Novel ultrasound features for the identification of the vulnerable carotid plaque

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i. Dedications

To my mother Stella for all her sacrifices and love

Στη μητέρα μου Στέλλα για όλα όσα έχει για μένα

To my father Christos' memory

Στη μνήμη του πατέρα μου Χρήστου

To my grandparents Grigoris and Marina for always being there for me

Στους παππούδες μου Γρηγόρη και Μαρίνα που ήταν πάντα κοντά μου

ii. Declaration

This dissertation is submitted for the degree of Doctor of Philosophy at Imperial College of London. It is a result of my own work and contains nothing, which is the outcome of work done with others, apart from where specifically indicated in the text.

This thesis did not exceed 100,000 words in length.

The total number of:

Tables 20, Figures: 16 and Images: 6

There is no conflict of interest to declare.

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iii. Publications related to this thesis

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2. Kyriacou E, Nicolaides A, **Makris G**, Griffin M, Pattichis CS, Petroudi S, Pattichis M Measurement of motion of carotid bifurcation plaques IEEE 12th International ***Conference on BioInformatics and BioEngineering, BIBE 2012***, pp. 506–511, 2012

3. **Makris GC**, Lavida A, Griffin M, Geroulakos G, Nicolaides AN. Three dimensional ultrasound imaging for the evaluation of carotid atherosclerosis. ***Atherosclerosis. 2011 Dec;219(2):377-83.***

4.Kyriacou E, Nicolaides A, Pattichis CS, Petroudi S, Pattichis M, Griffin M, Kakkos S, **Makris G**. First and second order statistical texture features in carotid plaque image analysis: preliminary results from ongoing research. **Conf Proc IEEE Eng Med Biol Soc**, 2011.

5. **Makris GC**, Geroulakos G, Nicolaides AN. Letter by Makris et al Regarding Article, "Carotid Artery Stenting Versus Carotid Endarterectomy: A Comprehensive Meta-Analysis of Short-Term and Long-Term Outcomes". ***Stroke***. 2011 Apr 28. [Epub ahead of print]

6. **Makris GC**, Nicolaides AN, Geroulakos G. Histological Analysis of the Carotid Plaque Post-endarterectomy: A Waste of Time or a Wasted Piece of Information? ***Eur J Vasc Endovasc Surg***. 2011 Apr 15. [Epub ahead of print]

7. **Makris GC**, Nicolaides AN, Geroulakos G. The Management of Asymptomatic Carotid Plaque Disease: Our Assumptions when we are Less Radical. ***Angiology***. 2011 Feb 8. [Epub ahead of print]

8. **Makris GC**, Lavida A, AN Nicolaides, Geroulakos G. The effect of statins on carotid plaque morphology: a LDL-associated action or one more pleiotropic effect of statins? ***Atherosclerosis***. 2010 May 7. [Epub ahead of print]

9. **Makris GC**, Nicolaides AN, Xu XY, G Geroulakos. Introduction to the biomechanics of carotid plaque pathogenesis and rapture: review of the clinical evidence. ***British Journal of Radiology***. 2010 January 10. [Epub ahead of print]

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iv. Conference Presentations related to this thesis

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Progression of carotid artery stenosis is associated with the occurrence of subsequent ipsilateral ischemic events and stroke: Results from the ACSRS study.

Kakkos S, Charalambous E, Sabetai M, Griffin M, Georgiou N, Geroulakos G, **Makris GC,** Andrew Nicolaides**.**

2. 5/2012: Cardiovascular Technology Network Symposium, London, UK

Analysis of carotid plaque biomechanics based on 3D ultrasound imaging.

Zhongjie Wang, **Gregory C. Makris** , Nigel Wood, Andrew. N Nicolaides, George Geroulakos, Xiao Yun Xu (poster presentation-1st Price)

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3-Dimensional Ultrasound for the investigation of carotid plaque disease.

GC Makris (oral presentation)

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***GC Makris**, M Griffin, G Geroulakos, AN Nicolaides(Oral presentation)*

v. Book chapters related to this thesis

1. **Makris, Gregory C**., Andrew Nicolaides, Anthi Lavida, and George Geroulakos. "Effect of Statin Therapy on Carotid Plaque Morphology." Ultrasound and Carotid Bifurcation Atherosclerosis (2012): 595-600, Springer editions

2. Nicolaides, Andrew, Maura Griffin, **Gregory C. Makris**, George Geroulakos, Dawn Bond, Efthyvoulos Kyriacou, Antonios A. Polydorou, and Victoria Polydorou. "Carotid Plaque Texture Analysis Using 3-Dimensional Volume Ultrasonic Imaging." Ultrasound and Carotid Bifurcation Atherosclerosis (2012): 299-324, Springer editions

3. Geroulakos G, **Makris GC**, Nicolaides AN. Asymptomatic carotid stenosis. Can we identify the high risk patient of stroke? The 5 years follow up of the ACSRS study on more than 1000 patients. CACVS 2010 Book (Controversies and Updates in Vascular Surgery 2010). Minerva editions 2010

vi. Common abbreviations

ICA: Internal carotid artery

CCA: common carotid artery

CEA: carotid endarterectomy

TIA: transient ischemic attacks

IMT: intima-media thickness

AF: amaurosis fugax

baPWV: brachial-ankle pulse wave velocity

CWT: circumferential wall tension

TS: tensile stress

WSS: wall shear stress

YEM: Young's elastic modulus

FSI: Fluid-structure interactions

CFD: computational fluid dynamics

FEA: finite element analysis

CWS: circumferential wall stress

MRI: magnetic resonance imaging

2D: two-dimensional

3D USS: Three-dimensional ultrasound

CS: circumferential stress/strain

ACSRS: Asymptomatic Carotid Stenosis and Risk of Stroke

LRNC: lipid rich necrotic core

WV: wall volume

LDL-C: Low Density Lipoprotein cholesterol

HDL-C: High Density Lipoprotein cholesterol

TG: Triglycerides

JBA: juxta-luminal black area

ROC: Receiver operating curve

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IQR: interquartile range is the box height from Q1 to Q3. Lower/Upper Whisker: The smallest/largest observed value within 1.5×IQR below/above the Q1/Q3.

S-Outlier: Suspected outliers shown by unfilled circles are between 1.5×IQR to 3×IQR below/above Q1/Q3. Outlier: Outliers shown by 5-pointed stars are 3×IQR or more below/above the Q1/Q3.

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viii. Abstract

Background: The identification of the vulnerable carotid plaque is of paramount importance in order to prevent the significant stroke-related mortality and morbidity. Currently the clinical decision-making around this condition is based on the traditional ultrasound evaluation of the degree of stenosis. However, there is emerging evidence supporting that this is not sufficient for all patients.

Aim of this thesis: The evaluation of novel carotid plaque features for the characterisation of plaque composition, volume and motion using 2 and 3 dimensional ultrasound technology. The ultimate goal is to identify novel sensitive imaging markers for carotid plaque characterisation and stroke-risk stratification.

Methods: The Asymptomatic Carotid Stenosis and Risk of Stroke (ACSRS) Study was a large prospective multicentre trial that was recently completed. A post-hoc analysis of the sonographic and clinical data from this study was performed in order to evaluate the effectiveness of novel ultrasound texture features, such as second order statics, on stroke-risk prediction. In addition, the change of specific texture features and degree of stenosis during the ACSRS follow-up time (8 years) and their importance for stroke prediction was evaluated. In order to assess the potential of 3D ultrasound carotid imaging we also developed a special methodology using a 3D broadband, linear array probe and the Q-lab software. This methodology was then applied in a clinical, cross-sectional study of patients with symptomatic and asymptomatic carotid disease. Finally we developed a carotid plaque motion analysis methodology that we tested on a feasibility study.

Results: The post-hoc analysis of more than 1, 000 patients from the ACSRS database showed that there are novel ultrasound features of plaque homogeneity that can contribute to plaque characterisation and improve stroke-risk prediction. Similarly our results suggest that the change of degree of stenosis or plaque's composition through time might have significant predictive value when combined with the above novel features. The study in 3D ultrasound prospectively assessed more than 80 people with symptomatic and asymptomatic carotid disease with both 2 and 3D

carotid ultrasound without, though, revealing any significant benefit from the use of 3D imaging in terms of stroke-risk prediction. Finally, our feasibility study on plaque motion analysis showed that it is possible to objectively characterise plaque motion, using ultrasound and dedicated software without complicated reconstructions.

Conclusion: The use of novel 2D ultrasound texture features in combination with traditional ones can improve the stroke-risk stratification. 3D ultrasound is a promising new approach, however, the current technology does not appear to offer a significant benefit in comparison to cheaper traditional 2D ultrasound for carotid plaque evaluation. Further research is warranted on this issue.

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PART I

REVIEW OF LITERATURE

Chapter 1

Carotid plaque pathogenesis, biomechanics and imaging

1.1 Introduction

Carotid plaque disease is considered to be one of the main of cerebrovascular events such as stroke and transient ischemic attacks. The incidence of cerebrovascular events is continuously rising in the developed world and poses a significant source of morbidity and mortality, which is globally associated with rising socioeconomic costs.

The midterm overall risk reduction with carotid endarterectomy (CEA) in patients with asymptomatic carotid stenosis is minimal. The ACAS study reported that in patients with stenosis >60%, CEA reduced the annual risk of stroke from 2% to 1%, which implies that approximately 20 procedures need to be performed to prevent one stroke in 5 years. [Executive Committee for the Asymptomatic Carotid Atherosclerosis Study, 1995] For this reason, there is considerable debate on the appropriateness of this procedure in all patients with asymptomatic carotid stenosis, and restriction to patients who are at high risk of developing neurologic events might be more cost-effective.

There has been significant effort in the past in the direction of developing new diagnostic algorithms that would enable physicians to distinguish between high and low risk patients according to the degree of stenosis of carotid lumen [Barnett HJ,1998] [European Carotid Surgery Trialists Collaborative Group,1998], or the intima-media thickness (IMT) [Hollander M, 2003], or even according to the grey scale median (GSM) of the atheromatic plaque.[Kakkos SK & Stevens JM,2007] As a result of this, plaque classifications -such as the Geroulakos' classification [Geroulakos G& Ramaswami G,1993] -have been developed in order to provide us with more specific and clinically orientated algorithms that could be used in the daily routine. However, there is still a grey area of asymptomatic patients that cannot be adequately stratified with regard to their risk due to insufficient evidence.

The current advances in medical imaging, image analysis and biomechanics provide us with promising new tools in the quest for more sensitive markers of plaque vulnerability.

The development of an atherosclerotic plaque as well as its rupture can be illustrated and explained as a complicated, dynamic system that involves a thixotropic fluid (blood) that flows within an elastic tube –with various and interacting properties- and encounters a surface irregularity along its course (plaque) and a variety of physical forces that interrelate and cause structural changes on both the wall of the tube and the surface of the plaque.

In bioengineering terms plaque rupture is equivalent to the structural failure of a certain material upon which a number of different forces are applied. The prediction of the amount of stress that a certain material can withstand before it reaches the crucial “break-limit” is a problem commonly encountered in the development of improved industrial materials.

1.2 Basic principles

1.2.1 Rheological and mechanical properties of carotid blood flow

Blood is a non-Newtonian fluid whose flow properties are not described by a single constant value of viscosity. It is widely accepted that blood is a shear thinning fluid whose viscosity increases non-linearly with reduction in shear rate. While in a Newtonian fluid the relation between shear stress and shear rate is linear – and the constant of proportionality is the coefficient of viscosity- that is not the case for non-Newtonian fluids. Nevertheless, the effect of non-Newtonian behaviour on blood flow in large arteries is insignificant and hence has been ignored in the majority of carotid hemodynamics studies.

Blood flow is usually described by Navier-Stokes equations, which describe the motion of fluid substances. They are differential equations that do not explicitly establish a relation among the variables of interest (such as pressure and velocity) but instead establish relations among the rates of changes. This is in contrast to a simple case of an ideal fluid (not compressible or viscid) flowing through a rigid tube for which equations can state that the rate of change of velocity (acceleration) is proportional to the pressure gradient in the direction of flow.

The arterial wall of medium and large arteries has elastic properties that resemble that of an elastic tube. However, factors such as hypertension, diabetes, smoking and aging cause significant changes to the arterial wall through time. (Milio G & Corrado E, 2006) These contribute to the dynamic and changing nature of this tube, which cannot be easily simulated. The Young's elastic modulus and the brachial-ankle pulse wave velocity (baPWV) (Hung CS & Lin JW, 2008) have been used to assess the arterial stiffness. The Young's modulus can be measured as the ratio of the uniaxial stress over the uniaxial strain in the range of stress in which Hooke's Law holds and the baPWV can be measured automatically using a volume plethysmographic apparatus.

The structure of atherosclerotic plaques is variable and may consist of different predominant substances. For example some plaques are more lipid-rich and, thus, unstable whereas others are more fibrotic and stable. Different types of plaque are associated with different biomechanical behaviour to stress and interact differently to the blood flow and changes in shear stress during the cardiac circle. In addition, due to the local stenosis and according to Bernoulli's principle there is an associated pressure gradient with energy loss, which is responsible for additional local stress. (Milio G & Corrado E, 2006)

1.2.2 Biomechanical parameters of carotid arteries

In the literature, a number of hemodynamic parameters have been used to describe the biomechanical behaviour of the carotid arteries. These include:

- the circumferential wall tension (CWT) which can be estimated from the systolic blood pressure and the internal diameter of the common carotid artery (CCA),
- the tensile stress (TS) which is proportionate to the CWT and inversely related to intima media thickness,
- the wall shear stress (WSS) which, in the simplest cases, is considered proportional to the blood viscosity and blood velocity and inversely associated with the internal diameter of the CCA and
- the Young's elastic modulus (YEM) which is representative of the stiffness of the artery (Carallo C , 1999) (Table 1.1)

Considering the above it is clear that each spot of the arterial wall and atherosclerotic plaque is subjected to a load of stresses with different directions and magnitude at different times within a cardiac cycle. The Von Mises criterion is a formula, which can be used to calculate whether the stress combination at a given point will cause failure. This theory is simply one of the several structural-

failure theories that combine principle stresses (in the x, y, or z direction) into an equivalent applied stress, which is compared to the tolerable stress of the material.

The complexity of the above described interactions between rheological and structural factors –like blood, arterial wall and plaque- and biomechanical factors –such as CWT, TS, and WSS- is obvious and calculations without using over-simplifying assumptions demand increased technical knowledge and powerful software. Fluid-structure interactions (FSI) describe the interactions of a deformable structure with an internal or surrounding fluid flow and can be achieved using the recent advances in computational fluid dynamics (CFD) and finite element analysis (FEA). CFD is one of the branches of fluid mechanics that uses numerical methods and algorithms to solve and analyse problems that involve fluid flows. Computers are used to perform the millions of calculations required to simulate the interaction of fluids and gases with the complex surfaces used in engineering. Finite element analysis method (FEA) is a numerical technique for solution of boundary-value problems. In its application, the system is represented by a geometrically similar model consisting of multiple but linked representations of discrete regions—i.e. finite elements. (Birchall D,2006).

The previously mentioned techniques have been combined with magnetic resonance imaging (MRI) to simulate the blood flow and provide us with information about the morphological features and the hemodynamic effect within stenotic carotid arteries (Birchall D,2006).

1.3 Mechanical stress and the association with the carotid plaque pathogenesis

The right and left carotid arteries are not affected to the same extent by atherosclerosis and investigations of the pathological process have revealed that these lesions are most commonly encountered in areas of low wall shear stress. (Gnasso A, 1997) Areas of bifurcation or curved arteries are portrayed as areas where low or oscillatory shear stresses occur (Cheng C, 2006) and this observation corresponds with the clinical experience of plaque disposition mainly at these sites. The theory behind the mechanisms of plaque creation, indicates that it is the oscillatory shear stress in combination with the low shear stress that is mainly responsible for the endothelial dysfunction, whereas the unidirectional shear stress may be protective for the endothelial layer (Gambillara V,2006; Melchionna R,2005 and Slager CJ, 2005).

1.3.1 Effect of stress on the arterial endothelium

It is possible that the key factor is the interaction between low or/and oscillatory shear stress and the subjacent endothelium, which responds by up-regulation of vaso-occlusive agents and down-regulation of vasodilating agents such as endothelial nitric oxide synthetase (eNOS) gene expression (Gambillara V,2006; Melchionna R,2005 and Slager CJ, 2005). In porcine carotid artery models that were exposed for three days to unidirectional high and low shear stress or to oscillatory shear stress there was an increase in the expression levels of metalloproteinase-2,-9 and a decrease in the plasminogen activator inhibitor-1 expression in the latter group (Gambillara V,2005) These results indicate that such a plaque-prone hemodynamic setting may cause wall remodeling of the artery and this is also supported by other experimental studies. (Cheng C, 2006) High shear stress is generally considered as an important destabilizing factor through the induced changes to the endothelium and smooth muscle cells of the plaque matrix as they are summarized in figure 1.2.

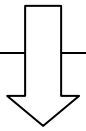
Table 1.1: Simplified formulas of the various types of stresses encountered during the wall stress analysis. *(Makris GC, 2010; doi: 10.1259/bjr/49957752.)*

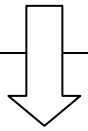
Type of stress	Simplified formula	Description of action
1. Circumferential Wall Tension (CWT)	CWT *(dynes/cm)* = SBP x (ID/2)	adjacent parts of the material tend to press against each other through a typical stress plane
2. Tensile Stress (TS)	TS *(dynes/cm^2)* = CWT/IMT	two sections of material on either side of a stress plane tend to pull apart or elongate
3. Wall Shear Stress (WSS)	WSS = 8 x μ x (MV/ID)	two parts of a material tend to slide across each other in any typical plane of shear upon application of force parallel to that plane
4. Young's Elastic Modulus (YEM)	YEM *(dynes/cm^3)* = (SBP-DBP) x (ID_M / [(ID_T-ID_R) x IMT]	describes arterial wall stiffness
5. Stress Phase Angle (SPA)	SPA= CWS/WSS	combination of CWS and WSS

SBP: systolic blood pressure, **ID:** Internal diameter in cm, **IMT**: Internal media thickness **,** **μ**: blood viscosity, **MV**: mean velocity **DBP**: diastolic blood pressure, **ID_M**: ID_R + (ID_T − ID_R)/3, **ID_R**: Internal diameter during R wave of cardiac cycle, **ID_T**: internal diameter during T wave of cardiac cycle**, CWS:** circumferential wall stress/strain.

