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SPECIFIC AIMS

Depression has been shown to increase the risk of a first coronary heart disease (CHD) event, independent of traditional risk factors that include age, male sex, hypertension, hypercholesterolemia, low high-density lipoprotein (HDL) cholesterol, cigarette smoking, and diabetes.[8-11] The biological candidate(s) that explain depression's excess risk of incident CHD remain unknown despite exploration of many biological candidates.[12] **25(OH)D insufficiency/deficiency is an unexplored biological candidate that could explain how, or for whom, depression is linked with incident CHD events.**

Low levels of **Vitamin D** (25(OH)D) and the associated rise in **parathyroid hormone levels** (PTH) have been implicated in the premature development of CHD.[13-15] Recent animal models show that 25(OH)D deficits or knockouts dysregulate the renin-angiotensin system,[16] cause cardiac hypertrophy,[17, 18] promote vascular and coronary calcification,[19] all of which are known to influence CHD risk.[20] Prospective human studies have found that 25(OH)D levels are associated with elevated CHD factors,[21] and peripheral vascular disease,[22] and predict incident coronary disease, stroke,[23] and death.[24-26] Importantly, low 25(OH) levels are highly prevalent.[21, 27]

Recent evidence has also suggested that 25(OH)D insufficiency is associated with increased depressive symptoms,[28-31] and influence factors that are involved in the pathogenesis of depression, including levels of nerve growth factor, acetylcholine acetylase, serotonin, testosterone, and thyroid hormone.[32] Thus, depression may be associated with low leves of 25(OH), and these low levels appear to predict incident CHD events. Therefore, our understanding of the pathways linking depression and incident CHD risk will be enhanced by determining the potential role of 25(OH)D in this association. **No prospective study, however, has assessed these two candidate CHD risk markers** simultaneously when predicting CHD incidence. It is possible that statistically controlling 25(OH)D *reduces* depressive symptoms' prediction of CHD incidence (**a mediator/prior cause model**). Alternatively, low 25(OH)D levels might more precisely identify those depressed who are at increased CHD risk. Ruling out co-existing medical conditions is required for evidence-based depression treatment;[33-37] it is possible that low 25(OH)D levels could be a target for remediation in depressed patient, and our study will be the first to explore this. Thus, we will also test if the depressive symptom—CHD risk is modified by 25(OH)D levels—(**with an effect modifier model**) an exciting possibility that has not been tested.

Specific Aim: To determine if elevated 25(OH)D explains some of the depressive symptoms' excess risk for CHD incidence or if it more precisely identifies those with depressive symptoms at CHD risk.

To successfully complete this aim, we propose to investigate the best fitting model for predicting **15-year incident CHD** that simultaneously assesses baseline depression, 25(OH)D, and the standard CHD risk factors in a population-based, randomly-selected study (**n=1,794**; 1995 Nova Scotia Health Study) of persons over the age of 18 years and with documented absence of CHD. As shown below, we have already demonstrated that depression predicts incident CHD events in this sample, independent of Framingham CHD risk factors. At the time of entry into the population-based study, participants gave written consent for the drawing, storage, and future use of their stored biospecimens and linkage to all their future medical records. Funding will enable us to assess baseline 25(OH)D levels from frozen samples and evaluate its contribution to CHD and its role in the depression-incident CHD relation.

Mediator/Prior Cause Model

Hypothesis 1a: Baseline depressive symptoms is associated with decreased 25(OH)D levels, controlling for age, sex, region, body mass index (BMI), seasonal variation, skin color, and the Framingham risk factors.

Hypothesis 1b: Decreased 25(OH)D levels is associated with increased 15-year risk for incident CHD, controlling for age, sex, region, BMI, seasonal variation, skin color, and the Framingham risk factors.

Hypothesis 1c: The 15-year incident CHD risk associated with depression is substantially decreased after controlling for 25(OH)D levels and further covariate adjustment.

Exploratory Mediator/Prior cause Hypothesis: Increased parathyroid hormone levels further accounts for some of the effects of the depression–CHD association, over and above 25(OH)D levels.

Effect Modifier Model

Hypothesis 2: 15-year CHD risk associated with baseline depressive symptoms is modified by 25(OH)D. Additional analyses of both models will control for lifetime pack-years, physical activity, medical comorbidities, inflammatory biomarkers, and cardiovascular and antidepressant medication use.

