the authors have dealt with the problem of multiplicity in a reasonable fashion and have specified the subgroups that will be assessed in the first instance.

**A:** Thank you.

**R1:** The sample size calculation is somewhat optimistic, particularly with regards to the power available to assess mortality. With regards to the estimates for mortality, a baseline mortality of 40% seems a little high, especially in comparison to the ADRENAL study results. A 20% absolute risk reduction is beyond what has been found for any treatment in this field.

**A:** The study was sized to have adequate power to detect a difference in the primary outcome (i.e. change in SOFA score). We agree that an absolute risk reduction of 20% is optimistic, although would be less than that seen in the original study by Marik et. al. Of note, a patient level meta-analysis including subjects from a number of ongoing trials of ascorbic acid, corticosteroids, and thiamine is planned and will be adequately powered to detect smaller mortality differences.

**R1:** The section on concomitant therapy is unusual. Why are trial participants allowed vitamin C/hydrocortisone/thiamine in the first 4 days? Patients who have an absolute indication for these medications are already excluded. This could be a big problem for the investigators if a large proportion of trial participants in the control group receive the intervention.

**A:** While we did exclude patients with a clear indication for any study drug at the time of enrollment, clinical trajectory changes (e.g. worsening shock), new information (e.g. a previously unknown history of alcoholism), or change in clinical team sometimes resulted in a decision to give a study article open label. For patient safety, we did not discourage clinical teams from providing a therapy they felt would be beneficial to the patient.

**R1:** As a minor point, the term “expired” in is used in places throughout the manuscript. Do the authors mean “died”?

**A:** This has been changed to ‘died’ as suggested.

**R1:** The current enthusiasm for the use of this cocktail of medications in patients with sepsis certainly warrants critical review and more rigorous evidence and the investigative team are to be commended for improving the evidence base in this field. I wish them all the best with the trial.

**A:** Thank you!

**Reviewer 2 (R2):**

**R2:** I appreciate the opportunity to review the manuscript of SAP for the ACTS trial. The ACTS trial will provide valuable information on this topic, as such I read the manuscript with great interest. I would like to comment mainly on the consistency throughout the manuscript and the published information in the trial registration and the trial protocol published through the registration website, i.e. ClinicalTrials.gov.

**A:** Thank you for the thoughtful comments.

**R2:** As for the primary outcome, the protocol mentioned to the imputation method for the missing SOFA score for the patients who died before 72 hours. However, in the manuscript of SAP, the authors said multiple imputation with chained equations would be performed in case of missing data are >15%. This should be consistent.

**A:** Thank you. The SAP included in the protocol version uploaded to CT.gov was developed at the time of initial protocol drafting. The current SAP under review has been updated with additional input from study statisticians. Of note, updates to the SAP have been done prior to any unblinding of the data and prior to completion of study enrollment.

As you note, the initial analysis plan called for direct imputation of SOFA scores in those cases where a patient died prior to 72 hours (~9% of the present cohort). This was removed as it was felt unlikely that there would be large group imbalances in early mortality and there was no clear ‘best’ approach to SOFA score imputation in the event of early death. However, as described above, the publication of CITRUS-ALI while the present manuscript was under review has led us to rethink this approach and we now include the direct imputation approach previously described as a sensitivity analysis.

**R2:** The lists of secondary outcomes are variably presented across the registration site, study protocol, and the SAP. Furthermore, one of the key secondary outcomes in the SAP, Renal Failure, is reported differently between the text on page 8 and Table 2 in the SAP manuscript. I would suggest the authors make sure the list of secondary outcomes and the definitions including the observation period being consistent.

**A:** Thank you. As above, the present manuscript will represent the final SAP upon its publication. The analysis plan presented will be made consistent across all published study documents prior to unblinding.

**R2:** The authors assume different standard deviations in the two study groups to estimate the sample size. Would the authors be able to provide any rationale for this assumption and also the larger expected effect, i.e. delta SOFA -6 in treatment group, than that in the Marik's before-after study?

