**Introduction to Revised Application**

We greatly appreciate the many constructive comments in the initial TL1 submission, including comments about the candidate (“excellent clinical training”), the mentorship team (“extensive experience”), the training plan (“key MSCR courses”), the research plan (“innovative”), and the environment (“strong”). The review committee raised several important points regarding institutional support, project feasibility, training, and study implementation. As a result, we have refocused the application to include specifics about the academic home, departmental support, study methods, career plan, and project focus. The application was shortened in parts to allow for the requested changes. Revisions have been marked by blue font.

1. **Academic Home**: This has been addressed, as per the letters. In review: I am completing my internal medicine residency within the Department of Medicine (DOM) at the end of June 2019. Starting July 2019, I will be appointed as a post-doctoral fellow within the Department of Epidemiology at Rollins School of Public Health (RSPH). Letters of support documenting this have been sent by Drs. Law and Dressler from DOM, and from Drs. Shah and Alonso from RSPH.
2. **Letter of Support from Department Chair**: The prior departmental letter was from my current position within the Department of Medicine. Attached is a supporting letter from Dr. Timothy Lash, the current Chair in the Department of Epidemiology at RSPH. This position is dedicated to research, and requires no clinical obligations.
3. **BioStamp Patches**: We appreciate the need to clarify the funding support for these patches, as they are critical for the support of the project. The reusable BioStamp patches have already been obtained by Dr. Amit Shah, and additional supplies are being ordered with his discretionary funding.
4. **Sample Size Calculations**: Because of the exploratory nature of the study, they are designed as pilots – we recognized the effect sizes may be too small to calculate group differences, but feel that the data will be very helpful for future grant applications and for more definitive evaluations of *Dyx,* neuropsychatric function, and coronary artery disease. We appreciate the importance of understanding the power from the pre-selected sample size (based on feasibility). I have met with the BERD consultant for assistance and he will help us to calculate appropriate power.
5. **Specific Aims**: Reviewer 2 raised the concern that our aims could have improved focus, particularly our focus on neuropsychological factors. We have reordered the aims, as seen in the modified Figure 3, and separated depression and cognitive impairment as individual outcomes.
6. **Research Project Funding**: Reviewer 2 requested further information into how the project would be funded. As the project is an ancillary study on the EmCAB, under the mentor Dr. Arshed Quyyumi (PI), the necessary resources including study staff are already available, which we have now added into the application.
7. **Candidate Training Timeline**: Reviewer 2 requested specifics on training timeline, which has been added to section D8, including research products and step-wise career goals.
8. **Human Subjects Protection**: Both reviewers requested further documentation of human subjects and inclusion of women, children, and minorities, which has now been included.
9. **Project Feasibility**: Reviewer 1 raised the concern that the MSCR and the research obligations may limit project feability. We will address this by increasing the amount of delegation to the current study staff, and increasing the effort towards the rigors of the MSCR.
10. **Research Experience**: Reviewer 1 noted the lack of published research products. I am continuing to address this by submitting additional manuscripts, including 1 research paper and 2 review articles, related to the field of neurocardiology.

**Disturbances of the Neurocardiac Axis: Using Heart Rate Variability to Measure Disease in the Brain and Heart**

**Principal Investigator:**

Anish Shah, MD

Resident

J. Willis Hurst Internal Medicine Residency

Department of Medicine

Phone: 469-835-7606

Email: [anish.shah@emory.edu](mailto:anish.shah@emory.edu)

**Lead Mentor:**

Amit Shah, MD, MSCR

Assistant Professor of Epidemiology

Assistant Professor of Medicine (Cardiology)

1518 Clifton Rd. NE, Rm 3053  
Atlanta, GA 30322  
Phone: 404-727-8712

Email: [ajshah3@emory.edu](mailto:ajshah3@emory.edu)

**Co-Lead Mentor:**

Alvaro Alonso, MD, PhD

Associate Professor

Department of Epidemiology

Rollins School of Public Health

Emory University

Phone: 404-727-8714

Email: [alvaro.alonso@emory.edu](mailto:alvaro.alonso@emory.edu)

