PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Key Dimensions of Posttraumatic Stress Disorder and Endothelial Dysfunction: A Protocol for a Mechanism-Focused Cohort Study
AUTHORS	Cleveland, Shiloh; Reed, Kristina; Thomas, Jordan; Ajijola, Olujimi; Ebrahimi, Ramin; Hsiai, Tzung; Lazarov, Amit; Montoya, Amanda; Neria, Yuval; Shimbo, Daichi; Wolitzky-Taylor, Kate; Sumner, Jennifer

VERSION 1 – REVIEW

REVIEWER	Christopher Celano Massachusetts General Hospital / Harvard Medical School United States of America
REVIEW RETURNED	24-Sep-2020

GENERAL COMMENTS	The authors present the protocol for an observational cohort study to examine predictors of endothelial dysfunction in individuals with PTSD. This is an interesting and timely topic that would be of interest to the readers of BMJ Open. The protocol is presented clearly overall, and the study design seems reasonable and well-powered to accomplish the aims set out by the authors. However, a few minor revisions may help to strengthen the manuscript prior to publication. General: • It may be helpful to describe how/why the authors chose their control group (i.e., individuals with trauma but no PTSD diagnosis). What is the rationale for not including a healthy control group, either in place of or in addition to the group of individuals with trauma but no PTSD. Introduction: • The authors state that endothelial dysfunction is a "malleable risk marker responsive to intervention." I am curious what interventions are available for endothelial dysfunction and why they would not be applied to all patients at risk of CVD (rather than just patients with PTSD), if endothelial dysfunction is involved in the pathogenesis of CVD/CAD. It seems like the purpose of this study really involves the identification of targets for psychological interventions for this patient population. If that is the case, a discussion of treatments for endothelial dysfunction seems a bit tangential. Participants: • MMSE score should be <=18, not >=18, as this refers to an exclusion criterion. • It would be helpful to provide a rationale for excluding patients receiving psychotropic medications. One could argue that since all patients are still having symptoms of PTSD, they are not receiving

	adequate treatment and therefore should be included in the analyses. Alternatively, if you argue that individuals receiving treatment for PTSD should be excluded, then it seems like individuals in psychotherapy for PTSD should also be excluded. I am not sure there is a "right" answer regarding this, but some rationale for this would be helpful. • Given that inflammatory markers are being measured, it may be useful to consider excluding individuals with inflammatory disorders (e.g., systemic lupus erythematosus) or taking anti-inflammatory medications (e.g., steroids). Outcome measures: • Though the study is likely underpowered for this, was there a consideration of evaluating for incidence of CVD during the follow-up period? Statistical analyses: • As depression is associated with markers of endothelial dysfunction, it might be useful to control for that in the analyses. If the authors choose not to do so, it would be helpful to provide a rationale for that decision.

REVIEWER	Neeti Mehta Emory University United States
REVIEW RETURNED	08-Jan-2021

GENERAL COMMENTS	This manuscript by Cleveland et al. details a protocol for a trial assessing the relationship between PTSD dimensions of fear and dysphoria and endothelial dysfunction in trauma-exposed participants with and without a diagnosis of PTSD both cross-sectionally and longitudinally in a subset of patients. The manuscript adequately outlines methods by which the study will occur, including assessment of PTSD and PTSD symptom severity using the CAPS, and measures of endothelial dysfunction via flow-mediated vasodilation (FMD) of the brachial artery and measuring endothelial cell-derived microparticles (EMPs). Additionally, measures of parasympathetic tone, oxidative stress, and inflammatory markers will be measured as potential pathway variables related to PTSD and endothelial dysfunction. Some minor clarifications are needed, as stated below: 1. The guidelines for protocol reviews for this journal state that dates of the study should be included in the manuscript; however, no dates of the study are included. Therefore, it is not clear whether the trial has already begun or is yet to begin. 2. Please clarify – will family history of CVD be exclusionary for the study? 3. Will the analyses control for comorbid depression or depression symptom severity measured by the PHQ-8? How will the PHQ-8 scores be used? 4. Please clarify the what is meant by “propensity score”. Is this evaluating the propensity for developing CVD? 5. For the eye-tracking paradigm, please further explain the use of only happy and sad face stimuli. Do you expect to see the same results with this paradigm as seen in depressed patients? 6. The participants will be asked to “refrain from taking medications and vitamins (except diabetes medication, blood thinners, statins, and birth control)” – will their concomitant medications be controlled for in analyses? These medications may impact the biologic measures of interest.

