

**Accelerated Atherosclerosis in High Risk Population Groups: An Assessment by
Magnetic Resonance Imaging**

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Full Title:

Accelerated Atherosclerosis in High Risk Population Groups: An Assessment by Magnetic Resonance Imaging

Short Title:

Accelerated Atherosclerosis Assessment by MRI

Protocol Version History:

Original Protocol: Version 1, dated 02/14/2013

Protocol Amendment 1: Version 2, dated 04/07/2014

Protocol Amendment 2: Version 3, dated 05/01/2015

Précis /Abstract:

The objective of this project is to accurately determine the constituents and characteristics of atherosclerotic plaques in carotid arteries by magnetic resonance imaging (MRI) techniques in cancer survivors; at different data intervals: before and after administration of treatment (medical and life style modification) and then correlate contrast agent dynamics with serum markers of inflammation and other tests of cardiac or vascular dysfunction where available.

The proposed study involves 100 asymptomatic patients who received prior chest or head and neck radiation therapy (HNXRT). MRI data (direct assessment of atherosclerosis) would be correlated with indirect measures of atherosclerosis (blood surrogate markers, metabolomics, Echocardiographic assessment and CT coronary calcium score).

Atherosclerosis is a chronic inflammatory process in the walls of arteries leading to plaque formation. Broadly, this process leads to stenosis of the arterial lumen by formation of atheromatous core (smooth muscle thickening, lipid accumulation such as cholesterol, lipid laden macrophages, calcium etc.) between the intimal layer and media layer of the arterial wall. Over the course of time, necrosis in these fat cells occurs, which leads to extracellular accumulation of lipids. At this stage, plaque is separated from the lumen of the vessel wall by a thin fibrous cap (FC) tissue and endothelial layer. Thin fibrous cap is a weak structure and is vulnerable to rupture resulting in either thrombus formation or a thromboembolic event. Thrombus formation can lead to complete occlusion of an arterial lumen in spite of having mild or moderate stenosis. Besides ruptured plaque constituents might embolize (break away) and occlude a smaller downstream vessel leading to tissue damage. Research studies have demonstrated that necrotic lipid core with a thin fibrous cap presents a significant increased risk for plaque rupture resulting in a thromboembolism (1-6). Several scientific studies and publications have demonstrated that, of all etiologies of non-cancer mortality, cardiovascular disease (CVD) remains the most significant cause of morbidity and mortality in cancer survivors (7-11).

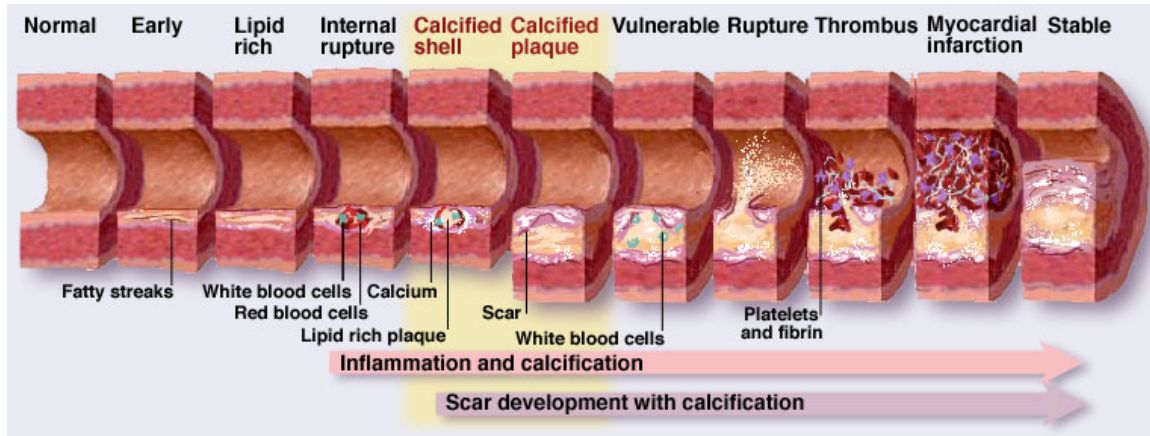
We hypothesize that non-invasive MRI techniques have the potential to accurately characterize the plaque constituents thereby indicating vulnerability of plaque. We intend to utilize 3 point Dixon (3 PD) and ultra short time to echo (UTE) MRI techniques to identify and measure plaque components such as hemorrhage, necrotic core and fibrous cap in carotid arteries. We intend to conduct an initial baseline MRI, blood tests (to correlate with surrogate markers of inflammation) and other tests whenever available of cardiac or vascular dysfunction (echocardiography and computerized tomography studies). This cohort will be followed up with medication and/or life style modification regimen for a period of initially 18 months and subsequently at 36 months. A repeat of all baseline studies (MRI and blood tests) would be performed as part of the 18 and 36-month follow-up.

We believe these studies would help in identifying the vulnerable plaque features in HNXRT patients and would provide us with a novel opportunity to identify and treat the disease early and hence in long run would reduce health related complications and health care costs.