**ABSTRACT**

Ischemic heart disease (IHD) is a highly prevalent and one of the leading causes of mortality, yet in the majority of affected individuals, their diagnosis is not detected by clinical exam. As such, the majority of sudden cardiac deaths occurs in those not previously diagnosed with IHD. This suggests the need for better IHD screening mechanisms, and a growing body of literature suggests autonomic dysfunction may be a novel risk factor. Ambulatory electrocardiography can be used to study autonomic dysfunction through heart rate variability. Recently, a novel marker of heart rate variability (*Dyx*) was found to be predictive of myocardial ischemia based on abnormal nuclear stress test. Our group also found that low *Dyx,* when measured in the early morning, was predictive of abnormal myocardial perfusion imaging as well. It is not known, however, how *Dyx* correlates with coronary angiography findings and the need for coronary intervention. Additionally, *Dyx* is influenced by central neuropsychological mechanisms, such as depression. Neurologic pathways may influence coronary microvascular function and lead to ischemia even in the absence of obstructive coronary artery disease. Assessment of depression and cognitive impairment and their relationship with *Dyx* may help to elucidate additional neurocardiac mechanisms on how autonomic function may lead to adverse outcomes, even in the absence of coronary artery disease. We will examine the relationship of neuropsychological metrics (mood, cognitive function) and ischemic heart disease with *Dyx* to better understand these effects in a high-risk cohort of patients with stable angina who are undergoing cardiac catherization. Aim 1 will evaluate the relationship of low *Dyx* with obstructive coronary artery disease, based on cardiac catherization. Aim 2 will determine the effect of neuropsychological pathology, as determined by depression, stress, and cognitive impairment, on autonomic dysfunction as measured by low *Dyx*. The goal of this study is to further evaluate the utility of *Dyx* as a measure of autonomic dysfunction that can help risk-stratify patients for obstructive coronary artery disease, and also determine the influence of brain-related factors on it as well. This research will improve our understanding of the clinical importance of disturbances of the neurocardiac axis through a quantified measurement of autonomic dysfunction as it relates to clinically actionable coronary artery disease. As such, it may help to yield very important, low-cost assessments of risk with widespread public health implications.

**A. SPECIFIC AIMS**

Four out of five patients with ischemic heart disease (IHD) are unrecognized by clinical exam.1 The majority of sudden cardiac death (SCD) still occurs in those without diagnosed IHD.2 Although the overall rate of cardiovascular mortality is declining, the rate of community events has not declined proportionally, suggesting the need for more public health interventions.3 A growing body of literature suggests that autonomic dysfunction is not only of prognostic value in cardiovascular mortality,4 but may serve as a novel risk factor for IHD. Recently, a new ECG-based biomarker of autonomic dysfunction based on heart rate variability (HRV), named *Dyx*, was found to be an important predictor of myocardial ischemia.5 *Dyx* is calculated from an hour-long recording of ambulatory ECG, and low values (< 2.0 units) are associated with an 8-fold increased odds of abnormal nuclear stress test findings (suggesting altered regulation of coronary blood flow and possibly IHD).5 While promising, the study was limited by a small sample size, it did not assess for relationship with angiographic findings; the outcome used, single photon emission tomography (SPECT), is only 80% sensitive and specific for obstructive coronary artery disease (CAD).6 In an analysis I independently conducted that was highlighted at the 2018 American Heart Association Scientific Sessions, we found that that low *Dyx* in the early morning was predictive of myocardial perfusion imaging (MPI) deficits in a cohort of 276 veteran twins without known CAD.7 We found for the first time that the time of day in which HRV is measured is a critical step in measuring heart disease risk. Nonetheless, we were unable to differentiate whether this relationship is due to obstructive CAD (requiring revascularization) and/or abnormal vascular reactivity (likely microvascular).8 This is important when considering the clinical implications of low *Dyx*.

Low *Dyx*, as with other HRV metrics, is influenced by central neurologic mechanisms, and in an unpublished analysis in our twins dataset, we found a robust association between depressive symptoms and reduced *Dyx*. This suggests neurologic pathways that may influence coronary microvascular function and lead to ischemia (even in absence of obstructive CAD).9 Neurovisceral integration theory describes a network of brain regions that influence cognitive function, mood, and autonomic regulation that help understand the anatomy of these connections.10 Neuropsychiatric disturbances, such as depression and cognitive impairment, have well-known effects on autonomic regulation,11,12 and confer a worse prognosis in coronary artery disease (CAD),13,14 and an increased risk of SCD.15,16 Low *Dyx* values may be due to brain-related factors, rather than (or in addition to) obstructive CAD.10,17 By studying the relationship of brain-based metrics (mood, cognitive function) and CAD with *Dyx*, we can better evaluate these effects.

I hypothesize that disturbances of the neurocardiac axis, assessed by both heart and brain metrics, associate with autonomic dysfunction, which can be measured by *Dyx*. As such, Dyx is a useful metric in both efforts to risk stratify for CAD, as well as detect neurovisceral dysfunction. We propose to study *Dyx* by measuring HRV through ambulatory ECG patches (BioStamp®, MC10 Inc.) in subjects with chronic stable angina undergoing evaluation in the Emory Cardiovascular Biobank. The Biobank is a prospective cohort study of individuals undergoing clinically indicated cardiac catherization in which conducts validated neuropsychological assessments are also made.18 The data we collect from this proposal will allow us to assess the relationship between autonomic function, neuropsychiatric status, and cardiac catherization. Our aims are:

