# D. APPROACH

## DX. Study Overview



This training grant proposes an ancillary study on an ongoing prospective registry of patients undergoing cardiac catherization, the Emory Cardiovascular Biobank (EmCAB) ﻿which was established to identify novel factors associated with the pathobiological process and treatment of cardiovascular disease.1 The EmCAB is enriched for patients with high suspicion for obstructive CAD, which provides ample statistical power for studies of risk prediction, and is led by one of the advisors, Dr. Arshed Quyyumi, and has been studied before by the mentoring team. The registry has over 7000 unique patients from three Emory Healthcare sites in Atlanta (Emory University Hospital, Grady Memorial Hospital, and Emory Midtown Hospital) and is supported by the Woodruff Foundation at Emory University, several NIH and other grants. 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. The EmCAB continues to consent and enroll over 40 patients per month, allowing this applicant to collect novel data. **Figure 1** shows an overview of the proposed aims. The proposed research will fund my training to obtain and analyze ECG data specific to autonomic function (through HRV indices), collect and interpret questionnaire-based data on depression, stress, and cognitive impairment, and gain proficiency in the statistical analysis.

# DX. Study Population

The EmCAB has had approximately 3000 major cardiovascular events thus far, and has had multiple studies that have identified additional predictive factors for cardiovascular disease outcomes, including protein biomarkers, oxidative stress markers, circulating progenitor cells, and inflammatory markers that may improve risk prediction beyond traditional risk factors. Participants are interviewed to collect demographic, medical history, family history, medication usage, health behaviors, psychological factors, cognitive impairment, and neuropsychological functioning prior to cardiac catherization. All patients aged 18 years and older undergoing cardiac catherization at the three listed Emory Healthcare sites are asked to participate. ﻿Patients 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 specifically evaluate subjects who are clinical stable, and exclude those with acute coronary syndrome.

## DX. Research Design

General Protocol: The enrollment, consent, and detailed phenotyping of the patients has been described in multiple prior studies using the EmCAB.1 ﻿Patients are enrolled and interviewed by study staff on the same day of their cardiac catheterizations, with most interviews occurring prior to catheterization. Other 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 sample for study will be collected daily over the course of the first several months of this study, with an estimated 20 patients enrolled per week. Coronary angiographies will be evaluated by 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. Semi-quantitative angiographic scoring will be performed using the Gensini score, which quantifies CAD severity by a non-linear points system for degree of luminal narrowing weighted by a multiplier for specific anatomical locations of any lesions.

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 we have acquired through my mentor Dr. Shah, for the collection of time series data of RR intervals for further analysis. I will assist with the consent and incorporation of the additional ECG data collection into the larger study protocol. The consent will occur in the early AM (7AM-9 AM), after which the patch will be applied until their angiogram. This follows from our recent findings that the most important time for detecting autonomic dysfunction is between 7 AM and 10 AM.2 If possible, however, additional data will be collected. We will use the commercial HeartTrends algorithm (Lev-El Diagnostics Ltd., Israel) to generate the *Dyx* measure, as well as an internally developed HRV analysis toolbox to generate additional HRV indices for comparison.3 The HRV toolbox was developed here at Emory with consultation from the Dr. Shah, due to his expertise in ECG measures of autonomic dysfunction.

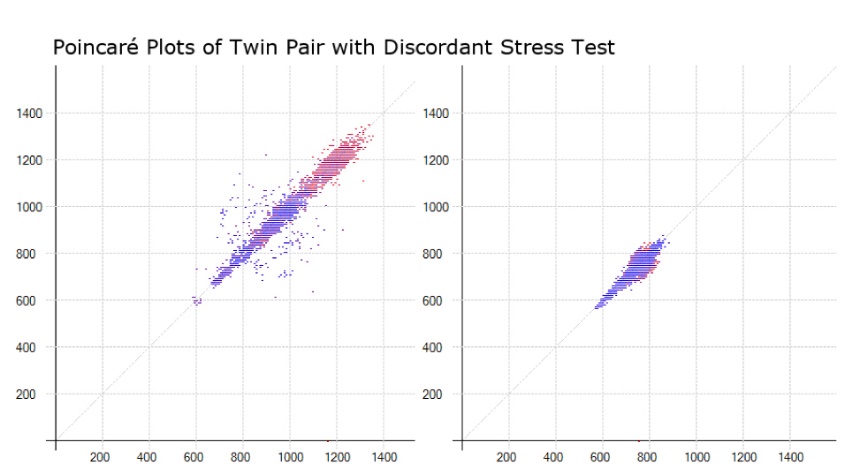
Neuropsychological Measures: As describe, the enrollment protocol includes patient interviews by study staff. Depressive symptoms will be assessed via the 9-question Primary Care Evaluation of Mental Disorders Brief Patient Health Questionnaire (PHQ-9).4 Moderate depression is seen at a cutoff of 10 points or higher (out of 27), which has a sensitivity and specificity of 88% for major depression.5 In addition, questionnaires are included on perceived life stress and chronic burden. Cognitive impairment will be measured using 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 (MCI) using a cutoff score of 26 (out of 30).6