Figure 1.1 Summary of the effect of Wall Shear Stress on atherosclerotic plaque development.
(Makris GC, 2010; doi: 10.1259/bjr/49957752.)

The effect of low wall shear stress on plaque pathogenesis	The effect of high wall shear stress on plaque instability
Enhancement of lipid oxidation and accumulation in the intima	Stimulation of endothelium proliferation and production of TGF-β /NO/plasmin
Endothelial dysfunction and decreased function of NO synthetase	NO will stimulates macrophages to secrete promatrix metalloproteinases (MMPs) which will be further activated by plasmin to transform to active MMPs
Smooth muscle cell proliferation and matrix synthesis	Increased smooth muscle apoptosis
Decrease of anti-oxidant proteins and increase of oxidative stress.	Increased expression of antioxidant enzymes
Increase of vascular cell adhesion molecules and increased recruitment of inflammatory cells	
Gene activation and vessel remodeling (if low WSS being sustained)	





Eccentric atherosclerotic plaque build-up and Outward vessel remodelling	Fibrous-cap matrix breakdown combined with increased vulnerability (upstream of plaque) Plaque progression is delayed or absent

1.3.2 Effect of stress on plaque progression and severity

Apart from the effect of wall stress on the initial mechanisms of plaque formation there is clinical evidence suggesting its important role in plaque progression and the observed severity of carotid atherosclerosis. Lee and his colleagues evaluated the IMT and plaque burden in bilateral CCA bifurcations, external and internal carotid arteries using duplex ultrasound in 80 untreated hypertensive patients. (Lee MY, 2008). They presented evidence suggesting that circumferential wall stress (CWS) and tensile stress (TS) are related to the severity of carotid plaque disease in this specific patient group. Additionally, Tang and colleagues combined in vivo MRI-based 2D/3D multi-component models with fluid structure interactions to study twenty-one human carotid plaques. (Tang D,2008) Lower plaque wall stress and lower flow wall shear stress were shown to contribute significantly to continued eccentric plaque progression.

Intraluminar stresses are affected by a variety of different factors. The carotid geometry itself was found to have an important effect on blood flow and on the magnitude of wall shear stress. (Nguyen KT,2008) Moreover in a previous study it was also shown that flow, diameter and blood viscosity have significant effect on wall shear stress. (Box FM, 2005). Finally factors like age, blood pressure, body mass index and IMT have been independently associated with changes in wall shear and circumferential wall stress (O'Leary DH,1999 and Zhao SZ,2002).

The combined effect of low wall shear stress and high tensile stress has also been evaluated by different groups and is believed to pose an important determinant of carotid plaque formation. (Zhao SZ,2002) This combined effect can be expressed using the phase difference between the solid circumferential stress/strain (CS) and the fluid wall shear stress curves or in other words the stress phase angle. (Tada S,2005)

1.4 Mechanical stress and carotid plaque vulnerability: the clinical evidence

It has been generally accepted that the degree of carotid lumen stenosis is not a sufficient marker for the underlying risk of plaque rupture. (Johnsen SH,2009) A number of trials have been recently launched in order to identify more reliable markers based on the plaque morphology and hemodynamic properties. Baseline evidence originating from in vitro (animal or artificial models) or even from computational simulation studies suggest that there are certain plaque characteristics that could be incriminated for plaque destabilization. The geometrical features of a stenotic lumen – such as eccentricity [Steinman DA,2000 and Tang D,2004] and lumen curvature- [Steinman DA,2000], the lipid pool size [Tang D,2004] and the fibrous cap thickness [Li ZY,2006] have been associated with instability in these experimental settings. Histological data from CEA specimens suggest that a combination of cap thickness of less than 500μm and minimum cap thickness of less than 200μm can be independently associated with cap rupture according to the Oxford plaque study. [Redgrave JN,2006]

1.4.1 The effect of carotid plaque characteristics on stress distribution in clinical settings

The application of the results of the above studies in clinical practice might be more challenging than initially expected due to the complexity and the dynamic nature of the carotid system. The current clinical evidence is rather limited but corresponds well to the previous experimental evidence. Fibrous cap thickness appears to be strongly associated with the stress levels applied on the plaque and consequently with the risk for rupture and thromboembolic events. [Gao H,2008; Li ZY,Tang T, 2008; Kock SA,2008] Lumen curvature was another characteristic associated with instability [Li ZY, Tang T,2008] in clinical settings. In addition, the presence of calcium deposition within plaque [Li ZY, Howarth S,2007] or the presence of ulceration [Groen HC,2007] may alter the local stress patterns or become altered by them, respectively, affecting the structural behaviour of the plaque.

1.4.2 The effect of different types of stress on carotid plaque stability

Attempts have been made to evaluate the effect of different types of stress in clinical and experimental settings. The current clinical evidence suggests that the various types of hemodynamic stress (WSS, TS, and CWT) may have a different association with plaque stability. High shear stress especially on the upstream of the plaque is several times lower in magnitude than tensile stress but it is possibly the factor leading to the formation of unstable plaque characteristics. [Groen HC,2007; Trivedi RA,2007; Slager CJ, 2005] High tensile stress [Tang D, Yang C, 2003], induced by blood-pressure pulse, is proposed as the most important factor eventually causing plaque rapture; repetitive inward bending [Paini A,2007; Beaussier H, 2008] of stiff carotid arteries may also be considered among potential contributors to the plaque structural fatigue and thrombogenic complications. However, so far there is limited or sometimes even conflicting clinical evidence about whether one of these factors poses a more important role or it is a matter of combined effect.

1.5 Limitations of the clinical evidence

Despite the number of experimental and computational studies there are still doubts with regard to the clinical implications of these novel and promising biomechanical characteristics. The current clinical evidence is rather limited and the number of studied patients is insufficient to help us reach safe conclusions. In addition, there is considerable variation in the type of stresses or wall/plaque characteristics analysed as well as to the methods applied for the stress analysis and imaging. In the majority of the studies magnetic resonance imaging has been used combined with fluid-structure interactions simulations contributing to the increased cost and complexity of the studies.

Finally each study –apparently due to the technical complexity- focused on a different type of stress or arterial wall/plaque characteristic and thus the potential combined effect of various stress types on an anisotropic carotid plaque model has not been adequately explored.

1.6 New techniques

The increased cost and technical complexity of 3-dimension MRI reconstruction for the analysis of the wall-stress relation is an important limitation of this approach. Other non-invasive ultrasound-based techniques could be applied, taking into consideration the recent technological progress. Non-invasive vascular elastography, motion plaque analysis and 3-dimension ultrasound imaging could be alternative potential approaches.

Non-invasive vascular elastography is a method to evaluate the vulnerability and the composition of a plaque creating elasticity maps, potentially enabling investigators to differentiate lipid pools and fibrotic plaques. [Maurice RL,2004] In contrast to the intravascular technique the radial strain and the ultrasound beam are not aligned in non-invasive acquisitions. [Ribbers H,2007] It has been suggested that in patients with non-homogenous atheromatic plaques, shear and stress elastograms may reveal interesting information regarding tissue composition, plaque size and mechanical interactions. [Schmitt C,2007]

Motion analysis of carotid plaques during systole and diastole may also reveal different mechanisms that contribute to plaque fatigue and rupture. A computerized method that objectively analyses such motion patterns using ultrasound images has been developed using 2-dimensional displacement vector maps. Motion analysis theory advocates that distinguished plaque regions may present different motion patterns. This may lead to internal structural stress and thus to the formation of fissures and ultimately plaque disruption. [Bang J,2003]

Finally there is increasing amount of evidence regarding the 3-dimensional ultrasound imaging and its potential applications in arterial wall and plaque image reconstruction, as well as to the direct evaluation of plaque volume. [Chiu B,2009; Fenster A, 2006; Ainsworth CD,2005] The potential of combining 3-dimensional ultrasound imaging and computational fluid dynamic analysis has not been

adequately explored with regard to the clinical effect of stress patterns to plaque vulnerability and poses a significant challenge for future studies. However, there is preliminary evidence that wall shear stress patterns derived from 3-dimensional ultrasound imaging can be both well reproducible and potentially comparable ,if not superior, to those acquired from MRI. [Glor FP,2004; Augst AD,2003]

1.7 Conclusions

Carotid plaque stress analysis appears as a promising diagnostic approach based on established mechanic and hemodynamic properties. Prospective, clinical studies are warranted to investigate in depth the applicability of the different aspects of this advance into daily, clinical practice. Established imaging techniques –such as MRI or 2-dimensional ultrasound- could be further developed. In addition novel, ultrasound-based techniques like elastography, plaque motion analysis and especially 3-dimensional ultrasound could be investigated in order to reduce cost and complexity and increase the quality of information regarding wall stress-carotid plaque interactions. A multidisciplinary approach –including vascular surgeons, radiologists and bioengineers- is considered as an essential element for the accomplishment of this challenging task.

Chapter 2

Current status in surgical management of carotid plaque disease and the ACSRS study

2.1. The debate regarding the management of significant asymptomatic carotid plaque disease

The debate regarding the conservative or surgical management of patients with significant asymptomatic carotid plaque disease [Spence JD,2010; Roffi M, 2010], has significant clinical implications. Current evidence suggests that the incidence of stroke is rather low at approximately 1% per year in individuals with asymptomatic carotid plaque disease who are on the appropriate medication and follow a healthy lifestyle [Abbott AL, 2009]. Nevertheless, the previous sentence includes 3 assumptions that generate reservations regarding ruling out more radical treatment options.

The first assumption is the term "asymptomatic" which can be interpreted as equivalent to "low risk". There is evidence to suggest that some asymptomatic carotid plaques are not as benign as may be assumed. A thin fibrous cap [Li ZY&Howarth SP,2006] seen on ultrasound as a juxtaluminal black area [Griffin MB,2010], silent embolic infracts on CT [Kakkos SK & Sabetai M,2009] or the detection of microemboli using transcranial Doppler [Spence JD, Tamayo A,2005] are associated with a 3-5 fold increase in risk of stroke. In addition, other factors such as decreased glomerular filtration rate <60 ml/min/1.73m^2 have been independently related to the risk of stroke as recently reported in a meta-analysis (284,672 participants) [Lee M, Saver JL,2010]. Novel plaque features in combination with routine clinical factors may provide more information in the future regarding the risk of stroke. The point here is that we are still underperforming in stratifying asymptomatic patients into risk categories, although as the Asymptomatic Carotid Stenosis and Risk of Stroke (ACSRS) Study highlighted, this may now be possible if both clinical and plaque features are considered. [Nicolaides A,Kakkos SK, 2010]

The second assumption is that asymptomatic patients are on adequate medication (statins, antiplatelet agents and/or antihypertensive drugs). Nevertheless, we know that the effectiveness of this approach can be affected by compliance or the failure to achieve blood pressure or low-density lipoprotein (LDL)-cholesterol targets [Daskalopoulou SS,2008]. Achieving treatment goals is challenging since, as recent evidence suggests, the ability of statins to stabilize the carotid plaque is LDL-cholesterol level-dependant [Makris GC, Lavida A,2010]. In other words, poor control may result in less protection. Moreover, the importance of the "healthy-user" effect [Brookhart MA,2007] on trials evaluating pharmacological approaches should also be taken into consideration since the socioeconomic status of the patient may impact on the accessibility of appropriate medical care in the long run.

The last assumption is achieving radical lifestyle modifications in long-term smokers and those who have never exercised. Smoking cessation clinics may have unsatisfactory results, especially in the long run [Robles GI,2008]. In addition, there is not enough data regarding the effect of risk factor modification clinics [Daskalopoulou SS,2008] and these are also not widely available. Nevertheless, there is evidence supporting a beneficial effect of risk factor management in patients with carotid artery disease [Liapis CD,2010].

Conservative management for patients with significant but asymptomatic carotid plaque disease should always be considered first but we should keep in mind that the success of this decision depends on certain assumptions before becoming a reality. The introduction of long-term risk factor modification may be one way of ensuring a decrease in cardiovascular risk [Lee M, Saver JL, 2010]. We are still learning about what defines an unstable plaque. The presence of symptoms or just the degree of stenosis, are not perfect "markers". Patients can be asymptomatic and at low-risk one day and the next they may suffer a stroke. Indeed, the risk category has changed but it is too late.

It appears that further improvement in diagnostic approaches for risk stratification and the introduction of a more structured network of risk factor modification clinics is the way to be equally effective when we are less radical [Liapis CD,2010].

2.2. Can we identify the patients at increased risk of stroke? Introduction to the ACSRS study.

The degree of stenosis has been for years an established measurement in order to assess the risk of ipsilataral embolic stroke and is a major determinant for eligibility for carotid endarterectomy. Studies performed more than a decade ago illustrated that the risk for stroke in asymptomatic patients with internal carotid artery stenosis <75-80% (NASCET criteria) was 0.1-1.6% per year and 2.0-3.3% per year when the stenosis was greater. (European Carotid Surgery Trialists Collaborative Group, 1995; Inzitari D, 2000)

Two randomized controlled trials, the ACAS in 1995(Executive Committee for the Asymptomatic Carotid Atherosclerosis Study, 1995) and ACST in 2004 (Halliday A, 2004) reported that in patients with asymptomatic ICA >60-70% (NASCET) carotid endarterectomy reduced the risk of stroke from 2% to 1% per year. This means that approximately 20 operations need to be performed to prevent one stroke in 5 years or 100 operations to prevent one stroke in one year. However, even this benefit was not without risks. In these trials CEA was associated with 2-3% perioperative rate of stroke and death. On the other hand medical therapy was left to the discretion of the local research teams and only 25% and 80% of the patients were on statins and antiplatelet therapy, respectively, when recruited. The definition of best medical treatment has changed dramatically since then with lipid lowering and blood pressure control to predefined targets and with life style changes so that currently the estimated average annual risk of ipsilateral cerebral stroke is approximately 1%. (Abbot A, 2009) As a result many authorities are questioning the need for carotid endarterectomy in asymptomatic individuals. There is continuous research for the development of diagnostic approaches in order to identify those characteristics, which define the vulnerable carotid plaque. If a specific patient-subgroup with asymptomatic

carotid stenosis with sufficiently higher than average risk of stroke could be identified then carotid surgery may still be justified.

In the past ultrasound had been the standard examination for the evaluation of patients with carotid disease and several studies have shown that hypoechoic and heterogenous plaques are more often associated with cerebrovascular symptoms than hyperechoic (echogenic) plaques. [Geroulakos G, Ramaswami G,1993] More recently, plaque area and plaque volume as well as texture features such as entropy, homogeneity and energy have also been shown to be associated with symptomatic plaques.[Spence JD,2006] However, the use of the above ultrasonic markers in combination in order to achieve better risk-stratification has not been properly investigated.

2.2.1. Study design and quality control

The ACSRS study was an international, multicentre, prospective, observational natural history study of asymptomatic patients with ICA stenosis >50% (ECST criteria) run by our group and a number of international experts. It was designed to overcome fundamental methodological problems of the previous risk-stratification trials such as small numbers, short follow-up and poor reproducibility of plaque types or texture features because of lack of image normalisation. The principal aim of the ACSRS study was to identify subgroups of patients with stroke-risk less than 1% and more than 4% annually using local (plaque characterization and texture analysis) and systemic factors in addition to the degree of stenosis.

Bilateral carotid ultrasound scanning was performed on admission and the entire duplex investigation was recorded on S-VHS videotape and sent to the coordinating centre. The degree of stenosis was graded according to established velocity criteria and expressed as both ECST and NASCET percentages (Mansfield A, 2004; Nicolaides A, Sabetai M,2003). A

high frequency linear array transducer (4 -7 MHz) was used and the following technical ultrasound settings were observed to ensure optimum image quality for plaque type classification and texture analysis.

Ultrasonographers from participating centers attended for two day training at the coordinating center. They were trained not only on equipment settings and method of image recording but also on the method of image normalization. Although ultrasonographers were not expected to perform image analysis, it was felt that knowledge of how it was done would ensure that all prerequisites for image analysis described above would be included. A specially prepared video recording with instructions how to perform the examination was provided. Video recordings of the examination and frozen images of plaques were sent to the coordinating centre for image analysis. Continuous feedback ensured quality (Nicolaides A, Sabetai M,2003).

Analysis of the baseline carotid plaque characteristics was performed with the use of the "Plaque Texture Analysis" software (version 3.2 –Incosoft International Ltd, PO Box, Greenford, London UB6 9ZN [Griffin M,2007]) after image normalization and segmentation.

The primary outcome measures were ipsilateral cerebral or retinal stroke (fatal and non-fatal), transient ischemic attacks and retinal embolic events (i.e amaurosis fugax) whereas secondary outcomes were death and all other neurological events.