	7. The methods for EDTA plasma isolation should be better described (including temperature of collection/spinning, amount of time from blood collection to plasma isolation, etc). 8. Will the effect of potential treatment (i.e. medication, psychotherapy, etc) between the baseline visit and 2-year follow-up be examined or controlled for?

VERSION 1 – AUTHOR RESPONSE

Reviewer #1: Dr. Christopher Celano, Harvard Medical School

Critique #1: It may be helpful to describe how/why the authors chose their control group (i.e., individuals with trauma but no PTSD diagnosis). What is the rationale for not including a healthy control group, either in place of or in addition to the group of individuals with trauma but no PTSD.

Response #1: When designing this study, we considered whether to include a no trauma control group and ultimately decided to focus only on trauma-exposed individuals. A number of studies comparing individuals without trauma and with trauma and posttraumatic stress disorder (PTSD) has demonstrated that trauma-exposed individuals exhibit elevated risk of incident cardiovascular disease (CVD), with the greatest risk in those with greater psychiatric severity (e.g., Sumner et al., 2015; Sumner et al., 2016). Including a healthy control group as the only control group would make it challenging to disentangle whether differences in endothelial function were due to trauma exposure per se or to the psychological sequelae of trauma exposure. Given that the aim of the study is to better understand what aspects of psychopathology after trauma—namely PTSD—might be most cardiotoxic, we thus chose to focus on CVD risk in trauma-exposed individuals. Notably, this approach mirrors the prior literature examining PTSD and endothelial dysfunction. For example, Grenon et al. (2016) and Violanti et al. (2006) demonstrated that greater PTSD symptom severity was associated with lower flow-mediated dilation (FMD) in samples of trauma-exposed individuals. We now provide our rationale for including only a trauma-exposed control group in the Brief Study Overview subsection of the Methods and Analysis section on p. 8:

> **"No-trauma controls were not included, as this study focuses on identifying what aspects of PTSD symptoms are linked to endothelial dysfunction."**

Critique #2: The authors state that endothelial dysfunction is a "malleable risk marker responsive to intervention." I am curious what interventions are available for endothelial dysfunction and why they would not be applied to all patients at risk of CVD (rather than just patients with PTSD), if endothelial dysfunction is involved in the pathogenesis of CVD/CAD. It seems like the purpose of this study really involves the identification of targets for psychological interventions for this patient population. If that is the case, a discussion of treatments for endothelial dysfunction seems a bit tangential.

Response #2: The purpose of this study is to identify a potential mechanism through which CVD risk may occur in trauma-exposed individuals; this mechanism may then be a treatment target for psychosocial intervention in future research. We thus note that endothelial dysfunction is a "malleable risk marker responsive to intervention" in order to emphasize why endothelial dysfunction is a promising marker for research focused on cardiovascular risk mechanisms and prevention in trauma-exposed individuals. As described in the protocol paper, the first step in this proposed line of research is to identify key manifestations of PTSD that may be most linked to endothelial dysfunction. Our goal is not to determine who should receive interventions for endothelial dysfunction. A next step will be to then test if intervening upon these manifestations of PTSD can bring about improvements in endothelial dysfunction.

Conducting this research with a cardiovascular marker that is malleable and responsive to intervention such as endothelial dysfunction is key in order to be able to document potential improvement. Given that endothelial dysfunction has been shown to respond to interventions such as prescribed exercise, lipid-lowering medication, and vitamin D (e.g., Lee et al., 2018; Pedralli et al., 2020; Stroes et al., 1995; Sugden et al., 2008), we hypothesize that it may also respond to psychological interventions and corresponding improvements in PTSD symptoms. Given the focus of the current study, we do not go into detail on existing interventions for endothelial dysfunction.

