CAROTID PLAQUE AND INTIMA MEDIA THICKNESS IN THE ASSESSMENT OF CARDIOVASCULAR RISK

by

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DEDICATION

This dissertation is dedicated
to my family and friends with cardiovascular disease
Robert M. Thompson
John E. Shaffer
David Moen
C. Robert Imler
Etta Imler
John Raugh
G. Fred Kensinger
Shirley Kensinger
Elsie Kensinger
George D. Kensinger
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Preface

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Over the past decade significant advances have been demonstrated in the prevention and treatment of cardiovascular disease (CVD). Despite these major strides, CVD continues to be our nation's most significant cause of morbidity and mortality. The risk status of asymptomatic persons varies greatly and thus requires a range of intense screening and interventions. This dissertation focuses on subclinical CVD measures and a new methodology that will improve CVD assessment in research and eventually improve primary prevention efforts.

There are three related projects, each of which uses noninvasive carotid ultrasound to assess cardiovascular risk. Project one focuses on an elderly population and evaluates the association of calcified carotid plaques with cardiovascular outcomes. Plaque characterization is a new research interest with the aim of identifying what makes one plaque more dangerous than another. As plaques age, they often become more complicated and calcify. However, the significance of calcification in the carotid arteries is poorly understood. In this project, I assess if carotid calcification is predictive of cardiovascular outcomes.

For project tow, a different high cardiovascular risk population, women systemic lupus erythematosus (SLE) is studied. Women with SLE have significantly high risk of myocardial infarction compared to women without SLE. The role that lupus-related risk factors play in cardiovascular disease progression above traditional risk factors is unclear. With carotid ultrasound, associations between intima-media thickness and plaque with both cardiovascular and SLE-specific risk factors are assessed.

The final project documents development of new computerized assessment of carotid artery plaques. Over the past decade both ultrasound and computerized assessment tools have improved which creates opportunity for improved plaque assessment in vivo. This methodology will characterize plaques, possibly identifying which plaques are dangerous. A novel plaque characterization software is paired with ultrasound scans to execute this methodology in the Ultrasound Research Laboratory. Included are software considerations, protocol development, staff training, worksheet design, quality control procedures, and a pilot study to evaluate the reproducibility of the measure. This research has public health significance by developing new cardiovascular risk assessment techniques which may lead to improved primary prevention and research methods.

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1 INTRODUCTION

Atherosclerotic plaques are the cause of strokes and heart attacks, but we still know little about why one arterial plaque causes an event and another one does not. While degree to which a plaque narrows a vessel and reduces blood flow was initially thought to be the cause of symptoms, newer research suggests that smaller plaques cause more events because of their unstable composition. Technologies to study plaque characterization are just emerging and carotid ultrasound is a medically acceptable, low cost, widely accessible method to study plaque characteristics. In the last decade, we have learned that certain carotid plaque characteristics are associated with increased risk of cerebral events. Yet relationship between carotid plaque composition and coronary events has not been studied.

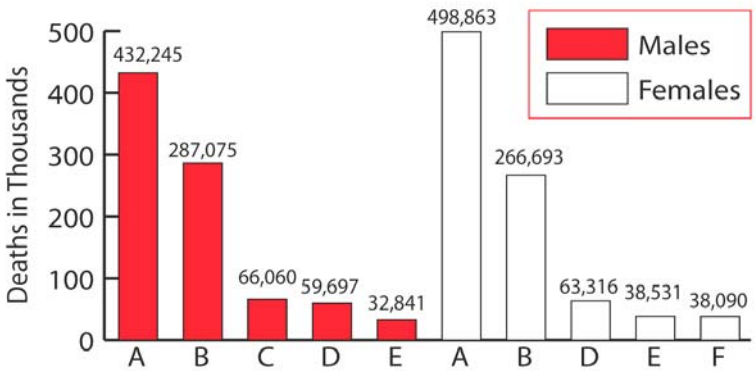
1.1 FOCUS OF DISSERTATION

This dissertation uses various noninvasive subclinical plaque measures to evaluate risk of cardiovascular disease in high risk populations. In the first project, the relationship of calcified carotid plaques is evaluated with CVD outcomes in an elderly population. In project two, the relationship of plaque presence, number and progression with cardiovascular risk factors is evaluated in a high risk group of women with lupus erythematosus. And finally, in the third project, the use of a new plaque analysis software is planned and executed for research use. This software is cutting-edge

technology that will lead to better understanding of plaque composition and plaque vulnerability. A formal reproducibility study is executed and analyzed as part of project three.

1.2 PUBLIC HEALTH SIGNIFICANCE

Cardiovascular disease (CVD) continues to be the leading cause of death in the Nation for both men and women, with >64 million people afflicted.(1;2) (Figure 1-1) CVD includes hypertension, myocardial infarction, chest pain (angina), congestive heart failure and cerebrovascular events (CVA and TIA). The risk for heart attack and death among persons with CVD is five to seven times higher than among the general population. The risk of CVD increases with age and also in certain diseases such as systemic lupus erythematosus, polycystic ovary disease and diabetes mellitus.



A. Total CVD
B. Cancer
C. Accidents
D. Chronic Lower Respiratory Diseases
E. Diabetes Mellitus
F. Alzheimer's Disease

Source: CDC/NCHS

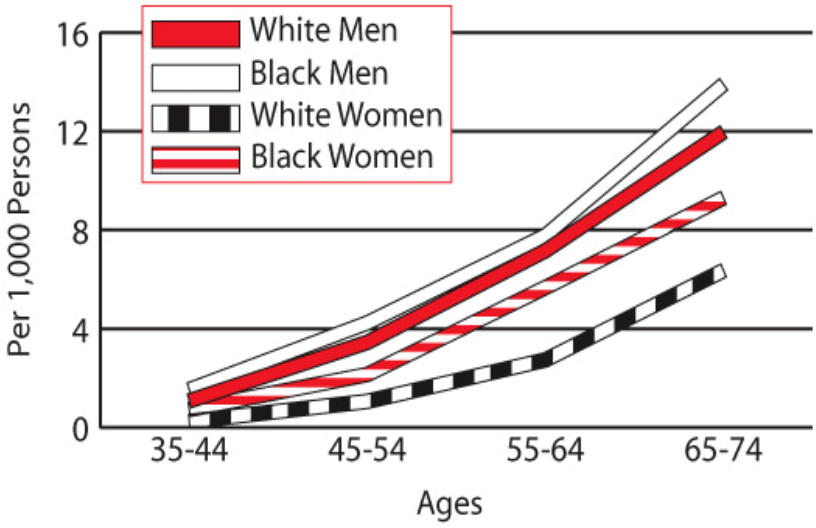
Figure 1-1: U.S. Causes of Death

The annual cost of CVD to the nation is projected to exceed $351 billion in 2003.(3) Yet these estimates do not convey the full impact of CVD from cognitive impairment after stroke and dementia.(4) Trends of the global burden of CVD over the next two decades support the prediction that heart disease and stroke will persist as the leading causes of death and disability worldwide unless effective public health action is taken to prevent them.(5;6)

Several factors accentuate this health concern. First is our aging population because CVD events and deaths increase with age (Figure 1-2). Over the next two decades, the number of Americans over 65 will increase significantly, from ~34 million in 2000 to more than 53 million in 2020.(7) Heart disease deaths are projected to increase sharply between 2010 and 2030, and the population of heart disease survivors is expected to grow at a much faster rate than the U.S. population as a whole. Marked increases in numbers of stroke deaths are also predicted.(8) Second, overweight and obesity is an increasing concern and known risk factor. Obesity affects a large proportion of the U.S. population—55 percent of adults—and 15% children(9). Obesity increases risk of illness from high blood pressure, high blood cholesterol and other lipid disorders, type 2 diabetes, CHD, stroke, and other diseases. Lastly, due to the obesity epidemic, the prevalence of diabetes mellitus (DM) is increasing, DM is a high risk disease for CV complications. These changes collectively will constitute a major increase in the nation's CVD burden, accompanied by increasing demands for related health care services, increases in health care expenditures, lost income and productivity, and prevalence of disease, disability, and dependency.

Cardiovascular disease is termed the silent killer because many cardiovascular events occur without warning. Fifty percent of MI's occur without any symptoms and 10% of strokes occur without warning. Many of the MI's lead to death before medical interventions occur. The standard

Framingham Risk Assessment fails to identify many at risk when they are asymptomatic. Therefore, additional new techniques are needed to identify individuals at high risk for CV events.



Source: NHLBI's ARIC surveillance study, 1987-2000.

Figure 1-2: Annual Rate of First Heart Attacks by Age, Sex and Race

Past research has provided the first steps into effective CVD prevention and mortality reduction. Included in this research have been subclinical measures such as intima-media thickness using B-mode ultrasound and ankle arm index. The results of these measures have led to treatment and modification of important risk factors. For example the reduction of high blood pressure and cholesterol, increased exercise, smoking cessation, and antiplatelet therapy has impacted cardiovascular disease. Newer strategies focus on weight control, initiating preventative measures earlier (beginning in childhood), and increasing public education. However, still half of all heart attacks occur in individuals with normal risk factor levels.

Healthy People 2010 has recommended using noninvasive methods to identify at-risk people for heart disease.(6) This research will contribute directly to the accomplishment of that goal

by testing the usefulness of subclinical measures in two high-risk populations and by the development of a novel protocol to assess carotid plaque characteristics.

1.3 LITERATURE CITED INTRODUCTION

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2 INTRODUCTION TO PROJECT 1

In early research, degree of stenosis with resulting altered blood flow was identified as a risk factor for stroke. Today we know that the components of plaques may be more important than the stenosis created by the plaque. One component of plaques that is poorly understood is the presence of calcification. In the first project of this dissertation, calcification of carotid plaques is used to assess the relationship with CV outcomes in an elderly, high CVD risk population.

CALCIFIED CAROID ARTERY PLAQUES PREDICT CARDIOVASCULAR OUTCOMES IN THE ELDERLY

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2.1 ABSTRACT

Background: Plaque composition using ultrasound is getting attention to identify patients at high risk for cardiovascular disease. Calcification of plaques prevents complete assessment with ultrasound and thus are overlooked as useful predictor. We explored whether calcified carotid plaques are able to predict cardiovascular outcomes in older adults.

Methods and Results: Participants included 187 hypertensive and 187 normotensive adults ≥ 60 years who underwent a duplex scan to identify the presence of calcified carotid plaques. Participants were followed for incident cardiovascular events and death for 11 years.

Participants with calcified carotid plaques had higher mortality and cardiovascular event rates than those without plaque or without calcification of plaques ($p<0.001$).

Using Cox univariate regression and controlling for age and male sex, higher systolic blood pressure, pulse pressure, glucose and the presence of calcified carotid plaques were associated with.mortality. Calcified plaques and untreated hypertension were significantly associated with to time-to-any CVD event ($p <0.001$).

Conclusions: Calcified carotid plaques predict mortality and cardiovascular outcomes above other common risk factors in the elderly. Carotid calcification may serve as an additional cardiovascular risk factor in the elderly.

2.2 INTRODUCTION

A key approach in combating cardiovascular disease is identifying the factors that put patients at highest risk and detecting them early in the course of disease. One of the factors that is gaining attention is the evaluation of plaque composition.(1) Investigators have focused on identifying plaque characteristics that make them vulnerable to rupture. Studies of coronary plaques have identified vulnerable plaques as having a lipid core with a thin fibrous cap and often the presence of thrombus.(2) Calcification is an integral part of the evolution of plaques and may also contribute to plaque vulnerability.(2-4)

Because disease in the carotid arteries is a marker of disease in the coronary arteries, investigators have begun to use carotid ultrasound as a way to non-invasively study plaque characteristics. Carotid plaque characteristics have consistently been associated with cerebrovascular outcomes.(5-9) However, one major problem with these studies is that calcified plaques are often excluded, primarily because shadowing caused by calcification makes plaque analysis difficult.(6;8;10;11) Thus, the ability to evaluate calcification as a marker of subsequent events is lost. This is of particular concern when evaluating populations where vascular calcification is common, such as patients with diabetes (12), end-stage renal disease,(13-15) the elderly.(2;16) The purpose of this study is to test whether calcified carotid plaques specifically are able to predict CV outcomes in a population of older adults.

2.3 METHODS

Participants

This study includes hypertensive (n=187)and normotensive (n=187) participants who underwent carotid duplex scans at baseline. The hypertensive participants were recruited from the Systolic Hypertension in the Elderly Program (SHEP) at the Pittsburgh field center.(17;18) SHEP was a muticenter randomized clinical trial designed to test the efficacy of treating isolated systolic hypertension in adults ≥60 years old. Screening was carried out in retirement centers, churches and other locations where healthy elderly adults could be identified. To be eligible, participants were ≥60 years old, had systolic blood pressure (SBP) of 160-219 mmHg, and diastolic blood pressure of <90 mmHg. Exclusions included recent myocardial infarction (MI), stroke (CVA) with residual paresis, uncontrolled congestive heart failure (CHF), peripheral arterial disease (PAD) with evidence of tissue injury or loss, transient ischemic attacks (TIA) with associated carotid bruit, and contraindication to study medications. Detailed screening and exclusion criteria have been previously reported.(17;19) The hypertensive group was randomly assigned to receive either stepped care BP treatment (active arm) or matching placebo medication (placebo arm) using a double-masked design. Complete description of medication therapy was previously reported.(20)

A normotensive group (N=187) was recruited during the last year of SHEP from the Pittsburgh site. The same SHEP screening process was used for the normotensives, except systolic blood pressure (BP) criteria was <160mmHg. Nomotensive participants were stratified by age to

allow adequate adjustment for age between the hypertensive and normotensive groups to ensure the same distribution between the hypertensive and normotensive groups was maintained.

Both groups were followed for 11 years by telephone contact. Only 10 (5%) SHEP and 3 (2%) normotensives were lost to follow-up. All participants signed informed consent approved by the institutional review board at the University of Pittsburgh.

Outcome Measures

The outcome measures were any cardiovascular event (CVD), any cerebrovascular event and death from any cause. Any CVD event included cardiovascular and cerebrovascular events. The cardiovascular events included CVA, TIA, MI, hospitalization for unstable angina, coronary revascularization, congestive heart failure, and death from cardiovascular cause. Any cerebral event was limited to TIA or CVA. Deaths were verified by review of hospital records and death certificates and were categorized as cardiovascular or other.

Scanning Protocol

A 5 MHz linear array transducer on a Toshiba 270 (Tustin, CA) was used to obtain B-mode images at the Ultrasound Research Laboratory at the University of Pittsburgh, in Pittsburgh. The presence of calcified lesions in the proximal and distal common carotid artery (CCA), the carotid bifurcation, and the proximal internal carotid arteries (ICA) bilaterally were recorded. Calcification was considered present when acoustic shadowing (blackness) was seen behind a bright plaque. Shadowing occurs when the ultrasound waves cannot penetrate a dense lesion and the waves scatter leaving a void behind the plaque. Two variables served as outcomes 1) a three-level categorical variable to indicate

absence of plaque, presence of noncalcified plaque, and presence of calcified plaque and 2) a binomial variable indicating the presence of no or noncalcified plaque and calcified plaque.

Statistical Methods

Descriptive data were assessed for the total group as well as stratified by hypertension. Continuous variables were reported as mean (+/- SD) and categorical variables as percent. Two-sided Student t-tests (for normally distributed data) and Wilcoxon (not normally distributed) were used to assess differences in subclinical risks factors in each group. Kaplan-Meier life-table methods were used to estimate mortality, any event, cardiac event, and any cerebral event for those with no plaque, non-calcified plaques, and calcified plaques, separately. In multivariate analyses, the presence of calcified plaques was assessed using a two level variable, because there were too few participants with no plaque. Thus plaque categorizes were 1) no or noncalcified plaques and 2) calcified plaques. Analysis of mortality included all deaths regardless of the cause. The log rank statistic was used to determine whether the survival curves differed, with $p<0.05$ considered statistically significant. Univariate Cox associations were used to select the covariates for building the final Cox regression model. Age and sex were controlled for in the final multivariate Cox regression model. Statistical analysis was performed using SAS version 8.02 (SAS Institute Inc., Cary, NC).

2.4 RESULTS

Participant Population

Baseline characteristics at the time of entry were compared for the hypertensive and normotensive groups (Table 2-1). As previously reported, age and race distributions were comparable: each were about 40% male and 99% Caucasian.(18) The hypertensive participants had higher mean diastolic blood pressure and BMI, and were less likely than normotensive participants to have ever smoked (all p values < 0.01). With respect to laboratory values, the hypertensive group had higher mean total cholesterol ($p < 0.001$), LDL, triglycerides, and creatinine, (all p values <0.01) and lower HDL ($p < 0.03$).

Plaque prevalence

The prevalence of plaque in the overall study participants was 93% (344/370). Plaque prevalence was 96% (177/184)in the hypertensive group and 90% (167/186) in the normotensive group ($p < 0.016$). Calcified plaque was more prevalent in the hypertensive group (74%) than in the normotensive group (55%) ($p<0.001$). The mean age of those with calcified plaques was 73 years and of those with noncalcified plaques was 69.5 years ($p<0.001$). Overall mortality was 25% (n=93) over the 11 year follow-up period. Of those who died, 84% (n=78) had calcified plaques and 16% (n=15) had none or non-calcified plaques ($p<0.001$).

Mortality

Participants were followed for vital status and CV events. The 11-year survival rates were 91% among those with no plaques, 82% among those with noncalcified plaques, and 55% among those with calcified plaques (**Figure 2-1**). Risk factors associated with mortality by Cox univariate regression analysis were age, male sex, higher systolic blood pressure, pulse pressure, glucose and the presence of calcified carotid plaques (Table 2-2: Project 1 Univariate associations of risk factors to time-to-mortality and time-to-any CVD event). After controlling for age and sex, the presence of calcified plaques, glucose and the untreated hypertensive group remained significant for mortality ($p < 0.003$ for all). Calcified plaque was significantly associated with mortality, independent of hypertension. The relative risk in the hypertensive group was 2.7 and in the normotensive group 3.7 ($p < 0.008$ for both) (data not shown).

Time to any Cardiovascular (CVD) events

Participants with calcified carotid plaques had a higher event rate than those without plaque or calcification of plaques ($p<0.001$) (**Figure 2-2**). The 11-year event-free rates were 86% among those with no plaques, 67% among those with noncalcified plaques, and 42% among those with calcified plaques (Figure 2). As expected, traditional risk factors associated with time-to-any CVD event were age, male sex, higher systolic blood pressure, pulse pressure (all p values < 0.001), total cholesterol, LDL, triglycerides (p values 0.01), and lower HDL ($p<0.001$) (Table 2-2). After controlling for age and male sex, calcified plaques and the hypertensive control group remained significantly associated with to time-to-any CVD event ($p < 0.001$) (Table 2-3). Calcified plaque was significantly associated with any CVD event, independent of hypertension. The relative risk in the hypertensive group was 1.9 and in the normotensive group 2.8 (data not shown).

Time to any Cerebrovascular event

When cerebral and coronary events were analyzed separately, only cerebrovascular event rates remained significantly different between the calcified vs. no plaque and noncalcified plaque groups ($p<0.002$) (**Figure 2-3**). Using multivariate Cox regression, after controlling for male sex and age, calcified carotid plaques (RR 3.3) and the hypertensive participants on placebo (RR 3.5) were at highest risk of developing a cerebrovascular event ($p <0.01$) (data not shown).

2.5 DISCUSSION

This study shows that among older adults, the presence of calcified carotid plaque predicts cardiovascular outcomes. Calcified plaques were associated with death, overall CVD events, and cerebrovascular events specifically. These data clearly show that even in the absence of clinically important stenosis, calcified lesions provide prognostic information.

This is the first ultrasound study to show that the presence of calcified carotid plaques in an elderly population is related to CV outcomes other than stroke. Among older adults in the Cardiovascular Health Study (CHS), hyperechoic and heterogeneous carotid plaques were related to transient ischemic attacks and strokes, using ultrasound, but associations with other cardiovascular outcomes were not evaluated.(21) In chronic renal patients, peripheral vascular calcification was associated with myocardial infarction measured radiographically(12)and with ultrasound(14) yet, no study has looked at carotid calcification specifically.

There are numerous reasons why calcified plaques may be related to outcome. First, calcified carotid plaques are likely a marker for coronary artery calcification which has been shown to be predictive of overall plaque burden (22-24)(25) as well as CV outcomes.(26;27) Second, it is possible that calcification may identify a subset of plaques that are vulnerable. Naghavi described two types of vulnerable plaques that contain calcification 1) a calcified nodule within or very close to the fibrous cap that can protrude through and rupture the cap and 2) severe calcification within a chronically stenotic plaque with old thrombus and an eccentric lumen. (1) It is hypothesized that some plaques go through repeated cycles of rupture, bleeding, and healing complicated by inflammatory reactions(2;28;29) which favor complicated plaque composition and calcification. (2)

Carotid ultrasound can characterize these different plaque features.(5;6;10;30-42) Studies report differing plaque characteristics for symptomatic and asymptomatic patients, (8;9;43) but the exact plaque composition that is thought to be associated with vulnerability is inconsistent.(6;21;41;44;45) On ultrasound, complicated plaques present as heterogeneous which often contain calcium(5;46) and are associated with CV outcomes.(21;29;45;47-49)

A third mechanism linking calcified plaques to outcome relates to plaque age and the developmental stages of plaque growth. Calcification occurs in the later stages of plaque development after going through multiple cyclic changes. Thus calcification probably indicates plaques that are older. Calcification may develop as plaques go. Among older adults, the high prevalence of glucose intolerance, subclinical cardiovascular disease, and reduced renal function that alters calcium and phosphorus metabolism may all contribute to calcified plaques. Thus, calcium may indicate adverse metabolic processes that are indicative of aging and would be associated with events. More research is needed to understand the role of calcified carotid plaques but this study shows that carotid calcification should not be dismissed because it predicts CV events in the elderly.

We found that calcified carotid plaques were strongly associated with stroke outcomes. This is consistent with findings from the Cardiovascular Health Study where both heterogeneous and calcified plaques were more prevalent among older adults with strokes and transient ischemic attacks.(50) One reason for this may be that calcified plaques are a marker for vascular stiffness. People with calcified plaques may also be at risk for medial wall calcification which has been linked to vascular stiffness in animals (51;52) and diabetic patients.(53) As the aorta stiffens, its critical cushioning effect is lost, exposing the brain and kidneys to damaging cyclic pressure.(54) Central artery stiffening also leads to increases in cardiac afterload and low coronary filling. Thus if calcified

carotid plaques are a marker for arterial stiffening, then this may explain the link with CV outcomes as well.

