

CORONARY ARTERY CALCIFICATION AND ITS PROGRESSION IN CANCER
PATIENTS

BY

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LIST OF ABBREVIATIONS

CAC	coronary artery calcium
CAD	coronary artery disease
CDC	Center for Disease Control
CHS	Cardiovascular Health Study
CI	confidence interval
CT	computed tomography
CV	cardiovascular
EBCT	electron beam computed tomography
HDL	high density lipoprotein
HU	Hounsfield unit
ICD	International Classification of Diseases
LDL	low density lipoprotein
MDCT	multi-detector computed tomography
MESA	Multi-Ethnic Study of Atherosclerosis
MI	Myocardial infarction
MR	magnetic resonance
mSv	milliSievert
SIR	standardized incidence ratio
TDW	Translational Data Warehouse

ABSTRACT

Research over the last several decades has demonstrated that cancer and its corresponding therapies are associated with an increased risk of ischemic heart disease in cancer survivors as compared to the general population. Though several mechanisms have been proposed, the reasons for this association have not been completely elucidated. Atherosclerosis, a complex pathologic process that can involve the coronary arteries, is the most common cause of ischemic heart disease and research has suggested cancer and its therapies may be involved in its development.

Coronary artery calcification is a surrogate marker of coronary atherosclerosis with a large amount of research demonstrating its ability to quantify burden of disease as well as predict cardiovascular events. Non-invasive detection and quantification of CAC is performed with computed tomography and has been used in both the clinical setting as well as large cohort studies serially monitoring cardiovascular risk factors and events. This thesis explores the novel application of CAC as a marker of atherosclerosis to investigate the association of CAC progression over time with cancer and its therapies in cancer survivors.

CHAPTER 1: BACKGROUND AND INTRODUCTION

Cancer and Cardiovascular Disease

Over the last two decades, the medical community has recognized that cancer and its treatment can result in undesired complications and toxicities leading to increased cardiovascular morbidity and mortality.¹ With improvements in diagnosis, treatment and subsequent cancer free survival, there are over 14 million cancer survivors in the United States in 2013.² Given the increasing size of this population, adverse cardiovascular effects from the cancer or from its therapies are a growing concern.

As compared to the general population, several cancers and their associated therapies have been implicated as increasing the risk of coronary artery disease and myocardial infarction.³⁻⁵ In a study following 4,414 long term survivors (greater than 10 years after diagnosis) of breast cancer, rates of myocardial infarct, angina pectoris and congestive heart failure were compared to the general female population.³ The standardized incidence ratio (SIR) for acute myocardial infarction in breast cancer was 1.23 (95% CI 1.08 to 1.39) and for angina pectoris was 1.30 (95% CI 1.16 to 1.45). These increased ischemic risks were associated with breast irradiation even after accounting for traditional risk factors such as diabetes, hypertension, and hyperlipidemia.

Hodgkin lymphoma survivors also have an increased burden of ischemic cardiovascular disease as compared to the general population. In a study of 1,474 individuals diagnosed with Hodgkin lymphoma on average 18.7 years earlier, the standardized incidence ratio for coronary heart disease was 3.2 (95% CI 2.7 to 3.7) in the lymphoma population indicating a greater than 3 times risk of angina or myocardial

infarction.⁴ Similar to the findings in the breast cancer study, treatment with radiation therapy increased the risk of ischemic events ($p < 0.05$). Interestingly, young age at the start of treatment was also a risk factor for ischemic cardiac events.

A third type of cancer and subsequent treatment regimen associated with increased ischemic disease is testicular cancer. In a study of 2,512 men who had survived more than 5 years beyond their cancer diagnosis, the SIR of coronary heart disease (myocardial infarction or angina pectoris) was increased at 1.17 (95% CI 1.04 to 1.31).⁵ Important risk factors included the combination of radiation and chemotherapy, recent smoking, and nonseminomatous cancer type ($p < 0.05$).

Potential Mechanisms of Increased Atherosclerosis

With increased incidence of ischemic heart disease in cancer survivors, these studies indicate that cancer and its associated therapies are associated with an increased risk of symptomatic coronary artery atherosclerosis. Coronary atherosclerosis is a pathologic process that leads to disease in the vasculature supplying blood to the myocardium and is influenced by multiple factors.

The earliest manifestation of atherosclerosis is called a fatty streak and is relatively common in individuals starting in childhood.⁶ This fatty streak is composed of macrophages high in lipids called foam cells, that are located below the endothelial lining of the artery, within the intima of the coronary vessels. Endothelial cell injury and dysfunction promotes this process. In addition, smooth muscle cells also migrate and accumulate in the intima resulting in an area of plaque and early atheroma.⁷ With time, the early atheroma may progress to a more complex lesion with a core of extracellular

lipids in a soft plaque and fibrous cap (called a fibro-atheroma). Eventually, the lesion may progress further with the deposition of calcium, called an advanced calcified atheroma.⁸ An individual may have multiple lesions at various stages of progression. Coronary atherosclerosis increases the risk of myocardial infarction via rupture of the plaque or symptomatic angina due to an atherosclerotic stenosis.⁸

Multiple pathways contribute to the pathogenesis of atherosclerosis including endothelial dysfunction, dyslipidemia, inflammatory processes and immunologic factors.⁹ The proposed mechanisms for cancer and its therapies increasing the risk of coronary atherosclerosis are broad and are dependent upon the type of cancer and therapy delivered. Hypothesized mechanisms include direct endothelial injury, metabolic and hemodynamic derangements, induction of prothrombotic state and acceleration of atherosclerotic and arteriosclerotic processes.¹ For example, radiation is thought to promote atherosclerosis by multiple connected mechanisms including endothelial damage, inflammation, fibrosis, and an increase in thrombosis.¹⁰ Another example of a potentially cardiotoxic therapy is aromatase inhibitors, a type of endocrine therapy commonly used in hormone receptor positive breast cancer.¹¹ Proposed mechanisms include elevations in serum lipid levels as well as inhibition of key enzymes within the vascular wall both of which promote atherosclerosis. Anti-metabolites 5-fluorouracil and its prodrug capecitabine have been associated with acute cardiac ischemic events with an incidence ranging from 3-9% with capecitabine and 1-68% with fluorouracil.¹ A broad range of mechanisms have been proposed including vasospasm, arteritis, thrombosis, direct myocardial and endothelial damage, and myocarditis¹². Newer, small molecule tyrosine kinase inhibitors have also been implicated with acute ischemia. Cardiac

ischemia or infarct occurred in 3% of individuals treated with sorafenib compared to <1% of placebo in a trial treating renal cell carcinoma.¹³ Finally, the progression of the atherosclerosis may be independent of the therapies to treat cancer and may be due to the intertwined processes of chronic inflammation and cancer¹⁴ as inflammation has been associated with the development of coronary atherosclerosis.⁶

Quantification of atherosclerosis by coronary artery calcium

The ability to non-invasively detect and quantify the coronary artery atherosclerosis has been a key goal in cardiovascular research. One such measure, coronary artery calcium (CAC), has been shown in multiple studies to have this capability.

Non-invasive assessment of coronary vascular calcification using fluoroscopy was first described in the 1950s. Initial studies described the association of the extent of calcification as seen on fluoroscopy cine with pathology specimens and subsequently linked quantification with clinically manifested ischemic heart disease.¹⁵ There was a high correlation between coronary calcification and incidence of myocardial infarction and symptoms of angina. Autopsy studies also noted that calcifications on fluoroscopy were strongly associated with clinically significant atherosclerotic lesions at death.¹⁶ In the late 1970s, a large cohort study demonstrated that calcification observed on fluoroscopy was associated with hemodynamically significant coronary stenoses detected on coronary angiography.¹⁷ This study by Margolis et al of 800 patients referred for cardiac catheterization demonstrated that 250 patients had coronary calcification and 236 (94%) of these had greater than 75% stenosis in one or more major epicardial coronary

vessels. The participants were followed longitudinally and increased calcification independently predicted worse outcomes as compared to individuals without calcification both in those with coronary stenoses as well as those without.

With advancements in imaging technology, improved quantification techniques were developed and employed. In the 1980s, the advent of ultrafast computed tomography (also called electron beam computed tomography, EBCT) allowed for the assessment of coronary artery calcification which was found to be much more sensitive than traditional fluoroscopy.¹⁸ Electron beam CT technology allowed for an extremely rapid scan of the heart which was necessary to overcome the motion of a beating heart. In an attempt to better quantify coronary calcium, Agatston et al. developed a quantitative scoring system for CAC which employed both the area of the calcium and the density of the calcium as calculated from the Hounsfield units of the lesion.¹⁹ This scoring system, though somewhat arbitrary with its initial design, became widely accepted and utilized in further studies. In order to decrease the interference from noise, thresholds of greater than 1 mm³ in volume and greater intensity than 130 Hounsfield units were set. A density score was assigned to each qualifying calcification based upon the maximum Hounsfield unit within the lesion: 1 for 130 to 199 HU, 2 for 200 to 299, 3 for 300 to 399 and 4 for greater than 400. The lesion score was calculated by the area of the lesion times the density. A total score is calculated as the sum of all the lesions in each of the 4 major vessel distributions. Other scoring systems including calcium mass and calcium volume have subsequently been developed and assessed though the Agatston score remains the most widely known and reported.²⁰

In a similar fashion to the autopsy studies and fluoroscopy, histopathology studies further confirmed that the calcium burden as measured by EBCT highly correlated with coronary plaque quantified at autopsy.²¹ Furthermore, calcified plaque was found to only be a small portion of the overall plaque burden (ie. lesions were at various stages including early atheroma, fibro-atheroma and advanced calcified atheroma) and importantly, absence of calcium did not completely exclude the presence of atherosclerotic coronary disease. Nonetheless, this research demonstrated that coronary artery calcium was quantifiable by EBCT and reflected the burden of coronary atherosclerosis.