**A:** The absolute difference in delta SOFA score between groups seen in the study by Marik et. al. was 81.3%, as compared to 33.3% predicted in the present study. We expect larger overall changes in SOFA score than seen in the Marik study as we anticipate higher baseline SOFA scores with more room for improvement. Notably, the study by Marik et. al. included patients with severe sepsis and <50% were on vasopressors. This contrasts with our study wherein all included patients are receiving vasopressors as the time of enrollment. The difference in predicted standard deviations reflects our expectation that some patients (e.g. those with underlying vitamin deficiency) might respond more robustly than others to the intervention, resulting in a wider spread of delta-SOFA in the intervention arm.

**R2:** The investigators mentioned to the sample size adjustment after 100 patient enrolments in the protocol. As it appears the ACTS trial has already recruited >150 participants, the result of the sample size adjustment could be included in the SAP manuscript.

**A:** The results are now included. No change in sample size was made as baseline SOFA scores and 30-day mortality were in line with those predicted at study outset. Specifically, at the time of the second DSMB meeting (after enrollment of 100 patients), 30-day mortality was 30% and 72-hour delta SOFA was 4.4 (± 3.7). If the Reviewer feels this information should be included in this published protocol and SAP, we will be happy to include it.

**R2:** Minor comment. Page 25, line 1. The methods or procedures were not explained in the manuscript. Thus, 'as above' would not be appropriate.

**A:** Thank you. This has been deleted.

**R2:** Minor comment. Page 11, line18–19. The trial protocol allowed open-label hydrocortisone, but it appeared open-label thiamine and ascorbic acid are not allowed. This should be consistent with the trial protocol.

**A:** For the sake of manuscript length, some elements of the full trial protocol are included only in the version uploaded to clinicaltrials.gov referenced in the manuscript. As described in response to Review 1, clinical teams were allowed to administer any open label medication that they felt was clinically indicated. This was primarily pertinent to the administration of open label corticosteroids to patients in septic shock.

## VERSION 2 – REVIEW

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| **REVIEWER** | Anthony Delaney<br>Royal North Shore Hospital |
| **REVIEW RETURNED** | 15-Nov-2019 |

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| **GENERAL COMMENTS** | I am satisfied that the authors have addressed the queries adequately.<br><br>I wish them all the best for the completion of the study and |

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| | dissemination of the results. |

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| **REVIEWER** | Tomoko Fujii<br>ANZIC-RC, Monash University<br><br>I am an investigator of the VITAMINS trial, which is similar to the ACTS trial. This might be a non-financial competing interest in relation to this paper. |
| **REVIEW RETURNED** | 11-Nov-2019 |

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| **GENERAL COMMENTS** | I would appreciate another opportunity to review the revised manuscript of SAP for the ACTS trial. I would acknowledge the authors' effort to address my previous comments. I would agree that the SAP to be published should be the final, but prior-to the un-blinding of the allocation in the trial data, plan for this trial. Please find some minor comments below.<br><br>1. As for the consistency in the list of secondary outcomes, I still found some secondary outcomes were not listed in table 2 or defined inconsistently in the manuscript. Please check thoroughly to avoid confusion.<br><br>2. Page 10, line 15. It is unclear what the word 'evaluable' means here. Would the authors be able to provide an explanation if the investigators recruit more than 200 patients and exclude some of them from the analysis? If the recruitment will be > 200, please provide the target number with explanations.<br><br>3. Page 10, line 18. Please explain the reason the investigators assume a larger effect than that observed in Marik's study in the manuscript.<br><br>4. Page 14, line 8. Would the authors be able to explain where the number of '20%' comes from?<br><br>5. The authors will apply the LOCF method to impute the missing SOFA score at the endpoint. As the SOFA score is the primary outcome of this trial, the limitation in the assessment of the score should be noted. |

## VERSION 2 – AUTHOR RESPONSE

**Reviewer 1 (R1):** I am satisfied that the authors have addressed the queries adequately. I wish them all the best for the completion of the study and dissemination of the results.

