Heterogeneity and Predictors of Stress Reactivity in Takotsubo Syndrome

The Broken Heart Study -II

Study Protocol

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Tool Revision History

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Summary of Revisions:

- Refined procedures for stress reactivity test
- Procedures for the collection of hair samples for the assessment of hair cortisol added
- Poor echocardiographic window added to exclusion criteria
- Perceived Stress survey added to study assessments
- Personnel changes

Version Number 3
Version date: 6-25-2020
Summary of revisions:

- Personnel added to study team
- Section on Covid-19 safety measures added

Version Number 4
Version date: 8-15-2020
Summary of revisions:

- Phlebotomist, and new research assistant added to study team
- Clarification of who will conduct trigger interview in visit 1

Version Number 5
Version date: 9-15-2020
Summary of revisions:

- Changes to stipend amounts given to participant at each study visit; minor typos

Version number 6
Version date: 10-5-2020
Summary of revisions:

- Minor changes to eligibility criteria and mental stress protocol; clarification of time window for study assessment; added a risk for Echo/Holter procedure; weight, height, waist/hip ratio no longer collected.

Version number 7
Version date: 03-29-2021
Summary of revisions:

- Change recruitment window to include up to 12 weeks.

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- Minor changes for clarification and typos.

Version number 8
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Summary of revisions:
Add student interns to research team

Version number 9
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Summary of revisions:
Changes to research team, add echocardiograms analyst, deletion of research assistant, change student intern to student research assistant.

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Summary of revisions:
Sample size and minor modifications to aim 3
Holter software used and location of Holter analysis
Location of echocardiogram analysis.

Version number 11
Version date: **03.23.2022**
Summary of revisions:
Add student intern
Modify consenting process to allow remote enrollment

Version number 12
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Summary of revisions:
Add co-investigator and psychology intern and remove student Intern

Version number 13
Version date: 01.16.2023
Summary of revisions:
Add co-investigator

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2 LIST OF ABBREVIATIONS

AE	Adverse Event
AHA	American Heart Association
BOMC	Blessed Orientation Memory Concentration test
CBPM	Centers for Behavioral and Preventive Medicine
Co-I	Co-Investigator
DBP	Diastolic blood pressure
DSMB	Data Safety and Monitoring Board
HADS	Hospital Anxiety and Depression Scale
HIPAA	The Health Insurance Portability and Accountability Act
HR	Heart rate
HRV	Heart rate variability
IRB	Institutional Review Board
LV	Lest ventricular
MACCE	Major adverse cardiac and cerebrovascular event
PCP	Primary Care Provider
PD	Project Director
PHI	Protected Health Information
PI	Principal Investigator
SAE	Serious Adverse Event
SBP	Systolic blood pressure
DBP	Diastolic blood pressure
RA	Research Assistant
TMH	The Miriam Hospital
TS	Takotsubo syndrome

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3 BACKGROUND AND RATIONALE

3.1. Background

3.1.1. Epidemiology. Takotsubo syndrome (TS), also known as apical ballooning syndrome and 'broken heart syndrome', is characterized by acute, reversible systolic heart failure accompanied by ischemic electrocardiographic changes and cardiac enzyme elevation. While initially described in a Japanese population, TS occurs worldwide, and it has been increasingly recognized over the past two decades. Data from the National Inpatients Sample (NIS) show that the incidence of TS in the United States has increased over 3-fold from 2007 to 2012.[2] TS accounts for nearly 10% of annual emergency room admissions for a suspected acute coronary syndrome (ACS) among women, although these figures are likely underestimated due to the under-diagnosis of less severe cases.[3-6]

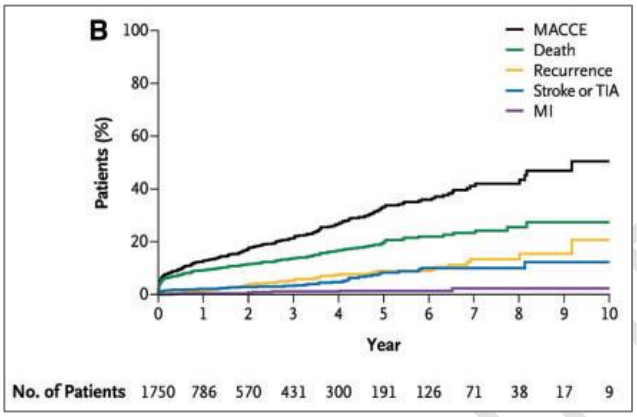


Figure 1. Kaplan–Meier Estimates of 10-Year Outcome Events. MACCE was a composite of death from any cause, recurrence of takotsubo cardiomyopathy, stroke or transient ischemic attack (TIA), or myocardial infarction (MI).[1]

3.1.2. Prognosis. Despite initial reports of a benign in-hospital prognosis, more recent data from multi-center registries have shown that rates of in-hospital complications and mortality are similar to those of ACS (5-7%). [1,2,7,8] Furthermore, TS survivors have a second event (usually occurring between 3 weeks to 4 years after the first episode)[7,9,10] in approximately 3-5% of case. As for the long-term prognosis of TS, recent data from the Nationwide Readmissions Database show that almost 25% of TS patients are readmitted within 90 days of discharge,[11] and both large registries and a comprehensive meta-analysis have documented rates of major adverse cardiac and cerebrovascular event (MACCE) of 10% per patient-year and all-cause death rates of 6%[1,7] (Figure 1). Lastly, a recent study has shown that TS survivors have long-term symptomatic and functional impairments of the left ventricular (LV) function,[12] possibly mediated by a chronic inflammatory state.[13]

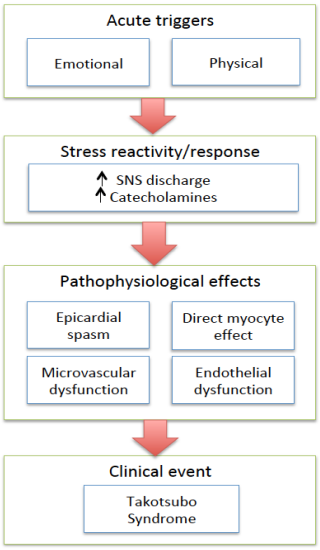
3.1.3. Clinical characteristics. The hallmark of TS is acute, severe heart failure due to an acute systolic dysfunction of the left ventricle. Other unique characteristics of TS include the almost exclusive involvement of older women (> 90% of cases) and the presence of an acute emotional (e.g., receiving bad news, unexpected loss, illness or injury of a close relative, angry arguments, and intense fear) or physical (e.g., surgery, sepsis, stroke, acute respiratory insufficiency) trigger preceding the onset of TS in about 2/3 of cases, while in the remaining 1/3 of cases no trigger is identified.[1,1,14] Symptoms include chest pain, dizziness, syncope, and dyspnea. The electrocardiogram (ECG) shows ST elevation or negative T waves in the precordial leads,[4] while the echocardiogram shows profound wall motion abnormalities that do not correspond to the distribution of a single coronary vessel and usually involve the apical and/or mid-portion ballooning of the left ventricle.[15-18] During systole, the bulging ventricle resembles a "tako-tsubo" (i.e., the octopus trap used by Japanese fishermen to trap octopuses) – hence, the syndrome's name. Although coronary artery disease may co-exist with TS, the coronary angiogram shows no

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evidence of obstructive coronary plaques, plaque rupture, or thrombosis.[19-21] Serum troponin is elevated in 85% of patients,[4] with peak elevations usually smaller than those observed in acute myocardial infarction (AMI). Although a number of diagnostic criteria have been recently proposed,[22] the Mayo Clinic criteria[23] are most commonly used to confirm a diagnosis of TS.

3.1.4. Pathophysiology. Genetic, hormonal, psychological, and pharmacological factors may play a role in the onset of TS. Studies looking at *genetically determined variations* in catecholamine sensitivity found that TS patients have an increased genotype frequency of beta1-adrenergic receptor (b1 AR) polymorphism, beta2 AR, and G protein coupled receptor kinase 5 (GRK5) polymorphism compared to controls,[24-26] but others did not confirm these results.[27,28] Taken together, this preliminary evidence suggests that the role of genetic factors in the onset of TS is still unclear.[29]

A role for *hormonal factors* was hypothesized to explain the disproportionate involvement of older women.[30-33] This hypothesis has been supported by animal studies showing that estrogen supplementation reduces responses to emotional stress.[33-35] Findings in human samples, however, are far from conclusive, with recent studies showing higher, or similar, concentrations of estradiol in TS women compared to controls.[36,37] Preliminary work from our group and others also found no differences in reproductive characteristics between female TS cases and controls.[38,39]



Data from large registry-based studies consistently indicate that patients with TS are more likely to have a history of *anxiety and depression* compared to controls with AMI or healthy controls.[1,14,40-42] Both anxiety and depression have been associated with decreased catecholamine re-uptake in other populations.[43-46] As for psychological factors, two studies (including our own) have examined if patients who display certain "negative" personality traits (e.g., type D personality; pessimism; hostility) could have a higher risk of developing TS but found no conclusive evidence that such traits could play a role in the onset of this condition.[47,48 47,48] Low stress resilience and poor coping skills could also contribute to the physiopathology of TS. Although evidence is preliminary even in other cardiac populations, studies have shown that low resilience is associated with increased risk of coronary heart disease, independent of confounding and mediating factors.[49]

Finally, several case reports have also linked the onset of TS with the use of *medications* that affect catecholamine uptake (e.g., SSRIs) or have sympathomimetic effects (e.g., beta-2 receptors agonists, nasal decongestants).[50]

There is strong evidence that the acute systolic heart failure which is the hallmark of TS is caused by an exaggerated discharge of the sympathetic nervous system (SNS) via local norepinephrine release at neuronal synapses along with secretion of adrenaline from the adrenal gland.[51,52] A seminal study showed extremely high levels of plasma catecholamines in TS patients compared to AMI controls and the endomyocardial biopsies from TS patients showed a form of myocyte damage (contraction band necrosis) that is uniquely associated with catecholamine excess.[53,54] According to the model proposed by Wittstein et al.,[54] exposure to an acute trigger elicits a profound response of the SNS with release of catecholamine at nerve terminals and into the bloodstream, which in turn activates the cascade of pathophysiological effects (epicardial spasm,

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myocyte injury,[55] impairment in coronary flow reserve,[56,57] and endothelial dysfunction),[58-60] which then lead to the acute systolic heart failure and to the full clinical syndrome (Figure 2).