A more recent development in CAC research was the validation of using multi-detector computed tomography (MDCT or simply CT) to quantify coronary artery calcification. Much more widely available and less expensive than EBCT, the study demonstrated that MDCT and EBCT produced similar scores.²² For these reasons, use of CT has now become the standard for clinical and research assessment of coronary artery calcium. Additional benefits of MDCT over EBCT include improved image quality and better spatial resolution.²³

Over the last two decades and with the transition to the more widely available MDCT, clinical research with coronary artery calcium has grown dramatically. The question of coronary artery calcium and its association with obstructive coronary artery disease was investigated in the ACCURACY trial. In this trial using 64 slice MDCT in a referred, symptomatic population, the sensitivity of CAC for obstructive disease (>50% narrowing) was very high (CAC score > 0 captured 98% of obstructive disease) but its

specificity is only moderate (42% of those with CAC score > 0 have obstructive disease but specificity improves to 88% for CAC score > 400).²⁴ Importantly, the investigators determined a negative predictive value of 98.6% with a score of zero for predicting absence of significant stenoses. Thus, with a high degree of certainty, a score of zero indicated an extremely low likelihood of obstructive coronary disease.

The role of ethnicity and gender in the prevalence and severity of CAC is important as well. In the Multi-Ethnic Study of Atherosclerosis (MESA) study comprised of individuals without a history of cardiovascular disease, the prevalence of CAC was higher in self-identified whites as compared to black, Asian-American or Hispanic individuals, a finding in both men ($p < 0.001$) and women ($p < 0.0001$).²⁵ These differences remained significant even after adjusting for traditional cardiovascular risk factors and socioeconomic co-variates. In a similar analysis of the MESA cohort, men had increased prevalence of CAC compared to women and in individuals with pre-existing calcium, men had greater calcium amounts as compared to women.²⁶

Additional research has been focused on the prognosis of CAC and future cardiac events. In asymptomatic individuals, CAC score has been demonstrated to have incremental and independent prediction for hard events of CAD mortality or myocardial infarction. A pooled analysis of over 27,000 asymptomatic individuals demonstrated that CAC scores equal to zero resulted in an 3-5 year risk of 0.4% as compared to 7.1% for scores greater than 1000.²⁷ CAC also is predictive of all cause mortality. In a large registry of over 25,000 participants followed for a mean of 6.8 years, CAC score provided incremental and independent risk prediction of all cause mortality beyond

traditional cardiovascular risk factors.²⁸ Ten year survival, after adjustment for age and risk factors, was estimated to be 99.4% for a CAC of 0 whereas survival was reduced to 87.8% with CAC of > 1000. CAC has also been demonstrated to be a useful addition to well established risk prediction calculations. In the MESA cohort of 5878 participants, the addition of CAC to traditional CV risk factors significantly improved the prediction of risk (area under ROC curve of 0.81 vs. 0.76 $p < 0.001$) and resulted in reclassification of risk in 26% of the sample.²⁹

Assessment of CAC has also been included in the recommendations for clinical practice. In the 2010 ACCF/AHA practice guidelines considered measurement of CAC reasonable to better risk stratify asymptomatic individuals at low to intermediate risk (6-10% within 10 years) as well as individuals with an intermediate risk (10-20% within 10 years) of cardiovascular events.³⁰ However, the guidelines recommend against CAC assessment in individuals with a low (<6%) ten year risk of events.

The longitudinal change of coronary artery calcification has recently become of interest both clinically and in research. Ideally, physicians would like to be able to determine in a non-invasive manner which patients have worsening atherosclerosis or which patients are responding to therapies aimed at atherosclerosis. A study of 495 asymptomatic participants without events indicated that mean increase in CAC per year was 17% when followed for 3.2 years.³¹ In individuals who subsequently experienced a myocardial infarction, mean progression prior to the event was 42% ($p = 0.0001$). Progression of CAC of greater than 15% conferred a relative risk of having an MI of 17.2 as compared to non-progressors. A study by Min et al followed 422 individuals without

CAC at baseline with annual CAC scans and found an incidence of 25.1% converting to CAC > 0 over the mean 4.1 years of followup³² Risk factors for conversion included age, hypertension, diabetes, and smoking ($p < 0.01$). In MESA, Kronmal et al determined an annual incidence of converting from 0 CAC to greater than 0 CAC of 6.6%.³³ Median annual change of Agatston score for those with pre-existing calcium was 14 and 21 for women and men, respectively. Interestingly, analysis of the risk factor contribution to development of CAC suggest a complex relationship with some risk factors (eg. LDL, HDL) only associated with incidence of CAC and others (eg. age, hypertension, diabetes and family history) associated with both incidence and progression of pre-existing CAC.

Progression of CAC and its ability for risk prediction has also been investigated. In a study of 4,609 asymptomatic individuals who underwent repeat CAC assessment on average 3.1 years apart, progression of CAC predicted all cause mortality above and beyond the baseline CAC score as well as traditional cardiovascular risk factors.³⁴ Budoff et al also analyzed CAC progression in 6,778 individuals in MESA and demonstrated that an increase in CAC was associated with an increase in future coronary heart disease events.³⁵ Individuals with the greatest increase in CAC (annual of progression of greater than 299) had an adjusted hazard ratio of 3.8 (95% CI of 1.5 to 9.6) compared to those without progression.

Coronary artery calcium and Cancer

As detailed previously, studies have demonstrated an increase in ischemic cardiovascular events in individuals with a history of cancer and therapy as compared to the general population. However, research investigating the association of subclinical

coronary atherosclerosis with cancer patients prior to therapy is relatively sparse. A small study in women diagnosed with breast cancer suggested that CAC was higher in women aged 55 to 64 years as compared to a control population age matched from MESA ($p<0.01$).³⁶ In another study that investigated atherosclerosis prior to cancer treatment, colo-rectal adenoma and coronary heart disease was associated in a cross-sectional fashion.³⁷ In this study of 488 Korean men undergoing screening colonoscopy and CT coronary angiography on the same day, the presence colorectal adenoma was significantly associated with the presence of obstructive and non-obstructive atherosclerosis of the coronary arteries ($p=0.001$). Even after adjusting for age, smoking and other cardiovascular risk factors, the relationship between obstructive ($> 50\%$ stenosis) disease and presence of adenoma was still present ($p=0.013$).

Research into the association of subclinical atherosclerosis with cancer patients post-therapy has also been performed. One series of 9 Hodgkin lymphoma patients, median age of 45 years, treated with mediastinal radiation an average 26 years earlier, reported that 6/9 were in the 90% for CAC score of published reference values.³⁸ Another series of 47 Hodgkin lymphoma patients treated with radiation found abnormally high CAC scores as compared to published values for CAC.³⁹ The CAC prevalence of 83% in this group was much higher than a previously published prevalence of 54% in an older referred cohort. Based upon their findings, the authors advocated for increased screening in Hodgkin survivors.

However, these positive findings were not seen in a recent study by Tjessem et al. This study investigated a cohort of 236 breast cancer survivors who had undergone

radiation +/- chemotherapy and compared their CAC with published distribution of a large, asymptomatic cohort.⁴⁰ They found that the age adjusted distribution was no different between the breast cancer survivors and the published control group (p=0.47).

Longitudinal assessment of coronary artery calcification in cancer patients has not been previously reported. The benefit of longitudinal assessment of CAC in cancer survivors is that if a relationship is discovered, it provides a stronger case for causality as compared to cross-sectional analysis. The Multi-Ethnic Study of Atherosclerosis (MESA) provides this ability to assess CAC in a longitudinal manner. The large cohort study of over 6000 participants enrolled individuals without known cardiovascular disease and followed the participants with repeat examinations, serial testing, and adjudication of cardiac events.⁴¹ Specifically, serial assessment of coronary artery calcium by CT and EBCT and validated cardiovascular outcome measurements are a strength of this study. Preliminary work with this dataset has demonstrated feasibility to investigate cancer and its effect on longitudinal change of coronary artery calcification.

Specific Aims

Accordingly, we plan to use the MESA study to achieve the following specific aims:

Specific Aim 1. To determine the relationship between cancer and its therapies with a subclinical measure of atherosclerosis, coronary artery calcium.

Hypothesis: Cancer patients will have accelerated calcification as compared to individuals without cancer even after adjusting for traditional atherosclerotic risk factors.

This research question is important for several reasons. First, it adds to the basic science knowledge regarding cancer and ischemic events and may provide insight whether atherosclerotic pathways are responsible for the increase in cardiovascular events in cancer patients. Second, coronary artery calcification has been shown to have profound cardiovascular morbidity and mortality and this research lays the groundwork to use CAC specifically in cancer patients to assess cardiovascular risk and identify those that may be particularly susceptible to cardiovascular events due to their cancer diagnosis and/or treatment. Finally, this research is a pilot investigation that has the potential to be used in clinically relevant decision making. For example, if a particular cancer type or therapy is found to be particularly harmful in terms of atherogenesis, the physician may want to choose alternative therapies or treat with preventative, risk lowering therapies. It is not expected that this research will accomplish all of these goals but as a pilot investigation, it will lay the groundwork for future research in these areas.