1. **To determine the effect of neuropsychiatric disturbances, as determined by depressed mood and cognitive impairment, on autonomic dysfunction.***Hypothesis: (A) High levels of depressive symptoms (Patient Health Questionnaire-9, PHQ-9), and (B) cognitive impairment (Montreal Cognitive Assessment, MoCA) will be associated with low Dyx.*
2. **To evaluate the relationship of autonomic dysfunction, measured by abnormal HRV, to the spectrum of progressive CAD.** *Hypothesis:* *Low Dyx (<2.0 units) in the morning hours associates with an increased odds of finding obstructive CAD (stenosis > 70%). When evaluated as a continuous exposure, Dyx negatively associates with CAD plaque burden (Gensini score) in a dose-response relationship.*19

The mentored research and structured didactics of the MSCR will prepare me for my goal of becoming a physician-scientist. My future goals include the pursuit of a K grant focusing on translational studies in neurocardiology, risk stratification, and prevention. Under the guidance of my mentorship team (Amit Shah, MD, MSCR (co-lead); Alvaro Alonso, MD, PhD (co-lead); Marc Thames, MD; Viola Vaccarino, MD, PhD; Arshed Quyyumi, MD) I will gain invaluable training in study design, primary data collection, data analysis, manuscript preparation, and grant-writing. This will help prepare me for my long-term goals in a career as a clinical investigator in cardiovascular epidemiology and translational research.

# SIGNIFICANCE

## B1. The Burden of Ischemic Heart Disease: a Hidden Epidemic with Unrecognized Risk Factors

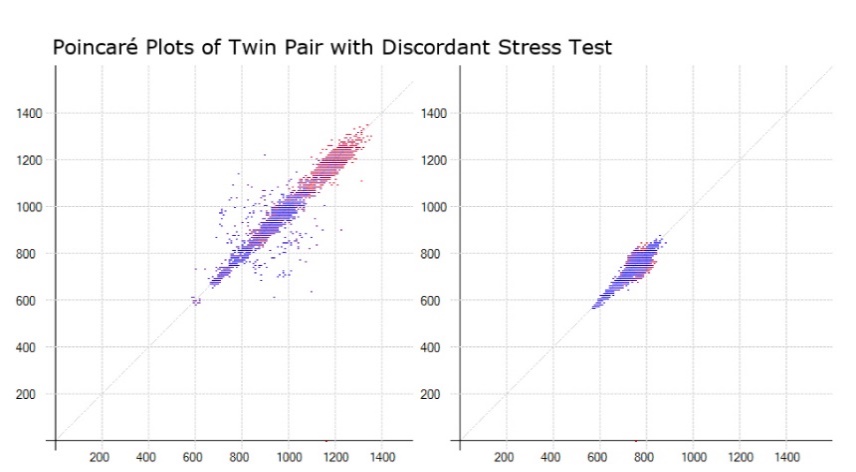
Ischemic heart disease (IHD) accounts for in 1 in every 7 deaths in the United States, with a prevalence of 3% or 7.9 million US adults.20 Over 700,000 new heart attacks occur annually, with annual costs of heart attacks ($12.1 billion). The estimated direct and indirect costs of IHD were $204 billion, and these medical costs are projected to double by the year 2030.20 However up to 20% of MIs are silent and up to 80% of IHD is unrecognized by standard ECG and clinical parameters.1 Although there has been a decline in cardiovascular mortality overall, the rate of out-of-hospital events has not decreased in proportion to the rate of in-hospital events.3 Mental stress plays a role in the complications of IHD, and is an under-recognized and important risk factor. More research on the brain-heart connection may help unlock some of the difficult roadblocks in reducing IHD in the community.21 New integrative and/or holistic strategies to approach IHD and prevent its associated complications may help lead to a downward frameshift in event rates.

## B2. Autonomic Nervous System Metrics Can Help Examine CVD Risk from Neuropsychiatric Factors

Heart rate variability (HRV) is an inexpensive biomarker derived from ambulatory electrocardiographic monitoring.22 It allows for continuous, non-invasive assessment of autonomic function, which is influenced by neuropsychological factors, such as mental stress, and heart disease.23,24 The neurovisceral integration theory provides the anatomical basis for this relationship. This theory links cognitive and affective networks to autonomic regulation by identifying the differential activation of important brain structures (prefrontal cortex, cingulate cortex, and insula).10,25 Psychological stress, including depression, leads to changes in autonomic function that are historically known to increase to the risk of ventricular ectopy, SCD, and cardiovascular disease.13,26–30 Cognitive impairment, particularly as it relates to executive function, also is related to abnormal HRV, and leads to an increased odds of IHD independent of other cardiovascular risk factors.31,32 These interesting findings suggest that neurovisceral dysfunction may contribute greatly to autonomic dysfunction as measured by HRV. Exploring this relationship may provide important insight into why those with dysautonomia have worse psychological, neurological, and cardiovascular outcomes.

