Carotid Atherosclerotic Plaque Instability as Assessed by Texture Analysis of Ultrasonic Images

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Abstract

BACKGROUND: Internal carotid artery stenosis is a major etiological factor causing cerebrovascular events. Carotid endarterectomy (CEA) has been shown to reduce the risk of future cerebrovascular events. It is increasingly recognized that in addition to stenosis, plaque morphology plays an important role in determining plaque stability. The evolution of imaging modalities such as ultrasound and of computer-assisted image analysis has led to the development of objective, reproducible methods to assess plaque morphology. Previous studies have identified differences in plaque morphology between asymptomatic plaques and those causing different cerebrovascular events and between men and women. Moreover, similarities in morphology between plaques in the carotid arteries have been reported. However, methods used in these studies were mostly subjective, and no study has evaluated these findings using digital image analysis in individuals referred for CEA.

METHODS: Eighty-four neurologically symptomatic and asymptomatic men and women referred for CEA underwent duplex ultrasound examinations and completed a questionnaire regarding their current medications, family history of cardiovascular disease and past medical history, including their most recent cerebrovascular event. We normalized image brightness, outlined atherosclerotic plaques and used computer software to extract 12 histogram/textural features from the outlined plaques. Three substudies were performed.

1) First, we compared ipsilateral plaques features between asymptomatic patients and those with different cerebrovascular symptoms (amaurosis fugax, transient ischemic attack (TIAs) and stroke).

2) Second, we evaluated the degree of correlation of these plaque features between ipsilateral and contralateral carotids in the various symptom subgroups and in the overall population.

3) Thirdly, we compared these plaque features between men and women, on both ipsilateral and contralateral sides.

RESULTS: **1)** Ipsilateral plaques from asymptomatic patients were more echogenic, as described by plaque type, grayscale median (GSM; echolucency), percentage of pixels below grayscale values of 10, 30 and 50 (PPCS1, bel_30, bel_50; echolucency), compared with all symptom groups except TIA. Asymptomatic plaques trended toward greater homogeneity, as described by runlength short-run-emphasis (runl SRE; homogeneity)) and skewness (heterogeneity) than symptomatic plaques as a group and amaurosis fugax plaques. Amaurosis fugax plaques were characterized by significantly greater echolucency than all other groups and also trended toward a significantly more homogeneous appearance than TIA plaques, as described by spatial gray level dependence angular second moment (SGLD ASM; homogeneity) and SLGD homogeneity (homogeneity).

2) The strongest correlations between the carotids were observed in spatial gray level dependence matrices information measure of correlation-1 (SGLDM IMC-1; heterogeneity), SGLDM correlation (heterogeneity), bel_50, skewness, and GSM.

3) Ipsilateral plaque echolucency was significantly greater in men (lower GSM, higher percentage of pixels between grayscale values of 10 and 20 (PPCS2; echolucency) and bel_30). Ipsilateral plaques from men were significantly more heterogeneous (higher SGLDM IMC-1 and skewness), and yet significantly more homogeneous (lower SGLDM correlation). Contralateral plaques demonstrated similar trends.

CONCLUSION: Using digital image analysis of ultrasonic plaque images, we determined significant differences in histogram/textural features between asymptomatic plaques and those causing different symptoms, in a high-risk cohort of CEA referrals and including some novel texture features. Next, we demonstrated that certain imaging features previously associated with plaque instability correlate well between the carotid sides. Finally, men had more unstable plaques than women in both carotid sides.

Large prospective studies are required to evaluate the prognostic value of digital image analysis.

Abstrait

INTRODUCTION: La sténose de l'artère carotide est une cause majeure d'évènements vasculaires cérébraux (EVC). L'endartérectomie carotidienne (EAC) réduit le risk de futur EVC. Il est de plus en plus reconnu que, en plus de la sténose, la morphologie de la plaque est un déterminant important de la stabilité de la plaque. L'évolution des modalités d'imagerie incluant l'échographie et de l'analyse d'image assistée par ordinateur a mené au développement de méthodes objectives et reproductibles pour évaluer la morphologie de la plaque. Des études antérieures ont identifié des différences dans la morphologie de plaque entre les plaques asymptomatiques et ceux qui causent différents EVC et entre les plaques d'homme et de femme. En outre, des similitudes morphologiques entre les plaques des deux carotides ont été rapportés. Cependant, les méthodes utilisées dans ces études étaient pour la plupart subjectives, et aucune étude n'a évalué ces résultats en utilisant l'analyse d'image numérique chez les personnes envoyées pour EAC.
MÉTHODES: Quatre-vingt quatre individus neurologiquement symptomatiques et asymptomatiques envoyés pour EAC ont subi des examens échographiques duplex et ont rempli un questionnaire détaillé concernant leurs médicaments actuels, les antécédents familiaux de maladies vasculaires et les antécédents médicaux, incluant leur EVC le plus récent. Nous avons normalizé la luminosité des images, établit le contour des plaques et nous avons extrait 12 fonctions d'histogramme/texture décrivant les plaques athéroscléreuse. Trois sous-études ont été réalisées.
1) Premièrement, nous avons comparé les caractéristiques des plaques ipsilatérales entre les patients asymptomatiques et ceux ayant subi différents symptômes vasculaires cérébraux (amaurose fugace, accident ischémique transitoire (AIT) et accident vasculaire cérébral).
2) Deuxièmement, nous avons évalué le degré de corrélation de ces caractéristiques entre la plaque des carotides ipsilatérales et

controlatérales parmi les sous-groupes de symptômes divers et la population entière.
3) Troisièmement, nous avons comparé ces caractéristiques entre les sexes, incluant les côtés ipsilatéraux et controlatéraux.
RÉSULTATS: 1) Les plaques asymptomatiques étaient plus échogène (*plaque type, GSM, PPCS1, bel_30* et *bel_50*) comparées à tous les groupes de symptômes, sauf AIT. Les plaques asymptomatiques ont démontré une tendance vers une homogénéité significativement plus grandes (*runl SRE* et *skewness*) que les plaques symptomatiques toutes ensemble et que les plaques amaurose fugace. Les plaques amaurose fugace ont été caractérisées par une échogénicité significativement plus grande (*plaque type, GSM, PPCS1, bel_30* et *bel_50*) que tous les autres groupes et aussi ont démontré une tendance vers une homogénéité significativement plus grande *(SGLD ASM* et *SLGD homogeneity)* que les plaques AIT.
2) Les plus fortes corrélations entre les carotides ont été observés dans *SGLDM IMC-1* (hétérogénéité), *SGLDM corrélation* (hétérogénéité), skewness, *bel_50* et *GSM*.
3) L'échogénicité de la plaque ipsilatérale était significativement moins élevé chez les hommes (*GSM*, *PPCS2*, *bel_30*). Plaques ipsilatérales des hommes étaient significativement plus hétérogène (*SGLDM IMC-1* et *skewness*), et pourtant significativement plus homogène (*SGLDM correlation*). Plaques contralatérales ont démontré des tendances similaires.
CONCLUSION: En utilisant l'analyse d'image numérique d'images ultrasonores de plaques, nous avons déterminé des différences significatives dans les caractéristiques histogrammes/texturales entre plaques asymptomatiques et ceux ayant causé des symptômes différentes, dans une cohorte à haut risque d'individus subissant EAC, et incluant certaines caractéristiques de texture de nouveaux. Ensuite, nous avons démontré que certaines caractéristiques qui étaient précédemment

associés avec l’instabilité de la plaque sont bien corrélés entre les carotides. Enfin, les hommes avaient des plaques plus instables que les femmes dans les deux carotides. Les grandes études prospectives sont nécessaires pour évaluer la valeur pronostique de l'analyse d'image numérique.

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Abbreviations

AHA – American Heart Assocation
ACAS – asymptomatic carotid atherosclerosis study
AmF – amaurosis fugax
ASM – angular second moment
Asymp - asymptomatic
BMI – body mass index
CAD – coronary artery disease
CAS – carotid artery stenosis
CCA – common carotid artery
CVD – cardiovascular disease
CEA – carotid endarterectomy
CTA – computed tomography angiography
DBP – diastolic blood pressure
DSA – digital subtraction angiography
ECA – external carotid artery
ECST – European Carotid Surgery Trial
EDV – end diastolic velocity
GSM – gray scale median
ICA – internal carotid artery
IMT – intima media thickness
JGH – Jewish General Hospital
MI – myocardial infarction
NASCET – North American Symptomatic Carotid Enderectomy Trial
MRA – magnetic resonance angiography
CE-MRA – contrast-enhanced magnetic resonance angiography
PAD – peripheral arterial disease
PET – positron emission tomography
PET/CT – positron emission tomography with computed tomography
PPCS1 – percentage of pixels with grayscale values less than 10
PPCS2 – percentage of pixels with grayscale values between 10-20
PSV – peak systolic velocity
Runl – runlength
Runl SRE – runlength short-run-emphasis
RVH – Royal Victoria Hospital
SBP – systolic blood pressure
SGLD – spatial gray level dependence
SGLDM COR– spatial gray level dependence matrices correlation
SGLD HOM – spatial gray level dependence matrices homogeneity
SGLDM IMC-1 – information measure of correlation -1
Symp - symptomatic
TIA – transient ischemic attack

1 - Background

1.1 - Stroke Epidemiology

Stroke represents a major health problem and is an important cause of long-term disability in several developed countries [1]. In Canada, 50,000 men and women suffer new or recurrent stroke each year, resulting in 15,000 deaths [2]. Moreover, for each of these annuals strokes, it is estimated that up to ten individuals suffer silent strokes as they produce subtle cognitive deficits rather than the more obvious motor, visual or language disturbances [3]. Mortality from stroke ranges between 10% and 30%, and its survivors remain at a high annual risk of recurrent ischemic events and mortality, both from myocardial infarction (MI) and repeated stroke [1]. After age 55, the risk of stroke doubles each decade [4] and the growth in the aging population will be a source of increasing disability. According to a recent survey, 300,000 Canadians are living with the effects of stroke, with 59.5% of stroke victims reporting the need for help with daily activities and 83.6% reporting feeling limited in performing activities that they had previously enjoyed [3]. Moreover, one in 5 stroke victims will suffer from a major depressive episode, markedly impairing recovery [3]. The long course of rehabilitation after a stroke contributes to the enormous financial burden of stroke carried by Canadians at $3.6 billion per year in hospital costs, physician services and long-term disability, with the average cost of acute (<3 months) care of stroke totaling $27,500 [3].

1.2 - Types of Stroke

There are two types of cerebrovascular accidents: ischemic stroke and hemorrhagic stroke. Ischemic stroke, representing 85% of strokes [3], is caused by the interruption of blood flow which produces cerebral ischemia, results in neurological symptoms typically lasting longer than 1 hour and associated with cerebral infarct [5]. When symptoms resolve in less than 1 hour and there is no evidence of cerebral infarction, the cerebrovascular

event is called a transient ischemic attack (TIA) [5]. Amaurosis fugax consists of monocular blindness usually lasting between a few seconds and 10-15 minutes [5]. Hemorrhagic stroke, also referred to as intracerebral hemorrhage, represents 15% of strokes and involves direct bleeding into the brain parenchyma that produces neurological symptoms [3].

1.3 - Etiology of stroke

There are many different causes of ischemic stroke, with cardioembolism, artery-to-artery embolism and thrombosis representing the most common types (Table 1). The etiology of stroke is often uncertain despite thorough investigation, with 30% of stroke cases unable to be attributed specific causes [6].

1.3.1 - Cardioembolic stroke

Ischemic strokes are caused by cardioembolism in 20% of stroke cases [6], whereby thrombi forming primarily on either the walls of the left atrium, left ventricle or the left heart valves (mitral or aortic) detach, embolize and occlude distal arteries supplying the brain. Depending on the duration of the occlusion, the embolism may cause a TIA (with early lysis) or a stroke (with later lysis).

The most common cause of cardioembolism is non-rheumatic atrial fibrillation [6], with thrombi forming on the atrial wall or in the atrial appendage. The average yearly risk for stroke in patients with atrial fibrillation is 5% [7] ranging between 1.5% and 23.5%, depending on the presence of certain risk factors, such as age, hypertension, poor left ventricular function, prior cardioembolism, diabetes and thyrotoxicosis [8].

Other sources of cardioembolism include recent MI, which may pose a particularly high risk when transmural and affecting the anteroapical ventricular wall [9]. Prosthetic valves, rheumatic heart disease may lead to endocarditis involving the mitral or aortic valves, promoting thrombus

formation, and subsequent embolization [10]. Paradoxical embolism includes the crossing of venous thrombi to the arterial circulation either by atrial septal defect or patent foramen ovale. However, as only 15% of the population suffers from these congenital anomalies, the significance of paradoxical embolism as etiology for stroke is controversial [6].

1.3.2 - Artery-to-artery embolic stroke

Unstable atherosclerotic plaque may cause thrombus formation and eventual embolism, occluding downstream intra-cranial arteries and causing an artery-to-artery embolic stroke. The most frequent source of artery-to-artery embolism is the carotid artery (10% of ischemic strokes), including the bifurcation (main source) and the common and internal carotid arteries (minor sources) [6]. Emboli may also originate from the aortic arch, vertebral and basilar arteries [11-13].

1.3.3 - Other causes of artery-to-artery embolic stroke

Intracranial atherosclerosis may cause a stroke either by embolism or by thrombus formation at the site of the diseased vessel [14]. More common in younger patients (<60 years), dissection of either ICA or vertebral arteries or vessels beyond circle of Willis can also lead to stroke, usually preceding the onset of symptoms by a few hours to days [15].

1.3.4 Small vessel stroke

Small vessel stroke, also known as lacunar infarction, represents 20% of all stroke cases and is caused either by atherothrombotic or lipohyalinotic occlusion of small cerebral arteries (30 to 300μm) branching off the middle cerebral, the basilar or the vertebral arteries [6].

Table 1 – Common Causes of Stroke [6]

1. Embolic occlusion	**1b) Artery-to-artery**
1a) Cardioembolism	Carotid bifurcation
Atrial fibrillation	Aortic arch
Mural thrombus	Internal carotid
Dilated cardiomyopathy	Common carotid
Valvular lesions	Vertebral artery
Mitral stenosis	Basilar artery
Mechanical valve	Arterial dissection
Bacterial endocarditis	**2. Thrombosis**
Paradoxical embolus	Small vessel stroke (lacunar)
Atrial septal defect	Large vessel thrombosis
Patent foramen ovale	Dehydration
Atrial septal aneurysm	
Spontaneous echo contrast	

1.4 - Risk Factors for Stroke

Several factors have been associated with increased risk for stroke. These include non-modifiable factors such as advanced age, male sex, personal or family history of cardiovascular disease and modifiable risk factors such as hypertension, hypercholesterolemia, diabetes mellitus, cigarette smoking, obesity, physical activity, atrial fibrillation and carotid artery stenosis (CAS).

1.4.1 - Age

Each year of life brings damage to the cardiovascular system, with injury increasing with the number and severity of risk factors (many of which are progressive in nature). Studies have shown that stroke risk doubles with every decade after the age of 55 years [4, 16].

1.4.2 – Sex

Although male sex has been associated with increased risk of stroke [4, 17], the greater life expectancy in women has led to a greater prevalence of stroke in women [18]. While women have been reported to suffer their first stroke at an older age than men [17-19], women had a higher stroke incidence above age 85 [19].

1.4.3 - Family history of cardiovascular disease

Family history of cardiovascular disease (CVD) predisposes individuals for stroke [20], whether through the perpetuation of similar lifestyles, or through the inheritance of genes predisposing to the development of CVD risk factors or those directly regulating vascular function [21]. Family history of stroke has been associated with increased stroke risk [22, 23]. Twin studies have supported the genetic basis for stroke inheritance [21, 24, 25], with one study reporting an increased risk of 4.3 ($p<0.05$) in the concordance for stroke in monozygotic twins stroke compared with dizygotic twins [25]. The children of parents suffering premature MI (age <45 years for men, <55 years for women) are also at increased risk for stroke [20, 26], although the link may be stronger with coronary heart disease [26]. Individuals with family history of premature MI also demonstrate more advanced early atherosclerosis, as represented by carotid intima-media thickness (IMT) [27, 28], and more endothelial dysfunction [28]. Moreover, family history of peripheral artery disease (PAD) predisposes to atherosclerosis, as shown by its association with a greater risk for PAD [29].

1.4.4 - Prior cardiovascular disease

Prior cardiovascular disease is another risk factor for stroke. After adjustment for other stroke risk factors, men and women with a history of CVD have a 73% and 55% greater stroke risk, respectively, (RR 1.73, 95% CI 1.68-1.78, RR 1.55, 95% CI 1.17-2.07), than those without a

history of CVD [30]. A history of stroke or TIA is another important risk factor for stroke [31]. In the first six months after a first-ever stroke, the risk of a second stroke is 8.8% (95% CI, 5.4-12.1%) [32], while at 5 and 10 years, the cumulative risks are 22.5-30% [31, 33] and 43% (95% CI, 34-51%) [33]. Furthermore, after a first-ever TIA, cumulative stroke risk has been reported as 8.6% at 7 days, 12.0% at 30 days, up to 14.5% at 90 days and as high as 30% at 5 years after the event [34-37].

1.4.5 - Hypertension

Hypertension is a well-established risk factor for stroke. In Canada, one in four individuals over the age of 20 have been given the diagnosis [38]. Both systolic blood pressure (SBP) and diastolic blood pressure (DBP) have been associated with risk for stroke [39]. Blood pressure lowering has been shown to effectively improve stroke risk by 30-40%, so far without a drug-specific effect, according to recent meta-analyses of randomized controlled trials [40-42].

1.4.6 - Hypercholesterolemia

Hypercholesterolemia is a common risk factor for stroke, with more than 40% of Canadians age 20 to 79 reporting high levels of total cholesterol [43]. A large cohort study reported that for every 1 mmol/l increase in total cholesterol, there was a 20% (95% CI, 1.16-1.24) increase in stroke risk [44]. Recent studies have also shown that increased triglycerides and decreased high-density lipoprotein cholesterol (HDL-C) have both been linked with elevated risk for stroke ([45-47]. A large meta-analysis evaluating statin trials found that for every 1mmol/l decrease in low-density lipoprotein cholesterol, there was a 21% decrease (OR 0.79, 95% CI, 0.73-0.85) in stroke risk [48]. Statins, however, may also reduce stroke risk by mechanisms other than lowering cholesterol levels through their pleiotropic effects [49].

1.4.7 - Diabetes mellitus

Diabetes mellitus affects approximately 2.4 million Canadians [50] and is a well-established risk factor for stroke. The Honolulu Heart Program [51] demonstrated a significant relationship between increased stroke risk and subgroups of increasing glucose intolerance, and after adjusting for other risk factors, reported that there was increased stroke incidence in both asymptomatic individuals with high glucose intolerance (> or = 225 mg/dl) and those with known diabetics, with relative risks of 1.43 (95% CI 1.00-2.04) and 2.45 (95% CI, 1.73-3.47), respectively [51]. Other studies have supported these results [52-54].

1.4.8 - Cigarette smoking

Many studies have demonstrated cigarette smoking to be an important risk factor for stroke, nearly doubling its risk after adjusting for other factors [55-57]. Furthermore, 12-14% of all stroke deaths have been attributed to smoking [58]. Other studies suggest that even exposure to environmental cigarette smoke has been linked with increased risk for stroke [59, 60].

1.4.9 - Obesity

Definitions of obesity have been based on the body mass index (BMI), whereby individuals with BMI greater than 30 are classified as obese [61]. Compared with individuals with low-normal weight (~BMI<23), those with BMI >30 have been reported to have risks for stroke between 1.78-1.95 [62-64], and for every unit increase in BMI, there is an adjusted increase in stroke risk of between 4% to 6% [63, 64]. A large epidemiological study including more than 439,000 Korean women found a strong linear correlation between stroke risk and BMI, which weakened, however, at more advanced ages [65]. Adjustment for major risk factors, such as

hypertension, hypercholesterolemia and diabetes attenuated but did not eliminate the increased risks associated with obesity measured by BMI [63-66]. Studies have also shown increased risk for stroke with increasing abdominal obesity, measured by waist-to-hip ratio [67].

1.4.10 - Atrial fibrillation

Atrial fibrillation is a common cardiac arrhythmia that increases the risk of cardioembolism and stroke 5-fold and is reported by the Framingham Study to affect 1.0-1.5% of individuals between age 50 and 59 and 23.5% of individuals between age 80 and 89 [8]. Although anti-arrhythmic therapy has been ineffective at decreasing stroke risk, warfarin treatment and aspirin treatment have been shown to reduce the risk for stroke by 60% and 20%, respectively [68, 69].

1.4.11 - Carotid Artery Stenosis (CAS)

CAS has been associated with increased risk for stroke. Natural history studies have demonstrated that increasing severity of ICA stenosis leads to higher risk of stroke. Accordingly, while stenosis less than 75-80% (in neurologically asymptomatic patients) represents a yearly risk of 0.1-1.6%, stenosis greater than 75-80% (in symptomatic patients) is associated with an annual risk of 2.0-3.3% [70-77].

1.5 - Management of CAS

1.5.1 - Medical management

Medical management of CAS includes risk factor reduction and antiplatelet therapy [78]. Risk factor reduction includes achieving specific blood pressure and lipid levels, maintaining appropriate glucose levels in diabetics and promoting smoking cessation. Although physical inactivity has an attributable risk for stroke of 30%, the risk reduction associated with increased activity remains to be determined [78]. The most widely used antiplatelet agent is aspirin (75-325mg daily) [79], although

clopidogrel (plavix) is a common alternative for symptomatic patients and in those with an aspirin allergy or failure [78].

1.5.2 - Surgical management

Surgical interventions for persons with carotid stenosis include carotid endarterectomy (CEA) and carotid angioplasty and stenting [80]. Current guidelines depend on the degree of stenosis, the presence of a recent history (<6 months) of cerebrovascular events (stroke, TIA, amaurosis fugax) and risk factors for peri- and post-operative complications [78].

The efficacy of CEA in individuals with asymptomatic carotid stenosis was established by the Asymptomatic Carotid Atherosclerosis Study (ACAS) which randomized 1662 individuals with 60-99% carotid stenosis to receive CEA and daily aspirin or aspirin alone [81]. The surgical group benefited from a decreased incidence of ipsilateral stroke and perioperative stroke or death compared with the aspirin alone group (5.1% vs. 11.0%), with an absolute risk reduction of 53% (95% CI, 22-72%). Two other trials support this benefit of CEA in asymptomatic carotid stenosis [82, 83]. A meta-analysis of the two larger trials found the absolute risk reduction to be 3% over three years, and that the number needed to treat (NNT) was 33 [84].

There is stronger evidence for CEA in individuals with symptomatic carotid stenosis, as demonstrated by the North American Symptomatic Carotid Endarterectomy Trial (NASCET) [85]. The study randomized 659 recently (<120 days) symptomatic patients with 70-99% stenosis to medical treatment, with or without CEA. The surgical patients experienced approximately half the rate of any ipsilateral stroke or death (15% vs. 32%), and approximately one third of the rate of ipsilateral stroke (9% vs. 26%) compared with those only receiving medical treatment [85]. Compiling data from two other large trials [77, 82], the absolute risk reduction of CEA in symptomatic carotid stenosis was 16%, and the NNT was 6.3 [86].

In addition to symptomatology, recommendations for CEA are based on the degree of stenosis caused, but not on plaque morphology [87]. However, it is increasingly recognized that, in addition to stenosis, plaque morphology is a risk factor for plaque instability and rupture [88, 89]. Accordingly, while some plaques causing high-grade stenosis remain stable and do not produce symptoms, other plaques causing moderate stenosis may become unstable and rupture. Moreover, histological and immunohistochemical studies of carotid specimens obtained during CEA have determined that certain morphological features typically characterize *unstable* plaques (plaques having caused cerebrovascular symptoms). In particular, features of unstable plaques include large extracellular lipid-rich core, a thin fibrous cap, ulceration, lumen thrombosis, and intraplaque hemorrhage [88, 90, 91]. Furthermore, infiltrations of inflammatory cells (mostly macrophages and T-lymphocytes) are often observed within the cap of the unstable plaque, which is characterized by fewer smooth muscles cells and less collagen than caps of stable plaques [88, 90, 91].

Histological and immunohistochemical analysis of CEA specimens have reliably distinguished stable from unstable plaques. Otherwise, unstable plaques may only be identified once they have ruptured and caused symptoms. However, invasive, costly and potentially life-threatening surgery performed on a patient with stable carotid plaque is dangerous and an improper management of resources and time. In contrast, recognizing plaque instability only after a crippling or fatal stroke does not allow for primary preventive intervention. Consequently, many investigators have focused on non-invasive imaging techniques in order to identify the unstable plaque *in vivo*, before the development of cerebrovascular symptoms.

1.6 - Non-invasive imaging modalities used in carotid stenosis

Several invasive and non-invasive imaging methods have been developed to study CAS and plaque morphology [92, 93]. Due to potential

complications arising from invasive procedures, patients are increasingly evaluated with non-invasive imaging modalities [94]. The most studied methods include magnetic resonance angiography, magnetic resonance imaging, computed tomography, and duplex ultrasound [93].

Magnetic resonance angiography (MRA) is used to evaluate stenosis. Contrast-enhanced-MRA (CE-MRA) boasts the highest sensitivity and specificity (94% and 93%, respectively) [94] of any non-invasive imaging method when compared with digital subtraction angiography (DSA), the 'gold standard' of stenosis measurement. Moreover, CE-MRA is more cost-effective than DSA and does not involve ionizing radiation [95]. However, as MRA is not available to all medical centers, it is often not the method of choice to evaluate stenosis in the everyday clinical practice [93].

MRA is also used in the study of plaque morphology. Good correlation between MRA imaging and histology has led to the development of a modified version of the American Heart Association (AHA) plaque classification [96]. MRA has been employed in longitudinal studies to evaluate atherosclerotic progression [97] and responses to pharmacotherapy [98]. However, MRA involves lengthy image processing and is not routinely used in clinical practice [93].

Computed tomography angiography (CTA) is used to measure stenosis, however it may also detect features of plaque instability such as surface ulceration [93]. Although it is highly specific (94%) when compared with DSA, it has poor sensitivity (76%) [94] and often underestimates stenosis [93]. It does, however, provide a broad view of the vasculature surrounding the carotids, from the aortic arch to the circle of Willis [93]. However, the use of CTA is limited by its associated radiation and the potential for contrast nephropathy in vulnerable patients.

The combination of positron-emission tomography (PET) with CT (PET/CT) represents a promising new imaging method in the characterization of carotid plaque morphology through the evaluation of

plaque inflammation [99], which has shown significant associations with unstable plaques visualized using ultrasound [100].

Duplex ultrasound, combining Doppler and B-mode ultrasound, has been the most widely employed imaging method in the study of atherosclerotic disease [89, 101]. Doppler ultrasound enables the evaluation of stenosis, and despite being operator dependent, it has a pooled sensitivity and specificity of 86% and 87%, respectively, when compared with DSA [102]. B-mode ultrasound permits the assessment of area and diameter reduction, IMT and atherosclerotic plaque morphology [89].