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CHAPTER 2: MANUSCRIPT

Cancer and its association with the development of coronary artery calcification: An assessment from the Multi-Ethnic Study of Atherosclerosis

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Abbreviations

CT	computed tomography
CAC	coronary artery calcium
MESA	Multi-Ethnic Study of Atherosclerosis
LDL	low density lipoprotein
HDL	high density lipoprotein
CI	confidence interval
SIR	standardized incidence ratio

Abstract:

Introduction Although cancer and its corresponding therapies are associated with increased ischemic heart disease, the temporal relationship between cancer and the development of coronary artery calcium (CAC), a marker of subclinical atherosclerosis, is unknown.

Methods Over a ten year period, 85 men (age 63.6 ± 8.3 years) and 50 women (age 62.1 ± 9.8 years) diagnosed with cancer during this period and 2987 cancer-free subjects (age 59.6 ± 9.2 years for men, age 59.7 ± 9.4 years for women) who enrolled in the Multi-Ethnic Study of Atherosclerosis (MESA) underwent serial assessments of CAC. We measured CAC prior to cancer diagnosis and approximately 10 years later. The incidence of new CAC (baseline Agatston score of zero converting to detectable CAC at followup) was modeled with relative risk regression and compared for cancer versus no cancer. Increase in pre-existing CAC was compared in these groups using linear regression of log transformed CAC.

Results: Cancers in women were predominantly breast, lung and uterine (52%) and in men, cancers were prostate and colorectal cancers (78%). The incidence of CAC was independently associated with cancer history (relative risk 1.32 ($p=0.04$) and 1.29 ($p=0.01$) for women and men, respectively). In participants with CAC at baseline, a clear difference of CAC progression was not observed between cancer and non cancer participants ($p=0.6$ for women and $p=0.2$ for men).

Conclusions A diagnosis of cancer is associated with the development of CAC even after accounting for atherosclerotic risk factors. However, in individuals with pre-existing CAC, it is not clear that the presence of cancer accelerates CAC over time.

Keywords: cancer, coronary artery calcium, subclinical atherosclerosis, cardiotoxicity

Introduction

In comparison with the general population, survivors of several cancers including breast cancer, lymphoma and testicular cancer experience an increase in the risk of coronary arterial atherosclerotic-related events including myocardial infarction, coronary artery disease, and angina (1–3). Coronary artery calcium (CAC), quantified by multi-detector computer tomography (CT) and the Agatston score, has become a well established, quantifiable marker of coronary arterial atherosclerotic burden and predictor of cardiovascular events (4). Prior studies have demonstrated in small series of Hodgkin's lymphoma survivors that CAC is elevated (5,6) but these findings were not replicated in breast cancer survivors (7). In addition, no prior studies have investigated the longitudinal change of CAC with a cancer diagnosis as compared to the general population.

Accordingly, we performed this cohort analysis to investigate the relationship of cancer and its treatment to incidence and progression of subclinical atherosclerosis. To achieve this objective, we utilized the Multi-Ethnic Study of Atherosclerosis, a large cohort followed for approximately 10 years with serial assessments of cardiovascular risk factors as well as quantification of subclinical vascular disease (i.e., coronary artery calcification). We hypothesized that a diagnosis of cancer would be associated with increased progression of CAC over time when compared to cancer free participants.

Methods

Study Design and Population

The Multi-Ethnic Study of Atherosclerosis (MESA) is a prospective cohort study designed to study the prevalence, risk factors and progression of subclinical cardiovascular disease. A detailed description of the study design and methods has previously been published (8). MESA enrolled a cohort of 6,814 participants aged 45 to 84 years from four race/ethnic groups in six US communities (Forsyth County, NC; New York, NY; Chicago, IL; Baltimore, MD; St. Paul, MN; and Los Angeles, CA) between 2000 and 2002. All participants were free of clinically diagnosed cardiovascular disease (heart attack, angina, stroke, transient ischemic attack, heart failure, undergone prior cardiac procedure including angioplasty, bypass surgery, valve surgery, pacemaker implantation) and were not undergoing active treatment for cancer. Each site attempted to recruit equal numbers of men and women with prespecified age and race/ethnicity proportion goals. The MESA study was approved by the institutional review boards of each study site, and written informed consent was obtained from all participants.

The MESA study conducted a baseline examination that included CAC scans (henceforth referred to as “baseline”) between 2000 to 2002 on 6,814 participants with 6,421 reporting no prior history of cancer. A repeat CT CAC scan was obtained at the 5th followup exam, conducted between years 2010 to 2012, (henceforth referred to “followup”) and was performed on a random 50% sample of the original MESA cohort (n=3305) who were part of the MESA Air ancillary study investigating air pollution and heart disease (9). Of the 6,421 participants without cancer at the baseline exam, 3,122 had a CT CAC scan performed at the follow up exam. During the nearly ten years between exams, 135 participants developed cancer (see Figure 1).

At both the baseline and the follow up examinations, participant demographics, medical history, medication use, laboratory data, and anthropometric data were collected. Diabetes mellitus was defined as fasting glucose ≥ 126 mg/dl or use of hypoglycemic medications (10). Use of antihypertensive and lipid lowering medications were based on the review of prescription medication containers. Current smoking and former smoking history were combined to define a dichotomous variable of current/former smoking history versus never. Resting blood pressure was measured 3 times in a seated position, and the average of the second and third readings was used. Total and high-density lipoprotein cholesterol were measured from blood samples obtained after a 12 hour fast.

Measurement of CAC Score

Details of the MESA computed tomography scanning and interpretation methods have been reported elsewhere (11). At the baseline examination, scanning centers assessed CAC by either an electron-beam CT scanner (Chicago, Los Angeles, and New York field centers) or a multidetector CT system (Baltimore, Forsyth County, and St. Paul field centers). Certified technologists scanned all participants twice with corrections using phantoms of known physical calcium concentration. A radiologist or cardiologist read all CT scans at a central reading center (Los Angeles Biomedical Research Institute at Harbor–University of California Los Angeles, Torrance, CA), blinded to all clinical and demographic information of the patients. The amount of coronary artery calcium was quantified with the Agatston score method (12) and a total score was determined by summing all individual lesions from the coronary vasculature (left main, left anterior descending, circumflex, and right coronary arteries). The mean score between the two

scans was utilized in the analysis. At the followup examination, CAC was assessed using multidetector CT and each participant was scanned once with corrections using a phantom of known physical calcium concentration.

Ascertainment of Cancer Status

Cancer history was determined in two ways. At the baseline examination, the questionnaire included questions regarding history of cancer and the type of cancer (“breast”, “prostate”, “colon”, “non-melanoma skin”, “blood (leukemia, lymphoma or other)”, or “other”) with the opportunity to free-text “other” types of cancer. Individuals with cancer prior to their MESA enrollment were excluded from analysis in order to ensure a baseline CAC scan prior to cancer and a follow up scan after cancer diagnosis. Non-melanoma skin cancers, owing to their superficial nature, were re-coded as the participant not having a cancer unless other cancers were also indicated. Participants selecting “Don’t Know” in regards to their cancer history were also excluded from the analysis (See Figure 1).

The diagnosis of cancer during the MESA study was determined utilizing data gathered during pre-specified MESA followup procedures after baseline examination. During the study, contact with the participants every 9 to 12 months regarding events including hospitalizations prompted a request for hospitalization records. The hospitalization records contained ICD-9 diagnosis codes pertinent to that hospitalization. ICD 9 codes associated with cancer (140.-209.) were extracted from the records and re-coded into types of cancer (see Table 1). Non-melanoma skin cancers were re-coded not

a cancer owing to their superficial nature. The time of cancer diagnosis was assumed the same as the hospitalization date.

Statistical Analysis

All analyses were stratified by gender due to expected gender differences in cancer type (see Table 1) and gender specific difference in distribution of coronary artery calcium (13). Means and standard deviations were calculated for normally distributed variables; medians and interquartile ranges were reported for raw CAC scores. Counts and percentages were calculated for categorical variables. Differences in continuous variables were compared using Student's t-tests (for normally distributed variables) or the Kruskal-Wallis test (for non-normally distributed variables). Categorical variables were compared using chi-square test or Fisher's exact test in the appropriate situations.

Longitudinal progression of coronary artery calcium and its association with intervening cancer diagnosis was performed in a two-part model as recommended for CAC due to the highly skewed nature of the variable (14,15). Analysis for the association of cancer diagnosis during follow-up and baseline prevalence and magnitude of pre-existing CAC with adjustments for risk factors was performed in a similar manner. The first analysis modeled the probability of the incidence of CAC as it relates to cancer history. Incidence was defined as progressing from no CAC (Agatston score= 0) to detectable CAC at followup (Agatston score > 0). Due to the magnitude of CAC incidence, the rare disease criterion allowing for the assumption that the relative risk is closely approximated by odds ratios was not met and thus, relative risk regression with log link with Gaussian error (the preferred binomial error failed to converge) was

implemented (14). Robust standard errors were used to ensure valid statistic inferences. Multivariable regressions were modeled for CAC incidence utilizing known risk factors associated with subclinical atherosclerosis. Model 1 included the continuous variable of age and the categorical co-variate of race/ethnicity (Caucasian, Black/African American, Hispanic, Chinese American) and cancer history. Model 2 (based upon the risk factors in 2013 ACC/AHA Guidelines (15)) included age and race/ethnicity as well the continuous covariates of total cholesterol, high density lipoprotein, and systolic blood pressure. Categorical variables in Model 2 included use of anti-hypertension medications, use of lipid lowering medication, current or former smoking status, history of diabetes and cancer history.