3.2. Gaps in knowledge.

Although we have reached a reasonable understanding of the major pathophysiological process involved in TS – namely, the role played by catecholamine surge in creating myocardial damage and acute systolic heart failure as well as the role of some likely predisposing factors**, *important knowledge gaps still exist regarding the unique pathophysiological milieu that contributes to the occurrence of this important condition*.**

- **Heterogeneity in TS triggers.** While exposure to an emotional trigger was thought to be a defining precipitant of TS, more recent data indicate that there is considerable heterogeneity in regard to triggers. Data from large international registries show that emotional triggers are present only in 1/3 of cases, while physical triggers are identified in another 1/3, and no triggers are identified in the remainder.[22] ***A critical weakness in these registries is a lack of precision in determining the triggering event, which is typically assessed from medical records reviews several years after the index episode. We will address this evidence gap by using the more rigorous interview method previously utilized by others to study triggers of AMI.*[61-63]**
- **Heterogeneity in TS pathophysiology**. The pathway linking distinct triggers to the clinical onset of TS remains to be defined. To the best of our knowledge only three small studies have examined this issue, and such studies focused specifically on stress reactivity. The first found that patients with TS (n = 18) had a greater catecholamine response during the performance of a mentally stressful task compared to both healthy controls (n = 19) and chronic heart failure patients, but found no differences in hemodynamic responses;[64] the second (19 TS, 20 AMI and 20 healthy controls) found that TS women had greater heart rate and higher nervousness scores in response to this mental stress compared to healthy controls;[65] the third [66] found no significant differences at rest or during mental stress in myocardial performance, diastolic function, or in heart rate variability between TS patients (n=22) and healthy controls (n=22). Limitations of these studies include small sample size, enrollment of TS patients a few months to several years removed from the index TS event, inadequate identification of TS triggers, and lack of catecholamine assessment before and during mental stress.[65,66] Further, the echocardiographic approaches were limited to standard echocardiography and tissue Doppler imaging.[67] ***The study we propose will address this evidence gap by a) Rigorously assessing the full range of circumstances proximal to TS onset, b) Assessing the constellation of physiological elements that define TS (autonomic nervous system activity and LV function) under controlled conditions, and then by c) Linking the type of trigger to physiology. Furthermore, by using more advanced speckle tracking echocardiography (STE), which captures LV dysfunction more accurately than tissue Doppler imaging, we will be better able to capture both reactive and long-term subtle anomalies of LV function in TS patients.***
- **Psychological characteristics and triggers**. When TS occurs consequent to a physical trigger (e.g., surgery, sepsis, stroke, acute respiratory insufficiency) the SNS response with its associated catecholamine surge is explained by the underlying acute medical or physical pathology. It remains unclear, however, why such an exaggerated catecholamine surge is precipitated by an emotional trigger (e.g., receiving bad news, unexpected loss, illness or injury of a close relative, angry arguments, and intense fear). Comparable life experiences eventually affect all individuals at some point throughout the lifespan with no dramatic health consequences and in fact, the large majority of individuals do not develop TS when exposed to emotional

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triggers. ***We will address this evidence gap by examining whether specific psychological characteristics can be identified among TS patients with emotional vs. physical vs. no triggers. We hypothesize that patients with emotionally triggered TS, compared to those with physical triggers or no triggers, will present a psychological profile characterized by higher depression and anxiety, poor coping skills, and low resilience, which, if identified, could guide therapeutic and preventive choices.***

- **Relationship of trigger status to prognosis**. While there is some evidence of differential prognosis according to identified trigger,[22] no study has examined whether the heterogeneity described above (i.e., emotional vs. physical triggers vs. no triggers and associated stress response under controlled conditions) has prognostic significance. Current studies are also seriously limited by nature and scope (registries) and imprecision in the ascertainment of triggers. ***We will address this evidence gap through longitudinal follow-up of the TS patients who undergo our rigorous and comprehensive assessment.***

3.3. Specific aims.

1) To rigorously establish and characterize heterogeneity in the pathophysiology of TS. We hypothesize:

a) Under controlled conditions, TS patients with emotional triggers vs. those with physical or no triggers will evidence a more robust response and slower recovery to mental stress, as evidenced by autonomic and functional ventricular indices;

b) Patients with emotionally triggered TS will also present a unique psychological profile characterized by higher levels of depression and anxiety, poor coping skills, and low resilience on comprehensive interview and questionnaires.

2) To rigorously test the contribution of TS triggering events and mental stress responsiveness to 1-year prognosis after TS event. We hypothesize that emotional triggers, along with autonomic and functional ventricular response to stress, will be associated with worse indices of ventricular function at 1 year of follow-up. We will also explore the contribution of this constellation to 1-year incident MACCE.

4 STUDY SAFETY

4.1. Overview.

Potential untoward events that may occur during this study include:

1) Transient, mild psychological discomfort during the completion of study surveys
2) Side effects of the stress reactivity test
3) Side effects from the venipuncture
4) Breach of confidentiality

These events are rare and the level of risk for each of these events is minimal.

The level of risk for each of these events is minimal.

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There are no risks associated with the collection of a small hair sample to measure hair cortisol levels (described in section 9.3, page 21).

4.2. Expected Risks and Protection Against Risks

1) Emotional discomfort during survey completion.
There is a very low risk for the occurrence of mild and transient psychological discomfort during the completion of study surveys (specifically, those assessing psychological distress). Should this problem arise, survey completion will be stopped immediately.

2) Side effects of mental stress protocol.
The major risk is the potential hazard of provoking another TS episode, myocardial ischemia or related cardiac consequences through the administration of the laboratory stress protocol. We have found in over 800 studies with both ischemic heart disease patients and cardiac arrhythmia patients that the hazards of performing this protocol are minimal, with no serious complications having occurred during such testing at Dr. Burg's institution. Additionally, no serious adverse effects of this protocol in cardiac populations have been reported in the literature. We also note that participants will be screened carefully and those who are clinically unstable will be excluded from the study. In addition, the study cardiologist Dr. Abbott will review eligibility criteria and give the final clearance for participation.

For patient safety, we will follow protocol termination guidelines similar to those used for clinical exercise stress testing: severe angina, systolic blood pressure greater than 220 mm/Hg, diastolic blood pressure greater than 120 mm/Hg, drop in systolic blood pressure greater than 20 mm/Hg. Risks will be minimized by performing stress reactivity tests within the Cardiac Research Office, a facility located within RIH hospital premises. Nursing and medical staff is available 24/7. All tests will be performed by staff certified in basic CPR. In the unlikely event of an adverse event, the test will be immediately stopped and the Research Assistant will page Dr. Abbott (study cardiologist) or the cardiologist on call for advice. If symptoms appear to be severe or in the rare case of a cardiac arrest, a code blue will be called and the patient will be admitted to the Emergency Department of RIH for immediate treatment.

3) Side effects from the venipuncture.
Blood samples will be taken by venipuncture, a procedure commonly performed in general medical care which involves inserting a needle into a vein in the arm and withdrawing a sample of blood. Participants may experience mild discomfort associated with blood sample collection, including pain during blood drawing and subsequent bruising. In very rare circumstances participants may experience fainting or local infection. These risks will be minimized by having an experienced certified phlebotomist performing the venipuncture.

4) Risks due to Echo cardiography and Holter Monitor: There is a minor risk of skin irritation (redness, itching) from the patches used to monitor your heart rate during the echocardiogram and the Holter. These are usually mild and do not require treatment. We may find a condition on your Echocardiogram which previously was not known. If we find out about new information that may affect your health, safety, or willingness to stay in this research, we will let you know

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as soon as possible. The tests done in the study, however, are performed for research purposes only and will not be available to you or your doctor.

5) *Breach of confidentiality.*
The likelihood of loss of privacy or confidentiality is rare and its impact on participants is likely to be minimal. Risks to privacy and confidentiality associated with this study are described in the consent form along with our procedures to minimize this risk.