2.2.2. Previously published results from the ACSRS study

A total number of 1,121 patients were recruited. The available results from a number of interim analyses of the ACSRS data can be summarized as follows:

A. Mortality-associated factors in patients with asymptomatic carotid stenosis.

Factors such as age, cardiac failure, male gender, myocardial ischemia and left ventricular hypertrophy on electrocardiogram (ECG) were independent predictors of increased risk for cardiovascular and all cause mortality according to Cox multivariate analysis. On the other hand antiplatelet therapy was associated with reduced risk. [Kakkos SK, Nicolaides A,2005]

Using the above risk factors it was possible to identify a high-risk group consisting of one third of the studied population with 66% all cause death rate and 40% cumulative cardiovascular death rate at 7 years. In comparison the other two thirds of the population were at low-risk with a 21% all cause mortality rate and a 10% cumulative cardiovascular death rate at 7 years. No significant difference in the cumulative ipsilateral stroke rate was observed between the two groups (Log Rank P>0.05). [Kakkos SK, Nicolaides A,2005]

B. Severity of asymptomatic carotid stenosis and risk of ipsilateral hemispheric embolic events.

The relation between the severity of ICA stenosis and other risk factors (such as serum creatinine levels) with the risk of ipsilateral embolic neurologic events was also reported in the first 1115 patients recruited with a mean follow-up period of 37.1 months. The ICA degree of stenosis was linearly associated with the event rate when expressed by the ECST method but was S-shaped when the NASCET criteria were used. Serum creatinine levels of more than 85 micromols/L (RR 2.1; 95%CI 1.23-3.65) and history of contralateral TIAs (RR 3.0; 95% CI 1.90-4.73) were identified as independent predictors of ipsilateral neurologic events including stroke. [Nicolaides AN, Kakkos SK,2005]

When these three risk factors were combined a low-risk (2.3% annual event and 0.7% annual stroke rates) and a high-risk group (7.3% annual event and 4.3% annual stroke rates) could be identified. The identification of novel risk factors in addition to the degree of ICA stenosis

may help one achieve a better risk stratification and refine the CEA indications. [Nicolaides AN, Kakkos SK,2005]

C. Effect of image normalization on carotid plaque classification and the risk of ipsilateral hemispheric events

One hundred and sixteen ipsilateral embolic events were documented in the first 1,115 patients recruited to the ACSRS study (mean follow-up 37.1 months). Plaques were classified as type 1-5 according to the Geroulakos classification. Before normalization all types of plaques were associated with a high event rate. After image normalisation more than 50% of the plaques were reclassified mainly to a higher or lower type and 94% of the events occurred in patients with type of plaque 1 to 3. [Nicolaides AN, Kakkos SK,2005] Patients with plaque types 4 and 5 had a cumulative stroke rate of 0.9% at 7 years (0.14% per year) but patients with plaque types 1 to 3 had a cumulative stroke rate of 14% at 7 years (2% per year). These results indicate that the risk of stroke in patients with plaque types 4 and 5 is low irrespective of the underlying degree of stenosis. [Nicolaides AN, Kakkos SK,2005]

D. Risk of ipsilateral hemispheric events and silent embolic infarcts on brain CT scan.

From the 1,121 patients with asymptomatic CPD who were included in the trial, 821 had baseline CT-Brain scans which were reported centrally by a neuroradiologist. In the baseline CT scans 17.8% of the patients (146) had silent embolic infarcts which were classified as: 15 small cortical, 8 large cortical, 72 discrete subcortical and 51 basal ganglia ipsilateral infarcts. [Kakkos SK, Sabetai M, 2009]

During the follow-up period (6 months to 8 years) 102 ipsilateral hemispheric neurologic events occurred (47 strokes, 38 TIAs and 16 amaurosis fugax), 24 patients were lost in follow-up and 134 died.

In patients with stenosis of less than 60% (mild stenosis) (n=359) the silent embolic infarcts were not associated with increased risk (log-rank P=0.65). In patients with ICA stenosis ranging from 60% to 99% stenosis (NASCET criteria) the cumulative annual stroke rate was 1% when silent embolic infarcts were not present and 3.6% when present (log-rank P=0.002). [Kakkos SK, Sabetai M,2009] Therefore the presence of silent embolic infarcts may prove useful in the identification of a high-risk group for TIAs and stroke and in the management of patient suffering from moderate ICA stenosis.

Chapter 3

Medical management of atherosclerosis and current imaging of disease progression

3.1.Introduction

In recent year the medical management of patients with carotid atherosclerotic disease has become the mainstay of management for stroke prevention. Especially with regard to the beneficial effect of statin administration to patients with cardiovascular disease, which been well established since the first large placebo-controlled trial was published almost fifteen years ago [The Scandinavian Simvastatin Survival Study Group, 1994] .Pooled data from two large meta-analyses provide significant evidence illustrating the beneficial effect of statin-treatment [Baigent C,2003; Amarenco P,2004]. The relative risk reduction rate for stroke was 21% [(OR) 0.79 (0.73-0.85)] with no heterogeneity between studies [Amarenco P,2004] while the 5-year incidence of major coronary events, coronary revascularization and stroke was reduced by about one-fifth per mmol/l reduction in LDL cholesterol [Baigent C,2003].

In addition, in a recent meta-analysis of statins combined with other strategies for stroke prevention which included 165, 792 patients, there was a reduction in relative risk for stroke of 21.1% (95% CI 6.3-33.5, p=0.009) for every 1 mmol/L decrease (39 mg/dl) in LDL cholesterol [Amarenco P,2009]. Reduction rates of 16% and 26% for fatal/ non-fatal strokes and total vascular events respectively, were also observed in another study after intensive treatment with atorvastatin. This was the only randomized trial (SPARCL-Stroke Prevention by Aggressive Reduction in Cholesterol Levels), which included patients who had suffered stroke or transient ischemic attacks (TIA) but without manifestations of heart disease after a median follow-up of 4.9 years [Amarenco P,2006].

The effect of statins on peripheral artery disease (PAD) and particularly on carotid plaque disease has been a matter of increasing importance due to the socioeconomic burden, which arises from stroke or TIAs. Two systematic reviews indicate that there is clinical evidence supporting the beneficial effect of statins on the development of PAD, though the quality of

the evidence was insufficient as most of the studies were non-randomized [Stalenhoef AF, 2009; Paraskevas KI, Liapis CD,2006]. In parallel, preliminary evidence exists with respect to the potential reductions of perioperative morbidity and mortality in patients undergoing non-cardiac vascular surgery. Further appropriately designed trials, however, are warranted in order to establish the true effect and define the duration and dosage of statin treatment needed to achieve maximum benefit [Paraskevas KI, Liapis CD,2006].

Plaque progression and vulnerability are invaluable prognostic markers of cerebrovascular events irrespectively of the studied vascular bed. Nevertheless the majority of studies are currently focused on the coronary system. The REVERSAL (Reversal of Atherosclerosis with Aggressive Lipid Lowering) [Nissen SE,2008] and ASTEROID (A Study to Evaluate the Effect of ROsuvastatin on Intravascular Ultrasound Derived Coronary Atheroma Burden) trials [Nissen SE,2006] are two of several randomized studies which concluded that the atheroma volume within the coronaries can be significantly reduced by statin therapy.

In this chapter the clinical evidence on the effect of medical management with HMG-CoA reductase inhibitors on carotid plaque morphology (size and composition) and the related serum inflammatory markers is reviewed.

3.2. Results from systematic review of the literature

Our recent systematic review of the literature [Makris GC, Lavida A 2010] showed that there is preliminary evidence supporting the notion that statin treatment may have beneficial effects on plaque morphology, which may be adequately assessed by ultrasound, MRI, and PET scans. There is also limited, supporting data for the anti-inflammatory potential of statins mainly based on the down-regulation of CRP production.

Paraskevas and colleagues [Paraskevas KI,2007] recently published an extensive review about the effect of statins on carotid IMT and the incidence of cerebrovascular events. The presented evidence supports the fact that statins reduce the rate of progression of the IMT and that the perioperative outcome in symptomatic patients undergoing carotid endarterectomy may be improved in statin users. However, despite the extensive use of IMT as a surrogate marker of atherosclerosis, there is uncertainty as to whether or not it is currently the best marker to quantify changes in atherosclerotic plaques. The carotid plaque and the IMT are considered genetically and biologically different and, thus, their association may be weaker than originally thought [Spence JD,2006; Coll B,2008].

3.2.1. The effect of the type of statin

Our systematic review [Makris GC, Lavida A 2010] was the first to accumulate evidence supporting that statin treatment not only beneficially alters carotid IMT but also results in plaque remodelling and regression. The majority of the studies found, used simvastatin and atorvastatin, since they have been the standard treatment for lipidaemic control, although there was no direct comparison between them with regard to their effectiveness in plaque stabilization. Recently, new statins ,such as rosuvastatin, have been licensed for lipid-lowering treatment around the world. No solid evidence supporting the superiority of a specific statin exists due to the lack of comparative trials and the heterogeneity between the existing ones. Nevertheless, a previous metanalysis from Pilote and colleagues suggested that simvastatin, pravastatin and atorvastatin, when used at their standard dosages, have no statistically significant difference in their effect on the long-term prevention of cardiovascular events [Zhou Z, 2006].

3.2.2.The effect of statin dosage on carotid plaque imaging features

The intensity of the statin-dosage scheme is another area of considerable controversy due to potential side effects. However, from a recent metanalysis of HMG-reductace inhibitor trials and other preventive strategies including more than 160,000 patients it was evident that the lower the LDL level, the lower the relative risk for stroke. [Amarenco P, 2004] The conclusions were the same regarding secondary prevention for non-cardioembolic stroke. Unfortunately, only a few studies were identified from our review, investigating the effect of intense statin treatment on plaque morphology and even fewer examined the effect of targeting LDL levels<100mg/dL. [Makris GC, Lavida A, 2010]

In a recent experimental study in fat apolipoprotein E-deficient mice it was found that fluvastatin may have an LDL-independent, pleiotropic effect on plaque stabilization [Nakamura K,2009]. The methodology applied was however, debated by researchers and further research on this issue appears to be necessary. Until then, the existing clinical evidence favours the fact that it is the effective lowering of LDL rather than the aggressive use of statins, which stabilizes carotid plaques [Corti R,2005; Watanabe K, 2005; Kadoglou NP,2008; Kadoglou NP,2009].

3.2.3. The use of combined lip lowering treatment.

Two large systematic reviews suggested that targeting higher HDL levels and/or lower triglyceride levels could be beneficial in terms of stroke prevention [Amarenco P, Labreuce J,2008; Labreuce J,2009]. This effect could be anticipated since the atherosclerotic process is not only associated with LDL levels but also with the function and levels of HDL and triglycerides. These are also considered major determinants of cardiovascular risk [Grundy SM,2004; Adult Treatment Panel III, 2001]. Niacin, fibrates and omega-3 fatty acids are the main agents that could be considered for combination treatment with statins. This could

establish better total lipidaemic control. There is accumulating evidence that combined lipidaemic treatment with niacin [Brown BG,2001], fibrates [Corti R,2007] and/or omega-3 fatty acids [Iso H,2001; He K,2002] may further increase plaque stability , thus,potentially reducing the rates of stroke. However, only two studies have reported the direct effect of combined treatment with statin and niacin [Zhao XQ,2001; Phan BA,2007]. In addition only one study measured the effect of statin and omega-3 fatty acids using carotid IMT [Mita T,2007]. More studies are required in order to establish if there is any clinical benefit from the use of lipid-lowering agents other than statins in terms of primary and secondary stroke prevention.

3.2.4. Imaging techniques and markers for carotid plaque evaluation after medical treamtment.

In the majority of the included trials in our systematic review [Makris GC, Lavidas A,2010] , MRI and 2-dimensional ultrasound imaging have been applied to evaluate plaque changes in relation to statin treatment. The imaging features that were evaluated ranged from plaque size to composition but only a few of them investigated the effect on both these characteristics [Zhao XQ,2001; Phan BA,2007; Yamagami H,2008, Underhill HR,2008]. In addition, information about the effect of statins on intraplaque haemorrhage, ulceration and neovascularisation was scarce and no significant data about the response of these characteristics to statin treatment was identified. Recent studies have shown that these characteristics are associated with plaque instability and rupture [Gao P,2007; Fisher M, 2005; Gao P, Chen ZQ,2007] and therefore further evaluation of their relation to statin administration is needed.

Despite the evidence that IMT is a strong predictor of future cardiovascular events [Lorenz MW,2007], the Tromso study showed that in a general population carotid plaque area can

be a stronger predictor of myocardial infarction than IMT [Johnsen SH,2007]. New markers of extra-coronary atherosclerosis have been proposed for the evaluation of plaque composition and area/volume (size). A small number of studies evaluating the effect of statin administration on both plaque size and composition were identified [Zhao XQ,2001; Phan BA,2007; Yamagami H,2008; Underhill HR,2008]. However, due to the lack of clinical end points, such as stroke or TIA rates and appropriate duration of the follow-up, no direct comparisons can be made between the predictive values of plaque size versus plaque composition. There are potential benefits in measuring 3-dimensional volume instead of composition- as significant effects of therapy can be detected in just 3 months with relatively small sample sizes [Ainsworth CD, 2005].

3.3 Limitations of the current evidence

There is considerable heterogeneity between the clinical studies evaluating the effect of medical management on carotid plaque characteristics. This heterogeneity is mostly due to the variation in selected patient-populations, imaging modalities used, and the duration and type of statin treatment. Additionally, only nine randomized studies were found, three of which were of high quality. The number of included patients was generally small in the majority of the randomized and non-randomized trials and there was no common primary endpoint between most of them. Although the literature search was performed systematically publication bias should be taken into account when interpreting the results of this review. [Makris GC, Lavida A 2010]

Van Bortel and his group [Kips JG, 2008] reviewed the imaging techniques currently used to identify vulnerable plaques, including those utilized in the studies we reviewed. Each technique has limitations, which must be taken into consideration when evaluating the effects of treatments on atherosclerotic plaque. 2D ultrasound can only provide limited views (longitudinal and transverse), making image quality and information content highly variable and operator dependent. Moreover, once an image is captured, additional views in other planes cannot be extracted. Preliminary results from 3D ultrasound studies suggest that some of these issues can be addressed using this novel technique [Chiu B,2008; Landry A,2005]. Nevertheless, it is also a heavily operator dependent technique which warrants further assessment and standardization.

More recently the introduction of angio-MRI (MRA) and angio-CT (CTA) have revolutionized volumetric vascular imaging. However their limited accessibility, increased cost, invasive nature, increased patient inconvenience and the possibility of contraindications (in patients

allergic to the contrast or with renal impairment) limit their use. Particularly with MRA, a major limitation is the poor spatial (400μm) and temporal resolution [Hatsukami TS,2000]. Plain MRA can be utilized to assess the arterial anatomy, severity of stenosis and spatial resolution but only high-resolution MRI can identify crucial plaque components [Fuster V,2005] such as calcification, haemorrhage and lipid rich core.

PET may also provide us with information regarding inflammation within plaques (i.e. macrophage activity), a useful marker for more metabolically active vulnerable plaques [Landini L,2003]. Nevertheless, due to its limited resolution, an adequate description of anatomical information cannot be provided. Thus, it must be combined with another imaging technique, like MRI, with obvious cost implications. Finally the exposure to radiation has to be significantly decreased before long-term follow-up studies can be performed.

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3.4.Conclusion

The current evidence regarding the effect of medical management on carotid plaque morphology and serum inflammatory markers suggests that carotid plaque morphology (size and composition) may be beneficially affected by statin administration.

However, due to the significant heterogeneity between the studies, the lack of large, high quality randomized trials and the possibility of publication bias, solid conclusions are hard to reach. In addition the clinical significance of the attributed changes in carotid plaque morphology remains unknown, along with the question of whether this is an LDL associated effect or a separate pleiotropic phenomenon of statins.

Properly designed studies that will investigate the sole or combined effect of statins with other lipid-lowering treatments on carotid plaque features and on the rate of cerebrovascular events, are warranted. Advanced imaging techniques such as high-resolution MRI and 3-dimensional ultrasound in combination with sophisticated image analysis software may also have a role in this direction.

Chapter 4

Emerging imaging techniques for the evaluation of carotid atherosclerosis: The MRI advances

4.1. Introduction

MR imaging is a relatively new tool that has found significant applications in vascular imaging due to the excellent soft tissue characterisation capabilities that it provides. A small number of studies have shown that vessel and plaque quantification measurement using MR can has be reproducible but there is still significant variability that should be taken into account in the design of future prognosis studies and clinical trials. This is especially true with regard to specific features such as the reproducibility of fibrous cap identification that needs to be improved. [Touzé E,2007]

Novel MR-defined plaque features of vulnerability are emerging and appear promising for the identification of the vulnerable plaque. In a recent systematic review in a total of 779 subjects it was demonstrated that the presence of plaque features such as intraplaque haemorrhage, lipid-rich necrotic core, and thinning/rupture of the fibrous cap is linked to an increased risk of future stroke or transient ischemic attack in patients with carotid atherosclerotic disease. Dedicated MRI of plaque composition can offer stroke risk information beyond measurement of luminal stenosis in carotid atherosclerotic disease. [Gupta A, 2013].

Although MRI is promising with regard to its clinical application for plaque characterisation, it will require further consensus regarding MRI settings and confirmation by histology. In a recent systematic review of the literature where more than 17 studies were included only two studies were performed on a high-resolution 3.0-T MRI scanner whereas the majority was performed on a 1.5-T scanner. This review of the available evidence suggested that it is probably premature for to routinely use MRI as an imaging modality to assess carotid atherosclerosis characteristics, which can be associated with plaque vulnerability. At the moment no well established predefined protocols for histology and MR imaging are in place

and further consensus will be needed with evidence provided by future studies.[den Hartog AG,2013]

Purpose of this chapter is to review the most important recent advances in MRI technology allowing better imaging and modelling of carotid atherosclerosis.

4.2.Novel MRI techniques

4.2.1. High resolution MR imaging above the 1.5Tesla

Currently the majority of MR based carotid imaging is performed using 1.5T magnets. The continuous advances in MR technology have allowed carotid imaging at higher magnetic strengths. Preliminary experience with eight-channel or higher neurovascular coils at 3T indicates an increase in signal to noise ratio (SNR) to contrast to noise ratio (CNR) compared with 1.5T, which is likely to improve resolution of carotid MR angiography. In a study of 18 subjects with carotid atherosclerosis 1.5 and 3 T systems using phased-array coils were used for imaging. [Young VE, 2012] T(1) weighted (T(1)W), T(2) weighted (T(2)W) and proton density-weighted (PDW) images were obtained and multiple slices were prescribed to encompass both the carotid bifurcation and the plaque. In this study the mean improvement in SNR in plaque was 1.9, 2.1 and 2.1 in T(1)W, T(2)W and PDW images, respectively, which were statistical significant. In a similar study by Furie et al.,[Hinton-Yates DP,2007] in a total of 10 subjects, the use of 3.0 T for carotid plaque imaging resulted in an increase in SNR and CNR compared to 1.5 T.