In conclusion, calcified carotid plaques predict mortality and cardiovascular outcomes, above other common risk factors. The presence of calcified carotid plaques may be a marker of complicated and vulnerable plaques in other vascular beds, especially the coronary arteries. The presence of calcified carotid plaques may serve as an additional CVD risk factor in the elderly.

Table 2-1: Project 1 Population Characteristics

Risk Factor	Hypertensive **s** **n=187**		Normotensive **s** **n=187**		**Combined N=374**	**p-value for difference between cases and controls**
Male sex %	40%		41%		151 (40%)	0.75
White race %	99%		98%		370 (98.7%)	0.51
Ever Smoke %	40%		54%		176 (47%)	**0.007**
Current Smoke %	10%		12%		40 (11%)	0.50
Calcified plaques %	74%		55%		65%	**<0.001**
	Mean	**SD**	**Mean**	**SD**	**Mean**	
Age	72.3	6.2	71.3	6.32	71.8	0.12*
Systolic blood pressure (mmHg)	170.4	8.7	126.8	13.1	148.6	**<0.01**†
Diastolic blood pressure (mmHg)	78.6	6.8	69.3	8.4	74.0	**<0.001**†
Total cholesterol mg/dl	241.8	42.5	219.5	39.9	230.6	**<0.001**†
High density lipoprotein mg/dl	51.9	12.7	55.1	14.6	53.6	**0.03***
Low density lipoprotein mg/dl	157.4	39.4	140.1	35.9	148.5	**<0.01***
Triglycerides mg/dl	166.3	96.6	123.4	72.1	144.7	**<0.01**†
Glucose mg/dl	100.8	29.3	109.4	19.6	105.2	**<0.01**†
Body mass index	28.0	6.1	25.6	4.5	26.8	**<0.01**†
Creatinine mg/dl	1.0	0.2	0.91	0.21	0.98	**<0.01**†
Lipoprotein (a) mg/dl	14.2	16.8	12.4	16.6	13.2	0.31*

* t-test † Wilcoxon

Table 2-2: Project 1 Univariate associations of risk factors to time-to-mortality and time-to-any CVD event

	Time to Death N=374			Time to Any CVD Event N=374		
Variable	**RR**	**95% CI**	**p-value**	**RR**	**95% CI**	**p-value**
Age per 5 yrs	**1.56**	**1.33-1.82**	**<0.001**	**1.32**	**1.16-1.50**	**<0.001**
Male sex	**2.64**	**1.74-4.00**	**<0.001**	**2.62**	**1.88-3.65**	**<0.001**
Systolic blood pressure per 10 mmHg	**1.21**	**1.11-1.33**	**<.001**	**1.16**	**1.08-1.25**	**<0.001**
Diastolic blood pressure per 10 mmHg	1.11	0.87-1.40	0.408	1.11	0.92-1.34	0.264
Pulse Pressure per 10 mmHg	**1.27**	**1.15-1.41**	**<0.001**	**1.20**	**1.11-1.30**	**<0.001**
Chol 25 mg/dl	1.0	0.88-1.13	0.932	**1.12**	**1.02-1.23**	**0.015**
HDL per 10 mg/dl	**0.59**	**0.39-0.88**	**0.01**	**0.57**	**0.41-0.79**	**<0.001**
LDL per 25 mg/dl	1.01	0.87-1.17	0.891	**1.14**	**1.02-1.27**	**0.017**
Triglycerides per 25 mg/dl	1.01	0.96-1.10	0.656	**1.05**	**1.01-1.09**	**0.017**
Glucose per 25 mg/dl	**1.27**	**1.07-1.50**	**0.007**	1.15	0.98-1.35	0.080
Basal Metabolic Index per 5 unit	1.11	0.93-1.32	0.265	1.12	0.97-1.30	0.117
Ever Smoke	0.67	0.45-1.01	0.055	0.73	0.52-1.04	0.082
Calcified plaque y/n [§]	**3.89**	**2.23-6.77**	**<0.001**	**2.65**	**1.78-3.95**	**<0.001**
Plaque						
None	**1.0**			1.0		
Uncalcified	1.80	0.41-7.96	0.441	2.67	0.81-8.81	0.106
Calcified	**6.30**	**1.55-25.69**	**0.010**	**6.22**	**1.97-19.61**	**0.002**
Blood Pressure Status						
Controls	1.0			1.0		
Hypertensive, active arm *	**1.68**	**1.04-3.02**	**0.037**	1.45	0.95-2.22	0.088
Hypertensive, placebo arm **	**3.11**	**1.92-5.04**	**<0.001**	**2.97**	**2.03-4.35**	**<0.001**

§ yes= calcified plaques no=includes no plaque and noncalcified plaques

* Hypertensive group taking antihypertensive medication ** Hypertensive group taking placebo

Table 2-3: Project 1 Multivariate associations of CVD risk factors with time-to-mortality and time-to-any CVD event

	Time to Death N=374			Time to Any CVD Event N=374		
Variable	**RR**	**95% CI**	**p-value**	**RR**	**95% CI**	**p-value**
Age per 5 years	1.43	1.21-1.70	<0.001	1.17	1.02-1.34	0.018
Male sex	2.46	1.60-3.77	<0.001	2.64	1.88-3.69	<0.001
Calcified plaque y/n§	2.77	1.57-4.86	<0.001	2.14	1.42-3.22	<0.001
Blood Pressure Status						
Normotensives	1.0			1.0		
Hypertensive, active arm*	1.64	0.95-2.83	0.077	1.34	0.87-2.08	0.186
Hypertensive, placebo arm**	2.15	1.31-3.52	0.003	2.20	1.49-3.26	<0.001
Glucose per 25 mg/dl increase	1.27	1.08-1.48	0.003	——	——	——

§ yes= calcified plaques no=includes no plaque and noncalcified plaques

** Hypertensive group taking antihypertensive medication ** Hypertensive group taking placebo

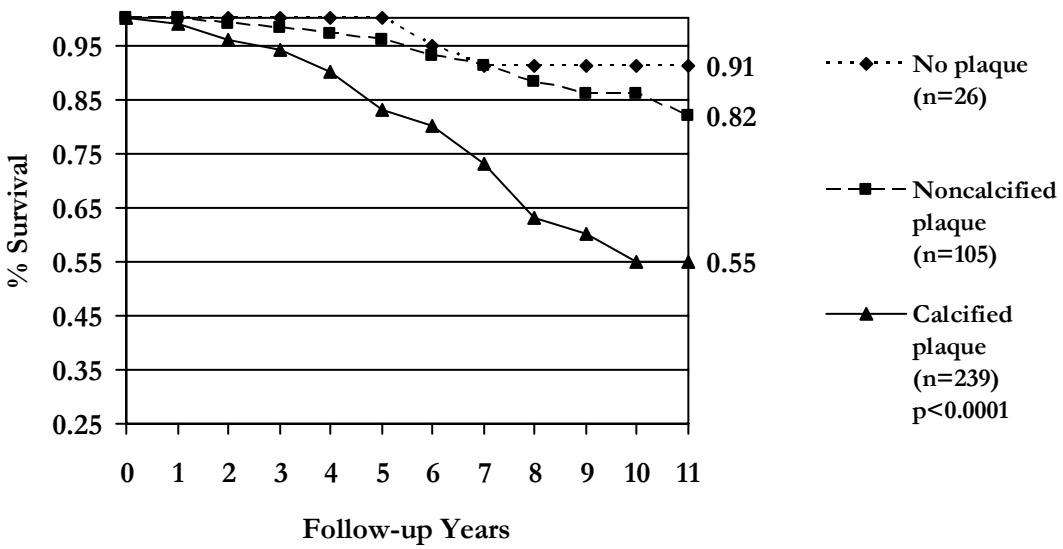

Figure 2-1: Kaplan Meier Estimates of 11 Year Survival by Presence and Type of Carotid Plaque

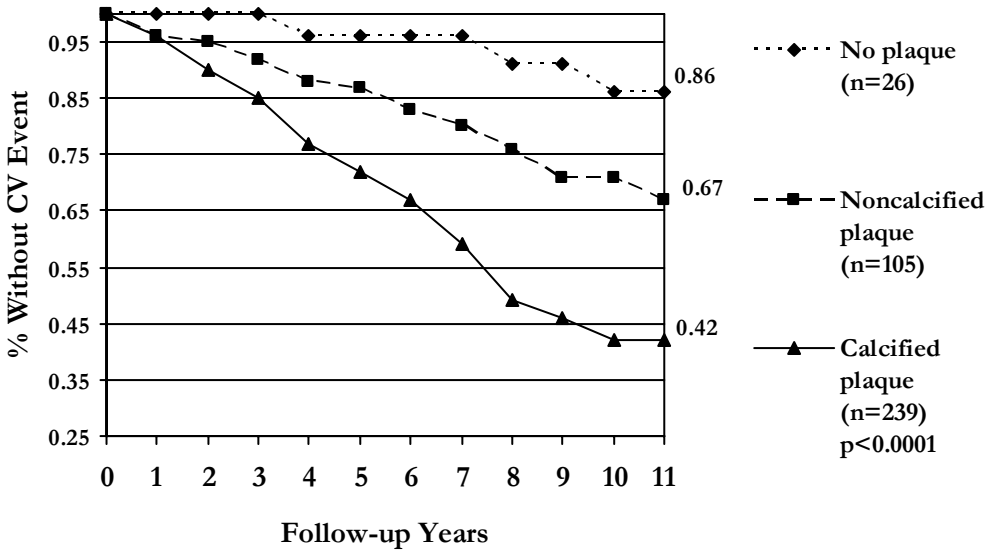

Figure 2-2: Kaplan Meier Estimate for Any CVD Event Over 11 Years by Presence and Type of Carotid Plaque

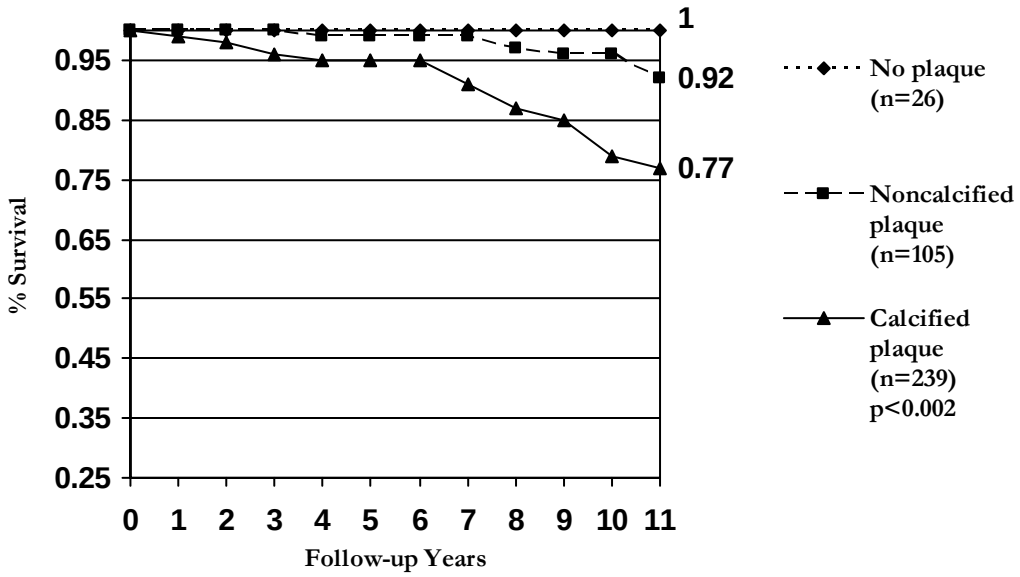

Figure 2-3: 11 Year Kaplan Meier Estimates for Cerebral Event by Presence and Type of Carotid Plaque

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3 INTRODUCTION TO PROJECT 2

Systemic Lupus Erythematosus (SLE) is a chronic, autoimmune disease which causes mutisystem organ damage through chronic inflammation and episodes of inflammatory exacerbations. The disease affects mostly women and is known to increase risk for death and morbidity due to heart attacks and strokes. The reason for higher risk of heart attack and stroke is not clear. Cross-sectional studies have shown that both traditional and SLE-related risk factors are related to the elevated risk, but no longitudinal study has evaluated the association between risk factors and subclinical measures. In project two, a population of women with SLE undergo baseline and follow-up ultrasound testing to evaluate the association between traditional and SLE related risk factors with subclinical outcome measures.

In this project, two ultrasound measures are utilized. One measure is the intima media thickness and the other is a measure of plaque burden, the number of plaques in the carotid system.

PROGRESSION OF CAROTID INTIMA-MEDIA THICKNESS AND PLAQUE IN WOMEN WITH SYSTEMIC LUPUS ERYTHEMATOSUS

Submitted to Journal of American Medical Association

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3.1 ABSTRACT

Background: Women with systemic lupus erythematosus (SLE) are at higher risk of cardiovascular disease and death from atherosclerosis than women without SLE. We conducted a longitudinal study to determine the relationship of traditional and SLE-related risk factors with atherosclerotic progression in this population.

Methods: Carotid ultrasound was performed at baseline (N=282) and after an average of 4.1 years of follow-up (n=214) on SLE women ≥ 18 years recruited from the Pittsburgh Lupus Registry. Clinical, serological, SLE-related factors and disease treatment were evaluated. Outcomes were carotid intima-media thickness (IMT) and plaque presence.

Results: Mean age was 45 years (standard deviation (SD) 10.9), mean IMT was 0.64 mm (SD 0.14) at baseline and 0.68 mm (SD 0.15) at follow-up. Mean IMT progression was 0.01 mm (SD 0.07) per year. In multivariate regression analysis, age and modified SLICC (lupus disease damage index) were associated with baseline IMT. Serum creatinine and age were strongly associated with IMT progression ($p < 0.01$) and modified SLICC and immunosuppressant non-use were borderline significant (p 0.04 and 0.08, respectively). Plaque prevalence was 33% at baseline and 40% at follow-up. Plaque progression occurred in 27% of the women. In multivariate regression, age, ever smoking, higher systolic blood pressure, longer steroid use and antidepressant use were associated with plaque presence at baseline ($p \leq 0.04$ for all). The primary factors associated with plaque progression were older age and higher triglycerides ($p < 0.01$). Higher serum complement C3 values and duration of SLE disease were borderline significant (p 0.05 and 0.07, respectively).

Conclusion: Both SLE-specific and traditional risk factors are associated with progression of carotid IMT and plaque. These subclinical measures may be useful managing women with SLE and as surrogate endpoints in SLE intervention trials.

3.2 INTRODUCTION

Systemic lupus erythematosus (SLE) is an autoimmune inflammatory disease that primarily affects women. It manifests with frequent exacerbations of inflammatory flares that may ultimately cause organ failure. Treatment consists of anti-inflammatory agents and immunosuppressive therapies. Patients with SLE are at higher risk of cardiovascular disease (CVD) compared to women of similar ages (1-5). Women with SLE under the age of 45 were found to have a 50 fold higher risk of myocardial infarction (MI) compared to women of similar age in the Framingham Offspring study(6). Cross-sectional and retrospective studies have shown that traditional risk factors including hypertension, obesity, diabetes mellitus, smoking, hyperlipidemia, hyperhomocysteinemia and sedentary lifestyle play a role in this accelerated atherosclerosis.(3;7;8) However, the diagnosis of SLE remains a strong risk factor for CVD, even after controlling for traditional risk factors. The most important SLE factors contributing to premature CVD remain unknown.

Noninvasive imaging techniques have been used to explore why SLE predisposes women to excess CVD burden. Using these modalities, increased rates of carotid focal plaque (37.1% vs. 15.2%)(7) and coronary calcium (30.7% vs. 8.7%)(9) have been reported in women with SLE compared to controls. Other studies have also found higher than expected rates of subclinical atherosclerosis in women with SLE.(4;5;10;11) To date, intima media thickness (IMT) and plaque assessment have not been used in longitudinal studies of women with SLE to evaluate rates of change and the factors that predict change in subclinical atherosclerosis. The ability to measure change in subclinical atherosclerosis among these women would provide the option of using surrogate cardiovascular endpoints in clinical trials of SLE therapies. The purpose of this study is to evaluate

progression of carotid atherosclerosis in SLE women and determine the relative contribution of both traditional and SLE-related risk factors.

3.3 METHODS

Patient Population

Women were recruited from the Pittsburgh Lupus Registry Cardiovascular Study as part of a longitudinal NIH funded study of CVD in SLE. The registry includes patients who have been seen either at the University of Pittsburgh Medical Center inpatient and outpatient facilities or by practicing rheumatologists in the Pittsburgh Metropolitan area. Thus, the sample represents a community based spectrum of mild to severe SLE with minimal tertiary care center referral bias. These women fulfilled the 1982 American College of Rheumatology revised criteria for the classification of definite or probable SLE (12). The updated criteria for SLE(13) had not yet been published when the study began. All women 18 years of age or older were invited to participate regardless of a previous history of a cardiovascular event. Baseline carotid duplex scans were obtained in 282 women with SLE of which 214 had follow-up scans. Of the 282, ten women declined the follow-up exam, 43 were lost to follow-up, one woman had a carotid endarterectomy between baseline and follow-up, and 15 had technically inadequate scans. Each participant provided written informed consent and authorization for release of medical information. The study was approved by The University of Pittsburgh's Institutional Review Board. At baseline, each participant completed an interview, a physical examination, laboratory tests and a carotid duplex scan. The follow-up scans were repeated an average of 4.2 years (range 1.8-9.7 yrs) later.

Measurement of Covariates/Traditional CVD Risk Factors:

At the baseline clinic examination, age, race, education, smoking habits, family history of cardiovascular disease, diagnosis of diabetes, and post-menopausal status were documented. Post menopause status was determined by a history of total hysterectomy or amenorrhea for ≥ 1 year in women in the peri-menopausal age group. If post-menopausal status was uncertain, follicle-stimulating hormone levels were measured.

This visit also included anthropometric measurements (height, weight, and waist and hip circumference), two consecutive seated blood pressures (averaged) and a 12 hour fasting blood draw. Blood samples were used to measure total cholesterol, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol and triglycerides. Lipid assays were performed at the Heinz Lipid Laboratory in the University of Pittsburgh Graduate School of Public Health, which is certified by the Centers for Disease Control and Prevention. The Friedewald equation was used to estimate LDL cholesterol.(14) Plasma glucose levels were determined by enzymatic assay, and plasma insulin levels were measured by radioimmunoassay. Hypertension was defined as an average systolic blood pressure of ≥ 140 mmHg , an average diastolic blood pressure of ≥ 90 mmHg, or the use of antihypertensive agents. Metabolic syndrome (MBS) was defined using the National Cholesterol Education Program (NCEP) Adult Treatment Panel (ATP) III clinical guidelines(15) as the presence of three or more of the following components: waist circumference >88 cm, triglycerides $\geq$ 150 mg/dl, HDL <50 mg/dl, BP $\geq$ 130/85 mmHg, fasting glucose $\geq$ 11 mg/dl.

SLE-related disease risk factors:

SLE disease activity and cumulative organ damage were measured by the same physician using the Systemic Lupus Disease Activity Measure (SLAM)(16) and the Systemic Lupus International

Collaborating Clinics/American College of Rheumatology (SLICC) damage index (17). The SLICC index measures irreversible damage from lupus or its treatment in approximately nine organ systems. Because the disease endpoint for this study is carotid atherosclerosis, the SLICC damage score was modified (SLICC mod) to remove values for cardiovascular and peripheral vascular disease to avoid higher scores for women with known cardiovascular damage. The modified version results are reported.

Women also provided information on corticosteroid usage (past/current, maximum dose, and duration), and current use of hydroxychloroquine, immunosuppressants, and antidepressants. Immunosuppressant medications included cyclophosphamide, azathiaprine, cyclosporine and methotrexate. Renal disease was defined using the SLICC renal variables, which requires the presence of nephrotic-range proteinuria ($\geq$3.5 grams/24 hrs) or renal insufficiency (glomerular filtration rate <50%) for at least 6 months. Laboratory studies included tests for lupus anticoagulant (partial thromboplastin time or dilute Russell Viper Venom time with mix), serum complement 3 (C3), complement 4 (C4), anticardiolipin antibodies (IgG >15 standard IgG phospholipids units, IgM >10 standard IgM phospholipids units (Incstar, Stillwater, MN), and native DNA (dsDNA) antibodies (by *Crithidia luciliae*). Complement (C3, C4) activation is considered a critical and final common pathway of the immune and inflammatory processes leading to tissue destruction in SLE.

Inflammatory markers:

Serum albumin, C-reactive protein, and fibrinogen levels were measured at baseline. Fibrinogen was measured using a modified clot-rate assay, while an enzyme-linked immunosorbent assay was used for determination of C-reactive protein (CRP), and a dye binding assay for albumin.(18) These assays were done at the University of Vermont.

Carotid atherosclerosis measurements:

Carotid ultrasound was performed at the University of Pittsburgh Ultrasound Research Laboratory (URL) and has been previously described.(1;19) High resolution ultrasound machines from Toshiba (Model 140, Tustin, CA) equipped with a 5mHz linear array transducer were used. Vascular technologists were certified annually by performing overscans and overreads on ten participants per year. IMT intraclass correlation between sonographers was ≥ 0.90.

Sonographers imaged the near and far walls of the common carotid artery (CCA), the far walls of the carotid bifurcation and the proximal internal carotid (ICA) artery bilaterally. For each segment, the sonographer imaged multiple planes, selected the area of thickest IMT including any plaque, and digitized the image. IMT readings were performed for both baseline and follow-up exams, one month apart with a consistent reader using an automated edge-detection software (AMS, Sweden).(20) The IMT measurements were averaged across 1 cm of each vessel segment bilaterally. Values from the 8 measures were then averaged to obtain the mean average IMT. Reproducibility within readers was assessed across 12 participants. The intraclass correlation was 0.87. IMT change was assessed on a continuous scale.

Plaque was defined as a focal projection within the intima-media layer that was at least 50% greater than adjacent areas. The CCA, bulb and proximal ICA were used to report the number and grades of plaque, an estimate of severity. The number of plaques was totaled bilaterally and grades were also summed to create the plaque index (PI), an estimate of overall plaque burden. Previously reported intraclass correlation for plaque measures was 0.93.(21)

Plaque progression was defined as any increase in number or size of plaques. When progression was based on increased plaque size, scans were reviewed and progression was verified.