Public Health Significance: 25(OH)D may be a novel biological candidate that partially explains the link between elevated depressive symptoms and incident CHD, all three of which are highly prevalent in general populations. If accounting for 25(OH)D decreases depression's prognostic risk, or identifies more precisely those with depression who are at CHD risk, then interventions can test if 25(OH)D supplementation in patients with depressive symptoms reduces the risk for CHD. If 25(OH)D does not decrease risk, then we will have added to the evidence-base by showing it is *not* a biological candidate to be pursued further. Thus, positive or null findings will contribute to our knowledge about how depression is associated with incident CHD.

A. SPECIFIC AIMS

In patients with an acute coronary syndrome (ACS) event, depression predicts Major Adverse Cardiac Events (MACE; non-fatal myocardial infarction, urgent coronary revascularization, and unstable angina requiring hospitalization), and all-cause mortality (ACM). Studies have linked exaggerated platelet aggregation to both depression, and MACE or ACM in cardiovascular disease patients. However, whether increased platelet aggregation partially explains the relation between depression and subsequent MACE/ACM, and the mechanism by which platelet aggregation is increased in depressed patients, remain unknown.

Depressed patients exhibit a number of platelet serotonergic abnormalities including a down regulation of serotonin (5-HT) transporter density, and also an upregulation of 5-HT_{2A} receptor density. Given the important role of the platelet 5-HT_{2A} receptor in 5-HT-induced platelet aggregation, depressed patients may be particularly susceptible to increased 5-HT-mediated platelet reactivity. Our pilot data suggest that depressed patients have higher levels of 5-HT-induced platelet aggregation compared to nondepressed patients, even after therapy with aspirin and clopidogrel, evidence-based antiplatelet therapy for ACS patients. **Therefore, increased platelet aggregation specifically to 5-HT may partially explain the link between depression and excess MACE/ACM after an ACS event.**

In the application, we propose to assess 5-HT-specific platelet aggregation measures in a large NIH-sponsored cohort study of 1,400 post-ACS patients in which blood is obtained for biomarkers (during hospitalization and again at 1 month post-discharge), and adherence to cardiovacular medications including aspirin and clopidogrel is monitored. Patients will be followed for 1 year for MACE/ACM events. Funding of this ancillary study will enable us to evaluate the role of abnormal 5-HT-platelet pathways (which would not otherwise be evaluated in the parent cohort study) in the depression-MACE/ACM relation.

Primary Aim: To determine the degree to which levels of 5-HT-induced platelet aggregation at hospitalization contribute to the relation between baseline depression and 1-year MACE/ACM.

Hypothesis 1: Depression is associated with higher levels of 5-HT-induced platelet aggregation at hospitalization in post-ACS patients, despite antiplatelet therapy with aspirin and clopidogrel.

Hypothesis 2: Higher levels of 5-HT-induced platelet aggregation at hospitalization is associated with increased 1-year MACE/ACM after an ACS event.

Hypothesis 3: There is a reduction in the hazard ratio for the relation between depression status and 1-year MACE/ACM, controlling for other prognostic factors, when 5-HT-induced platelet aggregation at hospitalization is added to the model.

Secondary Aim: To determine the degree to which levels of 5-HT-induced platelet aggregation at 1-month follow-up contribute to the relation between baseline depression and 1-year MACE/ACM. Analyses will additionally control for aspirin and clopidogrel adherence from hospital discharge to 1-month.

Exploratory Aims: We will repeat the analyses substituting 5-HT-induced platelet aggregation with total peripheral 5-HT levels, as well as 5-HT-induced platelet ATP release, a marker of dense granule exocytosis and platelet aggregation amplification. We will additionally examine the relation between changes in depression symptomatology and changes in 5-HT platelet measures from baseline to 1-month.

Finally, analyses will control for anti-depressant medication use, and will then be repeated excluding patients on antidepressants.

Public Health Significance: Despite advances in therapy for ACS patients, MACE/ACM risk is still high. If depressed patients have higher levels of 5-HT-mediated platelet aggregation despite antiplatelet therapy with aspirin and clopidogrel (which is the standard of care for ACS patients), and this accounts for a substantial proportion of their excess risk, mechanistic studies could investigate platelet as well as brain 5-HT receptor density and signaling in depression for cardiovascular prognosis. Further, our study could inform the design of therapeutic targets in the area of depression and post-ACS prognosis.