However, improved resolution may not be the most exciting application of 3T MR imaging. Several plaque components potentially identified on 3T MR imaging are correlated with recent ipsilateral carotid thromboembolic symptoms. The combination of 3T and sensitive surface coils can improve the evaluation of carotid plaque composition, which would contribute towards the shift of MR imaging away from a strict evaluation of luminal narrowing to a dedicated evaluation of plaque morphology and texture. This notion was demonstrated in a study by Demarko et al, in which 19 patients were imaged at 3T. In this study there were significant associations between the presence of thin/ruptured fibrous cap (100% versus 36%, P = .006) and lipid-rich necrotic core (100% versus 39%, P = .022), with marginal association with haemorrhage (86% versus 33%, P = .055). [Demarco JK,2010]

4.2.2. 2D versus 3D MRI

The use of traditional 2 dimensional (D) MR imaging versus 3D for carotid plaque evaluation is still a matter of considerable debate. In a 2 dimensional sequence, each radiofrequency (RF) pulse excites a only narrow slice, while in a 3D one, each RF pulse excites the whole imaging volume using phase encoding to discriminate spatially. For general MR use, 3D sequences have shown to achieve greater sensitivity since each acquisition represents an average of the entire sampled volume. In a study by Balu et al, a total of 18 subjects with significant carotid stenosis were scanned with 2D (2-mm slice thickness) and 3D (1-mm/0.5-mm slice thickness). [Balu N,2008] Morphological measurements, signal-to-noise ratio (SNR) in the wall and lumen, and wall-lumen contrast-to-noise ratio (CNR) were compared between 2D and 3D images. The lumen SNR, wall SNR, and CNR were comparable between the two methods. There was also no difference in average volumetric measurements between 2D/3D. In contrast the distributions of small plaque components such as calcification were better characterized by the 3D acquisition while there was a higher sensitivity to motion artifacts with 3D imaging, resulting in a small number of examinations with low image quality. The conclusion from this study was that 2D and 3D protocols can provide comparable morphometric and volumetric measurements of the carotid artery with the 3D imaging offering an advantage in terms of small plaque component visualization but with the price of lower reliability for image quality.

In another study [Takano K, 2012], twenty-two patients scheduled for carotid endarterectomy underwent carotid plaque MR imaging with both 2D and 3D MR sequences and the quality of images as well as the SNR was evaluated. These observations were compared for each plaque component according to the histological category of the plaque between 2D and 3D sequences. No significant differences were observed among the overall imaging quality scores of the two modalities, although 3D sequences allowed visualization in

random orientations, as well as better depiction of small plaque components such as ulcerations and calcifications. The SNR of the plaque to the submandibular gland on T1-weighted 3D sequence was significantly higher than that on 2D sequence. In addition it was shown that the SNR of the plaque to the submandibular gland of histology-defined soft plaque components were significantly higher on T1-weighted 3D sequence than on 2D sequence. There were no significant differences between the two T1-weighted sequences for hard components. From this study it was concluded that 3D variable-flip-angle turbo spin-echo is a promising tool for the diagnosis of carotid plaques and can improve the characterisation of certain plaque components. Further research is warranted.

4.2.3. Dynamic contrast enhanced MR imaging

The theory of intraplaque neovascularisation being associated with active intraplaque inflammation and increased risk for rupture has gained popularity the last decade. In the past, dynamic contrast enhanced (DCE) MRI was extensively utilised to study the vascularity and neovascularisation within tumours. Recently though, further applications came to light with delayed and dynamic contrast enhanced (CE) MRI being introduced as non-invasive tools to assess the extent of plaque neovascularization in animals and patients with atherosclerosis. [Calcagno C, 2010]. Gadolinium is a contrast agent typically administered for routine MR angiography and can be used to improve plaque characterization. Gadolinium has favourable properties that allow enhancement of the cap, more reliable vessel wall measurements, and better visualisation of the intraplaque neovessels. The above properties combined with the pre-contrast series can help in the identification of intraplaque haemorrhage. At the moment there is a limited number of studies evaluating the usefulness of these features in stroke risk prediction.

In a study by a Yuan et al [Sun J,2013] , symptomatic patients with carotid plaque disease underwent bilateral carotid artery MRI examination, which included multi-contrast

sequences for detecting intraplaque haemorrhage (IPH) and a DCE MRI sequence for characterizing adventitial perfusion. Kinetic modelling of the dynamic contrast-enhanced MRI time series was performed to estimate adventitial perfusion and K(trans). The presence of IPH was linked to a significantly higher value in adventitial K(trans) (0.142 ± 0.042 vs 0.112 ± 0.029 min(-1); P<0.001). This relationship remained significant after adjusting for other confounding factors. This study demonstrated an independent pathophysiological link between the adventitia and IPH and related it to the microcirculation of the adventitial vasa vasorum. Adventitial perfusion imaging may be useful in studying plaque pathogenesis, but further studies are deemed as necesary.

In another study [Gao T, Zhang Z,2009], it was shown that different types of stroke can be identified by brain MRI detection before invasive therapies. In this study 102 consecutive subjects with and without cerebrovascular event were studied with contrast-enhanced carotid MRI, brain MRI and magnetic resonance angiography. This study demonstrated that from 63 patients with mild to moderate stenosis (< or =70%), 44 (69.8%) had vulnerable plaques, which was significantly more than those in patients with severe stenosis (>70%; p < 0.001).

Finally in a study by Millon et al., sixty-nine patients scheduled for a carotid endarterectomy underwent a 3-T contrast enhanced MRI. [Millon A,2012] The carotid plaque enhancement was assessed on T1-weighted images performed pre and post contrast administration. Histological analysis was performed of the entire plaque and of the area with matched contrast enhancement on MR images. Gadolinium enhancement was observed in more than 50% of the patients. There were three types of carotid plaques that were identified depending on the enhancement pattern. In other words they were grouped according to whether it was the shoulder region, the shoulder and fibrous cap or the central part of the plaque enhancing more. Fibrous cap rupture, intraplaque haemorrhage, and plaque

gadolinium enhancement was significantly more frequent in symptomatic than in asymptomatic patients (P=0.043, P<0.0001, and P=0.034, respectively). After histological analysis, gadolinium enhancement was significantly associated with vulnerable plaque (American Heart Association VI, P=0.006), neovascularization (P<0.0001), macrophages (P=0.030), and loose fibrosis (P<0.0001). The presence of neovessels, macrophages, and fibrosis in the enhancing area was 97%, 87%, and 80%, respectively, and was different depending on the enhancement location in the plaque. From this study it appears that the enhancement of carotid plaque is associated with vulnerable plaque phenotypes and related to an inflammatory process.

4.2.4. Ultrasmall superparamagnetic iron oxide (USPIO)

Sinerem is an ultrasmall superparamagnetic iron oxide (USPIO), which has significant uptake by macrophages and thus it, can act as a marker of inflammation. The application of USPIOs in MRI imaging has helped us to track the macrophage activity in carotid atherosclerosis. In a study Tang et al., twenty symptomatic patients underwent multi-sequence MRI before and 36 hours after USPIO infusion. [Tang T, Howarth SP,2006]. The images were manually segmented into quadrants, and the signal change in each quadrant was assessed after USPIO administration. All symptomatic carotid plaques appear to have inflammatory components with an increase in USPIO uptake. This study highlights the argument that carotid atherosclerosis can be a truly systemic disease. In addition, in another study by Tang et al., the relationship between the degree of MR-defined inflammation using USPIOs particles and the severity of luminal stenosis in asymptomatic carotid plaques was explored. It was shown that there was no significant relationship between the degree of inflammation and the degree of luminal stenosis, supporting the theory that the currently used as gold standard, degree of stenosis, is not the best marker for stroke risk evaluation. [Tang TY, Howarth SP,2008]

In another new promising application of MRI-USPIOs, it was combined with PET-FDG for the evaluation of the carotid atheroma inflammation. In a small feasibility study, in two patients, it was shown that the imaging results from the two types of imaging were complementary and concordant, implying that both techniques mirror similar metabolic processes. The main conclusion of this study was that the inflammatory activity identified by these two possibly complimentary methods, might act as a possible surrogate risk marker for the identification of patients who are suitable for carotid surgery. Obviously solid evidence is still lacking regarding the practical clinical outcomes and further research is warranted regarding its cost effectiveness. [Tang TY, Moustafa RR,2008]

4.3.Combination of MRI with PET for the investigation of carotid plaque disease

In recent years hybrid scanners such as PET/CT have emerged as a useful modality in clinical routine as well as an important research tool. The newly developed fully integrated PET/MR scanners combine excellent soft tissue contrast and functional imaging capabilities. Their combination can quantify plaque neovascularization and inflammatory infiltrate, respectively. Previous studies have shown that plaque (18)F fluorodeoxyglucose (FDG) uptake correlates with macrophage content and that it may be an alternative modality to directly identify the inflamed plaque in carotid artery stenosis. In this section we are presenting the available evidence on the combination of these two techniques for the identification of the vulnerable plaque. [Davies JR,2005]

In a study by Kwee et al., 50 patients with symptomatic carotid stenosis were assessed with CT, MR and FDG-PET imaging and the agreement between them was compared. It was shown that correlations between (18) F-FDG PET and CT/MRI findings are weak, however, correlations between CT and MRI measurements appear to be moderate to strong, but with considerable variation in absolute differences. [Kwee RM,2009].

Finally in a study by Davies et al.,only in 7 out of the 12 patients waiting for an endarterectomy, high FDG uptake was seen in the lesion targeted for endarterectomy. In the remaining 5 patients, FDG uptake in the targeted lesion was low. [Davies JR,2005] In these 5 patients, 3 had non-stenotic lesions identified on HRMRI that exhibited a high level of FDG uptake. All 3 of the highly inflamed non-stenotic lesions were located in a vascular territory compatible with the patients' presenting symptoms. This was one of the first studies to suggest that angiography may not always identify the culprit lesion. From this preliminary data it appears that combined FDG-PET and HRMRI might be able to assess the degree of inflammation in stenotic and non-stenotic plaques, which could potentially be used to

identify lesions responsible for embolic events even when the degree of stenosis is less than 70%.

Again it seems that the combination of MRI and PET may add significant information useful for plaque characterization. However, the especially high cost of these two techniques combined makes the evaluation of their cost effectiveness mandatory before any safe conclusions can be reached. Further studies are warranted.

4.4. Conclusion

MR imaging has revolutionised many areas of clinical imaging and diagnosis. Lately there has been significant activity in the field of vascular imaging and especially that of carotid imaging and stroke. The identification of the vulnerable carotid plaque has proven to be a particularly complicated problem with many parameters and limitations. Ultrasound has been for years the mainstay of diagnosis and is still the basis of every management plan, however, there is mounting evidence to suggest that it is not enough. MR is currently the most sophisticated imaging method with favourable imaging properties and a wide range of potential “plug inns” that could be very useful for the development of a customised approach with regard to the identification of the vulnerable plaque. At the moment there are many different promising approaches which are based on MR imaging, though, most of them are just at their beginning and further clinical research is need to determine clinical outcomes and cost effectives. Until then ultrasound will remain the first line investigation of choice.

PART II

Methods for 2D and 3D ultrasound image analysis and processing

Chapter 5

Established 2D ultrasound image analysis methodology for carotid plaque characterization

5.1. Introduction

The Asymptomatic Carotid Stenosis and Risk of Stroke (ACSRS) Study was an international, multicentre, natural history study of asymptomatic patients with internal carotid artery (ICA) stenosis >50% (ECST criteria). The principal aim of the ACSRS study was to identify subgroups of patients with annual stroke risk of less than 1% and more than 4% using local (plaque characterization and texture analysis) and systemic factors in addition to the degree of stenosis [Liapis CD,2009].

Bilateral carotid ultrasound scanning was performed on admission to the ACSRS study and the entire duplex investigation was recorded on VHS videotape and sent to the coordinating centre. The degree of stenosis was graded according to established velocity criteria and expressed as both ECST and NASCET percentages. Our group over the years has developed a unique methodology and software for objective characterisation of carotid plaque composition.

The exact image acquisition technique will be described in this chapter.

5.2.Methods

5.2.1.Ultrasound duplex examination

Two dimensional Ultrasound image capture Ultrasound examinations were performed using duplex scanning and colour flow imaging (IU2200,Phillips Ultrasound System, USA). Duplex scans and plaque images were recorded on S-VHS videotapes and later on DVDs discs. A S-VHS "Panasonic" video cassette recorder (model AG-7350-B, Matsushita electric Ind. Co. Ltd, Japan) and an external capturing device-frame grabber (Snappy v2, Play Inc., Ca, USA) were used to transfer the images from the videotapes to a computer, saved as Tag Image File Format (Aldus TIFF) files at the initial stages of the ACSRS study.

5.2.2. Standard ultrasound imaging settings and degree of stenosis assessment

The degree of stenosis was graded according to established velocity criteria and expressed as both ECST and NASCET percentages [Liapis CD,2009; Nicolaides AN, Kakkos SK,2010]. A high frequency linear array probe (4 -7 MHz) was used and the following technical ultrasound settings ensured optimum image quality for plaque type classification and texture analysis:

- Maximum dynamic range was used to ensure the greatest possible display of gray scale values.
- Persistence was set on low and frame rate on high, the latter ensuring good temporal scale values.
- The time gain compensation curve (TGC) was sloping through the tissues but was positioned vertically through the lumen of the vessel because to ensure that the brightness of the adventitia of the anterior and posterior walls was similar.
- The overall gain was adjusted to give optimum image quality. This was achieved by adjustment of the gain control to minimize but not abolish noise.
- The ultrasound beam was at 90^0 to the arterial wall.
- The minimum depth was used so that the plaque occupied a large part of the image.

5.2.3. Computer-assisted plaque analysis

Analysis of the baseline carotid plaque characteristics was performed with the use of the "Plaque Texture Analysis" software (version 3.2 –Incosoft International Ltd, PO Box, Greenford, London UB6 9ZN) after image normalization and segmentation. The black and white image and the corresponding colour image (using the colour or power Doppler) of the area of interest were used for the identification of the plaque outline during segmentation (image 5.1) as it has been shown in a recent publication [Fenster A, 2004]. Fifty-one histogram and ultrasound texture features of the grey tones were produced from the outlined carotid plaque area and were extracted (image 5.2) using the following algorithm methods (Appendix I):

(i) Histogram measures.

(ii) First order grey level parameters, including grey scale median (GSM)

(iii) The Spatial Gray Level Dependence Matrices (SGLDM) .

(iv) Gray level difference statistics (GLDS).

(v) Gray level run length statistics (RUNL) .

(vi) The Fourier power spectrum (FPS).

Methods (iii)-(vi) are novel texture analysis algorithms used to study heterogeneity, which will be studied in this thesis. All methods were applied on the region of interest (ROI) selected by the expert physician; pixels used for the analysis were only the pixels of the ROI, and not the pixels of the rectangle that includes the ROI, as several other applications do.

5.2.4. Visual plaque classification

Carotid plaques were classified into five types according to their overall echodensity in:

- Type 1 (completely echolucent lessons, sometimes a thin fibrous cap is visible)

- Type 2 (predominantly echolucent lesions having less than 50% echogenic components)
- Type 3 (predominately echogenic with less than 50% echolucent components)
- Type 4 (uniformly dense echogenic lesions with less than 10% echolucent components)
- And type 5 (calcified plaques with excessive acoustic shadow).

The presence of discrete echogenic plaque components or the presence of 15% or more of the total plaque area as an echolucent plaque component near the lumen (juxta-luminal) was also recorded. [Kakkos SK, Griffin MB,2012]

The above settings were essential prerequisites for plaque texture analysis, which was performed at the coordinating center. Participating sonographer to the ACSRS study had to attend a two-day training course at the coordinating center.

Video-recordings of the examination and snapshot images of plaques were sent to the coordinating center for image analysis. Continuous feedback was provided to the participating centers to ensure quality [Heliopoulos J,2008].

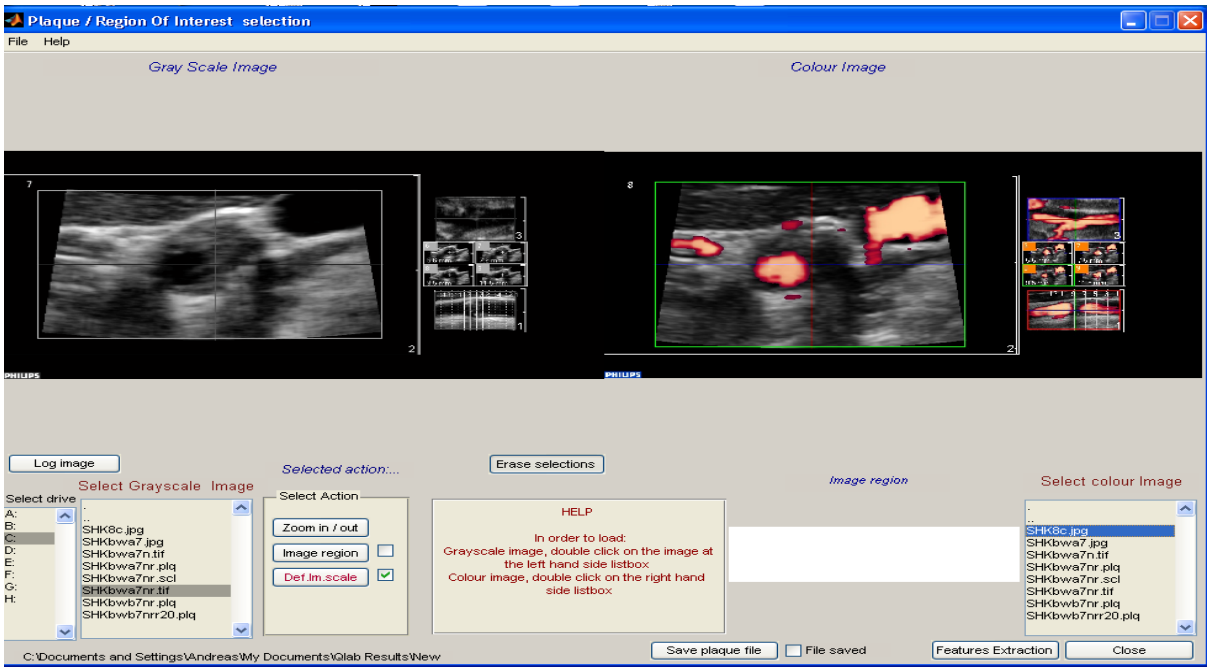


Image 5.1: B-mode and corresponding Doppler image in Inconsoft Carotid Plaque Texture analysis programme as they were acquired by the 2D ultrasound scan.