Introduction:

Radiation therapy (XRT) is critical for the treatment of many benign and malignant neoplasms. It is well established that cancer survivors who have undergone XRT exhibit a long-term survival rate that significantly lags behind age and gender-matched population controls (7-11). Several scientific studies and publications have demonstrated that, of all etiologies of non-cancer mortality, cardiovascular disease (CVD) remains the most significant cause of morbidity and mortality in cancer survivors (7-11). Until recently radiation vasculopathy has attracted little research attention, because patients would often succumb to their malignancy before the vascular conditions manifested clinically (8). This is no longer the rule as more patients are now "outliving" their malignancies and are presenting with the long-term sequelae of cancer treatments (9). Sequelae and manifestations of accelerated atherosclerosis have been identified in the vasculature of almost every organ system (12-15). Atherosclerotic plaques that are susceptible to rupture, termed "vulnerable" or "unstable" consist of a large necrotic core made substantially of lipids and cell debris and a thin, collagen-poor fibrous cap (3, 16-18). Another characteristic of unstable plaques is that they contain numerous inflammatory cells. It has been documented that inflamed plaque and those with a large lipid core and thin fibrous cap are more likely to progress and cause clinical events than those without these features (19-21). Inflammation plays a major role in the onset, progression and destabilization of arterial plaque (22-27). Fig 1. Shows a brief overview of pathobiology and progression of arterial atherosclerosis.

Fig 1: Overview of pathobiology and progression of arterial atherosclerosis



Therefore imaging the vasculature for both its inflammatory state and presence of type of disease is very important (28-30). The potential of magnetic resonance imaging (MRI) to characterize arterial plaques has been extensively studied (1, 31-33). MRI due to its ability of tissue characterization, superior resolution, lack of radiation, non invasiveness is an ideal modality for in vivo study of arterial plaques.

There are no studies performed to date that have evaluated MRI arterial plaque characteristics in HNXRT and correlated with serum markers of inflammation, metabolomics, echocardiographic cardiac dysfunction assessment and computerized tomography (CT) coronary calcium scores. Furthermore, no studies have been performed to look into associations between plaque contrast kinetics and morphology using anatomical MRI before and after initiation of medical and-/or life style modification therapy.

Aims

The purpose of this study is to assess carotid artery MRI plaque characteristics in 100 asymptomatic cancer survivors with history of chest, head and neck radiotherapy (HNXRT) and with subclinical atherosclerosis. Another objective is to analyze plaque changes in the same group after initiation of medical treatment for 18 and 36 months.

The following specific aims (A), research questions (RQ) and hypotheses (H) will be addressed.

A 1: To characterize the plaque constituents including fibrous cap (FC), lipid core and hemorrhage within the carotid plaque by MRI

RQ 1a: What are the predominant plaque constituents?

RQ 1b: Is there a predominance of complex and vulnerable plaque features in the neck arterial vasculature of cancer survivors?

H 1: We hypothesize that there is a predominance of vulnerable plaque in carotid arterial vasculature of HNXRT patients due to the phenomenon of radiation vasculopathy.

A 2: Determine the correlation between plaque inflammation (direct measure by MRI) with serum markers of inflammation through blood tests (hsCRP, LpPLA2, IL-6) and metabolomics

and other tests of cardiac or vascular dysfunction (echocardiography and computerized tomography coronary calcium score studies).

RQ 2: Is there any correlation between the direct and surrogate markers of inflammation?

H 2: We hypothesize that there is a predictable correlation between the direct and surrogate measures of inflammation.

A 3: To assess the longitudinal change in the artery plaque characteristics (inflammation and morphology) with treatment.

RQ 3: Is there a predictable relationship between inflammation (pattern of enhancement-contrast kinetics) and vulnerable features within a plaque?

H 3: We hypothesize that changes in plaque characteristics pre and post treatment would correlate with contrast agent dynamics over time.

A 4: To determine the efficacy of comprehensive treatment (life style modification + statin) versus (life style modification + placebo).

RQ 4: Is one regimen better than the other in cancer survivors with subclinical atherosclerosis?

H 4: We hypothesize that comprehensive treatment (life style modification + statin) would have better inflammation control and favorable plaque modification than life style modification alone as measured by parameters in (A2 & A3).

Research Strategy:

Subjects:

We intend to recruit 100 asymptomatic cancer survivors who had prior history of chest, head and neck radiotherapy (HNXRT). Our potential participants are patients of The Winship Cancer Institute who are being invited to return to The Survivorship Clinic as part of an ongoing funded trial at Emory Survivorship clinic (Novel testing for subclinical cardiovascular disease in lymphoma survivors, IRB 00057029).

Inclusion criterion:

Age 22 and above with prior head and neck or chest irradiation, six months or more post head and neck irradiation, documented subclinical cardiovascular disease (inflammatory markers in the serum, pre-existing plaques [detected by ultrasound, CT or MRI], asymptomatic major arterial stenosis, and not being considered for arterial surgery or endovascular treatment.

Exclusion criterion:

Recurrence of cancer (with or without treatment), planned surgical or endovascular intervention for revascularization of carotid arteries at the time of enrollment, renal failure, eGFR < 45 (calculation based on serum creatinine levels, race, age and gender), medically unstable or hematologic, renal, or hepatic dysfunction, non-atherosclerotic arterial stenosis (dissection), presence of stents or external clips that can cause artifacts impairing accurate interpretation of MRI data, contraindications to MRI: cardiac pacemaker, metal implants, metal in eyes, pregnant

or nursing women, claustrophobia, allergy to MRI contrast; physical or mental impairment that would limit the patient's ability to comply with the medical instructions or study procedures.

Setting:

The research will be coordinated through the Emory School of Medicine, Department of Radiology in partnership with The Winship Cancer Institute, Survivorship Clinic, Department of Cardiology and The Stroke Center. All the aspects of clinical and research assessment and follow-up will be provided within The Emory Healthcare System.