## B3. Advances in Electrocardiography are Independently Prognostic of Cardiovascular Disease Risk

Advanced ECG analysis conveys information about autonomic function that is an independent marker for cardiovascular mortality.22,33,34 Traditional methods including exercise stress testing have limited sensitivity,6 but HRV analysis has led to novel risk markers for IHD that need further investigation.5,35 Information within traditional HRV indices was thought to be explained by heart rate itself,36 but newer indices capture additional information.37 A novel method for HRV analysis is througha non-linear method named *Dyx*, which uses the multipole method in Poincaré plots where RR intervals are plotted as a function of prior RR intervals (**Figure 1)**.38–40 *Dyx* measures overall variability in heart rate, but also indicates how erratic and non-linear the heart rate trends are as well. After MI, low *Dyx* has an independent hazard ratio of 2.4 (CI 1.5 to 3.8) for mortality in a recent study.41 Very few studies however have studied the prospective value of autonomic dysfunction in predicting obstructive CAD as measured by coronary angiography. A single, recent study examined *Dyx* with no known IHD and found that low *Dyx* predicted abnormal MPI with an improved sensitivity and specificity to exercise stress test.5 We demonstrated in a cohort of 276 individuals with no known IHD from the Emory Twin Study, low *Dyx* in morning hours had a 12-fold increase in the odds of abnormal MPI. 7 These findings validate the prior work on *Dyx* but also contextualize autonomic balance within the circadian rhythm.42,43 These important findings have helped to place measures of autonomic dysfunction in a potentially clinically useful role. Given the low costs and risks associated with this measure (requires only ambulatory ECG), there is a pressing need for more translational research in the area. Such technologies may ultimately reduce the need for invasive testing with coronary angiography as well as identify asymptomatic, high-risk individuals.



**Figure 1. Poincaré Plot for *Dyx***

## B4. Clinical Implications and Utility of Measuring Autonomic Dysfunction

Our research program will pursue clinical relevance for an HRV-based biomarker, and potentially help to fill an important gap that has prevented HRV from becoming a mainstream clinical test for cardiovascular disease diagnosis.22 This potential is gaining momentum. Based on recent research findings,5 HeartTrends was recently established as a company to offer *Dyx* as a clinical tool to risk-stratify individuals for CAD. Their work is only supported by small, industry-sponsored studies of non-invasive cardiac imaging however, and therefore not currently being utilized in most clinics. Our project will provide an independent evaluation of their metrix, *Dyx*, as a potentially measure of *obstructive CAD* as measured by *coronary angiography*. This additional test of its clinical relevance may help evaluate its clinical potential; if we find that it is predictive, this may have widespread clinical implications when deciding who to send for coronary angiography. *Dyx* is not solely a metric of obstructive CAD risk, however. It may also help measure the complex relationship between the heart and the brain, and explain stress-related microvascular disease dysfunction as well.9 This is important because studies showing low *Dyx* as predictive of abnormal myocardial perfusion imaging (MPI), including our own, are inherently limited: abnormal MPI findings cannot distinguish between the larger epicardial coronary arteries and smaller myocardial resistance vessels.7,35 *Dyx*, as a marker for neurovisceral dysfunction, may also shed light into the mechanism of increased non-cardiac risk. As such, it may have potential in predicting psychiatric outcomes such as future depression,44 as well as future cognitive decline.45

**C. INNOVATION**

Innovation is central to this proposal, which seeks to validate a new, low-cost ECG-based measure (*Dyx*) as an alternative diagnostic test for obstructive CAD. It may also provide a robust measure of autonomic regulation in disturbances of the neurocardiac axis that has additional relevance to psychiatric and neurologic diseases. This is a paradigm shift towards metrics that have a focus on the brain-heart connection, as opposed to focused tests on anatomy or self-reported symptoms. This new way of seeing IHD as connected to the neurocardiac axis may lead to clinical practice changes in disease management as well. For example, it may promote stress management and exercise therapy in management of IHD.46 *Dyx* is relatively new and unexplored compared to other HRV indices. For the first time, we are also taking into close consideration the time of day when measuring HRV. 42,47 Most previous studies, on the other hand, do not evaluate this at all, or average all of the HRV metrics over and entire 24 hour period.35,48,49



**Figure 2. BioStamp**

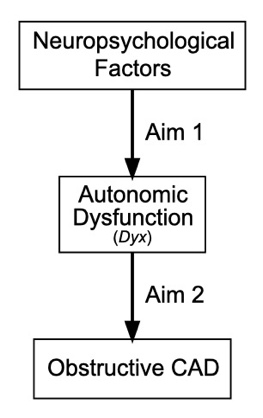
The technology utilized for this study is also cutting edge and more easily collected in clinical settings than previous methods. We will utilize the BioStamp patch (**Figure 2**), which has a much lower patient burden than traditional Holter monitoring (smaller than a credit card).

Our study design also allows us to achive breakthrough in the assessment of Dyx; as opposed to previous studies, we can now evaluate its predictive potential with with coronary angiography findings. Future studies (may be included in K23) may also evaluate the relationship of *Dyx* with secondary clinical outcomes. Additional evaluation of *Dyx* with depression and cognitive function will be the first studies of their kind. It will lead to better a mechanistic understanding of the neurocardiac axis, and future work may help to evaluate non-cardiac outcomes such as depression. Overall, our rigorous, holistic evaluation of *Dyx* will help provide critical assessment of its value in IHD risk prediction and evaluation of neuropsychological pathology.