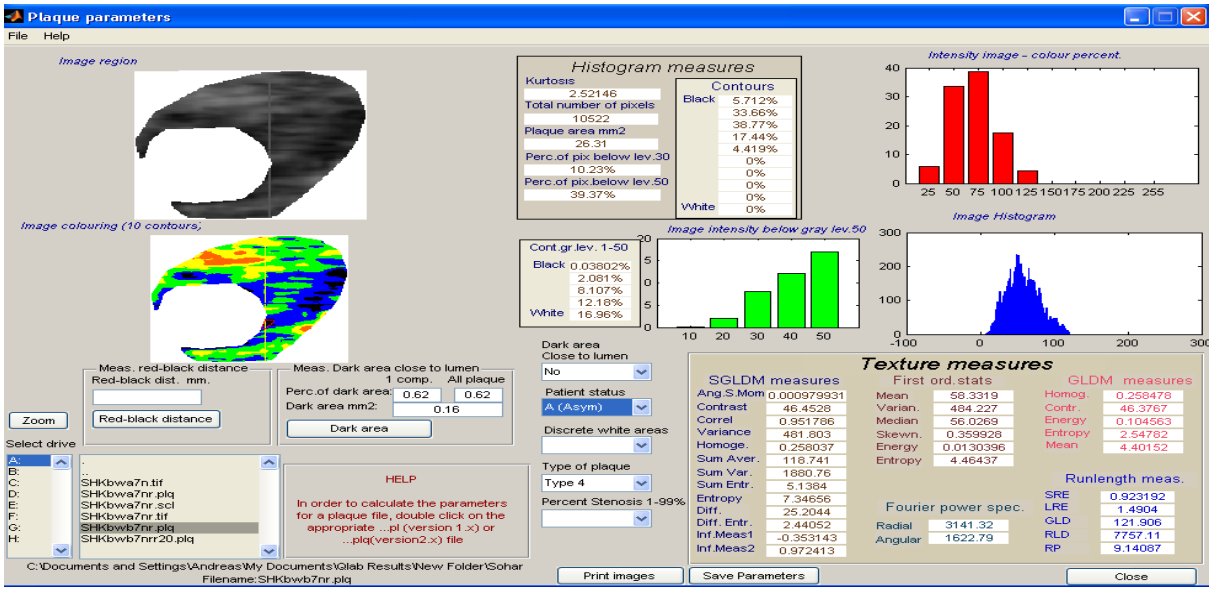


Image 5.2: Example of feature extraction from a specific carotid plaque slice using the image analysis software.

Chapter 6

Development of the 3D image acquisition and analysis protocol

6.1. Introduction

Three-dimensional ultrasound (3DUS) may combine the advantages of volumetric vascular imaging of MRI with those of ultrasound imaging (increased availability, non-invasive nature and decreased cost) [Makris GC, 2011]. The new volumetric acquisition techniques may facilitate the storage of multiple, consecutive and equidistant views, which are subsequently converted in a matrix of points, which are representative of the volume scanned.

The purpose of this study was the development of a protocol to obtain and analyse the composition and volume of an entire carotid plaque using 3DUS.

Our hypothesis is that 3DUS may simultaneously provide us with carotid plaque characteristics such as volume and composition for the entire plaque with the use of a simple, semi-automated method of plaque analysis and commercially available software.

6.2. Methods and protocol development

6.2.1. *2D Ultrasound scanning*

Bilateral carotid ultrasound scanning was performed using a high frequency linear array transducer (4 -7 MHz, Phillips IU22). The ultrasound beam was at 90^{o} to the arterial wall and the minimum depth was used so that the plaque occupied a large part of the image. This way optimum image quality for plaque type classification and texture analysis was obtained (image 6.1).

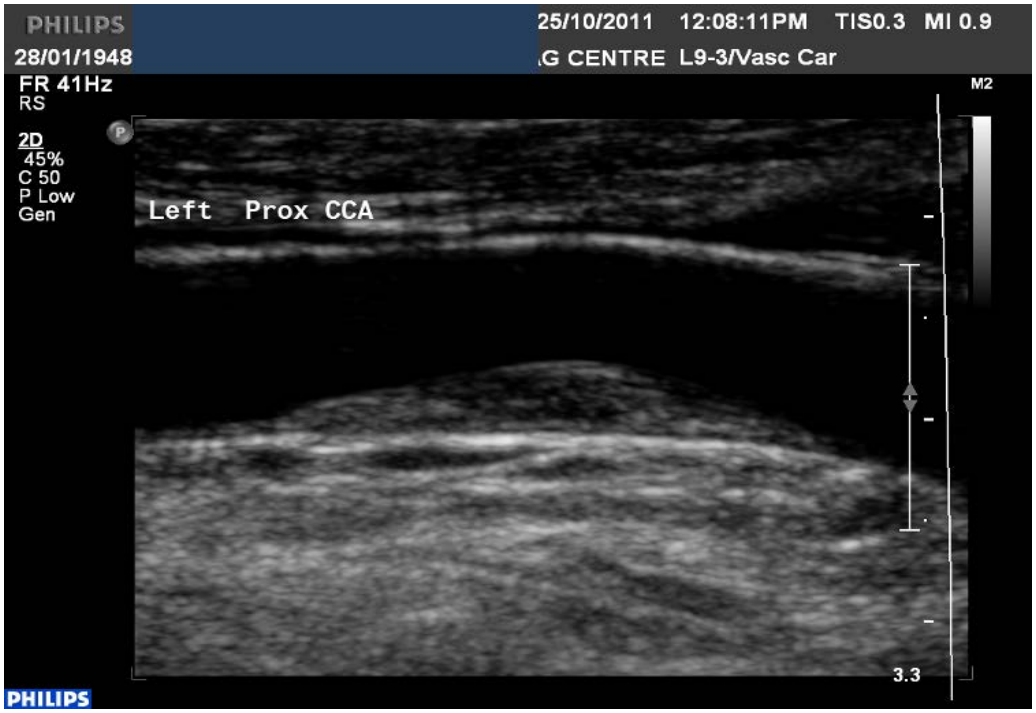


Image 6.1: Common carotid echogenic plaque in B-mode, 2 dimensional ultrasound imaging.

6.2.2.Three dimensional image acquisitions

The VL13-5, 3D broadband, linear array probe (Phillips Co) on an iU22 ultrasound system (Philips Co.) was used to obtain 3D images. After the carotid plaque was identified with the 2D probe the 3D probe was positioned above the lesion. A set of multiple B-mode and the corresponding colour Doppler 3D images of the carotid plaque were automatically acquired.

6.2.3. Segmentation using Q-Lab software

The Qlab software (Philips Co.) is commercially available quantification software with various applications in cardiac, 3D ultrasonography. However certain applications of this software can be applied for the analysis of carotid plaque images. The application “General Imaging 3DQ (GI -3DQ)” provides tools for measuring angles, 2D distance, area and volume The obtained 3D images of the entire carotid plaque volume were uploaded to this software using a regular personal computer. The inter-slice distance was set to 2mm and the number of slices to 13 in order to cover the entire plaque volume (Image 6.2). The software, with the use of the “iSlice” application, automatically produced the corresponding transverse sections for the entire plaque volume (image 6.3). The same procedure was followed for both the B-mode and the corresponding colour images.

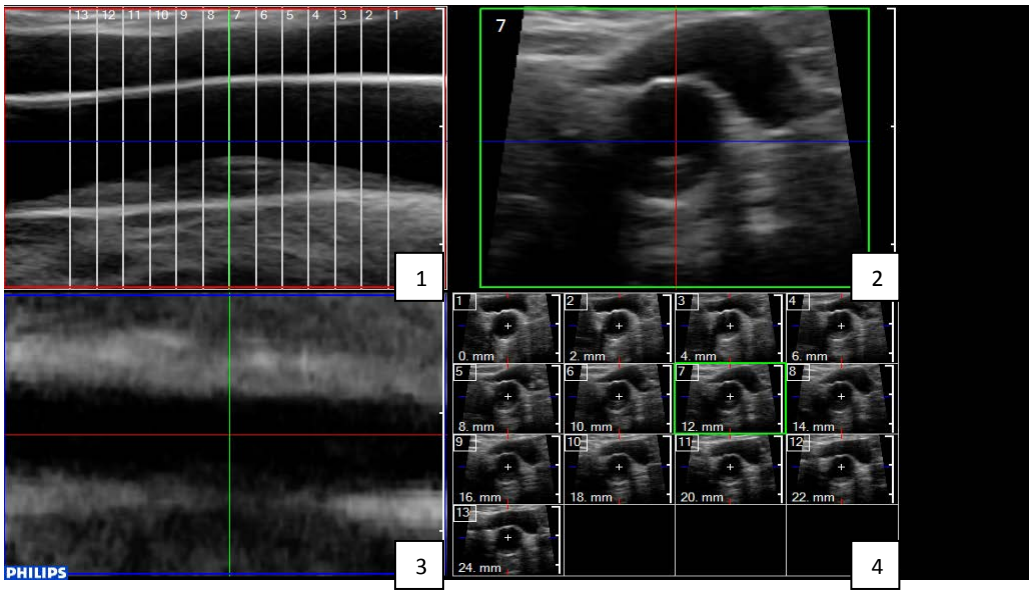


Image 6.2: Q-lab interface: (1) longitudinal section of the carotid plaque as obtained by 3D ultrasound probe; the vertical lines represent the acquired transverse sections, (2) an example of a transverse section of the plaque as acquired by 3D ultrasound, (3) horizontal view of the common carotid, (4) Acquired transverse sections of the entire carotid plaque.

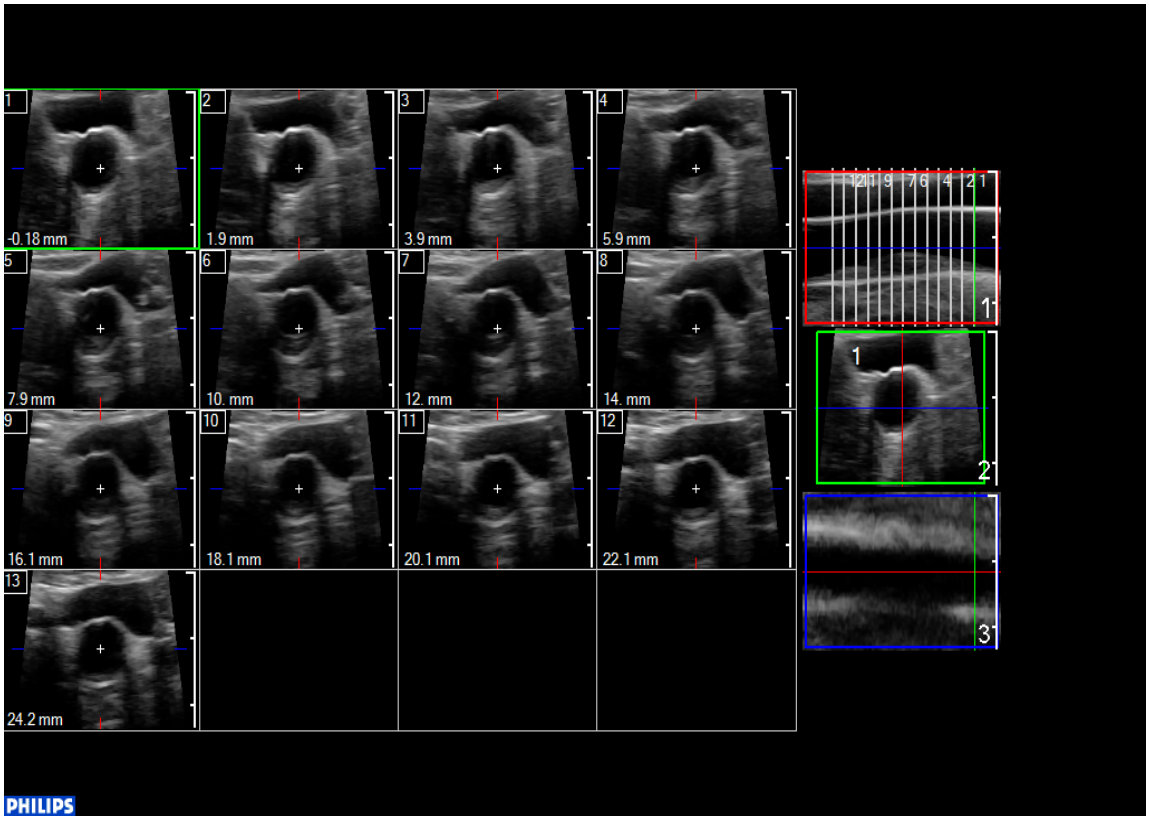


Image 6.3: Thirteen transverse sections as they were acquired from the 3D ultrasound probe. The inter-slice distance was 2mm.

6.2.4. Image analysis

The image analysis was performed using the commercially available "Iconsoft Carotid Plaque Texture analysis" software as described in the previous chapter. For each pair of the colour and the corresponding B-mode transverse images produced by the Qlab software, image normalisation for gray scale, image standardisation to 20 pixels per mm, image segmentation (cropping of the plaque) and texture features extraction were performed as it has been described in previous publication by our group [Kakkos SK, 2009]. The same procedure was repeated for all the 13 3D transverse sections as well as for the 2D longitudinal section.

6.3. Calculation of volume and texture features of the 3D images

Plaque Volume and Volume Texture Feature were then calculated using the following, simple, mathematical formulas: For transverse sections at n mm intervals:

$$\text{Volume} = (\text{Area1} \times n) + (\text{Area2} \times n) + \ldots + (\text{AreaN} \times n).$$

For the mean GSM of the entire plaque the value was calculated as follows:

$$\text{GSMvol} = (\text{GSM1} * \text{Area1}) + (\text{GSM2} * \text{Area2}) + \ldots + (\text{GSMn} * \text{Arean}) / \text{Area1} + \text{Area2} + \ldots\ldots + \text{Arean}.$$

The latest version of the statistical software SPSS (IBM) was used for the analysis of the data.

6.4. Preliminary results and proof of concept

To test this methodology we analysed the carotid plaque (image 6.1), which was obtained from a volunteer during a regular outpatient appointment and after verbal consent was obtained.

6.4.1. Pilot 2D measurements

The GSM and plaque area of the longitudinal section of the plaque were 35.82 and 59.8mm2 respectively. According to these measurements the plaque belongs to a plaque type III.

6.4.2. Pilot 3D measurements

Thirteen transverse sections with an inter-slice distance of 2mm were automatically produced and analysed from the obtained 3D volume (Image 6.3). The mean GSM and plaque area from the transverse sections were 24.7 (SD: 9.5) and 18.8 mm2 (SD: 6.17) respectively. The variability of GSM and plaque area along the various plaque transverse segments is obvious as it is illustrated in figures 6.1 and 6.2. For the entire plaque the GSM and plaque volume were 23 and 490.6 mm^3, respectively.

There was a significant difference between the GSM of the entire plaque as this was acquired during the 3D imaging and the GSM of the ultrasound slice, which was captured with the 2D probe. The echogenicity of the plaque is almost doubled when the whole volume of the plaque is considered. This isolated finding may indicate that, indeed, there can be significant differences in plaque characterization when evaluating a random 2D longitudinal slice of the plaque and when the whole volume of the carotid plaque is being assessed using 3D ultrasound.

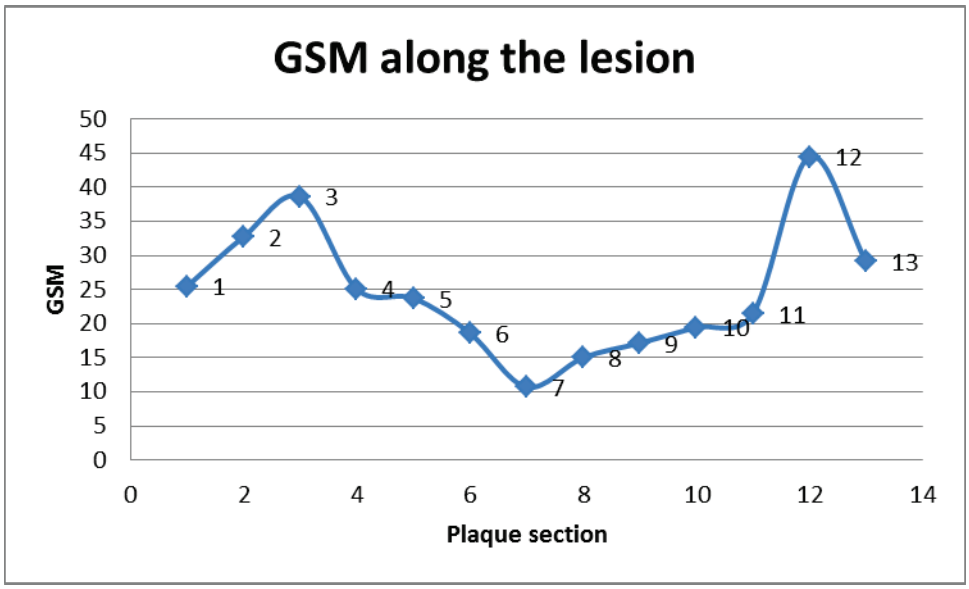


Figure 6.1: Grey scale median (GSM) change of the transverse images long the course of the carotid plaque. The interslice distance is 2mm.

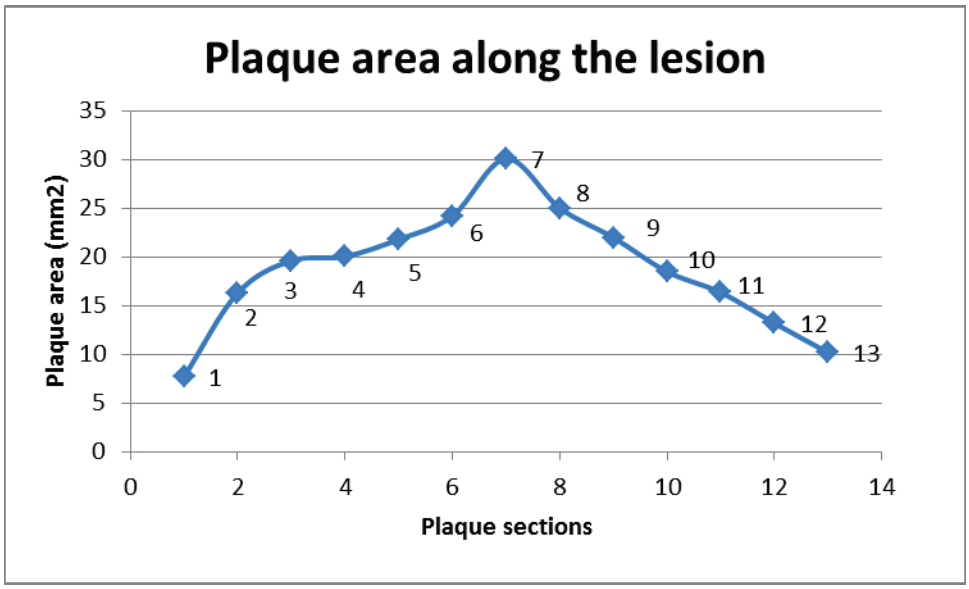


Figure 6.2: Transverse plaque area measurements along the course of the carotid plaque. The inter-slice distance is 2mm.