- Confidentiality of study data will be maintained by numerically coding all data, by disguising identifying information, and by keeping all data in locked file drawers. All information obtained from participants will be accessible only to research staff. In weekly staff meetings when study procedures are reviewed and issues of compliance are raised, participants will not be identified by name unless necessary, and then, only by first name and only to the study personnel who require this information.
- Study staff will maintain participant privacy by conducting phone interviews in private offices, by securing records in locked cabinets and by using password-protected databases. Project staff meetings will be held in private conference rooms.

4.2.2. Suicide safety protocol. If suicidal thoughts or severe distress are reported at screening or at any study visit, the RA or PD will immediately initiate an assessment of suicidality risk following a standard protocol that we have used in previous studies (attached to this application/section of SOP).

4.3. Expected Benefits

The proposed research does not result in direct benefits to participants. By participating, patients may benefit others because findings from this study will increase our understanding of the pathophysiology of this important condition. The novel knowledge generated from this work will inform the development of preventive and therapeutic strategies designed to improve outcomes in patients with TS.

5 DATA AND SAFETY MONITORING PLAN

5.1. Monitoring entities

The following entities will safeguard the participants' safety and the integrity and quality of the data:

- **Institutional Review Board (IRB)**: The Human Subjects Committee/Institutional Review Board (IRB) at our institution (The Miriam Hospital) will provide oversight to the current study. The IRB functions in compliance with the congressional statutes governing human research. The study is submitted for approval and will meet all IRB requirements and directions.
- **A Core Group** consisting of the Principal Investigator (Salmoirago-Blotcher), the Project Director, and Co-Investigators Burg, Dunsiger, and Abbott will be responsible for the ongoing monitoring of the study and for reporting to the IRB and NIH any safety issues or threats to data integrity. The core group will meet regularly (twice a month) to monitor recruitment procedures, consistency with eligibility criteria, protocol deviations, accrual and retention,

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participant safety, and data integrity in order to take the necessary measures in a timely fashion (Table 1).

- **The Data and Safety Monitoring Board (DSMB)** will be the entity ultimately responsible for monitoring the safety of participants and the validity and integrity of the data. The DSMB will include a senior biostatistician and a cardiologist with expertise in takotsubo syndrome and heart failure. Neither will be in any way associated with the study. The DSMB will formally meet once a year in person or via video-conference to review accrual and retention rates, data quality, possible adverse events related to the performance of stress reactivity tests, re-evaluate measures for the protection of human subjects if needed, and re-assess the risk/benefit ratio of the study. A report will be compiled after each meeting and will include (1) a list of adverse and serious adverse events classified by severity and likelihood of being related to the study procedures; (2) whether adverse event rates are consistent with pre-study assumptions; (3) rates and reasons for study withdrawal; (4) whether all participants met entry criteria; and (5) whether continuation of the study is justified. Summary reports of DSMB meetings will be sent to the PIs, to the IRB, and to the NIH officer and will be included in the annual NIH report for the project.

5.2. Monitoring timelines

Study safety and progress will be monitored according to the schedule outlined in table 1 (more frequently if needed).

Table 1

Data Type	**Frequency of Review**		
	PD, Data manager & PI	Core group	DSMB
Subject accrual (n screened, n eligible, accrual rates, adherence to eligibility criteria)	Weekly	Bi-monthly	Annually
Adverse Events*	Weekly	Bi-monthly	Annually
Compliance with assessment protocols (echo, mental stress protocol, catecholamine collection)	Weekly	Bi-monthly	Annually
Data integrity	Weekly	Bi-monthly	Annually

***Please note**: serious adverse events will be reported within 24 hours to the IRB and to the sponsor (NIH) in accordance with their requirements. their requirements.

The DSMB will meet annually to review data concerning safety and conduct of the trial. The DSMB will address:

Safety: patterns of recruitment, drop out, reasons for study drop out and withdrawal and the frequency of AEs and SAEs. The DSMB will make recommendations to the Investigators regarding whether to continue, amend or terminate the trial based on analysis of the safety data.

Conduct of the study: the DSMB will also evaluate the performance of the study including any protocol violations, improper entry criteria, accrual rate, participation rate, and inadequate follow-up, and will make recommendations for improvement or termination. The Chair of the DSMB will send a copy of signed recommendations and comments from the DSMB to the NHLBI Program

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Officer within 1 month of each monitoring review. A copy will be attached to the Annual IRB review.

5.3. DSMB reports

Our study team will generate an Annual Report for the DSMB Chair. The Annual Report will address:
(1) whether AE rates are consistent with pre-study assumptions;
(2) reason for dropouts from the study;
(3) whether all participants met entry criteria;
(4) whether continuation of the study is justified; and
(5) conditions whereby the study might be terminated prematurely.

Report tables will be generated only from aggregate baseline and safety data for the study population and will not contain data on primary or secondary endpoints.

5.4. Adverse Events and Serious Adverse Events

a. Definition. An adverse event (AE) is defined as any untoward medical occurrence in a participant temporally associated with participation in the clinical study. An adverse finding can include a sign, symptom, abnormal assessment (laboratory test value, vital signs, electrocardiogram finding, etc.) or any combination of these. A Serious Adverse Event (SAE) is any adverse event that results in one or more of the following outcomes:
   - Death
   - A life-threatening event
   - Inpatient hospitalization or prolongation of existing hospitalization
   - A persistent or significant disability/incapacity
   - Important medical event based upon appropriate medical judgment

   Note: A birth defect is a highly unlikely event considering the age of our population. This outcome has been excluded.

b. Classification of AE Severity. AEs will be labeled according to severity, which is based on their impact on the patient. An AE will be termed 'mild' if it does not have a major impact on the patient, 'moderate' if it causes the patient some minor inconvenience and 'severe' if it causes a substantial disruption to the patient's wellbeing.

c. AE Attribution Scale. AEs will be categorized according to the likelihood that they are related to the study intervention. Specifically, they will be labeled either definitely, probably, possibly or unrelated to the study intervention. The determination of whether an AE is related to the study intervention will be made by the PI. If relatedness is unclear, the PI will seek the guidance of the study cardiologist.

Reportable and non-reportable events. The IRB requires reporting of all SAEs as well as of those AEs that are unanticipated and related to the study protocol or procedures (that is, they are "unlikely", "possibly", "probably" or "definitely" related). Unanticipated AEs are events, signs and symptoms that are not listed as risks on the consent form. Please note that AEs that are either anticipated or are "definitely not" related to the intervention procedures ***are not reportable to the IRB at RIH.***.

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5.5. AE Reporting Procedures and Follow-Up

All *reportable* (see 6.1. for definition) *adverse events (AEs*) will be reported by the PD to the IRB at RIH. Serious adverse events (SAEs) are reported to the IRB within 24 hours as described below ("SAE reporting"). All other reportable adverse events will be reported during the routine annual continuation reports to the IRB. In addition, all AEs (reportable and non-reportable) will be provided to the Chair of the DSMB prior to each meeting.

All study visits and follow-up assessments will include proactive assessments of potential adverse events. The RA will record all AEs and SAEs in a secure database using the format in the example below:

Subject Identifier	AE Onset	AE End	AE Code	Severity	SAE? (y/n)	Relatedness	Action Taken	Outcome	Comments
Subj001	11/1/15			1	N	2	1	1	Subject felt anxious during MT; resolved within 15 minutes
Subj002	12/3/2015			2	N	3	1	1	Dizziness during exercise session
Subj003	12/18/15			3	N	0	4	4	Subject fell while hiking; broke leg; withdrew from study

Severity of AE:	**Relatedness to intervention**:	**Action Taken**:	**Outcome**:
1 = Mild 2 = Moderate 3 = Severe 4 = Life threatening or disabling	0 = Definitely unrelated 1 = Unlikely 2 = Possibly related 3 = Probably related 4 = Definitely related	0 = None 1 = Intervention modification 2 = Medical intervention (specify in comments) 3 = Hospitalization 4 = Intervention discontinued 5 = Other (specify in comments)	1 = Resolved 2 = Recovered with minor sequelae 3 = Recovered with major sequelae 4 = Continuing treatment 5 = Condition worsening 6 = Subject death

Participants will be asked about all AEs at each intervention session and at each follow up assessment. AEs will be recorded on the AE log and noted on the CO form. The RAs will report all AEs to the PIs as soon as possible. All AEs will be assessed to determine their classification and whether they meet criteria for an SAE. The study RAs will refer participants to their own physician for assistance with adverse symptoms. Each week the PIs will review AE reports from the previous week for events that were reported as new or continuing and will follow all AEs to the point of a satisfactory resolution. The PIs will be available at all times to discuss other adverse symptoms with the RA and the study participants.

SAE Reporting. SAEs that are unanticipated, serious, and/or possibly related to the study intervention will be reported to the DSMB, the IRB at RIH, and to the sponsor (NHLBI) in accordance with their requirements (see 5.1 and following).

Stopping rules. Considering the minimal risk of this study stopping rules have not been established. However, the DSMB will closely monitor the risk/benefit ratio and the progress of the study and should issues in accrual, retention, data quality or safety arise, will take prompt action including considering the interruption of the study. Given the low risk of this study, no interim analyses are proposed.