In those with prevalent CAC at baseline (defined as an baseline Agatston score > 0), the linear association between cancer history and the change in coronary artery calcium was analyzed using a technique previously described (16). The dependent variable in the linear regression model is $\text{Log}(\text{CAC}_{\text{Followup}} + 25) - \text{Log}(\text{CAC}_{\text{Baseline}} + 25)$ where log is the natural logarithm. The constant of 25 was chosen based upon prior literature demonstrating its benefit of resulting in improved normality (15) (Thus throughout the study, any reference to log transformed score implies that a constant of 25 was added prior to the natural logarithm calculation). Multivariable regression was also employed using the same co-variate models as described above. All statistical analyses were performed using SAS statistical software version 9.3 (Cary, North Carolina).

Results

Fifty women and 85 men with a cancer diagnosis occurring during the MESA follow up period and 1,583 women and 1,404 men without a cancer history underwent CAC assessment at both baseline and followup. The cancer types between the men and women differed as shown in Table 1. The time between the scans at baseline and followup was not different in participants with and without cancer (Table 2). The mean time from cancer diagnosis to followup CT scan was 4.8 years for women and 4.2 years for men. Baseline demographics and characteristics of the MESA participants with and without cancer, stratified by gender, are also shown in Table 2. At baseline, women in the cancer subgroup were less likely to be Hispanic, more likely to have received antihypertensives, and had higher mean systolic blood pressure as compared to women without cancer. The prevalence of CAC at baseline was higher in women in the cancer group. After adjusting for baseline covariates shown in Table 2, the difference in CAC prevalence remained significant ($p=0.03$). The men with cancer were older, with a trend towards more participants receiving anti-hypertensive medications as compared to the men without cancer group. The unadjusted prevalence of CAC at baseline was also higher in men in the cancer group but not significant with adjustment for baseline covariates ($p=0.4$).

In the analysis of incidence of CAC, there were 27 women without CAC at baseline in the cancer group and 16 (59%) developed detectable CAC at followup. In the cancer free group, 1,084 women had CAC=0 at baseline and from these, 456 (42%) developed CAC at follow up. In the unadjusted model, the relative risk of progressing

from no CAC to detectable CAC was 1.41 ($p=0.03$) in those with cancer as compared to those without (see Figure 2). Table 3 demonstrates that even after adjustments for risk factors, there was a 32% ($p=0.04$) increased risk of transitioning from a zero CAC score to detectable CAC if a woman had been diagnosed with cancer between the two scans.

Of the 23 men without CAC at baseline in the cancer group, 18 (78%) went on to develop a non-zero score at follow up. In the cancer free group, 630 men had CAC=0 at baseline. Of these, 323 (51%) developed CAC at followup (see Figure 2). In men without prevalent CAC, the cancer group was older ($p=0.004$), had increased receipt of antihypertensives ($p=0.001$) and were current or past smokers ($p=0.01$). The unadjusted relative risk of progressing from no CAC to detectable CAC was 1.52 ($p=0.003$) in those men with cancer as compared to those without (Table 3). Even after adjusting for risk factors, there was a 29% ($p=0.01$) increased risk of transitioning from a zero CAC score to detectable in men with cancer as compared to non-cancer participants.

Twenty three women in the cancer group and 499 without cancer had detectable coronary artery calcium at the baseline examination. In women, both the baseline CAC score (log transformed) and the followup CAC score were higher in the cancer compared the cancer free participants with cancer group (Table 4). However, after adjusting for risk factors, the baseline difference in CAC score was no longer significant ($p=0.07$). After adjustment for risk factors, the difference in the log transformed CAC scores between followup and baseline (ie. the progression of pre-existing CAC) was not statistically different between the two groups ($p=0.5$). This finding is also reflected in the lack of significance in the beta coefficient associated with cancer in the unadjusted linear

regression model results ($\beta=-0.10$, 95% confidence interval (CI) of -0.40 to 0.18).

Furthermore, with risk factor adjustments in Models 1 and 2, the relationship between CAC change and cancer was not significant (β for Model 1=-0.05, 95% CI of -0.34 to 0.24; β for Model 2=-0.09, 95% CI of -0.37 to 0.20) as the confidence interval contains 0 in all analyses.

In men with pre-existing CAC, there were 62 in the cancer group and 774 without cancer. The men in the cancer group were more likely to be using anti-hypertensive medications ($p=0.05$) and a lower HDL ($p=0.02$). There was no difference in the baseline transformed CAC score, the followup transformed CAC score or the subtracted difference (ie. the progression of CAC) of transformed values between the men cancer and non cancer groups (Table 4). Similarly, this is also reflected in the lack of significance in the beta coefficient associated with cancer in the unadjusted linear regression model ($\beta = 0.08$, 95% CI of -0.25 to 0.08). After risk factor adjustments in Models 1 and 2, the relationship between CAC progression and cancer as represented by the beta coefficient associated with cancer status was not significant (β for Model 1 = 0.13, 95% CI of -0.04 to 0.29; β for Model 2 = 0.11, 95% CI of -0.05 to 0.27).

Discussion

There were three important findings in this study. First, the baseline prevalence of coronary artery calcium was higher in women in the cancer group as compared to those without cancer, a finding that persisted even after adjusting for baseline risk factor differences. Second, a diagnosis of cancer and its treatment was associated with the increased incidence of developing coronary artery calcification in men and women even

after accounting for atherosclerotic risk factors. Finally, a significant association was not observed between a cancer diagnosis and progression of pre-existing CAC in men or women. These findings suggest that cancer, its therapies or a common antecedent risk factor may contribute to the development of subclinical atherosclerosis.

To the best of our knowledge, this is the first longitudinal study investigating the relationship of incident cancer with progression of coronary artery calcium. The study was performed in an ethnically diverse patient population with a large comparator group who underwent concurrent examinations and CT scans. In addition, the cancer diagnoses (Table 1) and assumed treatments were contemporary, using data from 2000 to 2012.

In this study, both women and men who were diagnosed with cancer during the study had a higher baseline prevalence of coronary artery calcium as compared to individuals who did not. This increased prevalence maybe in part explained by the difference in baseline risk factors such as age and race/ethnicity (17). In women in the cancer group, there was a trend towards increased age, fewer Hispanics, increased use of anti-hypertensive medication and a higher mean systolic blood pressure. However, upon adjusting for these differences, the prevalence of CAC remained significantly higher in the cancer group suggesting an unrecognized subclinical vascular disease difference between the two groups even before the diagnosis of cancer. This raises the possibility of a common antecedent process between the development of cancer and the development of cancer. In men with cancer, increased average age, a strong risk factor for CAC, was at least in part responsible for this baseline difference in CAC prevalence because after

accounting for these difference, the prevalence of CAC in the two groups was no longer present .

Despite the baseline differences in prevalence, an increased incidence of developing CAC over the next decade was found in both men and women diagnosed with cancer as compared to individuals without cancer (Figure 2). Previous research has demonstrated that age, race/ethnicity, smoking, diabetes, and hypertension are known risk factors for increased incidence of CAC (15). Even after accounting for these risk factors in the two models, a history of cancer (diagnosed prior to follow up CT by a mean of 4.8 years for women and 4.2 years for men) was associated with an increased incidence of CAC (see Table 3).

For those with CAC at baseline, we assessed whether the progression of pre-existing CAC was associated with cancer diagnosis. Though both the initial and followup CAC scores in women with cancer were higher than individuals without cancer, the unadjusted change between the two groups was not different. With adjustments of known cardiovascular risk factors , the change in CAC remained similar in those with and those without incident cancer. In men with prevalent CAC, there was no difference in the baseline CAC scores between the groups nor was there a difference in the change in CAC between examinations. With accounting for the CV risk factors, cancer was not found to be a factor in the progression of pre-existing CAC.

In individuals with pre-existing CAC, we did not demonstrate a significant increase in CAC in men or women with cancer. There are several potential explanations for this complex relationship. First, other typical risk factors for atherosclerosis have also

shown an increase in the incidence of CAC over time but not progression of pre-existing CAC. Low density lipoprotein (LDL) and high density lipoprotein (HDL) have a similar relationship to the one found here for cancer: they both are associated with the incidence of CAC but not the progression of pre-existing CAC (15). Another possible explanation for the significant association of cancer and CAC incidence with no significant increase in pre-existing CAC progression is that the statistical approach utilized may affect the outcomes. Recent publications have demonstrated that varying statistical definitions of CAC progression can lead to divergent associations with risk modifiers (18,19). Similarly, the relatively low number of participants with cancer limited the power of this analysis to detect a difference.

Previous research investigating coronary artery calcium and its association with cancer and/or its therapies has been focused on cross-sectional data and obtained differing results. Two cross-sectional studies have associated cancer, prior to therapy, with increased CAC scores. In a cohort of breast cancer patients prior to chemotherapy or radiation, Mast et al demonstrated increased CAC in middle aged women (55 to 64 years) as compared to MESA age-matched controls (20). In men, coronary stenoses as assessed by CT angiogram was associated with colorectal adenoma, a pre-cursor to cancer, in a Korean cohort study adjusting for cardiovascular risk factors (21). More advanced polyps were associated with more severe coronary obstruction.

In addition, several studies have investigated the potential impact of cancer and its therapies on CAC. One series of 9 Hodgkin's lymphoma patients, median age of 45 years, treated with mediastinal radiation an average 26 years earlier, reported that 6/9

were in the 90% for CAC score of published reference values (5). A similar series of 47 Hodgkin's lymphoma patients treated with radiation found abnormally high CAC scores as compared to published values for CAC(6). However, these positive findings were not seen in a recent study by Tjessem et al. This study investigated a cohort of 236 breast cancer survivors who had undergone radiation +/- chemotherapy and compared their CAC with published general population CAC scores showing a similar age-matched distribution (7).