Sample Size:

We base the power analysis on aim 2 and 3 as mentioned above. Aim 2 is based on correlation between plaque inflammation (direct measure by MRI) with serum markers of inflammation and other tests of cardiac function. As the data is not available for us at this point, we will assume the "true" correlation is ≥ 0.7 . We conducted the power analysis based on a correlation test using the fisher-Z transformation. We aim for a type-I error of 0.05 and also to achieve a power of 0.99. Based on these set conditions, on power analysis; if we have 64 measurements / subjects, then we will have a 99% power to detect the "true" correlation between measurements given a significant level (0.05).

Our goal or aim 3 is to check the change in the artery plaque characteristics where the interest is in the estimation of the association between the parameters for carotid artery plaque characteristics (Ktrans, ~~Within the plaque. There~~) and of inflammation will be 3 measurements (taken approximately 18 months apart of these parameters for each subject (initial/baseline measurement, second set at 18 months and third set at 36 months). We are interested in estimating the slopes between Ktrans for each of the subjects, and obtaining the weighted slope via the use of linear mixed effects model (34) where we model the within-subject measurements using the variance-covariance structure of autoregressive with lag 1.

Our goal 3 is to check the change in the artery plaque characteristics between 0 and 18 months, from 18 months to 36 months and also compare a difference between 0, 18 and 36 months. We used an ANOVA model to perform power analysis. We assume the effect size between the three measurements is 0.28. This implies that 92 subjects can achieve greater than 99% power to detect the true difference given the significant level is 0.05. To account for a potential attrition rate of ~10%, we will recruit 100 subjects.

We chose the larger sample size as the final sample size. All the power calculation is calculated based on statistical software R with package "pwr".

Health Evaluation of Patients:

Antecedents and Screening: Demographic/clinical variables assessed include age, gender, marital status, education, ethnicity, occupation, type of lymphoma, chemotherapy agents used and dosage, and total RT dosage. During screening, the short BLESSED cognitive screening tool (BOMC) is used to exclude those with scores > 11 indicating cognitive impairment that would interfere with participation. The Shortened WHO Rose Angina Questionnaire (Rose) will

be utilized to screen for the presence of unrecognized coronary artery disease. This shortened screening questionnaire includes the three most predictive questions of angina, major coronary events, and coronary heart disease mortality. Assessment of patient heart disease knowledge will be made with the coronary heart disease (CHD) Knowledge Test, a 40 item multiple-choice instrument originally designed to assess self-care, risk factor management, and general knowledge of CHD. Scores are obtained by giving 1 point for each correct answer with higher scores indicating higher knowledge. Health evaluation and tests are conducted as part of 'The Novel Testing for Subclinical Cardiovascular Disease in Lymphoma Survivors' (ongoing funded trial at Emory Survivorship clinic, IRB 00057029).

Variables and Measures:

Blood will be drawn for testing in the MRI Research Laboratory at EMORY by a research nurse. The blood specimens will be drawn immediately before each MRI examination to measure routine laboratory testing of serum markers of inflammation (hsCRP, LpPLA2, IL-6) and metabolomics. Creatinine will be measured to calculate eGFR. In addition, the Framingham Risk Assessment Tool for General Cardiovascular Disease (FRAT) will be employed to determine the 10-year risk prediction for general cardiovascular events using the assessed values of age; history of diabetes, smoking, and hypertension; systolic blood pressure; and measured total and HDL cholesterol levels.

Other tests:

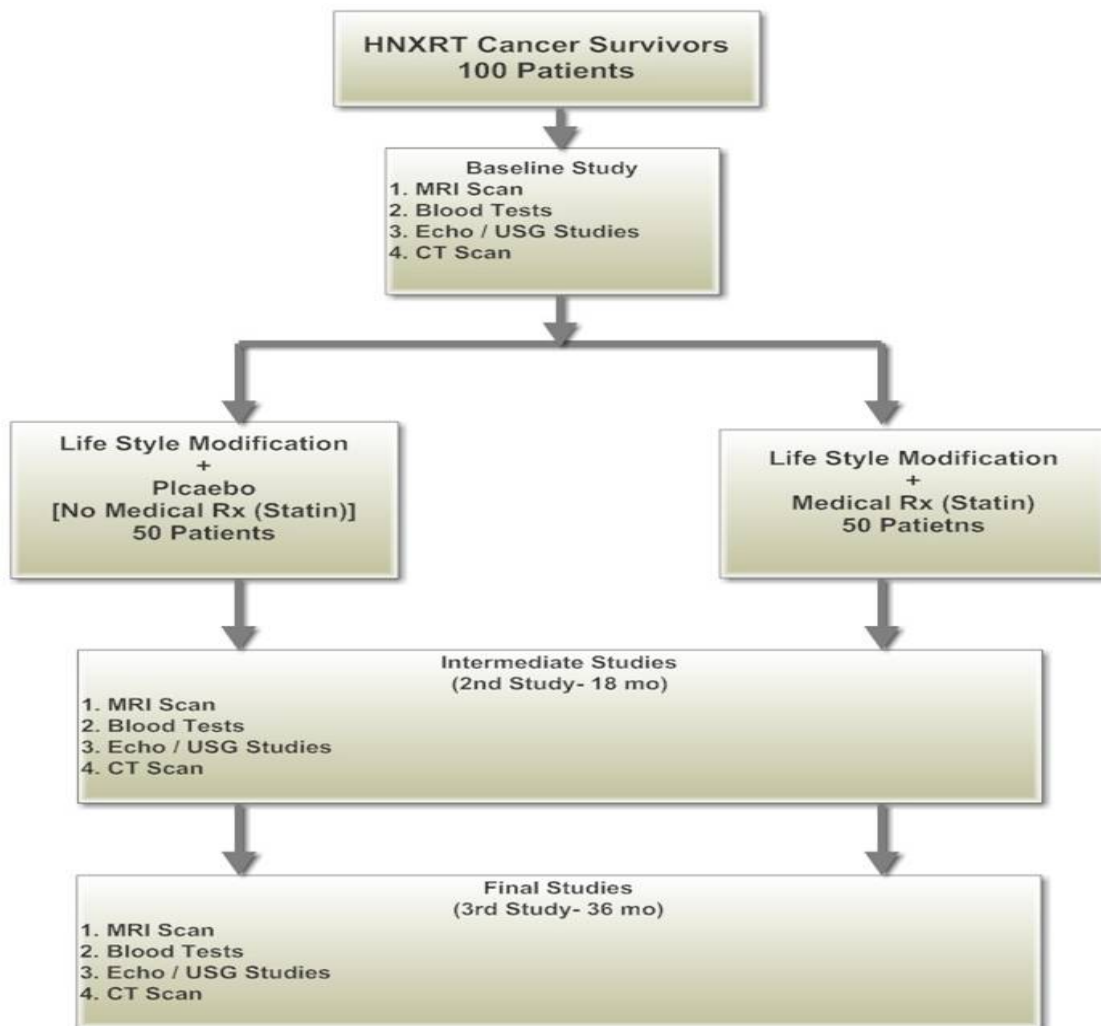
The following novel markers that will be assessed include measurement of serum levels of BNP, HS-CRP, and cTnI, Echocardiography for the assessment of systolic and diastolic function and structural defects, the presence of coronary artery calcium (CAC) and calcium score with the CT. For all cancer survivors with HNXRT, the above tests (except hsCRP, LpPLA2, IL-6 and metabolomics) will be conducted as part of 'The Novel testing for subclinical cardiovascular disease in lymphoma survivors' (ongoing funded trial at Emory Survivorship clinic, IRB 00057029). We intend to apply for a grant / fund to cover for tests not covered by ongoing funded trail at Emory Survivorship clinic, IRB 00057029.

Examination Plan Overview:

All participants will be given IRB approved informed consent. Those who decide to take part and give consent for the study would be enrolled in the research study. We would be taking snap shots with MRI at three data points and monitor treatment effects. Overview of the research study is shown in Fig 2. A baseline MRI will be performed to assess arterial atherosclerotic constituents. Once the initial MRI has been performed, and in the case where subclinical disease is present i.e. elevated blood markers or arterial plaque on MRI. HNXRT cancer survivor patient with abnormal tests will be started on treatment, which includes life style modification and-/or lipid lowering medication. Patients with positive tests would be divided randomly into two groups; one group will receive life style modification + statin therapy and the second group will receive life style modification alone. A repeat MRI and blood tests (2nd study) will be performed at 18 months after initiation of such treatment to assess changes in plaque morphology and enhancement characteristics. MRI findings of baseline and final would be

correlated with surrogate markers of inflammation, Echo and CT findings. A final set of MRI and blood tests will be performed at 36 months after initiation of such treatment to assess changes in plaque morphology and enhancement characteristics. MRI findings of baseline and final would be correlated with surrogate markers of inflammation, Echo and CT findings.

Fig 2. Shows overview of the research study



Treatment and Clinical Follow-Up:

Patients enrolled in the “Accelerated Atherosclerosis in High Risk Population Groups: An Assessment by Magnetic Resonance Imaging” study will be evaluated in the cardiology clinic by Khusrow Niazi, MD,

FACC, FSCAI who is the Director of Peripheral Vascular Intervention at Emory University and treats patients with carotid artery disease. All these patients will be randomized based on the last digit of their medical record number. If the last digit will be an even number they will then be given a statin, which they will take and will also be instructed about regular exercise. Patients will be made aware of the side effects of statins and about the procedures to report these side effects. Patients with the last digit being an odd number will ONLY be advised about exercise. All patients will have regular visits and patients on a statin will be followed up and evaluated for any adverse effects of the medication and for and for compliance at 18 and 36 months by the study preventive cardiologist. The preventive cardiologist or any other physician involved with care of the patient may elect for more frequent follow up as well as modification or discontinuation of the study prescribed therapy if deemed necessary based on his or her best clinical judgment.

These patients will get routine clinical follow-up in the oncology (The Winship Cancer Institute Clinics) and cardiology clinics (Emory Hospital) as part of the ongoing routine clinical care.

Within five weeks of initial MRI evaluation, our recruited research that demonstrate increased blood markers and/or atherosclerosis on MRI would be subjected to life style modification ± medical (statin) therapy. Department of Cardiology would prescribe and monitor statin therapy: Rosuva (20 mg once a day) with target LDL goal < 70; non-HDL goal < 100 and Aspirin (81 mg once a day). The rationale for using rosuva 20 mg is because this dosage has already been successfully used in JUPITER Trial (35). The follow-up by Cardiology department would comprise educating the patients on life style modification and also how to monitor and report any adverse drug affects which if expected would be further assessed with laboratory testing with LFT's and CBC. Any patients who suffer clinical side effects of treatment as part of our research would be taken off our study. Patients will continue to follow-up in the survivorship clinic and with the preventative cardiologist as indicated.