1.7 - Ultrasonic Image analysis of carotid plaques

1.7.1 - Early work: visual classifications

Several groups developed classification systems of ultrasonic plaque appearance in order to systematically assess carotid plaque morphology. Three main binary classifications have been created: homogeneous or heterogeneous [103], dense or soft [104, 105] and echolucent or echogenic (relating to overall level of echo patterns) [106]. Gray-Weale et al. proposed a classification including four types, with type 1 plaques as predominantly echolucent, type 2 as predominantly (>75%) echolucent but with some echogenic components, type 3 as predominantly echogenic (>75%) with small echolucent areas (<25%), and type 4 as entirely echogenic [107]. The most widely used classification was developed by Geroulakos et al., who modified the Gray-Weale classification by defining *predominant* as greater than 50% rather than 75% and by describing a fifth type of plaque, which cannot be accurately classified due to its calcified surface [108].

1.7.2 - Early work: The unstable plaque on ultrasound

Subsequent ultrasonic studies demonstrated that unstable plaques typically produce high-grade stenosis, possess surface ulcerations and appear echolucent (classified as plaque types 1 and 2) [108-110].

1.7.3 - Correlation between echolucency and plaque histology

Studies have shown that plaque echolucency is associated with rapidly progressing lesions [110, 111] and histological features of plaque instability [91, 112-114]. For example, echolucent plaques (type 1 and 2) have been associated with larger necrotic core volume [115] and increased macrophage density than echogenic plaques (type 3 and 4) ($p=0.02$) [112]. Moreover, studies have shown echogenicity to be inversely related to the proportion of intraplaque hemorrhage and lipid-rich core in the plaque ($p=.005$), and directly related to collagen content and calcification ($p=.0001$) [113, 116].

1.7.4 - Echolucent plaques associated with increased risk for stroke

Studies have found plaque echolucency to contribute to stroke risk independently of degree of stenosis [117]. One study found that in symptomatic patients, the relative risk (RR) of ipsilateral ischemic stroke for echolucent versus echogenic plaques was 3.1 (95%, CI, 1.3-7.3), whereas for 80-99% versus 50-79% stenosis, the RR was only 1.4 (95% CI, 0.7-3.0) [117]. Moreover, relative to symptomatic patients with echogenic 50-79% stenotic plaques, those with echogenic 80-99% stenotic plaques, echolucent 50-79% stenotic plaques, and echolucent 80-99% stenotic plaques had RRs of ipsilateral ischemic stroke of 3.1 (95% CI, 0.7-14), 4.2 (95% CI, 1.2-15), and 7.9 (95% CI, 2.1-30), equivalent to absolute risk increases of 11%, 18%, and 28%, respectively [117]. Furthermore, another study demonstrated that among plaques with stenosis of 70-99% (ECST criteria[77]), the RR for ipsilateral stroke in

individuals with plaque types 1, 2 and 3 versus those with types 4 and 5 was 11.7 (95% CI, 1.63-84.5)[118].

1.7.5 - The problem: low reproducibility of subjective plaque characterization

However, although these studies demonstrated echolucency to be an important risk factor for plaque instability, their methods were highly subjective, using visual inspection alone to categorize plaque echolucency into different plaque types. Attempts were made at improving the reproducibility of methods by evaluating plaque echolucency according to three reference structures, namely blood (termed hypoechoic) sternocleidomastoid muscle (isoechoic) and bone (hyperechoic) [119]. Polak et al. assessed plaque echolucency using these methods and determined that, in plaques causing greater than 50% stenosis, hypoechoic plaques were associated with significantly greater incidence of ipsilateral ischemic stroke than isoechoic and hyperechoic plaques (relative risk of 2.78) [120]. The authors then suggested that the use of quantitative, computer-assisted methods would be necessary for more objective plaque characterization [120].

1.7.6 - The solution: computer-aided analysis of plaque morphology

The need for greater reproducibility resulted in the development of the grayscale median (GSM), which serves to quantify plaque echodensity (overall plaque brightness) and is defined as the median of the grayscale values (scale: 0-255, 0=black, 255=white) [121]. Histological studies demonstrated that plaques with low GSM (more echolucent) were associated with large necrotic core volume [115] and increased macrophage infiltration on histology (p=.002) [112]. Moreover, while one study demonstrated that plaque with a GSM of less than 32 had a 5-fold increase in the prevalence of silent brain infarcts on CT brain scans [122],

another group found a GSM cut-off point of 50 to have a 4.6 fold increase [123].

1.7.7 - Advent of normalization

Thus, although the use of computers enabled the quantification of echolucency, the variability in the value of GSM cut-off points [122-126] revealed the need for ultrasonic image normalization, which could render comparable all images captured under different instrument settings, from different scanners, by different operators, and through different peripherals such as DVD, video or magnetooptical disk [109, 127]. Images are normalized by assigning GSM values of 0 and 180 to the blood and adventitia, respectively [109]. The standardization of image brightness resulted in the reclassification of 60% of the plaques in one large trial [118], whereby before normalization, all types were associated with events, and after normalization, 94% of events occurred in patients with plaque types 1-3 [118].

1.7.8 - Beyond echolucency: the controversial role of plaque heterogeneity

Although there is good agreement regarding echolucency as risk factor for plaque instability, the role of heterogeneity in plaque instability has been less clear, with different studies suggesting either heterogeneity or homogeneity to be associated with instability [121, 128-134].

These studies, however, used subjective methods to assess heterogeneity. Some defined heterogeneous plaques as having two or three types of echoes (dark, intermediate, bright) compared with homogeneous as having only one type of echo [135]. Others developed semi-quantitative methods, with plaques needing to fulfill all four prescribed criteria to be considered heterogeneous [121]. The poor agreement between studies concerning methods assessing heterogeneity and the resulting unclear association between heterogeneity and symptom development revealed the need to quantify heterogeneity.

1.7.9 - Digital Image Analysis: quantification of echodensity and heterogeneity

Recent advances in digital image analysis have led to more sophisticated computer-assisted methods of plaque characterization, able to *quantify* not only the echodensity (overall brightness), but also the *texture* (heterogeneity) of the plaque [109, 136-139].

1.7.9 - Digital image analysis: well-established in the study of solid organs

Digital image analysis is a well-established method initially developed by Electrical and Electronic Engineers [127]. In fact, ultrasonic texture characterization has previously been used by multiple groups on echocardiographic images and ultrasonic images of the breast and liver [140]. Moreover, ultrasonic texture studies in liver disease have successfully identified texture features that can distinguish between normal liver, malignant disease and diffuse parenchymal disease, distinguishing several different echopatterns in the latter which cannot be achieved by conventional liver ultrasound [141, 142].

1.7.10 - Digital image analysis: new software designed for carotid atherosclerosis

Based on digital image analysis, new software designed for carotid atherosclerotic plaque has been developed. This software, based on the texture analysis tool platform for Matlab, Math-Works Inc., produces 51 histogram and texture features of the gray tones of the pixels within the plaque (see Appendix – Figures 8-11).

1.8 - Cerebrovascular symptoms and plaque echomorphology

There is evidence that different cerebrovascular symptoms may be associated with different types of plaques. Tegos et al. [143] reported that amaurosis fugax was caused by very echolucent and highly-stenotic

plaques (90% stenosis), TIA and stroke were produced by plaques with intermediate echolucency and intermediate stenosis (80%) and plaques causing no symptoms were echogenic, and caused moderate stenosis (70%); $p<0.05$).

Symptomatology may also be related to textural features. Comparing 10 symptomatic with 9 asymptomatic plaques, one study found 3 texture features to be significantly associated with the presence of symptoms [144]. Using the same software as the present study, Kakkos et al. demonstrated that in addition to GSM, two measures of heterogeneity, spatial gray level dependence matrices (SGLDM) correlation and SGLDM information measure of correlation-1 (SGLDM IMC-1) were significantly associated with ipsilateral embolic brain infarcts on logistic regression [137]. Furthermore, the study found that the combination of SGLDM IMC-1 with GSM significantly improved the value of GSM in distinguishing embolic from non-embolic CT-brain infarctions ($p=0.02$), with the area under the ROC curve increasing from 0.62 to 0.81 (representing good diagnostic accuracy) [137]. Recently, Kakkos et al. [138] used texture analysis to distinguish asymptomatic plaques from those causing different symptoms. Amaurosis fugax was independently associated with severity of stenosis, percentage of pixels with gray levels 0-10 (PPCS1; echolucency and texture) and SGLDM IMC-1. TIAs were independently associated with PPCS1, SGLDM correlation and skewness (texture) and strokes with PPCS1, SGLDM correlation, and percentage of pixels with gray levels 11-20 (PPCS2; echolucency and texture). The area under the curve of the predicted probability for amaurosis fugax, TIA and stroke was 0.92, 0.82 and 0.85, respectively [138].

Other texture features that have shown promise in characterizing plaque heterogeneity include SGLD angular second moment (SGLD ASM), SGLD homogeneity and gray level runlength statistics (RUNL) [119]. *Further studies are required to confirm the textural features and echolucency of plaques causing different types of symptoms.*

1.9 - Plaque instability – a systemic condition

Studies have suggested that atherosclerosis is a systemic disease [145]. Accordingly, plaque burden and instability within different arteries are likely to be similar. Rothwell et al. reported that patients with irregular (unstable) plaques in both carotid arteries were more likely than those with smooth plaques to have suffered a previous MI (hazard ratio (HR) 1.82, 95% CI, 1.23-2.64), p=0.007) [145]. Brevetti et al. found that femoral artery plaque GSM lower than the median was a significant predictor of echolucent carotid plaques (3.87; 95% CI, 1.53-9.83). Moreover, patients with PAD and those with concomitant PAD and coronary artery disease (CAD) were more likely to have echolucent carotid plaques than patients with CAD alone (OR 5.13; 95% CI, 1.27-20.67, p=0.21, and OR 4.16; 95% CI 1.68-10.28, respectively).

There is strong evidence that plaques in carotid arteries have similar morphology [145-147]. Adams et al. (2002) found that total carotid wall volumes were moderately correlated (concordance correlation coefficient r_c=0.71) and calcification volumes were well correlated (r_c=0.94) between the two sides [146]. Furthermore, irregular plaque in one carotid artery was more likely than smooth plaque to have an irregular plaque in the contralateral artery (OR 2.21; 95% CI, 1.62-3.01, p<0.001) [145]. In addition, Paraskevas et al. reported that in patients with contralateral ICA occlusion, there was a similar distribution of carotid plaque types 1-4 between the two carotids [147]. *However, no studies have quantitatively correlated the plaque morphology in one carotid artery with plaque morphology in the contralateral* artery.

1.10 - Sex differences in carotid atherosclerosis

Many studies have aimed to uncover the differences in CVD between men and women [148]. It is well established that prior to menopause, the risk of cardiovascular disease in women is significantly less than that of

men [149]. Many studies have focused on determining sex differences in carotid atherosclerosis [150-155]. Epidemiological studies have shown that men are prone to have a greater number of cardiovascular risk factors than women [66, 154]. Moreover, step-wise increases in the number of risk factors in men results in greater progression of carotid stenosis than in women [153]. More specifically, the proportion of men vs. women who had greater than 50% stenosis with no risk factors was 2.4% vs. 0.6% ($p=0.01$), with one risk factor; 6.7% vs. 1.5% ($p<0.001$), with two risk factors; 10.7% vs. 2.7% ($p<0.001$) and with all three risk factors; 18.6% vs. 5.0% ($p=0.01$). Other studies found male gender to be an independent risk factor for carotid stenosis progression [156].

There is evidence that gender may not only contribute to the development of carotid stenosis but also to a more unstable plaque morphology. For example, the European Carotid Plaque Study Group found a significantly larger amount of soft tissue (lipid core and hemorrhage) in CEA specimens from men than from women ($p=0.0006$), and that the quantity of soft tissue was inversely related to plaque echogenicity on B-mode images ($p<0.0001$) [133].

Furthermore, a population-based study by Joakimsen et al. *(*1999) that included 3016 men and 3404 women aged 25 to 84 years, evaluated plaque morphology with B-mode ultrasound [150]. Overall, a greater proportion of men had carotid plaques than did women (55.4% vs. 45.8%, respectively), and there was a greater prevalence of ‘soft’ plaques (consisting of grades 1 and 2) in men than in women [150].

However, the methods used in these two studies to compare plaque morphology in men and women were subjective in nature and required further investigation using objective methods such as GSM and features of texture analysis.

1.11 - Research objectives and hypotheses

Objective 1**:** To estimate the differences in plaque types (more subjective) and in the degrees of echogenicity and heterogeneity (more objective) characterizing the ultrasonic images of asymptomatic carotid plaques and those having caused different cerebrovascular symptoms (amaurosis fugax, TIA and stroke).

Hypothesis 1: More asymptomatic patients will have type 4 plaques, more echogenic and more homogeneous than those causing symptoms. Amaurosis fugax patients will have type 1 plaques, more echolucent than all other plaques, and more homogeneous than plaques causing either TIA or stroke.

TIA and stroke patients will have type 2 and 3 plaques, more echolucent than asymptomatics, more echogenic than amaurosis fugax, and more heterogeneous than asymptomatic plaques and those causing amaurosis fugax.

Objective 2: To estimate the degree of correlation of plaque morphology between in carotid arteries in terms of plaque types, as well as histogram/textural features describing plaque echogenicity and heterogeneity.

Hypothesis 2: Plaque echogenicity and heterogeneity in plaque from one carotid artery will be similar to that of the contralateral carotid artery.

Objective 3: To estimate the differences in plaque morphology between men and women referred for CEA in terms of plaque types, and histogram/textural features describing plaque echogenicity and heterogeneity.

Hypothesis 3: Lower plaque type and greater echolucency and heterogeneity will be associated with male sex.

2 - **Methodology**

2.1 - Recruitment

In this hospital-based cross-sectional study, we recruited consecutive neurologically symptomatic (amaurosis fugax, TIA, stroke) and asymptomatic men and women who were scheduled to undergo CEA at the Royal Victoria Hospital (RVH) (part of McGill University Health Center) and the Jewish General Hospital (JGH) (McGill University-affiliated hospital). The vascular surgery departments at these two centers perform a large number of CEAs each year (RVH: approximately 60, JGH: approximately 100; estimates are based on hospital statistics over the two years prior to the start of the current study). All patients underwent CEA performed using a standard surgical protocol.

2.2 Inclusion/Exclusion criteria

Patients were referred for CEA if they:

a) had recently (previous six months) developed hemispheric symptoms ipsilateral to an ICA stenosis of 60-99% (NASCET criteria) [87]
b) were neurologically asymptomatic on both hemispheres and have ICA stenosis of 70-99% (NASCET criteria) [87]

Such eligible patients would be excluded if they had previously undergone CEA or carotid angioplasty and stenting on the same carotid artery or if they could not understand the protocol.

Subjects were recruited from the pre-operative vascular surgery clinics at the RVH and the JGH. Having obtained written, informed consent from each patient (see Appendix – Figure 3), we scheduled an appointment prior to their CEA during which an ultrasonic examination and anthropometric measurements were performed. Participants were also given a questionnaire to complete and return to the research team at their ultrasound appointment.

2.3 - Questionnaires (see Appendix – Figures 1 and 2)

2.3.1 - The main questionnaire

The main questionnaire queried medication use, family history of CVD, past medical history and history of tobacco consumption (see Appendix – Figure 1).

Patients were considered positive for a *family history of CVD* if a first-degree relative (parent, sibling, child) had a premature MI (men < age 45, and women < age 55), an ischemic stroke or PAD. Patients with no knowledge of their family history for any reason were not included in related analyses.

The route, dosage, frequency and start date of each *medication* was obtained. Medication was considered current and significant to the study if it had been consumed for over a month prior to the ultrasound examination. Medications targeting vascular risk factors such as hypertension, hypercholesterolemia and diabetes were recorded.

Past medical history of interest included prior CVD and vascular risk factors. The presence of previous MI, coronary intervention, congestive heart failure, angina, PAD or any intervention targeting peripheral atherosclerotic arteries were questioned. Information regarding risk factors included the presence of hypertension, dyslipidemia or diabetes, the duration of the disease and the treatment (if treated). Risk factor status was positive with prior risk factor diagnosis by a physician and/or current prescription for medications treating these conditions.

History of tobacco consumption included smoking status, classified into current smokers, former smokers (having quit for longer than one month) and never smokers (having smoked fewer than 100 cigarettes total [157]. The number of pack-years smoked was defined by the product of total years of smoking and the number of cigarette packs smoked each day. One pack of cigarettes was equivalent to 20 cigarettes. In former smokers, the time elapsed since smoking cessation was recorded.

2.3.2 - Symptom questionnaire

Using a symptom questionnaire (see Appendix – Figure 2), patients recorded the date, duration and nature of their most recent and severe cerebrovascular symptoms. Patients with a history of neurological symptoms associated with CAS were considered symptomatic. Cerebrovascular events causing symptoms lasting more than 24 hours were considered strokes. Symptoms resolving in fewer than 24 hours were considered to represent TIAs unless the only symptom was transient monocular blindness, which would represent amaurosis fugax. When a patient had suffered more than one type of cerebrovascular event, the most recent and severe event dictated the symptom status of the patient.

2.3.3 - Data verification using of medical records

All medical information provided by the patients was verified using medical records at the RVH and at the JGH, including admissions, progress notes, emergency room consults done by neurology or vascular surgery services.

2.4 - Duplex ultrasound: machine settings

Participants underwent ultrasound assessments of both carotid arteries, performed by the same ultrasonographer, who was blinded to patient clinical status. Ultrasonic examinations were performed at the Cardiology Research Laboratory at the RVH (S6.10) using General Electric Vivid 7 ultrasound machines (General Electric, Waukesha, WI, USA) and standard settings ensuring optimum image quality for plaque type classification and texture analyses [158]. In particular, transcutaneous real-time grey scale mode (real-time B-mode) with a M12L linear probe was used with the following specifications [158]:

- **Time Gain Compensation curve**: sloping through the tissues but positioned vertically through the lumen of the vessel as there was little attenuation of the ultrasound beam as it passed through blood.

This ensured similar brightness of the adventitia of the anterior and posterior walls.

- **A linear post-processing curve** was used.
- **Persistence:** minimal (set on low).
- **Frame rate:** high frame rate was used to ensure satisfactory temporal resolution with accurate depiction of motion.
- **Overall gain:** adjusted to give optimum image quality and set to minimize but not abolish noise.
- **Dynamic range**: set to maximum (60 dB).
- **Depth**: minimized so that plaque occupied a large part of image

2.5 - The exam

2.5.1 - Preparing the Patient for the Exam

The examination was performed after a 10 minute rest in the supine position on the examination bed while three electrocardiogram (ECG) leads were placed in three locations: immediately inferior to the jugular notch, on the lower part of the sternum and midway along the patient's left costal margin. The leads were connected to the ultrasound machine in order to obtain an ECG tracing synchronized to the images and cineloops acquired. The lights were turned off and the window blinds pulled down for the entire duration of the exam to allow for consistent, optimal lighting and minimal variation in the gain. Blood pressure (BP) was then taken twice on each arm by cuff sphygmomanometry, at intervals of 2-3 minutes. The mean systolic BP and mean diastolic BP were recorded from the arm with the higher BP. If BP values varied by more than 5mmHg, BP would be taken a third time and the average of the two most similar measurements was recorded.

2.5.2 - ICA Identification and Initial Scan

To begin the ultrasound exam, the head was rotated from the anterior-posterior position, 15-45 degrees away from the side to be examined. The

sonographer followed a standard protocol, beginning with an initial scan of the carotid in the transverse view, beginning proximally at the common carotid artery (CCA) and finishing at the most distal point of the internal carotid artery (ICA). Identification of the external carotid artery (ECA) enabled the identification of the ICA. The ECA Doppler waveforms are characterized by a high-resistance flow pattern, with sharp systolic peaks and relatively little flow in diastole. The ECA was confirmed if, by tapping the temporal artery, notches in the Doppler waveform were observed.

2.5.3 - Identifying plaque causing maximal stenosis, measuring velocities and calculating stenosis

The initial scan was used to identify plaque causing significant stenosis, and was recorded as a black and white cineloop in the transverse projection (ultrasound video). The plaque causing maximal stenosis was identified through blood flow velocity measurements obtained by Doppler ultrasound. Peak systolic and end diastolic velocities (PSV and EDV) were measured at the middle of the lumen of both mid CCA and mid ICA. These velocities were used to calculate specific ratios that have been correlated with degrees of stenosis measured with DSA, the 'gold standard' of stenosis measurement. Accordingly, different values of ratios: PSV_{ICA}:PSV_{CCA}, EDV_{ICA}:EDV_{CCA} and PSV_{ICA}/EDV_{CCA} have been associated with specific ranges of degrees of stenosis, as described previously [159] (see Appendix - Figure 4). Degree of stenosis was expressed according to NASCET criteria, which represents the percentage obtained when subtracting from 1 the ratio of the maximally stenosed lumen diameter to the diameter of the distal normal ICA [159].

2.5.4 - Capturing the plaque causing maximal stenosis

Longitudinal plaque visualization using anterior, lateral and posterolateral projections served to determine the projection in which the plaque caused maximal diameter reduction. Cineloops lasting two cardiac

cycles were captured in this view, in both color and black and white. In addition, transverse cineloops of the point of maximal stenosis were recorded, both in color and black and white.

2.5.5 Contralateral carotid artery

The same procedure was repeated on the contralateral carotid artery to identify and capture an image of the plaque causing maximal stenosis.

2.5.6 - Anthropometric measurements

Following the ultrasound assessment, height, weight and waist circumference were measured and recorded using resources of the clinic.

2.5.7 - Exam storage

The cineloops and measurements captured were then immediately available on the ECHO PACS system, accessible at the cardiac ultrasound lab at the Montreal General Hospital (D17.153), where image selection was performed.

2.6 - Image selection protocol

2.6.1 - Image selection

Image selection was performed using the ECHO PACS software, which enabled frame-by-frame viewing of the cineloops. Black and white longitudinal images of plaques producing maximum stenosis in each carotid artery were selected for analysis from the cineloops acquired during the exam. Two observers selected the images independently. In the case of disagreement, a third observer (supervisor) enabled a consensus to be reached.

2.6.2 - Image Selection Criteria: Plaque thickness

Moreover, plaques were defined as focal protrusions into the lumen that were thicker than 1.5mm upon measurement from edge of adventitia, as previously described [160]; thickenings of less than 1.5mm were considered IMT and were not analyzed. The measurements were performed using appropriately programmed commercially available software (texture analysis tool platform for Matlab, Math-Works Inc.).

2.6.3 - Image Selection Criteria: Image quality and plaque visibility

Images with sharply defined plaque contours and adventitia were chosen in order to ensure subsequently reproducible outlining of the plaque during image cropping. If acoustic shadow partially masked the plaque (<50% hidden), the image frame containing the largest area of visible plaque was selected. Images of plaques more than 50% masked by acoustic shadow were excluded from analysis.

2.6.4 - Additional images selected for simultaneous visualization

In addition to the black and white longitudinal image to be analyzed, additional images and cineloops were selected, not for analysis, but for more thorough visualization of plaque shape. These included the longitudinal, black and white cineloop, the corresponding longitudinal color cineloops and the corresponding transverse, black and white, and color images.

2.7 - Image Normalization

Once selected, the images were then normalized using texture analysis software (the same software used to verify plaque thickness during image selection). Normalization was accomplished using linear scaling with blood and adventitia serving as two reference points, given grayscale values of 0 and 190, respectively, as previously described [118]. First, a noiseless area of “blood” is selected from the vessel lumen near the plaque. Next, the most echogenic portion of adventitia nearest to the plaque was

enlarged 4-fold using the zoom function and the inner two-fourths were selected (see Appendix – Figure 7). The image scale was then established by selecting two depth markers separated by 10mm increments, using appropriate zoom to choose vertically aligned points.

2.8 - Image Cropping

2.8.1 - Visual aids

The “Image Crop” module displayed two images: the normalized black and white image to be cropped and the corresponding image with color. The color image aided in defining the outline of darker plaques, which are more difficult to discern in the black and white image (see Appendix – Figures 5 and 6). During analysis, two cineloops would run continuously: the cineloop from which the analyzed image was taken, and the corresponding cineloop in color. The visualization of cineloops allowed accurate distinction of adventitia from similarly echogenic adjacent soft tissue through the different motion of the two components, facilitating the exclusion of the adventitia from the plaque outline.

2.8.2 - Outlining the plaque

Using the “Image Crop” facility, the plaque was outlined with individual mouse clicks, marking points along the plaque contour that would then be automatically connected by straight lines.

2.8.3 - Inclusion and exclusion

Plaques on near and far walls were included in the outline, with plaque defined as any protrusion into the lumen from the adventitia larger than 1.5mm, as described previously [160]. Acoustic shadow was also excluded from the plaque outline, and was defined as an area completely devoid of any gray pixels that extended to the bottom of the image.

2.9 - Image Analysis

Once saved, clicking the “Feature Extraction” produces the 51 textural and histogram features describing plaque morphology (see Appendix – Figure 9). These values were automatically inputted into a text database that could be opened with “Microsoft Excel” (Microsoft Inc, Redmond, Wash). The program color-coded the plaque pixels according to grey level value. More specifically, pixels with gray values 0-24 were represented by black, 25-29 by blue, 50-74 by green, 75-99 by yellow, 100-124 by orange, and pixels brighter than gray value of 124 were red (see Appendix – Figure 8).

2.9.1 - Plaque types (see Appendix – Figure 8)

The program has been trained through neural networks to identify plaque types according to a classification system based on the Geroulakos visual classification [108]. Subsequently confirmed visually by the M.Sc. student, plaque types were attributed by the program according to the following 5 definitions.

Type 1. Uniformly echolucent (black): <15% of the pixels in the plaque area were occupied by pixels with grayscale values >25.

Type 2. Mainly echolucent: pixels with grayscale values >25 occupy 15-50% of the plaque area

Type 3. Mainly echogenic: pixels with grayscale values >25 occupy 50-85% of the plaque area

Type 4: Uniformly echogenic: pixels with grayscale values >25 occupy >85% of the plaque area

Type 5: Plaque cannot be classified due to heavy calcification and acoustic shadow

2.9.2 - Continuous Image Analysis Features (see Appendix – Figures 9-12)

Texture features previously used in studying carotid plaque morphology on ultrasound were employed in the present study [119, 122, 132, 137, 138, 161].

1. **GSM**. The median of all grayscale values within the plaque outline.
2. **PPCS1**. The percentage of pixels with grayscale values <10.
3. **PPCS2**: The percentage of pixels with grayscale values between 10 and 25.
4. **Bel_30**: The percentage of pixels with grayscale values <30.
5. **Bel_50**: The percentage of pixels with grayscale values <50.
6. **SGLD ASM** (Spatial gray level dependence matrices correlation angular second moment): Measure of homogeneity of the plaque, evaluating the number of dominant gray-tone transitions. Larger values of ASM indicate a more *homogeneous* plaque. [138]
7. **SGLD HOM** (Homogeneity)**:** Measure of homogeneity, with higher values indicating a more *homogeneous* plaque [162].
8. **SGLDM correlation** [137, 138]: Measure of heterogeneity, with higher values indicating a more *heterogeneous plaque.*
9. **SGLDM IMC-1** (Information Measure of Correlation-1) [137, 138]: Measure of heterogeneity, with higher values indicating a more *heterogeneous plaque.*
10. **Skewness** characterizes the degree of asymmetry of the distribution of gray values around the mean [138], with higher values indicating a more *heterogeneous plaque.*
11. **RunI SRE** (Runlength short-run-emphasis) Measure of homogeneity, with higher values indicating a more *homogeneous plaque, with finer texture [163].*

2.10 - Inclusion criteria for correlation studies

Any plaque which met the criteria for analysis (as detailed above under 'Image Selection') was included in correlation studies between the two carotids.