We demonstrated an increase in the incidence of CAC over time in individuals diagnosed with cancer compared with non-cancer controls. Our study differs from prior studies in several ways. First, the present study included a wide range of cancers though predominated in women by breast, lung and uterine (52% of the women) and in men, prostate and colo-rectal (78%). Additionally, our comparison group was selected from the same study as opposed to using published previously published CAC values or historical controls. Finally, our study was longitudinal and was able to evaluate incident CAC.

There are many potential mechanisms for the association between cancer and progression of coronary arterial calcification. Prior studies have shown that certain chemotherapies for cancer are associated with an increase in atherosclerotic risk factors including hypertension (22) and metabolic syndrome (23). Several chemotherapeutic regimens have been suggested to cause acute ischemic events. Anti-metabolites 5-fluorouracil and its prodrug capecitabine have been associated with acute cardiac ischemic events with an incidence ranging from 3-9% with capecitabine and 1-68% with fluorouracil (24). A broad range of mechanisms have been proposed including

vasospasm, arteritis, thrombosis, direct myocardial and endothelial damage, and myocarditis (25). Newer small molecule tyrosine kinase inhibitors have also been implicated with acute ischemia. Cardiac ischemia or infarct occurred in 3% of individuals treated with sorafenib compared to <1% of placebo in a trial treating renal cell carcinoma (26). Radiation therapy has also been associated with ischemic cardiovascular events (1–3) in cancer survivors with hypothesized mechanisms of endothelial injury, arteriosclerosis and fibrosis of the coronary vasculature, and prothrombotic state (27). Finally, the progression of the atherosclerosis may be independent of the therapies to treat cancer and may be due to the intertwined processes of chronic inflammation and cancer (28) as inflammation has been associated with the development of coronary atherosclerosis (29).

Study Limitations: There are some notable limitations to this study. First, the followup CT CAC score (performed in the ancillary MESA Air study) was performed on approximately 50% of the original MESA population. Those that were not chosen for the MESA Air substudy or those who opted not or were unable to have the scan performed may have been different from those who did participate. Furthermore, the method of diagnosing cancer during the course of the study depended upon using ICD 9 codes which required hospitalization for any cause. Participants diagnosed with cancer who were never hospitalized would be incorrectly classified. Finally and importantly, this study does not have details regarding the patients' cancer diagnosis including the specific histological subtype of cancer or its staging. The cancer treatment is unknown including whether chemotherapy was given, which chemotherapy and whether surgery and radiation were also part of the treatment plan. Due to this limitation, we are unable to

determine associations with specific treatments or cancers and thus, determination of cause is speculative. However, this study, as an initial investigation, raises some important questions about cancer and its relationship to progression of subclinical atherosclerosis.

In conclusion, this cohort analysis demonstrated that a diagnosis of cancer is associated with the development of subclinical atherosclerosis as determined by coronary artery calcification. This relationship is complex as demonstrated by the association of increased prevalence of CAC in women prior to a cancer diagnosis. Further research is needed to delineate the details of this relationship and determine which cancers and/or therapies or shared cancer-atherosclerosis risk factors/pathways are responsible for the onset of subclinical atherosclerosis in these patients.

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Figure 1:

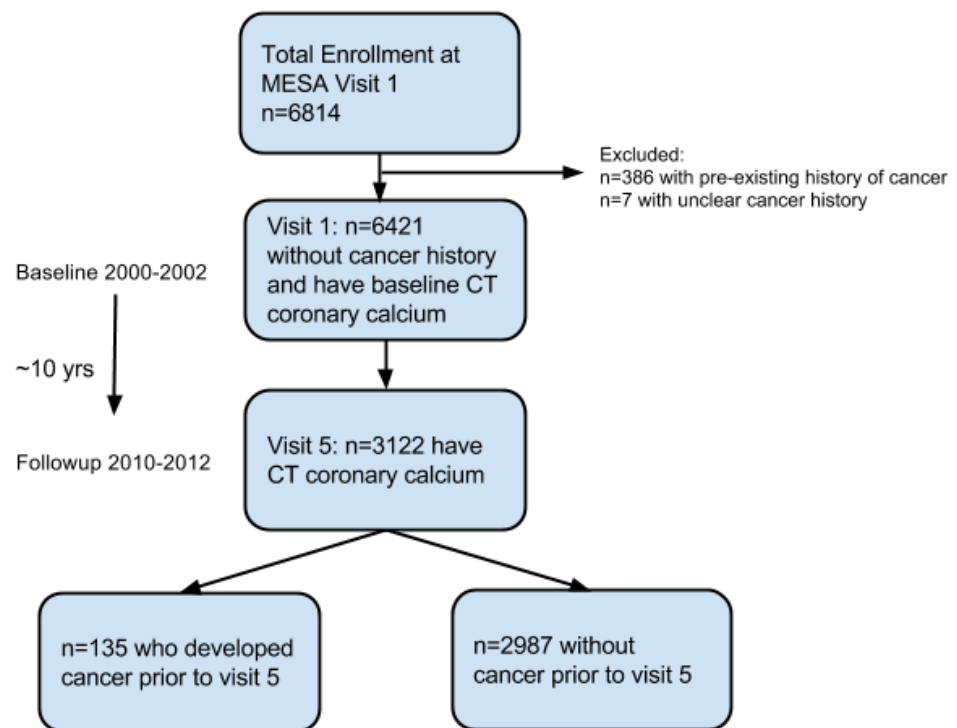


Figure 2:

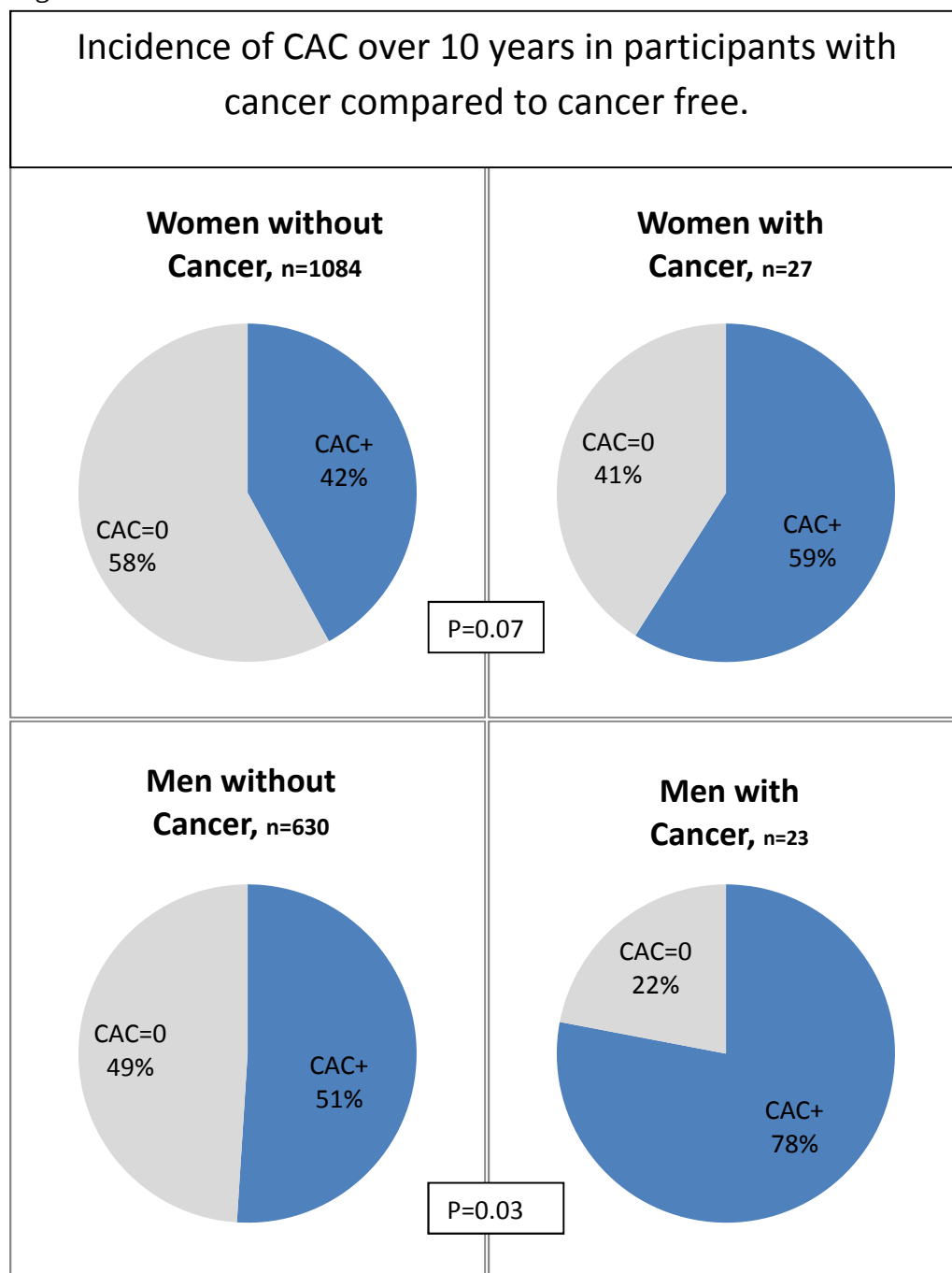


Figure Legend

Figure 1: Flow diagram of MESA cohort who underwent serial coronary artery calcification assessments divided into cancer and no cancer subgroups

Figure 2: Unadjusted incidence (defined as undetectable CAC at baseline transitioning to presence of CAC at followup, indicated by CAC+) of coronary artery calcification over 10 years, grouped by cancer history, stratified by gender. Both women and men with cancer experience higher incidence of CAC as compared to participants without a cancer history.