The randomized therapy may be modified or discontinued at the discretion of any physician involved with a study patients' care or the study-designated preventative cardiologist based on clinical judgment. This may occur during scheduled follow up with the study-designated preventative cardiologist or at any point.

Life style modification will be tailored, implemented and followed up by Emory HeartWiseSM Risk Reduction Program. This Program will use several analytical tools including questionnaire (BOMC, Rose, FRAT, CHD Knowledge Test). These analytical tools, laboratory as well as imaging tests will help EmoryHeartWise Risk reduction Program devise the best life style modification and exercise strategy for our patients.

Emory HeartWiseSM Risk Reduction

Joint Proposal WCI/Preventive Cardiology- Emory HeartWiseSM Risk Reduction:

Lifestyle Modifications in our high-risk patients with Subclinical Atherosclerosis

12 week/3 months; 3 x / week, total 36 visits

Recommend 6 Minute Walk Test (MWT) on Telemetry for each Patient: functional screen

Evaluation:

1. A 6 Minute walk test on telemetry – screening tool for function and cardiovascular/hemodynamic response.
2. PHQ-9 (Patient Health Questionnaire) depression questionnaire
3. Risk stratification – ACSM (American College of Sports Medicine) guidelines

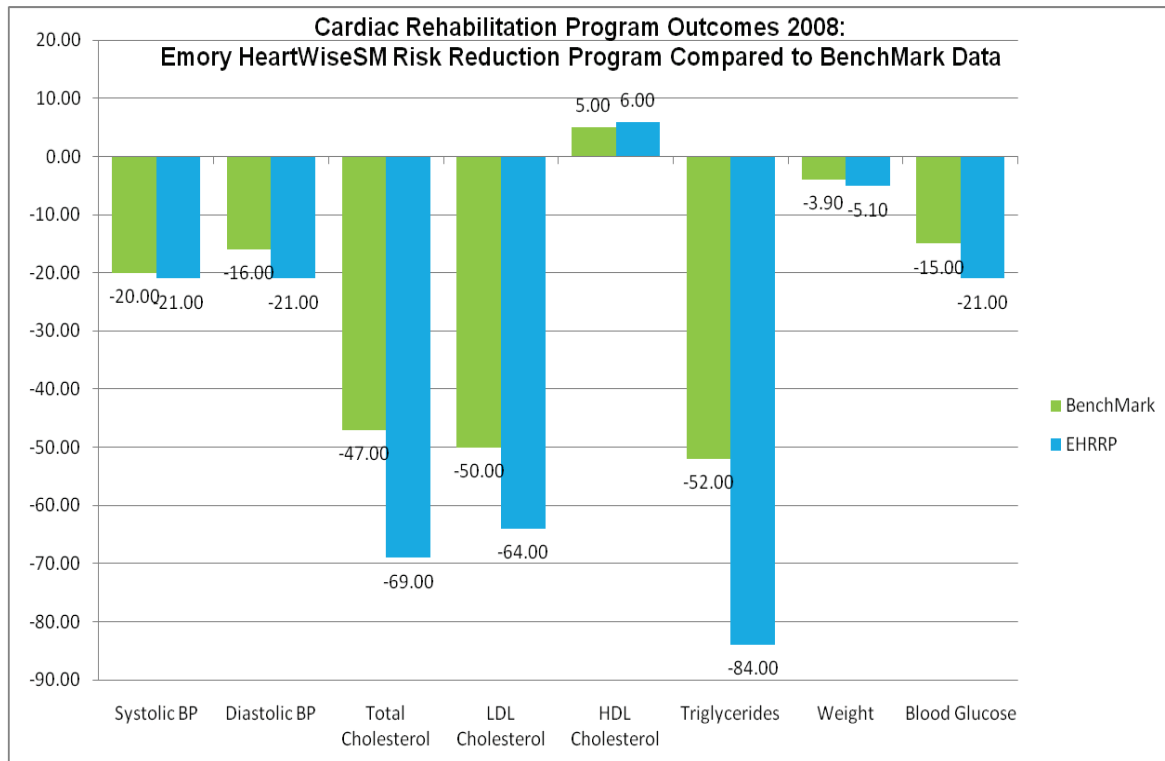
Exercise components:

1. Create exercise prescription based on ACSM guidelines and results of 6 MWT.
2. Orientation: safety principles of exercise, waist hip circumference; BMI (Body Mass Index); risk factor identification.
3. Brief meeting with Registered Dietitian (RD) for review of food intake/diary.
4. 1x/month 10 -15 minute meeting with RD for review of food intake and plan.
5. Minimum of 3x/week exercise program – based on FITT (frequency, intensity, time, and type) principles.
6. Individualized exercise prescription created and progressed by staff; tracked on flow sheets; monitor blood pressure; if diabetes - Blood Glucose (BG) pre/post/during if necessary.

The Healthy Exercise for Lymphoma Patients (HELP) trial was a randomized controlled trial looking at the benefits of 12 weeks supervised exercise program for cancer survivors compared to usual care (36). Improvements in health-related fitness and quality of life were maintained at the 6-month clinical follow up. The same model of secondary prevention through supervised, graded exercise as well as identifying and intervening with education and active participation in risk factor modification is being proposed to help high-risk patients identified with subclinical atherosclerosis.