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5.6. Monitoring of Study Progress

Accrual, retention. Review of the number of screened and eligible participants/month, accrual rates, adherence to inclusion/exclusion criteria will occur weekly. This will help evaluate the adequacy of the eligibility criteria and of the recruitment procedures and to address concerns in a timely fashion (table 1).

Compliance with the assessments' protocols. Compliance will be assessed weekly. At the end of each procedure a checklist will be completed in which consistency of assessment procedures will be evaluated, as well as any side effect occurring during the procedure (table 1).

Data accuracy (table 1). The PD and the data manager will be responsible for tracking participants to ensure that all data are collected in a timely and efficient fashion; for developing and generating monitoring reports; and for providing timely and relevant feedback to the project leadership regarding the accuracy and precision of data.

6 ELIGIBILITY CRITERIA

6.1. Study Population

Participants will be recruited from 3 hospitals – Rhode Island Hospital (RIH), The Miriam Hospital (TMH), and Newport Hospital.

- Study assessments, including mental stress protocol, will be conducted at the Cardiac Research Office at RIH.
- Project coordination, data abstraction, data management and analyses will be performed at the Centers for Preventive and Behavioral Medicine (CBPM) at TMH.

Vulnerable populations. This study will not recruit individuals from vulnerable populations (i.e.: adults unable to consent, Infants, children, teenagers or pregnant women). Subjects with severe cognitive impairment (and thus, unable to provide informed consent) are excluded from participation in the study (see 9.2 for details).

6.2. Inclusion Criteria

Participants must meet all of the following inclusion criteria to participate in this study (see sections 9.1 and 9.2 for a description of the screening visit and of the screening assessments:

a) Age ≥18;
b) A new diagnosis of TS fulfilling Mayo clinic criteria;[23]
d) Ability to understand and speak English.

6.3. Exclusion Criteria

Participants will be excluded if they meet any of the following exclusion criteria:

a) Inability or unwillingness to give informed consent;
b) Severe cognitive impairment;
c) Uncontrolled hypertension;
d) Acute psychosis;
e) High risk of suicidality;
f) Pregnancy;

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g) If the patient is clinically unstable;
h) Poor echocardiographic window;
i) Conditions that would interfere with adherence to study requirements (e.g., ongoing alcohol or substance abuse).

7 ENROLLMENT

7.1 Participants' Recruitment

Recruitment approaches will include the following:
1) Every week the RA will identify patients admitted with a diagnosis of TS through reviews of EMRs at our referral hospitals for appropriate ICD codes (ICD-10 code I51.81) and text searches for terms including takotsubo syndrome, apical ballooning syndrome, and similar (a HIPAA waiver and HIPAA preparatory to research requests are attached to this application). The RA will conduct a preliminary eligibility screening from the EMR (to exclude diagnoses of acute psychosis and ascertain that a poor echocardiographic window is not noted in the participant's chart); we will ask IRB permission to access patient data at this stage of our research (HIPAA waiver). Before discharge, a study staff will approach eligible patients to describe the study, assess interest and provide a study package including a letter inviting the patient to participate and a copy of the consent form. The package will be sent by mail to patients that cannot be approached (i.e., because they are sent home over the weekend). Patients will be instructed to communicate their interest (or lack thereof) via a dedicated phone number or email address. If no contact is made, the Project Director (PD) will follow-up by phone within one week after initial contact (this "opt-out" procedure has been IRB-approved and successfully implemented in our pilot study). During this call, the PD will confirm interest and schedule the first study visit between 2 and 12 weeks after discharge. This window allows sufficient time for participants to recover from the acute event.
To capture patients that might have escaped EMR searches we will also use the following recruitment approaches:

1) Flyers placed in clinics and in local public venues (e.g., community centers, libraries, supermarkets)
2) Social media (e.g. Facebook and Twitter) and on-line resources (hospital intranet; Craig's List)
3) Lifespan physician referrals

For items 1 & 2 a written physician's confirmation of TS diagnosis will be required per study inclusion criteria.
All study recruitment materials will be submitted to IRB for approval.
We will track returns from the different recruitment venues in order to identify the most efficient recruitment strategies.

7.2 Screening and Consenting Procedures

7.2.1. Interest assessment phone call: Initial contact will be made via telephone by interested individuals using a dedicated phone number that will be included in the invitation letter and all recruitment materials. Individuals calling about the study will be screened by the study PD in a phone conversation to determine initial eligibility. The PD will briefly describe the study and its requirements and enquire whether the caller is still interested. The PD will explain the need to

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collect some information from the caller to determine whether they are eligible to participate and request verbal permission to proceed. A scripted screening instrument will be used to standardize this phase of enrollment. Upon obtaining verbal consent the PD will complete the phone screen. A standardized formula will guide the PD to determine whether the caller is (a) eligible, (b) potentially eligible-pending or (c) not eligible.
If the caller has been recruited via options 1 and 2, the PD will request contact information for the participant's physician and obtain verbal permission to send the participant's physician a letter to obtain a confirmation of TS diagnosis. Once the confirmation is received, the PD will contact the individual to schedule the first study visit. If the physician does not confirm a diagnosis of TS, the individual will be called and notified that they are not eligible for the study.

7.2.2. Informed consent. *This study will involve only ONE informed consent process.* Once the potential participant has confirmed interest in participating, he/she will receive a consent form to read discussing the purpose of the study, its risks and benefits and clearly stating that he/she can withdraw at any time without any adverse consequences. Full informed consent will be obtained in person by a study staff in a private room at the RIH or via remotely (phone or video chat) after a thorough explanation of the study design, of the study intervention, and of the risks and benefits involved. Since the study requires access to protected health information (PHI), HIPAA authorization will be required from each individual in order to access his or her medical records. Participants will receive a copy of the consent for their records. *Once informed consent procedures are completed*, participants will undergo a battery of screening assessments (described in section 9.2). The RA will complete a screening log checking that all eligibility criteria are met at this point.

7.2.3. Screening failures: Microsoft ACCESS© will be used for tracking all participants who expressed interest in participating and were screened for eligibility. We will collect data from all individuals assessed for eligibility (i.e., individuals who consented but then resulted non-eligible at the completion of the screening process) so that we can provide a diagram and description of the recruitment process both for publication purposes and as an indicator of future generalizability of the study. Based on data from our pilot study, [39,48] we estimate that screen failure rates will be ~50%, and that 50% of screened participants will enroll in this study.

7.2.4. Randomization. n/a: although this study meets the NIH definition of clinical trial, no randomization is involved.

7.3. Retention plan

Patients will be asked to provide two different telephone numbers (home and mobile phone number) and an email address. The study tracking system will identify participants due for a visit. We will send mail/email reminders or phone messages to remind patients of their follow-up appointments. We will also maintain contact by sending birthday and holiday cards. Participants who wish to drop out of the study will be queried as to their reasons for doing so and every attempt will be made to address their concerns. To encourage compliance with assessment visits, participants will receive a $ 200 stipend ($ 50 for visit 1, $ 75 for visit 2, $ 75 for visit 3). Compensation will be provided as cash vouchers on the day of the visit once all procedures are completed.

8 STUDY DESIGN AND PROCEDURES

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8.1. Study Outcomes

Aim 1: To rigorously establish and characterize heterogeneity in the pathophysiology of TS. We hypothesize**:**

1a) Under controlled conditions, TS patients with emotional triggers vs. those with physical or no triggers will evidence a more robust response and slower recovery to mental stress, as evidenced by autonomic and functional ventricular indices;

1b) Patients with emotionally triggered TS will also present a unique psychological profile characterized by higher levels of depression and anxiety, poor coping skills, and low resilience on comprehensive interview and questionnaires.

Aim 2: To rigorously test the contribution of TS triggering events and mental stress responsiveness to 1-year prognosis after TS event. We hypothesize that emotional triggers, along with autonomic and functional ventricular response to stress, will be associated with worse indices of ventricular function at 1 year of follow-up. We will also explore the contribution of this constellation to 1-year incident MACCE.

8.2. Overview

The study will be conducted among newly admitted patients with a validated diagnosis of TS[23] (n=106) from 3 large medical centers in RI (Rhode Island Hospital (RIH), The Miriam Hospital (TMH), and Newport Hospital)). Assessments will be conducted at baseline (two visits, 2 weeks apart), and 12 months after enrollment. At the first visit (2-12 weeks after discharge), clinically stable patients will undergo a comprehensive interview to identify the events proximal to the onset of TS symptoms and complete a battery of psychosocial questionnaires. 1-2 weeks thereafter patients will be invited for a second visit to undergo a laboratory mental stress following a protocol previously utilized by our group.[68-72] Baseline and reactive changes in measures of autonomic nervous system activity (circulating catecholamines, cardiac vagal control) and LV function (Ejection fraction; Global Longitudinal Strain assessed with speckle tracking echocardiography)[67,73,74] will be assessed. Twelve months thereafter assessments of LV function will be repeated and information on MACCE will be collected (from the medical record).
The overall duration of this study will be 5 years; each individual (n=106) will be involved for 12 months.

8.2.2. Study visits

Study visits involving mental stress protocol and hair and blood samples collection will be conducted at the Cardiac Research Office located on the 9th floor of the Ambulatory Patient Care building of Rhode Island Hospital, 593 Eddy Street, Providence, RI.