Table 1: Cancer type stratified by gender

Cancer in Women	Number in group , (% of group)	Cancer in Men	Number in group (% of group)
1. Breast	13 (26%)	1. Prostate	55 (65%)
2. Lung	8 (16%)	2. Colon/Rectal	11 (13%)
3. Uterine	5 (10%)	3. Kidney	3 (4%)
4. Colon/Rectal	4 (8%)	4. Lymphoma	2 (2%)
5. Lymphoma	2 (4%)	5. Leukemia	2 (2%)
All Others	18 (36%)	All Others	12 (14%)
Total cancer participants	50	Total cancer participants	85

Table 2: Baseline characteristics according to future cancer status and stratified by gender

Characteristic	No Cancer Women , n=1583	Cancer Women, n=50	P Value	No Cancer Men, n=1404	Cancer Men, n=85,	P Value
Race/Ethnicity: White	579 (36%)	23 (46%)	0.2	572 (41%)	40 (47%)	0.3
Chinese	185 (12%)	3 (6%)	0.2	182 (13%)	6 (7%)	0.1
Black	452 (29%)	20 (40%)	0.08	329 (23%)	26 (31%)	0.1
Hispanic	367 (23%)	4 (8%)	0.009	321 (23%)	13 (15%)	0.1
Age, years	59.7 (9.4)	62.1 (9.8)	0.08	59.6 (9.2)	63.6 (8.3)	<0.0001
Current/Former Smoker	639 (40%)	22 (44%)	0.6	789 (56%)	53 (62%)	0.3
On antihypertensives	884 (56%)	26 (72%)	0.02	750 (53%)	54 (64%)	0.07
Diabetes	144 (9%)	6 (12%)	0.5	154 (11%)	12 (14%)	0.4
On lipid meds	225 (14%)	9 (18%)	0.5	241 (17%)	14 (16%)	0.9
Total cholesterol ,mg/dl	200.3 (34.7)	201.5 (44.9)	0.8	188.9 (33.3)	186.8 (40.1)	0.6
HDL ,mg/dl,	56.5 (15.3)	55.4 (16.4)	0.6	44.6 (11.3)	43.3 (9.7)	0.3
Systolic blood pressure,mmHg	124.1 (21.7)	131.2 (23.7)	0.02	123.9 (17.9)	127.2 (19.3)	0.1
Time from CAC Baseline to CAC Followup, years	9.7 (0.6)	9.7 (0.6)	0.7	9.6 (0.6)	9.5 (0.5)	0.1
Time From Cancer diagnosis to CT Scan, years	.	4.8 (3.1)		.	4.2 (2.7)	
Prevalent CAC at baseline	499 (32%)	23 (46%)	0.03	774 (55%)	62 (73%)	0.001
Baseline CAC Scores median, (IQR)	0 (0, 9.6)	0 (0, 109.1)	.	5.3 (0.0, 107.8)	48.7 (0, 168.8)	.

Values are mean ± standard deviation or n (%).

Abbreviations: CAC: coronary artery calcium, HDL: high density lipoprotein, IQR: interquartile range

Table 3: Unadjusted and adjusted relative risk of incidence of coronary artery calcification (i.e. score = 0 at baseline visit progressing to detectable at followup) in participants with cancer (n=27 women, n=23 men) as compared to those without cancer (n=1084 women, n=630 men), stratified by gender.

Relative Risk	Unadjusted Model (95% Confidence Interval)	Model 1, (95% Confidence Interval)	Model 2, (95% Confidence Interval)
Women	1.41 (1.02 to 1.94)	1.42 (1.09 to 1.87)	1.32 (1.02 to 1.71)
Men	1.53 (1.21 to 1.92)	1.32 (1.08 to 1.61)	1.29 (1.05 to 1.59)

Model 1: age and race/ethnicity. Model 2: Model 1 and lipid medication, total cholesterol, high density lipoprotein, on hypertension medication, systolic blood pressure, current or former smoker, and history of diabetes

Table 4: Progression of Coronary Artery Calcium (log transformed) in those with prevalent CAC at baseline (CAC score > 0) according to cancer history and stratified by gender, unadjusted.

Characteristics	No Cancer Women, n=499	Cancer Women, n=23	P Value	No Cancer Men, n=774	Cancer Men, n=62	P Value
Baseline CAC	46.9 (12.3, 152.3)	111.2 (30.8, 409.1)	-	87.0 (20.8, 295.1)	87.6 (27.8, 310.2)	-
Baseline Log (CAC)	4.5 (1.0)	5.1 (1.2)	0.007	4.9 (1.2)	4.9 (1.1)	0.8
Followup CAC	247.9 (102.3, 598.6)	456.1 (172, 982.7)	-	390.7 (147.7, 902.8)	505.9 (190.7, 994.0)	-
Followup Log(CAC)	5.6 (1.1)	6.1 (1.1)	0.04	6.0 (1.1)	6.1 (1.1)	0.4
Difference in Log Transformed Scores	1.1 (0.7)	1.0 (0.7)	0.5	1.2 (0.6)	1.2 (0.6)	0.3

Abbreviations: CAC: Coronary artery calcium score, Log: natural logarithm
 Values are mean ± SD or median (interquartile range)
 Log (CAC) represents Log (CAC score + 25)
 Difference in Log Transformed Scores is Log (Followup CAC score + 25) - Log(Baseline CAC score + 25)

CHAPTER 3: REVIEW OF PRIOR RESEARCH APPROACHES AND APPLICATION OF LESSONS LEARNED TO A PROPOSAL FOR FUTURE STUDY

The cohort study presented in the Chapter 2 manuscript demonstrated that cancer and/or its treatments are associated with the development of coronary artery calcification. This pilot investigation, however, does not allow for further characterization of this relationship and is unable to answer questions about potential mechanisms and specific risk factors associated with cancer and its treatments. Further research is needed to better understand these relationships and to better delineate which cancers and/or therapies are responsible for promoting the progression of subclinical atherosclerosis. Once this is determined, the research can begin to be translated to clinical practice with the ultimate goal of reducing the risk of cardiovascular events in cancer survivors.

This final chapter describes research aims and a proposal designed to better understand the relationship between cancer, its treatments and atherosclerotic cardiovascular disease. However, to better design future research studies, a brief review of approaches previously investigated will be useful as it will demonstrate some of the hurdles and limitations encountered and attempts to overcome these. This review of four previously investigated approaches aids in directing and designing future prospective investigations as the pitfalls and limitations of prior attempts can be ameliorated and designed out of the study.

Approach 1: De-identified local Wake Forest Health System data

The Wake Forest Translational Data Warehouse (TDW) is a large database that integrates clinical and research data including demographics, vitals, diagnoses, procedures, medications, lab results, and visit details from the medical records of the Wake Forest system from both inpatient and outpatient visits dating back to 1984. The TDW has consolidated this data into a large database that can be utilized for research purposes. Investigators are able to perform preliminary queries of anonymized data for cohort identification without the need for IRB approval..

The benefits of utilizing the TDW to investigate the association of cancer and atherosclerosis included rapid startup to assess feasibility (no need for IRB approval initially for cohort quantification) and that large amounts of data (essentially the participants' entire medical records) were potentially available on the specified cohort. Details regarding the patient's cancer history, treatment, and cardiovascular history were available in an organized format via the TDW that could be easily manipulated into a statistical software package. The main limitation to this approach was the retrospective nature of the data collection. Typically, the data available was ordered for clinical indications (ie. the CAC score was done in conjunction with a CT coronary angiogram which was ordered based upon clinical concerns of chest pain and atherosclerotic disease) and therefore, not all data would be consistently available on all cancer patients. This is an inherent limitation in retrospective chart review type research and that could be addressed with careful selection of the control cancer free population to match the cancer cohort.

However, the final hurdle that prevented further progress with this approach was the small cohort size. Initial queries were performed on this database and revealed that very few cancer survivors within the Wake Forest system (on the order of a couple dozen) underwent formal CAC scoring over the past 20 years. This number would be further reduced once the data was limited to cancer survivors who underwent CAC scanning post cancer diagnosis and treatment. Even more limiting was that analysis would be limited to cross-sectional investigation only because repeat longitudinal CAC scores were not clinically justifiable at the time and thus, exceedingly rare.

Approach 2: Use of pre-existing imaging to calculate CAC

A second, rather novel approach to answering the specific aims of this research involved the calculating coronary artery calcium from non-dedicated studies. In other words, the approach would use non-gated chest computed tomography (ordered for a clinical indication such as cancer staging) to calculate CAC scores (henceforth, called modified CAC scores). Coronary calcium currently is quantified using standardized scan protocol.¹ The biggest difference between a CAC scan and a “normal” CT of the chest is a CAC scan is electrocardiographically gated study (ie. takes the xray picture at a certain time during the cardiac cycle so the heart is effectively stationary) whereas a chest CT is not. The use of non-dedicated CT scans to quantify subclinical atherosclerosis in cancer patients, if feasible, would overcome several of the limiting factors of the retrospective study in Approach #1 and offers the benefit of using pre-existing imaging to answer the research questions, a significant cost savings. A preliminary investigation into the feasibility of this approach was undertaken and the lessons learned are discussed here.

The use of non-dedicated thoracic CTs for calculation of CAC has been demonstrated and validated to a limited extent in the literature. A study by Budoff et al in 2011 investigated the concordance of a dedicated CAC scan (64 slice detector, gated at 75% of cardiac phase, 2.5 mm slice thickness, tube voltage of 120 peak kilovolts and tube current of 430 milliamps) versus a non gated CT scan obtained in a COPD lung research study investigating lung parenchyma in 50 participants.² The settings on the lung scan were helical mode without gating, with similar tube voltage, current and slice thickness. Agatston scoring and CAC volume were both utilized to quantify CAC in the scans and compared separately. The results demonstrated 100% concordance in detecting CAC. Results demonstrated excellent correlation between both the two scans ($r=0.96$ for Agatston protocol, $p<0.0001$). Bland-Altman analysis demonstrated that there was a bias of ungated studies having greater values on average than gated studies (95% confidence interval of the difference 168 to 538) with greater discordance at higher average CAC scores.