Change in number of plaques ranged from -1 to 4. The 11 women with negative change values were considered non-progressors.

Statistical analysis:

Descriptive statistics were reported as means [with standard deviations (SD)] and medians (with range) for continuous variables and as percentages for categorical variables. Univariate linear and logistic regression analyses were used to determine the association between baseline risk factors with IMT and plaque and change in these measures. Univariate associations were used to select the covariates for stepwise regression. The significantly associated variables ($p \leq 0.05$) were then used to build the final regression models. The multivariate progression models were adjusted for the baseline value (IMT or plaque) and time between scans. Progression outcomes were reported as both overall and annual changes. When multiple candidate variables were available to measure related characteristics (e.g. SBP and pulse pressure), the factor most significant (lowest p-value) in univariate analysis was selected for inclusion in the stepwise selection procedure. If distributional assumptions of the regression procedures were not met, variable transformations were considered. Statistical analysis was performed using SAS version 8.02 (SAS Institute Inc., Cary, NC). Standardized beta coefficients were reported.

3.4 RESULTS

Population Characteristics at Baseline:

The 282 women studied were predominantly White (89%), 10% were African American and 1% Asian or Eastern Indian (Table 3-1). This is a reflection of the racial composition of the Pittsburgh Metropolitan Area. They had a mean age of 45 years (SD 10.4). The average time between scans was 4.2 years (range 1.8-9.7) and average SLE duration was 10.5 years (range 0.5-41 years). Ninety-three (33%) were hypertensive (92% of these treated), 41 (14.5%) had a history of CVD, and 124 (44%) were postmenopausal at baseline. Among postmenopausal women, 59 (48%) were on hormone replacement therapy (HRT). Thirty-six percent of women had elevated total cholesterol levels (≥ 200 mg/dl) and 57% had elevated LDL levels (≥ 100 mg/dl). Of those with elevated cholesterol or LDL, less than 5% were being treated with cholesterol lowering medication. The modified SLICC damage score ranged from 0-8 (median 1) and the SLAM score ranged from 0-21 (median 6). The higher the SLAM and SLICC scores the more disease activity and organ damage, respectively. These values demonstrate a broad range of disease activity and damage in this relatively young cohort of women with SLE.

Prevalence and Progression of IMT and Plaque

Among women with baseline scans (n=282) the mean IMT was 0.64 mm (SD 0.14). Of those with two scans (n=214), the baseline mean IMT was 0.63 (SD 0.14) and follow-up was 0.68 mm (Table 3-1). The average total IMT change was 0.05 mm with an average annual IMT progression rate of 0.01 mm/year (SD 0.074mm, $p < 0.01$).

Prevalence of any plaque was 33% at baseline and 40% at follow-up. The majority of women showed no change in number of plaques (68%). An increase in number of plaques was seen in 27% while 5% of women (n=11) had a decrease of one plaque.

Risk Factors associated with IMT and IMT Progression

Numerous traditional as well as SLE-related risk factors were associated with higher baseline IMT (Table 3-2). The strongest ($p<0.001$) were older age, hypertension, higher blood pressure, larger waist circumference, longer years of steroid use, and higher modified SLICC damage index. In multivariate analysis (Table 3-3), the majority of variation in baseline IMT was explained by older age and higher modified SLICC index. No other risk factors added further predictive value to the model after these two variables were included.

A number of traditional and SLE-related risk factors were also associated with change in IMT including older age, years of steroid use, immunosuppressant non-use, and lower serum albumin (Table 3-2). In multivariate analysis (Table 3-3), independent predictors of greater IMT progression were older age, lower diastolic blood pressure, higher serum creatinine, and higher modified SLICC. Immunosuppressant non-use was borderline significant (p 0.08). Because kidney function is incorporated in the modified SLICC the model was re-run without creatinine. When this was done, the association between SLICC and IMT progression was highly significant (standardized beta 0.20, p 0.005).

Risk Factors for Plaque and Plaque Progression

Traditional and SLE-specific risk factors were also related to plaque at baseline (Table 3-2). The strongest ($p<0.001$) traditional risk factor associations were older age, postmenopausal status, higher

systolic blood pressure and higher pulse pressure. The strongest SLE-related risk factors associated with plaque were longer duration of SLE, longer years of steroid use, antidepressant use, and higher modified SLICC index. In multivariate analysis, factors independently associated with plaque presence at baseline were older age, ever smoking, higher systolic blood pressure, longer steroid use, and antidepressant use (Table 3-3). Many traditional and SLE-related factors were associated with progression of carotid plaque in univariate analyses (Table 3-2). Primary factors independently associated with plaque change in the multivariate analysis were older age and higher triglycerides (Table 3-3). Higher serum C3 values and duration of SLE disease were borderline significant (p 0.05 and p 0.07, respectively) (Table 3-3).

3.5 DISCUSSION

The average rate of IMT progression observed among women with SLE was 0.01 mm (SD 0.03) per year. SLE specific risk factors were related to both IMT and IMT change. This rate of IMT progression (0.01 mm per year) appears to be high in comparison to normal populations. Among age-similar women in the Women's Healthy Lifestyle Project (WHLP), rates of IMT progression were less (0.007 mm/yr).(22) SLE women in this group have rates closer to those seen among older women in the Atherosclerosis Risk in Communities (ARIC) study (aged 45-65). IMT progression rates were 0.0086 mm per year (23) for women in ARIC. Thus, the higher CVD event rates seen in women with SLE appear to be preceded by higher rates of subclinical disease progression. Because IMT is known to be predictive of subsequent CVD outcomes (8;24;25) these data suggest that measures such as IMT progression might be used as surrogate endpoints in clinical trials of SLE therapy.

Both higher serum creatinine and higher modified SLICC damage index were independent predictors of IMT progression. The association of SLICC with IMT progression decreased when creatinine was also in the model because the SLICC score includes kidney function. These findings suggest several things. First, the vasculature is a target of the generalized organ damage that occurs with SLE. Second, the renal damage caused by SLE either contributes to or is a marker of cardiovascular disease progression. The health of the kidneys and the vasculature are intimately entwined and the kidneys are particularly susceptible to the consequences of vascular aging.(26) Thus, monitoring kidney function likely provides useful information on the vascular effects of SLE. This idea is supported by cross-sectional data that have shown that impaired renal function is related to coronary artery calcification (11) and carotid atherosclerosis(27) among SLE women.

In univariate analysis, immunosuppressant use was associated with slower IMT progression. After controlling for age, this association became borderline, probably due to inadequate power. Only 37 women (13%) were on immunosuppressants at baseline. This is likely a reflection of the mix of university and community patients with SLE enrolled in the study resulting in many patients with mild disease. The association between immunosuppressants and vascular disease is consistent with cross-sectional data which found that cyclophosphamide non-use was associated with plaque among SLE women(7). These data together suggest that more aggressive treatment for SLE may slow vascular aging and premature CVD.

The 33% prevalence of carotid plaque found here is similar to other populations of SLE women (17%-40%).(1;5;7;8;27;28) These rates are higher than similar populations of non- SLE women where rates have been reported to be 11-25%.(7;8;29) The 33% prevalence found here is even higher than the 19.3% prevalence reported in an older population of women in The Aging Vascular Study (EVA, age 59-71 years).(30) Twenty-seven percent of our SLE women had plaque

progression, also higher than the 18.3% progression rate reported in EVA.(30) Thus, it appears that women with SLE develop lesions earlier and these lesions may progress faster than among normal women.

Both prevalence and progression of plaque were associated with SLE-related factors after controlling for age. It is likely that the heightened inflammatory process in SLE contributes to higher CVD. Elevated serum complement (C3) was found to be related to plaque progression, (p 0.05), while only of borderline significance. We have previously reported that high serum C3 levels are related to increased aortic stiffness in SLE women (28;31). Serum C3 has also been found to be associated with coronary calcification.(11). These observations are particularly surprising because decreased levels of C3 (precursor of complement activation) are traditionally associated with lupus pathogenesis. There are two possible explanations for our observation that increased serum levels of C3 are associated with carotid plaque progression and vascular stiffness in lupus. First, it may reflect an acute phase response. We believe this is unlikely because other acute phase proteins such as CRP and fibrinogen were not associated with plaque or vascular stiffness in the same patients. Second, activation of the complement system may actually contribute to plaque formation and increased vascular stiffness. It is recognized that inflammation is involved in all stages of atherosclerosis development and complement is the final common pathway in all physiologic and pathophysiologic inflammatory processes.(32-35) One direct mechanism includes increased endothelium permeability, leading to plasma protein influx into the arterial wall.(36) Indirectly, serum C3 may be involved through stiffening of the vasculature that later creates an environment where plaques are more likely to form. SLE-related inflammatory abnormalities may also function synergistically with traditional risk factors, making them particularly harmful.

Longer steroid use also increased risk of plaque and this is consistent with other studies.(1;4) Similar research has found longer steroid use to also be associated with higher IMT compared to controls(8) and with CAD(37). Conversely, Roman found less use of steroids to be associated with plaques(7) and Asanuma found no relation between steroid use and CAC.(9) Although the anti-inflammatory effects of steroids may be beneficial, these agents have atherogenic potential due to the effects on total cholesterol, lipoproteins, blood pressure, adiposity, or through modification of the pathogenesis of atherosclerosis.(38-40) Longer use of steroids did not influence plaque progression.

Psychosocial stressors may also influence CVD progression in SLE women. Our finding that anti-depressant use was associated with plaque is intriguing, but difficult to interpret, since antidepressants may be used for reasons other than treating depression, including pain management in patient with SLE. Data on depression was not collected at baseline. However, non-SLE studies have repeatedly shown evidence that depression significantly contributes to the pathogenesis and expression of coronary artery disease (CAD) (41-44) as well as carotid plaques (45) and coronary calcification. (46) Further investigation into the effects of depression on CVD in SLE is warranted.

It should be considered that our population represents mostly Caucasians with milder cases of SLE than seen in a rheumatology practice. Confirmation of SLE diagnosis was made by medical record review and the number of women on immunosuppressant therapy was low. It is probable that progression rates of carotid plaque and IMT may be higher in other SLE cohorts.

In conclusion, both carotid IMT and plaque progression can be measured using B-mode ultrasound in women with SLE. IMT and plaque progression appear to be greater than non-SLE historical controls. Rates of progression are related to both SLE-related and traditional risk factors and these two may work in concert to increase CVD risk. The clinical significance of this study impacts CVD prevention among SLE women. It appears that more aggressive treatment of SLE

results in slower progression of CVD. Additionally, monitoring kidney function likely provides useful information on the vascular effects of SLE, detected by changes in carotid IMT. This study provides data to support the use of carotid B-mode ultrasound as a surrogate endpoint in future clinical trials examining the efficacy of new biologic and immunomodulatory therapies for SLE.

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Table 3-1: Project 2 Population Characteristics at Baseline

N=282

Baseline Risk Factors	**n**	**Percent**			
White race %	248	88%			
Level of education					
≤ 12	102	33%			
13-16	125	44%			
>16	55	20%			
Postmenopausal, % **Hormone Replacement Therapy** (n=postmenopausal women)	124 59	44% 48%			
Ever Smoke, %	124	44%			
Steroid use ever, %	264	94%			
Current steroid use, %	159	44%			
Current Immunosuppressant meds % *	37	13%			
Current Antidepressant use	63	22%			
Hypertension, % *	93	33%			
Metabolic Syndrome, % *	64	23%			
	n	**Mean (SD)**		**Median**	**Range**
Age, years	282	45.0 (10.9)		44.8	20-75
SLE duration, years	279	10.53 (7.3)		9.3	0.5-41.4
Steroid use in years	256	6.07 (6.8)		3.75	0-36
Systolic Blood Pressure, mmHg	282	120.0 (18.2)		120.0	85-224
Diastolic Blood Pressure, mmHg	282	78.17 (10.5)		78.5	48-110
Pulse Pressure, mmHg	282	42.03 (13.0)		40.0	15-120
Cholesterol, mg/dl †	282	193.88 (40.8)		186.5	106-384
HDL, mg/dl †	282	56.56 (16.2)		54.9	21.3-119
LDL, mg/dl †	282	111.39 (33.6)		105.0	39-227
Triglyceride, mg/dl †	282	127.42 (83.9)		105.0	33-802
BMI, kg/m^2 †	280	27.73 (7.1)		26.34	17.03-68.97
Serum Creatinine, mg/dl †	282	1.03 (1.3)		0.80	0.4-13.7
Fibrinogen, mg/dl †	268	300.82 (72.6)		294.5	136-702
C-reactive protein, mg/ml	271	4.42 (8.2)		2.10	0.2-89.8
Complement C3, mg/dl †	281	94.81 (24.4)		94.0	30-177
Complement C4, mg/dl †	281	21.68 (7.9)		21.0	6-47
Albumin, mg/dl †	282	4.68 (0.4)		4.7	2.9-5.7
WBC, 1000/mm^3 †	281	5.87 (2.3)		5.4	1.8-15.0

Table 3-1 con't

Baseline Risk Factors	n	Mean (SD)	Median	Range
SLAM score	280	6.86 (3.7)	6.0	0.0-21.0
SLICC damage score, modified	281	1.28 (1.6)	1.0	0.0-8.0
Baseline mean IMT (mm)	277	0.643 (0.1)	0.603	0.44-1.48
Follow-up mean IMT (mm)	214	0.683 (0.2)	0.650	0.48-1.51
IMT yearly change (mm)	214	0.011 (0.023)	0.009	-0.17 -0.11
Time between scans (years)	214	4.19 (2.0)	3.99	1.81-9.70

*__Immunosuppressant meds__ included cyclophosphamide, azathiaprine, cyclosporine and methotrexate.

Metabolic syndrome as defined by Adult Treatment Panel (ATP) III clinical guidelines: Three or more: waist circ. >88 cm, triglycerides $\geq$ 150 mg/dl, HDL <50 mg/dl, BP $\geq$ 130/85 mmHg, fasting glucose $\geq$ 11 mg/dl.

Modified SLICC: excludes cardiovascular and peripheral vascular components. **Hypertension** was defined as average systolic blood pressure of $\geq$140 mmHg , an average diastolic blood pressure of $\geq$90 mmHg or the use of antihypertensive agents.

† To convert values to SI units, multiply: cholesterol, HDL, LDL by 0.0259 for mmol/L, triglycerides by 0.0113 mmol/L, creatinine by 88.4 μ mol/L, fibrinogen by 0.0294 μ mol/L, complement C3 and C4 by 0.01 g/L, albumin by 10 g/L, and WBC by 1.0 x10^9/L.

Table 3-2: Project 2 Univariate Relationship of Traditional CVD Risk Factors with IMT and Plaque

Baseline N=282, Change N=214

	Baseline IMT		IMT Change		Plaque Presence y/n			Plaque Change y/n		
Traditional CVD risk factors at baseline	**Standardized Beta coefficient**	**p-value**	**Standardized Beta coefficient**	**p-value**	**Odds Ratio**	**95% CI**	**P-value**	**Odds Ratio**	**95% CI**	**P-value**
Age	0.60	<0.01	0.14	0.04	1.12	1.09-1.16	<0.01	1.09	1.05-1.13	<0.01
SES, Level of education	-0.14	0.02	-0.05	0.49	0.92	0.81-1.03	0.14	0.77	0.66-.090	<0.01
Postmenopausal	0.45	<0.01	0.12	0.07	4.59	2.69-7.84	<0.01	3.66	1.94-6.90	<0.01
Current Smoke	-0.08	0.20	0.05	0.43	1.35	0.67-2.71	0.40	1.81	0.77-4.26	0.17
Ever Smoke	0.17	0.01	0.10	0.16	2.28	1.37-3.72	<0.01	1.44	0.78-2.64	0.24
Metabolic Syndrome	0.12	0.04	0.12	0.07	1.78	0.65-2.13	0.58	1.59	0.79-3.20	0.19
Diabetes	-0.02	0.77	<-0.01	0.96	0.68	0.14-3.44	0.64	4.78	1.10-20.69	0.03
Hypertension	0.24	<0.01	<-0.01	0.94	2.07	1.25-3.46	<0.01	2.16	1.67-4.01	0.01
Systolic Blood Pressure	0.31	<0.01	-0.08	0.23	2.59	1.79-3.74	<0.01	2.15	1.40-3.31	<0.01
Diastolic Blood Pressure	0.12	0.05	-0.13	0.06	1.03	1.01-1.06	0.01	1.04	1.01-1.07	0.01
Pulse Pressure	0.34	<0.01	-0.02	0.76	1.06	1.04-1.09	<0.01	1.04	1.02-1.07	0.002
Cholesterol	0.21	0.01	-0.02	0.75	1.01	1.00-1.02	0.01	1.01	1.00-1.02	<0.01
HDL	0.07	0.26	-0.02	0.73	1.01	0.99-1.02	0.27	1.00	0.98-1.02	0.64
LDL	0.14	0.01	-0.02	0.78	1.01	1.00-1.02	0.06	1.01	1.00-1.02	0.01
Triglyceride	0.15	0.01	-0.01	0.92	1.00	1.00-1.01	0.07	1.01	1.00-1.01	0.01
Insulin	0.12	0.04	0.08	0.23	1.02	0.99-1.05	0.15	1.02	0.99-1.06	0.16
BMI	0.19	<0.01	0.12	0.09	1.05	1.02-1.09	0.01	1.04	1.00-1.09	0.05
Waist	0.21	<0.01	0.10	0.14	1.02	1.01-1.04	0.01	1.02	1.00-1.04	0.01

Table 3-2 con't

SLE - Related	Standardized Beta coefficient	p-value	Standardized Beta coefficient	p-value	Odds Ratio	95% CI	P-value	Odds Ratio	95% CI	P-value
SLE duration, years	0.20	<0.01	0.11	0.11	1.05	1.01-1.08	0.01	1.05	1.01-1.10	0.01
Steroid use ever	-0.02	0.74	0.07	0.33	2.54	0.72-9.02	0.14	1.26	0.34-4.77	0.72
Current steroid use	0.03	0.62	-0.01	0.84	1.18	0.72-1.95	0.51	1.07	0.58-1.96	0.83
Steroid use in years	0.29	<0.01	0.15	0.04	1.08	1.04-1.12	<0.01	1.02	0.99-1.07	0.35
Immunosuppressant meds	-0.09	0.15	-0.15	0.03	0.66	0.29-1.46	0.29	1.78	0.79-4.05	0.16
Antidepressant use	0.14	0.02	0.02	0.80	2.39	1.34-4.26	<0.01	0.83	0.38-1.83	0.65
Serum Creatinine	0.12	0.04	0.12	0.07	1.73	0.80-3.75	0.16	2.39	0.52-11.11	0.26
C-reactive protein	0.09	0.14	-0.04	0.60	1.01	0.98-1.04	0.46	1.01	0.98-1.04	0.55
Complement C3	0.18	<0.01	0.03	0.69	1.01	1.00-1.02	0.38	1.02	1.01-1.03	<0.01
Complement C4	0.17	<0.01	0.07	0.29	1.02	1.00-1.05	0.23	1.04	1.00-1.08	0.03
Albumin	-0.09	0.12	-0.14	0.04	0.76	0.40-1.42	0.38	0.99	0.46-2.13	0.98
SLAM score	-0.03	0.65	-0.05	0.51	0.93	0.86-1.00	0.04	1.06	0.98-1.15	0.15
SLICC (modified)	0.21	<0.01	0.11	0.09	1.20	1.03-1.40	0.01	1.23	1.01-1.48	0.03

Table 3-3: Project 2 Age-Adjusted Relationships of CVD Risk Factors to IMT and Plaque

	IMT				PLAQUE			
Risk Factors	**Baseline (N=282)**		**Change * (n=214)**		**Presence at Baseline**		**Progression y/n***	
	STB †	**p-value**	**STB**	**p-value**	**OR‡ (CI)**	**p-value**	**OR**	**p-value**
Age per 5 yrs	0.587	<0.001	0.318	<0.001	1.61 (1.35-1.21)	<0.001	1.30 (1.06-1.58)	0.012
Ever smoke	——	——	——	——	1.94 (1.06-3.55)	<0.001	——	——
Systolic blood pressure per 10 mmHg	——	——	——	——	1.24 (1.03-1.50)	0.02	——	——
Diastolic blood pressure per 10 mmHg	——	——	-0.172	0.01	——	——	——	——
Serum Creatinine mg/dl	——	——	0.189	0.005	——	——	——	——
Steroid use per 5 years	——	——	——	——	1.34 (1.06-1.68)	0.01	——	——
Current Antidepressant use	——	——	——	——	2.05 (1.03-4.08)	0.04	——	——
Complement C3 per 25 units							1.43 (1.00-2.05)	0.05
Current Immunosuppressant use	——	——	-0.124	0.08	——	——		
SLE duration per 5 years							1.23 (1.00-1.57)	0.07
Triglycerides per 25 mg/dl	——	——	——	——	——	——	1.67 (1.03-1.32)	0.014
Modified SLICC per 1 unit	0.131	0.007	0.145	<0.05	——	——	——	——

*adjusted for baseline value and time between scans †STB=standardized beta coefficient ‡OR=Odds Ratio

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4 INTRODUCTION TO PROJECT 3

Plaque rupture is the primary cause of coronary events. Thus, plaque characterization is a new way to identify characteristics of vulnerable plaques. With the recent advances in ultrasound technology and customized software, plaque components are able to be identified and have been correlated with histology. Subjective scales and noncustomized software have been used in the first attempts to study plaque characteristics. These methods have suggested that plaque characteristics are more important than percent stenosis in predicting events, yet more studies are needed. The computer assessment methodology to characterize plaques needs further development and will likely be beneficial for analyzing carotid artery plaques noninvasively in the future. A first step to using computer assessment methods is to develop and standardize protocols for use in population-based research. At the Ultrasound Research laboratory at the University of Pittsburgh, carotid ultrasound is used to study atherosclerosis. Plaque characterization is not yet included in the carotid ultrasound protocol and would add a new dimension to our research efforts. This section describes the components of implementing plaque assessment in the lab. It includes the following: 1) development and use of scanning, and reading protocols 2) training of staff in use of scanning and reading protocols 3) development and refinement of reading software, and 4) design and analysis of reproducibility study for the new measures.