2.11 - Reproducibility studies

Inter- and intra-operator reproducibility studies were performed. A random selection of 17 images (~10% of total 162 images) was blindly analyzed by the primary examiner (M.Sc. student) and a second examiner (PhD student under same supervisor). In case of disagreement, the final decision was made by consensus.

2.12 - Statistical analyses

Univariate differences in population characteristics such as age and BMI were evaluated using the student's T-test. Covariates were adjusted for depending on the size of the subpopulations being compared.

2.12.1 - Objective 1 – association between symptomatology and texture features

In the univariate analysis, differences in the 11 texture analysis features between asymptomatic and symptomatic patients and between the five symptom groups: asymptomatic, amaurosis fugax, TIA, stroke and 'TIA or stroke' were tested using the student's T-test. Fisher's exact test (2-sided) was used due to the small sample size to evaluate the relationships between symptom group and plaque type (1-2 vs. 3-4).

In the multivariate analysis, logistic regression was used to evaluate the texture features that were significantly different between groups on univariate analysis. Differences were adjusted for age and BMI in all comparisons involving the asymptomatic group (we did not adjust for sex because there were no women within the asymptomatic group), and for age, sex and BMI for comparisons between all other symptom groups.

Moreover, analysis involving differences in total number of modifiable risk factors (hypertension, hypercholesterolemia, diabetes and smoking history) between the symptom groups was conducted with Fisher's exact test (2-sided).

2.12.2 - Objective 2 – Correlation of texture features between carotids

Pearson's correlation was used to estimate the relationship between the two carotids concerning the 11 features of texture analysis.

2.12.3 - Objective 3 – Sex differences in carotid atherosclerosis

In the univariate analysis, differences in the 11 texture features between men and women were evaluated using the student's T-test. Fisher's exact test (2-sided) was used to evaluate the relationship between sex and plaque type (1-2 vs. 3-4).

In the multivariate analysis, logistic regression was used to calculate odds ratios associated with the texture features that were significantly different between groups on univariate analysis. Differences were adjusted for age and BMI. These tests were performed for both ipsilateral and contralateral carotid arteries.

In addition, Fisher's exact test (2-sided) was used to evaluate the relationship between sex and risk factor number (various combinations of risk factor numbers: eg., 1-2 vs. 3-4 and 1-3 vs. 4). Logistic regression was used to calculate odds ratios representing differences between the sexes regarding number of modifiable risk factors (listed under Objective 1).

2.12.4 - Statistical software

Statistical analysis was performed using PASW (Predictive Analytics Software) Statistics 18 and SAS (Statistical Analysis System) statistical 9.1.

3 - Results

3.0 - Baseline characteristics of whole population

Eighty-four patients aged 45-89 years were recruited between 2009 and 2011, with 81% of patients recruited from the RVH and 19% of patients from the JGH. Recruitment rate was 74% and 2 patients were excluded due to excessive acoustic shadowing (>50% plaque area). Baseline characteristics, symptom status, vascular risk factors and medication use are displayed in Table 1.

More men were recruited than women, and most patients were overweight. The vast majority of the cohort had suffered a cerebrovascular event during their lifetime, and vascular risk factors were frequently observed. Patients receiving antihypertensive medication were mostly commonly prescribed 1-2 antihypertensive medications, with the major classes of medication nearly equally favored. Statins and biguanides were the most frequently prescribed cholesterol-lowering and antidiabetic medications, respectively. Most treated patients had been receiving treatment for more than 5 years. Moreover, 63 (75%) and 24 (28.6%) of all patients were on acetylsalycylic acid (ASA) (80mg) and clopidogrel (75mg), respectively, with 13 (15.5%) patients taking both ASA and clopidogrel. Seven (8.5%) patients were taking warfarin.

Table 1 – Baseline characteristics of whole population

Patient Characteristics	All
Hospital (RVH:JGH)	68:16
Age (years)	69.4 (9.94)
Sex (women)	21 (25.0%)
BMI (kg/m^2)	27.1 (4.2)
Waist circumference (men) (cm)	100 (12.0)
Waist circumference (women) (cm)	94.5 (11.0)
Systolic Blood Pressure (mmHg)	138.3 (16.9)
Diastolic Blood Pressure (mmHg)	69.6 (9.8)
Pulse pressure (mmHg)	68.7 (16.9)
Symptomatology	
Asymptomatic	14 (16.7%)
Symptomatic	70 (83.3%)
AmF	17 (20.2%)
TIA	23 (27.4%)
Stroke	30 (35.7%)
TIA or stroke	53 (75.7%)
Time since event	
AmF (n=14)	

<30 days	7 (50.0%)
30-90 days	4 (28.6%)
90-180 days	3 (21.4%)
>180 days	0 (0.0%)
TIA (n=16)	
<30 days	10 (62.5%)
30-90 days	5 (31.2%)
90-180 days	0 (0.0%)
>180 days	1 (6.3%)
Stroke (n=26)	
<30 days	13 (50.0%)
30-90 days	9 (37.0%)
90-180 days	3 (10.0%)
>180 days	1 (3.0%)
Family history of vascular disease	31 (36.9%)
Early MI	9 (10.7%)
Stroke	15 (17.9%)
PAD	12 (14.3%)
History of vascular disease	
CAD	32 (38.1%)
PAD	15 (17.9%)
Either PAD or CAD	23 (27.4%)
Both PAD and CAD	12 (14.3%)
Modifiable risk factors	
Hypertension	72 (85.7%)
Hypercholesterolemia	69 (82.1%)
Diabetes	26 (31.0%)
Smoking history	66 (78.6%)
Medication	
Antihypertensives (type)	
Diuretics	26 (31.0%)
ACEI	23 (27.4%)
ARBs	30 (35.7%)
β-Blockers	29 (34.5%)
CCBs	28 (33.3%)
Direct-acting smooth muscle relaxant	1 (1.2%)
α-2 Agonists	1 (1.2%)
Direct renin blockers	1 (1.2%)
Antihypertensives (#)	
0	16 (19.0%)
1-2	50 (59.5%)
3-4	17 (20.2%)
5-6	1 (1.2%)
Duration of treatment (n=81)	
0 – 2 months	2 (3.1%)
2 months – 1 year	9 (13.8%)
1 – 5 years	12 (18.5%)
>5 years	42 (64.6%)
Cholesterol lowering	
No cholesterol lowering medication	18 (21.4%)
Statin	65 (77.4%)

Ezetimibe	4 (4.8%)
Fenofibrate	1 (1.2%)
Niacin	2 (2.4%)
Combination therapy (with statin)	6 (7.1%)
Duration of treatment (years) (n=74)	
0 – 2 months	2 (3.6%)
2 months – 1 year	11 (19.6%)
1 – 5 years	13 (23.2%)
> 5 years	30 (53.6%)
Antidiabetic	
No antidiabetic medication	60 (71.4%)
Biguanides	18 (21.4%)
Sulphonylureas	15 (17.9%)
Dipeptidyl peptidase-4 inhibitors	1 (1.2%)
Insulin	5 (5.9%)
Combination therapy (with insulin)	4 (4.8%)
Duration of treatment (n=82)	
0 – 2 months	0 (0.0%)
2 months – 1 year	4 (18.2%)
1 – 5 years	2 (9.1%)
> 5 years	16 (72.7%)

*Variables presented as mean (standard deviation).
RVH: Royal Victoria Hospital, *JGH:* Jewish General Hospital, *BMI:* body mass index, *AmF*: amaurosis fugax, *TIA*: transient ischemic attack, *MI*: myocardial infarction, *PAD*: peripheral artery disease, *CAD*: coronary artery disease, *ACEI*: Angiotensin cleaving enzyme inhibitor, *ARBs*: Angiotensin II receptor blockers, *CCBs*: Calcium channel blockers

3.1 - Objective 1

To determine whether cerebrovascular symptomatology is associated with different plaque types (more subjective), degrees of echogenicity and heterogeneity (more objective).

3.1.1 - Baseline characteristics of different symptom groups (Tables 2-4)

A total of 14 asymptomatic patients and 70 symptomatic patients were recruited, of which 17 had experienced episode(s) of amaurosis fugax, 23 had TIA(s), 30 had stroke(s) and 53 had either stroke(s) or TIA(s). The baseline characteristics of the patients grouped according to their symptomatology are presented in Table 2.

The asymptomatic group was significantly younger than the symptomatic group and all symptom groups ($p<.04$) except amaurosis fugax ($p=.12$), likely due to sample size as the mean age of amaurosis

fugax patients was similar to other symptomatic groups. The asymptomatic group consisted solely of men and was characterized by the most elevated mean BMI, although no significant differences in BMI were observed between the symptom groups. Among the different symptomatic groups, asymptomatic patients most commonly had a family history of vascular disease and a personal history of coronary artery disease (CAD) and of vascular disease (CAD or PAD).

Compared to symptomatic patients, asymptomatic patients were more often treated with β-blockers and were more frequently prescribed multiple antihypertensive medications, likely related to a higher proportion of patients with CAD. Stroke patients were more commonly treated with statins and both oral antidiabetic agents and insulin. The majority of patients receiving medical treatment in each symptom group had been prescribed these medications for at least 5 years.

Table 2 – Baseline characteristics of patients with different symptomatology

Patient characteristics	**Asymp (n=14)**	**Symp (n=70)**	**AmF (n=17)**	**TIA (n=23)**	**Stroke (n=30)**	**Tia or Stroke (n=53)**
Age* (Years)	64.5 (8.1)	70.3 (9.1)	69.8 (10.1)	70.4 (8.6)	70.4 (9.4)	70.4 (9.0)
Sex* M:W	14:0	49:21	11:6	17:6	21:9	38:15
BMI* (kg/m^2)	28.6 (3.8)	26.7 (4.3)	26.4 (3.5)	26.9 (5.4)	26.9 (3.8)	26.9 (4.5)
Waist circ* (men) (cm)	99.7 (15.4)	100.9 (11.9)	100.2 (7.7)	97.3 (13.7)	104.4 (11.4)	101.0 (12.9)
Waist circ* (women) (cm)	-	94.4 (12.2)	93.8 (10.4)	93.2 (13.9)	99.1 (9.3)	95.4 (12.0)
SBP* (mmHg)	141.6 (20.8)	137.5 (15.9)	136.0 (18.4)	137.8 (16.7)	138.1 (14.3)	138.1 (15.8)
DBP* (mmHg)	71.1 (7.4)	68.4 (17.1)	71.2 (9.4)	67.8 (9.7)	71.2 (9.4)	68.6 (9.9)
PP* (mmHg)	68.8 (15.5)	68.6 (17.3)	64.8 (18.6)	70.0 (17.9)	68.9 (15.8)	69.6 (17.2)
Family hx for vascular disease	8 (57.1%)	23 (32.9%)	5 (29.4%)	9 (39.1%)	9 (30.0%)	18 (34.0%)
Early MI	2 (14.3%)	7 (10.0%)	0 (0.0%)	2 (8.7%)	5 (16.7%)	7 (13.2%)
Stroke	4 (28.6%)	11 (15.7%)	3 (17.6%)	4 (17.4%)	4 (13.3%)	8 (15.1%)
PAD	2 (14.3%)	10 (14.3%)	2 (11.8%)	3 (13.0%)	5 (16.7%)	8 (15.1%)
Hx of vascular disease						

CAD	7 (50.0%)	25 (35.7%)	7 (41.2%)	8 (34.8%)	10 (33.3%)	18 (34.0%)
PAD	3 (21.4%)	12 (17.1%)	4 (23.5%)	4 (17.4%)	4 (13.3%)	8 (15.1%)
Either PAD or CAD	6 (42.9%)	17 (24.3%)	3 (17.6%)	6 (26.1%)	8 (26.7%)	14 (26.4%)
Both PAD and CAD	2 (14.2%)	10 (14.3%)	4 (23.5%)	3 (13.0%)	3 (10.0%)	6 (11.3%)
Modifiable risk factors						
HTN	13 (92.9%)	59 (84.3%)	14 (82.4%)	20 (87.0%)	25 (86.2%)	45 (84.9%)
Hchol	12 (85.7%)	57 (81.4%)	11 (64.7%)	20 (87.0%)	26 (89.7%)	46 (86.8%)
Diabetes	5 (35.7%)	21 (30.0%)	4 (23.5%)	8 (34.8%)	9 (31.0%)	17 (32.1%)
Smoking hx	12 (85.7%)	54 (77.1%)	14 (82.4%)	19 (82.6%)	21 (72.4%)	40 (75.4%)
Medication						
Anti-HTN (type)						
Diuretics	4 (28.6%)	22 (31.4%)	6 (35.3%)	7 (30.4%)	9 (30.0%)	16 (30.2%)
ACEI	4 (28.6%)	19 (27.1%)	3 (17.6%)	6 (26.1%)	10 (33.3%)	16 (30.2%)
ARBs	6 (42.9%)	24 (34.3%)	7 (41.2%)	6 (26.1%)	11 (36.7%)	17 (32.1%)
β-Blockers	9 (64.3%)	20 (28.6%)	3 (17.6%)	8 (34.8%)	9 (30.0%)	17 (32.1%)
CCBs	5 (35.7%)	23 (32.9%)	6 (35.6%)	6 (26.1%)	11 (36.7%)	17 (32.1%)
Direct-acting smooth muscle relaxants	1 (7.1%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
α-2 Agonists	1 (7.1%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Direct renin blockers	1 (7.1%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Anti-HTN (#)						
0	1 (7.1%)	15 (21.4%)	4 (23.5%)	4 (17.4%)	7 (23.3%)	11 (22.6%)
1-2	8 (57.1%)	42 (60.0%)	9 (53.0%)	15 (65.2%)	18 (60.0%)	33 (62.2%)
3-4	5 (35.7%)	12 (17.1%)	4 (23.5%)	4 (17.4%)	4 (13.3%)	8 (15.1%)
5-6	0 (0.0%)	1 (1.4%)	0 (0.0%)	0 (0.0%)	1 (3.3%)	1 (1.9%)
Duration of treatment	(n=13)	(n=68)		(n=21)		(n=51)
0 – 2 months	0 (0.0%)	2 (3.8%)	1 (7.7%)	0 (0.0%)	1 (4.3%)	1 (2.5%)
2 months–1 yr	1 (8.3%)	8 (15.1%)	1 (7.7%)	2 (11.8%)	5 (21.7%)	7 (17.5%)
1 – 5 yrs	1 (8.3%)	11 (20.8%)	1 (7.7%)	3 (17.6%)	7 (30.4%)	10 (25.0%)
>5 yrs	10 (83.3%)	32 (60.3%)	10 (76.9%)	12 (70.6%)	10 (43.5%)	22 (55.0%)
Cholesterol lowering						
No cholesterol lowering medication	2 (14.3%)	16 (22.9%)	7 (41.2%)	6 (26.1%)	3 (10.0%)	9 (17.0%)
Statin	11 (78.5%)	54 (75.7%)	10 (58.8%)	17 (73.9%)	27 (90.0%)	44 (83.0%)
Ezetimibe	3 (21.4%)	1 (1.4%)	1 (5.9%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Fenofibrate	1 (7.1%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)

Niacin	2 (14.3%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Combination therapy (with statin)	5 (35.7%)	1 (1.4%)	1 (5.9%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Duration of treatment		(n= 60)	(n=13)		(n=24)	(n=47)
0 - 2 months	0 (0.0%)	2 (4.5%)	0 (0.0%)	0 (0.0%)	2 (9.5%)	2 (5.3%)
2 months-1 yr	2 (16.7%)	9 (20.4%)	0 (0.0%)	3 (17.6%)	6 (28.6%)	9 (23.7%)
1 – 5 yrs	4 (33.3%)	9 (20.4%)	3 (50.0%)	1 (2.5%)	5 (23.8%)	6 (15.8%)
> 5 yrs	6 (50.0%)	24 (54.5%)	3 (50.0%)	13 (76.4%)	8 (38.1%)	21 (55.3%)
Antidiabetic						
No antidiabetic medication	10 (71.4%)	50 (71.4%)	14 (82.4%)	16 (69.6%)	20 (66.6%)	36 (67.9%)
Biguanides	3 (21.4%)	15 (21.4%)	3 (17.6%)	4 (17.4%)	8 (26.7%)	12 (22.6%)
Sulphonylureas	2 (14.3%)	13 (18.6%)	1 (5.9%)	4 (17.4%)	8 (26.7%)	12 (22.6%)
DPP-4 inhibitors	1 (7.1%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Insulin	0 (0.0%)	5 (7.1%)	0 (0.0%)	2 (8.7%)	3 (10.0%)	5 (9.4%)
Combination therapy (with insulin)	0 (0.0%)	4 (5.7%)	0 (0.0%)	1 (4.3%)	3 (10.0%)	4 (7.5%)
Duration of treatment		(n=68)			(n=28)	(n=51)
0 - 2 months	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
2 months-1 yr	2 (50.0%)	2 (11.1%)	0 (0.0%)	1 (14.3%)	0 (0.0%)	1 (7.1%)
1 – 5 yrs	1 (25.0%)	1 (5.6%)	1 (33.3%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
> 5 yrs	1 (25.0%)	15 (83.3%)	2 (66.7%)	6 (85.7%)	7 (100%)	13 (92.9%)

*Variables presented as mean (standard deviation).
Asymp: asymptomatic, *Symp*: symptomatic, *AmF*: amaurosis fugax, *TIA*: transient ischemic attack, *BMI*: body mass index, *Circ*: circumference, *SBP*: systolic blood pressure, *DBP*: diastolic blood pressure, *PP*: pulse pressure, *Hx*: history, *MI*: myocardial infarction, *PAD*: peripheral artery disease, *CAD*: coronary artery disease, *HTN*: hypertension, *Hchol*: hypercholesterolemia, *anti-HTN*: anti-hypertensive, *ACEI*: Angiotensin cleaving enzyme inhibitor, *ARBs*: Angiotensin II receptor blockers, *CCBs*: Calcium channel blockers, *Yr:* year, *DPP-4*: Dipeptidyl peptidase-4

3.1.2 - Risk factor differences between symptom groups

The number of modifiable vascular risk factors (RFs) (hypertension, hypercholesterolemia, diabetes and smoking) was found to be similar between the 6 symptom groups (Tables 3-4). All symptom groups except the amaurosis fugax had over twice as many patients with multiple risk factors (3-4) as those with few risk factors (0-2), with the amaurosis fugax group consisting of similar proportions of individuals with multiple risk factors compared with few risk factors.

Table 3 - Number of modifiable risk factors among patients with different symptomatology

Modifiable RFs (#)	Asymptomatic (n=14)	Symptomatic (n=70)	AmF (n=17)	TIA (n=23)	Stroke (n=30)	TIA or stroke (n=53)
0	0 (0.0%)	4 (5.7%)	1 (5.9%)	2 (8.7%)	1 (3.3%)	3 (5.7%)
1	1 (7.1%)	4 (5.7%)	2 (11.8%)	0 (0.0%)	2 (6.7%)	2 (3.8%)
2	3 (21.4%)	14 (35.0%)	5 (29.4%)	4 (17.4%)	5 (16.7%)	9 (17.0%)
3	5 (35.7%)	37 (52.9%)	7 (41.2%)	13 (56.5%)	17 (56.7%)	30 (56.6%)
4	5 (35.7%)	11 (27.5%)	2 (11.8%)	4 (17.4%)	5 (16.7%)	9 (17.0%)
0-2 RFs	4 (28.6%)	22 (31.4%)	8 (47.1%)	6 (26.1%)	8 (26.7%)	14 (26.4%)
3-4 RFs	**10 (71.4%)**	**48 (68.6%)**	**9 (52.9%)**	**17 (73.9%)**	**22 (73.3%)**	**39 (73.6%)**

Table 4 (a, b, c) – Differences in number of modifiable risk factors among patients with different symptomatology

a)

Fisher's exact test	Modifiable RFs (#)	Symptomatic (n=70)	AmF (n=17)	TIA (n=23)	Stroke (n=30)	TIA or stroke (n=53)
Asymptomatic (n=14)	0-1 vs. 2-4	1.00	.62	1.00	1.00	1.00
	0-1 vs. 3-4	1.00	.60	1.00	1.00	1.00
	0-1 vs. 4	.62	.25	1.00	.59	1.00
	0-2 vs. 4	.28	.17	.66	.67	.45
	0-2 vs. 3-4	1.00	.47	1.00	1.00	1.00
	0123 vs. 4	.13	.20	.27	.26	.16

b)

Fisher's exact test	Modifiable RFs (#)	TIA (n=23)	Stroke (n=30)	TIA or stroke (n=53)
Amaurosis fugax (n=17)	0-1 vs. 2-4	.30	.65	.35
	0-1 vs. 3-4	.27	.35	.15
	0-1 vs. 4	.21	.54	.25
	0-2 vs. 4	.29	.33	.20
	0-2 vs. 3-4	.16	.20	.12
	0123 vs. 4	.36	.40	.43

c)

Fisher's exact test	Modifiable RFs (#)	Stroke (n=30)
TIA (n=23)	0-1 vs. 2-4	.63
	0-1 vs. 3-4	.63
	0-1 vs. 4	1.00
	0-2 vs. 4	1.00
	0-2 vs. 3-4	1.00
	0123 vs. 4	1.00

3.1.3 - Ultrasonic Imaging (Tables 5-15)

3.1.3.1 - Stenosis and plaque type

The mean stenosis values and the number of patients with certain plaque types were recorded and grouped according to patient symptomatology and are presented in Table 5. The mean ipsilateral stenosis values of each symptom group were similar (range: 85.0-85.7%). Contralateral stenosis is discussed under Objective 2.

Table 5 – Stenosis and visual classification of plaques from patients with different symptomatology

	Asymp (n=14)	**Symp (n=70)**	**AmF (n=17)**	**TIA (n=23)**	**Stroke (n=30)**	**TIA or stroke (n=53)**
Stenosis						
Ipsilateral	85.5 (9.0)	85.3 (9.4)	85.4 (9.2)	85.7 (8.4)	85.0 (10.6)	85.3 (9.6)
Contralateral	48.7 (28.6)	40.8 (31.1)	49.3 (34.0)	43.9 (34.3)	33.4 (25.8)	38.0 (30.1)
Plaque Type						
1	1 (7.1)	5 (7.1%)	3 (17.6%)	0 (0.0%)	2 (6.7%)	2 (3.7%)
2	5 (35.8%)	41 (58.6%)	12 (70.6%)	12 (52.2%)	17 (56.7%)	29 (54.7%)
3	7 (50.0%)	23 (32.9%)	2 (11.8%)	11 (47.8%)	10 (33.3%)	21 (39.7%)
4	1 (7.1%)	1 (1.4%)	0 (0.0%)	0 (0.0%)	1 (3.3%)	1 (1.9%)
5	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
1 and 2 (echolucent)	6 (42.9%)	**46 (65.7%)**	**15 (88.2%)**	**12 (52.2%)**	**19 (63.4%)**	**31 (58.4%)**
3 to 5 (echogenic)	**8 (57.1%)**	24 (34.3%)	2 (11.8%)	11 (47.8%)	11 (36.6%)	22 (41.6%)

3.1.3.2 - Texture features and plaque type

Eleven texture features of ultrasonic plaque images were determined for both ipsilateral and contralateral carotid arteries and are presented in Table 6 and Table 16, respectively. Differences between symptom groups in these imaging features were evaluated and presented in Tables 7 through 15.

Table 6 – Texture features of ipsilateral carotid plaques in patients with different symptomatology

	Asymp (n=14)	**Symp (n=70)**	**AmF (n=17)**	**TIA (n=23)**	**Stroke (n=30)**	**'TIA or stroke' (n=53)**

GSM	30.6 (19.2)	20.9 (16.3)	13.6 (9.2)	23.5 (11.6)	23.1 (21.2)	23.3 (17.5)
PPCS1	26.4 (24.6)	36.7 (19.8)	44.9 (17.8)	30.4 (17.0)	37.0 (21.3)	34.3 (19.8)
PPCS2	13.8 (6.8)	15.1 (5.6)	16.3 (6.3)	14.8 (4.2)	14.7 (6.3)	14.8 (5.5)
Bel_30	54.2 (23.81)	65.3 (19.9)	74.0 (11.8)	60.0 (17.2)	64.5 (24.1)	62.5 (21.3)
Bel_50	73.1 (20.4)	81.4 (15.6)	87.2 (8.7)	79.4 (11.9)	79.5 (20.1)	79.4 (16.8)
SGLD ASM	.057 (.162)	.042 (.062)	.059 (.074)	.023 (.036)	.047 (.069)	.037 (0.058)
SGLDM COR	.912 (.071)	.914 (.057)	.899 (.075)	.925 (.047)	.914 (.053)	.918 (0.050)
SGLD HOM	.256 (.200)	.290 (.126)	.327 (.146)	.261 (.095)	.291 (.133)	.278 (.119)
SGLDM IMC-1	-.317 (.084)	-.297 (.065)	-.293 (.075)	-.304 (.059)	-.294 (.064)	-.297 (.062)
Skewness	1.53 (.90)	1.74 (.66)	1.89 (.51)	1.69 (.49)	1.70 (.83)	1.72 (.68)
Runl SRE	.935 (.035)	.921 (.027)	.913 (.030)	.925 (.019)	.923 (.030)	.924 (.026)

Symptomatic vs. asymptomatic plaques (Table 7): The symptomatic group was characterized by significantly more echolucent plaque types (types 1-2) than echogenic plaque types (types 3-4) compared with the asymptomatic group (adjusted (adj.) p=.04). Symptomatic plaques had a significantly lower mean GSM (adj. p=0.020), and significantly higher values of PPCS1, bel_30 and bel_50 than asymptomatic plaques (adj. p=.04, adj. p=.03 and adj. p=.04, respectively). Symptomatic plaques were characterized by significantly lower values of Runl SRE when compared with asymptomatic plaques (adj. p=.07) after adjusting for age only.

Table 7 – Difference in ultrasonic texture features between asymptomatic (n=14) and symptomatic (n=70) plaques

Image features	**T-test (Sig.)**	**Unadjusted OR (Sig.)**	**Adjusted OR (Age only) (Sig.)**	**Adjusted OR (Age, BMI) (Sig.) (n=81)**
Plaque type (1-2 vs. 3-4)	.14	.39 (.12-1.26) .12	**.26 (.07-.97) .04**	**.21 (.05-.91) .040**
GSM	*.05*	*.97 (.94-1.00) .06*	**.96 (.93-.99) .02**	**.96 (.92-.99) .02**
PPCS1	*.09*	*1.03 (1.00-1.06) .09*	**1.04 (1.00-1.07) .03**	**1.04 (1.00-1.07) .04**
PPCS2	.44	1.04 (.94-1.16) .44	1.04 (.94-1.16) .42	1.05 (.94-1.17) .40
Bel_30	*.07*	*1.02 (1.00-1.053) .08*	**1.03 (1.00-1.06) .03**	**1.03 (1.00-1.06) .03**
Bel_50	*.09*	*1.03 (1.00-1.06) .09*	**1.04 (1.00-1.07) .04**	**1.04 (1.00-1.08) .04**

SGLD ASM	.57	.19 (<.01-61.24) .58	.47 (.01-184.16) .80	.44 (.01-144.33) .78
SGLDM COR	.94	1.43 (<.01->99.99) .94	.19 (<.01->99.99) .74	.06 (<.01->99.99) .58
SGLD HOM	.41	7.24 (.07-766.10) .40	18.47 (.14->99.99) .24	10.36 (.08->99.99) .44
SGLDM IMC-1	.30	88.00 (.02->99.99) .30	66.44 (.02->99.99) .32	73.66 (.02->99.99) .31
Skewness	.25	1.71 (.68-4.29) .25	2.08 (.77-5.64) .15	2.15 (.76-6.09) .15
Runl SRE	*.09*	*<.01 (<.01-33.40) .09*	**<.01 (<.01-.68) .04**	*<.01 (<.01-8.98) .07*

Trending values (.05<p<.10) are in italics.
Significant values (p<.05) are in bold.