6.5 Discussion

6.5.1. The value of 2- dimensional ultrasound and the dawn of the 3D era

For a significant number of patients with carotid plaque disease the 2D ultrasound and the subsequent analysis of the information that is provided are sufficient in order to determine the best possible plan of care. However, 2D ultrasound can only provide us with a limited number of views (longitudinal and transverse) and certainly their quality and information that they contain can vary due to its heavily operator-dependant nature. In addition, once the slices (2D) have been captured it is not possible to extract additional planes from the same plaque. The above may have significant implications if we intend to use the composition of the plaque as a risk stratification tool.

The 2D ultrasound provides us with the same information as if we were taking an imaging "biopsy" of a certain part of the plaque, which then we consider as representative of the entire plaque. The reason is that atherosclerotic plaques "grow" in a 3-dimensional environment in all directions both in the longitudinal and transverse axis of the artery. In addition, in the case of heterogeneous plaques the single capturing of one 2D slice of the carotid plaque may be not really representative in terms of composition. Of course, as it was mentioned before, even this approach may be sufficient and cost-effective for a significant number of patients, however, the detrimental effects of cerebrovascular events urge for the investigation of every possible improvement in the diagnosis and management of the vulnerable carotid plaque.

6.5.2.Principles of 3D ultrasound imaging for volumetric and composition measurements

The basic concept behind 3DUS imaging is that by combination and juxtaposition of non-contiguous 2 dimensional ultrasound slices to obtain a 3D volume of the image of the targeted structure. There are three necessary steps, which are required:

i. Acquisition of a series of images through manual or mechanical scanning

Manual scanning using a 2D ultrasound probe and a hand-free technique was utilized at the preliminary stages of 3D imaging development for image acquisition. This technique had an important limitation resulting from the uncontrolled scanning speed, which could affect the accuracy and reproducibility of the volume measurement. This is the reason why the new generation of 3D probes is able to perform mechanical scanning with constant speed. This way the acquired slices are deflected by the same angle and separated by a regular distance and thus there is increased accuracy and reproducibility of the measurements. The types of probes currently available for mechanical scanning are linear probes for linear scanning and convex probes for angular scanning both of which have peripheral vascular applications.

ii. Image transformation in a matrix of corresponding points and volume reconstruction.

Each point of every acquired 2D slice is then placed in space according to specific x, y and z coordinates creating a 3 dimensional matrix of the image. The interslice distance (which can be predetermined) and the interslice spaces are filled by points, which are automatically interpolated from the grey-scale average levels of the 2 adjacent corresponding views. When there is underlying movement of the targeted organ (i.e. during systolic and diastolic vessel movement) ECG synchronization may be required in order to avoid the corrugation of the image.

iii. Computerized processing of the data and volume digitalized illustration and navigation.

Sophisticated software such as the Q-Lab™ (Phillips) can provide us with the significant computer power that is essential for the analysis of the acquired 3D information. The basic concept behind this kind of software is the combination of surface geometric information and the 3D ultrasound representation of the tissue as voxels (3D) instead of pixels (2D). The main reconstructions can be performed off-line using a conventional, personal computer and after the scanning of the patient has been completed without any additional inconvenience for the patient.

There are certain limitations to our methodology mainly because the technique is based on ultrasound principles. Thus, very calcified plaques or very tight carotid stenosis (above 90%) may cause image degradation and make the image analysis of the plaque impossible. In addition because of the bulkier 3DUS probe manoeuvring can be more challenging in patients with high bifurcations. In these patients the use of MRI may be appropriate in order to get compositional information about the underlying carotid plaque.

6.6. Conclusion

Simultaneous analysis of the composition and volume of the entire carotid plaque is feasible using 3D ultrasound and a simple, semi-automated, computerized methodology for image analysis based on commercially available software.

Chapter 7

Development of plaque motion analysis methodology for the identification of the vulnerable carotid plaque.

7.1. Introduction

It has been suggested that plaques may rupture not only as a result of inherent instability, but also due to excessive mechanical forces during the cardiac cycle. Hennericci et al., proposed that plaque surface motion analysis maybe able to distinguish between high and low risk plaques. [Meairs,1999] This approach was based on reconstructions of plaque motions using temporal 3-D ultrasound imaging. This study showed that there were important differences in the maximal discrepant surface velocity motion between asymptomatic and symptomatic plaques. In another study by Murillo et al. a multi-scale motion approach was used to estimate 2-D plaque motion and differentiate between symptomatic and asymptomatic carotid plaque. [Murillo S,2012] Finally Golemati et al. in a more recent study reported on the differences found between the average motion measurements extracted from symptomatic versus asymptomatic cases [Golemati,2012].

In this chapter we will describe a new image analysis methodology for quantifying discordant plaque motion, which is the phenomenon of different parts of the plaque moving in different directions with different velocities during the cardiac cycle. Concordant plaque motion on the other hand is the phenomenon of all parts of the plaque moving in the same direction and the same velocity. Obviously, discordant motion could be related to higher strains, as opposed to concordant motion, which is associated with lower strain values.

Our aim is to develop this methodology for objective plaque motion analysis that will classify and quantify the carotid plaque motions and help clinicians to differentiate between plaques that are likely to rupture because of high internal strains from those with low strains.

7.2.Method

The plaque Motion Analysis (PMA) was studied using customised research software developed by Dr Hamed Nasrabadi and Professor Marios Pattichis from the University of New Mexico, USA.

7.2.1. Ultrasound video acquisition and visual classification

Before any processing the video loops were anonymised and studied blind without knowledge of symptoms. The size of the ultrasound videos was 568 x 448 pixels and the frame rate 40 frames per second (fps).

We extracted a series of 8-10 consecutive cardiac cycles from each video loop that did not include motion artefacts such from neck movement or swallowing. The loops were then categorised visually as with concordant or discordant motion. A carotid plaque was reported as discordant if it had components move in different directions, at certain parts of the cardiac cycle, especially in peak systole. Instead, it was classified as concordant if it had all of its components simultaneously move in the same direction throughout the cardiac cycle.

7.2.2 Video motion estimation

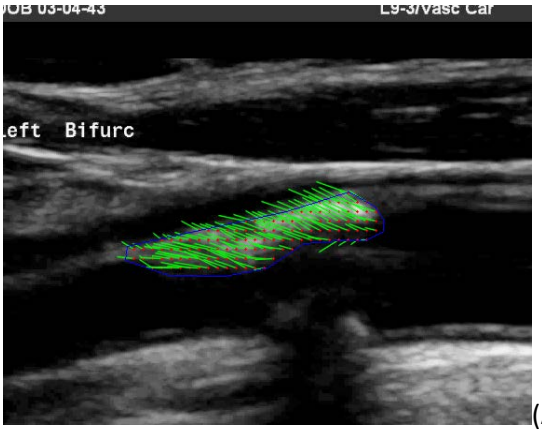
Motion estimation was based on Farneback's method [Farnebäck G,2002]. This method has been shown to perform slightly better than the method developed by Horn and Schunck's [Horn BKP,1981]. Motion was computed between any two video frames, in contrast to the traditional Horn and Schunck's approaches that uses the averages over several video frames [Horn BKP, 1981]. For this motion estimation protocol, we computed the overall motion over a time interval specified by the used as opposed to computing motion between successive video frames. This is specified as the $Two\ Frames\ Comparison\ Interval$ parameter. In this study the interval between different successive frames compared was set to 0.1 seconds.

7.2.3. Plaque motion visualization and analysis

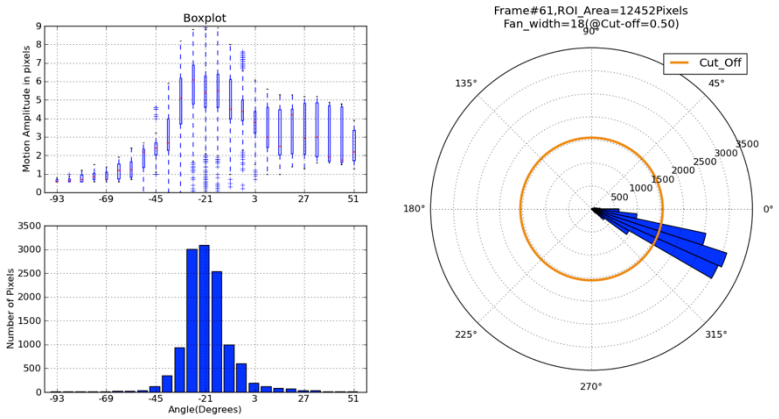
Unstable plaques were those that were seen to move in different directions in peak systole. Stable plaques on the other hand were those that had all their parts move simultaneously in the same direction throughout the cardiac cycle.

Between the different sequential frames the interval compared was 0.1 sec, the distance between the pixels with vectors was 10 pixels and the magnitude of pixel movement was set to 5. Histogram bar width was set at 6 and cut-off to peak ratio (polar-fan) at 0.5.

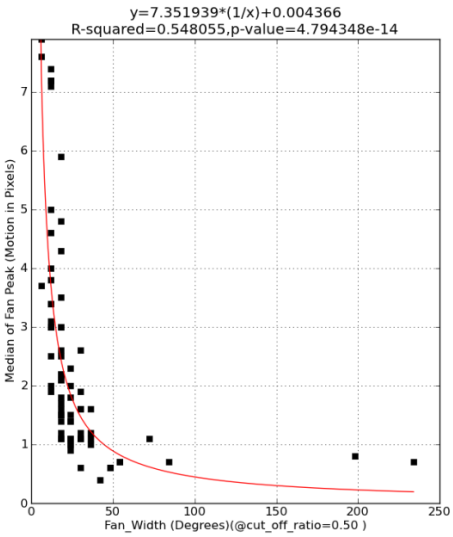
For every cine-loop a plot of peak motion against fan width was obtained (Fig 7.1). From each plot, the maximum fan-width (MFW) corresponding to pixel movement 5 (MFW5), 4 (MFW4), 3 (MFW3) and 2 (MFW2) were obtained. Also, the sum of the MFWs for pixel movements 5 to 3 (SMFW5-3) and the sum of the MFWs for pixel movements 5 to 2 (SMFW5-2) were calculated.



(A)



(B)



(C)

Figure 7.1. (A) Frame 61 at peak systole compared with the frame 57 (interval of 0.2 sec) shows that all the pixels move in the same direction. (B) The data of frame 61 show the histogram of the pixels and the motion amplitude. The narrow fan on the circular presentation confirms that all the pixels move in the same direction (C) Scattergram of peak motion against fan width. There is a characteristic narrow fan-width for peak motion of 7 pixels down to 2.

7.3. Conclusion

It appears that with the use of this methodology and software we can develop objective measures to analyse carotid plaque motion using B-mode ultrasound video loops. This way we can obtain dynamic information regarding plaque interaction with its surrounding environment, which might be useful for further plaque characterisation. The preliminary results from the further evaluation of this methodology will be present at Part V of this thesis.

PART III

The Asymptomatic Carotid Stenosis and Risk of Stroke (ACSRS) Study; post hoc analysis

Chapter 8

The effect of novel texture features of homogeneity and echolucency on carotid plaque characterization; Results from the ACSRS study

8.1.Introduction

The Asymptomatic Carotid Stenosis and Risk of Stroke (ACSRS) Study was an international, multicentre, natural history study of asymptomatic patients with internal carotid artery (ICA) stenosis >50% (ECST criteria). The principal aim of the ACSRS study was to identify subgroups of patients with annual stroke risk of less than 1% and more than 4% using local (plaque characterization and texture analysis) and systemic factors in addition to the degree of stenosis [Nicolaides A, Sabetai M,2003].Clinical factors such as age, cardiac failure, male gender, myocardial ischemia and left ventricular hypertrophy on electrocardiogram (ECG) were independent predictors of increased risk for cardiovascular and all cause mortality according to Cox multivariate analysis [Kakkos SK, Nicolaides A,2005].

Using the above risk factors it was possible to identify a high-risk group consisting of one third of the studied population with 66% all cause death rate and 40% cumulative cardiovascular death rate at 7 years. In addition serum creatinine levels of more than 85 micromols/L, history of contralateral transient ischaemic attacks (TIAs) [Nicolaides AN, Kakkos SK,2005] or silent embolic infarcts on the CT scan [Kakkos SK, Sabetai M,2009] were identified as independent predictors of ipsilateral neurologic events including stroke. The final results of the ACSRS study were recently published indicating that the degree of stenosis, history of contralateral TIAs or stroke, grey scale median (GSM), plaque area, and discrete white area (DWA) were independent predictors of ipsilateral cerebrovascular or retinal ischemic (CORI) events. [Nicolaides AN, Kakkos SK,2010]

Combinations of the above factors could stratify asymptomatic patients into different stratum of risk for ipsilateral CORI events with predicted risk close to observed risk. Of those 923 patients with ≥ 70% stenosis, the predicted cumulative 5-year stroke rate was less than 5% in 495, 5% to 9.9% in 202, 10% to 19.9% in 142, and ≥ 20% in 84 patients [Nicolaides AN, Kakkos SK, 2010].

One of the limitations of 2-dimensional (2D) ultrasound is that it is operator dependent. In the past plaque characterisation was considered as not reliable due to the subjective measures that were used. However, this problem was addressed by image normalisation using two reference points (blood and adventitia) and linear scaling [Griffin M, Nicolaides A,2007]. As a result of this, reproducible measurements of i.e. gray scale median could be performed when the same patients were scanned by different equipment, different operators in different locations [Griffin M, Nicolaides A,2007]. In the ACSRS study the image analysis after normalization resulted in more than 50% of the plaques being reclassified mainly to a higher or lower type and 94% of the events occurred in patients with type of plaque 1 to 3- according to the Geroulakos classification. Patients with plaque types 4 and 5 had a cumulative stroke rate of 0.9% at 7 years (0.14% per year) but patients with plaque types 1 to 3 had a cumulative stroke rate of 14% at 7 years (2% per year). These results suggested that the risk of stroke in patients with plaque types 4 and 5 can be low irrespectively of the underlying degree of stenosis [Nicolaides AN, Kakkos SK,2005].

On the basis of these findings the ACSRS study has shown that plaque classification and grey scale median (GSM) can be used to stratify patient-risk. Nevertheless, a number of other computer-generated texture features, such as first order statistics (FOS), grey level difference statistics (GLDS), grey level run length statistics (RUNL) and spatial gray level dependence matrices (SGLDM) have shown to be promising in differentiating symptomatic from asymptomatic plaques in cross-sectional studies [Kakkos SK, Stevens JM,2007]. Nevertheless, they have not been yet applied to prospective studies such as the ACSRS study. The potential for improved stratification using these novel texture features is due to their ability to provide combined information both for plaque composition, according to grey levels and plaque heterogeneity [Kakkos SK, Sabetai M,2009].

Our hypothesis is that some of these texture features will be associated with the unstable carotid plaques providing risk-stratification over and above that provided by stenosis and GSM alone. These novel features will be tested on the baseline ultrasonic images of the ACSRS study.

8.2.**Methods**

8.2.1. Study population

The study design, the quality control process as well as the baseline characteristics of the included population in the ACSRS study have been previously reported [Nicolaides A, Sabetai M,2003; Nicolaides AN, Kakkos SK,2010]. Professor Stavros Kakkos and the ACSRS collaborators' team under the supervision of Professor Andrew Nicolaides performed the recruiting, ultrasound scanning and data collection during an 8-year period. Briefly the study design was as follows:

8.2.2.Inclusion criteria

Newly referred (<3 months) patients with 50-99% ICA stenosis in relation to the carotid bulb diameter (ECST method) without previous ipsilateral (CORI) events and without neurological abnormality were recruited to the study after written informed consent. Patients who had had contralateral cerebral hemispheric/retinal or vertebrobasilar symptoms or signs of stroke/TIA were included if asymptomatic for at least 6 months prior to recruitment. For patients with bilateral asymptomatic carotid atherosclerosis the side with the more severe stenosis was considered ipsilateral (the study artery).

8.2.3. Exclusion criteria

Patients who could not attend for a six monthly neurological assessment and those with a limited life expectancy because of conditions such as severe cardiac failure or disseminated malignancy were excluded.

8.2.4.Recruitment sources and ethical approval

The participating centres, their eligibility criteria and quality control procedures have been described previously (Nicolaides A, Sabetai M,2003). The Multicenter Research Ethics Committee (North Thames, London, UK) and local ethics committees have approved the study.

8.2.5 Clinical and Biochemical Characteristics

At baseline all patients had a history taken and a physical examination by the local neurologist, electrocardiographic (ECG) examination and collection of fasting blood.

8.2.6. Two-dimensional Ultrasound image capture

Bilateral carotid ultrasound scanning was performed on admission to the ACSRS study and the entire duplex investigation was recorded on an S-VHS videotape and sent to the coordinating centre. The degree of stenosis was graded according to established velocity criteria and expressed as both ECST and NASCET percentages [The European Carotid Surgery Trialists Collaborative Group,1995; Inzitari D,2000]. The exact image acquisition technique has been described elsewhere [Griffin M, Nicolaides A, 2007] and was an essential prerequisite for plaque texture analysis, which was performed at the coordinating centre. Ultrasonographers from participating centres attended two day training at the coordinating centre. Video recordings of the examination and frozen images of plaques were sent to the coordinating centre for image analysis and continuous feedback ensured quality.

8.2.7. Grading of internal carotid stenosis

The velocities were measured at the point of maximum internal carotid stenosis and at the centre of the common carotid artery lumen with the beam of ultrasound at 60 degrees to the direction of flow. Because absolute velocity measurements could underestimate stenosis or overestimate stenosis the sonographers at each centre were trained to use a combination of velocity ratios and absolute velocity measurements [Nicolaides A, Sabetai M, 2003] (Table 8.1).

Table 8.1. Duplex velocity criteria used in the ACSRS study.