4.1 MECHANISMS OF VULNERABLE PLAQUES

Plaque composition and vulnerability have emerged as being the primary factor underlying cardiovascular events.(1;2) To date, soft and lipid-rich plaques have been reported to be more likely than fibrous or collagen-rich plaques and calcium to rupture or cause symptoms. (3-5) More research focusing on plaque morphology is needed to identify the types of plaques that cause events. However, tools to evaluate plaque characteristics obtained by noninvasive methods are just recently available.

What makes one lesion vulnerable compared to another is not completely understood and it is an ongoing focus of research. Acute coronary syndromes are caused by three main mechanisms that cause dangerous thrombi. Of these, plaque rupture (65-70%) is the most common, (6;7) plaque erosion (25-30%) is second and calcified nodules that protrude into the lumen (2-5%) are seen less often.(4) Plaques typically have a fibrous cap that covers the lesion and this often becomes thin, making it vulnerable to rupture. (1;4) Rupture activates two processes when the lipid core becomes exposed. First, a mechanical process occurs in which platelets quickly adhere to the injured vascular surface and fibrin and platelets rapidly accumulate to form a thrombus. The thrombus occludes the vessel lumen and restricts flow distally causing an acute coronary syndrome. The second process is an inflammatory process beyond the focus of this dissertation.

To prevent events, a tool which will allow identification of high risk plaques *before* symptoms occur is needed. Ultrasound and computer software used together have the potential to meet this goal. Because disease in the carotid arteries is a marker of coronary artery disease, investigators have begun to use carotid ultrasound as a way to noninvasively study plaque

characteristics. Carotid plaque characteristics have been studied preoperatively with ultrasound and compared postoperatively with histological assessment with good agreement. The scales have been primarily subjective and difficult to reproduce or track changes over time. Plaques that appear echolucent (black) are correlated with hemorrhage and lipid cores (8-11) and are often present with cerebrovascular symptoms. Other studies are similar, reporting that thrombus, fresh blood, and lipids are present in vulnerable carotid lesions.(6;7;12-17) Most of these studies have used subjective ultrasound classifications to characterize plaques. The reproducibility of subjective scales are fair and therefore, a few studies have begun using nonspecialized imaging software to assess plaque characteristics. One problem with these tools is that they are not able to discern normal ultrasound signals from ultrasound artifacts and signal noise. Computerized plaque analysis is a new tool that will improve plaque characterization in vivo and improve standardization of plaque assessment for research.Thus, this dissertation takes steps to improve and provide specialized quantitative software to evaluate carotid plaque characteristics for CVD research. Automated software will provide a way to monitor plaques for clinical trials of atherosclerotic interventions.

4.2 EVOLUTION OF PLAQUE MEASUREMENTS

Early in the 1980's, a few studies assessed carotid lesions from symptomatic patients who underwent a carotid endarterectomy. Ultrasound measurements were performed before the surgery, scored with a subjective scale and compared to the histology reports.(11;18) In the 1990's ultrasound technology improved resolution improving subjective scoring of plaques with histology. (6;19;20) Plaque composition was also being studied with invasive and other noninvasive technologies such as angiography, (20) magnetic resonance (MR) imaging,(21-24) and spiral CT(25) Studies were suggesting that hypoechoic carotid plaques were seen more often in patients with cerebrovascular symptoms than in those without symptoms. However, standardization and reproducibility remained unresolved issues in the use of these techniques. In the last several years, investigators have moved to commercially available, general image assessment software to analyze carotid plaques.(8;26-28) Although these packages were not made specifically for evaluating atherosclerotic lesions, they showed promise. With ultrasound and image software, the gray scale median (GSM) values have been used to quantify carotid plaque characteristics. The initial studies reported that lower GSM's are associated with cerebrovascular symptoms and now specialized software is available for plaque characterization. Project three of this dissertation implements the use of the software by 1) developing scanning, digitizing and reading protocols, 2) training and implementing computer software specifically developed to evaluate carotid plaque characteristics, and 3) developing and implementing a pilot study to assess the reproducibility of the new measures.

4.3 CHARACTERIZING PLAQUES

The *Gray-Weale score* (GWS) is a subjective scoring system used specifically to characterize the plaques. A basic understanding of how plaques are described is helpful to be able to understand the GWS. The basic descriptors are as follow:

Echolucent or hypoechoic = appear black on ultrasound
Echogenic or hyperechoic = appear white on ultrasound
Heterogeneous has mixed echogenicity = appear black and white, sometimes calcified
Homogeneous is uniformly echogenic = either dominantly black, white or gray

The GWS used the above descriptors and assigns a number (1-4) to each plaque according to the following guidelines: *type 1*, dominantly echolucent; (similar to blood) *type 2*, dominantly echolucent with small areas of echogenicity; *type 3*, substantially echogenic with few echolucent spots; or *type 4*, uniformly echogenic(11) and for this project, a *type 5* was added meaning, unclassified, due to artifacts or poor image quality. These will be described in more detail in the training section of this dissertation.

To improve plaque characterization research, the tests must be 1) valid 2) standardized and 3) reproducible. Computerized software is the logical choice to improve plaque assessment and it meets these criteria. In most image assessment programs, no mechanism exists to deal with sources of error such as artifacts, commonly seen on carotid ultrasound images. The software used in this project reduces error due to artifacts and partnered with carotid ultrasound, carotid plaque assessment may be a promising methodology to characterize plaques.

4.4 DEPARTMENT OF EPIDEMIOLOGY ULTRASOUND RESEARCH LABORATORY (URL)

The Ultrasound Research Laboratory (URL) at the University of Pittsburgh, has been performing carotid ultrasound in epidemiological studies since 1985, beginning with the Systolic Hypertension in the Elderly Program (SHEP) and the Cardiovascular Health Study (CHS). The Laboratory (URL) was established with the goal of providing a high volume of quality ultrasound studies for research purposes. Staffing includes three full-time registered vascular technologists, and two part-time technologists who read studies and provide additional support. The technologists are trained and certified in all testing procedures provided by the laboratory. The lab supports 13 ongoing studies funded by the NIH, the American Heart Association and the National Arthritis Foundation. In 2005, our total test volume was 1710 tests on 1100 participants.

The laboratory offers subclinical atherosclerosis measures using carotid ultrasound technology that focuses on intima media thickness (IMT) a well established subclinical measure. In the past identification of plaques was adequate but in light of newer research, more specific plaque assessment would benefit the studies. Thus, to add a measure of plaque characterization to the capabilities of the URL would improve research methods. We believe the addition of plaque assessment will lead to better assessment of CV risk and improve preventative and treatment of patients.

4.5 FINAL OVERVIEW OF PROJECT THREE

Better prevention is needed to identify people at risk for cardiovascular disease who are asymptomatic. Past research has provided the first steps into effective CVD prevention and mortality reduction with IMT measures. Yet, new risk assessment tools need to be developed since half of all heart attacks occur in individuals with normal risk factor levels. The study of plaques in vivo has emerged as a promising avenue to further identify at-risk patients for CV events. This project proposes to use the various subclinical atherosclerotic measures to assess two high risk populations and to develop protocols for a novel tool used to characterize carotid plaques. Successful use of this new tool will allow improved plaque assessment in clinical trials and move ultimately improve CVD prevention efforts.

A summary of the data collection that will yield analysis for a final assessment will result in the following measures for plaque analysis:

1. Gray-Weale Scale (GWS) (subjective measure)
2. Gray Scale Median (GSM) (quantitative measure)
3. Calcification prevelance
4. Echocucent vs. echogenic (based on computerized quantitative measure %WHITE)

4.6 ULTRASOUND INSTRUMENTATION

High resolution ultrasound (US) machines are used to obtain quality images for research purposes. Ultrasound transducers contain piezoelectric crystals that convert pressure waves into electrical signals and go through complex algorithms to display images on a monitor. Simplistically, the waves are emitted from the transducer into the tissue, and bounce back with varying degrees of intensity based on tissue density. The machine measures the intensity and an image is displayed using 255 shades of gray on a television-like monitor. Blood density, for example, is displayed with black pixels and soft tissue has varying degrees of black pixels that appear white or shades of gray.(**Figure 4-1**) This image is called the grayscale image or B-mode image (Brightness mode).

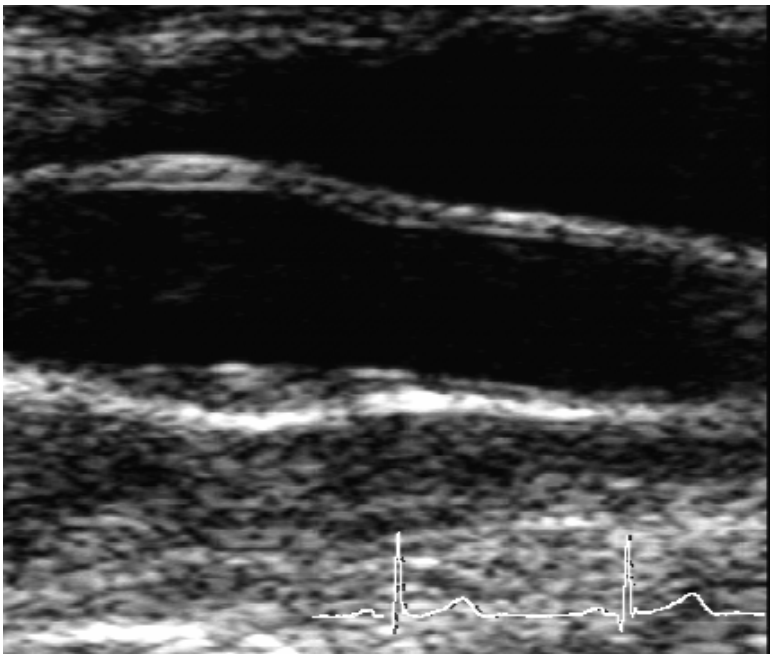


Figure 4-1 Different pixel intensities as tissue density changes

4.7 METHODS

Scanning Protocol

The participant is placed supine with the knees supported and the sonographer at the head of the bed. A standard lead II electrocardiogram rhythm is gathered throughout the exam. The carotid system is imaged from the base of the neck to the most cephalad portion possible. The regions of interest include the distal common carotid artery, the carotid bifurcation and the internal carotid artery (Figure 4-2) A carotid artery plaque is defined as a focal projection into the lumen that is 50% or greater than adjacent wall thickness or a thickness of >1.5mm. The sonographer completes the plaque worksheet as each segment or side is scanned.(Appendix D) The image containing the plaque is optimized using hand movements, time-gain-compensation, and head maneuvers until the full plaque is seen with intact shoulders. A cineloop (memory) is captured and replayed frame-by-frame. The optimum image is digitized in end diastole and saved for off-line scoring at a later time. One challenge for plaque assessment is to optimize the entire plaque for digitization while clearly displaying the interfaces. The shoulders are the areas before and after the plaque that attach to the intima-media surfaces and should be well visualized. (Figure 4-3)

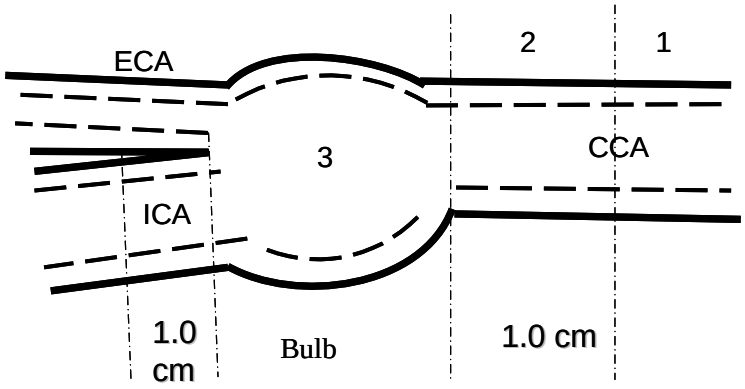


Figure 4-2: Schematic of carotid artery

Section 1, from the origin of the common carotid artery (CCA) to 2 cm proximal to carotid bifurcation; 2, from 2 cm proximal to carotid bifurcation to beginning of carotid bifurcation bifurcation (point where the near and far walls of artery are no longer parallel); 3, from beginning of carotid bifurcation to flow divider; 4, first centimeter of internal carotid artery (ICA), measured from tip of flow divider; 5, first centimeter of external carotid artery (ECA), measured from tip of flow divider

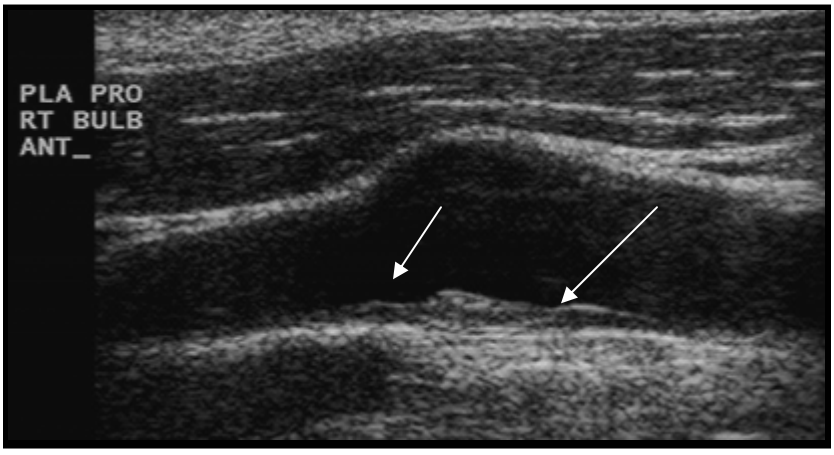


Figure 4-3: Plaque Shoulders

4.8 READING PROTOCOL

The image is imported into the Automated Measurement Software (AMS, Sweden) and calibrated to set the range of gray scale specific for that image. Calibration is accomplished by selecting an area that is black (anechoic) (typically within the lumen) and a second area that is the white (echoic), such as the adventitia. The software sets the scale with these two measures which is then used to assign the gray scale intensity for each pixel within the plaque. The reader zooms the image, outlines the plaque along the lumen-intima interface and along the media-adventitia interface. The plaque ends are identified where the plaque becomes 50% of the adjacent IMT and the region of interest is closed with vertical lines at each end. The plaque is now encapsulated and ready for assessment. The software is then prompted to assess each pixel within the plaque based on the initial scale and values are produced using complex algorithms. The quantitative scores are determined electronically and reported as *percent white* and *gray scale median*. Also, a descriptive (echogenic vs. echolucent) score is assigned based on the quantitative data. The reader also subjectively scores the plaque just prior to the software scoring. The full technical step-by-step protocol is in Appendix B.

The automated outcome measures generated by the software are 1) the gray scale median (GSM), 2) the percent white value and 3) plaque classification. The GSM and percent white values are continuous variables and the plaque classification is a binomial variable.

4.9 SOFTWARE CONSIDERATIONS

The AMS software is different than the conventional image software being used for research currently. These software use the normalized mean intensity but this does not work properly

because of the two types of artifacts seen with ultrasound. Based on an analysis of the distribution of pixel intensities inside the plaque, a grayscale threshold is automatically selected so that the plaque is segmented into white and dark points (a so called binary plaque image).

The percentage of white pixels is calculated and used as a feature for classifying the plaque as being echolucent (low percentage of white) or echogenic (high percentage of white). The classification of plaque into echolucent and echogenic is based on a detailed analysis of the pixel intensities inside and outside the plaque. There are three different aspects of this analysis. The first aspect concerns the percentage of "white" pixels inside the plaque. On ultrasound, noise is represented by snow that can be within the lumen and also within the plaque. The AMS software compensates for this artifact by "removing" the snow.

The second aspect concerns a measure of white artifacts inside the lumen, similar to the noise described above. The underlying idea is to compensate for this type of artifact. In short, if the plaque contains the same type of white artifacts as inside the lumen, it should not be taken into account in the calculation of percentage of white.

The third aspect concerns shadows under the plaque. If those shadows are caused by bright reflectors inside the plaque, the dark part inside the plaque and under the reflector should not be included in the calculation of percentage of white. The AMS software also compensates for this by "removing" the shadows. By eliminating these errors, the percent white value is more robust than using the gray scale median.

4.10 TRAINING AND CERTIFICATION

Image Capture

Image quality is of utmost priority when performing subclinical atherosclerosis measures with ultrasound. Images must be clear, showing continuous interfaces and distinct plaques. Gains must be carefully adjusted for each image to avoid missing any plaques and to prevent artifacts that can be mistaken for plaques. Hand maneuvers to that assure excellent images are not intuitive and must be taught, practiced and perfected. We changed our protocol to capture plaque images with clear interfaces and adequate distinct shoulders. While implementing this change, we reviewed captured images during the learning process until the staff was proficient acquiring plaque images. Over an eight month period the staff collected images of all plaques from any research study participant tested in the lab. The standard research protocol was used with the addition of digitized images of all plaques on each scan.

Subjective Plaque Scoring (Gray Weale Scale)

Plaque scoring involves two separate processes: subjective scoring and electronic scoring. I started with subjective scoring as a first step to understand plaque characterisitics. For that we used the Gray-Weale scoring system. Training included several sessions of didactic training and review of many sample images.

We adopted the GWS that includes the standard four categories and added a fifth category to indicate that the image was not able to be scored due to artifacts. Category 1 of the GWS was a plaque that is mostly echolucent, appearing mostly black **(Figure 4-4)** on ultrasound exam. Category 2 represents a plaque that is heterogeneous, consisting of more echolucency

than echogenicity **(Figure 4-5)**. Category 3 represents a plaque that is also heterogeneous, but consists mostly of echogenic material and has less than 25% echolucent areas within it **(Figure 4-6**) Category 4 represents a plaque that appears mostly echogenic (Figure 4-7). Category 5 is a plaque that cannot be categorized due to shadowing.

Figure 4-4: Schematic of plaque, category 1 of the Gray-Weale Scale. The plaque is mostly echolucent. For corresponding ultrasound image: **Figure 4-8**

Figure 4-5: Schematic of plaque, category 2 of the Gray-Weale Scale. The plaque is heterogenous and mostly echolucent with some echogenic material. For corresponding ultrasound image:**Figure 4-9**

Figure 4-6: Schematic of plaque, category 3 of the Gray-Weale Scale. The plaque is mostly echogenic with <25% of echolucency. For corresponding ultrasound image: **Figure 4-10**

Figure 4-7: Schematic of plaque, category 4 of the Gray-Weale Scale. The plaque is mostly echolucent. For corresponding ultrasound image: **Figure 4-11**

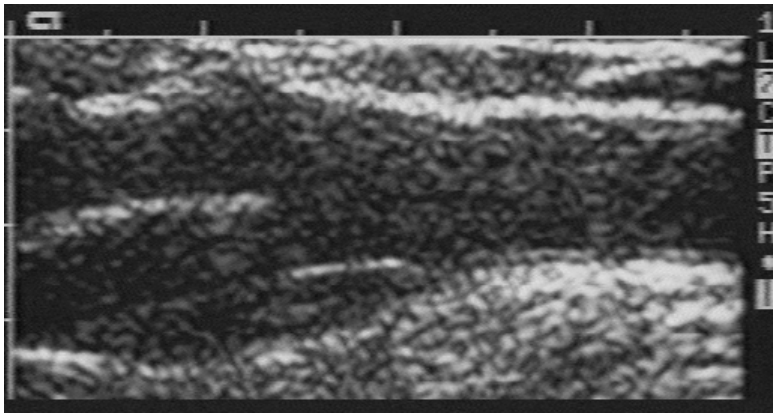


Figure 4-8: Category 1 of GWS: Echolucent plaque. Plaque would not be visible without high gains

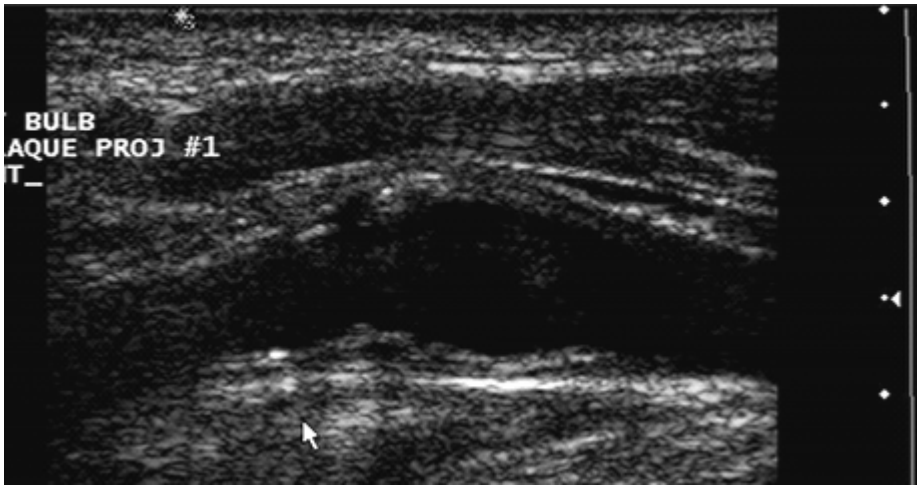


Figure 4-9: Category 2 of GWS: Mostly echogenic (white) but has less than 25% of echolucency

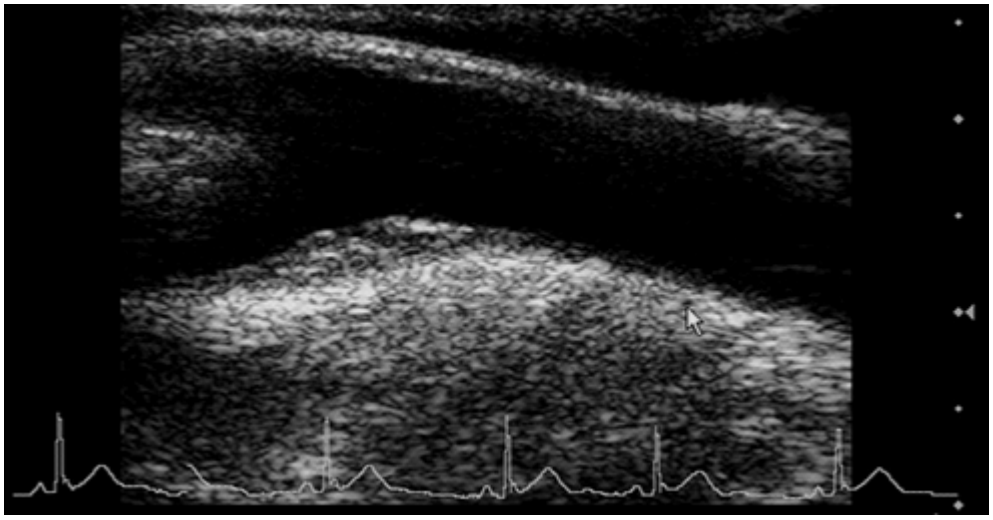


Figure 4-10: Category 3 of the GWS: Mostly echogenic with a small amount of echolucency

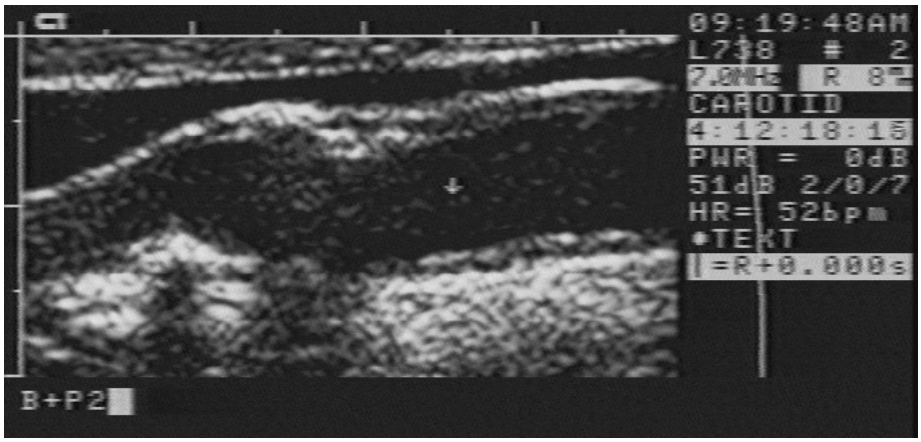


Figure 4-11: Category 4 of the GWS: Mostly echogenic. Notice the shadowing which indicates calcification**.**

After several training and practice sessions the two certified sonographers read several different groups of plaque images and the percent agreement was assessed. After reading each group, the staff met regularly to discuss their results and problems they faced. These meetings and ongoing scoring improved agreement over time from 27% to 77% (Table 4-1) over an 8 month period.