Amaurosis fugax vs. asymptomatic plaques (Table 8): The amaurosis fugax group was significantly more likely to have echolucent plaque types than echogenic plaque types compared with the asymptomatic group (adj. p=.02). Amaurosis fugax plaques had a significantly lower mean GSM than asymptomatic plaques (adj. p=.02). Amaurosis fugax plaques had significantly higher mean PPCS1, bel_30 and bel_50 (adj. p=.04, adj. p=.03 and adj. p=.04, respectively) than asymptomatic plaques.

Amaurosis fugax plaques were trending toward significantly greater values of skewness than asymptomatic plaques (adj. p=.09). Amaurosis fugax plaques were also trending toward lower values of Runl SRE (adj. p=.06) after adjustment for age only.

Table 8 - Difference in ultrasonic texture features between asymptomatic plaques (n=14) and those causing amaurosis fugax (n=17)

	T-test (Sig.)	Unadjusted OR (Sig.)	Adjusted OR (Age only) (Sig.)	Adjusted OR (Age and BMI) (Sig.)
Plaque type	**.02**	**.10 (.02-.62) .01**	**.07 (.01-.56) .01**	**.09 (.01-.68) .02**
GSM	***<.01***	**.91 (.84-.99) .02**	**.90 (.82-.98) .02**	**.90 (.83-.99) .02**
PPCS1	**.02**	**1.05 (1.00-1.09) .03**	**1.05 (1.01-1.10) .03**	**1.05 (1.00-1.10) .04**
PPCS2	.30	1.06 (.95-1.20) .30	1.06 (.93-1.20) .37	1.06 (.93-1.20) .40
Bel_30	***<.01***	**1.07 (1.0-1.12) .02**	**1.07 (1.01-1.04) .02**	**1.07 (1.01-1.13) .03**
Bel_50	**.02**	**1.07 (1.00-1.15) .04**	**1.08 (1.01-1.16) .03**	**1.07 (1.00-1.15) .04**
SGLD ASM	.96	1.16 (.00-486.47) .96	2.10 (<.01-967.24) .81	3.44 (<.01->99.99) .70
SGLDM COR	.63	.07 (<.01->99.99) .62	.02 (<.01-591.74) .44	<.01 (<.01-101.72) .25

SGLD HOM	.26	14.73 (.13->99.99) .26	25.28 (.18->99.99) .20	16.32 (.13->99.99) .25
SGLDM IMC-1	.40	61.82 (<.01->99.99) .39	28.46 (<.01->99.99) .50	27.94 (.01->99.99) .514
Skewness	.17	2.28 (.69-7.50) .17	2.71 (.75-9.74) .13	*3.08 (.83-11.35) .09*
Runl SRE	*.06*	*<.01 (<.01-18.27) .08*	*<.01 (<.01-5.88) .06*	<.01 (<.01-255.40) .11

Trending values (.05<p<.10) are in italics.
Significant values (p<.05) are in bold.
Highly significant values (p<.01) are in bold italics.

TIA vs. asymptomatic plaques (Table 9): The TIA plaques were characterized by a greater tendency toward lower plaque types and a lower mean GSM, both differences non-significant. The most likely reason for the difference in p-values between TIA (adj. p=.16) and stroke (adj. p=.07) groups is the smaller size of the TIA subgroup, as the mean GSM of the TIA group was very similar to that of the stroke group (23.5 vs. 23.1, respectively) and its standard deviation much lower (11.6 vs. 21.1).

Table 9 - Difference in ultrasonic texture features between plaques causing no symptoms (n=14) and those causing TIA (n=23)

	T-test (Sig.)	Unadjusted OR (Sig.)	Adjusted OR (Age only) (Sig.)	Adjusted OR (Age, BMI) (Sig.)
Plaque type	.74	.69 (.18-2.62) .58	.60 (.14-2.53) .48	.90 (.16-5.20) .91
GSM	.17	.97 (.92-1.02) .18	.96 (.91-1.01) .11	.96 (.91-1.02) .16
PPCS1	.57	1.01 (.98-1.05) .556	1.02 (.98-1.06) .36	1.01 (.98-1.05) .46
PPCS2	.57	1.04 (.91-1.18) .56	1.01 (.88-1.16) .93	1.00 (.87-1.15) .97
Bel_30	.40	1.02 (.98-1.05) .39	1.02 (.98-1.06) .34	1.02 (.98-1.05) .43
Bel_50	.25	1.03 (.98-1.07) .25	1.03 (.98-1.08) .22	1.03 (.98-1.08) .28
SGLD ASM	.34	.03 (<.01-90.42) .39	.12 (<.01-213.84) .57	.10 (<.01-392.12) .58
SGLDM COR	.50	61.25 (<.01->99.99) .49	998.90 (.02->99.99) .30	402.60 (<.01->99.99) .38
SGLD HOM	.92	1.29 (.01-158.84) .92	5.27 (.03->99.99) .54	3.43 (.02-654.52) .65
SGLDM IMC-1	.56	20.30 (<.01->99.99) .55	.16 (<.01->99.99) .74	.25 (<.01->99.99) .81
Skewness	.47	1.51 (.50-4.56) .46	1.54 (.50-4.71) .45	1.46 (.49-4.34) .50
Runl SRE	.25	<.01 (<.01->99.99) .25	<.01 (<.01->99.999) .20	<.01 (<.01->99.99) .24

Trending values (.05<p<.10) are in italics.
Significant values (p<.05) are in bold.

Stroke vs. asymptomatic plaques (Table 10): The stroke group was more likely to have echolucent than echogenic plaque types (adj. p=.07) compared with asymptomatic patients. Plaques associated with stroke had a lower mean GSM than asymptomatic plaques (adj. p=.07). Stroke plaques were characterized by significantly greater PPCS1 than asymptomatic plaques (adj. p=.04). Bel_30 was borderline significantly greater in stroke plaques than in asymptomatic plaques (adj. p=.05). Runl SRE was non-significantly greater in asymptomatic plaques than stroke plaques after adjustment for age only (adj. p=.09).

Table 10 - Difference in ultrasonic texture features between plaques causing no symptoms (n=14) and those causing stroke (n=30)

	T-test (Sig.)	Unadjusted OR (Sig.)	Adjusted OR (Age only) (Sig.)	Adjusted OR (Age, BMI) (Sig.) (n=41)
Plaque type	.32	.43 (.12-1.58) .21	*.25 (.05-1.14) .07*	*.23 (.05-1.15) .07*
GSM	.271	.98 (.95-1.01) .27	1.10 (1.01-1.20) .11	*.96 (.93-1.00) .07*
PPCS1	.139	1.02 (.99-1.06) .15	**1.04 (1.00-1.07) .04**	**1.04 (1.00-1.08) .04**
PPCS2	.662	1.02 (.92-1.13) .66	1.04 (.93-1.15) .51	1.04 (.94-1.17) .45
Bel_30	.196	1.02 (.99-1.05) .19	*1.03 (.99-1.06) .07*	*1.03 (1.00-1.06) .05*
Bel_50	.339	1.01 (.98-1.05) .33	1.03 (.99-1.06) .13	1.03 (.99-1.07) .11
SGLD ASM	.812	.48 (<.01-166.20) .81	1.07 (<.01-452.21) .98	1.61 (<.01-687.52) .88
SGLDM COR	.994	1.04 (<.01->99.99) .99	.07 (<.01->99.99) .66	.02 (<.01->99.99) .54
SGLD HOM	.485	5.05 (.06-442.84) .48	16.91 (.11->99.99) .275	14.92 (.11- >99.99) .28
SGLDM IMC-1	.290	156.98 (.02->99.99) .28	171.58 (.01->99.99) .29	162.29 (<.01->99.99) .31
Skewness	.446	1.38 (.61-3.14) .44	1.66 (.68-4.09) .27	2.00 (.76-5.21) .16
Runl SRE	.225	<.01 (<.01->99.99) .23	*<.01 (<.01-51.65) .09*	<.01 (<.01-164.48) .11

Trending values (.05<p<.10) are in italics.
Significant values (p<.05) are in bold.

TIA or stroke vs. asymptomatic plaques (Table 11): Grouped together, plaques causing TIAs or strokes had lower GSM than asymptomatic plaques (adj. p=.05), and non-significantly greater PPCS1 after adjustment

for age only (adj. p=.07). Plaques causing TIAs or strokes were characterized by greater bel_30 and bel_50 than asymptomatic plaques (adj. p=.09 and p=.09, respectively). Runl SRE was non-significantly greater in asymptomatic plaques than 'TIA or stroke' plaques after adjustment for age only (adj. p=.08).

Table 11 - Difference in ultrasonic texture features between plaques causing no symptoms (n=14) and those causing 'TIA or stroke' (n=53)

	T-test (Sig.)	Unadjusted OR (Sig.)	Adjusted OR (Age only) (Sig.)	Adjusted OR (Age, BMI) (Sig.) (n=64)
Plaque type	.37	.53 (.16-1.75) .30	.36 (.10-1.37) .14	.39 (.10-1.51) .17
GSM	.18	.980 (.950-1.01) .18	*.97 (.94-1.00) .07*	*.96 (.93-.1.00) .05*
PPCS1	.22	1.02 (.99-1.05) .22	*1.03 (.99-1.07) .07*	1.03 (.99-1.06) .10
PPCS2	.58	1.03 (.93-1.15) .57	1.03 (.92-1.15) .58	1.04 (.93-1.16) .54
Bel_30	.21	1.02 (.99-1.04) .21	*1.02 (.99-1.06) .08*	*1.03 (.99-1.06) .09*
Bel_50	.24	1.02 (.99-1.05) .24	*1.03 (.99-1.07) .09*	*1.03 (.99-1.07) .09*
SGLD ASM	.47	.12 (<.01-39.32) .48	.34 (<.01-134.13) .72	.34 (<.01-132.76) .73
SGLDM COR	.72	7.07 (<.01->99.99) .72	2.24 (<.01->99.99) .89	.77 (<.01->99.99) .96
SGLD HOM	.59	3.61 (.03-386.67) .59	14.87 (.08->99.99) .31	9.20 (.06->99.99) .39
SGLDM IMC-1	.33	88.86 (.01->99.99) .32	29.30 (<.01->99.99) .46	38.06 (.04->99.99) .43
Skewness	.39	1.48 (.61-3.58) .36	1.72 (.66-4.43) .26	1.81 (.68-4.84) .23
Runl SRE	.17	<.01 (<.01->99.99) .17	*<.01 (<.01-16.52) .08*	<.01 (<.01-126.51) .11

Trending values (.05<p<.10) are in italics.
Significant values (p<.05) are in bold.

Amaurosis fugax vs. TIA plaques (Table 12): The amaurosis fugax plaques were significantly more likely to have more echolucent plaque types than the plaques causing TIA (adj. p=.03). Amaurosis fugax plaques had a significantly lower GSM than TIA plaques (adj. p=.01) as well as a significantly higher PPCS1 (adj. p=.02). Moreover, amaurosis fugax plaques had significantly greater values of bel_30 (adj. p=.01) and bel_50 (adj. p=.04) and trended toward significantly greater values of SGLD ASM

than TIA plaques (adj. p=.08). Unadjusted SGLD HOM trended toward being significantly greater in amaurosis fugax plaques compared with TIA plaques (p=.09).

Table 12 - Difference in ultrasonic texture features between plaques causing amaurosis fugax (n=17) and those causing TIA (n=23)

	T-test (Sig.)	Unadjusted OR (Sig.)	Adjusted OR (Age only) (Sig.)	Adjusted OR (Age, Sex, BMI) (Sig.)
Plaque type	**.02**	**6.9 (1.3-37.14) .02**	**6.85 (1.27-37.09) .03**	**6.81 (1.22-38.15) .03**
GSM	***<.01***	**1.09 (1.02-1.17) .01**	**1.10 (1.02-1.18) .01**	**1.10 (1.02-1.19) .01**
PPCS1	**.01**	**.95 (.91-.99) .02**	**.95 (.91-.99) .02**	**.95 (.91-.99) .02**
PPCS2	.39	.95 (.83-1.07) .39	.94 (.83-1.07) .36	.94 (.83-1.07) .34
Bel_30	***<.01***	**.94 (.89-.99) .01**	**.94 (.89-.99) .01**	**.93 (.88-.99) .01**
Bel_50	**.03**	**.93 (.87-.99) .04**	**.93 (.87-.99) .04**	**.92 (.86-.99) .04**
SGLD ASM	*.08*	*<.01 (<.01-3.21) .07*	*<.01 (<.01-3.18) .07*	*<.01 (<.01-4.18) .08*
SGLDM COR	.19	>99.99 (.02->99.99) .20	>99.99 (.02->99.99) .21	>99.99 (.05->99.99) .14
SGLD HOM	.12	*<.01 (<.01-2.43) .09*	<.01 (<.001-2.56) .10	<.01 (<.001-3.48) .12
SGLDM IMC-1	.62	.08 (<.01->99.99) .61	.08 (<.01->99.99) .60	.02 (<.01-605.04) .46
Skewness	.22	.43 (.12-1.63) .22	.43 (.12-1.64) .22	.40 (.10-1.72) .22
Runl SRE	.12	>99.99 (.01->99.99) .13	>99.99 (<.01->99.99) .13	>99.99 (<.01->99.99) .15

Trending values (.05<p<.10) are in italics.
Significant values (p<.05) are in bold.
Highly significant values (p<.01) are in bold italics.

Amaurosis fugax vs. stroke plaques (Table 13): Amaurosis fugax plaques were more likely to be attributed echolucent plaque types than were stroke plaques (adj. p=.06), and had comparatively lower values of GSM (adj. p=.07), both differences trending toward significance. Amaurosis fugax plaques also trended toward significantly higher values of bel_30 and bel_50 (adj. p=.08 and adj. p=.09, respectively).

Table 13 - Difference in ultrasonic texture features between plaques causing amaurosis fugax (n=17) and those causing stroke (n=30)

	T-test (Sig.)	Unadjusted OR (Sig.)	Adjusted OR (Age only) (Sig.)	Adjusted OR (Age, Sex, BMI) (Sig.) (n=44)
Plaque type	*.09*	*4.34 (.83-22.64) .08*	*4.56 (.84-24.77) .08*	*6.15 (0.91-41.57) .06*
GSM	*.09*	1.04 (.99-1.09) .11	*1.04 (.99-1.10) .11*	*1.06 (.99-1.12) .07*
PPCS1	.20	.98 (.95-1.01) .20	.98 (.95-1.01) .23	.98 (.94-1.01) .17
PPCS2	.43	.96 (.87-1.06) .41	.96 (.87-1.06) .44	.95 (.85-1.07) .41
Bel_30	.14	.98 (.94-1.01) .15	.97 (.94-1.01) .14	*.96 (.91-1.00) .08*
Bel_50	.14	.97 (.92-1.01) .15	.96 (.92-1.01) .15	*.94 (.87-1.01) .09*
SGLD ASM	.63	.12 (<.01-569.58) .62	.13 (<.01-678.93) .64	.18 (<.01->99.99) .69
SGLDM COR	.50	32.97 (<.01->99.99) .56	40.69 (<.01->99.99) .50	170.78 (<.01->99.99) .43
SGLD HOM	.42	.16 (<.01-12.49) .41	.16 (<.01-13.54) .42	.18 (<.01-15.93) .45
SGLDM IMC-1	.98	1.13 (<.01->99.99) .98	1.51 (<.01->99.99) .93	1.47 (<.01->99.99) .94
Skewness	.49	.73 (.31-1.73) .48	.74 (.31-1.77) .49	.73 (.27-2.01) .54
Runl SRE	.29	>99.99 (.01->99.99) .29	>99.99 (<.01->99.99) .29	>99.99 (<.01->99.99) .23

Trending values (.05<p<.10) are in italics.
Significant values (p<.05) are in bold.

Amaurosis fugax vs. 'TIA or stroke' plaques (Table 14): Echolucent plaque types were more common in amaurosis fugax plaques than in plaques causing either 'TIA or stroke' (adj. p=.04). Plaques causing 'TIA or stroke' had a mean GSM of 23.3 while the mean GSM of amaurosis fugax plaques was 13.6 (adj. p=.03). Plaques causing 'TIA or stroke' were characterized by significantly lower values of PPCS1 and bel_30 (adj. p=.04 and p=.03, respectively) and trended toward lower values of bel_50 (adj. p=.06), when compared with amaurosis fugax plaques.

Table 14 - Difference in ultrasonic texture features between plaques causing amaurosis fugax (n=17) and those causing 'TIA or stroke' (n=53)

	T-test (Sig.)	Unadjusted OR (Sig.)	Adjusted OR (Age only) (Sig.)	Adjusted OR (Age, Sex, BMI) (Sig.) (n=67)
Plaque type	**.04**	**5.32 (1.10-25.66) .04**	**5.40 (1.11-26.37) .04**	**5.72 (1.12-29.09) .04**
GSM	**.03**	**1.05 (1.00-1.11) .04**	**1.06 (1.00-1.11) .04**	**1.06 (1.01-1.12) .03**
PPCS1	*.04*	*.97 (.94-1.00) .05*	**.97 (.94-1.00) .06**	**.97 (.94-.99) .04**
PPCS2	.34	.95 (.87-1.05) .34	.96 (.87-1.05) .35	.95 (.86-1.05) .31
Bel_30	**.04**	**.97 (.93-.99) .04**	**.96 (.93-.99) .04**	**.96 (.92-.99) .03**
Bel_50	*.07*	**.96 (.91-1.00) .03**	*.96 (.91-1.01) .08*	*.94 (.89-1.00) .06*
SGLD ASM	.22	<.01 (<.01-21.26) .22	<.01 (<.01-24.41) .23	<.01 (<.01-33.40) .26
SGLDM COR	.25	228.71 (.02->99.99) .25	246.85 (.02->99.99) .26	784.79 (.03->99.99) .20
SGLD HOM	.17	.05 (<.01-3.62) .17	.05 (<.01-3.89) .18	.06 (<.01-4.64) .20
SGLDM IMC-1	.81	.35 (<.01->99.99) .81	.39 (<.01->99.99) .83	.21 (<.01->99.99) .74
Skewness	.34	.66 (.28-1.54) .33	.66 (.28-1.56) .34	.66 (.26-1.65) .37
Runl SRE	.15	>99.99 (<.01->99.99) .16	>99.99 (<.01->99.99) .16	>99.99 (<.01->99.99) .135

Trending values (.05<p<.10) are in italics.
Significant values (p<.05) are in bold.

TIA vs. stroke plaques (Table 15): Features of echolucency and heterogeneity were similar between TIA and stroke plaques, with no significant differences.

Table 15 - Difference in ultrasonic texture features between plaques causing TIA (n=23) and those causing stroke (n=30)

	T-test (Sig.)	Unadjusted OR (Sig.)	Adjusted OR (Age only) (Sig.)	Adjusted OR (Age, Sex, BMI) (Sig.) (n=50)
Plaque type	.57	.63 (.21-1.91) .42	.62 (.20-1.91) .40	.53 (.16-1.74) .29
GSM	.93	1.00 (.97-1.03) .93	1.00 (.97-1.03) .93	.99 (.95-1.03) .55
PPCS1	.23	1.02 (.99-1.05) .23	1.02 (.99-1.05) .18	1.02 (.99-1.06) .16
PPCS2	.93	1.00 (.90-1.10) .92	1.00 (.90-1.10) .95	1.02 (.91-1.14) .75
Bel_30	.45	1.01 (.98-1.04) .44	1.01 (.98-1.04) .43	1.02 (.99-1.05) .26
Bel_50	.98	1.00 (.97-1.03) .98	1.00 (.97-1.04) .98	1.01 (.97-1.05) .60
SGLD ASM	.12	>99.99 (.05->99.99) .14	>99.99 (.05->99.99) .14	>99.99 (.05->99.99) .13
SGLDM COR	.36	<.01 (<.01-437.89) .36	<.01 (<.01-449.52) .35	<.01 (<.01-123.42) .226
SGLD HOM	.35	10.29 (.08->99.99) .34	11.83 (.08->99.99) .33	14.26 (.09->99.99) .31
SGLDM IMC-1	.53	18.85 (.03->99.99) .52	19.00 (<.01->99.99) .52	78.20 (<.01->99.99) .37
Skewness	.81	1.10 (.49-2.49) .81	1.11 (.49-2.51) .81	1.34 (.54-3.31) .52
Runl SRE	.73	.02 (<.01->99.99) .73	.02 (<.01->99.99) .72	.02 (<.01->99.99) .74

Trending values (.05<p<.10) are in italics.
Significant values (p<.05) are in bold.

3.2 – Objective 2

To estimate the degree of correlation between the texture features of ultrasonic images of atherosclerotic plaque between the two carotid arteries. A) overall and B) within individuals having experienced different symptomatology

3.2.1 – Stenosis, plaque type and texture features of both carotids

Ipsilateral stenosis and plaque types are discussed under objective 1 (Table 5). Greater mean contralateral stenosis was found in amaurosis fugax and asymptomatic patients, while stroke patients had the lowest mean contralateral stenosis. No significant differences in plaque type were observed. It is interesting, however, that only contralateral amaurosis fugax plaques were characterized by a majority of echogenic plaque types (Type 3-5). Texture features of ultrasonic plaque images were calculated

for both ipsilateral and contralateral carotid arteries and are presented in Table 6 and Table 16, respectively.

Table 16 – Stenosis, plaque type and texture features of contralateral carotid plaques in patients with different symptomatology

	Asymp (n=14)	**Symp (n=70)**	**AF (n=17)**	**TIA (n=23)**	**Stroke (n=30)**	**TIA or stroke (n=53)**
Stenosis (%)	48.7 (28.6)	40.8 (31.1)	49.3 (34.0)	43.9 (34.3)	33.4 (25.8)	38.0 (30.1)
Plaque type						
1	0 (0.0%)	6 (8.5%)	1 (5.9%)	1 (4.3%)	4 (13.3%)	5 (9.4%)
2	10 (71.4%)	32 (45.7%)	7 (41.1%)	13 (56.5%)	12 (40.0%)	25 (47.2%)
3	4 (28.6%)	30 (42.9%)	9 (53.0%)	8 (34.8%)	13 (43.3%)	21 (39.6%)
4	0 (0.0%)	2 (2.9%)	0 (0.0%)	1 (4.3%)	1 (3.3%)	2 (6.7%)
5	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
1-2 (echolucent)	**10 (71.4%)**	**38 (54.2%)**	8 (47.0%)	**14 (60.9%)**	**16 (53.3%)**	**30 (56.7%)**
3-5 (echogenic)	4 (28.6%)	32 (45.8%)	**9 (53.0%)**	9 (39.1%)	14 (46.7%)	23 (46.3%)
Texture Features						
GSM	26.1 (15.0)	27.0 (15.1)	26.6 (11.3)	26.0 (13.3)	28.1 (18.4)	27.9 (16.0)
PPCS1	26.9 (15.2)	26.9 (17.5)	27.2 (14.5)	25.4 (19.4)	28.0 (18.0)	25.6 (17.5)
PPCS2	17.2 (7.0)	15.6 (5.9)	15.6 (4.9)	15.4 (6.4)	15.7 (6.1)	15.7 (6.3)
Bel_30	59.5 (19.3)	58.0 (19.3)	58.5 (16.7)	57.8 (19.5)	57.8 (21.1)	57.1 (20.2)
Bel_50	77.0 (16.5)	77.6 (16.1)	78.3 (14.5)	79.2 (14.3)	76.0 (18.6)	77.0 (16.9)
SGLD ASM	.011 (.018)	.023 (.047)	.014 (.023)	.028 (.070)	.021 (.032)	.019 (.037)
SGLDM COR	.923 (.058)	.901 (.060)	.907 (.063)	.889 (.051)	.907 (.066)	.90 (.06)
SGLD HOM	.231 (.090)	.233 (.103)	.226 (.087)	.226 (.121)	.243 (.100)	.229 (.099)
SGLDM IMC-1	-.331 (.063)	-.295 (.063)	-.292 (.066)	-.287 (.057)	-.303 (.069)	-.295 (.065)
Skewness	1.46 (.47)	1.38 (.63)	1.37 (.49)	1.53 (.82)	1.27 (.51)	1.35 (.65)
Runl SRE	.934 (.022)	.933 (.024)	.934 (.023)	.935 (.025)	.932 (.024)	.935 (.022)

The degrees of correlation of texture features between ipsilateral and contralateral carotids in both the entire population and in the symptom subgroups were determined and presented in Table 17.

Whole population: Several features were significantly correlated in the whole population, including GSM (p=.02), bel_30 (p=.01), bel_50 (p<.001), SGLDM COR (p<.001) and IMC-1 (p<.001). PPCS2 was borderline significantly correlated (P=.050) within this group.

Asymptomatic patients: Bel_50, SGLDM COR and IMC-1 were significantly correlated (p=.02, p<.01 and p=.02, respectively), among

asymptomatic patients. GSM was borderline significantly correlated (p=.05).

Symptomatic patients: Plaque type, bel_30 and bel_50 were significantly correlated among symptomatic patients (p=.02, p=02, p<.01, respectively). SGLDM COR and IMC-1 were both highly significantly correlated (p<.001, p<.001).

Amaurosis fugax patients: Bel_50, SGLDM COR and IMC-1 were significantly correlated among amaurosis fugax patients (p=.02, p=.01 and p<.001, respectively).

TIA patients: Bel_30, bel_50, SLGD COR and IMC-1 were significantly correlated (p=.03, p=.01, p<.01, p=.01) among TIA patients. GSM trended toward significant correlation (p=.08).

Stroke patients: Plaque type was significantly correlated (p=.01) among stroke patients, and SGLDM COR and IMC-1 were highly significantly correlated (p<.001, p<.001 and p<.01, respectively).

TIA or stroke patients: Highly significant correlations were found in plaque type (p<.01), bel_50 (p<.01), SGLDM COR (p<.001) and IMC-1 (p<.001). GSM demonstrated significant correlation (p=.04) and skewness trended toward significant correlation (p=.09).