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SPECIFIC AIMS

In patients with an acute coronary syndrome (ACS) event, depression predicts an increased risk of recurrent myocardial infarction (MI) and mortality. The goal of this study is to identify the mechanisms that explain why depressed post-ACS patients have an increased risk for recurrent MI/mortality. Coronary plaque disruption with subsequent platelet-thrombus formation is essential for the onset of an ACS event. Antiplatelet medications including aspirin (ASA) and agents that block the adenosine diphosphate (ADP) P2Y12 receptor such as clopidogrel are the mainstays of therapy for ACS patients. The current thinking is that ACS events are caused by a co-occurrence of both a disrupted coronary plaque and the increased propensity of a blood clot to form over the disrupted plaque. After an ACS event in which a disrupted plaque is present, a pro-thrombogenic state, characterized by platelet-thrombosis-promoting factors, is essential for the initiation of the next clinical event. Patients with platelet-thrombosis-promoting factors are said to have **vulnerable blood**. **The goal of this study is to test the hypothesis that depressed post-ACS patients have vulnerable blood when exposed to acute psychological stress through activation of the sympathetic pathway.**

The "Perfect Storm" is our group's conceptualization of ACS in which events are not caused by a single or a few factors, but rather result from the unfortunate confluence of specific situations in the context of underlying risk factors. Our study challenges the existing paradigm in which mechanisms or potential treatment targets of depression and MI/mortality are examined without considering the role of acute stress and proposes a new paradigm wherein it is in the presence of acute stress that putative mechanisms are more clearly revealed. We propose that it is the confluence of depression **and** exposure to acute stress that will best reveal the biological mechanism(s) that underlie the link between depression and poor post-ACS prognosis.

Given that platelets are essential components of the thrombi that occlude the coronary arteries, prior studies have focused on examining whether an association exists between depression and exaggerated platelet aggregation. We and other investigators have shown that depression is associated with increased platelet aggregation while others have shown no association. Inconsistencies in the literature on depression and platelet aggregation may have arisen because acute stress exposure was unmeasured in prior studies. Furthermore, few prior studies have been conducted in post-ACS patients taking optimal antiplatelet agents.

The sympathetic pathway plays a pivotal role in the response to psychological stress. Despite being weak agonists of platelet aggregation, catecholamines (epinephrine and norepinephrine) are potent amplifiers of platelet aggregation induced by other agonists including ADP and arachidonic acid (AA), platelet aggregation pathways that are inhibited by clopidogrel and ASA respectively. **Therefore, in depressed post-ACS patients, exaggerated sympathetic activation to stress may lead to vulnerable blood despite treatment with clopidogrel and ASA.**

Design: We propose a state-of-the-art laboratory-based experiment to assess in vitro and ex vivo measures of vulnerable blood before and after a mental stress task in 140 post-ACS patients taking clopidogrel and ASA who are depressed (N=70) and non-depressed (N=70), none of whom are taking antidepressants.

Primary Aim: To examine whether depression after an ACS event is associated with increased mental stress-induced platelet aggregation in patients taking optimal antiplatelet therapy.

Hypothesis: At 2 months post-ACS, compared to non-depressed patients, depressed patients will exhibit a greater increase in in-vitro platelet aggregation induced by ADP and secondarily AA in response to an acute mental stress task.

Secondary Aims: (1) We will investigate the role of the sympathetic pathway, determined by circulating levels of epinephrine and norepinephrine, in mental stress-induced vulnerable blood in depressed vs. non-depressed post-ACS patients. **(2)** To examine the role of the platelet-fibrin pathway, in vitro platelet GP IIb/IIIa receptor activation, which mediates fibrinogen binding, will be assessed by flow cytometry. **(3)** To provide further insight into blood vulnerability in the presence of a disrupted plaque, ex vivo platelet-thrombogenicity and fibrin formation will be assessed using a novel perfusion chamber.

Exploratory Aim: We will sample an additional 10% of post-ACS patients who are taking antidepressants (i.e., serotonin reuptake inhibitors) to establish feasibility for conducting future studies with this group of patients.

Biomedical Significance: Despite advances in therapy for ACS patients, recurrent MI/mortality risk is still high in depressed patients. If depressed post-ACS patients have vulnerable blood due to acute stress exposure through increased sympathetic activation, then stress-induced vulnerable blood is a novel therapeutic target for reducing the increased cardiovascular risk in these high risk patients.

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