In the Discussion section, we note the potential for intervention work in future research on pp. 19-20:

> **"PTSD predicts incident CVD, but the field needs intervention targets and intermediary mechanisms to determine if PTSD interventions can offset CVD risk. This study will test whether endothelial dysfunction could be an early subclinical, modifiable mechanism by which PTSD increases CVD risk, and whether posttraumatic fear or another dimension could be the target to offset that risk in vulnerable, trauma-exposed individuals in future intervention studies."**

Critique #3: MMSE score should be <=18, not >=18, as this refers to an exclusion criterion.

Response #3: We thank the reviewer for pointing out this typo. We have now corrected this in the manuscript on p. 9.

Critique #4: It would be helpful to provide a rationale for excluding patients receiving psychotropic medications. One could argue that since all patients are still having symptoms of PTSD, they are not receiving adequate treatment and therefore should be included in the analyses. Alternatively, if you argue that individuals receiving treatment for PTSD should be excluded, then it seems like individuals in psychotherapy for PTSD should also be excluded. I am not sure there is a "right" answer regarding this, but some rationale for this would be helpful.

Response #4: We chose to exclude participants receiving psychotropic medications (except benzodiazepines taken as-needed), as research has demonstrated that these medications can affect key variables of interest in the current study, including FMD (our main outcome; Hantsoo et al., 2014), psychophysiological responses (Grillon et al., 2009; Ikawa et al., 2001), and attentional allocation (Wells et al., 2014; Zhang et al., 2020). Given this evidence, we chose to be more conservative with our approach and excluded individuals on these medications in an effort to minimize their impact on our measures. We now provide a brief rationale for our decision when describing this exclusion criterion on p. 8 of the manuscript:

> **"(b) current psychotropic medication use (except benzodiazepines taken as-needed), as this has been shown to influence key study variables including FMD, psychophysiological responses, and attentional allocation[57-61],,**

Critique #5: Given that inflammatory markers are being measured, it may be useful to consider excluding individuals with inflammatory disorders (e.g., systemic lupus erythematosus) or taking anti-inflammatory medications (e.g., steroids).

Response #5: When designing the study, we considered the balance of inclusion vs. exclusion criteria for our sample. Inflammatory markers will be explored as potential pathway variables linking PTSD with endothelial dysfunction. Given that this is an exploratory aim of the study, we decided not to exclude individuals with inflammatory disorders or taking anti-inflammatory medications. However, we are collecting comprehensive information on all medical conditions and medications as part of our medical history interview, and we can account for these variables in our analyses. Indeed, we note on p. 17 that medications will be considered as potential covariates.

Critique #6: Though the study is likely underpowered for this, was there a consideration of evaluating for incidence of CVD during the follow-up period?

Response #6: We are certainly interested in the links between key dimensions of PTSD and incident CVD, and we are collecting data on any medical conditions, including cardiovascular events, that onset over the follow-up period. However, given the small sample size, we do not anticipate having a sufficient number of cardiovascular events to analyze. Thus, we do not address this point in the protocol paper, although we believe that this is an important direction for future research.

Critique #7: As depression is associated with markers of endothelial dysfunction, it might be useful to control for that in the analyses. If the authors choose not to do so, it would be helpful to provide a rationale for that decision.

Response #7: We agree that it is important to account for depression in our analyses, especially given high comorbidity of PTSD and depression. We are collecting diagnostic and self-report symptom data related to depression. We now describe how we will incorporate these measures into analyses on p. 17:

> **"Even though posttraumatic fear has been found to be distinct from the core symptoms of depression,[98 99] we will also consider depression as a potential confounder given high comorbidity of PTSD and depression.[2] Data on depression diagnoses and symptoms will be examined as covariates, and we will explore how comorbidity of PTSD and other psychopathology (e.g., depression) relates to endothelial dysfunction."**

Reviewer #2: Dr. Neeti Mehta, Emory University School of Medicine

Critique #1: The guidelines for protocol reviews for this journal state that dates of the study should be included in the manuscript; however, no dates of the study are included. Therefore, it is not clear whether the trial has already begun or is yet to begin.