Table 17 – Degree of correlation between ipsilateral and contralateral plaques in patients with different symptoms

Imaging feature		All (n=84)	Asymp (n=14)	Symp (n=70)	AF (n=17)	TIA (n=23)	Stroke (n=30)	TIA or stroke (n=53)
Plaque type	R value Sig.	.14 .23	.41 .16	**.29** **.02**	-.28 .30	.32 .16	**.48** **.01**	***.43*** ***<.01***
GSM	R value Sig.	**.26** **.02**	*.54* *.05*	*.24* *.05*	-.07 .80	*.39* *.08*	.26 .20	**.29** **.04**
PPCS1	R value Sig.	.13 .27	.14 .65	.14 .27	-.16 .55	.32 .16	.14 .50	.21 .15
PPCS2	R value Sig.	*.22* *.05*	.41 .17	*.22* *.08*	.21 .43	.23 .32	.24 .23	.23 .12
Bel_30	R value Sig.	**.28** **.01**	.47 .10	**.28** **.02**	.28 .30	**.46** **.03**	.22 .28	**.30** **.04**
Bel_50	R value Sig.	***.43*** ***<.001***	**.65** **.02**	***.41*** ***.001***	**.57** **.02**	**.53** **.01**	*.36* *.07*	***.40*** ***<.01***
SGLD ASM	R value Sig.	-.02 .88	-.13 .66	.01 .94	.01 .98	-.15 .51	.25 .21	.03 .85
SGLDM COR	R value Sig.	***.60*** ***<.001***	***.73*** ***<.01***	***.60*** ***<.001***	**.62** **.01**	***.62*** ***<.01***	***.71*** ***<.001***	***.65*** ***<.001***

SGLD HOM	R value Sig.	.12 .30	.10 .73	.13 .30	.28 .29	.07 .77	.12 .56	.10 .50
SGLDM IMC-1	R value Sig.	***.64*** ***<.001***	**.62** **.02**	***.64*** ***<.001***	***.79*** ***<.001***	**.53** **.01**	***.65*** ***<.001***	***.58*** ***<.001***
Skewness	R value Sig.	.17 .13	.11 .72	.20 .11	-.05 .87	-.07 .77	***.52*** ***<.01***	*.25* *.09*
Runl SRE	R value Sig.	.04 .76	-.05 .88	.06 .64	-.01 .96	.28 .23	<.01 .99	.09 .54

Trending values (.05<p<.10) are in italics.
Significant values (p<.05) are in bold.
Highly significant values (p<.01) are in bold italics.

3.3 - Objective 3

To determine differences in textural features between the ultrasonic carotid plaques images of men and women referred for CEA.

3.3.1 - Baseline characteristics of men and women

The population consisted of 63 men and 21 women, whose baseline characteristics are presented in Table 18. Age and BMI were not significantly different between men and women. Diabetes was far more prevalent in men than in women. While there were proportionately more men with CAD, PAD was more common among women.

Women were more often prescribed multiple (3-4) antihypertensive agents than were men. β-Blockers were the most commonly prescribed agent among men, far more frequently than among women, while diuretics and ARBs were the most common antihypertensives among women. Statins and biguanides were the most frequently observed cholesterol lowering medications and hypoglycemic agents in both sexes.

Table 18 – Baseline characteristics of men and women

	Men (n=63)	**Women (n=21)**
Age* (Years)	69.5 (9.8)	68.3 (8.1)
BMI* (kg/m^2)	27.3 (4.0)	26.5 (4.8)
Systolic blood pressure* (mmHg)	136.9 (16.6)	141.2 (18.5)
Diastolic blood pressure* (mmHg)	69.7 (9.7)	69.4 (8.7)
Pulse pressure* (mmHg)	67.0 (17.4)	71.8 (16.9)
Symptomatology		
Asymptomatic	14 (22.2%)	0 (0.0%)
Symptomatic	49 (77.8%)	21 (100%)
AmF	11 (17.5%)	6 (28.6%)
TIA	17 (27.0%)	6 (28.6%)
Stroke	21 (33.3%)	9 (42.9%)

TIA or Stroke	38 (60.3%)	15 (71.4%)
Time since event		
AmF (n=14)		
<30 days	2 (14.3%)	5 (35.7%)
30-90 days	4 (28.6%)	0 (0.0%)
90-180 days	2 (14.3%)	1 (7.1%)
>180 days	0 (0.0%)	0 (0.0%)
TIA (n=16)		
<30 days	7 (43.8%)	3 (18.8%)
30-90 days	4 (25.0%)	1 (6.2%)
90-180 days	0 (0.0%)	0 (0.0%)
>180 days	0 (0.0%)	1 (6.2%)
Stroke (n=26)		
<30 days	9 (34.6%)	4 (15.4%)
30-90 days	6 (23.1%)	3 (11.5%)
90-180 days	2 (7.7%)	1 (3.8%)
>180 days	0 (0.0%)	1 (3.8%)
Family history of vascular disease	23 (36.5%)	8 (38.1%)
Early MI	7 (11.1%)	2 (9.5%)
Stroke	12 (19.0%)	3 (14.3%)
PAD	9 (14.3%)	3 (14.3%)
History of vascular disease		
CAD	26 (41.3%)	6 (28.6%)
PAD	10 (15.9%)	5 (23.8%)
Either PAD or CAD	18 (28.6%)	5 (23.8%)
Both PAD and CAD	9 (14.3%)	3 (14.3%)
Modifiable risk factors		
Hypertension	54 (85.7%)	18 (85.7%)
Hypercholesterolemia	53 (84.1%)	16 (76.2%)
Diabetes	25 (39.7%)	1 (4.8%)
Smoking history	50 (79.4%)	16 (76.2%)
Medication		
Antihypertensives (type)		
Diuretics	19 (30.2%)	7 (33.3%)
ACEI	18 (28.6%)	5 (23.8%)
ARBs	23 (36.5%)	7 (33.3%)
β-Blockers	26 (41.3%)	3 (14.3%)
CCBs	22 (34.9%)	6 (28.6%)
Direct-acting smooth muscle relaxant	1 (1.6%)	0 (0.0%)
α-2 Agonists	1 (1.6%)	0 (0.0%)
Direct renin blockers	1 (1.6%)	0 (0.0%)
Antihypertensives (#)		
0	11 (17.5%)	5 (23.8%)
1-2	41 (65.1%)	9 (42.9%)
3-4	10 (15.9%)	7 (33.3%)
5-6	1 (1.6%)	0 (0.0%)
Duration of treatment	(n=61)	(n=20)
0 – 2 months	0 (0.0%)	2 (13.3%)
2 months – 1 year	6 (12.0%)	3 (20.0%)
1 – 5 years	11 (22.0%)	1 (6.7%)
>5 years	33 (66.0%)	9 (60.0%)
Cholesterol lowering		
No cholesterol lowering medications	13 (20.6%)	5 (23.8%)
Statin	49 (77.8%)	16 (76.2%)
Ezetimibe	3 (4.8%)	1 (4.8%)
Fenofibrate	1 (1.6%)	0 (0.0%)
Niacin	2 (3.2%)	0 (0.0%)
Combination therapy (with statin)	5 (7.9%)	1 (4.8%)
Duration of treatment	(n=58)	(n=16)
0 - 2 months	1 (2.2%)	1 (9.1%)
2 months - 1 year	6 (13.3%)	5 (45.4%)

1 – 5 years	13 (28.9%)	0 (0.0%)
> 5 years	25 (55.6%)	5 (45.4%)
Antidiabetic		
No antidiabetic medications	40 (63.5%)	20 (95.2%)
Biguanides	17 (27.0%)	1 (4.8%)
Sulphonylureas	14 (22.2%)	1 (4.8%)
DPP-4 inhibitors	1 (1.6%)	0 (0.0%)
Insulin	4 (6.3%)	1 (4.8%)
Combination therapy (with insulin)	3 (4.8%)	1 (4.8%)
Duration of treatment	(n=61)	
0 - 2 months	0 (0.0%)	0 (0.0%)
2 months - 1 year	4 (15%)	0 (0.0%)
1 – 5 years	2 (10%)	0 (0.0%)
> 5 years	15 (75%)	1 (100%)

*Variables presented as mean (standard deviation).
AmF: amaurosis fugax, *TIA*: transient ischemic attack, *MI*: myocardial infarction, *PAD*: peripheral artery disease, *CAD*: coronary artery disease, *ACEI*: Angiotensin cleaving enzyme inhibitor, *ARBs*: Angiotensin II receptor blockers, *CCBs*: Calcium channel blockers, *DPP-4*: Dipeptidyl peptidase-4

3.3.2 - Risk factor differences between men and women

Before adjustment for age and BMI, men were more likely to have a greater number of risk factors (3-4 vs. 1-2) than women, trending toward significance (p=.07) (Tables 19-20).

Table 19 – Number of modifiable risk factors in men and women

Modifiable Risk factors (#)	Men (n=63)	Women (n=21)
0	3 (4.8%)	0 (0.0%)
1	2 (3.2%)	3 (14.3%)
2	10 (15.9%)	7 (33.3%)
3	32 (51.0%)	11 (52.4%)
4	16 (25.4%)	0 (0.0%)
0-2 RFs **3-4 RFs**	15 (23.8%) **48** (76.2%)	10 (47.6%) **11** (52.4%)

Table 20 – Differences in number of modifiable risk factors in men (n=63) vs. women (n=21) (reference group)

	Modifiable RFs (#)	Fisher's exact test (sig.)	Unadjusted OR (sig.)	Adjusted OR (Age, BMI) (sig.) (n=81)
Men vs. women (reference group)	0-1 vs. 2-4	.41	.53 (.11-2.42) .41	.48 (.10-2.32) .36
	0-1 vs. 3-4	.35	.40 (.08-1.93) .25	.41 (.08-2.09) .28
	0-1 vs. 4	**.03**	<.01 (<.01->99-99) .94	<.01 (<.01->99.99) .93
	0-2 vs. 4	***<.01***	<.01 (<.01->99.99) .95	<.01 (<.01->99.99) .95
	0-2 vs. 3-4	.10	*.38 (.14-1.07)* *.07*	.45 (.16-1.32) .15
	0123 vs. 4	***<.01***	<.01 (<.01->99.99) .95	<.01 (<.01->99.99) .96

Trending values (.05<p<.10) are in italics.
Significant values (p<.05) are in bold.
Highly significant values (p<.01) are in bold italics.

3.3.3 - Ultrasonic imaging (Tables 21-26)

3.3.3.1 - Stenosis and plaque type

Ipsilateral carotid stenosis values were similar between sexes (p=.82), and echolucent plaque types were more common in men than in women (p=.36) (Table 21).

Table 21 – Ipsilateral carotid stenosis and plaque types in men and women

	Men (n=63)	Women (n=21)
Stenosis (%)	85.2 (9.3)	85.8 (9.5)
Plaque Type		

1	5 (7.9%)	1 (4.8%)
2	36 (57.1%)	10 (47.6%)
3	21 (33.3%)	9 (42.8%)
4	1 (1.6%)	1 (4.8%)
5	0 (0.0%)	0 (0.0%)
1-2 (echolucent)	**41 (65.1%)**	**11 (52.4%)**
3-5 (echogenic)	22 (34.9%)	10 (47.6%)

Contralateral stenosis was similar between men and women (p=.53) (Table 22) and echolucent plaques were more common in men than in women, although non-significant (p=.22).

Table 22 – Contralateral carotid stenosis and plaque type in men and women

	Men (n=63)	**Women (n=21)**
Stenosis (%)	43.4 (30.5)	38.5 (32.0)
Plaque Type		
1	4 (6.3%)	2 (9,5%)
2	35 (55.6%)	7 (33.3%)
3	24 (38.1%)	10 (47.6%)
4	0 (0.0%)	2 (9.5%)
5	0 (0.0%)	0 (0.0%)
1-2 (echolucent)	**39 (61.9%)**	9 (42.9%)
3-5 (echogenic)	24 (38.1%)	**12 (57.1%)**

3.3.3.2 – Texture features

Men vs. women – ipsilateral plaques (Tables 23-24): GSM was non-significantly lower in men than in women (adj. p=.09) after adjustment for age only. PPCS2 was observed to be significantly greater in men than in women (adj. p=.04). Bel_30 (adj. p=.05) and bel_50 (adj. p=.04) were also greater in men compared to women, borderline significant and significant, after adjustment for age only. After adjusting for age only, plaques from men were characterized by significantly lower values of SGLDM correlation and by a trend toward significantly greater values of IMC-1 than women (adj. p=.04 and adj. p=.08, respectively). Adjusted for age only, values of skewness were trending toward being significantly greater in men than women (adj. p=.09).

We also performed additional analyses, only including symptomatic individuals, as there were no asymptomatic women. The significance of these trends increased, so that the differences in GSM (p=.02) and bel_30 (.01) became significant and those in bel_50 (p<.01) and PPCS2 (p<.01) became highly significant. As for texture trends, the differences in SGLDM correlation (p=.03), IMC-1 (p=.02) and skewness (p=.03) were all significant, albeit unadjusted.

Table 23 – Values of texture features in men and women

Texture Features	**Men (n=63)**	**Women (n=21)**
Plaque type	See Table 21	See Table 21
GSM	20.9 (14.9)	27.7 (21.8)
PPCS1	36.2 (21.0)	31.6 (20.3)
PPCS2	15.7 (6.0)	12.5 (4.7)
Bel_30	65.7 (20.3)	56.2 (21.3)
Bel_50	82.0 (15.4)	73.4 (18.6)
SGLD ASM	.046 (.092)	.039 (.066)
SGLDM COR	.906 (.059)	.936 (.056)
SGLD HOM	.284 (.143)	.286 (.136)
IMC-1	-.293 (.065)	-.323 (.073)
Skewness	1.78 (.67)	1.49 (.76)
Runl SRE	.923 (.030)	.926 (.026)

Table 24 – Texture features of ipsilateral carotid plaques in men (n=63) (reference group) vs. women (n=21)

Texture Features	T-test (Sig.)	Unadjusted OR (Sig.)	Adjusted OR (Age only) (Sig.)	Adjusted OR (Age, BMI) (Sig.) (n=81)
Plaque type	.44	.62 (.23-1.67) .34	.59 (.21-1.62) .30	.61 (.21-1.75) .36
GSM	.11	.98 (.95-1.01) .12	*.98 (.95-1.00) .09*	.98 (.95-1.01) .23
PPCS1	.38	1.01 (.99-1.04) .38	1.01 (.99-1.04) .32	1.01 (.99-1.04) .42
PPCS2	**.03**	**1.12 (1.01-1.24) .03**	**1.12 (1.01-1.24) .03**	**1.11 (1.00-1.23) .04**
Bel_30	*.07*	*1.02 (.99-1.05) .07*	*1.02 (.99-1.05) .05*	*1.02 (.99-1.05) .09*
Bel_50	**.04**	**1.03 (1.00-1.06) .04**	**1.03 (1.00-1.06) .04**	*1.03 (.99-1.06) .07*
SGLD ASM	.74	3.08 (<.01->99.99) .73	4.05 (<.01->99.99) .68	3.12 (<.01->99.99) .74
SGLDM COR	**.04**	**<.01 (<.01-.93) .04**	**<.01 (<.01-.60) .04**	*<.01 (<.01-1.28) .05*
SGLD HOM	.95	.89 (.03-30.07) .95	1.04 (.03-37.09) .99	.78 (.02-30.55) .90
IMC-1	*.07*	*832.99 (.48->99.99) .08*	*753.06 (.44->99.99) .08*	*816.93 (.38->99.99) .09*
Skewness	*.09*	*1.95 (.88-4.34) .09*	*2.03 (.90-4.60) .09*	1.83 (.78-4.29) .16
Runl SRE	.71	.04 (<.01->99.99) .71	.01 (<.01->99.99) .64	.13 (<.01-99.99) .83

Trending values (.05<p<.10) are in italics.
Significant values (p<.05) are in bold.
Highly significant values (p<.01) are in bold italics.

Men vs. women – contralateral plaques (Tables 25-26): Adjusted for age and BMI, values of PPCS2 and bel_50 trended toward significant differences between the sexes (adj. p=.08 and adj. p=.09, respectively). Adjusted for age only, mean GSM trended toward significantly lower values in men than in women (adj. p=.06).

It is interesting to note that, compared with the ipsilateral side, the contralateral plaques display similar yet attenuated trends in echolucency (GSM, PPCS2, bel_50) and heterogeneity (SGLDM correlation, IMC-1, skewness).

When asymptomatic patients were excluded, differences in bel_50 were borderline significant (p=.06), in GSM, trended toward significance (p=.08) and in SGLDM correlation, were significant (p=.02).

Table 25 - Texture features of contralateral carotid plaques in men and women

Texture Features	Men (n=63)	Women (n=21)
Plaque type	See Table 22	See Table 22
GSM	24.3 (11.9)	31.8 (19.9)
PPCS1	28.2 (16.5)	25.5 (18.1)
PPCS2	16.9 (5.8)	13.9 (5.8)
Bel_30	61.2 (16.7)	53.3 (22.0)
Bel_50	80.2 (13.4)	72.6 (19.8)
SGLD ASM	0.019 (0.043)	0.024 (0.046)
SGLDM COR	0.902 (0.051)	0.915 (0.079)
SGLD HOM	0.231 (0.100)	0.249 (0.101)
SGLDM IMC-1	-0.294 (0.061)	-0.313 (0.069)
Skewness	1.46 (0.52)	1.22 (0.78)
Runl SRE	0.933 (0.024)	0.933 (0.021)

Table 26 – Differences in texture feature of contralateral carotid plaques between men and women

Texture Features	T-test (Sig.)	Unadjusted OR (Sig.)	Adjusted OR (Age only) (Sig.)	Adjusted OR (Age, BMI) (Sig.) (n=81)
Plaque type	.21	.49 (.18-1.32) .16	.45 (.16-1.26) .13	.51 (.17-1.51) .22
GSM	**.04**	*.97 (.94-1.00) .07*	*.97 (.93-1.00) .06*	.97 (.94-1.01) .13
PPCS1	.53	1.01 (.98-1.04) .50	1.01 (.98-1.04) .48	1.01 (.98-1.04) .66
PPCS2	**.04**	*1.09 (.99-1.19) .07*	*1.09 (.99-1.20) .06*	*1.09 (.99-1.19) .08*
Bel_30	.12	1.02 (.99-1.05) .12	1.02 (.99-1.05) .11	1.02 (.99-1.05) .17
Bel_50	*.07*	*1.03 (.99-1.06) .08*	*1.03 (.99-1.06) .07*	*1.03 (.99-1.06) .09*
SGLD ASM	.72	.15 (<.01->99.99) .73	.11 (<.01->99.99) .69	.04 (<.01->99.99) .56
SGLDM COR	.33	.01 (<.01-80.43) .33	.01 (<.01-74.35) .32	<.01 (<.01-49.46) .27
SGLD HOM	.57	.25 (<.01-29.45) .57	.33 (<.01-28.98) .56	.09 (<.01-14.20) .35
SGLDM IMC-1	.29	64.25 (.03->99.99) .29	66.46 (.03->99.99) .28	309.27 (.08->99.99) .18
Skewness	.12	2.15 (.82-5.59) .12	2.14 (.82-5.58) .12	1.95 (.73-5.20) .18
Runl SRE	.86	.14 (<.01->99.99) .86	.12 (<.01->99.99) .85	.58 (<.01->99.99) .96

Trending values (.05<p<.10) are in italics.
Significant values (p<.05) are in bold.
Highly significant values (p<.01) are in bold italics.

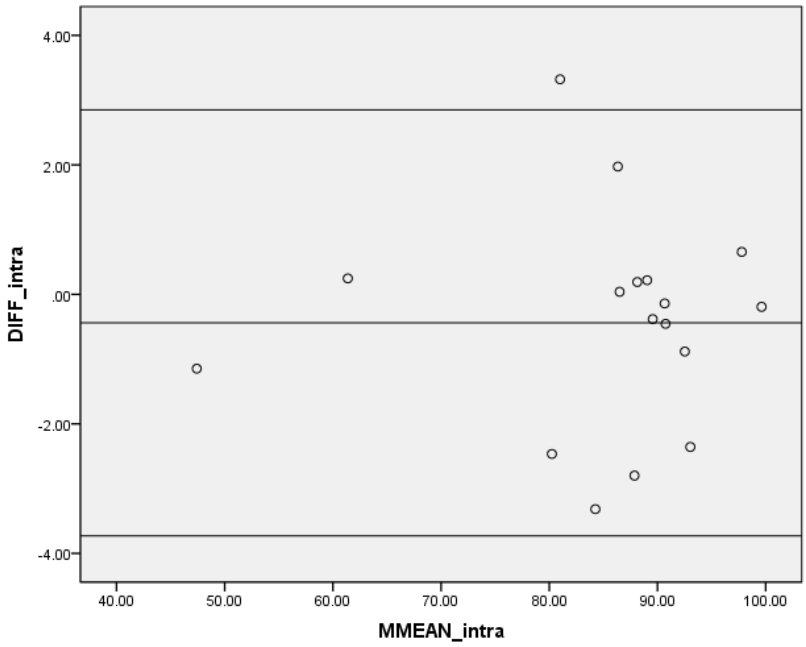
3.4 - Reproducibility Studies

Intra-operator and inter-operator reproducibility demonstrated small mean differences and standard deviations.

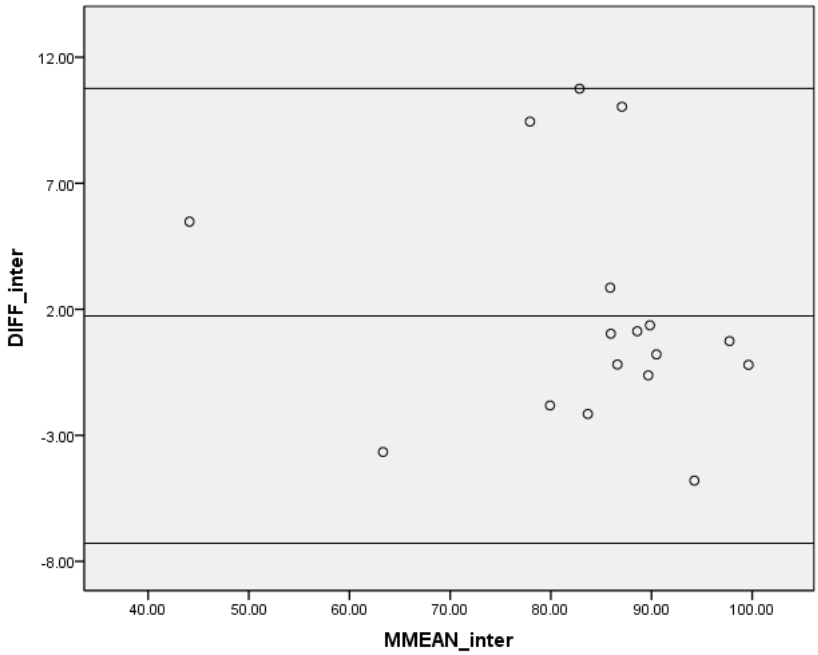
Table 27 – Intra-operator and inter-operator reproducibility

Difference	Intra-operator	Inter-operator
Mean	-0.44	1.74
Standard deviation	1.68	4.62
95% confidence interval	-1.30 0.42	-0.63 4.12

Plot 1 – Intra-operator reproducibility (Bland-Altman plot)



Plot 2 – Inter-operator reproducibility (Bland-Altman plot)



4 - Discussion

4.1 Texture differences between symptom groups

As part of objective 1, we used digital image analysis to assess differences in ultrasonic texture (echolucency (darkness) and heterogeneity) ipsilateral carotid atherosclerotic plaques in individuals referred for CEA, including men and women, both asymptomatic and those having suffered cerebrovascular symptoms, namely amaurosis fugax, TIAs and strokes. Plaques causing TIA or stroke formed an additional group in our analyses due to previously reported similarities of ultrasonic features [138, 143].

Plaques from the amaurosis group, the 'TIA or stroke' group and all-symptoms group were found to be significantly more echolucent (darker) than the asymptomatic group, with plaques causing strokes only trending toward greater echolucency. Although the TIA group exhibited nearly identical echolucency as the stroke group, it was insufficiently powered to reach a significant difference in comparison with the asymptomatic group. Among the symptom groups, the amaurosis fugax plaques were significantly more echolucent than the TIA group and the 'TIA or stroke' group, and trended toward greater echolucency than the stroke group.

In the amaurosis group and all-symptoms group, ipsilateral plaques were more heterogeneous than those in asymptomatic patients. Amaurosis fugax plaques were also shown to trend toward significantly greater homogeneity than those from TIA patients. Amaurosis fugax plaques were more homogeneous than stroke plaques, with the non-significance of this difference likely caused by the large variability within the stroke group (see *4.1.1.6: Echolucency differences – asymptomatic vs stroke*).

4.1.1 - Echolucency differences between symptom groups

These findings are consistent with previously reported differences in echomorphology between asymptomatic carotid plaques and those

causing different symptoms [137, 138, 143]. Tegos et al. [143] had demonstrated that amaurosis fugax and asymptomatic plaques were most echolucent and echogenic (echo-rich - brighter), respectively, with both TIA and stroke plaques found to have intermediate echolucency. These particular findings were observed without image normalization, a technique that dramatically improved the evaluation of plaque echolucency, leading to the reclassification of 652 (60%) of the plaques in one large study [119]. Furthermore, in this study, while plaque types 1-3 were associated with only 71% of the cerebrovascular events, image normalization increased this proportion to 94% [119]. In the present study, we performed image normalization on and extracted multiple features of echolucency from plaque images. Thus, we successfully reproduced the plaque echolucency differences found by Tegos et al. [143], and confirmed recent work with texture analysis done by Kakkos et al. [138].

4.1.1.1 - Echolucency differences - asymptomatic vs. symptomatic

In the present study, the difference in the grayscale median GSM (overall brightness of plaque) between asymptomatic and symptomatic patients (30.1 vs. 20.9, respectively) was not as large as differences in previous studies (in which GSM differences between symptomatic and asymptomatic patients ranged between 15 and 17.5) [121, 138, 164]. Moreover, previously reported GSM cut-off points for positive symptomatology include 40 [164] and 32 [161], which would not hold true in our population given that the GSM values below such cut-off points would include the mean GSM of our asymptomatic population.

4.1.1.2 - The high-risk asymptomatic plaque hypothesis

To our knowledge, we are the first group to use digital image analysis to evaluate plaque morphology differences between symptomatic and asymptomatic individuals referred for CEA. Previous studies comparing symptomatic and asymptomatic plaque morphology recruited all patients

with significant carotid stenoses (commonly greater than 50-70%) [121, 137, 138, 143, 161, 164], rather than high-risk referrals for CEA. Therefore, possible explanations for the above-mentioned discrepancies include the nature of our study population, namely, individuals referred for CEA, and the referral practices specific to the RVH and JGH. According to the AHA guidelines for CEA, individuals are referred for CEA when recent (<6 months) neurological symptoms are associated with a stenosis between 60-99%, or if stenosis is between 70-99% and unaccompanied by neurological symptoms [87]. These indications for CEA are based on the results of the NASCET and ACAS trials [81, 85], which compared the effectiveness of CEA and medical management to medical management alone, within symptomatic and asymptomatic patients with varying degrees of stenosis. Two systemic reviews combining these studies with other CEA trials have reported CEA to be more highly effective in symptomatic patients (NNT: 6.3) [86] than for asymptomatic patients (NNT: 33) [84].

In addition to a relatively decreased CEA benefit for asymptomatic patients, recent evidence has led to the understanding that the combination of statins, antiplatelet medication and aggressive lifestyle changes leading to improvement of risk factor burden have decreased the reported annual risk of stroke associated with asymptomatic carotid stenosis to 1%, rendering the acceptable <3% perioperative risk of morbidity and mortality associated with CEA unjustifiable [165]. Thus, asymptomatic patients are less likely to be referred for CEA than are symptomatic patients, at least in Canada. Therefore, asymptomatic patients ultimately referred for CEA are likely at a relatively higher risk than asymptomatic patients overall. Consequently, the asymptomatic patients included in our study might have been at higher risk than asymptomatic patients in previous studies who were included based solely on degree of carotid stenosis [121, 143, 161, 164] and not on the need for high-risk carotid intervention.