The Office has been actively involved in hundreds of research studies since 1979 funded by the NIH, industry, and investigator-funded studies. The clinical research space features fully-equipped examination rooms, an interview/consultation room, cardiac monitors and defibrillators, echo machines, and a fully equipped lab with -80°C freezer storage.

Baseline Visit 1 (1.5 - 2 hours). This visit will be scheduled between 2 -12 weeks after discharge. To provide convenience, the consenting process and components of visit 1 will be offered in-person or remotely of the participant's choice. Participants who choose to do remotely will receive a link to the consent form via REDCap prior to a scheduled phone call or video chat.
The sequence of events during this first visit will be as follows:

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1) Full informed consent (described in detail in 7.2.2) will be obtained after a thorough explanation of the study design and of the risks and benefits involved. Patients will be required to sign a HIPAA authorization to allow study staff to access information recorded in the EMR. After obtaining consent, study staff will confirm the participant's eligibility for the study as per criteria defined above. Participants who choose to sign electronically, the study staff will print and sign a copy of the consent form for the study records and will provide the participant a copy at their next visit. An electronic form will be emailed to the participant if they prefer.

2) Triggers interview will be conducted by a study staff and completion of self-report psychosocial questionnaires. Participants who chose to do remotely, will receive a link to the questionnaires via REDCap to complete electronically.

Baseline Visit 2 (3.0 hours). This visit will be scheduled between 1 and 2 weeks after visit 1. Participants will complete the stress reactivity protocol developed by Dr. Burg and colleagues (described in detail below in the Assessments section). At baseline, during mental stress, and during recovery, blood samples for catecholamine assessments, continuous digital ECG for assessments of cardiac vagal control by heart rate variability, and an echocardiogram for the assessment of LV function will be obtained. A small sample of the participant's hair will also be collected for assessments of chronic cortisol levels.

NOTE: Two separate baseline visits are planned to avoid excessive participant burden and limit emotional reactivity before the performance of the stress reactivity test.

Follow-up Visit (1.5 hour). This visit will be scheduled 1-year after baseline visit 1. Participants will undergo a final echocardiogram with the modalities described in the Approach section.

In case participants are not able to attend their study appointment, windows have been established for rescheduling the visit:

Assessment	Window before due date	Window after due date
Visit 1	n/a	2 weeks
Visit 2	n/a (has to be at least 1 week after visit 1)	2 weeks
Visit 3	2 weeks	2 weeks

8.3. Mental Stress Protocol.

Patients will report to the Cardiac Research Office at RIH on their scheduled date and will be instructed to eat a light meal and refrain from caffeine and tobacco products for the prior 3 hours. Patients will take their regular medications, including beta-blockers. About 80% of patients receive beta-blockers at discharge, and we are concerned about the safety of holding these medications close to the index episode. We note that Dr. Burg's (Co-Investigator on this study) group found that the use of beta-blockade does not affect hemodynamic and autonomic responses to mental stress (large group of more than 400 patients).[75-77]

Participants will be escorted to the laboratory, an indwelling catheter will be established for blood sampling, and the participant will be connected to an ECG monitor and an automated blood pressure cuff. Participants will then be instructed to rest quietly for 30 minutes in a recumbent

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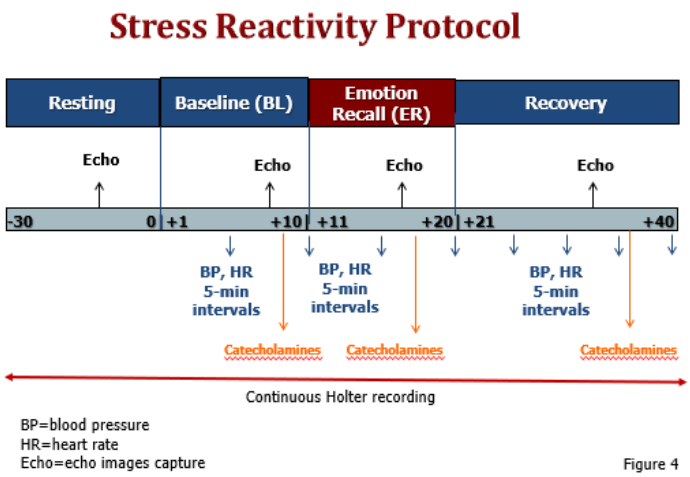


Figure 4

position to allow for the dissipation of the catecholamine response that accompanies the establishment of the indwelling line. The baseline echocardiogram is also performed during this period. At the completion of the 30-min rest period, participants will undergo a standard mental stress protocol that Dr. Burg' and his team have utilized for studies of cardiac populations with stable ischemic heart disease and cardiac arrhythmias (n>500).[68-72,76] Safety precautions and criteria for test interruption are described in section 5.2 . The protocol consists of three sequential phases: Baseline (BL), Emotion Recall (ER), and a Recovery Phase (Figure 4).

Baseline (BL) (10 min). This period serves as the formal baseline for all assessments. The participant is again instructed to rest quietly. Approximately 10 min into the BL phase, blood sample is drawn for baseline catecholamine assay. Heart rate (HR), and systolic and diastolic blood pressure (SBP and DBP, respectively) are recorded at 5- and 10-minutes during BL for validation. Holter recording is started and recorded continuously throughout the protocol. The baseline LV strain echocardiographic images are captured during this period.

Emotion Recall (ER - 5-10 min). This task was selected because of its potent, reproducible effect on cardiovascular hemodynamics and because, in contrast to other tasks used to assess reactivity in cardiac patients (e.g., mental arithmetic), it is more ecologically valid for our study population.[68-72,76] The participant is instructed to think about an incident during which the participant became irritated, aggravated, or frankly angry during the previous 2 weeks. The participants are told to inform the study staff when they have the incident clearly in mind. They are then instructed to relate the incident and their experience out loud; frequent questions to re-elicit the emotion are asked. Blood for catecholamine assay is sampled 10 minutes into the ER phase. HR, SBP, and DBP are measured every 5 min during ER; Holter is continuously recorded (see above), and an echocardiogram is also performed during his period.

Recovery Phase (20 min). Catecholamines are collected 18 min into recovery and echocardiographic images are captured again during his period. Upon completion of the recovery phase, the catheter is removed and participants are de-instrumented; heart rate and blood pressure are assessed every 5 minutes) before participants are released to ensure that hemodynamic parameters returned to baseline and that the patient is stable.

Validity checks. HR, SBP, and DBP are collected at 5- and 10-min during BL, every 5 min during ER, and every 5 minutes during recovery as validity checks as well as for safety during the baseline, stress, and recovery phases. Averages will be calculated for these parameters separately for the last three readings of BL and for all readings during ER. Change in HR, SBP and DBP (mmHg) from BL to ER are calculated by subtracting the BL average from the ER average. Patients will also complete assessment of state stress/emotion at the end of BL and ER, and again 10 and 20 min into Recovery on a 5-point Likert-Scale ranging from 1 (not at all) to 5 (extremely so) for angry/frustrated, anxious/tense, sad, and relaxed (e.g. "How stressed do you feel? How tense/anxious do you feel? How angry/irritated do you feel? How relaxed do you feel?"). Change

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scores are generated by subtracting the Emotion Recall and Recovery reports from the baseline report.

Safety procedures are described in section 4.2.

9 STUDY ASSESSMENTS

9.1. Study Assessments Schedule

This table diagrams the study assessments and the schedule of administration.

	Screening Call	Visit 1	Visit 2	Visit 3	Collection Method
Contact information	X				Survey
Cognitive impairment (BOMC)		X			Survey
Suicidality risk		X			Survey
Consent form		X			Form
HIPAA		X			Form
Blood pressure		X	X		At visit
Socio-demographics		X			Survey
Psychological surveys		X			Survey
Medical History, Comorbidities		X			EMR
Medications		X		X	EMR/survey
TS triggers interview		X			At visit
Mental Stress			X		At visit
Hair cortisol			X		At visit
LV function (Echocardiogram)			X	X	At visit
Vagal control (Holter ECG)			X		At visit
MACCE				X	EMR

Baseline visit 1 = 2-4 weeks post-discharge; visit 2 = 1-2 weeks after visit 1; Visit 3 =12 months after visit 1.
EF=ejection fraction; EMR= Electronic Medical Record abstraction. MACCE are collected at 12 months of FU.

9.2. Screening Assessments

These evaluations occur to determine if the candidate is eligible for the study (see also 7.2).

- ***Severe cognitive impairment*** will be evaluated using the Blessed Orientation Memory and Concentration test (BOMC). Patients with BOMC scores ≥10 will be excluded because they lack the cognitive skills to learn the intervention.
- ***Uncontrolled hypertension*** will be defined as blood pressure measurement ≥ 180/100 mmHg at baseline visit 1, measured using a Dinamap XL automated BP monitor according to current recommendations.[78] If visit 1 is done remotely, self-report BP will be collected and then assess at Visit 2 when patient arrives to assure eligibility before continuing with visit 2.
- ***Suicidality risk*** will be assessed by questioning participants on recent suicidal ideation using a validated instrument used in our previous studies (SDDS-PC[79,80]). Individuals endorsing 1 or

more items will be excluded from the study (procedures to be adopted in case high suicidality risk is detected are detailed in section 4.2.2).