# D. APPROACH

## D1. Study Overview

This training grant proposes an ancillary study on an ongoing prospective registry of patients undergoing cardiac catherization, the Emory Cardiovascular Biobank (EmCAB, PI Quyyumi) ﻿which was established to identify novel factors associated with the pathobiological process and treatment of cardiovascular disease.18 The EmCAB is enriched for patients with high suspicion for obstructive CAD, which provides ample statistical power for studies of risk prediction. The registry has over 7,000 unique patients from three Atlanta-based sites in the Emory University Hospital system. The EmCAB has ongoing enrollment (10-20 patients per week), with established facilities, staff, and data collection mechanisms in place, and has IRB approval for future research that includes analysis of de-identified data. **Figure 3** shows an overview of the scientific basis of the proposed aims. The ECG data needed to calculate *Dyx* will be added as an ancillary study using the existing study team. The current coordinator will apply the patch and retrieve the data. Processing of HRV is automatic and will be available from the Biostamp software suite.



**Figure 3. Specific Aims**

# D2. Study Population

The EmCAB has assessed approximately 3,000 major cardiovascular events thus far.14 It also evaluates additional biomarkers for inflammation, cardiac injury, and genetics, with the goal of predicting CVD outcomes.18 All patients aged 18 years and older undergoing cardiac catherization are recruited to participate by a full-time study coordinator. After informed consent, they are interviewed for health behaviors and neuropsychological functioning the same day, prior to cardiac catherization. They are excluded if they have congenital heart disease, severe valvular heart disease, severe anemia, a recent blood transfusion, myocarditis, history of active inflammatory disease, cancer or are unable or not willing to provide consent (approximately 5%). We will also exclude those with acute coronary syndrome.

## D3. Research Design

General Protocol: The enrollment, consent, and detailed phenotyping of the patients has been described in prior studies of the EmCAB.18 ﻿Additional measures, including lifestyle factors, medical comorbidities, revascularization during the index cardiac catheterization, and previous revascularization procedures are ascertained via patient interview and chart review. The study sample will be collected daily over the first several months, with an estimated 10-20 patients enrolled per week. Coronary angiography will be evaluated by the Gensini score, which is a visual estimation of luminal narrowing in multiple segments based on a modified form of the American Heart Association classification of the coronary tree by trained cardiologists.19

Heart Rate Variability: This study will add HRV to the data collected by the EmCAB. We will use non-invasive, continuous, ambulatory ECG patches (Biostamp®, MC10 Inc), which have already been acquired through my mentor Dr. Shah. I will assist with the consent and incorporation of ECG data collection into the larger study protocol. The consent will occur in the early morning (7 AM — 9 AM), after which the patch will be applied until their angiogram. Our recent findings demonstrate that the most important time for detecting autonomic dysfunction is between 7 AM and 10 AM.7 If possible, the ECG duration will be extended. We will use the commercial HeartTrends algorithm (Lev-El Diagnostics Ltd., Israel) to generate the *Dyx* measure, as well as an internally developed HRV toolbox to generate additional indices for comparison.50 A materials transfer agreement with the company is already in place with HeartTrends.

Neuropsychological Measures: The enrollment protocol includes patient interviews by study staff. Depressive symptoms will be assessed via the 9-question Primary Care Evaluation of Mental Disorders Brief Patient Health Questionnaire (PHQ-9).51 Moderate depression is seen at a cutoff of 10 points or higher (out of 27), with a sensitivity and specificity of 88% for major depression.52 Cognitive impairment will be measured by the Montreal cognitive assessment (MoCA), which is a measure of global cognitive function and is comprised of the sub-domains: memory, visuospatial function, executive function, sustained attention, language, and orientation. The MoCA has a sensitivity of 90% and specificity of 87% for detecting mild cognitive impairment with a score of 26 (out of 30).53

Power Calculation: In this exploratory pilot study, the expected cohort size is 150 patients, collected over the first 6 months. We anticipate a maximum of 20% loss of data due to poor ECG quality (>20% artifact), missing data, or other exclusion criteria as above. We do not expect attrition as this is a cross-sectional study. After these reductions, we expect a sample size of 120 patients. We expect a rate of 70% of CAD, and 15-20% of neuropsychiatric disease. After biostatistician consultation, we will use α=0.05, and 1-β=0.80. We would be adequately powered to detect a large effect size in for the primary outcomes for both specific aims (Cohen’s d = 0.5).54

**D4. Specific Aim #1: To determine the effect of neuropsychiatric disturbances, as determined by depressed mood and cognitive impairment, on autonomic dysfunction.**

Rationale: Depression and cognitive impairment are not only common is patients with CAD, but are also prognostic after MI, indepdent of traditional risk factors.31,56,58,59 Our preliminary analyses from the Emory Twin Study found *Dyx* to be a significant determinant of depressive symptoms (r= 0.14, p<0.001 in 276 individuals). Also, in a preliminary analysis from the Atherosclerotic Risk In Communities (ARIC) study, psychological life stressors such as exhaustion and anger are associated with abnormal HRV. Cognitive impairment also associates with decreases in HRV.57 We will look to elucidate the independent relationship Dyx, a marker of neuropsychiatric disturbance, with depression and cognitive impairment, which has not yet been studied.