4.1.1.3 - High-risk asymptomatic plaques due to progression of stenosis

Among the select few asymptomatic patients who were referred for CEA in our cohort, over half were primarily referred for CEA due to rapid progression of stenosis, according to surgical transcripts written by the vascular surgeons. Interestingly, progression of stenosis has been associated with increased echolucency [110] and greater risk for cerebrovascular symptoms [111, 166]. Therefore, it is possible that some of our asymptomatic patients would have soon become symptomatic had they not undergone surgery. Moreover, asymptomatic patients were, on average, more than 5 years younger than symptomatic patients. Thus, it is likely that these asymptomatic patients would have become symptomatic by the time they would have reached the age of the symptomatic groups. Consequently, some of the plaques in the asymptomatic cohort may have had unusually similar echomorphology to symptomatic plaques.

4.1.1.4 - High-risk asymptomatic plaques due to male predominance

Furthermore, in many centers (including the RVH and JGH), fewer women than men are referred for CEA because of reports of reduced CEA benefit for women [84, 86]. More specifically, analysis of pooled data from ACAS and the asymptomatic carotid surgery trial (ACST) demonstrated a highly significant difference in relative risk reduction in men compared with women (49% vs. 4%)[84]. This reluctance to refer women for CEA (especially if asymptomatic) was clearly evident in our cohort, which, despite consecutive recruitment of patients, did not include asymptomatic women. Given that previous studies have shown that men are more likely to have soft plaques than are women [133], a men-only asymptomatic cohort is likely to be at higher risk than an asymptomatic group including women.

4.1.1.5 - High-risk asymptomatic plaque hypothesis supported by clinical data

Clinical data from the present study support the notion that the asymptomatic patients in our cohort were at unusually high risk. Compared with all other symptom groups, the asymptomatic group had the greatest mean BMI and the greatest prevalence of family history of vascular disease (stroke especially), personal history of CAD, prescription of β-blockers and prescription of multiple anti-hypertensive medications.

4.1.1.6 - Echolucency differences – asymptomatic vs. stroke

In addition to the asymptomatic cohort being characterized by more echolucent plaques than previously reported in the literature, stroke plaques were unusually echogenic, only trending toward being more echolucent than asymptomatic plaques. Although this non-significance is partly the result of the small size of both asymptomatic and stroke groups, it is also caused by the larger standard deviations within the stroke group of the mean values of plaque echolucency features, especially when compared to the standard deviations of the markedly smaller cohorts of individuals with amaurosis fugax and TIA. This large variability within the stroke group is likely related to a process well-described in histological studies, whereby plaques causing symptoms experience remodeling with time post-event. More specifically, plaques removed less than 60 days after a stroke were more unstable than those removed less than 60 days after a TIA. However, the instability persisted in TIA but not in stroke; plaques removed more than 180 days after the event were significantly less unstable after a stroke than after a TIA [167]. Therefore, the recruitment of patients having suffered strokes with widely variable timing in relation to their ultrasound examination results in capturing different stages of plaque remodeling. In our stroke cohort, 50% became symptomatic within 30 days of their ultrasound exam, 37% within 30-90 days, 10% within 90-180 days and 3% within more than 180 days of their

ultrasound exam. In our TIA cohort, 62.5% became symptomatic within 30 days, 31.2% within 30-90 days and 6.3% within more than 180 days of their exam. Thus, exams accompanied by greater delay relative to the most recent events might reveal stabilized stroke plaques and persistently unstable TIA plaques. In our amaurosis fugax cohort, 50% became symptomatic within 30 days, 28.6% within 30-90 days and 21.4% within 90-180 days of their exam. However, post-event remodeling in plaques causing amaurosis fugax has yet to be studied.

4.1.2 - Heterogeneity – association with symptoms

Our study confirmed the association between heterogeneity with plaques causing symptoms reported in previous studies [128, 129, 135]. These studies classified plaque echogenicity into three components, hypoechoic, isoechoic and hyperechoic, and a plaque was deemed heterogeneous if it contained more than two components. Thus, heterogeneous plaques were more often associated with stenosis progression and neurological events. However, while these conclusions relied on qualitative methods to evaluate plaque heterogeneity, our study employed quantitative measures of heterogeneity, improving objectivity.

Other studies, however, have found symptoms to be associated with homogeneity rather than heterogeneity [121, 138, 143, 161, 164]. These studies were mostly performed using either qualitative or semi-quantitative methodology.

One recent study by Kakkos et al. employed the same digital image analysis software as the present study and found a significant association between plaques causing stroke or TIA and lower values of a feature of heterogeneity, SGLDM correlation (higher values indicate greater heterogeneity), comparing these symptomatic plaques to asymptomatic control plaques [138]. The authors therefore concluded that symptoms were associated with homogeneity. However, the same study revealed amaurosis fugax and TIA plaques to be independently associated with

greater values of other heterogeneity features, named SGLDM IMC-1 and skewness, respectively [138]. Thus, these results may suggest a more complicated association between symptoms and heterogeneity, and further studies are needed to confirm these associations.

4.1.2.1 - Comparison with the present study: significant texture features

Although the present study found no significant differences in SGLDM correlation between the different symptom groups, SGLDM IMC-1 most greatly differed between asymptomatic and amaurosis fugax plaques, supporting the independent association found by Kakkos et al. [138]. Skewness, found by Kakkos et al. to be associated with plaques causing TIA, was determined in the present study to be greater in every symptomatic group than in the asymptomatic group, with the greatest difference observed between asymptomatic plaques and amaurosis fugax, trending toward significance before adjustment. Differences are likely related to the different nature of our asymptomatic cohort, which included high-risk patients referred for CEA and did not include women, and to the variable timing between neurological event and ultrasound exam in the present study.

4.1.2.2 - Additional texture feature differences

Compared with asymptomatic plaque, symptomatic and amaurosis fugax plaques were characterized by lower runl SRE (measure of homogeneity – lower values indicating greater heterogeneity and coarser texture). This finding associates symptoms with heterogeneity, as described by runl SRE.

Our study also demonstrated amaurosis fugax plaques to be more homogeneous than TIA plaques in terms of greater values of SGLD ASM and SGLD HOM (both features of homogeneity – higher values indicating greater homogeneity), representing, to the best of our knowledge, the first comparison of texture features between plaques causing amaurosis fugax

and TIA. This finding suggests that TIA plaques are more heterogeneous than amaurosis fugax plaques, as described by these two features.

Although non-significant, values of SGLD HOM follow trends previously demonstrated by Elatrozy et al. [164], whose study demonstrated 60% of symptomatic plaques to have values of SGLD HOM greater than >0.2 vs. only 40% of asymptomatics. Likewise, albeit with large standard deviations, SGLD HOM in our asymptomatic cohort was 0.256 while symptomatic plaques are characterized by a mean SGLD HOM of 0.290, and with SGLD HOM of 0.327 in amaurosis fugax plaques. Thus, although non-significant, these differences may indicate a greater homogeneity of symptomatic plaques compared with asymptomatic plaques, as described by SGLD HOM.

These three features, runl SRE, SGLD ASM and SGLD HOM, have previously been incorporated into a diagnostic algorithm aiming to separate plaques according to their symptomatology [119], and are currently being tested in natural history studies such as the Asymptomatic Carotid Stenosis and Risk of Stroke (ACSRS) study [118, 168].

4.2 - Texture feature correlation between carotid arteries

As part of objective 2, we used digital image analysis to successfully identify texture features of ultrasonic carotid plaques that correlate significantly between plaques originating from the ipsilateral and contralateral arteries (Table 17). We included from our cohort all patients with contralateral plaques, defined as any protrusion greater than 15mm from the adventitia [160].

The best correlated feature was SGLDM IMC-1 (a measure of heterogeneity - higher values indicate greater heterogeneity), demonstrating a very good to excellent relationship (r >.75)[169] between the two carotid arteries among the amaurosis fugax group.

Five texture features exhibited moderate to good correlations (.50 < r < .75)[169] between various symptom groups. These included SLGDM

correlation (measure of heterogeneity – higher values indicate greater heterogeneity), which correlated among the whole population as well as within the 6 symptom groups, and SGLDM IMC-1, which demonstrated a moderate to good correlation among all groups except among amaurosis fugax patients, as mentioned above. Asymptomatic, amaurosis fugax and TIA patients had plaques with moderate to good correlations in bel_50 (measure of echolucency, percentage of pixels below grayscale value of 50). Skewness was moderately to well correlated among stroke patients only, while GSM was similarly correlated only in asymptomatics, although borderline significant (p=.05).

Plaque type and three features of echolucency, GSM, bel_30 (percentage of pixels below grayscale value of 30) and bel_50, demonstrated **fair correlations** (.25 < r < .50)[169] among various symptom groups. Plaque type correlated fairly within the symptomatic, stroke and 'TIA or stroke' groups, with stroke patients demonstrating the strongest correlation within this group (r=.48). Other notable fair correlations include bel_30 among TIA patients (r=.46) and asymptomatic patients (r = .47, p=.104).

4.2.1 - Plaque morphology between the carotids – similarities and differences

A relationship in plaque morphology between the carotid arteries has been previously suggested by several studies [145-147]. One study by Rothwell et al. demonstrated that the presence of an ipsilateral plaque with an irregular surface increased the likelihood of a contralateral carotid plaque with an irregular surface by a factor of 2.2 when compared to the presence of an ipsilateral plaque [145]. Moreover, Paraskevas et al. demonstrated that, among individuals with internal carotid artery occlusion, plaque type (1-5) distribution was similar between the two carotids, with 38.3% of the occluded carotid and 43.3% of the stenosed

contralateral carotid consisting of echolucent plaques (Plaque types 1-2) [147].

The present study is novel, as it is different from these two studies in that we demonstrated correlations of plaque morphology using quantitative measures of plaque image texture instead of the subjective methods described in these studies, namely the description of plaque surface irregularity [145], and the visual classification of plaques into different plaque types [147]. Moreover, the present study also included symptomatic as well as asymptomatic patients, as opposed to only symptomatic patients [145, 147], and also performed correlations within different symptom subgroups.

4.2.2 - Texture feature correlations in symptomatic vs. asymptomatic individuals

The poor correlation of certain texture features between symptomatic ipsilateral and asymptomatic contralateral plaques is supported by a study done by Saam et al. [170]. In this study, magnetic resonance imaging (MRI) was used to detect differences in plaque composition between the two carotid arteries in unilaterally symptomatic individuals [170]. While a non-significant difference was found in lipid core area (8.1 vs. 6.3mm^2) (p=.2), a significant difference was determined in hemorrhage area (3.5 vs. 1.1mm^2) (p=.003). These two elements of plaque composition are both represented by PPCS1 and PPSC2 (measures of echolucency - percentage of pixels between grayscale values of 0-10 and 10-20, respectively) [171], and differences in these features may explain the poor correlation of both PPCS1 and PPCS2 within our cohort of symptomatic patients. Interestingly, the strongest correlation of PPCS2 was found within the asymptomatic cohort, although non-significant (r=.41, p=.167). Moreover, despite representing the smallest subgroup, asymptomatic individuals demonstrated the best correlations among the subgroups in four other features: GSM, bel_30, bel_50 and SGLDM correlation.

Weaker correlation of echolucency within the symptomatic group compared with the asymptomatic group may be related to morphological changes associated with an acute event such as rupture, thrombosis or hemorrhage or possibly associated with plaque remodeling post-event as previously described in histological analyses [167, 172]. However, it is significant that, in symptomatic patients, despite the morphological changes that may been precipitated by an acute event, features of plaque morphology still correlate well between the carotid arteries.

4.2.3 - Correlation of texture features associated with particular symptoms

Certain texture feature correlations are particularly noteworthy due to their independent associations with plaques causing certain symptoms. The strongest correlation of our study was SGLDM IMC-1 among amaurosis fugax patients (r=.79) (although SGLDM IMC-1 demonstrated moderate to good correlations in all other groups as well). Interestingly, SGLDM IMC-1 was found by Kakkos et al. [138] to be one of the features independently associated with amaurosis fugax plaques when compared to asymptomatic plaques.

Another texture with particular significance in the literature is SGLDM correlation, which was the strongest correlated feature aside from SGLDM IMC-1 among the different symptom groups. Kakkos et al. [138] found SGLDM correlation to be independently associated with stroke plaques and TIA plaques when compared with asymptomatic plaques.

Skewness demonstrated a moderate to good correlation solely within the stroke group. Skewness was previously associated with symptoms by Kakkos et al., who observed that skewness was independently associated with TIA plaques [138].

4.2.4 - Systemic instability: Mechanisms

The present study has shown that certain plaque texture features demonstrate significant correlations between the carotid arteries. These

specific features have previously been associated with plaque instability, suggesting a relationship between the carotids concerning plaque instability. Common morphological features between plaques in the carotid arteries may indicate common systemic factor(s) affecting plaque morphology (instability). Certain vascular risk factors such as hypertension, hypercholesterolemia, diabetes, smoking and obesity have been shown to independently predict progression of carotid atherosclerosis burden [173-176]. While traditional risk factors predict plaque burden, Rothwell et al. found that the small risk factor differences in a population of 3007 patients could not account for the observed associations in plaque instability between the carotids, nor between the carotids and the coronaries [145]. Another study, following 1769 participants for 20 years, observed that traditional risk factors predicted plaque thickness but had no relation to plaque composition [177].

Other systemic factors suggested to be atherogenic include infection and inflammation. One study including 504 patients demonstrated that a history of exposure to higher numbers of different pathogens was associated with stenosis progression (OR 3.8, 95% CI 1.6-8.8) after adjustment for age, sex, cardiovascular risk factors, highly sensitive C-reactive protein (hs-CRP) and statin intake [178]. White blood cell count, neutrophil count, Interleukin-6 (IL-6) levels and hs-CRP have all been associated with greater plaque echolucency [179-181]. Plasma levels of IL-6 and vascular cell adhesion molecule-1 were found to be higher in patients with multi-vessel atherosclerotic disease compared with single-vessel disease, and these levels predicted new vascular episodes/vascular death more stronger in patients with multi-vessel compared with single-vessel disease [182].

4.3 - Sex differences in carotid atherosclerosis

As part of objective 3, we used digital image analysis to successfully identify differences in ultrasonic texture features of ipsilateral and

contralateral carotid plaques originating from men and women referred for CEA. Plaques from men were characterized by greater echolucency than women on *both* ipsilateral and contralateral sides. Concerning heterogeneity, men had ipsilateral plaques that trended toward higher mean values of two features of heterogeneity, SGLDM IMC-1 and skewness, and a significantly lower value of another feature of heterogeneity, SGLDM correlation, compared with women. Heterogeneity of contralateral plaques from men demonstrated the same trends compared with women concerning these features, although non-significant.

Interestingly, despite the asymptomatic cohort consisting entirely of men, plaques from men (77.8% symptomatic) were still more echolucent than those of women (100% symptomatic). In fact, a higher proportion of women than men suffered amaurosis fugax (the symptom group characterized by the most echolucent plaques), increasing the expected echolucency among women overall.

By nature of being referred for CEA, all recruited subjects were deemed to be at high risk, and yet significant sex differences exist concerning plaque morphology. The fact that symptomatic women may have brighter plaques than symptomatic and even asymptomatic men could have important implications for sex-based differences in the pathogenesis of atherosclerosis.

4.3.1 - Sex differences in clinical data

In addition to texture differences between the sexes concerning plaque morphology, there were also differences in the number of modifiable vascular risk factors (hypertension, hypercholesterolemia, diabetes and smoking history). In the present study, the odds of having multiple risk factors (3-4) rather than few risk factors (1-2) was 61.7% more in men compared with women. Furthermore, there were no women (0%) with all four risk factors, compared with 16 out of 63 men (~25%) who had all four.

The increased risk factor number in males has previously been reported in CEA populations as well as in the general population [66, 154]. Thus, compared with men, women were more likely to have fewer modifiable vascular risk factors in addition to having more echogenic plaques.

There was significantly more type 2 diabetes in men than in women (p=.002; Fisher's exact test). Studies have shown that diabetic status is associated with the presence of heterogeneous, ulcerated [131], echolucent plaques [131, 183, 184]. However, in our population, diabetics and non-diabetics had very similar echolucency values (GSM: 23.3 vs. 22.5; p=.838 – student's T-test).

The male predominance in β-blockers prescription (41.3% vs. 14.3%; p=.032, Fisher's exact test, 2-sided) may be related to the higher prevalence of coronary artery disease in men (41.3% vs. 28.6%; p=.437, Fisher's exact test), which itself reflects established sex differences in coronary disease observed in the greater population [148].

Women, however, were more frequently prescribed multiple (3-4) anti-hypertensives than were men, which is an unexpected finding given studies that have shown women to be undertreated compared with men in the context of coronary artery disease [185].

4.3.2 - Sex differences in plaque echolucency

Sex differences in plaque morphology have been reported in work by Sillesen et al. [133], which, through the analysis of 270 CEA specimens, demonstrated that plaques from men had greater quantities of "soft tissue" (primarily lipid core and hemorrhage) than those from women (p=.0006). More specifically, they found that soft tissue constituted 27% and 19% of the volume of plaques from men and women, respectively. Moreover, they found that plaque echogenicity was inversely related to the amount of soft tissue (p=.005) within the plaque [133].

The sex differences in plaque echolucency presented in the current study are further supported by a large population-based study including

3016 men and 3404 women aged 25-84 years, which found echolucent plaque types (1 and 2) to be more frequent among men than women [150]. It is interesting to note that while they found a male predominance in carotid plaque presence that significantly decreased with age, the male predominance in echolucent plaques actually increased with age [150]. Our study was insufficiently powered to assess this trend.

These studies that suggested a greater plaque echolucency in men were performed using subjective methods, by visually classifying plaques into 3 or 4 grades of echolucency [133, 150]. In our study, we used objective, quantitative measures of echolucency and we performed image normalization according to reference structures as previously described [118], further adding to the reproducibility of our data (see *4.1.1 Echolucency differences between symptom groups*). The mean GSM (with lower values indicating greater overall echolucency) was lower in men than women on both ipsilateral and contralateral sides to a similar degree (20.9 vs. 27.7 and 24.3 vs. 31.8, respectively). Although only the contralateral side GSM difference trended toward significance (adj. p=.06), other features of echolucency, bel_30 and bel_50, trended toward significance in favor of greater echolucency in men, with bel_50 significant before adjustment.

While Sillesen et al. quantified lipid core and hemorrhage histologically, the present study quantified plaque lipid core and hemorrhage from men and women using image analysis, specifically with the feature PPCS2 (measure of echolucency) [171], in both ipsilalateral and contralateral carotid arteries. Thus, we observed ipsilateral and contralateral plaques from men to be characterized by greater PPCS2 than those plaques in women (OR 1.12, 95% CI 1.01-1.24, adj. p=.03; and OR 1.09, 95% CI .99-1.20, p=.06, respectively). Imaging studies evaluating sex differences in the quantity of lipid core and hemorrhage had previously only been performed using the MRI [155].

4.3.3 - Sex differences in plaque heterogeneity

To the best of our knowledge, we are the first group to use texture analysis to compare the degree of heterogeneity of carotid plaques from men and women. In our cohort, men had plaques characterized by trends toward greater heterogeneity as evidenced by greater SGLDM IMC-1 and skewness, and also more homogeneous plaques as evidenced by significantly lower values of SGLDM COR than women.

Although no previous studies have linked texture features with sex, Kakkos et al. found that these same texture features distinguished symptomatic plaques from asymptomatic plaques [138]. More specifically, greater values of SGLDM IMC-1 were independently associated with amaurosis fugax plaques, greater values of skewness and lesser values of SGLDM correlation were independently associated with TIA and lesser values of SGLDM correlation were independently associated with stroke plaques [138]. It is interesting to note that while in the work by Kakkos et al., these texture features distinguished symptomatic plaques from asymptomatic plaques, in our study we found similar differences in these features between men, both symptomatic and asymptomatic, and women, only symptomatic, on both ipsilateral and contralateral sides.

4.3.4 - Sex differences in plaque echolucency: importance of symptomatology differences

While in past studies, there were no differences in symptomatology between men and women, the present study had no asymptomatic women. It is interesting, therefore, that this greater plaque echolucency (equivalent to lipid core and hemorrhage [91, 112, 114, 171]) in men was successfully reproduced in the present study, which compared both symptomatic *and* asymptomatic men with symptomatic women only.

The significance of this difference in symptomatology becomes evident through a study involving histological analysis of 450 CEA specimens, which demonstrated that there is an especially pronounced difference

between asymptomatic men and women in the prevalence of atheromatous plaques (plaques consisting of >40% fat) (39% vs. 9%, respectively, p=.02), when compared with the difference in prevalence of atheromatous plaques between all men and women (including symptomatic individuals) (40% vs. 22%, p<.001) [186]. The study also found a greater proportion of plaques with high collagen content in asymptomatic women compared with asymptomatic men (55% vs. 24%, p=.003) and symptomatic men (55% vs. 15%, p<.001). There was no significant difference between symptomatic women and men concerning collagen content [186].

4.3.5 - Accentuated sex differences among symptomatic patients

In additional analyses, to eliminate this difference in symptomatology we excluded the 14 asymptomatic men to compound symptomatic men and women, rendering significant the differences in several features of echolucency and heterogeneity between men and women that had previously only demonstrated trends. Noteworthy differences in echolucency features included GSM, which became significant (p=.02), and bel_50 and PPSC2, which became highly significant (p<.01 and p<.01, respectively). Concerning the three heterogeneity features of interest, SGLDM correlation, SGLDM IMC-1 and skewness, all three were significant (p=.02, p=.03, p=.02, respectively), whereas only SGLDM correlation had previously been significant in univariate analysis when the asymptomatic group was included.

Women and men were similarly distributed among the symptom groups, with a very slightly increased proportion of men with TIA and decreased proportion of men with amaurosis fugax compared to women. These minute differences would only serve to attenuate the observed texture feature differences.

4.3.5 - Sex differences in carotid atherosclerosis: Mechanisms

Although previous studies have established there are clear sex differences in features of carotid plaque instability on histology and on imaging, the mechanisms responsible for these differences remain unknown.

While differences in lifestyle factors exist between the sexes, these do not fully account for the established sex differences in CVD and so indicate an important role for genetics [187]. In a meta-analysis including 7941 patients having suffered strokes, women were more likely than men to have parental history of stroke, and among women alone there was a greater frequency of maternal rather than paternal stroke history, with men showing no association with maternal or paternal history [188]. Moreover, genes involved in lipid metabolism have emerged as potential sources for sex differences in carotid atheroslerosis.

Estrogen has also been a major focus of investigation for the mechanism of sex differences in vascular disease. Studies using animal models have suggested that estrogen may contribute significantly to arterial remodeling, as estrogen has been reported to reduce the proliferation of arterial smooth muscle cells [189] and the production of collagen and elastin [190, 191]. Furthermore, estrogen given to ovariectomized, lipoprotein(a) transgenic mice attenuated atherosclerotic vascular remodeling [192].

Epidemiology data also indicate a beneficial role of estrogen in CVD, with good evidence supporting a hormone therapy-induced reduction in rates of strokes, MIs and all-cause mortality [193, 194]. Moreover, the increased incidence in events reported in two clinical trials [195, 196] was no longer significant after four years, leaving only a reduction in vascular events [197].

Inflammation is another factor that may contribute to sex differences. In a study including 5341 individuals, markers of inflammation including WBC, fibrinogen and CRP were significantly associated with plaque area only in men, while WBC was significantly associated with plaque

echolucency uniquely in women [180]. Further research including genetic analysis and the effect of estrogen and markers of inflammation are required to establish the importance of their roles in sex differences in carotid atherosclerosis.

4.4 - Contribution

Objective 1**:** The current study confirmed that there are significant differences in carotid plaque echolucency between asymptomatic individuals and those having suffered cerebrovascular symptoms, namely amaurosis fugax, TIA or stroke [137, 138]. These particular findings were novel because they were reproduced from asymptomatics and symptomatics in a high-risk cohort. Furthermore, we found novel texture feature differences between symptomatic and asymptomatic plaques and between TIA and amaurosis fugax plaques.

Objective 2**:** To our knowledge, we were the first to identify texture features of ultrasonic plaque images that demonstrated good correlation between the carotid arteries. The strongest correlating features had previously been found to be independently associated with the development of amaurosis fugax, TIAs and strokes [138].

Objective 3: To our knowledge, we were the first to use digital image analysis to detect sex differences in plaque morphology, with features of heterogeneity demonstrating the same trends in men and women as previously observed between asymptomatic plaques and plaques causing amaurosis fugax, TIAs and stroke [138].

4.4.1 - Hypothesis Generation

These findings may encourage the use of quantitative, objective methods of image analysis in evaluating carotid plaque stability, with the particular objective of distinguishing high-risk, unstable plaques from low-risk, stable plaques, and how these high-risk plaques differ between men and women. More specifically, studies could evaluate whether the texture

features that distinguish symptomatic from asymptomatic plaques in men correspond those in women.

Correlation studies could evaluate the evolution of the degree of correlation between the carotids with increasing levels of contralateral stenosis, as plaque heterogeneity has been reported to increase with increasing stenosis [129].

4.4.2 - Clinical Impact

The reliable identification of the high-risk plaque through image analysis could lead to improvements in the management of patients with carotid stenosis, whereby solely individuals with high-risk plaques will undergo any high-risk carotid intervention, with only medical management required for appropriate treatment of low-risk patients. Revised indications for endarterectomy which would include plaque morphology criteria would especially benefit groups at otherwise low risk for cerebrovascular events, such as asymptomatic individuals and women, and might spare those at high perioperative risk. Moreover, morphological criteria necessary for CEA referral may be different for men and women.

In the present study, differences in plaque stability were successfully determined within a CEA cohort between symptomatic and asymptomatic individuals, despite being more homogeneously high-risk than the greater population with carotid stenosis. Consequently, patients with plaques causing *any* degree of carotid stenosis could therefore be monitored for the development of plaque instability before plaque rupture, leading to the more appropriate timing of an intervention, whether surgical or medical. Thus, not only would image analysis provide more accurate monitoring of plaque stability in symptomatic patients to prevent future events (secondary prevention), but it could also identify asymptomatic individuals with unstable plaques before these plaques rupture and cause cerebrovascular symptoms (primary prevention).

Furthermore, the effectiveness of medications targeting plaque stabilization could be monitored through the use of image analysis. Thus, image analysis could evaluate the effectiveness of new pharmacological agents in stabilizing plaques, as well as monitor plaque stability in patients receiving plaque stabilizing agents to ensure an adequate response to therapy. With the development of effective plaque stabilizing medications, patients with mild to moderate stenosis could be treated sooner to prevent early plaque progression and plaque rupture. Image analysis could also be used to evaluate the effect of particular medications on ipsilateral versus the contralateral carotid.

In addition, the close relationship between plaques in the carotid arteries may merit an aggressive bilateral carotid screening program of both carotid arteries and aggressive medical treatment aimed at stabilization of plaques in both carotid arteries. Large prospective studies are required to verify these possibilities.

4.5 - Limitations

Limitations of the current study include its cross-sectional nature. To be validated, the significance of the texture feature differences and correlations requires prospective natural history studies of both men and women not undergoing CEA.