Table 4-1: Project 3 Percent agreement of GWS between readers over 8 month training period

Group	Number Agreed	Number of Images	Percent Agreement
1	4	15	27%
2	7	15	47%
3	9	13	69%
4	8	11	73%
5	16	28	57%
6	23	30	77%

4.11 AUTOMATED PLAQUE CHARACTERIZATION

Automated plaque assessment training was performed in several phases. I obtained the software and was self-trained by reading the manual and experimentation. While experimenting I used email to pose questions to the developers. After I was familiar with the software and practiced reading images, I had two telephone calls with the developer to answer additional questions, discuss problems I encountered, and review the results. I wrote and standardized the capturing and reading protocols and then trained the staff on these protocols.

To standardize plaque length I decided at what point the increase in IMT became plaque and this was used as the longitudinal plaque endpoints. We standardized closing the region of interest with vertical lines at each end of the plaque. Last, we standardized the posterior portion

of the ROI to be as close to the pixel above the hyperechoic demarcation of the adventitial border (Figure 4-12)

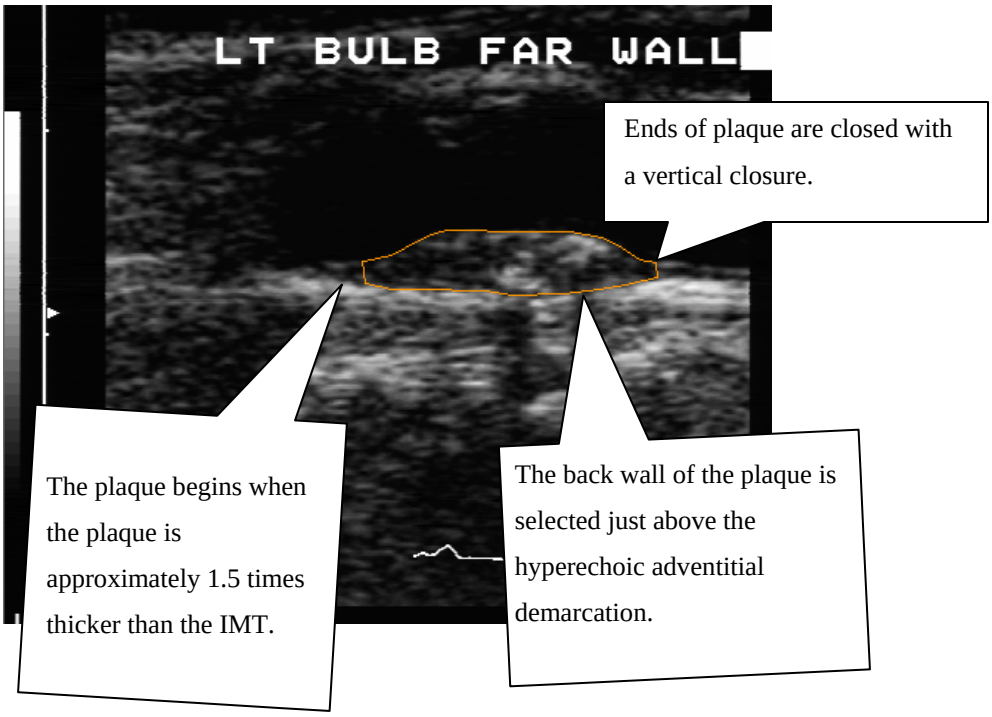


Figure 4-12: Selection of plaque region of interest

4.12 REPRODUCIBILITY OF PLAQUE MEASURES

A pilot study to assess the reproducibility of the automated plaque assessment between readers was executed (see Appendix G). The automated reading was performed on the same 50 plaque images by three readers. Two were full-time certified sonographers and one was a nonsonographer but trained in subclinical methodology. Both the sonographer and nonsonographer repeatability was tested to see whether a specially trained sonographer was required to read plaque images. The results were analyzed to test agreement between the readers. Briefly, spearman correlation was assessed for the gray-scale median and the percent

white variable. Comparison of the two full-time sonographers yielded a Pearson correlation of the GSM 0.88. The intraclass correlation (ICC) showed that 86% of the variation in GSM is due to the differences between participants. For the more robust percent white variable, the Pearson correlation was 0.89 with 89% of the variable due to the difference between participants. The subjective Gray-Weale scores correlated 82% of the time. The kappa statistic for between readers for the automated plaque categorization was 0.61, which represents good agreement.

After analysis, the readers reviewed each image and discussed problems they experienced. As a group, we discussed the differences in reading and I conducted remedial training to further standardize the protocol. During this training, we discovered several ways to standardize the technique to improve replication. First, the plaque images were not consistently zoomed before encapsulating the plaque for analysis. We now require the images to be zoomed to see the plaque borders more clearly. Second, the media-adventitia line was sometimes drawn one pixel inside the medial border and other times it was drawn one pixel outside the medial border, thus including the adventitial wall. When the latter was done, the percent white value was erroneously higher. After analyzing how this affected one of the main outcome measures (percent white), we decided to draw the media-adventitia line of the plaque, just inside the medial border, thus avoiding a false increase in the percent white value, by including the pixels from the adventitial interface. The third change was when the user selected the region of interest for setting the threshold value. Some users used a large box while others made a small box, just large enough to include the vessel and plaque. The software developers recommended using the largest box possible to improve setting the threshold value, based on the algorithms. We standardized each of these steps and the two full-time sonographers plan to reread the same 50 images.

The automated software improved the agreement between readers for the plaque categorization 87% vs. 27% when the staff categorized the plaques. The percent white quantification is slightly more robust than the grayscale median.

The tentative goals for plaque reproducibility are: ≥ 90 for both Pearson correlation and the intraclass correlation for the GSM and percent white measures. A Kappa statistic of >.75 will be the tentative goal for the automated computerized classification for plaques. This value represents excellent agreement.(29)

4.13 QUALITY CONTROL

Quality control (QC) processes help prevent minor drifts in scanning and reading techniques that have been known to jeopardize image quality and progression data. QC is an essential component of any research facility and the URL has several processes setup. First we assess the scanning process by one sonographer over scanning five participants for all the other sonographers on a quarterly basis. A constant reader reads all images. We can evaluate if any plaques are missing and if the plaque characterization is similar. As a second piece of QC we review the tapes of 10 scans quarterly and give direct feedback to the sonographers regarding plaque identification and protocol adherence.

Sonographers are recertified yearly for both scanning and reading. All sonographers scan the same participants and each sonographer reads all the images which allows between sonographer correlations as well as between reader correlations for all measures.

5 WORK TO BE DONE AFTER FINAL DEFENSE

Several goals are planned for the next year. First, the remaining 50 images will be read to complete a reproducibility analysis between and within reader (total N=100). I plan to publish these results after the software developers publish the validity study for the plaque module of the AMS software.

Second, I plan to include plaque assessment in several upcoming grants. One study is in a population of women with systemic lupus erythematosus (SLE). A comparison of plaque composition between women with SLE and controls may identify different plaque characteristics in SLE women, helping to understand why their CVD risk is so high. The draft proposal for this grant is included in this dissertation (see Appendix H).

In the URL, we also plan to assess the relationship of plaque characteristics with cardiovascular risk factors in other populations. I plan to write a grant to fund this work. We also have the potential to evaluate plaques in different diseased populations as we educate other investigators about this new methodology.

The URL provides ultrasound tests on ongoing research projects of many populations and providing plaque assessment to study subclinical cardiovascular disease will expand our knowledge of plaque morphology.

APPENDIX A: PLAQUE ASSESSMENT TECHNICAL PROTOCOL

Date: 02/2006

Software: AMS Plaque Assessment Module

Note: This protocol is for vascular sonographers familiar with reading IMT images

Software: AMS Plaque Assessment Module

Step by step process:

1. Open Plaque Assessment Software
2. Select the image to be analyzed:
   a. File… load image… select the image to be read
3. **To calibrate** the image…Click on CALIBRATE. Click the left mouse button on a measurement tick typically on the side of the image. This sets the first tick. If you need to move it, use the up/down arrows for proper placement.
   a. To set the second cursor repeat the same process on the tick that indicates 1 cm
   b. Click OK to finish the calibration process
4. **Set Reference** points**:** This is how the computer will know the range of gray scale for this particular image.
5. **Set gray scale range**:
   a. Click BLACK… You will now select a part of the US image that is the blackest. The lumen should be the backest part of the image. Move the mouse cursor to the lumen and click once with the left mouse and drag to make a small region of interest (ROI) box. This sets the scale to the blackest pixels. At the top of the screen the black reference is seen.

b. Click WHITE… move mouse cursor to the adventitia and click once with the left mouse and drag to make a small region of interest (ROI) box on the whitest area of adventitia. This sets the scale to the whitest pixels. (Figure 5-1)

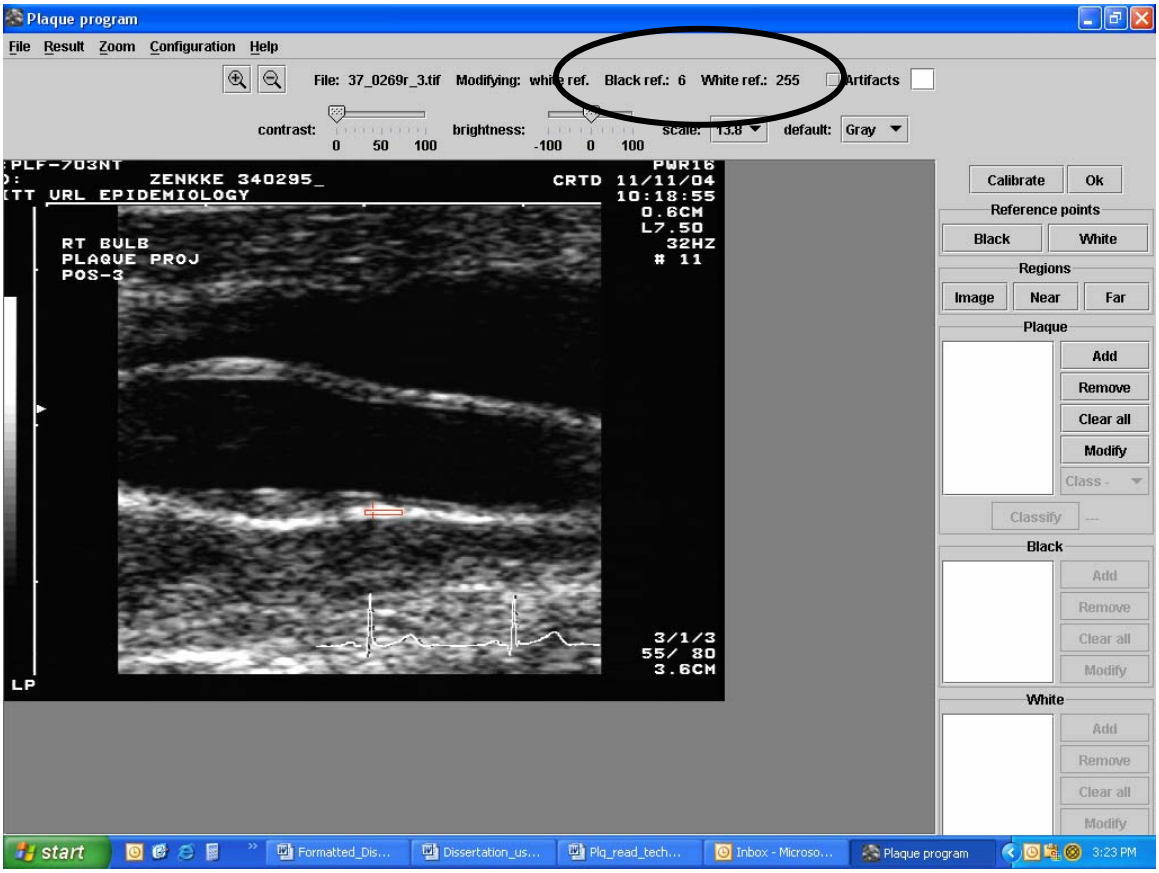


Figure 5-1: Black and white reference values for plaque characterization.

6. Select the area on the screen that is ultrasound data:

a. Select IMAGE… Draw a box around the portion of the picture that contains ultrasound data. Be sure to exclude any words and EGG. Keep the borders inside the image a little bit so you don't accidentally select non-ultrasound data.

7. **Prepare to score the first plaque**

   a. Select ADD under PLAQUE. The first plaque will appear in writing in the box. Using the left mouse button, outline the plaque. Stop the ends where the IMT is about 50% of the adjacent ‘reference ‘ IMT.

8. Subjectively score the plaque

   a. Click on CLASS drop-down arrow and select the Gray-Weale score that you think applies to this plaque.

9. Score the plaque electronically

   a. Click on CLASSIFY and the system will score the plaque. It will assign either echogenic or echolucent to describe the plaque.

10. Save the data

   a. Select FILE… SAVE PLAQUE

11. **View the results**

   a. select RESULTS (at the top) … VIEW RESULTS

   b. A window will open. To view all the results, double click on the blue icon to the left of the data lines. This will expand the subdirectory.

   c. Locate the line that begins with FEATURES. The main values of interest for plaque assessment are the percent WHITE value and the MEDIAN. (Figure 5-2)

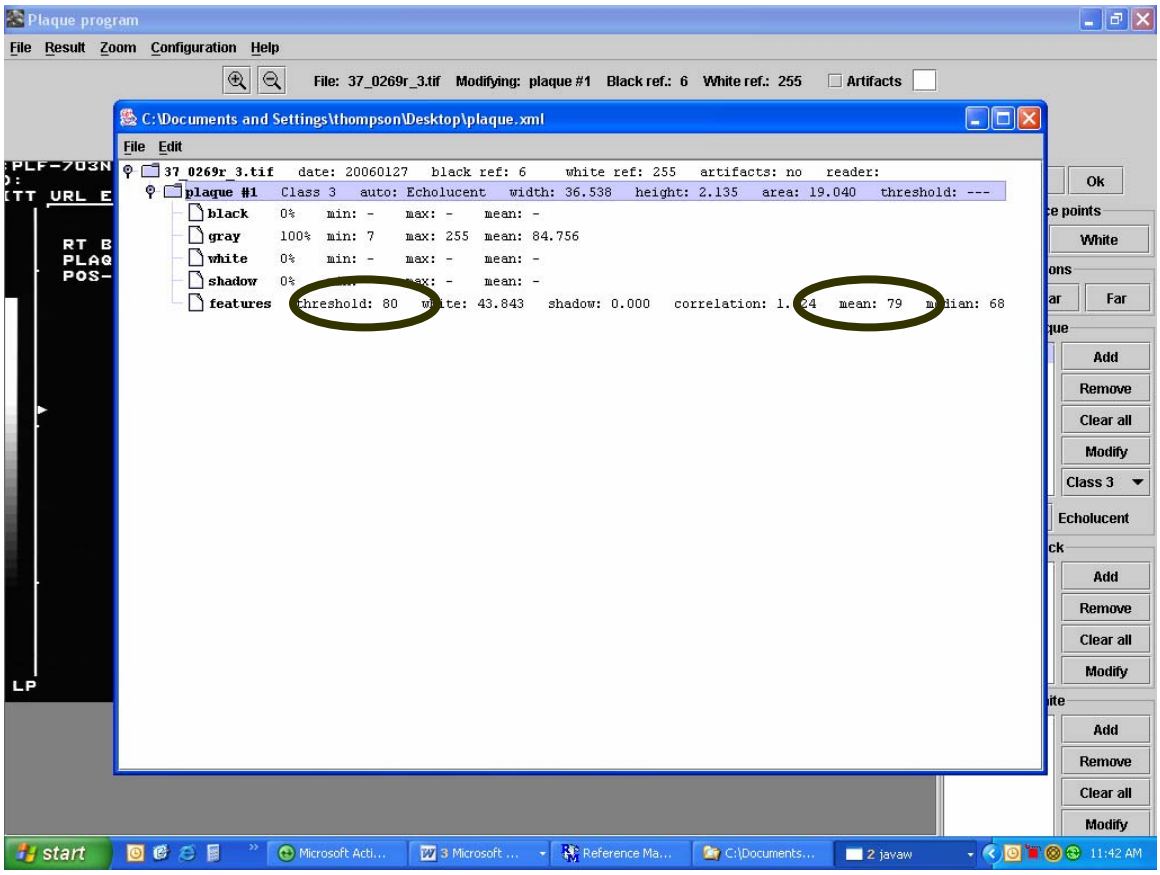
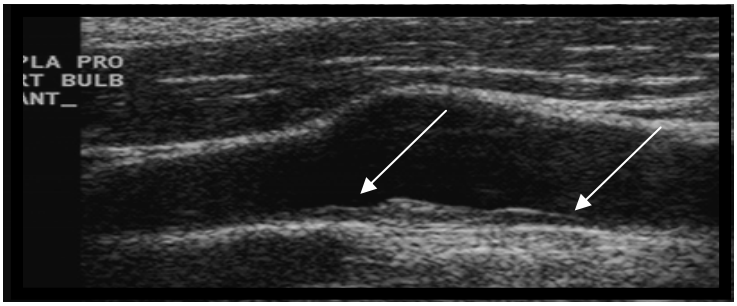


Figure 5-2: Main values of interest in output window for plaque assessment.

APPENDIX B: GRAY-WEALE SCALE SCORING PROTOCOL

1. Assess each image individually for the following
    - Is there a plaque? Confirm its presence in at least 2 projections. A plaque is defined as an area that is greater than the adjacent IMT by 50 %.
    - Capture the plaque displaying the shoulders. The shoulder is where the plaque begins and ends and is connected to the intima-medial segment. The plaque should be continuous as shown in figure 3-5.