**A:** Thank you! We greatly appreciate your time and important contribution to the review of our protocol and SAP.

**Reviewer 2 (R2):** I would appreciate another opportunity to review the revised manuscript of SAP for the ACTS trial. I would acknowledge the authors' effort to address my previous comments. I would agree that the SAP to be published should be the final, but prior-to the un-blinding of the allocation in the trial data, plan for this trial. Please find some minor comments below.

**A:** Thank you again for your review and thoughtful comments. We have addressed your notes in the revised manuscript.

**R2:** As for the consistency in the list of secondary outcomes, I still found some secondary outcomes were not listed in table 2 or defined inconsistently in the manuscript. Please check thoroughly to avoid confusion.

**A:** We have identified a few inconsistencies as noted by the reviewer and corrected them. We appreciate the reviewer's careful attention to these discrepancies, which will prevent any confusion at the time of analysis. After multiple reviews, we did not identify any remaining inconsistencies. Clinicaltrials.gov has been updated accordingly and is presently released for review.

**R2:** Page 10, line 15. It is unclear what the word 'evaluable' means here. Would the authors be able to provide an explanation if the investigators recruit more than 200 patients and exclude some of them from the analysis? If the recruitment will be > 200, please provide the target number with explanations.

**A:** An evaluable patient is one who received at least one dose of study drug and will therefore be included in the modified intent-to-treat analysis. We will enroll 200 evaluable patients. Any patient who is randomized but not ultimately given study drug will be included in the CONSORT diagram and reasons for not receiving study drug provided. This information has been added to the manuscript.

**R2:** Page 10, line 18. Please explain the reason the investigators assume a larger effect than that observed in Marik's study in the manuscript.

**A:** As we are primarily concerned with between group differences in change in SOFA score over time, the relative change is what will be used to determine the effectiveness of the intervention and formed the basis of our power calculations. In that respect, our estimates are quite a bit more conservative than those put forth in the study by Marik et. al. Specifically, Marik found a 4-point difference (81.3% relative difference) in SOFA score change between groups whereas we are powered based on an expected 2-point difference (33.3% relative difference). As we are enrolling a sicker population and expect a higher baseline SOFA score, the same percent change will result in a larger absolute decrease in SOFA score. This information has been added to the manuscript.

**R2:** Page 14, line 8. Would the authors be able to explain where the number of '20%' comes from?

**A:** In a review of the existing literature, there is no standard imputation strategy for SOFA score data missing due to death. In discussion with the trial steering committee and statisticians, a 20% 'penalty' for death was planned for this sensitivity analysis. In collected data thus far, few patients (<10%) expire prior to the 72-hour time point.

**R2:** The authors will apply the LOCF method to impute the missing SOFA score at the endpoint. As the SOFA score is the primary outcome of this trial, the limitation in the assessment of the score should be noted.

**A:** One strength of this study is that SOFA score will be collected (including blood draws) at each time point, regardless of whether the patient is in the ICU or on the medical floor. Thus, non-death related SOFA missingness will be quite low and is expected to occur at random (e.g. due to laboratory error). As suggested however, we have added a note regarding potential bias resulting from SOFA missingness and plan to report SOFA missingness by group.

## VERSION 3 – REVIEW

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| **REVIEWER** | Tomoko Fujii<br>Monash University, Australia<br><br>I am an investigator of the VITAMINS trial, which is similar to the ACTS trial. This might be a non-financial competing interest in relation to this paper. |
| **REVIEW RETURNED** | 20-Nov-2019 |

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| **GENERAL COMMENTS** | The authors have addressed my comments largely and I do not have further comments.<br>Thank you. All the best with the trial. |