One of the main limitations of the study was the small size of each symptom subgroup, limiting the power to adjust for certain demographic parameters and clinical characteristics. However, vascular risk factors have been reported to bear little influence on plaque stability [145]. The number of women and asymptomatic patients was especially small due to certain studies indicating reduced benefit from CEA in these populations [83, 165]. Moreover, the lack of women within this asymptomatic group prevented sex adjustment in any comparisons involving asymptomatic patients. In addition, as a result of fewer patients, there were few plaque types 1 and 4, reducing the statistical power of plaque type comparisons.

However, it is noteworthy that despite the small sample size, there were many significant findings, and expected trends were observed.

The self-reported nature of patient medical history and medication duration would have been a limitation, however all information was verified using patients' medical charts.

Multiple methods of image documentation may sometimes represent a source of pixel loss, however we used a single method, digital images, and were able to directly transfer images between the ultrasound machine and the workstations where image selection and analysis were performed.

Although the use of cineloops allows for the rapid capture of multiples images, it entails the additional challenge of choosing the ideal image. Thus, there was potential for subjectivity in image selection, however two individuals chose images separately and followed a standard protocol with image selection criteria (see Methods: 'Image Selection'). The process of image normalization could entail subjectivity, however a standard protocol was followed, whereby, after magnifying the image by a factor of four, the inner two fourths of the brightest area of adventitia nearest to the plaque was selected. Plaque outlining is operator dependent but the present study was supported by very good intraoperator and interoperator reproducibility.

During plaque outlining, acoustic shadow (where no pixels are visible due to heavy calcification) was not included, however a protocol was followed in ordered to be consistent.

Recruited symptomatic patients underwent ultrasound examination at varying times following their events, according to the vascular surgery schedule at the RVH and JGH. As plaque remodeling occurs following different events, varying durations between events and ultrasound examinations may possibly result in variable plaque morphology. With ongoing recruitment, a larger population will enable the stratification of patients according to duration since event.

We did not take measures to ensure a cerebrovascular event was not caused by an aortic arch/atrial appendage embolus. However, by nature of the subjects being referred for CEA, they are closely followed by vascular surgeons who themselves need to confirm the carotid as the embolus source.

4.6 - Future work

Ongoing recruitment will increase statistical power and will enable adjustment for multiple co-factors, notably sex.

Future work will also include digital image analysis of plaques from common femoral artery of patients included in this study with the purpose of establishing correlations between carotid and femoral ultrasonic plaque texture. Previous studies have shown that echolucent carotid plaques were more frequently observed among patients with echolucent femoral plaques rather than those with echogenic femoral plaques [181].

Moreover, we will also use texture analysis of ultrasonic plaque images to monitor plaque remodeling following different cerebrovascular events. Thus far, large histological studies have demonstrated that plaques remodel differently depending on whether they have caused a TIA or a stroke, indicating potential differences in the underlying pathology [167, 172]. In addition, we will use texture analysis to assess whether plaque remodeling exhibits sex differences and how remodeling alters carotid plaque texture correlation with time.

5 - Conclusion

In conclusion, specific texture features describing echolucency and heterogeneity differ between ultrasonic images of carotid plaques in men and women and in those with different cerebrovascular symptomatology. Furthermore, texture features previously associated with specific cerebrovascular events demonstrate good correlation between the carotid arteries. These findings encourage the use of digital image analysis to

objectively evaluate ultrasonic carotid plaque texture between different populations. Large prospective studies are required to establish the prognostic role of texture analysis.

6 - Appendix

Figure 1 – Main questionnaire

PATIENT COORDINATES

This first section refers to some personal information for administrative use only. This information will remain confidential.
(If you do not have this information write "Do not know")

Name (Last, first): _____
Provincial Medical Insurance card number:_____ Expiry date:_____
❒ Man ❒ Woman
Birth date: ___/____/___
Day/Month/Year
Address
Number and street and apartment: _____
City:_____
Postal Code: _____
Telephone
Home: (__) _____
Office: (__) _____
Cellular Phone: (__) _____
E-mail: _____
Alternate contact person:

Name (Last, first): _____
Address
Number and street and apartment: _____
City:_____
Province: _____
Postal Code: _____
Telephone
Home: (__) _____
Office: (__) _____
Cellular Phone: (__) _____
Your family physician:

Name (Last, first): _____
Address
Number and street: _____
City:_____
Province: _____
Postal Code: _____
Telephone
Office: (__) _____

ETHNIC BACKGROUND

1. Were you born in Canada?
   ❒ Yes ❒ No, specify country of birth: _____
2. If no, when did you move to Canada? Year:_____
3. People living in Canada come from many different cultural and geoethnic backgrounds. Are you?
   - ❒ South Asian (e.g. East Indian, Pakistani, Sri Lankan, etc.)
   - ❒ Southeast Asian (e.g. Cambodian, Indonesian, Laotian, Vietnamese, etc.)
   - ❒ European (Anglo-Saxon, French, Italian, Greek, Spanish, German, Slavic, Scandinavian, etc)
   - ❒ Chinese
   - ❒ Filipino
   - ❒ Latin/Hispanic American
   - ❒ African (African /African American)
   - ❒ Arab
   - ❒ Japanese
   - ❒ Korean
   - ❒ Indigenous / Aboriginal / Native American
   - ❒ Middle Eastern / North African (Afghan, Algerian, Moroccan, Egyptian, Lebanese, Iranian, Iraqi, Israeli, Palestinian, Syrian, Tunisian, Turkish etc.)
   - ❒ Caucasian

   Other (specify): _____
4. What is the cultural and geoethnic background of your mother? _____
5. What is the cultural and geoethnic background of your father?
6. What is your mother tongue? _____

HEALTH IN GENERAL

7. How would you rate your health in general, compared to people your age?
   ❒ Excellent ❒ Very Good ❒ Good ❒ Poor ❒ Very Poor

MEDICATION USE

8. Do you take aspirin? ❒ Yes ❒ No
   If yes, when did you start? ____/____
   Month/Year
9. Check the aspirin that you are taking:
   - ❒ Adult (325 mg)
   - ❒ Baby (80 mg)
   - ❒ Other (specify): _____
10. How often do you take the aspirin?
    - ❒ Once per day
    - ❒ Number of times per week: _____
    - ❒ Other (specify): _____

11. List all **prescribed** medications that you are currently taking (including aspirin, contraceptives and hormonal replacement therapy)
    ❒ I am not taking any prescribed medications

Name of medication	Dose	Route*	Number of times per day	Start Date (month/year)

*by mouth, by injection, patch, syrup, pills, suppository, etc.

12. What other **non-prescription** medications are you currently taking?
(Check all that apply)

❒ I am not taking any non prescribed medications including nutritional supplements,
vitamins or herbal remedies

❒ Tylenol
❒ Allergy medication
❒ Cough syrup
❒ Multiple vitamins
❒ Vitamin A
❒ Vitamin B
❒ Vitamin C
❒ Vitamin E
❒ Folic acide
❒ Calcium
❒ Omega-3
❒ Ginkgo biloba
❒ Garlic
❒ Selenium
❒ Ginseng
❒ Chamomile
❒ Other(s) (specify): _____
_____

FAMILY MEDICAL HISTORY

13. Are your parents alive?
Mother: ❒Yes Age: _____ ❒ No Age at time of death: _____
Father: ❒Yes Age: _____ ❒ No Age at time of death: _____

14. If they have passed away, indicate the cause of death:
Mother: _____
Father: _____

15. How many brothers and/or sisters do you have that are alive?
Brother 1 Age: _____ Sister 1 Age:_____
Brother 2 Age: _____ Sister 2 Age:_____
Brother 3 Age: _____ Sister 3 Age:_____

16. If you a have sibling (s) who have passed away, indicate:

Cause of death of brother(s)	Age at time of death
1.	
2.	
3.	
Cause of death of sister(s)	Age at time of death
1.	
2.	
3.	

17. For each of your natural parent(s) and sibling(s), check the appropriate box with respect to each of the following disorders that they have or have had in the past:

	Brothers		Sisters		Mother	Father
	Yes	Number of brothers	Yes	Number of sisters	Yes	Yes
Heart attack/Angina						
Heart attack/Angina **before** 55 years old for women in the family or 45 for men in the family						
High blood pressure						
Stroke						
High cholesterol						
Diabetes						
Circulation problems in legs						

YOUR PAST MEDICAL HISTORY

18. In the past, have you had any of these **diseases or procedures**? (Check all that apply)

	Yes	No	Year		Yes	No	Year
Heart attack or myocardial infarction				Irregular heart rhythm			
Angina or chest pain from heart disease				Heart defects from childhood			
Congestive heart failure				Blocked arteries / neck or brain			
	Yes	No	Year		Yes	No	Year
Heart valve problems				Blocked arteries in legs			
Rheumatic fever				Thrombophlebitis / clot in leg veins			

Stroke				Aortic aneurysm			
Clot in lungs							
Coronary angioplasty or stent placement				Coronary artery bypass surgery			
Valve surgery (which valve)?				Artificial pacemaker or defibrillator			
Surgery of your carotid arteries If yes, which side? ___				Surgery of arteries of the legs, arms, or your aorta			
Angioplasty for carotid arteries If yes, which side? ___				Angioplasty of arteries of the legs, arms, or your aorta			

19. In the past, have you had any of the following **<u>medical conditions</u>**? (Check all that apply)

	Yes	No	Year		Yes	No	Year
Neurological disease (muscle or nerves)				Lung disease (emphysema, bronchitis or asthma)			
Thyroid disease				Migraine headache(s)			
Peptic ulcer/stomach problems				Bowel disease (colitis, diverticulitis or irritable colon)			
Kidney disease				Arthritis (joint pain)			
Cancer, (specify):				Osteoporosis (fragile bones)			
Digestive system (cirrhosis, hepatitis, pancreatitis Gallbladder)				Depression, anxiety or other emotional problems			
Blood disorders/ anemia				Alcoholism			

Others (describe): _____
_____

HYPERTENSION

20. Has a health professional ever told you that you have had high blood pressure or hypertension? ❒ Yes ❒ No, **if no, go to question # 25**
21. When did he/she first tell you? _____/____
Month / Year
22. Have you ever been treated for high blood pressure with medication?
❒ Yes ❒ No, **if no go to question # 25**
23. When did you start taking medication for high blood pressure? _____/____
Month / Year
24. Are you still taking medication for high blood pressure?
❒ Yes ❒ No, when did you stop? _____/_____
Month / Year

HIGH CHOLESTEROL

25. Has a health professional ever told you that you have had high cholesterol?
    ❒ Yes ❒ No, **if no go to question # 30**
26. When did he/she first tell you? _____/____
    Month / Year
27. Have you ever been treated for high cholesterol with medication?
    ❒ Yes ❒ No**, if no go to question # 30**
28. When did you start taking medication for high cholesterol? _____/____
    Month / Year
29. Are you still taking medication for high cholesterol?
    ❒ Yes ❒ No, when did you stop? _____/_____
    Month / Year

DIABETES

30. Has a health professional ever told you that you have diabetes?
    ❒ Yes ❒ No, **if no go to question # 36**
31. When did he/she first tell you? _____/____
    Month / Year
32. Have you ever been treated for diabetes with medication?
    ❒ Yes ❒ No, **if no go to question # 36**
33. What kind of medication did you take to treat diabetes?
    ❒ Pills ❒ Insulin injections ❒ Both
34. When did you start taking this medication for diabetes?
    ❒ Pills _____/_____ ❒ Insulin injections _____/_____
    Month / Year Month / Year
35. Are you still taking medication for diabetes?
    ❒ Yes, what kind: ❒ Pills ❒ Insulin injections ❒ Both
    ❒ No, when did you stop?
    ❒ Pills _____/_____ ❒ Insulin injections _____/_____
    Month / Year Month / Year

TOBACCO CONSUMPTION

36. Have you ever smoked?
    ❒ No**, if no go to question # 45**
    ❒ Yes, when did you start? _____/_____ ***OR*** at what age did you start? _____ years
    Month / Year
37. Do you smoke now?
    ❒ Yes ❒ No, when did you stop? _____/_____
    Month / Year
38. What types (s) of tobacco do you, or have you, use (d) the most?
    ❒ Cigarettes ❒ Pipe
    ❒ Cigars ❒ Other (specify): _____
39. If you smoke now, even if you have stopped smoking at several occasions in your life,
    a) how many years did you smoke all together? ___ year(s)
    b) on average, how many cigarettes do you smoke per day? ____
40. If you are currently smoking, where do you smoke the most?

❒ Home ❒ Social outing
❒ Work ❒ Other (specify): _____

41. How long after you wake up in the morning do you smoke your first cigarette?_
42. If you are currently smoking, have you ever tried to quit smoking? ❒ Yes ❒ No
If yes, a) how many times have tried to quit smoking? _____
b) how are you trying to quit?
❒ Cessation counseling clinic ❒ Acupuncture
❒ By yourself, no external help ❒ Hypnosis
❒ Patches ❒ Gum
❒ Other (describe): _____
43. If you have stopped smoking throughout your life,
a) how many years did you smoke all together? ___ year(s)
b) how many cigarettes have you smoked, on average, per day? _____
44. If you have stopped smoking, how did you manage to quit?
❒ Cessation counseling clinic ❒ Acupuncture
❒ By yourself, no external help ❒ Hypnosis
❒ Patches ❒ Gum
❒ Other (describe): _____
45. Are you exposed to second-hand smoke (to be in the presence of a smoker on a regular basis)? ❒ Yes ❒ No
If yes, where are you exposed to second-hand smoke and how often? (Check all that apply)
❒ Home _____ hours per day
❒ Work _____ hours per day
❒ Social ___ hours per week

RECREATIONAL DRUG CONSUMPTION

46. Have you ever taken illicit drugs? ❒ Yes ❒ No, **if no go to question # 50**
If yes, what type (s) of drugs? Describe_____
47. Do you take these illicit drugs now? ❒ Yes ❒ No
If yes, what type (s) of drugs? Describe _____

48. Have you ever taken cocaine? ❒ Yes ❒ No
If yes, when was the first time? _____/____
Month / Year
when was the last time? _____/____
Month / Year
49. During this period, how frequently did you use it?
❒ Daily ❒ Weekly ❒ Monthly ❒ Less than monthly
50. Have you ever taken Viagra? ❒ Yes ❒ No
If yes, when was the first time? _____/____
Month / Year
when was the last time? _____/____
Month / Year
51. During this period, how frequently did you use it?
❒ Daily ❒ Weekly ❒ Monthly ❒ Less than monthly

FOOD CONSUMPTION/DIETARY HABITS

52. Have you ever been on a special diet? ❒ Yes ❒ No
53. Are you following any special diet now? ❒ Yes ❒ No
    If yes, what type? _____
54. Did a doctor recommend that you change your diet? ❒ Yes ❒ No
55. Do you drink coffee regularly?
    ❒ Yes, how many cups per day? _____ cups / day ❒ No
56. Check usual coffee type:
    ❒ Caffeinated ❒ Decaffeinated ❒ Both
57. Do you drink caffeinated tea regularly?
    ❒ Yes, how many cups per day? _____ cups / day ❒ No
58. Not counting juice, how often do you eat fruits?
    Per week: _____ Never ❒
59. How often do you eat green salad and vegetables?
    Per week: _____ Never ❒
60. How often do you usually eat red meat?
    Per week: _____ Never ❒
61. How often do you usually eat fried food and/or high fat-content food?
    Per week: _____ Never ❒
62. How many times a week do you eat a "home cooked meal" (not including restaurants or frozen dinners)? _____
63. How many times a week do you eat frozen dinners, at restaurants, or fast food?_____
64. How often do you drink soft drinks?
    Per week: _____ Never ❒
65. Do you drink alcoholic beverages? ❒ Yes ❒ No **if no go to question # 67**
    If yes, how often? _____
    If yes, how many glasses of each per week:
    ❒ Wine, sherry, port (1 glass = 4 oz.) _____ Glasses/week
    ❒ Beer, ale, etc. (1 bottle = 12 oz.) _____ Bottles/week
    ❒ Spirits or hard liquor (1 drink = 1½ oz.) _____ Drinks/week
66. How many years have you been drinking these amounts?
    _____ years ❒ less than 1 year
67. Have you ever been a heavy drinker in the past?
    ❒ Yes, for how many years? _____ ❒ No

PHYSICAL ACTIVITY HABITS

68. How physically demanding is your daily job (including caregivers)?
    ❒ Not at all ❒ Mild ❒ Moderately ❒ Very (get out of breath)
69. How physically demanding are your usual daily activities (e.g. house work, getting to work)?
    ❒ Not at all ❒ Mild ❒ Moderately ❒ Very (get out of breath)
70. On average, how many floors do you climb up per day? (One floor equals ten steps)
    _____ number of floors/day
71. On average, how many city blocks do you walk per day?

_____ city blocks or _____km (s)

72. Do you engage in any regular exercise (e.g. brisk walking, jogging, bicycling, work out at the gym) long enough to work up a sweat?
❒ Yes ❒ No
If yes, how many times per week? _____ for how many minutes? _____

73. How long have you been doing this exercise? _____ months _____ years

74. In the past, have you been doing regular exercise? (e.g. brisk walking, jogging, bicycling, work out at the gym) (Check all that apply)
❒ Never
❒ On and off for _____ years
❒ Continually for _____ years

75. During which season(s) do you exercise? (Check all seasons that apply)
❒ Winter ❒ Spring ❒ Summer ❒ Fall

PERSONAL ASSESSMENT

76. In general, how do you feel about yourself?

I feel:	Strongly agree	Agree	Neither agree nor disagree	Disagree
That I have a number of good qualities				

I feel:	Strongly agree	Agree	Neither agree nor disagree	Disagree
That I am a person of worth, at least equal to others				
That I am able to do things as well as other people				
That I have a positive attitude toward myself				
All in all, I am satisfied with myself				
All in all, I am inclined to think of myself as a failure				

77. How often , during the past week, did you:	Never	Once in a While	Fairly Often	Very Often
Feel hopeless about the future?				
Feel lonely?				
Have your mind go blank?				
Feel discouraged or “down”?				
Feel tense or under pressure?				
Lose your temper?				
Feel bored or have little interest in things?				
Feel fearful or afraid?				
Have trouble remembering things?				
Cry easily or feel like crying?				

Feel nervous or shaky inside?				
Feel critical of others?				
Feel easily annoyed or irritated?				
Get angry over things that are not too important?				

78. On a scale of 1-10 with 10 being severe stress, how do you rate your level of stress?

Level of stress	Does not apply	No Stress 1	2	3	4	5	6	7	8	9	Severe Stress 10
At work	❒										
At home											
Overall											

79. How confident do you feel in managing your stress?
❒ Not confident ❒ A little confident
❒ Moderately confident ❒ Very confident

EDUCATION

80. What is your level of education? _____

OCCUPATION

81. What is your current occupation?_____

MARITAL STATUS

82. What is your marital status? _____

REPRODUCTIVE HISTORY AND MENOPAUSE
For women only
For men, the questionnaire is complete, Thank you

83. At what age did you begin having your period? Age: ____
84. What was the first date of your last menstrual period?___/____/___
Day/Month/Year
85. Do you have regular periods? _____ ❒ Yes ❒ No ❒ Not always
86. If you have regular periods, how long is your cycle? Number of days __
87. If you do not have regular periods, what is the minimum and maximum number of days of your periods in the past year? Minimum number of days _
Maximum number of days _____
88. Are you pregnant right now? _____ ❒ Yes ❒ No ❒ Do not know
89. How many times have you been pregnant (including abortions and miscarriages)? _____
90. How many deliveries have you had in total? ___
a) Of your total deliveries, how many still births have you had? ___
b) Of your total deliveries, how many premature babies have you had? ___

During one or more of your pregnancies	Yes	No	Unsure
91. Did you have high blood pressure?			
92. Did you have pre-eclampsia/eclampsia?			
If yes, a) at how many weeks of your pregnancy? _____			
b) were there proteins in your urine?			

93. Did you develop diabetes or pre-diabetes during your pregnancies?			
94. Did you have thrombosis (clots) during or after your pregnancies (including abortions or miscarriages)?			

BIRTH CONTROL USE

	Yes	No	Unsure
95. Have you ever taken birth control pills?			
96. Are you currently using birth control pills?			

97. If you are using another form of birth control other than the pill, (describe):_____

MENOPAUSE

98. Are you menopausal?
- ❒ Yes, when was your last period? ____/___ Month/Year
- ❒ No **(the questionnaire is complete, thank you)**
- ❒ Unsure

99. If you are menopausal, what kind of menopause?
- ❒ Natural menopause
- ❒ Hysterectomy:
  - ❒ Uterus only
  - ❒ Ovaries only
  - ❒ Uterus, one ovary
  - ❒ Uterus, both ovaries
  - ❒ Unsure

HORMONE REPLACEMENT THERAPY

100. Are you currently taking hormone replacement therapy?
- ❒ Yes
  - If yes, a) what kind? ❒ Estrogen ❒ Progesterone ❒ Combination ❒ Unsure
  - b) what form? ❒ Orally ❒ Vaginal cream ❒ Patch ❒ Other (specify): _____
  - c) when did you start? ____/___ Month/Year
- ❒ No
  - If no, have you ever taken hormone replacement therapy?
    - ❒ No **(if no, the questionnaire is complete, thank you)**
    - ❒ Yes, if yes, when did you start:____/___ when did you stop:____/___ Month/Year

101. What is the longest length of time you have used hormone replacement therapy?
___ years ❒ less than 1 year

THANK YOU!

Figure 2 – Cerebrovascular symptom questionnaire

Symptom Checklist

Patient ID: _____ Date:_____ **Site of Plaque**: ☐ Left ☐ Right

Amaurosis Fugax

□ Vision Problems (loss to one eye) – may last for seconds until minutes, and appears as a black/gray shade closing

Frequency:_____ Date of First Event:_____ Date of Latest Event:_____

Stroke/TIA (Transient Ischemic Attack)

Symptom	Side	Duration	Frequency	Severity (1-10)	Date of First Event	Date of Latest Event
□ Numbness						
□ Lip/Face Drooping						
□ Weakness						
□ Blindness/Double Vision						
□ Difficulty Speaking						
□ Confusion						
□ Difficulty Swallowing						
□ Difficulty Walking						
□ Loss of Balance/Coordination						
□ Headaches						
□ Changes in Hearing/Taste						
□ Loss of Memory						
□ Difficulty Reading and Writing						
□ Mood Changes						
□ Clumsiness						
□ Changes in Alertness						
□ Problems with Bowels						
□ Dizziness (vertigo)						
□ Response to Pain Altered						
□ Nausea						

Other:

Conclusion: □ **Symptomatic** □ **Asymptomatic**

Figure 3 – Consent Form

Identification of the Unstable Atherosclerotic Plaque: From Bench to Clinical Practice

RESEARCHERS

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SPONSOR

The Research Institute of McGill University Health Centre (RI MUHC) is sponsoring this study. The research will be conducted at the Royal Victoria Hospital (RVH) and the Jewish General Hospital (JGH).

RELEVANT TERMS

Atherosclerosis: is the condition in which an artery wall thickens as the result of a build-up of fatty materials.

Carotid Artery: an artery that is located in the neck and supplies the brain with blood.

Carotid Endarterectomy: is a surgical procedure used to prevent stroke, by clearing the carotid artery of the build-up of fatty material that reduces blood flow.
Cardiovascular: what is related to the heart and blood vessels of the body.

INTRODUCTION

You are being asked to participate in a research study designed to better evaluate the severity of atherosclerosis in individuals with and without stroke symptoms, referred for carotid endarterectomy (CEA).

Before you decide to participate, it is important to carefully read through and understand the content of this consent form. Make sure all of your questions are answered and take your time making a decision. If you decide to participate in this study, you will be asked to sign this consent form, and your treating physician will be notified regarding your involvement in the study.

BACKGROUND

Atherosclerosis of the carotid arteries is a disease caused by the build-up of fat (plaque) in these arteries. This plaque may harden and narrow the arteries, and further restrict blood flow. It may also worsen to become more dangerous, leading to a stroke. This disease is a leading cause of death in Canada and worldwide. For this reason, it is important to study the appearance of carotid plaques. It is essential to find out when the plaques become unstable, ready to break off and cause strokes.

PURPOSE OF THE STUDY

In this research study, we will compare the appearance of the plaques on ultrasound and under the microscope after your surgery. We aim to find out how dangerous plaques on microscope appear on ultrasound. This will help us recognize dangerous plaques before they break off and cause strokes. The results of this research may lead to a better understanding and treatment of this condition and other cardiovascular diseases. In the initial phase of this research a total of 150 subjects referred for CEAs will take part in this study at the Royal Victoria Hospital (RVH) and the Jewish General Hospital (JGH).

STUDY PROCEDURES

If you agree to take part in this research study, you will be asked to undergo the following procedures:

<u>At the time of recruitment</u>

- You will be asked to fill out a questionnaire that will take approximately 20 minutes to complete. The questionnaire includes questions about your past medical history, medications, health status, and family history. You will also be asked if you have had any stroke symptoms in the past. In addition, your medical file will be consulted by the study staff for any information relevant to the study.

<u>At your clinical visit before surgery</u>

- You will undergo a physical examination including measurements of your blood pressure, heart rate, and pulse. We will measure your weight and height, as well as your waist and hip circumference.
- An ultrasound of the arteries in your neck and legs will be done, and the images will be saved. We will then analyze the images using a computer program to find out what your plaques look like. Ultrasound is a widely used, safe and non-invasive procedure that will take approximately 60 minutes.
- The function of your cardiovascular system and hardening of your arteries will be measured using applanation tonometry while you are resting using a simple and safe technique. This will take approximately 60 minutes.
- You will not be asked to discontinue any medication you take during the duration of the study.

<u>On the day of surgery</u>

- A blood sample (approximately 30 mL) will be taken. This will be taken along with the standard blood collection on the day of surgery.
- Plaques removed on the day of surgery will be stored for later analysis.

<u>At your regular appointment approximately 6 weeks after the surgery</u>

- The function of your cardiovascular system and hardening of your arteries will be measured again using applanation tonometry, while you are resting.

<u>At 8, 16 and 24 months after your surgery</u>

- An ultrasound of the arteries in your neck and legs will be repeated, and the images will be saved. We will then analyze the images using a computer program to find out if the plaques have become worse.
- The function of your cardiovascular system and hardening of your arteries will also be measured again using applanation tonometry to find out if there are any changes.
- A blood sample (approximately 30 mL) will also be collected at this time, as well as a questionnaire to see if there any relevant changes.

USE OF BLOOD SAMPLES

It is possible that tests will be developed in the future which may help people who are at risk for atherosclerosis and cardiovascular disease. However, it is not possible to predict which additional tests may become of value in the near future. Therefore, in addition to seeking your participation in this study, we are seeking your consent to collect and store a blood samples (30 mL) for future analyses that might be related to atherosclerosis and cardiovascular disease. The samples will be frozen and stored in Dr. Jacques Genest's laboratory at the RVH for 5 years and then destroyed if not used.

The use of your blood samples or medical information is not intended to provide you or your physician with test results. The study sponsor will not make any results available to you, any insurance company, your employer, your family, the study doctor, or any other physician who treats you now or in the future. Access to your samples will be restricted to the research team only. No other testing will be done on the samples.

USE OF RESULTS FROM OTHER TESTS

The images from the ultrasound tests of your neck and leg arteries will be stored and analyzed in the main study. These results may be used for additional related studies in the future.

Plaques obtained will be processed through the Surgical Pathology Department at the McGill University Health Center, and used to be analyzed under the microscope. These specimens will be stored at the Montreal General Hospital for 5 years and then destroyed if not used.

Access to your results from all of the above mentioned tests will be restricted to the research team. No other testing will be done.