A study by Jacobs et al in 2010 investigated the inter-scan variability of CAC derived from ungated thoracic CTs.³ Again, the scans were performed in the setting of a lung cancer research study in which 584 participants underwent two scans approximately 4 months apart. Three techniques of assessing CAC score were utilized including Agatston score, volume score and calcium mass score. Results demonstrated good interscan agreement (Kappa of 0.67) for Agatston score categorization. However, interscan variability was approximately twice as high as the gated cardiac CT CAC derived values when compared with previously published data. This is important because a large interscan variability impedes the ability to make longitudinal comparisons in

individuals and requires significantly larger groups in order to be statistically powerful enough to make comparisons. Nonetheless, previously published research demonstrated that CAC scoring from non-dedicated scans was feasible and reasonably represented true CAC values.

Prior research at Wake Forest has resulted in a medium sized cohort of cancer patients whose medical history and research results have been organized into a structured database. The patients in this database have been diagnosed with breast cancer, lymphoma or leukemia and often required multiple computed tomographic images of the chest before and during their cancer treatments. The indications and types of studies performed on these patients included: 1. cancer staging in the form of helical non-gated thoracic CTs with or without intravenous contrast, 2. cancer staging, monitoring and recurrence surveillance using registration CTs from positron emission tomography scans and 3. radiation planning and simulation using non-contrast chest CT. These scans were obtained at various time points during cancer diagnosis and therapeutic treatments including pre and post chemotherapy. There were several benefits to this research approach. First, no additional imaging was required and pre-existing imaging could be used to glean beneficial cardiovascular information without risks (ie. radiation). Additionally, the imaging was performed in the participants independently of clinical concerns for cardiovascular complications or symptoms which eliminates the selection bias described in approach #1.

A pilot project was initiated to investigate the feasibility of determining modified CAC scores from previously acquired CT scans on a cohort of cancer survivors at Wake

Forest. The goals of the pilot investigation were three-fold: 1. Using the software available at Wake Forest, could modified coronary artery calcium scores be obtained? 2. What type of scans were done and when were they performed in relation to the cancer diagnosis and chemotherapy? and 3. Were there sufficient numbers of patients to proceed with answering the specific aims of our research?

The cohort utilized for this pilot project was a subset of from the cohort of cancer patients at Wake Forest who had previously participated in research utilizing cardiac MR imaging to monitor cardiac function and pathology post treatment for cancer.⁴ The original cohort comprised of 53 patients diagnosed with breast cancer, leukemia or lymphoma who were treated at Wake Forest Baptist Medical Center and received anthracycline based chemotherapy. The participants underwent serial measurements of left ventricular function and scar assessment by cardiac MR and had serum cardiac biomarkers taken at baseline (prior to chemotherapy), 1, 3 and 6 months post chemotherapy. CT scans were available if clinically indicated (most commonly for the 3 reasons discussed earlier) and performed at Wake Forest.

A chart review was performed on the 53 participants to determine if eligible imaging was available for calculation of modified coronary artery calcification at baseline. (Note: the feasibility of longitudinal evaluation was not investigated at this time). Of the 53 participants, 39 had imaging that that was capable of producing a modified CAC score. Of these, 29 had imaging that was within 6 months of starting chemotherapy. Table 1 summarizes the demographics of the participants that met this

criteria. Figure 1 shows 4 examples of varying coronary artery calcification and the calculated modified CAC score.

Table 1: Characteristics of cancer survivors with adequate imaging to calculate a modified CAC score

Characteristic	
Gender: Male	11 (38%)
Female	18 (62%)
Race: White	25 (86%)
Black	4 (14%)
Cancer Type: Breast	14 (48%)
Leukemia	7 (24%)
Lymphoma	8 (28%)
Type of CT Scan: CT chest	12 (41%)
CT for radiation planning	7 (24%)
CT/PET	10 (35%)
Age, years	53.6 (15)
Modified CAC score, median (IQR)	3.1 (0, 42)
Zero CAC score	14 (48%)
Chemotherapy to CAC score time, days	53.4 (74.3)

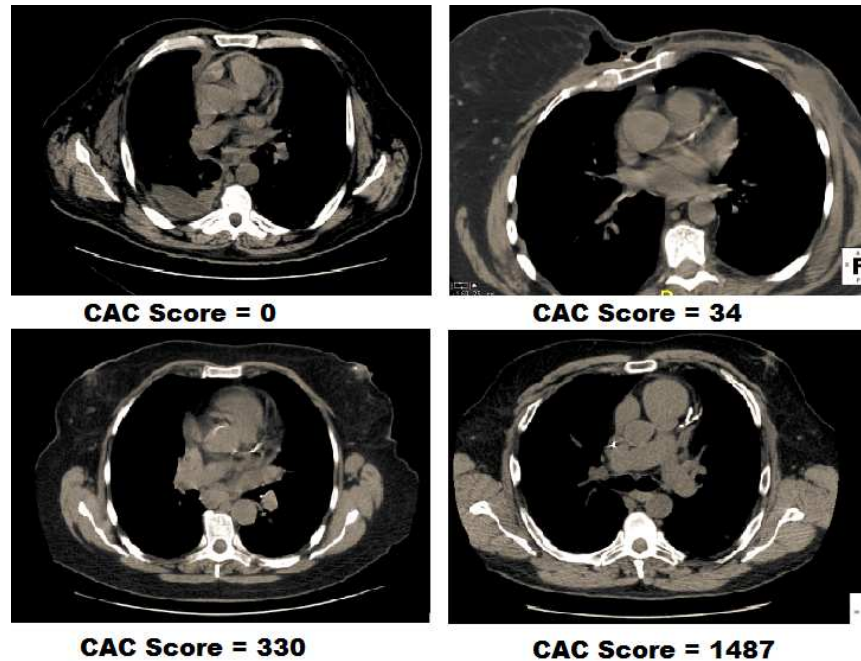
Values are mean $\pm$ standard deviation, n (% of group) or median (interquartile range)

In assessing the feasibility of pursuing this research at Wake Forest, several conclusions were made. First, calculating a modified CAC score from non-dedicated CT scans was feasible and produced results that were grossly similar to previously published CAC values for this population.⁵ However, there were significant limitations to this approach that ultimately proved insurmountable.

First, unlike the previously published research described previously^{2,3}, there were actually three different ungated CT scan protocols (CT chest, CT for radiation planning, and CT/PET) and the modified CAC scores between the 3 different types of scans were not necessary interchangeable. For example, slice thickness varied between the 3

different types of scans which could lead to significantly varying modified CAC scores and preclude the ability to combine and compare scores. Limiting the inclusion criteria to only one type of scan would reduce the group size by two thirds resulting a study that likely was too underpowered to properly test the hypothesis. Furthermore, as shown in published research, the increased variability of the modified CAC score would require greater numbers of participants as compared to using a more reproducible dedicated CAC scan. The second limitation was that investigating the longitudinal change in CAC and its association with cancer would not be possible given the small numbers of patients with consistent repeat CT imaging. In this cohort, repeat imaging was occasionally performed on lymphoma patients to monitor response to therapy and recurrence, but as shown in Table 1, this cohort was only 8 patients. Additionally, there is likely a significant selection bias associated with using individuals with modified CAC appropriate imaging. For example, individuals with more advanced cancer are more likely to undergo additional imaging to monitor for spread. Despite promising initial success, the limitations to this approach proved too great to overcome.

Figure 1: Example modified coronary artery calcium scores (Agatston method) from non-gated thoracic CTs in patients with cancer



Approach 3: Link local clinical data with MESA Data

One of the main limitations of the research presented in the Chapter 2 manuscript is the lack of details regarding the diagnosis of cancer and its therapies in the cancer group. One hypothesized approach to overcome this limitation was to utilize the MESA database and combine it with local clinical data available from Wake Forest. The strength of this approach was that the cardiovascular data from MESA could be supplemented by the clinical cancer data from Wake Forest clinical records. The medical record from Wake Forest would include specific cancer diagnoses, staging information, and histology. Other vital information gathered from the clinical records would include treatment information such as surgery, chemotherapy regimen, quantity of chemotherapy, and

whether adjunctive radiation was utilized. One significant assumption with this approach is that the MESA participant from Forsyth County was receiving their cancer care from a Wake Forest Baptist facility. Ultimately, this approach was abandoned when we determined that only 30 individuals enrolled in MESA from Forsyth County developed cancer in between their two CAC scans. This number would likely be even smaller given that our assumption regarding all Forsyth participants in MESA received their cancer care at Wake Forest was likely false.

In an effort to increase numbers of MESA cancer participants, one could apply for local IRB approval at each of the research institutions associated with MESA (total of 6) and proceed with a chart review of individuals diagnosed with cancer during MESA to obtain additional clinical details of their cancer diagnosis and treatment. However, beyond the significant amount of efforts required to obtain approval and subsequent access to clinical records, the assumption that MESA participants received cancer care at the MESA institution would likely prove even more incorrect (for example, in Los Angeles there are several large hospital systems besides UCLA that a MESA participant may receive cancer care).