- A negative pregnancy test will be requested for all women who are not post-menopausal (>5 years since menopause at enrollment, unless they had a prior hysterectomy/ovariectomy).
- Clinical information relevant for other eligibility criteria (e.g., acute psychosis) will be obtained from the electronic medical record (EMR). The study cardiologist Dr. Abbott will review eligibility criteria and give the final clearance for participation. Patients who are clinically unstable during hospitalization but otherwise eligible will be contacted once they become clinically stable.

9.3. Baseline Assessments

Timeline and source of data are shown in 9.1.

- ***Socio-demographics*** (age, sex, race/ethnicity, education, marital status, income, insurance status) will be self-reported using standard forms validated in other studies
- ***Medical history*** will be abstracted from medical records using standard abstraction forms that have been validated in previous studies medical history and will include date of index event, coronary risk factors, referral hospital, ECG characteristics at admission, troponin and CK levels at admission, admission LV EF, coronary angiographic characteristics, TS echocardiographic type, cardiovascular procedures during hospitalization; the Charlson co-morbidity index (a validated measure of the number and the seriousness of comorbidities),[81] and medications pre-admission and at discharge (e.g., use of hormone replacement therapy, beta-blockers, ACE-inhibitors, antidepressants and anxiolytics).
- ***TS Triggers*** will be assessed using the protocol and interview script used in the Determinant of Myocardial Infarction Onset Study, modified specifically for our study to capture triggers that are relevant to the onset of TS.[61-63] During the interview we will obtain information on the time and place of onset of the TS episode, type of symptoms, and potential triggering factors. To be considered a trigger, an event will have to occur within a 26 hours window preceding the index TS event.[61-63,82] Triggers will be coded as emotional, physical, or no triggers if none is identified (see section 12 for Quality Assurance).
- The ***Hospital Anxiety and Depression Scale (HADS)***, a 14-items self-report instrument validated in cardiac patients[83-85] with two sub-scales (range=0-21) measuring anxiety and depression and higher scores (overall score and each sub-scale) indicating the presence of more severe symptoms) will capture the severity of anxiety and depression symptoms. Scores will be calculated immediately after completion. If at any time during the study a participant scores >14 on the depression subscale, the RA will start a suicidality assessment (see 4.2 for suicidality protocol).
- The ***PTSD checklist*** (PCL Civilian version, a standardized self-report scale comprising 17 items that correspond to the key symptoms of PTSD) will be used to assess PTSD symptoms severity.[86,87] Respondents indicate how much they have been bothered by a symptom over the past month using a 5-point Scale ranging from 1 -Not at All to 5-Extremely; a higher total severity score indicated more severe symptoms.
- The ***Brief Resilience Scale***,[88] is an instrument specifically created to assess the ability of adults to bounce back or recover from stress that has been validated in cardiac patients.[89]
- The ***Brief COPE***, a 28-item multidimensional measure assessing the frequency with which a person uses different coping strategies, each rated on a Scale from 1 (I haven't been doing this at all), to 4 (I've been doing this a lot) will be used to measure coping strategies.[90,91]

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- The ***Perceived Stress Scale***, [92] a widely accepted instrument that investigates how often experiences of stress occurred during the previous month, and focuses on the subject's cognitive appraisal of stress, perceived control and coping skills, will be measured to assess stress. A higher total score reflects higher stress burden.
- ***Hair Cortisol***. In addition to markers of acute stress response, we are interested in collecting a marker of chronic stress. Scalp hair grows at an average rate of 1 cm per month. We will obtain samples of 3 cm length that represent cortisol deposition over roughly the 3-month period prior to collection. We will collect 10 milligrams of hair from an area approximately 5 mm in diameter (pencil width) from the posterior vertex of the scalp, immediately above the root, using fine scissors. The scalp-side of the samples will be marked, and samples will be kept in airtight plastic bags in a dry, dark location until analysis. The first 3 cm (approximately 1 inch) of hair will be cut from the scalp-side of each sample and ground into a fine powder in a ball mill. Cortisol is then extracted in methanol, dried, re-suspended in a phosphate buffer, and assayed using the ELISA method (Arbor Assays for Human Hair). De-identified samples will be stored and shipped to Dr. Meyer's lab at UMass Amherst for analysis. [93-97]
- ***Plasma catecholamines*** (epinephrine (E) and norepinephrine (NE)) will be collected three times during mental stress (10-min into BL, 10-min into ER, and 10 minutes into recovery). Consistent with procedures adopted in previous studies, blood is drawn and samples immediately placed on ice. Clear tubing is covered and collection tubes contain reduced glutathione to prevent oxidation. Samples will be spun and stored at -70°. Samples will be shipped and then analyzed at the chemistry laboratory of the Yale Center for Clinical Investigation (YCCI) by high-performance liquid chromatography (ESA Inc, Chelmsford, MA) using electrochemical detection (Coulochem II) after alumina extraction.
- ***Digital Holter ECG***. Holter data for the determination of cardiac vagal control will be collected using the Digital SEER Light Holter ECG Recorder - General Electric Health (24HR, 5 Lead, 2 Channel). Holter ECG data is collected during the mental stress protocol starting at baseline and is recorded continuously into recovery. The event marker on the Holter monitor is used to designate the start and end of each period (e.g., BL, ER, Recovery). De-identified ata will be downloaded at CBPM and analyzed by Dr. Salmoirago-Blotcher. All Holter data will be analyzed using GE CardioDay V2.5 Hoter Analysis system software. Methods for analysis of HRV are those used by Dr. Burg's group previously,[76,98] and are based on standards established in the literature. Holter recordings are digitally sampled and analyzed, with each Holter file manually processed and edited to ensure accurate identification of QRS complexes. A list of R-R intervals with annotations denoting normal beats, types of ectopic beats, and noise is saved for further processing and analysis with customized software. For frequency domain determination, the heart rate spectrum is computed using a fast Fourier transform with a Parzen. For all analyses, the power spectrum is integrated over discrete frequency bands as employed by Bigger et al:[99,100] very low frequency (VLF) 0.0033 to < 0.04 Hz; low frequency (LF) 0.04 to < 0.15 Hz; high frequency (HF) 0.15 to 0.40 Hz, and total power (TPow) 0 to 0.40 Hz. For purposes of the proposed study we will focus specifically on the high-frequency band as an index of cardiovagal (parasympathetic) control.[101] High frequency power (hf-HRV) in Ln $msec^2$ measures a demonstrated index of vagal activity,[102] will be determined separately for BL and ER by averaging of HF values for 4-min segments within each phase, and change will be calculated (ER minus BL).

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- ***Left Ventricular Function*** will be evaluated via echocardiograms performed at the Cardiac Research Office (ICAEL accredited laboratory) by certified cardiac sonographers. The echocardiographs are GE state-of-the-art portable machines, with full digital recording of all principal cardiac ultrasound modalities: linear (M-mode) 2-D, color flow, spectral, and tissue Doppler, as well as strain imaging capabilities, all performed using standard protocols. Study recordings will be reviewed and quantitated by experienced echocardiographers. From these echocardiographic data, 2-D volumes and ejection fraction (EF) will be computed using the biplane Simpson's method, according to the ASE guidelines.[103] Measurements will also include Global Longitudinal Strain (GLS) using off-line analysis software (TomTec imaging systems) by Dr. Song at RIH. . GLS will be calculated as the average LV longitudinal strain across the segments obtained using apical two, three, and four-chamber views.

9.4. Follow-up Assessments

The following assessments will take place 12 months since enrollment:

- ***Ventricular function (Echocardiogram)*** (described in 9.3)
- ***Medications & other therapies*** information will be self-reported using a standardized form.
- ***Major adverse cardiac and cerebrovascular event (MACCE).*** This will be a composite of death from any cause, TS recurrence, stroke, transient ischemic attack (TIA), and myocardial infarction. This operationalization is consistent with that of the largest available retrospective analysis of TS to date (Templin et al.) and will allow us to compare our outcome rates with those of the literature.[1] We will also collect information on each component of the composite outcome, as well as on disease-specific mortality, angina, coronary revascularization, supraventricular and ventricular cardiac arrhythmias, and heart failure. If information is not available in the EMR because the patient is followed elsewhere, we will contact the patient's PCP to obtain this information. MACCE information will be collected from the EMR and adjudicated by two blinded cardiology fellows. See 12.4 for quality assurance.

9.5. Process Measures.

Assessments will include n of screened and eligible participants, n of those who refused participation and reason(s) for refusal; retention rates; n of participants lost to follow up. This information is necessary for the determination of study generalizability.

9.6. Blinding

The outcome adjudicators (cardiology fellows) will be blinded to study hypotheses and to the trigger status of each participant.

10 DATA COLLECTION

10. 1. Data sources

Data will be collected and using pre-tested and validated forms, which have been found to be acceptable by the type of individuals who will participate in this project. Information about medical history and MACCE will be collected from EMR or directly from the patient's primary care physician or cardiologist using previously designed abstraction forms. We will develop electronic versions of each study form and data abstraction form for data entry using RedCap technology.