The premise of inclusion of exercise regimen in our study is that regular, moderate levels of exercise have been shown to be beneficial at lowering blood pressure, total cholesterol, low-density lipoprotein, triglycerides, increasing high density lipoprotein, metabolic syndrome, insulin sensitivity, and all-cause mortality (37). Intensive lifestyle modification through education, support, and supervised exercise are the core components directed at risk factor reduction (38). Fig 3. (below) demonstrates risk factor modification for patients who completed a 12 weeks cardiac rehabilitation program compared to a larger cohort of cardiac rehabilitation patients in other parts of the country (39). The same model of prevention through supervised, graded exercise as well as identifying and intervening with education and active participation in risk factor modification is being proposed to help high-risk patients with subclinical atherosclerosis.

Fig 3: Shows Cardiac Rehabilitation Program Outcomes 2008



MRI Examination:

Pre-MRI screening and informed consent:

Upon arrival at the MRI Research Center, a trained MRI technologist will screen each study participant for metal objects and implants that may be hazardous in the MRI environment. The subject will be given a copy of the informed consent form. The PI or the co-investigator will be responsible for ensuring that the subject fully understands the procedure, for answering any questions the subject may have and for obtaining written informed consent. The technologist will then guide the subject to the MRI scanner room.

Imaging protocol:

All scans will be performed on a 3.0T MRI scanning system using 4-channel dedicated coil. A fast gradient echo 3-plane localizer will be performed to locate the major artery and its bifurcations. The location and extent of the lesion will be ascertained or verified from the 3D-TOF angiographic images (described below). The site of imaging will be selected to coincide with the location of a selected known lesion. The acquired axial images and the MIP formatted images will both be used to verify the region of stenosis and will be used to prescribe the high-resolution anatomical images (described below). Five to ten imaging locations will be prescribed depending on the known extent of the lesion. Double-oblique sections will be acquired for optimal visualization of the artery plaques for all the sequences described below.

Three-Dimensional time-of-flight MR angiography:

An axial three-dimensional spoiled gradient echo (3D-SPGR) MR angiogram will be used for scout images and to locate regions of stenosis. The acquisition will consist 40 to 60 contiguous slices. A field-of-view (FOV) of 12 cm to 16 cm at the region of interest will be used. Additional scan parameters are: Slice thickness = 2.0 mm, matrix = 256 x 256, receiver bandwidth = 32 KHz, flip angle = 45°, TR = 32 ms, TE = 4.7 msec, number of signal averages = 2. Maximum-intensity pixel projection (MIP) images will be created from all the image sections obtained during the 3D-SPGR acquisition.

High Resolution Anatomical Images:

T1 weighted (T1W), T2 weighted (T2-W) and proton density weighted imaging (PDWI) using a double inversion-recovery fast spin echo sequence and 3-point Dixon (3-PD) imaging will be performed. All black-blood scans will be cardiac gated. The following scanning parameters will be common to all three of the contrast-weighted scans: Section thickness = 2.0 mm, matrix = 512 x 384, receiver bandwidth = 62.5 KHz, ETL = 24, FOV: right-left - 12 cm to 16 cm; anterior-posterior – 0.75 * right-left dimension, Number of signal averages = 2. The specific weighting parameters are: T1-W: TE = 10 ms, TR = 1 R-R interval; T2-W: TE = 45 ms, TR = 2 R-R intervals; PDW: TE = 10 ms, TR = 2 R-R intervals.

DCE 2D SPGR:

Dynamic contrast-enhanced (DCE) two-dimensional T1-W SPGR pulse sequence will be used for dynamic contrast-enhanced imaging of the arterial plaque. The following scan parameters will be used: Slice thickness = 2.0 mm, FOV: right-left - 12 cm to 16 cm; anterior-posterior – 0.75 * right-left dimension, matrix = 256 x 160, receiver bandwidth = 32 KHz, flip angle = 60°, TR = 120 ms, TE = 2.9 msec. Twelve to fifteen double-oblique slices will be acquired during each scan. The orientation of the slices will be the same as those of the black-blood images. A baseline non-contrast scan will first be acquired. Then 0.1 mmol/kg of Gd-DTPA will be injected by a power injector at a rate of 2 mL/sec simultaneous to the contrast enhanced acquisition. A total of 20 similar image acquisitions will be performed with no time elapsing between scans after the injection.

Baseline, Intermediate and Final MRI:

Each subject will undergo similar scan protocol at baseline, intermediate and final MRI. Anatomical landmarks will be used to match images (baseline, intermediate and final) in the same patient from the three time points spread over an intervals of 18 months. These landmarks include arterial bifurcations, vertebrae, proximity of other vessels and patterns of fat and muscle. Since the slice volume would be planned centered at the area of interest for all the studies (initial and repeat); the exact Z-location of each slice would be known and reproducible for acquisition and comparative measurements.

Specific Aims:

A # 1: Characterization of plaque constituent's i.e. fibrous cap (FC), fat and hemorrhage within the carotid artery plaque by multiple MRI sequences: 3-point Dixon (3PD) and MCW sequences (3-D TOF, T1W, T2W & PDW)].

Rationale: Using MRI we will determine the plaque characteristics, which may help understand the increase risk of stroke in this patient population.

Data analysis: The plaque size and morphology (vessel wall area) will be determined as described in section below.

Expected results: We anticipate that the plaque morphology will be accurately and more importantly reliably determined and followed up by a combination of MCW and 3PD sequences.