Response #1: Thank you for bringing this to our attention. Enrollment for the study began in 2019. We now include the following information in the Brief Study Overview subsection of the Methods and Analysis section on p. 8:

> **"Enrollment began in 2019, and the study is projected to continue through 2023."**

Critique #2: Please clarify – will family history of CVD be exclusionary for the study?

Response #2: Only a personal history of CVD is exclusionary for the study. We have now clarified that exclusions include "individual CVD history" on p. 8 of the manuscript.

Critique #3: Will the analyses control for comorbid depression or depression symptom severity measured by the PHQ-8? How will the PHQ-8 scores be used?

Response #3: Please see the response to Critique #7 from Reviewer #1.

Critique #4: Please clarify what is meant by "propensity score". Is this evaluating the propensity for developing CVD?

Response #4: The propensity scores are calculated during recruitment to balance sociodemographic and trauma-related characteristics across participants in the PTSD and trauma-exposed control groups. We now clarify that the scores reflect propensity of PTSD group membership; they do not indicate the propensity for developing CVD. The following information is now provided in the manuscript on p. 11 when describing the propensity scores:

> **"Propensity scores are used in recruitment to improve balance of sociodemographic and trauma-related characteristics across the PTSD and trauma-exposed control groups.[78] Scores reflecting propensity of PTSD group membership are calculated using age, gender, race/ethnicity, trauma type, time since trauma, and two-way interactions among these variables. Matching during recruitment is based on quintiles of the propensity score distribution, with scores re-estimated weekly throughout recruitment. Participants are recruited if there are <3 unmatched individuals from their group in the same quintile."**

Critique #5: For the eye-tracking paradigm, please further explain the use of only happy and sad face stimuli. Do you expect to see the same results with this paradigm as seen in depressed patients?

Response #5: This study examines two broad dimensions of PTSD: fear and dysphoria. Given that we include objective measures of posttraumatic fear responses with our psychophysiological indicators, we wanted to have an objective measure of posttraumatic dysphoria as well. The eye-tracking paradigm described in the manuscript is an established and reliable task-based measure of dysphoria-related attentional allocation. Although eye-tracking paradigms have been developed that measure attention to a variety of affective stimuli, we specifically chose this task because it has been found to distinguish between individuals with and without depression (Lazarov et al., 2018; Hoffman & Subramaniam, 1995; Deubel & Schneider, 1996).

Critique #6: The participants will be asked to "refrain from taking medications and vitamins (except diabetes medication, blood thinners, statins, and birth control)" – will their concomitant medications be controlled for in analyses? These medications may impact the biologic measures of interest.

Response #6: We comprehensively document all medications and can account for these as covariates in analyses. We ask participants to refrain from taking medications and vitamins,

except for those listed above, because the FMD measure is sensitive to medication use, among other factors.

Critique #7: The methods for EDTA plasma isolation should be better described (including temperature of collection/spinning, amount of time from blood collection to plasma isolation, etc).

Response #7: We now describe our methods for EDTA plasma isolation in more detail on pp. 11-12 of the manuscript.

> **"EDTA tubes are centrifuged within one hour of collection at 1500g at 4°C for 10 minutes to isolate plasma; aliquots are stored at -80°C for inflammatory marker assays."**

Critique #8: Will the effect of potential treatment (i.e. medication, psychotherapy, etc) between the baseline visit and 2-year follow-up be examined or controlled for?

Response #8: History of psychiatric treatment and medication use are assessed at both baseline and follow-up. We plan to account for this in our analyses. Due to word limits, we do not describe this in the manuscript, as they are not central to our aims.

VERSION 2 – REVIEW

REVIEWER	Neeti Mehta Emory University United States
REVIEW RETURNED	28-Feb-2021

GENERAL COMMENTS	All critiques have been adequately addressed by the authors.