Power Calculation: The expected cohort size from this ancillary study is 150 patients, which we should be able to collect over the course of 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. After these reductions we estimate a sample size of 120 patients. Using α=0.05, and 1-β=0.80, we would have the power to detect a different of magnitude as small as Cohen’s d=0.32. The preliminary data from the Emory Twin Study, and based on the increased event rate in the EmCAB, this minimum magnitude of difference will likely be surpassed for most analyses.

**DX. Specific Aim #1:** To evaluate the relationship of abnormal HRV during morning hours with obstructive 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.2 We are looking to, for the first time, evaluate *Dyx* during morning hours and the relationship between angiographic findings of obstructive CAD.

Data Collection and Analysis: I will be directly involved with the study coordinators to enroll patients and help consent for the use of ambulatory ECG. I will be responsible for working with the BioStamp company for the extraction of ECG data into usable RR intervals and evaluation for arrhythmia (which would be excluded from analysis). I will also communicate with the HeartTrends company to ensure appropriate and timely generation of the *Dyx* index. The algorithm uses the multipole method of HRV analysis through Poincaré plots, in which RR intervals are plotted as a function of prior RR intervals as seen in **Figure 2**.7,8 I will learn to use the HRV toolbox under the guidance of Dr. Shah to generate frequency and geometric domain indices of HRV, which is critical for my training to familiarize myself with the mathematical principles and technical skills underlying signal processing. 3 All HRV indices will be generated for each hour for comparison. I will compare the exposure of HRV indices to the outcome of the presence of obstructive CAD (stenosis >=70%) on cardiac catherization using logistic regression models, with multivariate analysis to evaluate the performance of HRV against traditional risk factors. I will perform these analyses independently, under the guidance of Dr. Alonso and Dr. Shah, to help apply the biostatistics skills strengthened by formal coursework during the MSCR.



**Figure 2. Poincaré Plot for *Dyx***

Potential Problems and Solutions: This patient population is likely to have a higher proportion of patients on beta-blockers, which has a known effect on heart rate and spectral HRV.9 For patients on beta-blocker therapy in an additional subgroup analysis to prevent confounding of the results. 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 obstructive coronary artery disease (stenosis >= 70%).

**DX. Specific Aim #2:** To determine the effect of depressive symptoms and stress on autonomic dysfunction.

Rationale: Depression is common in patients with CAD, and is an independent risk factor for increased mortality after MI.10 Psychological stress, including depression, is well known to lead to increased risk of SCD.11 We found in preliminary analysis that in a cohort from the Emory Twin Study that *Dyx* had a robust association with depressive symptoms. We also found in preliminary analysis that in a cohort from the Atherosclerotic Risk In Communities Study other psychological life stressors, such as exhaustion, were associated with abnormal HRV. We will look to elucidate the relationship and effect size of depression and stress with autonomic dysfunction through *Dyx.*

Data Collection and Analysis: The primary exposures will be depressive symptoms and stress. The primary outcome will be autonomic function, measured by *Dyx* and other HRV indices. I will create linear regression models for the continuous *Dyx* measure, and logistic regressions for *Dyx* using the previously established cutoff of <2 units. As in **Aim #1**, I will also create models that adjust for cardiovascular risk factors, with additional focus on age and gender due to their impact on depression and HRV. As part of my training, I will also use structured equation modeling to help quantify effect size and the influence of other comorbid conditions, which I will learn to interpret and model through advanced biostatistics coursework and independent study through the MSCR.

Potential Problems: As patients are undergoing cardiac catherization, some patients may have acute reasons for presentation, for which the PHQ-9 is not validated. We will exclude patients with a diagnosis of acute MI for that reason. Treatment of depression may lead to favorable changes to autonomic function, and thus antidepressant use may affect HRV. We will control for antidepressant use through additional subgroup analysis.