Measurement of Changes in Carotid Plaque Size, Characteristics and Morphology Using Multiple-Contrast-Weighted High-Resolution MR Imaging:

To minimize errors and to improve contrast to noise ratio, five contiguous images centered at the point of interest will be analyzed and the results will be averaged. The vessel wall area will be calculated by measuring the area of the lumen and the total artery area (vessel wall area = total artery area – lumen area). The vessel wall area and total artery area will be determined by tracing contours along the boundaries. The areas will be calculated using a semi-automated analysis tool (MASS Analytical Software, Medis Medical Imaging Systems, Leiden, The Netherlands). In the selected images, the maximum, minimum and average wall thickness will also be measured.

Identification of plaque constituents will be facilitated by multiple contrast-weighted MR images and also 3-point Dixon (selective water/fat) (17, 40). Table 1 & 2 shows the MR signal intensities that are associated with the various plaque components depending on the type of weighting that is used. The different signal intensities that result from the use of MCW (table 1) and 3-point Dixon technique (table 2) images improve the accuracy of identifying the plaque constituents.

Table 1. Criteria for identifying intraplaque hemorrhage and lipids using 3-point Dixon technique.

Plaque Feature	Water	Fat
Calcification	Hypo-intense	Hypo-Intense
Hemorrhage	Hyper-Intense	Hypo-intense
Lipid	Hypo-Intense	Hyper-intense

Table 2. Criteria for identifying intraplaque hemorrhage and lipids using MCW imaging.

Plaque Feature	TOF	T1W-BB-FSE	T2W-BB-FSE

Calcification	Hypo-Intense	Hypo-Intense	Hypo-Intense
Hemorrhage	Hyper-Intense	Hyper-Intense to Iso-Intense	Hypo-Intense to Iso-Intense
Lipid	Hypo-Intense	Hyper-Intense to Iso-Intense	Hypo-Intense to Iso-Intense

If complex plaques are present in any of the study subjects, the area of lipid core regions, the thickness of fibrous caps and the size of calcifications will be measured.

A # 3: To assess the longitudinal change in the carotid artery plaque characteristics (inflammation and morphology) as therapy progresses during the study period. Also to see if there is a predictable relationship between inflammation (pattern of enhancement) and vulnerable features within a plaque

Rationale: High-resolution MRI methods have shown that changes in the size and morphology of arterial plaques occur over time following the initiation of therapy (17, 40, 41). DCE-MRI parameters in plaques have been shown to correlate with histopathological measurement of inflammatory cell content and neovasculature (42, 43). For e.g. carotid artery wall thickness has been measured by MRI and has been associated with serum markers of inflammation (44). High resolution anatomical MRI has demonstrated that changes in the size of carotid artery plaques occur over time in patients who were receiving therapy (41). It is our hypothesis that the characteristics of arterial plaques in this patient population will change favorably over the duration of this study (36 months) and these changes will be measurable by DCE and high spatial resolution MRI.

Data analysis: The DCE-MRI dynamic enhancement parameters (Ktrans and λ_p) will be determined as described in section below. The plaque size and morphology (vessel wall area) will be determined as described in SA # 1.

Statistical analysis: The three parameters mentioned above (Ktrans, λ_p , vessel wall area) are continuous variables. Before performing the statistical modeling we will check first to see if the distributions of these variables are normal; otherwise, we will use log transformation prior to the modeling of these parameters. We will first compute the mean, median, and standard deviation at each time point for these parameters. We will plot these data by patient over the 3 time points to examine the longitudinal profiles. We will use the linear mixed effects model (random coefficient model with random intercept and slope) with these parameters serving as dependent variables. The independent variables will be the time indicator and other potential confounders that we may need to control (e.g. age, gender, race, and body mass index). The interest in this model is the coefficient associated with the time indicator, which allows us to obtain the adjusted population mean estimate of these parameters at each of these time points. Since we have the best linear unbiased prediction estimates of the random intercepts and slopes for each subject,

we will be able to examine individual subject profiles and assess the temporal trend for each subject. Thus this random coefficient linear mixed effects model will allow us to obtain the overall adjusted mean estimate (K_{trans} , for each of the individual subjects, overall across all above mentioned time points, and at each of the time points.

To assess the association between the vessel wall area, and K_{trans} and linear mixed effects models (random coefficient models) to obtain the estimates of fixed effects. We will carry out 2 analyses in these models, we will use vessel wall area as the dependent variable and K_{trans} and potential confounders if necessary. The slope estimate from these models is interpreted as a measure of adjusted association between the changes in plaque size and morphology, and DCE-MRI dynamic enhancement parameters.

Assessment of Arterial Plaques by Measuring Contrast Agent Dynamics Using MR Images:

The dynamics of the contrast enhancement will be quantified using a generalized kinetic model that was proposed by Tofts, et al (45) which is based on two compartments, the blood plasma and the extravascular extracellular space (EES). K_{trans} and model, which assumes that the signal intensity of the contrast agent in the tissue compartment and the blood plasma compartment are linearly proportional to the total concentrations of the contrast agent in the tissue, C_t and the blood plasma, C_p (42, 43, 45). This approach assumes that there is an exponential decay in the concentration of the contrast agent in the plasma described by the equation, where A_i is the initial plasma concentration and m_1 is the plasma signal decay rate.

For e.g. in case of carotid arteries, the values for A_i and m_1 will be determined by an ROI placed in the lumen of the jugular vein on each image in the time-course at each section location. C_t will be found by identifying regions of enhancement within the plaque. A sample of pixels large enough to cover the enhancing regions will be taken. The average of the sample pixels for each image at a given location will be used as input values for the kinetic model. The kinetic analysis will be performed using a workstation tool by the two co-investigators and repeated to assess for the inter-observer and intra-observer variability.