Figure 5-3: The shoulders of a plaque



- Assess the vessel lumen for echos. The lumen should be the blackest area of the image if the gains are set properly. Sometimes if the plaque is hypoechoic the sonographer needs to increase the gains to see the plaque. This is the case in the image above. The plaque was hypoechoic and the sonographer increased the gains to clearly display the plaque. The reader can assess this because the lumen has artifacts within it. If the gains are reduced completely to blacken the lumen, it is likely that the plaque would be difficult to see or may disappear entirely. This is important when assigning the GWS. If the gains need to be elevated to see the plaque, the plaque is likely more black than the image is reflecting. The reader may choose to drop the score down by 1 category in this case.
- Assess the plaque according to the following scale guidelines:

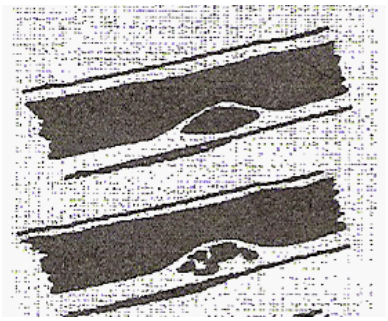
Type 1: dominantly echolucent (black), similar to blood (lumen)

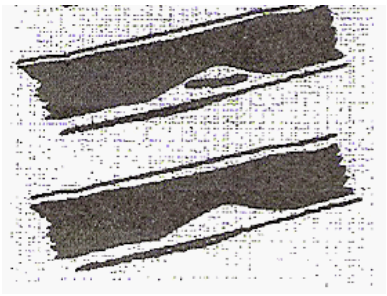
Type 2: dominantly echolucent with small areas of echogenicity

Type 3: substantially echogenic with few echolucent (black) spots

***Type 4*:** uniformly echogenic

Type 5: unclassified due to dense calcification and shadowing or unable to see whole plaque. (shoulders are missing, some other technical problem)





APPENDIX C: DUPLEX SCAN PLAQUE ASSESSMENT WORKSHEET

Study _____ Record#_____

URL ID:_____ Enter:_____

Study ID:_____ Vertify:_____

Scan Date_____/_____/__ __ __ __ Scan Category:_____

Reader:_____ Read date: *Repro only:* Scan Seq: _____ Read Seq _____

A. Plaque:

Right CCA Artery ☐ FW ☐ NW ☐ No plaque ☐ Thickening

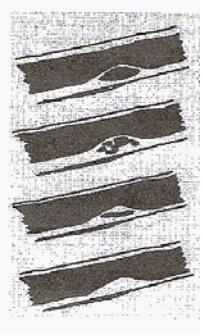
____ 1: Dominantly echolucent plaques, with a thin echogenic cap

____ 2: Substantially echolucent lesion with small areas of echogenicity

____ 3: Dominantly echogenic lesions with small areas of echolucency (<25%)

____ 4: Uniformly echogenic lesion

___ 5: Unclassified due to dense calcification and shadowing or no shoulder



Right BIFURCATION ☐ FW ☐ NW ☐ No plaque ☐ Thickening

____ 1: Dominantly echolucent plaques, with a thin echogenic cap

____ 2: Substantially echolucent lesion with small areas of echogenicity

____ 3: Dominantly echogenic lesions with small areas of echolucency (<25%)

____ 4: Uniformly echogenic lesion

___ 5: Unclassified due to dense calcification and shadowing or no shoulder

Right ICA Artery ☐ FW ☐ NW ☐ No plaque ☐ Thickening

____ 1: Dominantly echolucent plaques, with a thin echogenic cap

____ 2: Substantially echolucent lesion with small areas of echogenicity

____ 3: Dominantly echogenic lesions with small areas of echolucency (<25%)

____ 4: Uniformly echogenic lesion

___ 5: Unclassified due to dense calcification and shadowing or no shoulder

URLID: _____

A. Plaque – **Left CCA Artery** ☐ FW ☐ NW ☐ No plaque ☐ Thickening

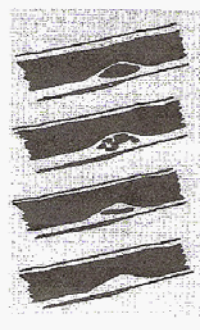
____ 1: Dominantly echolucent plaques, with a thin echogenic cap

____ 2: Substantially echolucent lesion with small areas of echogenicity

____ 3: Dominantly echogenic lesions with small areas of echolucency (<25%)

____ 4: Uniformly echogenic lesion

___ 5: Unclassified due to dense calcification and shadowing or no shoulder



Left BIFURCATION ☐ FW ☐ NW ☐ No plaque ☐ Thickening

____ 1: Dominantly echolucent plaques, with a thin echogenic cap

____ 2: Substantially echolucent lesion with small areas of echogenicity

____ 3: Dominantly echogenic lesions with small areas of echolucency (<25%)

____ 4: Uniformly echogenic lesion

___ 5: Unclassified due to dense calcification and shadowing or no shoulder

Left ICA Artery ☐ FW ☐ NW ☐ No plaque ☐ Thickening

____ 1: Dominantly echolucent plaques, with a thin echogenic cap

____ 2: Substantially echolucent lesion with small areas of echogenicity

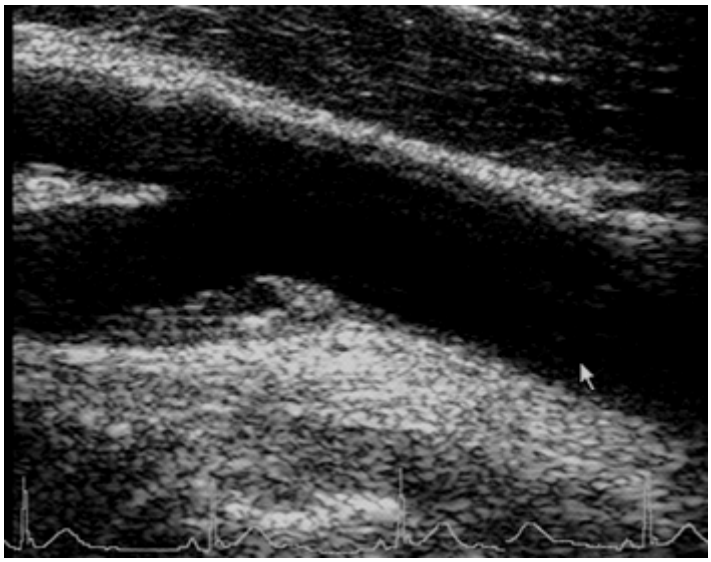
____ 3: Dominantly echogenic lesions with small areas of echolucency (<25%)

____ 4: Uniformly echogenic lesion

___ 5: Unclassified due to dense calcification and shadowing or no shoulder

APPENDIX D: PLAQUE CHARACTERIZATION FULL REPORT

N=50



Ultrasound Research Laboratory

Planned, Trained, Implemented, Analyzed and Interpreted

By

Trina Thompson MPH, BSN, RVT

February 20, 2006

D.1 TABLE OF CONTENTS

Reproducibility Analysis

Plaque Characterization using AMS

N=50

D.1.1 Plaque Characterization using AMS Reproducibility Analysis

Between readers

02/15/06

Purpose:

The purpose of this analysis was to assess the interreader repeatability of several values during plaque characterization using the AMS plaque module.

Sample: 50 images were collected from any study in the URL. These images reside at:

U:\URL\Plaque_repro_for LUPUS_grant_01_2006

Variables of primary interest:

Gray scale median Percent White of 'feature' (plaque)

Other variables compared:

Black reference value

White reference value

Area of plaque

Statistical Analysis:

Correlation analysis between PWV Read1 and Read2 was carried out using Spearman rank correlation. Repeatability of the 2 measures was assessed using the method described by Bland and Altman (1). Limits of agreement were calculated using a regression approach for nonuniform differences between measures recommended by the same authors (2). Additionally the variance component (VARCOMP) procedure in SAS was utilized to calculate the intraclass correlation coefficient of reliability.

D.1.2 Summary of Results:

Descriptive Statistics:

Measure N=50	**Reader 06**		**Reader 023**		**Reader 049**	
	Mean	**Range**	**Mean**	**Range**	**Mean**	**Range**
GSM	57 (18)	26 - 95	51.8 (16.7)	27 - 91	55.3 (17.7)	29 - 103
% White	36 (18)	5 - 74	33.9 (17.3)	2 - 71	36.9 (15.9)	3 - 76
Mean	65 (18)	30 - 101	59.6 (17.0)	30 - 95	64.5 (18.5)	38 - 113
Black Ref	8.5 (11.3)	0 - 74	8.6 (11.2)	0 - 74	9 (11)	1 - 74
White Ref	199 (46)	97 - 255	205.2 (44.1)	112 - 255	207 (45)	112 - 255
Area	20 (10.7)	6 - 48	17.7 (7.4)	3.7 – 35.9	21 (10)	5 - 46
Differences	**049-006**		**049-023**		**Eventually 049-049**	
GSM	-2.1 (10.9)	-25 - 19	3.5 (8.6)	-14 - 24		
% White	0.9 (7.9)	-19 - 23	3.0 (7.8)	-13 - 18		
Mean	-0.6 (12.5)	-27 - 25	4.9 (10.7)	-23 - 29		
Black Ref	0.6 (1.5)	-2 - 5	0.54 (1.4)	-2 - 5		
White Ref	8.7 (17.9)	-28 - 62	2. (11.9)	-21 - 45		
Area	0.7 (7.8)	-25 - 16	3. (5.4)	-7 - 16		
Spearman Corr **GSM**	0.79		0.84			
Pearson Corr **GSM**	0.82		0.88			
ICC **GSM**	0.82		0.86			
Spearman Corr **% White**	0.86		0.85			
Pearson Corr **% White**	0.89		0.89			
ICC **% White**	0.89		0.89			
Kappa Automated Class	0.61		0.41			

GSM

Random difference checks Tonee(49) – Dennis (23) = gsmdiffTD 19 (-'s)= 38%

Obs	ID	plqgsm049	plqgsm023	gsmdiffTD
1	30	63	77	-14
2	18	39	52	-13
3	37	38	50	-12
4	19	39	48	-9
5	21	32	39	-7
6	12	46	52	-6
7	38	75	81	-6
8	9	38	43	-5
9	43	51	56	-5
10	50	60	65	-5
11	17	53	57	-4
12	20	41	45	-4
13	41	37	40	-3
14	22	67	69	-2
15	33	77	79	-2
16	15	29	30	-1
17	23	38	39	-1
18	28	65	66	-1
19	46	88	89	-1
20	49	68	68	0
21	29	37	36	1
22	44	67	65	2
23	31	40	37	3
24	48	36	33	3
25	27	95	91	4
26	35	38	34	4
27	42	31	27	4
28	1	42	37	5
29	6	47	42	5
30	3	63	57	6
31	7	43	37	6
32	8	71	64	7
33	13	41	34	7
34	16	45	38	7
35	39	65	58	7
36	34	94	86	8
37	32	54	44	10
38	2	50	39	11
39	10	63	52	11
40	36	50	39	11
41	5	52	40	12
42	25	46	34	12
43	45	58	46	12
44	11	69	56	13
45	24	69	56	13
46	14	57	43	14
47	4	54	39	15
48	26	59	43	16
49	40	80	59	21
50	47	103	79	24

SPEARMAN CORR Procedure GSM

N=50

2 Variables: plqgsm049 plqgsm023

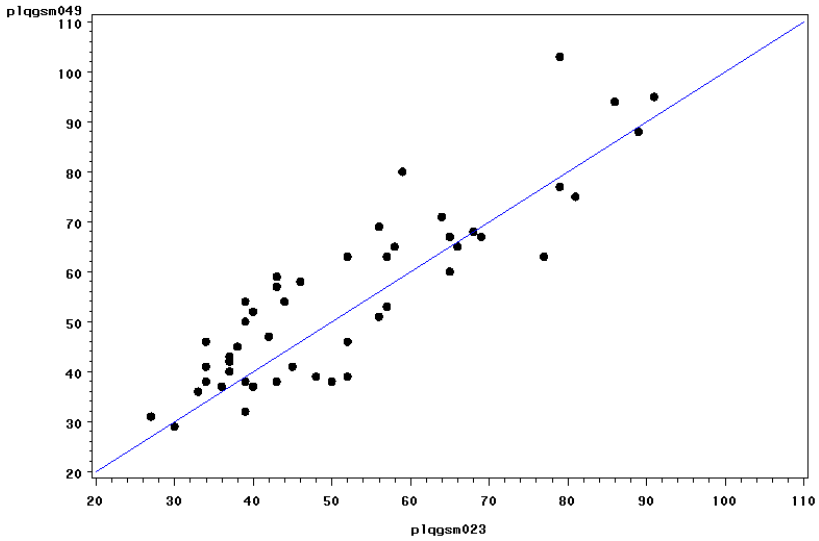
Variable	N	Mean	Std Dev	Median	Minimum	Maximum
plqgsm049	50	55.26000	17.70831	52.50000	29.00000	103.00000
plqgsm023	50	51.80000	16.65251	47.00000	27.00000	91.00000

Spearman Correlation Coefficients, N = 50
Prob > |r| under H0: Rho=0

	plqgsm049	plqgsm023
plqgsm049	1.00000	**0.84284** <.0001
plqgsm023	**0.84284** **<.0001**	1.00000

Spearman correlation between Tonee and Dennis is 0.84
p-value <0.0001

Pearson correlation is: 0.88



Intraclass correlation between Tonee and Dennis GSM

Variance Components Estimation Procedure

Class Level Information

Class	Levels	Values
id	50	1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50
tech	2	23 49

Number of observations 100

Dependent Variable: PlqGSM

Type 1 Analysis of Variance

Source	DF	Sum of Squares	Mean Square	Expected Mean Square
tech	1	299.290000	299.290000	Var(Error) + 50 Var(tech)
id	49	27143	553.947143	Var(Error) + 2 Var(id)
Error	49	1810.210000	36.943061	Var(Error)
Corrected Total	99	29253	.	.

Type 1 Estimates

Variance Component	Estimate
Var(tech)	5.24694
Var(id)	258.50204
Var(Error)	36.94306

Intraclass correlation:

ICC=var GSM between participants / Total variability= 258.50204 . 300.69203=0.86

Conclusion:

86% of the variation is GSM was due to differences between participants.

Bland Altman

Tonee (049) and Dennis (023), GSMDIFF

Normality of differences for GSMdiff between Tonee and Dennis 35

The REG Procedure
Model: MODEL1
Dependent Variable: gsmdiffTD

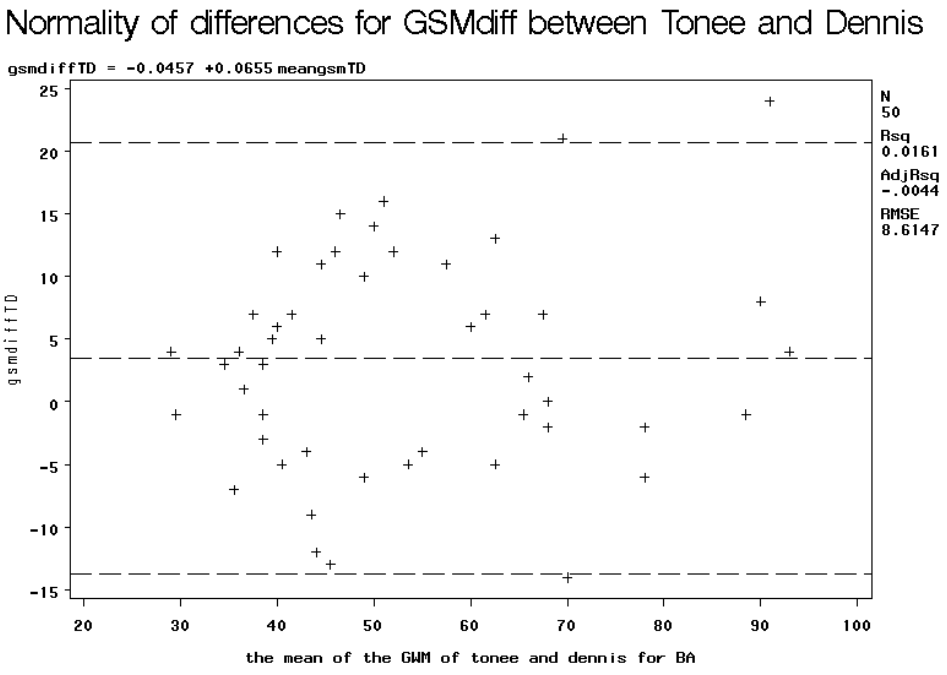
Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	58.20810	58.20810	0.78	0.3802
Error	48	3562.21190	74.21275		
Corrected Total	49	3620.42000			

Root MSE	8.61468	R-Square	0.0161
Dependent Mean	3.46000	Adj R-Sq	-0.0044
Coeff Var	248.97925		

Parameter Estimates

Variable	Label	DF	Estimate	Parameter Error	t Value	Pr > |t|
Intercept	Intercept	1	-0.04568	4.14164	-0.01	0.9912
meangsmTD	the mean of the GWM of tonee and dennis for BA	1	0.06549	0.07395	0.89	0.3802



Percent White

Random difference checks Tonee – Dennis = pwhdiffTD 17 (-‘s)= 34%

Obs	ID	plqpwh049	plqpwh023	pwhdiff TD
1	30	26.201	38.814	-12.613
2	28	45.074	56.319	-11.245
3	38	40.951	52.026	-11.075
4	37	34.705	42.761	-8.056
5	50	38.890	45.930	-7.040
6	18	28.714	34.321	-5.607
7	22	49.757	54.743	-4.986
8	12	29.927	34.883	-4.956
9	9	32.444	37.004	-4.560
10	43	34.288	37.870	-3.582
11	49	66.882	70.265	-3.383
12	23	32.100	34.887	-2.787
13	33	47.734	50.280	-2.546
14	21	23.947	26.371	-2.424
15	19	18.480	20.744	-2.264
16	20	20.847	22.841	-1.994
17	34	65.000	65.622	-0.622
18	46	62.602	62.429	0.173
19	31	26.411	25.449	0.962
20	10	45.814	44.842	0.972
21	48	3.311	2.335	0.976
22	41	17.212	16.131	1.081
23	25	13.507	11.914	1.593
24	29	28.105	26.449	1.656
25	6	36.859	35.040	1.819
26	44	45.999	44.155	1.844
27	1	32.145	29.695	2.450
28	8	57.088	53.855	3.233
29	15	11.853	8.464	3.389
30	17	28.519	24.698	3.821
31	35	16.658	12.479	4.179
32	47	72.802	68.441	4.361
33	27	76.229	71.013	5.216
34	7	19.896	13.945	5.951
35	16	32.743	24.250	8.493
36	2	31.641	22.650	8.991
37	3	50.432	41.391	9.041
38	42	21.655	12.340	9.315
39	40	52.700	43.364	9.336
40	11	38.945	29.140	9.805
41	5	32.168	21.543	10.625
42	14	46.361	34.403	11.958
43	13	41.296	29.224	12.072
44	26	44.249	31.917	12.332
45	32	19.922	5.070	14.852
46	4	37.093	21.668	15.425
47	45	38.313	22.400	15.913
48	24	54.250	38.325	15.925
49	39	43.465	27.199	16.266
50	36	31.112	13.571	17.541

The CORR Procedure
1 With Variables: plqpwh023
1 Variables: plqpwh049
Tonee and Dennis

Simple Statistics

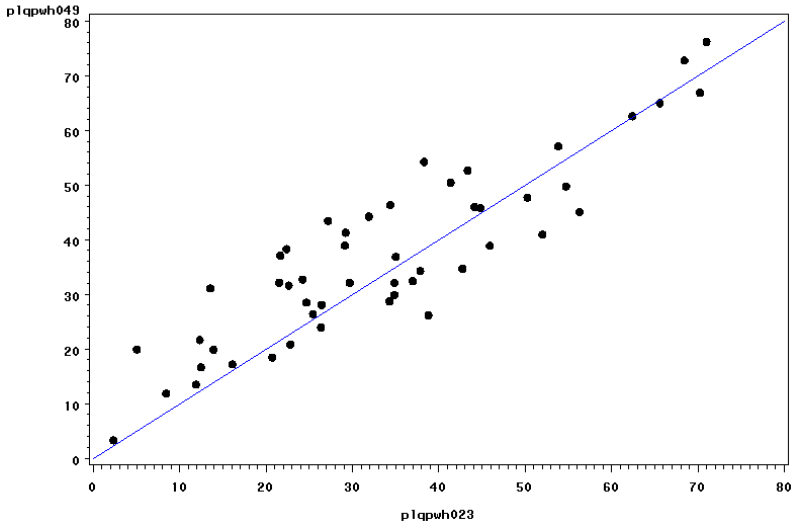
Variable	N	Mean	Std Dev	Median	Minimum	Maximum
plqpwh023	50	33.90940	17.29075	33.11900	2.33500	71.01300
plqpwh049	50	36.94592	15.91481	34.49650	3.31100	76.22900

Spearman Correlation Coefficients, N = 50
Prob > |r| under H0: Rho=0

	plqpwh049
plqpwh023	**0.84509**
	<.0001

Spearman correlation between Tonee and Dennis is 0.85
p-value <0.0001

Pearson correlation is: 0.89



ICC for Tonee and Dennis

Percent White variable

Variance Components Estimation Procedure

Class Level Information

Class	Levels	Values
id	50	1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50
tech	2	**23 49**

Number of observations 100
Dependent Variable: PlqPWh

Type 1 Analysis of Variance

Source	DF	Sum of Squares	Mean Square	Expected Mean Square
tech	1	230.511343	230.511343	Var(Error) + 50 Var(tech)
id	49	25575	521.935641	Var(Error) + 2 Var(id)
Error	49	1485.456400	30.315437	Var(Error)
Corrected Total	99	27291	.	.

Type 1 Estimates

Variance Component	Estimate
Var(tech)	4.00392
Var(id)	245.81010
Var(Error)	30.31544

Intraclass correlation:

89% of the variation of '% white' was due to differences between participants.