Data Collection and Analysis: I will be directly involved with the study coordinators to enroll and consent patients for ambulatory ECG. I will be responsible for working with the BioStamp company to retrieve raw ECG data, and will use the HRV toolbox to automatically extract and convert it into usable RR intervals and evaluate for arrhythmia (which would be excluded from analysis). I will communicate with the HeartTrends company to assist with the appropriate and timely generation of the *Dyx* index through Poincaré plot analysis.38,39 I will learn to use the HRV toolbox under the guidance of Dr. Shah to generate hourly frequency and geometric domain indices of HRV for additional HRV assessments.50 Familiarizing myself with the mathematical principles and technical skills underlying signal processing will be critical for my training. The primary exposures will be depressive symptoms (PHQ-9) and cognitive impairment (MoCA score). The exposures will be analyzed for correlation. The primary outcome will be autonomic function, measured by *Dyx* and other HRV indices. Each exposure will be included in individual regressions models for the continuous *Dyx* measure, and logistic regressions for *Dyx* using the clinical cutoff of <2 units.22 Secondary outcomes will adjust for sociodemographic and traditional cardiovascular risk factors. My training through the MSCR will provide me the necessary tools to conduct these analyses, with support from Dr. Alonso and Dr. Shah in evaluation and interpretation of the data.

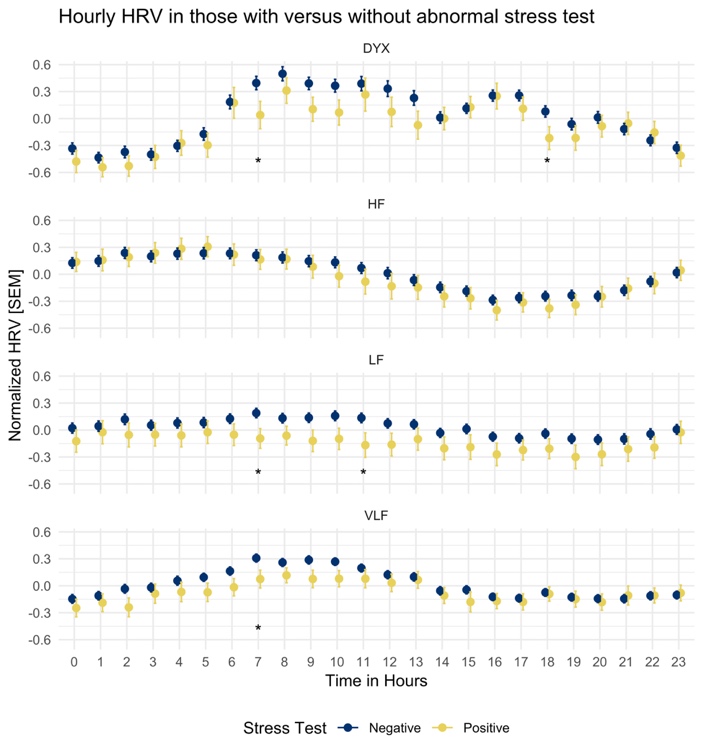
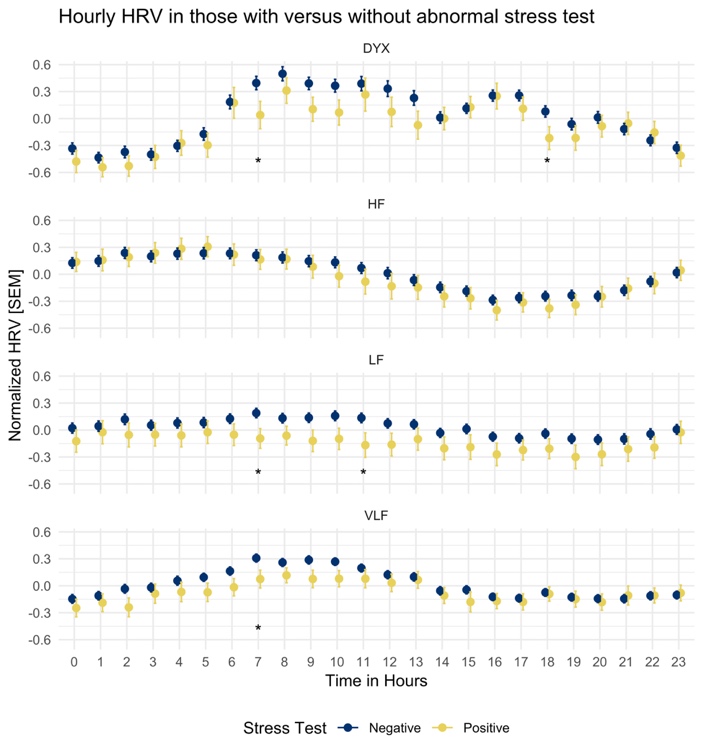
Potential Problems and Solutions: The PHQ-9 is not validated in the setting of acute stress, for which we will exclude patients diagnosed with acute coronary syndrome. Treatment of depression may lead to favorable changes in HRV. We will control for antidepressant use through additional subgroup analysis. The generalizability of this data is difficult, as patients with severe cognitive impairment may not be referred for catherization, and thus excluded from the study.