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10.2. Data safety

Electronic data will be stored in password protected data files accessible only to authorized project personnel including study staff, the Data Systems Analyst, and the study Statistician. All data files will be automatically backed up daily. Paper data will be stored in locked file cabinets. Data containing identifiers (e.g., name, address, phone number of participants from ACCESS files) will be stored on password-protected secure servers separately from de-identified study data (e.g., surveys, screeners).

10.3. Data collection

Research Electronic Data capture (REDCap) technology[108] will be used to for direct data entry in electronic study forms during the interviews; clinical data will be entered by the RA from the abstraction forms into electronic version of the abstraction forms in REDCap. Any ambiguity in the response to a question will be brought to the attention of the Data Systems Analysts for clarification. If the Data Systems Analyst is unsure how to code the response, the matter will be brought to the attention of the PI. The Data Systems Analyst will maintain a log so that future occurrences of problems will be handled in the same manner.

11 DATA MANAGEMENT

11.1. Tracking Systems

Prior to beginning subject recruitment; the Data Systems Analyst will develop a tracking system using Access software. The tracking system is essentially a reminder and participant tracking system to aid the study staff in ensuring that all procedures are followed and assessments administered in a timely fashion. The tracking system will include all steps included within the Recruitment, Treatment and Follow up phases of subject participation in the trial. Quantitative data will be entered into an Access database where they will be stored for cleaning.

11.2. Data entry and storage

Our major data management and analysis needs for the proposed project can be met by using a Pentium-based microcomputer. The data systems analyst will conduct data management under the supervision of the study biostatistician and the PI. De-identified data will be exported directly through the REDCap interface into study datasets. STATA©10, Statacorp-LP statistical software[72] will be used for statistical analyses.

Electronic data will be stored in password protected data files accessible only to authorized project personnel including study staff, the Data Systems Analyst, and the Statistician. All data files will be automatically backed up daily. Paper data will be stored in locked file cabinets. Data containing identifiers (e.g., name, address, phone number of participants from ACCESS files) will be stored on password-protected secure servers separately from de-identified study data (e.g., surveys, screeners) and will be destroyed at study completion.

The Data Systems Analyst will work with the project statistician Dr. Dunsiger to ensure that data are cleaned prior to analysis. When ready for analysis, he will provide a SAS database of collected data to the statistician.

12 QUALITY CONTROL AND QUALITY ASSURANCE

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12.1. Quality assurance.

A number of procedures have been planned to standardize data collection and limit bias. *A Standard operating procedures* (SOP) manual will be developed during the study start-up period of this study that explicitly describes the specific procedures related to quality assurance. These procedures will include:

- REDCap employs automatic checks of data that are out of range and invalid entries are automatically corrected.
- For Holter and mental stress protocol – all measurements are automated; de-identified information will be imported electronically from excel spreadsheets into final datasets.
- Personnel responsible for data abstraction and adjudication will be blinded to the study hypotheses and trigger status.
- We will use standardized interview scripts (modified from Determinant of Myocardial Infarction Onset Study)[61-63] to consistently elicit trigger information from study participants.
- Trigger interviews will be digitally recorded for randomly selected quality-control checks (30%).
- Participants will be blinded to study hypotheses.

12.2. Personnel Training

During the start-up phase, Drs. Burg and Salmoirago-Blotcher will train study personnel in all activities related to the establishment of the study protocol and associated documentation (e.g., SOP manual), management of specific equipment and supplies, participant instrumentation, timing and flow of each element of the stress reactivity protocol (e.g., establishment of IV access, resting baseline, stress procedures, recovery) along with timing of specific data acquisition (e.g., establishment of echocardiographic window, timing of blood draws and blood pressure measurement), transduction of data streams, preparation of study data sources (e.g., blood samples) for transmission to Yale sub-contract site, and training in the delivery of resting baseline and the mental stress task; as well as procedures for the collection of hair samples for the assessment of chronic cortisol levels. Personnel will be trained in data abstraction and receive formal EPIC certification. The psychologist during triggers interviews will be trained using the manual from the Determinant of Myocardial Infarction Onset Study.[61-63] Because she will attend mental stress tests, the RA will receive basic CPR certification. All personnel on the present application have received mandatory education in human research subject protection (Collaborative Institute Training Initiative hosted by the University of Miami) and Good Clinical Practice and will maintain certification throughout the study.

12.3. Data monitoring

Under supervision from the PI and the Biostatistician (Dr. Dunsiger), the Data Systems Analysts (Chris Breault) will ensure that entered data accurately represent data collected. She will conduct error-checking procedures quarterly on all data to ensure their accuracy. Data entry systems developed using Access will include appropriate skip patterns and parameter limits to ensure that data are entered accurately (e.g., item response formats from 1-5 will not permit entry of other numbers). Data entry systems will be tested to ensure that these constraints are working correctly

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prior to entering real data. REDCap employs automatic checks for values that are out of range or represent errors of logic. Outliers will be corrected if possible, with verification from participants. Quantitative data will be cleaned prior to analysis and then made available to the study statisticians for analysis. Dr. Dunsiger will be primarily responsible for the final outcome analyses and will assist in the preparation of manuscripts and report.

12.4. Intra- and inter-rater reliability. Reliability of the long-term outcomes data (MACCE) from the EMR will be assessed by calculating intra- and inter-rater variability between and within the ratings by the adjudicators. The goal is to assess the degree to which the two raters give consistent estimates of the outcome variables. Similar procedures will be followed for coding of triggers (emotional, physical, or no trigger). Echocardiographic data will be analyzed by two independent echocardiographers and inter-rater reliability will be estimated. Data will be analyzed by the same echocardiographer a second time for calculation of intra-class correlation coefficient (ICC) to estimate intra-rater variability both at baseline and during mental stress. A kappa statistic between 0.61-1.00 will be considered in the good-excellent range.

13 STATISTICAL CONSIDERATIONS

13.1. Preliminary analyses

Preliminary analyses will examine comparability of participants at baseline on demographic and other characteristics using Fisher or t-tests depending on the specific variable characteristics (categorical or continuous). We will examine the distributional properties of continuous variables to determine if normalizing transformations should be applied before conducting further analyses. After all the data have been collected, and before conducting the final outcomes analyses, we will explore the relationship of several key variables that could impact the outcome. If there are group differences on these variables and/or there are relationships of these variables to the outcomes, we will include them as covariates in our analyses (see sensitivity analyses, 13.3.4).

13.2. Aim 1 analyses.

Consistent with ***Aim 1a*** we will examine associations between trigger status and stress response using a mixed effect[1] regression model, in which we will simultaneously regress mean changes from baseline in plasma catecholamines on trigger status prior to TS event, time (10-min into baseline, 10-min into ER and 10-min into recovery), and interaction between trigger status and time, as well as confounders identified based on the literature. The model will include a subject-specific intercept to adjust for repeated measures within participant over visit time (baseline, ER, recovery phase) and subject-specific slopes which will characterize person-specific patterns of triggers. Mixed effects models allow for a wide range of outcome distributions (including normal and zero-inflated distributions) as well as specifications of the time effect (linear, non-linear). Specification of contrasts allow for the estimation of all-time effects using a single model. That is, we will be able to estimate the effect of trigger status on response to emotional recall as well as the slope of effect between emotional recall and recovery phase (this will be an indicator of recovery response). Using a parallel set of models, we will examine effects of trigger status prior to TS event on secondary outcomes (Vagal Control and GLS). Modeling will use a similar approach to specification of time effects, namely contrasts that will allow us to estimate the effect

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of trigger status triggers on vagal response (for example) to emotional recall as well as the slope of the change between ER phase and recovery. ***As per aim 1b***, we will examine the association between trigger status and psychological characteristic collected at visit 1 using a series of generalized linear models. Separate models will be used for each psychological construct considered. For both aim 1a and 1b, models will control for confounders identified from the literature, including age, sex, race, coronary risk factors, and beta-blocker use.[2,3]

13.3. Aim 2 analyses.

Consistent with ***Aim 2***, we will examine the contribution of TS trigger status and mental stress responsiveness to 1-year prognosis after TS event using a mixed effects model, in which we will regress ventricular function scores at 1-year follow-up (absolute values of EF and GLS score) on trigger status prior to TS event, as well as time (within-in visit)-varying mental stress responses during visit 2. This is a multivariate approach that allows for estimating the simultaneous effects of triggering events and mental stress on prognosis at 1 year. Mental stress variables will be indexed by time within-visit (ER, recovery), allowing for us to estimate the contribution of stress response during emotional recall and recovery post-emotional recall on ventricular function at follow-up, using contrasts. Interest is in estimating the effect sizes, to compare the relative contribution of each of the predictors on ventricular function at 1-year post baseline. To explore the effects of TS trigger status and mental stress responsiveness on MACCE, we will run a parallel analysis using a regression model implemented with Generalized Estimating Equations with robust standard errors to examine the contribution of TS trigger status and mental stress responsiveness (multivariate predictors over time) on MACCE at 1-year follow-up. All models will control for known confounders identified from the literature (age, gender, and TS type; medications (beta-blockers; ACE-inhibitors));[4-6] in addition, we will adjust for baseline LV function indices, Charlton co-morbidity index, and FU duration to control for right censoring. As this is exploratory, interest is in estimating effect sizes rather than strict statistical hypothesis testing.