Math:

ICC=var '% white' between participants / Total variability= 245.81010/280.12946=0.89

Bland-Altman

Tonee and Dennis Percent White DIFF

Normality for Percent White Diff between Tonee and Dennis

The REG Procedure
Model: MODEL1
Dependent Variable: pwhdiffTD

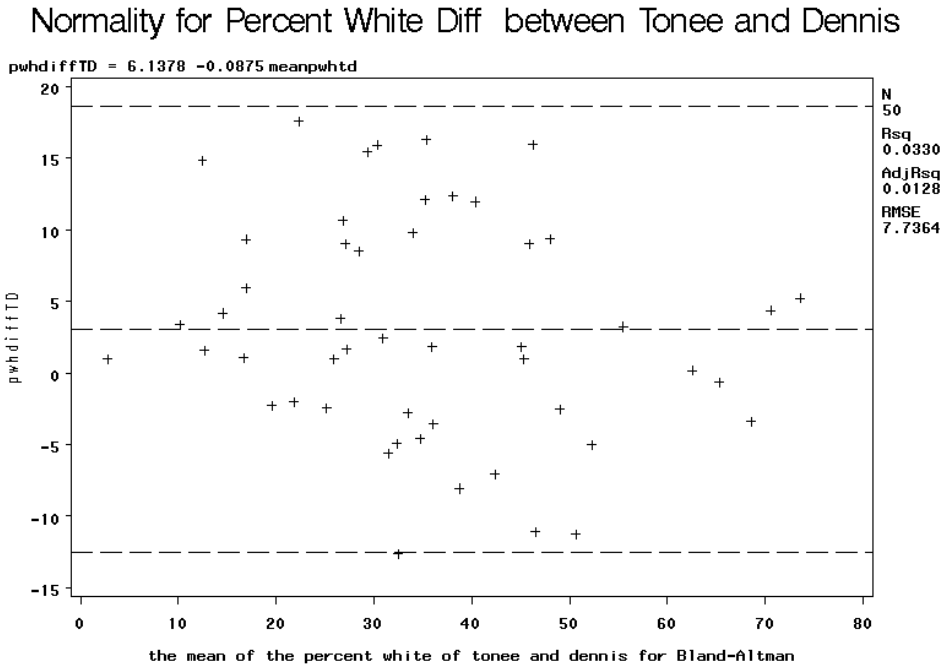
Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	97.98715	97.98715	1.64	0.2069
Error	48	2872.92565	59.85262		
Corrected Total	49	2970.91280			

Root MSE	7.73645	R-Square	0.0330
Dependent Mean	3.03652	Adj R-Sq	0.0128
Coeff Var	254.78006		

Parameter Estimates

Variable	Label	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|
Intercept	Intercept	1	6.13776	2.65928	2.31	0.0253
meanpwhtd	the mean of the percent white of tonee and dennis for Bland-Altman	1	-0.08754	0.06841	-1.28	0.2069



Kappa Statistic

Auto_class

KAPPA
Tonee and Dennis
Automated CLASS Variable
The FREQ Procedure

Table of **auto_c049 by auto_c023**

auto_c049 auto_c023

Simple Kappa Coefficient

Kappa	**0.4156**
ASE	0.1615
95% Lower Conf Limit	0.0991
95% Upper Conf Limit	0.7321

Sample Size = 50

Analysis between Tonee (49) and Holly (06) GSM

Very experienced reader vs. minimally experienced reader

Random difference checks Tonee–Holly=gsmdiffTH 27 (-'s) = 54%

Obs	ID	plqgsm049	plqgsm06	TH
1	3	63	88	-25
2	9	38	60	-22
3	7	43	64	-21
4	8	71	89	-18
5	24	69	85	-16
6	4	54	69	-15
7	18	39	53	-14
8	22	67	81	-14
9	29	37	50	-13
10	37	38	50	-12
11	38	75	87	-12
12	20	41	51	-10
13	41	37	47	-10
14	49	68	78	-10
15	21	32	41	-9
16	13	41	50	-9
17	48	36	44	-8
18	28	65	72	-7
19	46	88	95	-7
20	35	38	45	-7
21	6	47	52	-5
22	12	46	49	-3
23	43	51	54	-3
24	50	60	63	-3
25	17	53	56	-3
26	23	38	40	-2
27	15	29	30	-1
28	44	67	67	0
29	32	54	54	0
30	16	45	44	1
31	10	63	61	2
32	19	39	36	3
33	27	95	92	3
34	39	65	62	3
35	30	63	59	4
36	40	80	76	4
37	33	77	72	5
38	42	31	26	5
39	1	42	35	7
40	36	50	43	7
41	25	46	38	8
42	5	52	43	9
43	14	57	48	9
44	11	69	57	12
45	26	59	47	12
46	31	40	27	13
47	34	94	80	14
48	45	58	40	18
49	47	103	85	18
50	2	50	31	19

SPEARMAN CORR Procedure GSM
N=50
Tonne with Holly

2 Variables: plqgsm049 plqgsm06

Simple Statistics

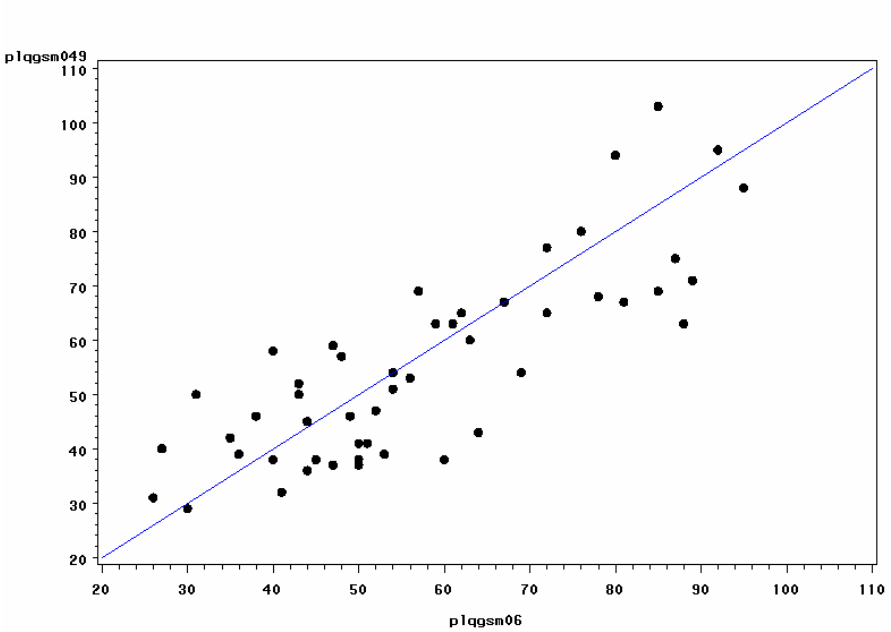
Variable	N	Mean	Std Dev	Median	Minimum	Maximum
plqgsm049	50	55.26000	17.70831	52.50000	29.00000	103.00000
plqgsm06	50	57.32000	18.46391	53.50000	26.00000	95.00000

Spearman Correlation Coefficients, N = 50
Prob > |r| under H0: Rho=0

	plqgsm049	plqgsm06
plqgsm049	1.00000	**0.78787** <.0001
plqgsm06	0.78787 <.0001	1.00000

Spearman correlation between Tonee and Holly is 0.79
p-value <0.0001

Pearson correlation is: 0.82



Intraclass correlation between Tonee and Holly GSM

Variance Components Estimation Procedure

Class Level Information

Class	Levels	Values
id	50	1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50
tech	2	6 49

Number of observations 100

Dependent Variable: PlqGSM

Type 1 Analysis of Variance

Source	DF	Sum of Squares	Mean Square	Expected Mean Square
tech	1	106.090000	106.090000	Var(Error) + 50 Var(tech)
id	49	29155	595.001837	Var(Error) + 2 Var(id)
Error	49	2915.410000	59.498163	Var(Error)
Corrected Total	99	32177	.	.

Type 1 Estimates

Variance Component	Estimate
Var(tech)	0.93184
Var(id)	267.75184
Var(Error)	59.49816
	328.18184

Intraclass correlation:

82% of the variation is GSM was due to differences between participants.

ICC=var GSM between participants / Total variability= 267.75184/328.18184=0.82

Bland Altman

Tonee and Holly GSMDIFF

Normality of differences for GSMdiff between Tonee and Holly

The REG Procedure
Model: MODEL1
Dependent Variable: gsmdiffTH

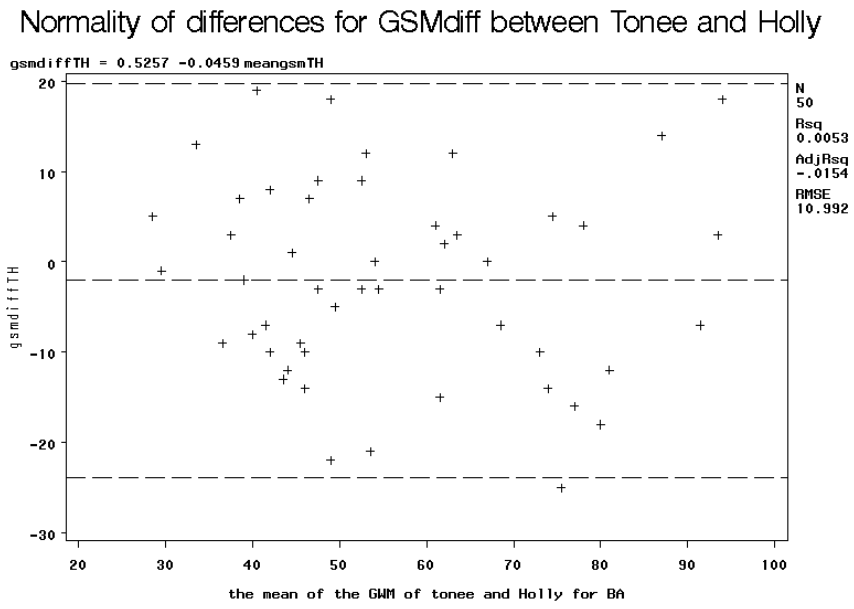
Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	30.75994	30.75994	0.25	0.6162
Error	48	5800.06006	120.83458		
Corrected Total	49	5830.82000			

Root MSE	10.99248	R-Square	0.0053
Dependent Mean	-2.06000	Adj R-Sq	-0.0154
Coeff Var	-533.61546		

Parameter Estimates

Variable	Label	DF	Estimate	Parameter Error	t Value	Standard Pr > |t|
Intercept	Intercept	1	0.52572	5.35548	0.10	0.9222
meangsmTH	the mean of the GWM of tonee and Holly for BA	1	-0.04594	0.09104	-0.50	0.6162



Check Difference values for Percent White Variable

Random difference checks Tonee–Holly = pwhdiffTH 22 (-‘s) = 44%

Obs	ID	plqpwh049	plqpwh06	TH
1	7	19.896	38.892	-18.996
2	41	17.212	28.765	-11.553
3	4	37.093	47.220	-10.127
4	38	40.951	50.670	-9.719
5	8	57.088	66.244	-9.156
6	3	50.432	58.600	-8.168
7	6	36.859	44.743	-7.884
8	28	45.074	52.800	-7.726
9	49	66.882	74.089	-7.207
10	12	29.927	37.018	-7.091
11	21	23.947	30.252	-6.305
12	13	41.296	47.543	-6.247
13	50	38.890	43.129	-4.239
14	43	34.288	38.042	-3.754
15	30	26.201	28.467	-2.266
16	37	34.705	36.403	-1.698
17	20	20.847	22.294	-1.447
18	48	3.311	4.685	-1.374
19	22	49.757	51.062	-1.305
20	19	18.480	19.708	-1.228
21	9	32.444	32.782	-0.338
22	46	62.602	62.891	-0.289
23	17	28.519	28.400	0.119
24	35	16.658	16.501	0.157
25	44	45.999	45.759	0.240
26	29	28.105	27.674	0.431
27	24	54.250	53.783	0.467
28	10	45.814	44.924	0.890
29	18	28.714	27.555	1.159
30	33	47.734	46.147	1.587
31	40	52.700	50.974	1.726
32	25	13.507	11.337	2.170
33	15	11.853	8.681	3.172
34	34	65.000	61.479	3.521
35	23	32.100	28.116	3.984
36	5	32.168	27.292	4.876
37	1	32.145	26.363	5.782
38	16	32.743	26.680	6.063
39	27	76.229	69.057	7.172
40	47	72.802	65.278	7.524
41	11	38.945	31.280	7.665
42	14	46.361	38.331	8.030
43	42	21.655	12.390	9.265
44	26	44.249	34.144	10.105
45	31	26.411	15.502	10.909
46	39	43.465	32.388	11.077
47	36	31.112	18.531	12.581
48	32	19.922	5.025	14.897
49	2	31.641	16.667	14.974
50	45	38.313	15.287	23.026

SPEARMAN CORR Procedure % White

N=50
Tonne with Holly
The CORR Procedure

1 With Variables: plqpwh06
1 Variables: plqpwh049

Simple Statistics

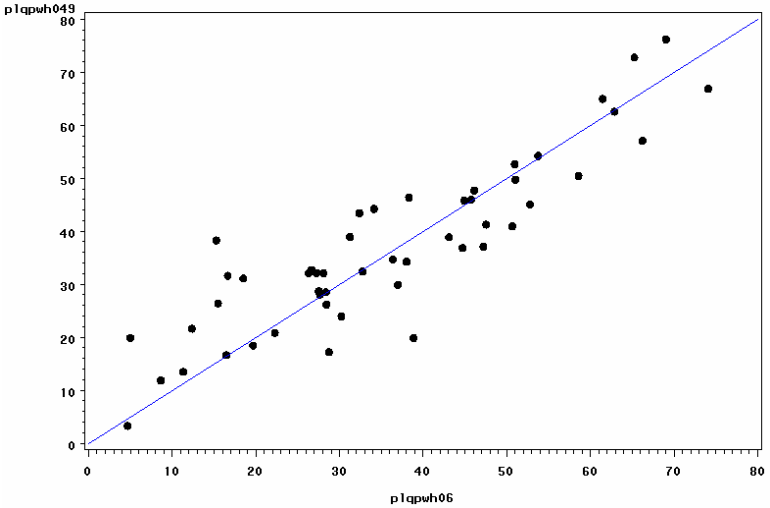
Variable	N	Mean	Std Dev	Median	Minimum	Maximum
plqpwh06	50	36.03688	17.53580	33.46300	4.68500	74.08900
plqpwh049	50	36.94592	15.91481	34.49650	3.31100	76.22900

Spearman Correlation Coefficients, N = 50
Prob > |r| under H0: Rho=0

	plqpwh049
plqpwh06	**0.86218**
	<.0001

Spearman correlation between Tonee and Holly is 0.86
p-value <0.0001

Pearson correlation is: 0.89



Intraclass correlation between Tonee and Holly Percent White

Variance Components Estimation Procedure

Class Level Information

Class	Levels	Values
id	50	1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50
tech	2	6 49

Number of observations 100

Dependent Variable: PlqPWh

Type 1 Analysis of Variance

Source	DF	Sum of Squares	Mean Square	Expected Mean Square
tech	1	20.658843	20.658843	Var(Error) + 50 Var(tech)
id	49	25933	529.238458	Var(Error) + 2 Var(id)
Error	49	1545.792072	31.546777	Var(Error)
Corrected Total	99	27499	.	.

Type 1 Estimates

Variance Component	Estimate
Var(tech)	-0.21776
Var(id)	248.84584
Var(Error)	31.54678
	280.17486

Intraclass correlation:

89% of the variation is % white was due to differences between participants.

ICC=var % White between participants / Total variability= 248.84584/280.17486=0.89

Bland-Altman

Tonee and Holly for Percent White Differences

Normality for Percent White Diff between Tonee and Holly

The REG Procedure
Model: MODEL1
Dependent Variable: pwhdiffTH

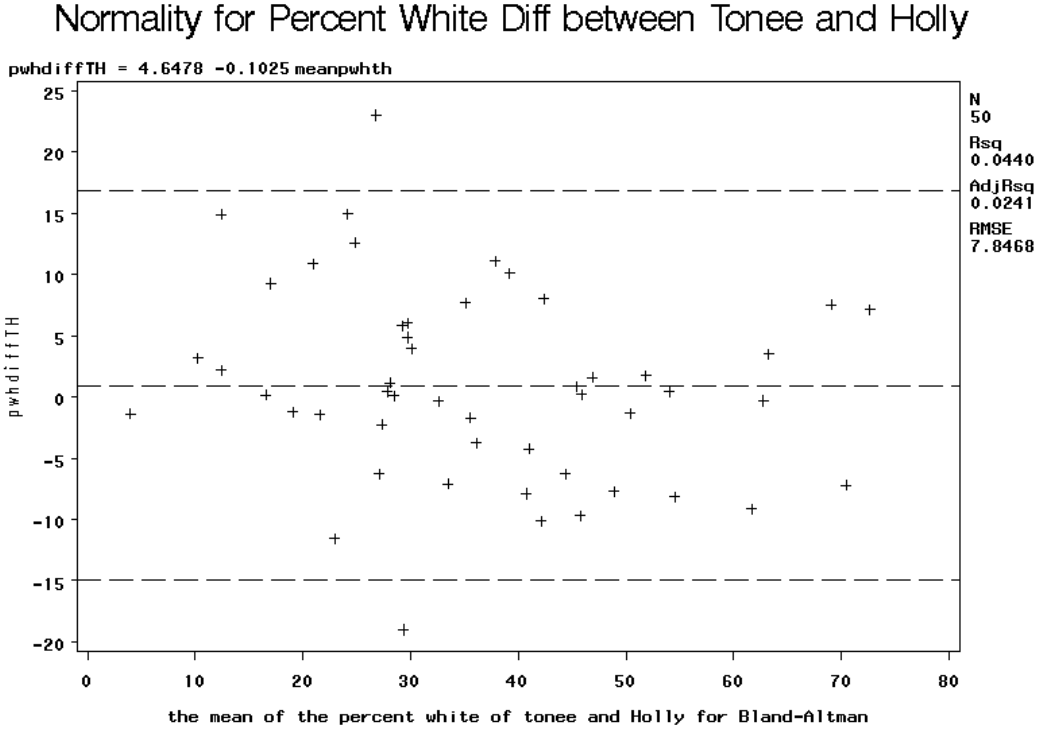
Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	136.10733	136.10733	2.21	0.1436
Error	48	2955.47682	61.57243		
Corrected Total	49	3091.58414			

Root MSE	7.84681	R-Square	0.0440
Dependent Mean	0.90904	Adj R-Sq	0.0241
Coeff Var	863.19748		

Parameter Estimates

Variable	Label	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|
Intercept	Intercep	1	4.64776	2.74861	1.69	0.0973
meanpwhth	the mean of the percent white of tonee and Holly for Bland-Altman	1	-0.10245	0.06891	-1.49	0.1436



KAPPA Statistic

Tonee and Holly

Automated Classification Variable

KAPPA
Tonee and Holly
Automated CLASS Variable

The FREQ Procedure

Table of auto_c049 by auto_c06

Simple Kappa Coefficient	
Kappa	**0.6067**
ASE	0.1329
95% Lower Conf Limit	0.3463
95% Upper Conf Limit	0.8672

Sample Size = 50

Appendix A.1

Distributions of the Difference variables for GSM and Percent White

The UNIVARIATE Procedure
Variable: **gsmdiffTD**

N	50	Sum Weights	50
Mean	3.46	Sum Observations	173
Std Deviation	8.59570372	Variance	73.8861224
Skewness	0.07758774	Kurtosis	-0.3403995
Uncorrected SS	4219	Corrected SS	3620.42
Coeff Variation	248.430743	Std Error Mean	1.21561608

Basic Statistical Measures

Location		Variability	
Mean	3.46000	Std Deviation	8.59570
Median	4.00000	Variance	73.88612
Mode	-1.00000	Range	38.00000
		Interquartile Range	14.00000

NOTE: The mode displayed is the smallest of 2 modes with a count of 4.

Tests for Location: Mu0=0

Test	-Statistic-		-----p Value------	
Student's t	t	2.846293	Pr > |t|	0.0064
Sign	M	5.5	Pr >= |M|	0.1524
Signed Rank	S	259.5	Pr >= |S|	0.0084

Tests for Normality

Test	--Statistic---		-----p Value------	
Shapiro-Wilk	W	0.987359	Pr < W	0.8663
Kolmogorov-Smirnov	D	0.078072	Pr > D	>0.1500
Cramer-von Mises	W-Sq	0.034034	Pr > W-Sq	>0.2500
Anderson-Darling	A-Sq	0.220882	Pr > A-Sq	>0.2500

Quantiles (Definition 5)

Quantile	Estimate
100% Max	24.0
99%	24.0
95%	16.0
90%	13.5

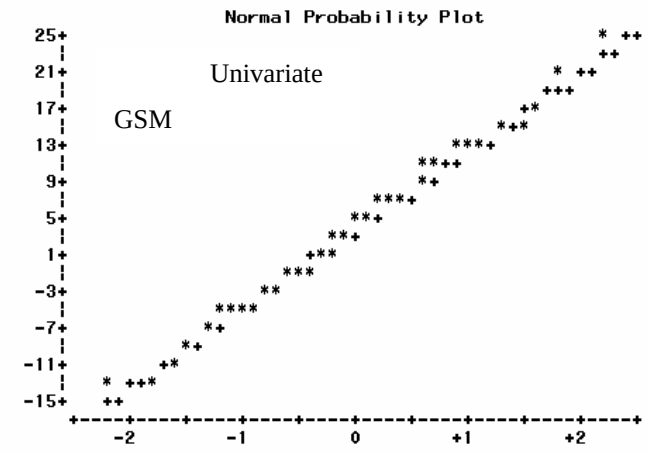
The UNIVARIATE Procedure
Variable: **gsmdiffTD**

Quantiles (Definition 5)

Quantile	Estimate
75% Q3	11.0
50% Median	4.0
25% Q1	-3.0
10%	-6.5
5%	-12.0
1%	-14.0
0% Min	-14.0

Extreme Observations

----Lowest----		----Highest---	
Value	Obs	Value	Obs
-14	1	14	42
-13	6	15	46
-12	4	16	44
-9	15	21	39
-7	14	24	32



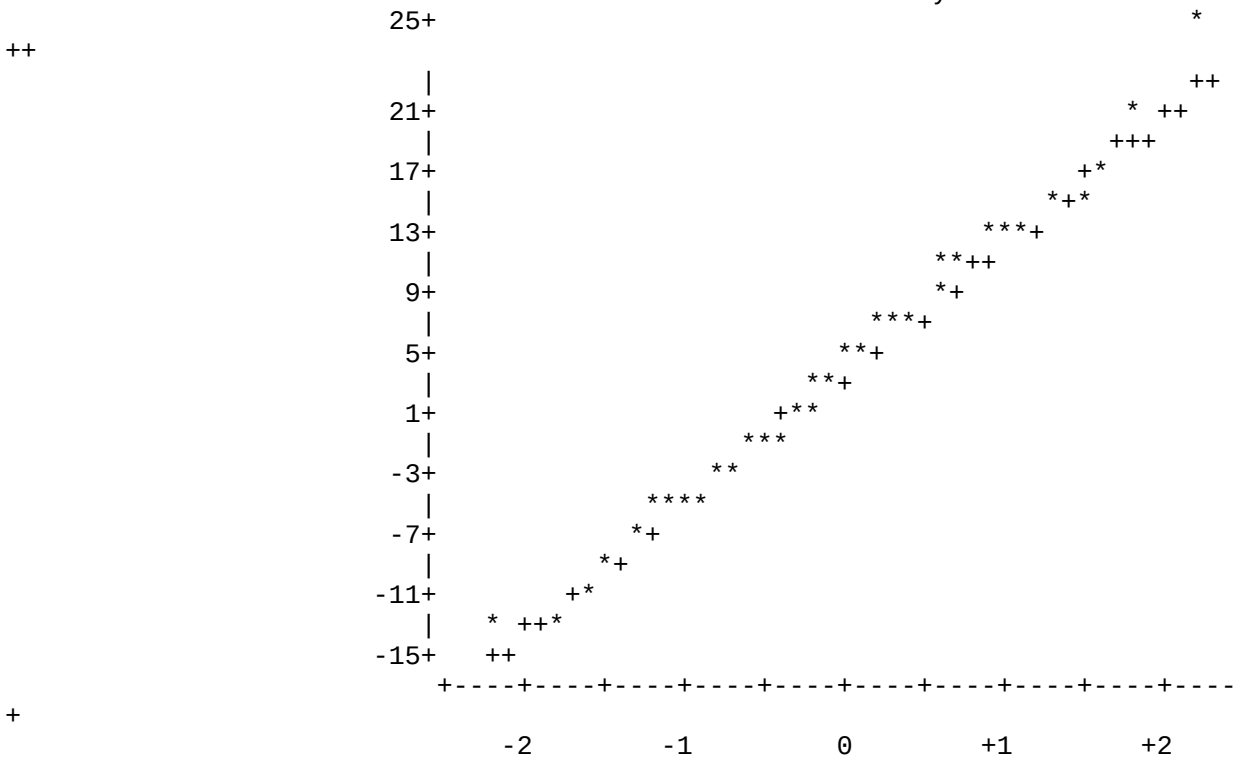
Univariate

GSM

The UNIVARIATE Procedure
Variable: gsmdiffTD

```
Stem Leaf                     #             Boxplot
  24 0                        1                |
  22                                           |
  20 0                        1                |
  18                                           |
  16 0                        1                |
  14 00                       2                |
  12 00000                    5                |
  10 0000                     4             +-----+
   8 0                        1             |     |
   6 000000                   6             |     |
   4 00000                    5             *-----*
   2 000                      3             |  +  |
   0 00                       2             |     |
  -0 0000                     4             |     |
  -2 000                      3             +-----+
  -4 00000                    5                |
  -6 000                      3                |
  -8 0                        1                |
 -10                                           |
 -12 00                       2                |
 -14 0                        1                |
     ----+----+----+----+
```