Angiographic Diameter Stenosis		Duplex Velocity Criteria				
N %	E %	PSV_{IC} (18,19)	EDV_{IC} (18-20)	PSV_{IC}/PSV_{CC} (21-23)	PSV_{IC}/EDV_{CC} (24,25)	EDV_{IC}/EDV_{CC}
		<120	<40	<1.5	<7	
11	50					<2.6
30	60					
47	70	120-150	40-80	1.5-2	7-10	
60	77		80-130	2-3.2		
65	80	150-250				
70	83		>130	3.2-4	10-20	2.6-5.5
82	90	>250		>4	20-30	
90	94				>30	>5.5
95	99			Trickle Flow		

Abbreviations: N= NASCET; E=ECST; PSV=Peak systolic velocity; EDV= End-diastolic velocity; IC=Internal carotid; CC=Common carotid

8.2.8. Image analysis using dedicated software

Analysis of the carotid plaque characteristics was performed with the use of the "Plaque Texture Analysis" software [Griffin M, Nicolaides A, 2007] (version 3.2 –Incosoft International Ltd, PO Box, Greenford, London UB6 9ZN) after image normalization and segmentation both for the baseline and the follow-up carotid images. More details regarding the plaque's composition image analysis can be found in chapter 5 of this thesis.

8.3 Statistical analysis

Data were analyzed using SPSS 17 statistical software (SPSS Inc, Chicago, Ill). The 51 texture features were evaluated within groups for high correlation (>0.8) using the non-parametric Spearman's rho in order to reduce multi-collinearity. Data were linearly transformed whenever possible before entering them into further analysis using appropriate transformations (i.e. logarithmic, square root – Appendix II). The Kolmogorov-Smirnov test as well as normal Q-Q plots and detrended Q-Q plots were used to assess deviations from normality.

Factor analysis was used to reduce the number of variables retaining only those with potential value to the model. The R-matrix determinant and the KMO and Barlett's values were assessed for R-matrix and sample size adequacy, respectively. Finally the Varimax orthogonal rotation was considered before the extraction of the final factors. Only the variables with loading factors above 0.8 were considered. Hazard ratios for the resulting texture features for ipsilateral CORI events were determined using an unadjusted Cox model. Those texture features that were significant at $p<0.05$ in the unadjusted models for CORI or stroke events were considered in the multivariate proportional hazard models.

Proportional hazards models were created including:

- (a) Only the novel texture features to predict CORI events without any adjustments for stenosis
- (b) Only the novel texture features to predict CORI events adjusted for the severity of ECST stenosis (mild: 50-69%, moderate: 70-89% and severe: >90%
- (c) Established (GSM, plaque area) or other emerging plaque features (discrete white areas) and the resulting novel texture features.

The aim was to assess if there was any improvement in the prediction model after the inclusion of the novel texture features. The diagnostic value of the various texture features and regression models were assessed with the receiver operator curve (ROC) method and expressed as area under the curve (AUC). The covariates included in each model were used to calculate the linear predictor score βχ (Xbeta- the sum of the product of mean-centred covariate values and corresponding parameter estimates) for every subject. ROC curves were constructed for βχ against observed 5-year CORI event rates.

These were compared between the models (a) to (c). Stratified Kaplan Meier curves were also constructed for the resulting texture features after they were divided into their quartiles with and without adjustment for stenosis.

8.4. Results

During the period 1998-2002, 1121 patients aged between 39-89 years (mean age 70±7.7SD, 61% male) were recruited with an average follow-up of 48 months (6-96 months). The baseline clinical characteristics have been previously reported [Nicolaides AN, Kakkos SK,2010]. A total number of 130 first ipsilateral CORI events occurred out of which 59 were strokes (12 fatal), 49 were TIAs and 22 were amaurosis fugax. The baseline ultrasound carotid images were analysed by dedicated software for plaque image analysis and resulted in the extraction of 51 novel ultrasound texture features. After multi-collinearity within the data was reduced to the minimum, 22 texture features were included in the analysis (Appendix II-table 1). Factor, principal component analysis of the resulting, variables selected only the transformed versions of the following 10 characteristics: bel_30, the GSM, SGLD_ASM, SGLD_CON, GLDM_ CON, RUNL_RLD, plaque area, CL_bel30, SGLD_COR and SGLDINM1 (Appendix I, A-C). The remaining features had loading factors of less than 0.8 and, thus, it was decided not to be included in the final analysis (Appendix II-table 4).

Survival analysis including only ultrasound texture features of the baseline images

The hazard ratios for each individual baseline clinical and biochemical features associated with patients with ipsilateral CORI , as well as their effect in the final prediction model, have been previously reported [Nicolaides AN, Kakkos SK,2010]. Therefore only the results from the hazard ratio analysis of the novel texture features will be demonstrated. The multivariate proportional hazard models for the resulting texture features (Table 8.2) showed that the only features that remained significant for this model (with and without adjustment for stenosis) were features SGLD_ASM (HR: 3.4 95%CI: 1.8-6.31,df:1, $p<0.0001$) and RUN_RLD (HR:1.02 95%CI: 1.0-1.04,df:1, $p<0.05$) . On the basis of the continuous variables the linear predictor scores $x\beta$ of this model were calculated for each patient resulting in Figure 8.1 with an area under the ROC curve of 0.836 (SE:0.018, $p<0.001$).

Table 8.2: Proportional hazards model including the resulting texture features from the factor principal component analysis after adjustment for the degree of stenosis.

Features	HR	SE	Wald Score	Sig.
GSM	1.923	3.3	0.03	0.84
SGLD_ASM	*3.450*	*0.3*	*16.13*	***0.000***
BEL-30	1.020	0.1	1.19	0.273
GLDM_CON	3.576	2.3	0.30	0.581
SGLDM_CON	0.846	0.9	0.29	0.864
RUN_RLD	*1.026*	*0.01*	*6.0*	***0.014***
PLAQUE AREA	0.915	0.1	0.31	0.572
CL-BEL30	1.082	0.2	0.15	0.698
SGLDM_COR	0.931	0.6	0.12	0.913
SGLDM_IMC1	5.183	5.4	0.9	0.764

Abbreviations: **SE**: Standard error, **GSM**: Grey scale median, **SGLDM**: Spatial Gray Level Dependence Matrices, **RUN:** Gray level run length statistics, **GLDM**: Gray level difference statistics, **ASM:** Angular Second Moment, CON: Contrast, **RLD:** Run length distribution, **COR:** Correlation, **IMC-1:** Information Measure of Correlation-1 , **HR**: Hazard Ratio

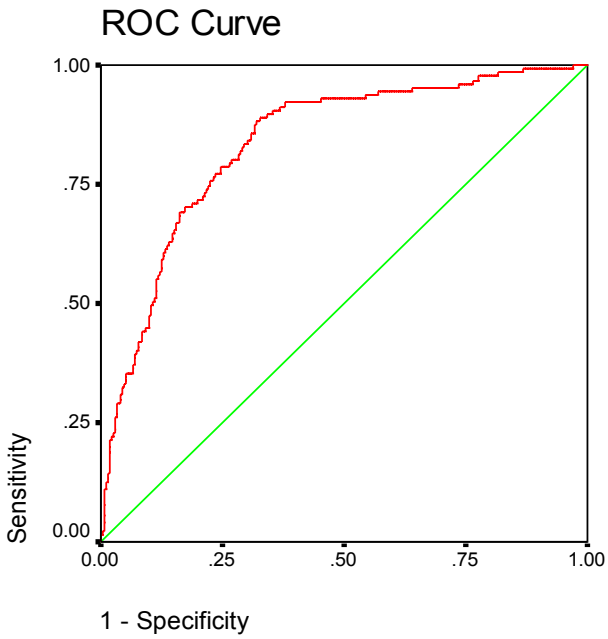


Figure 8.1: ROC curve for CORI events using all novel texture features resulting from the factor analysis. *The area under the curve is 0.836 (SE: 0.018, p<0.001)*

The new texture features SGLD_ASM and RUN_RLD were controlled in the same multivariable proportional hazard model for established plaque characteristics (such as GSM, plaque area in mm^2 and degree of ECST stenosis) and other emerging ones like the presence of discrete white area (DWA). The results (Table 8.3) demonstrated that both SGLD_ASM and RUN_RLD remained significant (HR: 3.3, 95% CI: 2.02 to 5.58, p<0.0001 and HR 1.0, 95% CI: 1.01 to 1.04, p<0.05 respectively). It is also worth noticing that the presence of discrete white areas within the plaque (DWA) was also significant in this model (HR: 0.48, 95% CI: 0.29 to 0.81, p<0.05). The ROC curve which resulted as the linear predictor $x\beta$ of a model including GSM, degree of stenosis and the 2 novel texture features had an AUC:0.829, SE:0.2, p<0.001. In comparison when the model included only the degree of stenosis and GSM the AUC was 0.746, SE: 0.2, p<0.001 (figure 8.2).

The cumulative ipsilateral CORI event free survival Kaplan-Meier curves for the quartiles of the significant, resulting variables SGLD_ASM and RUN_RLD are shown in figures 8.3 and 8.4. The pooled over strata, long-rank test statistic for equality of survival distributions for SGLD_ASM and RUN-RLD was 102. 28 (df: 3, p<0.0001) and 21.81 (df: 3, p=0.0001), respectively.

Table 8.3: Proportional hazard model including the significant, novel texture features (SGLD_ASM,RUN_RLD) controlled for previously established plaque features such as GSM, plaque area, discrete white areas (DWA) and degree of stenosis

Features	HR	SE	Wald Score	Sig.
PLAQUE AREA	0.825	0.138	1.9	0.166
GSM	0.140	1.774	1.2	0.268
STENOSIS	1.013	0.008	2.9	0.085
DWA	*0.487*	*0.261*	*7.6*	***0.006***
RUN_RLD	*1.030*	*0.009*	*9.8*	***0.002***
SGLD_ASM	*3.343*	*0.262*	*21.2*	***0.000***

Abbreviations: **SE**: Standard error, **GSM**: Grey scale median, **SGLDM_ASM**: Spatial Gray Level Dependence Matrices-Angular Second Moment, **RUN_RLD**: Gray levels run length statistics- Run length distribution, **DWA**: Discrete white areas, **HR**: Hazard Ratio

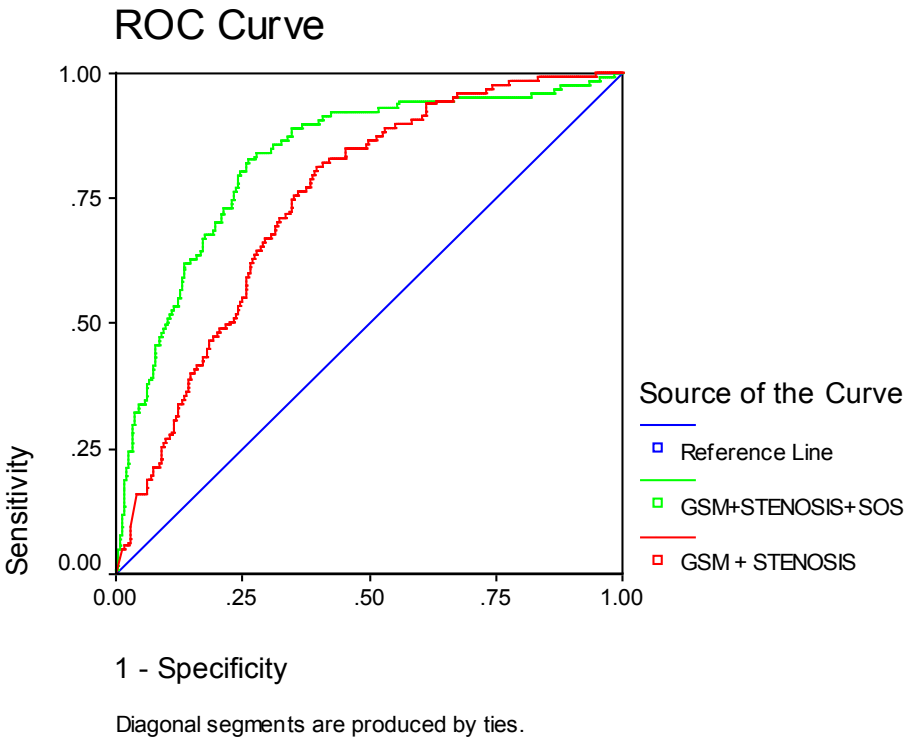


Figure 8.2: Comparison of the two models. The model (green line) including GSM, degree of stenosis and the novel texture features (SOS) appear to be superior than the model with GSM and stenosis alone (AUC:0.829, SE:0.2, p<0.001 vs. 0.746, SE: 0.2, p<0.001, respectively)

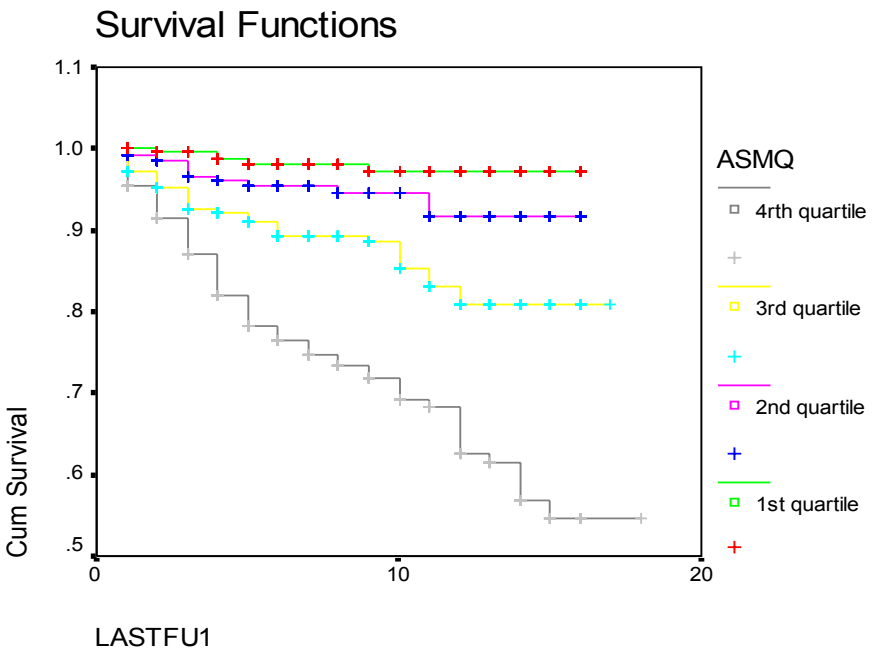


Figure 8.3: Ipsilateral CORI event-free survival Kaplan-Meier curves for SGLD_ASM quartiles (ascending order)

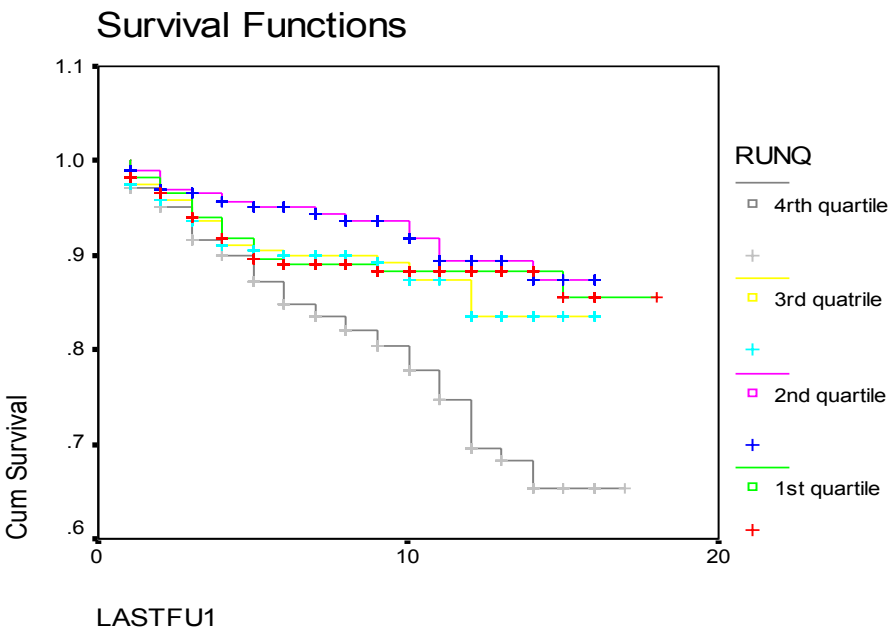


Figure 8.4: Ipsilateral CORI event-free survival Kaplan-Meier curves for RUN_RLD quartiles (ascending order)

8.5. Discussion

Purpose of this study was to investigate if certain SOS measures (alone or in combination with FOS measures), the degree of stenosis and the plaque area can improve the stroke-risk stratification of patients with carotid plaque disease.

The "Iconsoft Carotid Plaque Texture analysis" is dedicated software for carotid plaque image analysis [Griffin M, Nicolaides A,2007]. This software provides, apart from the GSM values (FOS measure) and plaque type, the above-mentioned measures of heterogeneity (SGLSM, GLDS, RUNL and FPS). The analysis of all these texture features of the plaque images from the ACSRS database, with and without adjustment for stenosis, revealed that only two of them (the SGLSM_ASM and the RUNL_RLD features) remained significant in the multivariate proportional hazard models for risk of stroke events. In addition when these two features where controlled for established plaque characteristics such as degree of stenosis, GSM values and plaque area (mm^2), they remained significant and in fact improved the predictive ability of the proportional hazard model for cerebrovascular events when compared with GSM and stenosis alone.

The SGLD_ASM feature belongs to the SOS-SGLD group and is a measure of homogeneity [Haralick RM,1973; Kadah YM, 1996; Wilhjelm JE,1998]. Heterogenous images have more grey-tone transitions, which result into lower readings. Indeed, it was shown, that groups with the different SGLD_ASM levels (i.e. 1^{st} quartile versus 4^{th} quartile) had different rates for cerebrovascular events in the survival analysis. In addition, the texture feature RUN_RLD –which is also a marker of image heterogeneity (Appendix I) showed potential regarding the differentiation between a high risk and low risk for cerebrovascular events event group. The above results in combination with the improved ROC curves ,when the new texture features were used, indicate that the use of both plaque homogeneity and echodensity features may further improve the predictive ability of the models for CORI events. These findings come into agreement with previous findings by Tegos and his

colleagues, who showed that predominantly homogenous plaques with decreased echogenicity could be associated with increased incidence of cerebrovascular events [Tegos TJ,2001].