Approach 4: Link MESA data with Cancer registries

Since the early 1990s, virtually every state in the United States has a cancer registry that tracks cancer information within their state.⁶ For example, in North Carolina, the North Carolina Center for Health Statistics (NCCHS) maintains the Central Cancer Registry (CCR).⁷ By law, all cases of cancer are to be reported by healthcare providers and/or organizations. Information sources include pathology data, hospital reporting, and

death certificate data. Though each state is responsible for collecting the cancer information, all the states' registries are consolidated via the National Program of Cancer Registries (NCPR) which is administered by the CDC.⁷ This data is then used to monitor cancer incidence, evaluate patterns, promote research and assist with allocating health resources.

Linking cardiovascular databases with cancer registries has been attempted before. The Cardiovascular Health Study (CHS) is a longitudinal cohort study of coronary artery disease and cerebrovascular disease designed to investigate risk factors related to the onset and progression of cardiovascular disease.⁸ Through an ancillary study, investigators linked CHS participant data with cancer registry data from the respective states. In one example study, the association between prostate carcinoma and certain serum proteins associated with diabetes was studied.⁹ The researchers estimated that the data in the cancer registries was 93-100% complete.⁹

Applying the registry data to MESA would have two main foreseeable benefits in the research investigating the relationship of cancer and CAC. First, the use of the cancer registry would provide more robust diagnoses of cancer as compared to the more error prone method employed in MESA that depended upon on hospitalization and ICD 9 coding. Secondly, cancer registries potentially contain limited cancer information though this could vary from state to state depending upon reporting requirements.

We discovered significant limitations to this approach which ultimately deterred further work towards combining MESA with a cancer registry. Though demonstrated to be feasible, significant effort would be required to acquire state IRB approval for each of

6 separate states (NC, MD, CA, NY, IL and MN) with communities studied in MESA. In discussions with investigators who had performed this type of research previously, the process was extremely time consuming. Additionally, the data in the registries from each state varies somewhat according the reporting rules in each state but in general, important data to our research such as chemotherapy regimens and radiation treatments would likely not be available.

Next Steps: Prospective Study Proposal and Design

As clearly demonstrated, investigating the association of cancer, its treatments and subclinical atherosclerosis using databases and pre-existing data is challenging and though important information can be gleaned, the results were limited by several factors. Though these limitations could be ameliorated to certain degree with the different approaches, new problems (eg. reduced numbers, additional bias, significant effort with reduced return, etc.) would be introduced. A prospectively designed cohort study could be performed to overcome these limitations and biases and provide significant insight into the relationship between cancer and its treatments with markers of subclinical atherosclerosis, its progression, and cardiovascular events.

The specific aims of this prospective study would be as follows:

1. To determine the incidence and progression of subclinical coronary atherosclerosis as measured by coronary artery calcium prior to treatment (chemotherapy, radiation, and/or surgery) and after cancer treatment.

- a. To determine the association of cancer and CAC with traditional risk factors for CVD disease (lipids, diabetes, hypertension) as well as serum biomarkers of inflammation and thrombosis.
 - b. To assess the independent and combined contribution of various types of chemotherapy and radiation to development of CAC
2. To determine the value of baseline (pre-treatment CAC) and CAC progression in predicting cardiovascular events in cancer survivors.

An independent, standalone study designed to investigate cancer and subclinical atherosclerosis by coronary artery calcium scoring would be expensive and resource intensive. If feasible, an ancillary study joined to a larger longitudinal study would be ideal. This would enable leveraging the infrastructure, demographic data, laboratory results, and outcomes information from the main study and adding serial CAC measurements from the ancillary study.

A recent proposed study submitted to the National Cancer Institute by a multidisciplinary group of collaborators would be well suited to be the main study. The overall goals of this study are to characterize the onset, progression and risk factors of cardiovascular dysfunction, exercise intolerance and CVD event in women treated for breast cancer. The proposed study is a multi-center cohort study of breast cancer survivors with an enrollment goal of 840 women with cancer and 160 controls without cancer. The participants, enrolled prior to cancer treatment, will undergo baseline evaluation with demographic collection, laboratory testing, cardiovascular imaging, exercise testing. Additionally, they will undergo serial assessment with imaging including

cardiac MR and echocardiography and followed for up to 7 years for cardiovascular events.

The ancillary study investigating CAC and cancer would require a subset of the 840 cancer patients to enroll for additional imaging. The comparison group of cancer free participants would be taken from MESA database. In order to be able to statistically account for treatment characteristics (chemotherapy and radiation), ideally there should be an attempt to selectively enroll based upon anticipated treatment. Though more advanced calculations with the assistance of a statistician are necessary, a simple power calculation using data from the prior manuscript to determine the size of the groups is as follows. To determine a difference in the incidence of CAC over time of 40% in the control and 60% in the cancer (estimates from manuscript), and assuming an α of 0.05 and desiring a power of 0.8, $n=97$ will be required. Assuming a dropout rate of 20%, we will need to enroll 122 individuals with cancer to see a difference.

Follow-up CAC scanning should be performed at 2 to 4 year time point which allows for comparison with the MESA population¹⁰ and accounts for the predicted rates of developing CAC. In the study by Budoff et al., the progression of CAC in individuals with pre-existing CAC had a median increase in Agatston score per year of 28.9 (quartile range of 60.9 to 529.1).¹⁰ In the study by Min et al., they found a conversion rate of no CAC to presence of CAC to be 25.1% over 4.1 years in a non-linear fashion (greatest conversion in year 5).¹¹ Typically, repeat clinically indicated scans are not part of treatment and monitoring of breast cancer survivors unless recurrence is suspected.

Therefore, repeat CAC scanning would be need to be part of the research protocol and scheduled independently.

Anticipated concerns with the study include approval from IRB due to the risk of increased radiation from the CAC scan. From the MESA and CARDIA studies, the radiation exposure from a single CAC scan ranged from 1.1 to 3.6 milliSievert (mSv) depending on the scanner, settings and body habitus.¹ To put this in perspective, the radiation exposure from natural background radiation is approximately 3 to 3.6 mSv per year. A two view chest x-ray is 0.1 mSv, mammography is 0.4 mSv, a chest CT is 7 mSv, and a PET scan for tumor localization is 14.1 mSv.¹² A single CAC scan at the age of 55 years old, assuming 2.3 mSv of exposure, results in an increase lifetime incidence 8/100,000 men and 20/100,000 women.¹³ However, newer protocols have been developed that decreased the exposure to 1.1 mSv and could be employed.¹⁴ This low dose CAC protocol CT could be added to the staging CT in individuals at risk for thoracic spread of the disease (eg. positive margins or nodes at time of surgical resection). The increase in radiation exposure is minimal as compared to the staging CT and same-time scheduling would increase participant compliance.

Conclusion

The study results from this thesis demonstrate that a cancer diagnosis is associated with the longitudinal development of coronary artery calcification. However, important questions remain including the specific risk factors for progression (cancer itself versus chemotherapy versus radiation) and further studies are needed. Learning from the difficulties encountered in developing this study, a prospective study investigating the

progression of subclinical atherosclerosis in breast cancer survivors, would offer insight into the potential causes of the increase in CAC. Overcoming the limitations of the previous study, the association between CAC and various chemotherapy regimens and radiation treatments and traditional risk factors could be better elucidated. The answers to these questions are very important in future management of breast cancer survivors and their increased risk of cardiovascular complications.

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Start-up cardiovascular diagnostics company that utilizes cardiac CT scans as an input to high performance computer algorithms based upon computational fluid dynamics and providing FFR_{CT}, a noninvasive method of improving the diagnostic accuracy of traditional cardiac CT interpretation.

Invasive Cardiologist

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Start-up company focused on designing and developing implantable device capable of detecting epileptic activity and responding with electrical stimulation to suppress progression.

Electronics Design Engineer

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Led team that designed and developed a high speed, ultra low power telemetry system for use in pacemakers and implantable cardiac defibrillators.

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PUBLICATIONS

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Sarah Graham Kenan Scholarship Medical academic scholarship.	UNC School of Medicine August 2003 – May 2005
Dr. Thomas Woodfin Sumner Memorial Scholarship Medical academic scholarship	UNC School of Medicine August 2002 – May 2003
Honors for Year End Grades Top 25% of class	UNC School of Medicine August 2004 – May 2005
Cum Laude Graduation Honors	Duke University May 2000
Dean’s List Honors, 3 semesters	Duke University Fall 1997,1999; Spring 2000

PRESENTATIONS

“Review of T2* and Iron Quantification” Cardiology Imaging Conference. Wake Forest Baptist Medical Center: March 18, 2015.

“Myocardial Fibrosis and Cardiac MRI” Cardiology Imaging Conference. Wake Forest Baptist Medical Center: February 19, 2014.

“Radiation from Cardiology Procedures.” Clinical Management Conference. Vanderbilt Heart Institute: November 30th, 2010.

Matthew Whitlock, MD

mattwhitlockmd@gmail.com

“Amiodarone Toxicity Monitoring: Vanderbilt Heart Institute Experience.” Quality Improvement Conference. Vanderbilt Heart Institute: June 22nd, 2010.

“Stent Thrombosis.” Cardiology Conference. Meharry Medical College: June 3rd, 2010.

“Who Watches the WATCHMAN?: Left Atrial Occluder Device” Clinical Management Conference. Vanderbilt Heart Institute: June 2nd, 2009.

“Heparin versus Lovenox in NSTEMI: TIMI 11b.” Journal Club. Vanderbilt Heart Institute: March 31st, 2009.

“Prescription Medication Abuse: Less Commonly Recognized Drugs.” Senior Resident Presentation. Oregon Health and Science University: January 11th, 2008.

PROFESSIONAL MEMBERSHIPS

Friesenger Society

July 2008 - present

Society of Cardiovascular Magnetic Resonance

July 2014 - present