POTENTIAL HARMS RISKS AND DISCOMFORTS

Associated with the study

- There are no known risks associated with your participation in this study.

Associated with the intervention

- Ultrasound - Ultrasound is a very safe imaging technique as it does not involve radiation. It is the same as used in pregnant women to assess baby growth.
- Blood tests - The taking of blood samples (as done by standard procedures) may cause some discomfort, fainting, formation of a small blood clot or swelling of the vein on surrounding tissue, bleeding from the puncture site, and/or rarely an infection. There is a possibility that you may faint, however, precautions will be taken to ensure your safety should this occur.
- Blood pressure and measurement of the hardening of the arteries - There are no risks and discomfort associated with measuring blood pressure and the hardening of the arteries.

POTENTIAL BENEFITS

You should not expect any direct benefit from participating in this study. However, the information from the study will help further our knowledge of this condition, and potentially help improve future patient care.

ALTERNATIVE TREATMENT

You do not need to take part in this study to receive treatment. You should discuss your options with the study doctor.

COMPENSATION

McGill University and the investigators would not be able to offer compensation in the unlikely event of any injury resulting from your participation in this research study, however, appropriate medical care will be provided through Quebec Medicare or private insurance programs. However, you are not giving up any of your legal rights by signing this consent form and agreeing to participate in this study.

CONFIDENTIALITY

The study staff (team of researchers) will consult your medical files to take notes of the relevant data to this research project relating to previous medications, lab results and hospitalizations.

All information will be kept strictly confidential by identifying you by a code to which only authorized personnel will have access. The data will be maintained until they are analyzed and then destroyed. The results from this study may be published, and other physicians participating in this research study may have access to your records related to this research study. Your identity will not be revealed in the combined results. Only the principal investigator and the clinical research team will have access to the coding list with your name on it.

In order to verify the research study data, monitors from the McGill University Institutional Review Board may review these records.

By participating in this study, you are agreeing to the sharing and releasing of information collected about you in this study with the individuals and entities indicated above. The study investigator will also inform your treating physician of your participation in this study. Your confidentiality will be protected to the extent permitted by applicable laws and regulations.

PUBLICATION

Your identity will not be released in any publications resulting from this study. Only the global results of the study will be published and your identity will not be revealed in the combined results.

COMMERCIAL ISSUES

The results of this research may have commercial or intellectual property value. Any products or processes that are developed as a result of this research will belong to the investigator. There are no plans to compensate you for any products developed from this research.

COST AND COMPENSATION FOR PARTICIPATION

You will be paid for expenses, i.e., parking and transportation costs, if you are asked to come for an additional visit, other than previously scheduled medical visits, up to a maximum of $17 per visit.

VOLUNTARY PARTICIPATION/PARTICIPANT RIGHTS

Your participation in this study is strictly voluntary, your decision will not affect your treatment in any way, nor will it in any way prevent you from receiving the routine care from the hospital where you were admitted. You may refuse to participate or you may discontinue your participation, and withdraw your consent at any time without explanation, and without penalty or loss of benefits to which you are otherwise entitled. If you decide not to participate or if you discontinue your participation you will suffer no prejudice regarding medical care or your participation in any other research studies. You do not give up any legal rights by signing this form. If you have any questions, now or later, we will be happy to answer them.

CONFLICT OF INTEREST

There are no known conflicts of interest that may influence any of the researchers involved in this study.

CONTACT INFORMATION AND/OR QUESTIONS

If you have any questions regarding the study, you should contact the investigator:

Dr. Stella Daskalopoulou at 514-934-1934, ext 42478.

If you have any questions regarding your rights as a research subject in the study, enquires are appropriately directed to the Ombudsman for the MUHC or the JGH (RVH: 514-934-1934, ext 35655, MGH: 514-934-1934, ext 48306, and JGH: 514-340-8222, local 5833).

DECLARATION OF CONSENT FOR BLOOD COLLECTION AND FOLLOW UP

Collected blood will only be used for the purposes of this study. After analyses, blood samples will be destroyed. All unused samples will be destroyed after 5 years.

I agree:□ I disagree:□ Participant's signature: _____

I will return at 8, 16, and 24 months after my surgery to continue participating in the study.

I agree:□ I disagree:□ Participant's signature: _____

DECLARATION OF CONSENT

I have read the contents of this consent form, and I agree to participate in this research study. I have had the opportunity to ask questions and all my questions have been answered to my satisfaction. I have been given sufficient time to consider the above information and to seek advice if I choose to do so. I will be given a copy of this signed consent form. By signing the consent form, I have not given up any of my legal rights.

Participant's Signature _____ Date _____

Printed Name _____

I have explained the research to the participant and, to the best of my knowledge; the participant has understood the proposed research and freely consented to research participation.

Investigator's (or Research Team Member) Signature _____ Date _____

Printed Name _____

ETHICS APPROVAL

The research project was approved by the research ethics committee of McGill University on January 6, 2010.

Figure 4 – Evaluation of carotid stenosis using velocity measurements captured with Duplex ultrasound. [159]

TABLE 1

Duplex Velocity Criteria Selected for Highest Accuracy*

Angiographic Diameter Stenosis		Size of Plaque a–h (See Fig. 2)	Duplex Velocity Criteria				
N%	E%		PSV_{IC}[23,24]	EDV_{IC}[4,23,24]	PSV_{IC}/PSV_{CC}[21,22,26]	PSV_{IC}/EDV_{CC}[27,28]	EDV_{IC}/EDV_{CC}**
11	50	a	<120	<40	<1.5	<7	<2.6
	60						
47	70	I b	120–150	40–80	1.5–2	7–10	
60	77	II c	150–250	80–130	2–3.2		
65	80	d			3.2–4	10–15	2.6–5.5
70	83	III e		>130		15–25	
82	90	f	>250		>4	>25	>5.5
90	94	g					
99	99	h	Trickle Flow				

* Minimum false positive and false negative tests.

** Baker JD. Standardized imaging and Doppler criteria for cerebrovascular diagnosis using Duplex sonography. Presented at AIUM, Las Vegas, NV, 1986.

Figure 5 – Longitudinal black and white ultrasonic image of an echolucent, heterogeneous plaque

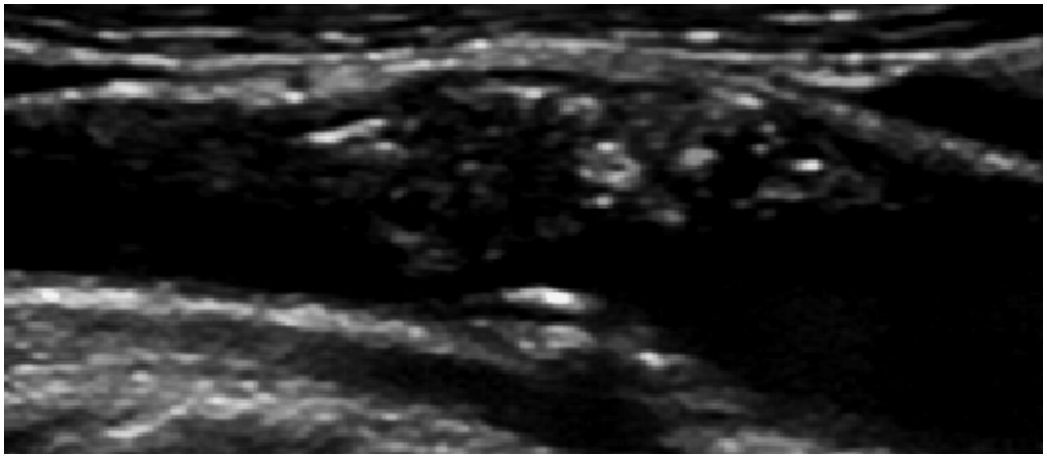

Figure 6 – Longitudinal color ultrasonic image of the above echolucent, heterogeneous plaque

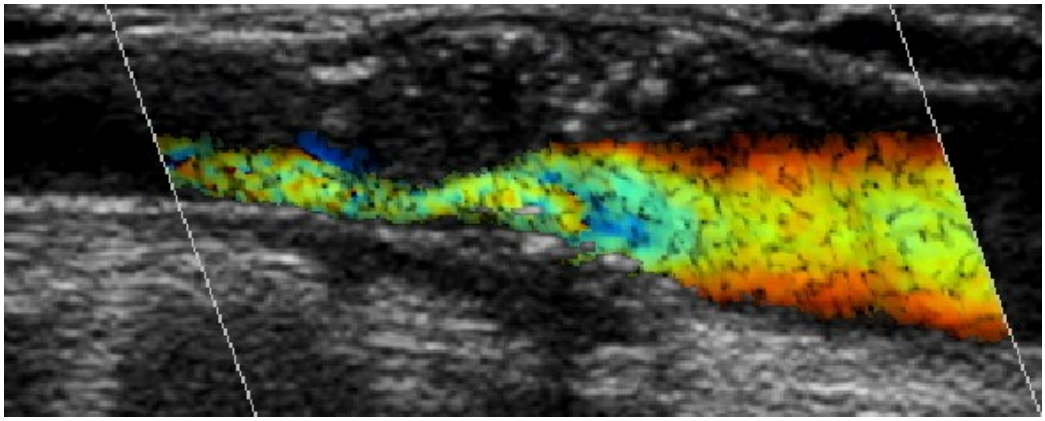

Figure 7 – Image Normalization [198]

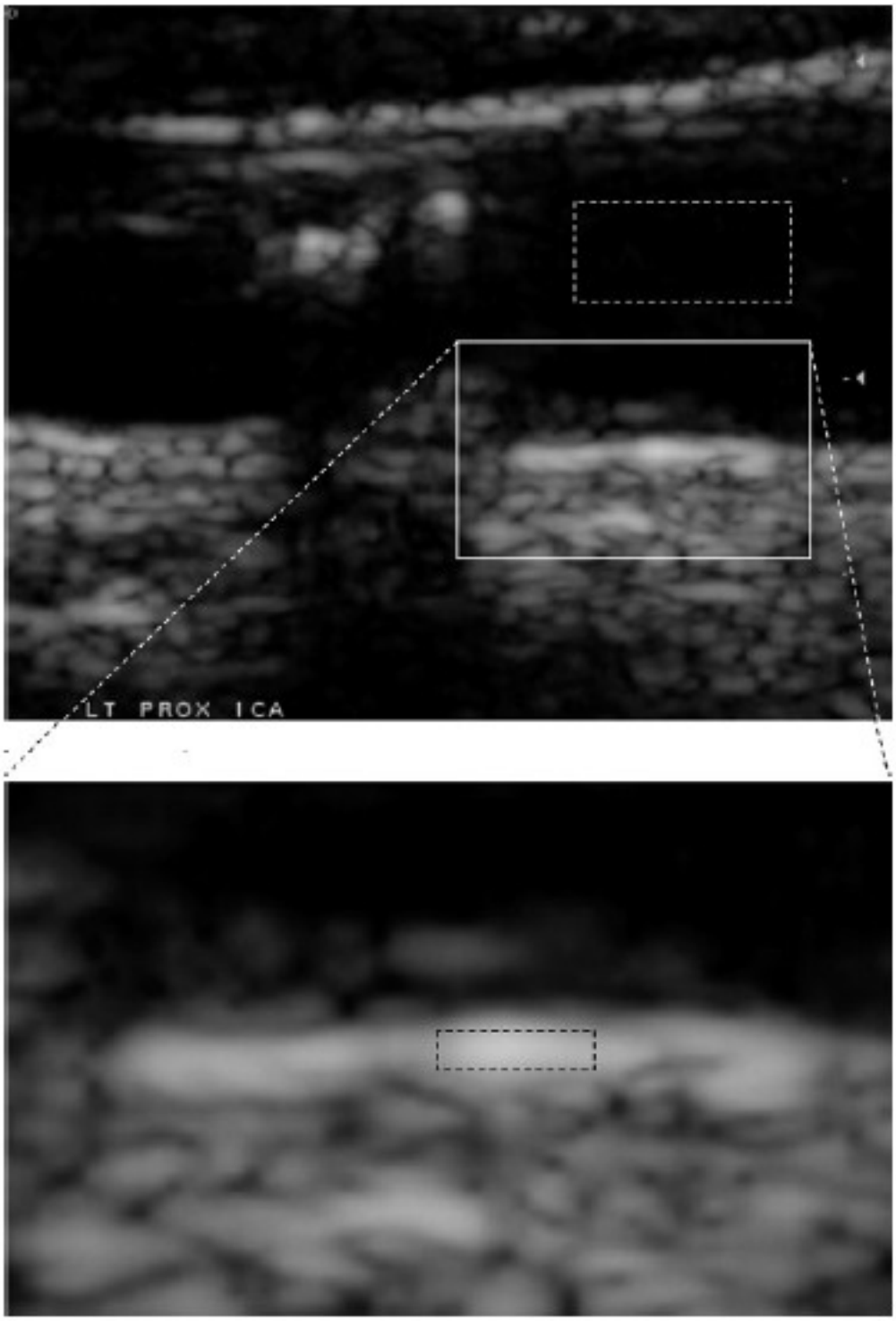

Figure 8 – Examples of plaque outlines in grayscale and color contoured A) Plaque type 1, GSM 2. B) Plaque type 2, GSM 18. C) Plaque type 3, GSM 28 D) Plaque type 4, GSM 98**.** [198]

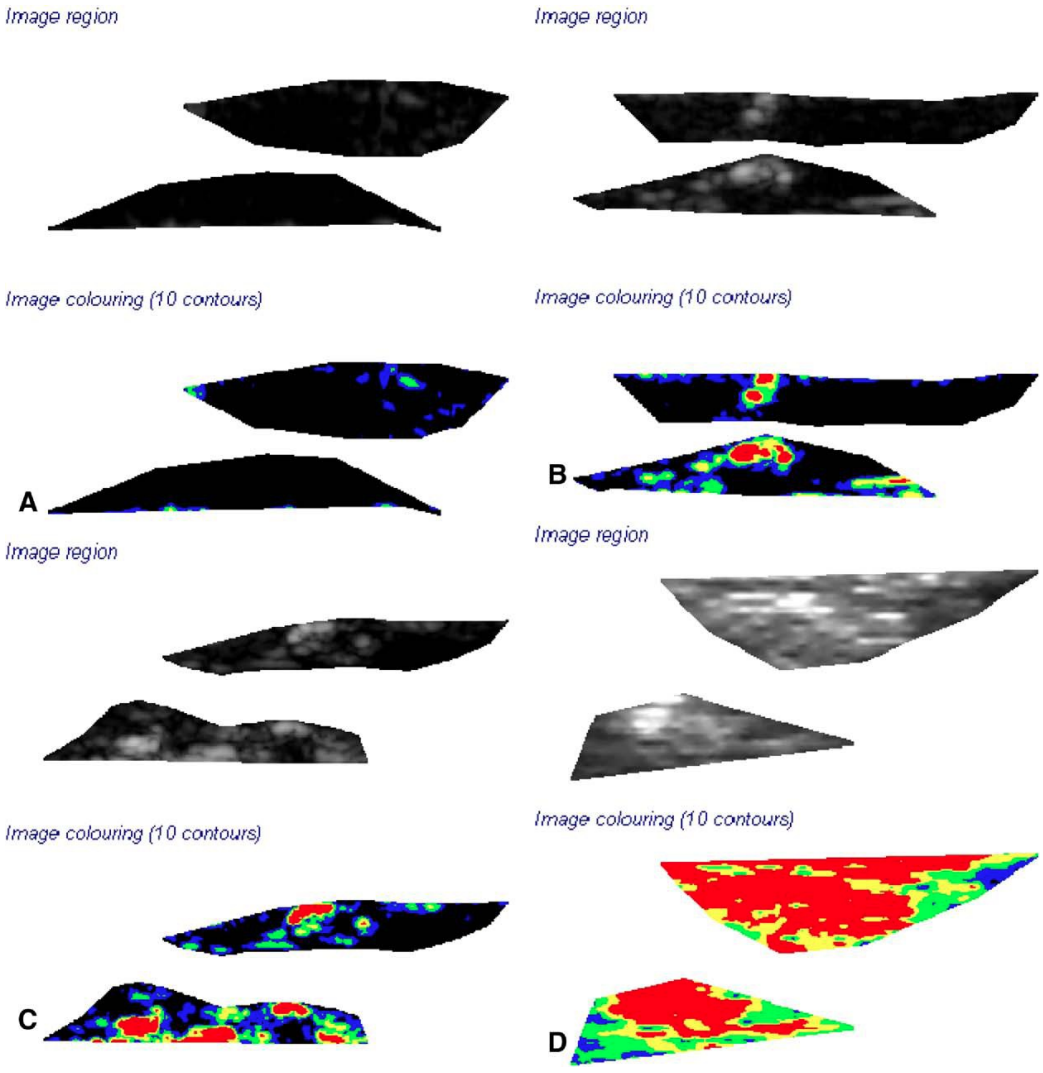

Figure 9 – List of variables produced by digital image analysis

(i) Histogram measures
1) Total number of pixels, 2) percentage of pixels below gray level 30 (PP< 30), 3) percentage of pixels below gray level 50 (PP< 50), 4) kurtosis, 5) percentage of pixels of each of the 10 contours of the 0-255 gray level spectrum: i) percentage of pixels with gray level less than 25 (PPC1), ii) percentage of pixels with gray level below 50 and above 25 (PPC2), iii) percentage of pixels with gray level below 75 and above 50 (PPC3), iv) percentage of pixels with gray level below 100 and above 75 (PPC4), v) percentage of pixels with gray level below 125 and above 100 (PPC5), vi) percentage of pixels with gray level below 150 and above 125 (PPC6), vii) percentage of pixels with gray level below 175 and above 150 (PPC7), viii) percentage of pixels with gray level below 200 and above 175 (PPC8), ix) percentage of pixels with gray level below 225 and above 200 (PPC9), x) percentage of pixels with gray level below 255 and above 225 (PPC10)]. The first 2 contours will be analyzed further into 5 sub-contours: a) percentage of pixels with gray level below 10 (PPCS1), b) percentage of pixels with gray level below 20 and above 10 (PPCS2), c) percentage of pixels with gray level below 30 and above 20 (PPCS3), d) percentage of pixels with gray level below 40 and above 30 (PPCS4), e) percentage of pixels with gray level below 50 and above 40 (PPCS5)
(ii) First Order Statistics on Gray Scale of pixels (FOS)
Mean value, 2) Median value, 3) Variance, 4) Mode, 5) Skewness, 6) Energy, 7) Entropy
(iii) Spatial Gray Level Dependence Matrices (SGLDM)
1) Angular second moment (ASM) 2) Contrast, 3) Correlation, 4) Variance, 5) Homogeneity, 6) Sum average (SAV), 7) Sum variance (SVA), 8) Sum entropy (SEN), 9) Entropy, 10) Difference variance (DVA), 11) Difference entropy (DEN), 12) Information measures of correlation-1 (IMC-1), 13). Information measures of correlation-2 (IMC-2)
(iv) Gray Level Difference Statistics (GLDS)
1) Contrast, 2) Energy, 3) Entropy, 4) Mean, 5) Homogeneity
(v) Fourier Power Spectrum (FPS)
1) Radial segmentation of Fourier space (FPS-frad) 2) Angular segmentation of Fourier space (FPS-fang)
(vi) Gray level run length statistics
1) Short Run Emphasis (SRE), 2) Long Run Emphasis (LRE), 3) Gray Level Distribution (GLD), 4) Run Length Distribution (RLD), 5) Run Percentage (RP)

Figure 10 – Histogram measures produced by the digital image analysis

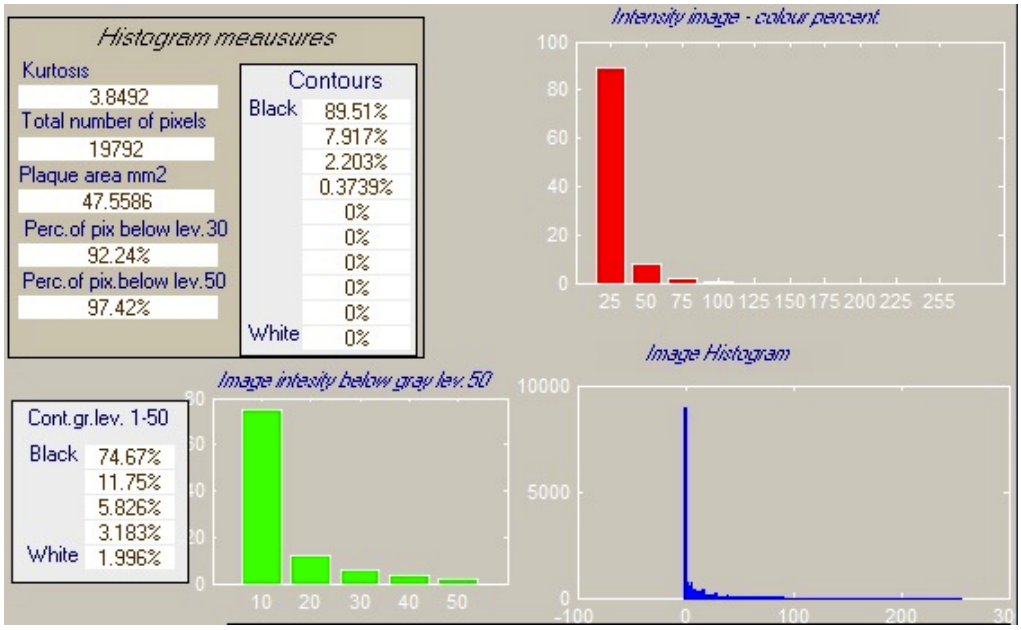

Figure 11 – Texture measures produced by the digital image analysis

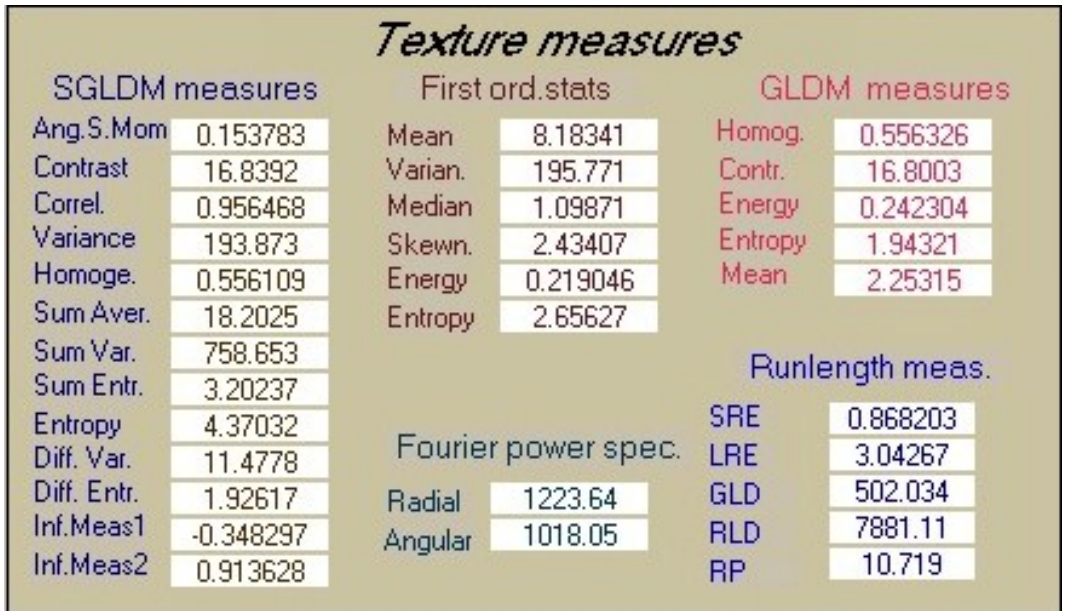

Figure 12 - Texture features

1. **GSM**. The median of all grayscale values within the plaque outline.
2. **PPCS1**. The percentage of pixels with grayscale values <10.
3. **PPCS2**: The percentage of pixels with grayscale values between 10 and 25.
4. **Bel_30**: The percentage of pixels with grayscale values <30.
5. **Bel_50**: The percentage of pixels with grayscale values <50.
6. **Four features based on spatial gray level dependence matrices (SGLDM):**

   Notation:

   *p(i,j): (i,j)*th entry in the normalized spatial gray level dependence matrix.

   = *P(i,j)/R,* where R is a normalizing constant.

   $p_x(i)$th entry in the marginal probability matrix obtained by summing the rows of *p(i,j),*

   $$= \sum_{j=1}^{N_g} p(i,j)$$

   N_g is the number of distinct gray levels in the quantized image.

   $$\sum_i \quad \text{means} \sum_{i=1}^{N_g}, \quad \text{and} \sum_j \quad \text{means} \sum_{j=1}^{N_g}$$

   [199]

   a. **SGLD ASM** (angular second moment):

   $$f_1 = \sum_i \sum_j \{p(i,j)\}^2$$

   Measure of homogeneity of the plaque, evaluating the number of dominant gray-tone transitions. Larger values of ASM indicate a more *homogeneous* plaque. [138, 199]

   b. **SGLD HOM** (homogeneity)**:**

$$Homogeneity = \sum_{i,j} \frac{p(i,j)}{1+|i-j|}$$

Measure of homogeneity, with higher values indicating a more *homogeneous* plaque [162, 199].

c. **SGLDM Correlation**:

$$f_3 = \frac{\sum_i \sum_j (i,j)p(i,j) - \mu_x \mu_y}{\sigma_x \sigma_y}$$

Where μ_x, μ_y, and σ_x, σ_y, are the mean and standard deviation values of p_x and p_y. [199]

SGLD correlation is a measure of heterogeneity, with higher values indicating a more *heterogeneous plaque* [137, 138].

d. **SGLDM** I**MC-1** (SGLDM Information Measure of Correlation-1):

$$f_{12} = \frac{HXY - HXY1}{\max\{HX, HY\}}$$

$$f_{13} = (1\text{-}\exp[-2.0(HXY2 - HXY)])^{1/2}$$

$$HXY = -\sum_i \sum_j p(i,j) \log(p(i,j))$$

where HX and HY are entropies of Px and Py, where entropy is defined as:

$$f_9 = \sum_i \sum_j p(i,j) \log(p(i,j))$$

and:

$$HXY1 = -\sum_i \sum_j p(i,j) \log\{p_x(i)p_y(j)\}$$

$$HXY2 = -\sum_i \sum_j p_x(i)p_y(j) \log\{p_x(i)p_y(j)\}$$

[199]

SGLDM IMC-1 is a measure of heterogeneity, with higher

values indicating a more *heterogeneous plaque* [137, 138, 199].

7. **Skewness:**

$$Skew = \frac{1}{N}\sum_{j=1}^{N}\left[\frac{I_j - \bar{I}}{\sigma}\right]^3$$

characterizes the degree of asymmetry of the distribution of gray values around the mean [138], with higher values indicating a more *heterogeneous plaque* [199].

8. **RunI SRE** (Runlength short-run-emphasis):

$$SRE = \frac{1}{n_r}\sum_{i=1}^{M}\sum_{j=1}^{N}\frac{p(i,j)}{j^2}$$

Each element p(i,j) corresponds to the number of runs with pixels of grayscale values equal to i and length of run equal to j along a particular orientation [163]. The size of the matrix P is n by k, where n is the maximum grayscale value in the image and k is equal to the possible maximum runlength in the corresponding image [163].

It is a measure of homogeneity, with higher values indicating a more *homogeneous plaque, with finer texture.*

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