Anticipated Results: We expect to find an independent association of depression and cognitive impairment with autonomic dysfunction, measured by low *Dyx*.

**D5. Specific Aim #2: To evaluate the relationship of autonomic dysfunction, measured by abnormal HRV, on the spectrum of progressive CAD.**

Rationale: Abnormal HRV indicates autonomic dysfunction and increases the risk of cardiovascular mortality. *Dyx* has been shown to predict myocardial perfusion defects during stress test. Our previous work suggests this finding is most robust during morning hours, as in **Figure 4**.7 We are looking to, for the first time, evaluate *Dyx* during morning hours and the relationship with obstructive CAD and overall plaque burden.

**Figure 4. Low Morning HRV and Abnormal MPI**



Data Collection and Analysis:I will compare the exposure of HRV to the outcome of coronary artery plaque burden, measured by the Gensini score, from cardiac catherization using linear regression models. Logistic regression models will be fit using a clinical cutoff point of *Dyx* (< 2.0 units)against obstructive CAD (stenosis > 70%).22 Secondary outcomes will evaluate the performance of HRV against traditional risk factors. I will perform these analyses independently to help apply the biostatistics skills strengthened by formal coursework during the MSCR.

Potential Problems and Solutions: This patient population is likely to have a high proportion of patients on beta-blockers, which has a known effect on heart rate and spectral HRV.55 For patients on beta-blocker therapy we will perform an additional subgroup analysis to prevent confounding. Continuous ECG data are susceptible to artifact, which may lead to erroneous data. Such data can be edited to remove noise and ectopy, and interpolated to fill gaps, which existing software techniques allow for the easy cleaning of raw RR interval data.

Anticipated Results: We expect to find an independent association with autonomic dysfunction in the morning hours, measured by low *Dyx*, with progressive coronary artery plaque burden.

## D7. Mentorship and Training Integration

Dr. Shah, as an expert in ECG signal processing and psychological stress, has been a dedicated mentor for the past three years. We will have frequent in-person and phone meetings, and he will provide guidance on data measurement, analysis, and interpretation of HRV metrics. Dr. Alonso stuies the effect of neurocognitive factors on arrhythmia risk and is an investigator in a large clinical cohort, the Atherosclerotic Risk In Communities study (ARIC). We will meet in-person every other week. He will provide guidance on study design, statistical analysis, and interpretation of neurocognitive impairment. Dr. Thames is a senior cardiologist with extensive research training in the cardiac autonomic nervous system, and has provided expert insight into mechanisms in prior manuscripts. He will continue to guide me in interpretation of basic and translational research, and share insight into autonomic mechanisms. Dr. Vaccarino, as the head of the Emory Program in Cardiovascular Outcomes Research and Epidemiology (EPICORE) and T32 program, conducts biweekly meetings that I will participate in by presenting my work, meeting with other trainees, and attending seminars by invited speakers on cardiovascular disease epidemiology. I will participate in dedicated teaching programs in vascular biology and biostatistics as a trainee in EPICORE. She will also provide feedback on manuscript drafting focused on epidemiology and biostatistics concepts. Dr. Quyyumi is the lead PI of the EmCAB, and has provided the research funding and study staff to allow for the integration of the new HRV component into the EmCAB. He will provide manuscript feedback, as well as provide guidance on the translational aspects of study design. The coure *MSCR 594 Scientific and Grant Writing* will give me exposure as for my F32 and future K23 grant in preventive cardiology. The basic and advanced biostatistics courses including *MSCR 534 Analytic Methods for Clinical and Translational Research* *and MSCR 596 Advanced Data Management in R* will enhance my ability to become an independent researcher.