Expected results:

We anticipate that the k_{trans} and the size of the plaques (vessel wall area) will change in a favorable way over the time course of the study due to institution of treatment (medicines and life style modification). We anticipate that changes in the vessel wall (plaque and vessel wall remodeling) as measured by high-resolution MRI will change in a way that is predicted by the change in the DCE-MRI inflammation parameters but that changes in the DCE-MRI inflammation parameters will precede those of the morphological changes. We expect this because previous studies have shown changes in inflammatory cell content occur within three months of the initiation of statin therapy, whereas changes in plaque morphology as measured by high-resolution anatomical

MRI occur at approximately twelve to eighteen months following the initiation of statin therapy (19, 41, 42).

A # 2: To investigate the relation between direct measures of plaque inflammation (MRI) and indirect measures of inflammation (blood serum markers of inflammation, Echocardiographic and CT studies) as therapy progresses.

Rationale: DCE-MRI studies have shown a correlation between the number of macrophages and amount of neovasculature and the patterns of dynamic contrast enhancement (42, 43). Our hypothesis is that over a longer period of time (36 months); there would be a correlation between these two (direct and indirect measures of inflammation).

Data analysis: The DCE-MRI dynamic enhancement parameters (Ktrans and λ_p) will be determined as described in section SA # 3 above. Samples will be drawn for serum markers of inflammation (hsCRP, LpPLA2, IL-6). Relationship if any between the inflammation within the plaque and serum markers will be observed.

Expected results: We expect a predictable correlation between the inflammatory markers in the blood and inflammation present within the vessel wall/plaque seen on the initial and post treatment sampling; this expectation is based on prior literature (19, 22, 46, 47).

We will carry out the modeling using SAS/STAT procedure MIXED.

A # 4: To determine the efficacy of comprehensive treatment (life style modification + statin) versus life style modification alone.

Rationale: The effects and efficacy of various combinations of treatment have not been studied in the context of radiation vasculopathy and accelerated atherosclerosis.

Data analysis: The techniques described in sections above (A1 to A3) would be used in combination to determine plaque-remodeling, inflammation within the plaque and serum markers of inflammation and compare efficacy of different treatment regimens. Each patient will get MRI examinations before and after treatment and therefore patients will serve as their own control.

Expected results: We anticipate that the efficacy of comprehensive treatment would outperform the results obtained with life style modification alone.

We will carry out the modeling using SAS/STAT procedure MIXED.

Project Timeline:

The equipment and resources are currently in place to begin patient examinations immediately upon receiving funding. We anticipate the recruitment of all 100 patients from the existing pool of patients at the Emory Clinics (Survivorship Clinic) within the 12 months of funding. The time from baseline MRI and initial follow-up MRI study will be 18 months which would give us time to observe therapy related changes in the plaque morphology and inflammation, observe correlation between plaque inflammation and inflammatory markers in the serum and also with

cardiac functional status as assessed by echocardiography and CT. The 2nd set of follow up MRI and blood tests at 36 months would serve as the end point for final analyses of the long term effects of the instituted treatment regimen. Thus, if the co-investigators responsible for statistical models receive the complete data 3 months after the end of the studies, it is expected to take another 3 months to complete the entire work. So realistically the project should be completed in 39 months.

Adverse Event Reporting:

We do not expect any serious physical adverse events from this research study. Participating in this study involves very few/minimal risks. Study staff will work with patients to minimize any stress or discomfort with the study procedures. The study involves a blood draw, which may cause slight bruising or pain at the site where the blood is drawn. In addition, bleeding, dizziness, fainting or feeling lightheaded, nausea, hematoma or infection may occur because of drawing your blood. The blood will be drawn from expert/trained staff so this will be less likely to happen.

The Computed Tomography (CT) for coronary artery calcification (CAC) and Echocardiogram are both non-invasive procedures. CT exams are generally painless, fast and easy. The risks associated with CAC screening are small (48). The echocardiogram is very safe, painless and uses ultrasound waves, for which there are no known risks.

We do not expect any serious physical adverse events from MRI examinations. Due to recent concerns related to the use of gadolinium-based contrast agents in patients with renal dysfunction and risk of nephrogenic systemic fibrosis (NSF), serum creatinine levels will be measured from blood drawn before each MRI examination (49). Patients with normal renal function values will be administered contrast and this approach virtually eliminates any possibility of NSF.

Statin treatment might cause some side effects in few individuals which would be closely monitored by symptoms and followed by blood tests; if any side effects occur, the medication would be immediately stopped; patients would be taken off the study protocol and they would continue their routine follow up in The Survivorship and Cardiology clinics.

Subjects will be provided with telephone numbers of the PI, co-investigators and the Emory University IRB. Any adverse events will be appropriately documented and reported through the Department of Radiology Safety Committee.

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Data and Safety Monitoring Plan (DSMP): There might be a minimal risk due to breach in confidentiality of the patient information, which we plan to overcome by keeping all personal facts and data private and confidential, and will use it strictly for research purposes. In order to maintain confidentiality, we will use a participant number rather than patient names on study records where we can. All patient records will be identified by an individual code number and the record of subject identifiers and corresponding reference codes will be stored in a locked cabinet in the protected and monitored research office building. All the results will be presented and published as group data. Agencies such as the Food and Drug Administration and the Emory University Human Investigations Committee have the right to review these records. We will keep the records private to the extent allowed by law. We will do this even if outside review occurs.

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