Normal Probability Plot



The UNIVARIATE Procedure
Variable: **pwhdiffTD**

N	50	Sum Weights	50
Mean	3.03652	Sum Observations	151.826
Std Deviation	7.78658291	Variance	60.6308735
Skewness	0.08029345	Kurtosis	-0.6967464
Uncorrected SS	3431.93549	Corrected SS	2970.9128
Coeff Variation	256.431142	Std Error Mean	1.10118912

Basic Statistical Measures

Location		Variability	
Mean	3.036520	Std Deviation	7.78658
Median	1.831500	Variance	60.63087
Mode	.	Range	30.15400
		Interquartile Range	11.86100

Tests for Location: Mu0=0

Test	-Statistic-		-----p Value------	
Student's t	t	2.757492	Pr > |t|	0.0082
Sign	M	8	Pr >= |M|	0.0328
Signed Rank	S	243.5	Pr >= |S|	0.0172

Tests for Normality

Test	--Statistic---		-----p Value------	
Shapiro-Wilk	W	0.973236	Pr < W	0.3118
Kolmogorov-Smirnov	D	0.08086	Pr > D	>0.1500
Cramer-von Mises	W-Sq	0.061436	Pr > W-Sq	>0.2500
Anderson-Darling	A-Sq	0.386716	Pr > A-Sq	>0.2500

Quantiles (Definition 5)

Quantile	Estimate
100% Max	17.5410
99%	17.5410
95%	15.9250
90%	15.1385
75% Q3	9.3150
50% Median	1.8315

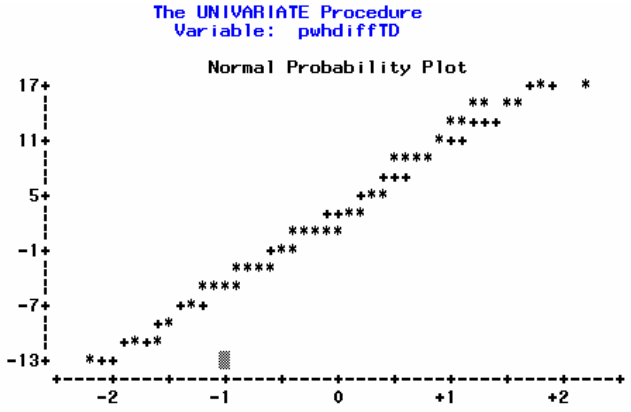
The UNIVARIATE Procedure
Variable: **pwhdiffTD**

Quantiles (Definition 5)

Quantile	Estimate
25% Q1	-2.5460
10%	-6.3235
5%	-11.0750
1%	-12.6130
0% Min	-12.6130

Extreme Observations

-----Lowest-----		-----Highest----	
Value	Obs	Value	Obs
-12.613	1	15.425	46
-11.245	2	15.913	47
-11.075	3	15.925	48
-8.056	4	16.266	49
-7.040	5	17.541	50



Normality for GSMDIFF between Tonee and Holly

The UNIVARIATE Procedure
Variable: **gsmdiffTH**

N	50	Sum Weights	50
Mean	-2.06	Sum Observations	-103
Std Deviation	10.9085437	Variance	118.996327
Skewness	0.00485671	Kurtosis	-0.637477
Uncorrected SS	6043	Corrected SS	5830.82
Coeff Variation	-529.54096	Std Error Mean	1.54270105

Basic Statistical Measures

Location		Variability	
Mean	-2.06000	Std Deviation	10.90854
Median	-2.50000	Variance	118.99633
Mode	-3.00000	Range	44.00000
		Interquartile Range	15.00000

Tests for Location: Mu0=0

Test	-Statistic-		-----p Value------	
Student's t	t	-1.33532	Pr > |t|	0.1879
Sign	M	-3	Pr >= |M|	0.4709
Signed Rank	S	-123	Pr >= |S|	0.2101

Tests for Normality

Test	--Statistic---		-----p Value------	
Shapiro-Wilk	W	0.985225	Pr < W	0.7812
Kolmogorov-Smirnov	D	0.074674	Pr > D	>0.1500
Cramer-von Mises	W-Sq	0.027694	Pr > W-Sq	>0.2500
Anderson-Darling	A-Sq	0.177757	Pr > A-Sq	>0.2500

Quantiles (Definition 5)

Quantile	Estimate
100% Max	19.0
99%	19.0
95%	18.0
90%	12.5
75% Q3	5.0

Normality for GSMDIFFbetween Tonee and Holly5

The UNIVARIATE Procedure
Variable: **gsmdiffTH**

Quantiles (Definition 5)

Quantile	Estimate
50% Median	-2.5
25% Q1	-10.0
10%	-15.5
5%	-21.0
1%	-25.0
0% Min	-25.0

Extreme Observations

----Lowest----		----Highest---	
Value	Obs	Value	Obs
-25	3	13	31
-22	9	14	34
-21	7	18	45
-18	8	18	47
-16	24	19	2

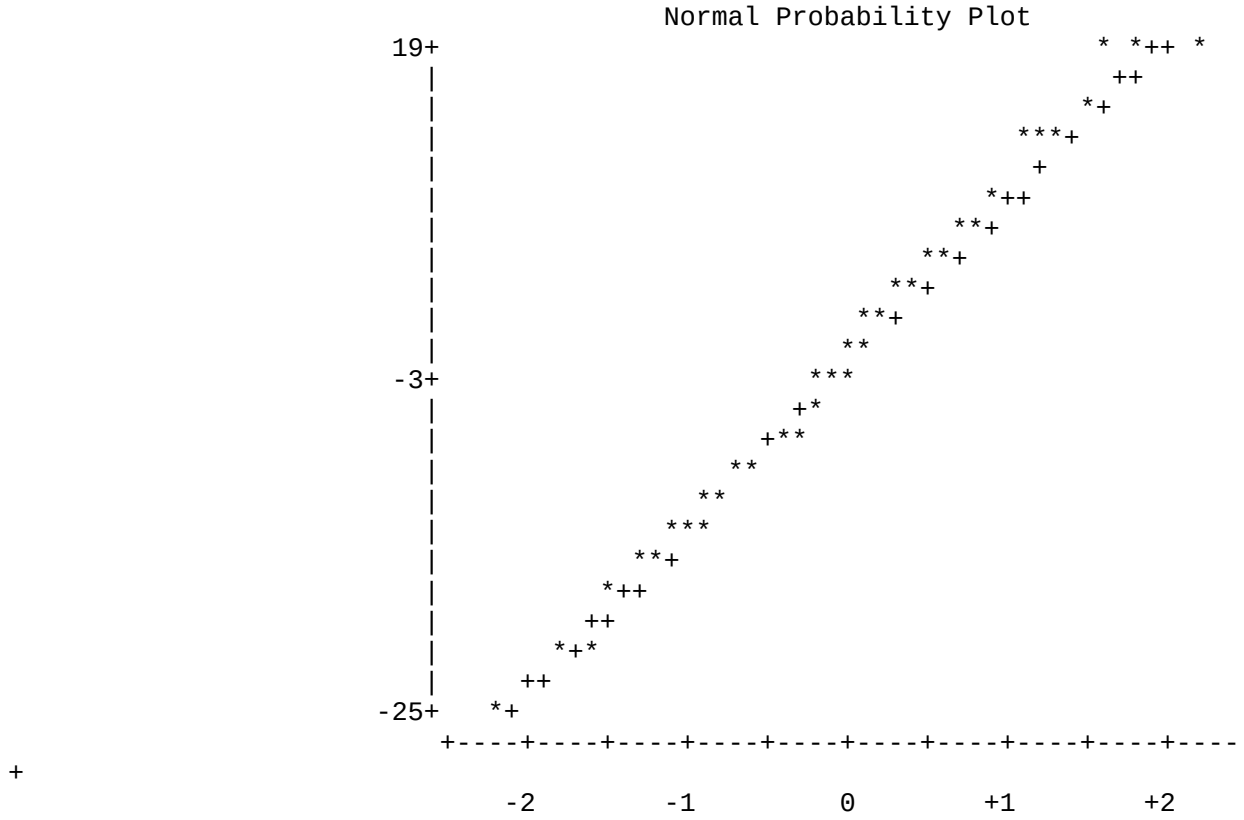
Normality for Percent White Diff between Tonee and Holly

The UNIVARIATE Procedure
Variable: gsmdiffTH

Stem	Leaf	#	Boxplot
18	000	3	
16			
14	0	1	
12	000	3	
10			
8	000	3	
6	00	2	
4	0000	4	+-----+
2	0000	4	| |
0	000	3	| |
-0	0	1	| |
-2	00000	5	*--+--*
-4	0	1	| |
-6	000	3	| |
-8	000	3	| |
-10	000	3	+-----+
-12	000	3	
-14	000	3	
-16	0	1	
-18	0	1	
-20	0	1	
-22	0	1	
-24	0	1	

Normality for Percent White Diff between Tonee and Holly

The UNIVARIATE Procedure
Variable: gsmdiffTH



Normality for Percent White Diff between Tonee and Holly

The UNIVARIATE Procedure
Variable: **pwhdiffTH**

Moments

N	50	Sum Weights	50
Mean	0.90904	Sum Observations	45.452
Std Deviation	7.94314509	Variance	63.093554
Skewness	0.22638338	Kurtosis	0.4592752
Uncorrected SS	3132.90183	Corrected SS	3091.58414
Coeff Variation	873.794893	Std Error Mean	1.12333035

Basic Statistical Measures

Location		Variability	
Mean	0.909040	Std Deviation	7.94315
Median	0.335500	Variance	63.09355
Mode	.	Range	42.02200
		Interquartile Range	10.30200

Tests for Location: Mu0=0

Test	-Statistic-		-----p Value------	
Student's t	t	0.809237	Pr > |t|	0.4223
Sign	M	3	Pr >= |M|	0.4799
Signed Rank	S	70.5	Pr >= |S|	0.5017

Tests for Normality

Test	--Statistic---		-----p Value------	
Shapiro-Wilk	W	0.986818	Pr < W	0.8461
Kolmogorov-Smirnov	D	0.079041	Pr > D	>0.1500
Cramer-von Mises	W-Sq	0.050191	Pr > W-Sq	>0.2500
Anderson-Darling	A-Sq	0.287525	Pr > A-Sq	>0.2500

Quantiles (Definition 5)

Quantile	Estimate
100% Max	23.0260
99%	23.0260
95%	14.8970
90%	10.9930
75% Q3	6.0630

Normality for Percent White Diff between Tonee and Holly

The UNIVARIATE Procedure
Variable: **pwhdiffTH**

Quantiles (Definition 5)

Quantile	Estimate
50% Median	0.3355
25% Q1	-4.2390
10%	-8.6620
5%	-10.1270
1%	-18.9960
0% Min	-18.9960

Extreme Observations

-----Lowest-----		-----Highest----	
Value	Obs	Value	Obs
-18.996	7	11.077	39
-11.553	41	12.581	36
-10.127	4	14.897	32
-9.719	38	14.974	2
-9.156	8	23.026	45

Normality for Percent White Diff between Tonee and Holly

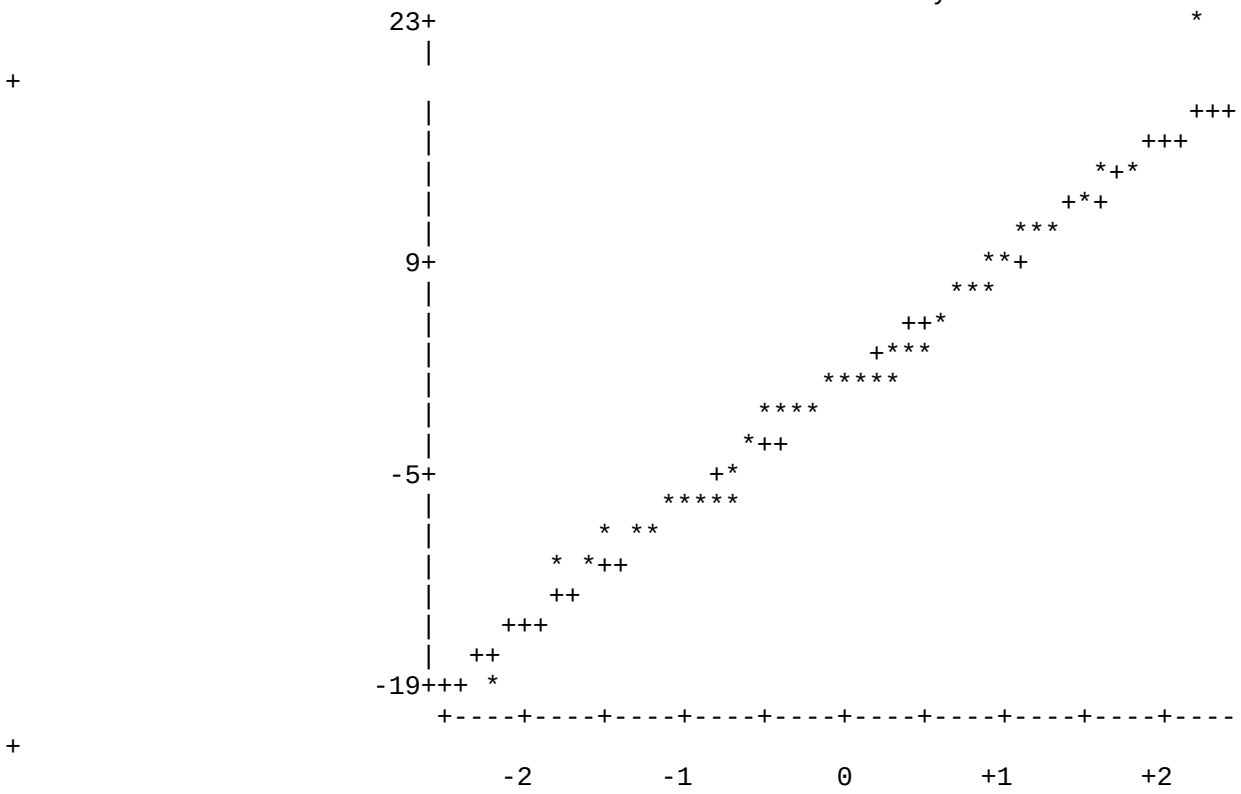
The UNIVARIATE Procedure
Variable: pwhdiffTH

Stem	Leaf	#	Boxplot
22	0	1	0
20			
18			
16			
14	90	2	|
12	6	1	|
10	191	3	|
8	03	2	|
6	1257	4	+-----+
4	098	3	| |
2	225	3	| |
0	122459267	9	*--+--*
-0	7443233	7	| |
-2	83	2	| |
-4	2	1	+-----+
-6	972132	6	|
-8	722	3	|
-10	61	2	|
-12			|
-14			|
-16			|
-18	0	1	|

Normality for Percent White Diff between Tonee and Holly

The UNIVARIATE Procedure
Variable: pwhdiffTH

Normal Probability Plot



APPENDIX E: FINAL PROTOCOL FOR NEXT GRANT SUBMISSION

E.1 SUBCLINICAL CARDIOVASCULAR DISEASE IN LUPUS

Very few investigators have examined the prevalence of carotid plaque and IMT in lupus using B-mode ultrasound.(1) (2) (3;4) We previously reported the presence of focal carotid plaque in a sample of CVD-free lupus women as 32% and the mean IMT as 0.71mm. In 175 unselected women with lupus, focal carotid plaque prevalence was 40%.(1) The inclusion of women with previous cardiovascular events in the second study may explain the higher prevalence. In a recent study by Roman et al.(4), 197 patients with lupus and 197 matched controls were evaluated with carotid ultrasonography and assessed for risk factors for cardiovascular disease.

The traditional risk factors for cardiovascular disease were similar among patients and controls. Carotid plaque was more prevalent among patients than the controls (37.1 percent vs. 15.2 percent, $P<0.001$). In multivariate analysis, older age, the presence of lupus (odds ratio, 4.8; 95 percent confidence interval, 2.6 to 8.7), and a higher serum cholesterol level were independently related to the presence of plaque. As compared with lupus patients without plaque, patients with plaque were older, had a longer duration of lupus and more disease-related damage, and were less likely to have been treated with prednisone, cyclophosphamide, or hydroxychloroquine. In multivariate analyses including patients with lupus, independent predictors of plaque were a longer duration of disease, a higher damage-index score, a lower incidence of the use of cyclophosphamide, and the absence of anti-Smith antibodies. These data support our finding that variables related to lupus disease activity are important predictors of cardiovascular disease, and that immunosuppressive therapy is associated with less progression of carotid IMT.

E.2 LUPUS FACTORS THAT MAY PROMOTE CARDIOVASCULAR DISEASE PROGRESSION

We believe that the pathogenesis of cardiovascular disease in lupus is multifactorial, due to an interaction between traditional cardiovascular risk factors and inflammation-induced and antiphospholipid antibody-mediated vascular injury/thrombosis from the underlying disease. Renal disease with resulting hypertension may accelerate the atherosclerotic process in lupus.

While we know that atherosclerotic plaques are the root cause of stroke and myocardial infarction, we still know little about why one arterial plaque causes an event and another does not. In the past, the degree to which a plaque narrows a given vessel and reduces blood flow was thought to be the primary determinant of its danger. However, in recent years, it has become clear that plaque rupture is the key determinant of a vascular event (5-9). Plaque rupture can occur in both large and medium plaques and thus the determinant appears to be plaque composition as opposed to solely plaque size.(6;10-12)

Women with lupus are known to be at high risk for cardiovascular disease.(11;13;14) Our own work and that of others has suggested that risk factors specific to the lupus disease process may have a particularly strong effect on plaque development. From a biological perspective, this makes sense because lupus brings with it abnormalities in platelet function, and thrombotic and inflammatory processes, all of which are related to plaque development and progression.

This raises the question of whether the high prevalence of CVD among women with lupus is related to a propensity to develop unstable plaques. Since the realization that plaque composition is likely the major driver of risk, researchers have been searching for a way to identify vulnerable or unstable plaques in vivo. Standard ways of evaluating plaques using ultrasound have been developed and automated software methods that are on the horizon are showing even more promise.(15-17) In general, individuals with echolucent or heterogeneous lesions have been shown to be at high risk for events relative to individuals with other plaque types.(10;18-21)

E.3 EVALUATION OF PLAQUE AND PLAQUE COMPOSITION:

During scanning, the sonographer will digitize images of all plaques. As part of our standard plaque evaluation, the number and size of plaques in each segment will be recorded. Plaque will be assessed in 4 areas: proximal common, distal common, carotid bulb and internal carotid. Plaques typically occur in the carotid bulb and proximal ICA where flow disturbances occur due to the carotid bifurcation. We expect plaques in the CCA to be rare.

We will use three separate methodologies to evaluate plaque in this study: The plaque index, a subjective measure of the degree of plaque, the Gray-Weale scale, a subjective scale of plaque quality, and finally an objective measure of plaque density using a computerized scoring system.

Plaque Index: The plaque index is reproducible(22) and has been used as a measure of focal plaque in numerous population.(23-26) In each carotid segment, the number of distinct plaques will be recorded. In addition, each segment will be given a grade, which corresponds to both the number and size of plaques. These grades will be defined as follows: Grade 0 = no observable plaque; grade 1 = one small plaque (less than 30% of the vessel diameter); grade 2 = one medium plaque (between 30% and 50% of the vessel diameter) OR multiple small plaques; grade 3 = one large plaque (greater than 50% of the vessel diameter) or multiple plaques with at least one medium plaque. The grades will then be summed to create the plaque index, a measure of extent of eccentric plaque.

Gray-Weale Scale: Studies using *subjective* scales from ultrasound have been useful in learning about lesion description and vulnerability. A subjective categorization was reported by Gray-Weale(27) and similar scales by other investigators.(11;28;29) The Gray-Weale scoring is as follows: *type 1*, dominantly echolucent; (similar to blood) *type 2*, dominantly echolucent with small areas of echogenicity; *type 3*, substantially echogenic with few echolucent spots; *type 4*, uniformly echogenic; *type 5* unclassified due to dense calcification and shadowing.

Computerized Scoring of Plaque Density : This will be accomplished using the AMS (Automated Measuring System) software developed by Dr. Thomas Gustavsson at the Chalmers University of Technology in Götenberg, Sweden. This software is similar to others that have been developed and used successfully.(19;30-32) Over the past year we have been working to evaluate the software and learn how to use it. Thus far we have scored 100 scans. Because this

will be the first formal study we have done with this software, we have begin with a reproducibility study to ensure that our readers are properly trained and are yielding reproducible results.

Plaque Scoring Protocol: If more than one plaque is present in a given segment, the largest plaque will be chosen. Images will be displayed on a monitor and calibrated. This will be accomplished by selecting an area on the image that is black (hypoechoic), such as the lumen and another area that is white (hyperechoic), such as tissue or plaque. The software will place these two extreme measures at opposite ends of a scale used to evaluate each pixel within the plaque. The reader then will outline the plaques with the cursor along the lumen-intima interface and along the media-adventitia interface. The system is prompted to electronically connect the dots to fully encompass the lesion. Each pixel within the plaque is identified on the initial scale and several measurements are obtained by applying various complex algorithms.

The gray-scale median (GSM) will be used to quantify overall plaque echogenicity. Lower GSM values have been shown to be associated with cerebral ischemia and symptoms .(20) (33;34) To date, no one has tested the association between carotid echogenicity and cardiovascular symptoms. It is possible that like IMT, vulnerable carotid plaques may be a predictor of coronary disease.

Authors use a variety of descriptors for plaque that are not yet standardized. For our purposes, we begin with the density of blood as seen on ultrasound. Blood appears black on an ultrasound image, when gains are set properly, and this is used as our baseline. Our key descriptors will be as follows:

Echolucent = less echogenic = hypoechoic = darker = appearing black like blood
Echogenic = more echogenic = hyperechoic = brighter =appearing whiter than blood
Heterogeneous = mixed echogenicity = both black and white echos
Homogeneous = mostly uniform echogenicity = dominantly black, white or gray

Most ultrasound studies report that plaques that resemble blood are most vulnerable and may contain thrombus compared to those on the other end of the scale (white).(15;27;35)

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