## D8. Timeline

This research proposal is intended to allow for completion of several projects over the course of the upcoming academic year, from July 2019 to June 2020, during which I am appointed as post-doctoral research fellow at the RSPH and will have no clinical obligations. I will spend 45-55% of my time completing academic coursework for the MSCR, including additional classes and seminars in advanced biostatistics and statistical programming languages. The remaining time will be dedicated to implementation of the study protocol, including enrollment, data collection, and primary data analysis. I will start to work with EmCAB coordinators, the MC10 BioStamp company, and the HeartTrends group by July 2019 or earlier. I will participate in direct enrollment only as time allows, as the EmCAB is already well-staffed, which wll allow me to focus on the MSCR and the direct research. The patient data collection will be completed by December 2019. From January 2020 onwards, statistical analysis and manuscript preparation will begin. By March 2020, I will dedicate time to completion of the dissertation and begin future grant proposals, including an F32 grant. I will present at local research meetings starting in the fall, submit at least 2 abstracts for national meetings by early spring, and have submitted 2 first-author manuscripts. I will apply for fellowship in academic cardiology subsequently, and apply for a K23 grant as I join faculty as a clinical investigator.

## D9. Future Directions

We expect to find a clinically meaningful (risk ratio >2) association with the non-linear HRV index, *Dyx*, and obstructive CAD, highlighting the utility of autonomic dysfunction as an independent risk factor for CAD. We also expect to find an independent association of *Dyx* with depression and neurocognitive impairment, demonstrating its role in quantifying neurocardiac health, and highlighting the importance of the brain in this relationship. I hope to use this research as the basis for screening for autonomic dysfunction through ECG to identify those at risk for obstructive CAD, and future grants would further elaborate on this work with larger sample sizes, clinical outcomes, and additional mechanisms such as inflammatory biomarkers. I also plan on using HRV as a tool to research the effects of neuropsychological factors on cardiovascular disease risk, such as directionality and effect size, with the intent to eventually direct interventions that may be protective.

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**PROTECTION OF HUMAN SUBJECTS**

This research plan proposes to perform an ancillary study on an ongoing prospective cohort study, called the Emory Cardiovascular Biobank (EmCAB, PI Quyyumi). This cohort is a registry of patients undergoing cardiac catherization to identify novel factors associated with pathobiological processes and treatment of cardiovascular disease. All patients are aged 18 years and older, and are at risk for coronary artery disease. The study was approved by the Emory University Institutional Review Board (IRB) and complied with federal, state, and internal regulations, including HIPAA. Recruited participants are men and women.

1. **Risks to Subjects**

This ancillary study will not involve any significant additional risks to the patients included in the EmCAB parent study. The patients will be asked to wear the Biostamp patch, which may cause discomfort, limitation in activity, and skin irritation upon removal. The patches will be applied by a trained personnel. The collected ECG data and analysis do not represent acute markers of illness that prompt referral to a medical provider.

1. **Adequacy of Protection against Risks**

All subjects included in the parent studies provided informed consent, approved by the IRB. Additional consent for the Biostamp will be integrated into the current study, with modifications to be approved of by the IRB. As part of the parent study, a supervising attending physician is available for evaluation of any events that may occur. Participants will be asked to contact the PI if there are any questions regarding the study.

1. **Potential Benefits of Proposed Research to the Subjects and Others**

This research is not specifically designed to provide a benefit to participants of the parent study. The potential benefits lie in identification of autonomic dysfunction that can be used to help in risk prediction and preventive strategies in the future.

1. **Importance of Knowledge to Be Gained**

By gaining an better understanding of disturbances of the neurocardiac axis, through measurement of autonomic dysfunction, we will be able to understand mechanisms behind cardiac and non-cardiac risk, and potentially develop preventive and treatment strategies.

1. **Data and Safety Monitoring Plan**

The parent studies from which data will be derived for this grant are observational studies that involve minimal additional risk to study participants. The EmCAB has an established Data Safety Monitoring Plan which have been used to report adverse events to the IRB and, if needed, stop the study in the event of an adverse reaction. There are no expected adverse events that will occur by the use of the Biostamp patch or in the data collected therein. Additionally, I will only use de-identified data that is hosued at Emory University. Therefore, the analyses propsed and data collected pose minimal risk to the participants.

**INCLUSION OF WOMEN, MINORITIES, AND CHILDREN**

This research plan proposes to perform an ancillary study on an ongoing prospective cohort study, called the Emory Cardiovascular Biobank (EmCAB, PI Quyyumi). This cohort is a registry of patients undergoing cardiac catherization to identify novel factors associated with pathobiological processes and treatment of cardiovascular disease. All patients are aged 18 years and older, and are at risk for coronary artery disease. The study was approved by the Emory University Institutional Review Board (IRB) and complied with federal, state, and internal regulations, including HIPAA. The planned enrollment expects a comparable number of men and women of diverse racial/ethnic profiles. Similar to the EmCAB cohort description, we expect the mean age at enrollment to be 63, with approximately 64% male, 72% white, 24% black, 2% Asian and 1% Hispanic. The planned analyses in this proposal will attempt subgroup analyses stratified by sex and race, but may not be powered to detect differences. The parent study does not include or recruit children, as the aim of the study was to evaluate subjects at risk for coronary artery disease, which rarely develops in persons <18 years of age.