13.3.4. Sensitivity analyses. For both aim 1 and 2, we will also conduct sensitivity analyses using models that additionally control for variables correlated with the predictor and outcome of interest (at a modest $p<.10$ level); this to ensure we control for confounders whose association with the outcomes of interest is unknown because they have not been collected in prior studies (because of their retrospective/registry-based design).

13.4. Missing data.

Characteristics of questionnaire and visit respondents and non-respondents will be compared using chi-square for categorical variables or t-tests or Wilcoxon rank sum tests for continuous variables. To reduce potential bias from non-response and to assume of missing at random more plausible, we will adjust for predictors of missing outcome data in multivariate modeling. The proposed models use a likelihood-based approach to estimation and thus makes use of all available without directly imputing missing outcomes, to produce consistent estimates of the regression parameters. ***We note that the proposed final sample size (n=106) accounts for 10% attrition,*** which is very conservative considering that in our previous study attrition was negligible and we had 0% missing data on information collected via in-person interviews and negligible missing data collected from the EMR (patients who denied permission to access their EMR). It is also important to note that due to the study timeline, there is potential for right censored data (namely that participants recruited at the end of the study may not reach 1-year follow-up because of late recruitment).

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Should this be the case, we will handle the censoring statistically and allow for length of follow-up to be treated as a covariate in the relevant outcome analyses.

13.5. Power calculations.

Power for **primary aims 1a and 1b** was calculated using G*Power and MPlus Monte Carlo estimation informed by preliminary studies in the literature. Effect sizes in the medium range are supported. To formally test power for the proposed Mixed Effects/GEE Models, Monte Carlo simulations were done in MPlus with 1000 replications and three seeds to confirm model stability for each model. To provide conservative estimates and minimize assumptions, simulation studies modeled patterns of attrition and missingness based on prior work. As this novel research is still very preliminary, we estimated effect sizes based on our prior work with TS patients as well as literature on TS and pathophysiology during mental stress.[7-9] Monte Carlo Models assumed (N=106) modest effects of covariates and converged with low parameter bias. For Aim 1, even with missing data, findings supported excellent power (80%) to identify medium effects (f^2=0.14). These effects are in line with what we saw in preliminary work showing associations between emotional triggers and TS, as well as psychological factors associated with TS. In addition, they are similar to published findings showing positive (non-significant) changes in HR variability pre- to during-stress among TS patients (median change 22 [-14-53])[7] as well as significant within group increases in HR from pre-stress to post-stress (median difference 3.5 [1-6.5]).[8] Thus, we are confident that we are more than adequately powered for our primary aim.

For **aim 2**, given the paucity of information in the literature, we are considering the MACCE outcome exploratory. However, we have conducted a power analysis to show we have sufficient power to even explore these associations. Given the large registry findings[5] that shows a MACCE rate of 9.9% patient-year at follow-up, if we assume a sample of N=106 at enrollment, we could detect effects of triggering events and mental stress reactivity on prognosis at follow up with effect sizes of approximately f^2=0.15, which are considered medium effects. Furthermore, with the given sample size we are powered for medium-sized effects for LV function outcomes (f^2=0.14) based on simulation models. In sum, for this study, with 106 participants enrolled we will yield 80% power to test both our primary and secondary study aims, using a two-tailed significance level α=.05.

14 COVID-19 SAFETY MEASURES

The study will follow current guidelines implemented by the RIH for access of patients to the hospital. RIH has resumed elective procedures as well as clinical trials. The following measures have been discussed with our study cardiologist Dr. Dawn Abbott.

- The day BEFORE their scheduled visit, participants will be screened for symptoms (cough, sore throat, fever, chills, diarrhea, shortness of breath, loss of taste or smell), and possible exposure (e.g., history of travel abroad) currently and during the previous 2 weeks before scheduling each of their study visits.
- Participants should confirm that they have not been ill, have not traveled in an area with a CDC Level 3 Travel warning (https://wwwnc.cdc.gov/travel/notices/warning/coronavirus-

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global), and have not had contact with a known or presumed COVID-19 patient for the 2 weeks before the visit.

- If any symptoms or exposure are present, participants will not be allowed to come for a study visit,and labeled as “pending eligibility” in the study tracking system.
- Participants will be screened by RIH security personnel at entrance to the APC building per RIH procedures.
- Study participants will be required to wear a surgical mask and gloves while in the hospital. If they do not have a personal mask, the study will provide one as well as gloves.
- Study staff will wear gloves and a surgical mask. Because the mental stress does not involve physical exercise, no special PPE is necessary for study staff (including the nurse performing blood draws) besides wearing a surgical mask and gloves.
- At entrance in the lab, upon completing all procedures for each participant, and when leaving the lab, the RA and all staff will wash hands with soap and water (for at least 20 seconds) or use hand sanitizer with >60%.
- At entrance in the lab, upon completing all procedures for each participant, and when leaving the lab, the RA will clean all surfaces and instrumentation using an RIH-approved disinfectant proven effective on SARS-CoV-2. Disinfectant bottles will be located in the lab. The instructions to use the disinfectant will be located with each bottle. Staff should saturate 2 to 3 paper towels with the disinfectant, wipe down surfaces, moving clean to dirty, and ensure that surfaces remain wet for 3 minutes after, which is the amount of time it will take in order to kill any pathogenic microorganisms.

15 REGULATORY REQUIREMENTS

14.1 IRB Review
14.2 Informed Consent, HIPAA Joint Privacy Notice
14.3 Subject Confidentiality
14.4 Unanticipated Problems

15.1 Institutional Review Board (IRB) Review

This protocol and any subsequent modifications will be reviewed and approved by the IRB, which is responsible for oversight of the study. The consent form and HIPAA forms are attached to this IRB submission.

15.2 Informed Consent and HIPAA

Consent will be obtained at one time point during this study (at baseline visit 1).
1) Individuals interested the study will be screened by a study staff in a phone conversation to determine initial eligibility. The study staff will briefly describe the study and its requirements and enquire whether the caller is still interested. If the caller is still interested, the study staff will explain the need to collect some preliminary information from the caller in order to determine whether they are eligible to participate. A script and screening instrument will be used to standardize this phase of enrollment.
2) Individuals who meet the initial eligibility criteria and who are interested in participating will be invited to a study visit (baseline visit 1) and provide written consent using an IRB approved consent form and HIPAA authorization form. In addition to describing the study and the

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participant's involvement in detail, the consent form also emphasizes that participation is voluntary, that consent may be withdrawn at any time, and that participants may withdraw from the study verbally or in writing by contacting the study PD and/or the Principal Investigator. Participants are given ample opportunity to ask questions before providing consent. Contact information for the PI, as well as the IRB (for complaints) is provided in the consent form. Signed informed consent forms and HIPAA forms will be kept in locked filing cabinets separate from all data. Participants will receive a copy of the consent and HIPAA document for their records.

15.3. Confidentiality

15.3.1. Protection of Subject Privacy. All phone contacts with the participants will take place in a private, locked office and will be conducted by staff trained and certified in the protection of human subjects in research studies and HIPAA requirements according to TMH policies.

15.3.2. Data safety

- Subjects will be identified with a randomly generated identification code unique to the subject and all personal identifiers will be removed from the study questionnaires and from datasets. Electronic communication with outside collaborators will involve only unidentifiable information. AE reports and annual summaries will not include subject-identifiable material.
- Data collected for screening (i.e. reasons for ineligibility and refusal) and tracking reasons (Access data base) that contain identifiers will be stored into a password-protected server separate from other study data and will be destroyed at study completion. This Access database is password accessible only to the study SRA, the data systems analyst, PD and the PI.
- Signed HIPAA forms and consent forms will be stored in locked filing cabinets separate from all other study data.
- Patients will be informed in the consent form that trigger interviews will be digitally recorded for quality assessments. Digital recordings will be destroyed as soon as quality assurance procedures are completed.
- All copies of de-identified study questionnaires (paper and/or electronic) will be destroyed at the earlier of two dates: 1) seven years after the end of the study; 2) after the manuscript based on the work is published.

15.4. Study Discontinuation

The study may be discontinued at any time by the IRB as part of their duties to ensure that research participants are protected.

15.5. Unanticipated Problems

The Office for Human Research Protections (OHRP) considers unanticipated problems involving risks to subjects or others to include, in general, any incident, experience, or outcome that meets **all** of the following criteria:

- unexpected (in terms of nature, severity, or frequency) given (a) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document; and (b) the characteristics of the subject population being studied;
- related or possibly related to participation in the research (in the guidance document, possibly related means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and

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- suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

An incident, experience, or outcome that meets the three criteria above generally will warrant consideration of substantive changes in order to protect the safety, welfare, or rights of subjects or others. Unexpected, serious and intervention-related SAEs will be immediately (< 24 hours) reported to the chair of the DSMB, the NHLBI officer, and the IRB in accordance with requirements.

Participants will be asked about all AEs at each intervention session and at each follow up assessment. AEs will be recorded on the AE log and noted on the CO form. Procedures for handling the reporting of AEs and SAEs to the IRB and the DSMB are detailed in the DSMP (section 5).

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