Anticipated Results: We expect that depressive symptoms and psychological stress independently associate with autonomic dysfunction, measured by low *Dyx*. We expect that this finding will be more robust than in other HRV measures.

**DX. Specific Aim #3:** To explore the relationship of cognitive impairment on autonomic dysfunction.

Rationale: There is an increased odds of developing cognitive impairment in IHD which is independent of cardiovascular risk factors. 12 HRV has been seen to independently associate with changes in cognitive function, even after adjustment for traditional risk factors.13 The increased risk may be mediated through autonomic dysfunction, and for the first time, we will look to identify the relationship of *Dyx* with cognitive impairment

Data Collection and Analysis: The primary exposure will be cognitive impairment per the MOCA, broken into its subdomains. The primary outcome will be autonomic function, measured by *Dyx,* along with other traditional HRV indices. Linear and logistic regression models will be used as described in **Aim #2**. Multivariable models will be adjusted for cardiovascular risk factors. I will analyze the MOCA by its subdomains in addition, as these may have different effects on autonomic function.

Potential Problems and Solutions: The generalizability of this data will be made complex as patients with more severe cognitive impairment may not be referred for catherization, and thus excluded from the study. This data will also be complicated by patients with preexisting cerebrovascular disease. To control for this, we will exclude patients that have known strokes.

Anticipated Results: We expect that lower MOCA scores, suggesting cognitive impairment, will independently associate with autonomic dysfunction, measured by low *Dyx*, and the association will be more robust than with other HRV measures.

## DX. Mentorship and Training Integration

The combination of direct human research experience (including patient enrollment and study design), statistical analyses and management of data, dedicated course work through the MSCR, and the clinical training from his residency are integral to building my foundation as a physician-scientist. Dr. Shah has an expertise in ECG signal processing and psychosocial stress, and 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 data. Dr. Alonso studies 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 on a biweekly basis, where he will provide guidance on study design and data interpretation as it relates to neurocognitive impairment, as well as direction on statistical analysis. Dr. Thames is a senior cardiologist with extensive research into the cardiac autonomic nervous system. He has provided expert insight into mechanisms and pathways in prior manuscripts, and will continue to teach me the interpretation of translational research in cardiac autonomic function. 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, allowing me to share my work, meet with other trainees, and attend seminars by invited speakers on cardiovascular disease epidemiology and health disparities. I will also 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 investigator of the EmCAB and has participated in a number of clinical trials. He will help me integrate this new HRV component into the EmCAB, giving me guidance on the practical steps needed to modify and design a clinical study. He will also provide feedback on manuscript drafting with a focus on translational and clinical aspects. Certain skills gained through the MSCR curriculum will be foundational to my success as a researcher. Of note, the *MSCR 594 Scientific and Grant Writing* will help as I prepare to apply for my first K grant in preventive cardiology. With my background in programming, particularly the statistical language R, 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 help formalize and enhance my ability to become an independent researcher.

## DX. 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. The applicant will spend 35-45% of his time completing academic coursework for the MSCR, including additional classes and seminars in advanced biostatistics and statistical programming languages. The applicant expects to split his remaining time between implementation of the proposed study protocol, including enrollment, data collection, and study design, and primary data analysis. No clinical work will be assigned. As the applicant has worked with Dr. Shah and the Rollins School of Public Health extensively over the past 3 years, he intends to start working with the EmCAB coordinators, the MC10 BioStamp company, and the HeartTrends group starting in April 2019 to facilitate a productive research year. In the spring of 2020, the applicant intends to use part of his time for the preparation of manuscripts to be submitted, along with grant proposals for future studies. This applicant is considering a career in academic cardiology, and will reach out to other faculty and researchers to gain additional training for future research throughout fellowship.

## DX. 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. I also expect to find an independent association of *Dyx* with depression, stress, and neurocognitive impairment, demonstrating its role in quantifying neurocardiac health, and highlighting the importance of the brain in this relationship. In the future, I hope to use this research as the basis for screening for autonomic dysfunction using ECG to identify those at risk for obstructive CAD, as well as understanding the heart-brain mechanisms better. Future grants would further elaborate on this work with larger sample sizes, clinical outcomes, and additional mechanisms such as inflammation. 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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