Despite the large number of patients recruited and the long-term follow-up of the ACSRS study, there are still certain limitations especially with regard to the operator-dependant nature of the ultrasound. Atherosclerotic plaques are 3 dimensional structures that can occupy the whole arterial lumen. This represents a rather challenging environment for the conventional 2D ultrasonography, since different sections of the plaque maybe be captured according to the operator's technique affecting the final outcome of the image analysis. In order to overcome this problem, the use of 3D ultrasound or MRI has been proposed since they may provide us with information regarding the whole volume of the plaque. However, further research is still needed in order to establish their cost-effectiveness [Makris GC, 2011].

In addition, digital image analysis of the carotid plaque is sensitive to the baseline ulltrasound-device settings. In other words, even small deviations from the described protocol for image capture or the use of despeckling filters may ruin the original information contained in the ultrasonound capture and impoverish the results from the image analysis. Further research is also warranted on this issue to determine the exact effect of these new despeckling technologies on carotid plaque characterization techniques.

8.6. Conclusion

This study showed for the first time that objective, computerized evaluation of novel plaque texture features, that incorporates both echogenicity and homogeneity information, may improve our predictive ability and the risk stratification between patients with asymptomatic carotid plaque disease.

Chapter 9

Change of novel texture features through time and implications for stroke risk prediction

9.1.Introduction

The current evaluation of carotid plaques only involves the assessment of the degree of stenosis and its progress through time to extrapolate from that the possible risk of stroke. The degree of stenosis though is not the best marker since it is not very sensitive and it can be difficult to accurately measure especially when very small changes may have occurred. [Nicalaides AN,2010]

The composition of the carotid plaque is another possible marker that may change in time and can be assessed using ultrasound. The use of medication, such as statins, has shown to be affecting plaque composition in different ways. [Makris GC, 2011] The same may happen after plaque rupture when thrombus is forming on the plaque surface. These changes could potential be followed and documented by regular ultrasound and possibly contribute in the identification of the vulnerable plaque.

There is very limited evidence regarding the use of monographic plaque texture change for the evaluation of carotid plaque vulnerability and risk of stroke. Using data from the ACSRS study we will try to explore this issue. It is possible that a combination of measurements through time and during the various follow-up visits can be a useful marker of plaque instability.

9.2.Methods

9.2.1. Study design

The study design, the quality control process as well as the baseline characteristics of the included population in the ACSRS study have been previously reported [Nicolaides A, Sabetai M,2003]
The significant, resulting texture features (from the above mentioned process) as well as GSM and the plaque area were assessed for the degree of their change during the follow-up period within a subgroup of the ACSRS cohort with no change in their degree of stenosis.

9.2.2. Study population

A random sample was selected with both symptomatic and asymptomatic individuals (ratio 1:2 respectively). Eligible for inclusion were patients participating in the ACSRS study with:

- follow-up time > 6months,
- follow-up ultrasound images of adequate quality and
- with no change in the degree of stenosis between the first and last visit.

The image analysis of the baseline and last follow-up visit's images was performed as previously described (chapter 5).

9.2.3. Statistical analysis

The Wilcoxon sign test was applied for non-normal distributions and dependant variables in order to compare the various texture features at baseline and last follow-up visit (i.e. GSMbase -GSMlast f/u). In addition the Mann-Whitney U test (non-parametric) was used to compare the change (delta) of the various texture features between asymptomatic and symptomatic patients (independant variables-i.e. DeltaGSM asympt versus DeltaGSM symptomatics).

9.3.Results

Sixty patients with no change in their degree of stenosis were randomly selected from the ACSRS study. However, 12 had to be excluded due to inadequate follow-up time or inadequate image quality of the follow-up images (i.e. presence of calcification or other artefacts). The baseline and last follow-up image of 33 asymptomatic and 15 symptomatic patients were included in the analysis. The baseline features of this subgroup are summarized in table 9.1.

The changes in GSM, plaque area and the resulting features SGLD_ASM and RUN_RLD were evaluated for a mean follow-up of 43.8 months. There was no change in the degree of internal carotid stenosis in this group of patients through the follow-up period.

When the change of the above features was assessed in all patients with and without symptoms [i.e. feature (baseline)-feature (last visit)] there was a significant change only in GSM and SGLD_ASM (Wilcoxon Signed Rank test, $p<0.05$). There was no change in plaque area and RUN_RLD features (table 9.2). When we separately assessed the change in the symptomatic group, again only the change in GSM and SGLD_ASM between the first and last visit remained significant (Wilcoxon Signed Rank test, $p<0.05$) despite the small sample size. In contrast, in the asymptomatic group there were not significant changes in any of the texture features or plaque area. Finally, there was no statistically significant difference in the degree of change in any of the studied characteristics between the two groups (i.e. DeltaGSM asympt vs. DeltaGSM Sympt), though the small sample size is not sufficient to reach sold conclusions.

Table 9.1: Baseline characteristics of the individuals included in the subgroup-analysis

Features	Mean		SD
(N=48)	Statistic	SE	
STENOSIS (%)	76.25	2.1	14.9
FOLLOW-UP (MONTHS)	43.8	3.3	23.4
GSM	22.5	2.3	16.1
RUN_RLD	12897.6	1045.9	7246.47
SGLDM_ASM	0.03	0.01	0.07

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Abbreviations: **SE**: Standard error, **SD**: Standard deviation, ***GSM***: Grey scale median, **SGLDM_ASM**: Spatial Gray Level Dependence Matrices-Angular Second Moment, **RUN_RLD**: Gray level run length statistics- Run length distribution

Table 9.2. Change of texture features between the first and last follow-up visit in the different groups.

Groups		GSM	Plaque area	SGLD_ASM	RUN_RLD
Symptomatics	**Z^**	-2.215	-0.738	-3.294	-1.079
(N=15)	**Asymp. Sig (2-tailed)**	***0.027****	0.46	***0.001****	0.281
Asymptomatics	**Z^**	-1.492	-1.188	-1.885	-0.027
(N=33)	**Asymp. Sig (2-tailed)**	0.136	0.235	0.059	0.979
ALL	**Z^**	-2.544	-1.456	-3.487	-0.595
(N=48)	**Asymp. Sig (2-tailed)**	***0.011****	0.145	***0.000****	0.552

Abbreviations: ^: Wilcoxon Sign Rank Test, *: Significant change between first and last follow-up visit, **GSM**: Grey scale median, **SGLDM_ASM**: Spatial Gray Level Dependence Matrices-Angular Second Moment, **RUN_RLD**: Gray level run length statistics- Run length distribution

9.4.Discussion

There are several natural history studies in patients with asymptomatic carotid stenosis, which investigated the association between stenosis progression and risk of ipsilateral cerebrovascular events. [Abbott AL, 2008] Despite the fact that most studies had limitations such as the small number of cerebrovascular events, limited duration of follow up and the lack of reporting TIAs and stroke as separate outcome events, they showed that progression to > 80% stenosis was linked to an increased risk of cerebrovascular events. However, again the majority of the included studies concluded that the value of serial screening to predict cerebrovascular events was limited by the low rates of progression but also by the low incidence rates of this outcome event. In our study we sought to assess the effectiveness of plaque composition progression for the characterization of carotid plaques.

The analysis of the follow-up images from a small number of patients with and without symptoms suggests that even when there is no change in the degree of stenosis, certain features such as GSM and SGLD_ASM may be altered in the symptomatic group through time.

This observation implies that composition changes may occur even when stenosis is constant leading to potentially more unstable plaques. The change of certain features during a short follow-up period instead of single, isolated measurements may be more sensitive in the identification of the vulnerable plaque. This observation is in contrast with the current clinical practice where the follow-up of carotid plaque disease is based on isolated measurements of the degree of stenosis and on a rough estimation of plaque composition (i.e echolucent or echogenic) without usually any measure of actual quantification. Nevertheless, more research is needed incorporating larger samples size and longer follow-up

9.5.Conclusion

The composition of the carotid plaque, as this is expressed by the above described texture features, may change through time even when the degree of carotid stenosis is constant. This observation may have significant implications on the way we evaluate the progression of carotid disease and the risk for stroke, since so far the only factor was the change in the degree of stenosis.

Chapter 10

Progression of asymptomatic internal carotid artery stenosis, associated factors and risk of stroke

10.1 Introduction

The main objective of the Asymptomatic Carotid Stenosis and Risk of Stroke (ACSRS) Study , was to assess the cerebrovascular risk stratification potential of combinations of baseline clinical and biochemical characteristics, sonographic degree of stenosis and plaque morphology. As previously described, indeed, the ACSRS study demonstrated that a combination of stenosis, plaque texture features (gray scale median-GSM, size of plaque area and presence of discrete white areas-DWA) and history of contralateral TIAs or stroke can stratify stroke risk from 1% to 10% per year. [Nicolaides AN, Kakkos SK,2010]

The above was achieved by using only the baseline degree of stenosis without any information regarding the progression or not of the disease. However, as mentioned previously a number of natural history studies in patients with asymptomatic carotid stenosis investigated the association between stenosis progression and risk of ipsilateral cerebrovascular events [Abbot A, 2009]. These studies suggested that the value of follow up screening to predict cerebrovascular events was limited. That was due to the low observed incidence rates of these outcome events as well as to the low rates of disease progression. Thus, a secondary objective of the ACSRS study was to assess the value of stenosis progression or regression using six monthly duplex scanning.

The aim of this chapter is to determine:

- The incidence of progression and regression,
- The association between changes in the severity of stenosis and ipsilateral cerebral events
- And finally the association of baseline clinical, biochemical and plaque features with change in the degree of stenosis.

10.2. Methods

The ACSRS study methodology including quality control, inclusion and exclusion criteria, recruitment sources, clinical and biochemical characteristics and duplex examination and image analysis have already been discussed in chapter 5. Only the relevant methodology for this chapter is presented here.

10.2.1.Grading of internal carotid stenosis

The velocities were measured at the point of maximum internal carotid stenosis and at the centre of the common carotid artery lumen with the beam of ultrasound at 600 to the direction of flow. Because absolute velocity measurements could underestimate stenosis or overestimate stenosis the sonographers at each centre were trained to use a combination of velocity ratios and absolute velocity measurements (Chapter 5).

10.2.2.Progression and regression of stenosis

Stenosis was graded into six groups using the above criteria: 50-59%, 60-69%, 70-79%, 80-89%, 90-95% and 95-99%. Progression or regression was considered to occur if there was progression to the next grade up or down of ≥10%, respectively, provided it was found on at least two consecutive visits.

10.2.3. Statistical analysis

Kaplan-Meier curves were initially used for the whole patient population of this study in order to determine the overall stenosis progression, occlusion and regression free survival over time. Then the hazard ratios for biochemical, clinical and ultrasonic features for progression, occlusion and regression were determined using an unadjusted Cox model for each variable. Continuous risk factors were converted to a normal distribution where possible using appropriate transformations.

Kaplan-Meier curves were also constructed for risk factors that were significant in the unadjusted models.

The risk factors that were significant at $P < 0.05$ in unadjusted models for progression, occlusion and regression were considered in multivariable Cox proportional hazards models. ROC curves were constructed to test the ability of each model to predict progression, occlusion or regression by using the linear predictor score ($x*\beta$) to construct. Kaplan-Meier curves were used for the whole cohort to determine overall ipsilateral cerebrovascular and in relation to the observed changes in ipsilateral stenosis.

The stroke predictive ability of progression or regression was assessed in a multivariate Cox models with cerebrovascular events (stroke) as the dependent variable.

SPSS statistical package (version 18, SPSS inc, Chicago, Ill, USA) was used for statistical analysis and production of graphs.

10.3. Results

10.3.1.Incidence of degree of stenosis change during follow-up.

Ipsilateral regression of stenosis by 10% stenosis (ECST% of stenosis) or more occurred in 3.8%. In the majority there was no change (76.4% or 856 patients). Progression by 10% or more occurred in almost 20% of the cohort in which progression without occlusion accounted for 16.9% and progression to occlusion for 2.9%. Progression by more than 20% stenosis units occurred in 27 (2.4%) patients. The incidence of progression was after one year was 4%, after 2 years 10%, after 3-4 years 6%, after 5 years 4%, after 6 years 3% and after 7 years 2%.

10.3.2.Ipsilateral stroke in relation to plaque progression.

From 130 ipsilateral cerebrovascular events (strokes, TIAs and AF) , more than half (67.7%) occurred in patients whose stenosis had not changed, almost one fourth (25.4%) in those with progression but not occlusion, and 9 in those who developed occlusion. There were no events in those with plaque regression. Regarding strokes alone there was a similar picture. From the 59 ipsilateral ischemic strokes again more than half (67.8%) occurred in those with no change in stenosis, 25.4% in those with progression but not occlusion and 6.8% in those that developed occlusion. As above no stroke were reported in the group with carotid plaque regression.

10.3.3.Incidence of stroke and the effect of stenosis and progression

With stroke as the dependent variable in a multivariate Cox model and with ipsilateral degree of stenosis and stenosis progression as covariates, both covariates were significant with hazard ratios of 1.3 (95% CI 1.068-1.687, P=0.01) and 1.85 (95% CI 1.072-3.0202, P=0.027) respectively (Table 10.1). With stenosis as the only covariate, the area under the ROC curve was 0.580 (95% CI 0.504 to 0.657) whereas when progression was added to the model, the area under the ROC curve increased to 0.629 (95% CI 0.555 to 0.704).

Table 10.1 Proportional hazards model with covariates the degree of stenosis and progression and stroke as the dependent variable.

Variable	β	HR	95% CI	P value
Degree of stenosis (10% increase)	0.295	1.343	1.068-1.687	.011
Progression	0.616	1.852	1.072-3.202	.027

10.3.4. Factors linked to progression

In Table 10.2 the hazard ratios for each individual baseline biochemical, clinical, and ultrasonic risk factors associated with ipsilateral progression of stenosis are listed. Male gender, elevated plasma creatinine, increasing plaque area, age and coronary artery disease were significant risk factors associated with increased incidence of progression ($p<0.05$). Lipid lowering therapy (statins) and increasing severity of stenosis appeared to be significant risk factors associated with a decreased incidence of progression.

Model 1-Carotid stenosis progression

In a multivariable Cox proportional hazards model with the significant features from table 2 as covariates, only creatinine, stenosis, plaque area, gender and lipid lowering therapy were independent predictors of occurrence of progression. (Table 10.3) The ROC curve constructed for this model had an area under the curve of 0.637 (95% CI 0.596 to 0.677); $P < 0.001$.

Table 10.2. Unadjusted hazard ratios (HRs) of the risk factors for ipsilateral carotid stenosis progression with or without occlusion, progression and regression.

Risk Factor	*Progression HR*	*95% CI*	*P*	*Occlusion HR*	*95% CI*	*P*	*Regression HR*	*95% CI*	*P*
Age (10 year increase)	**1.19**	**1.01-1.41**	**.044**	1.18	0.76-1.84	.452	**0.62**	**0.44-0.87**	**.006**
BMI (5 units increase)	1.03	0.82-1.15	.712	0.85	0.53-1.35	.486	1.15	0.81-1.63	.424
SBP (10 units increase)	1.08	0.99-1.16	.051	1.01	0.90-1.33	.351	1.00	0.85-1.19	.968
DBP (10 units increase)	1.07	0.93-1.22	.353	1.26	0.88-1.80	.214	1.37	0.99-1.88	.055
Creatinine (20% increase)	**1.22**	**1.13-1.32**	**<.001**	**1.27**	**1.05-1.53**	**.013**	0.89	0.69-1.15	.386
Ln(GSM+40)	0.78	0.51-1.20	.258	**0.24**	**0.07-0.76**	**.015**	0.74	0.28-1.96	.543
GSM < 23	1.21	0.93-1.59	.157	**2.62**	**1.29-5.30**	**.008**	1.01	0.52-1.95	.972
Plaque area $^{1/3}$ (mm^2)	**1.37**	**1.14-1.65**	**.001**	1.46	0.90-2.38	.125	0.82	0.53-1.26	.366
Fibrinogen	1.01	0.87-1.18	.890	1.19	0.82-1.72	.362	1.08	0.75-1.55	.669
Total cholesterol	1.05	0.93-1.18	.419	1.12	0.83-1.50	.463	1.21	0.95-1.56	.124
LDL cholesterol	1.05	0.92-1.20	.436	1.06	0.76-1.50	.721	1.29	0.82-1.45	.572
HDL cholesterol	0.74	0.52-1.05	.095	0.88	0.37-2.89	.770	1.49	0.85-2.64	.164
Triglycerides	1.03	0.90-1.17	.683	0.93	0.62-1.39	.711	1.11	0.84-1.47	.470
Ipsilateral Stenosis (10% increase)	**0.84**	**0.76-0.93**	**.001**	**2.58**	**1.69-3.93**	**<.001**	**1.47**	**1.11-1.96**	**.009**
Contr. Stenosis (10% increase)	1.05	0.95-1.16	.341	**1.39**	**1.11-1.74**	**.004**	1.06	0.91-1.23	.440
Plaque type 4+5	1			1			1		
3	1.16	0.77-1.75	.470	1.12	0.32-4.30	.798	0.95	0.40-2.26	.922
2	1.32	0.87-2.03	.195	2.58	0.74-8.97	.137	0.60	0.22-1.65	.322
1	1.57	0.89-2.76	.120	3.93	0.94-16.4	.061	2.36	0.82-6.73	.109
Male	**1.71**	**1.28-2.28**	**.001**	**3.18**	**1.31-7.74**	**.011**	1.56	0.81-3.01	.179
Smoking	0.91	0.64-1.28	.598	0.79	0.30-2.05	.631	1.34	0.66-2.73	.416
Coronary artery disease	**1.36**	**1.04-1.78**	**.023**	1.73	0.87-3.48	.120	1.14	0.60-2.14	.699
Atrial fibrillation	1.20	0.53-2.71	.656	1.36	0.18-10.0	.760	2.44	0.68-10.1	.219
Hypertension	0.86	0.66-1.13	.292	1.21	0.57-2.55	.622	0.61	0.33-1.13	.119

Diabetes		1.23	0.89-1.69	.201	0.61	0.22-1.76	.364
0.68	0.26-1.72	.412					
History of contr. TIAs or stroke		1.26	0.92-1.72	.146	1.63	0.76-3.53	.211
0.31	0.07-1.29	.109					
Antihypertensive therapy		0.87	0.67-1.14	.324	1.02	0.50-2.08	.959
0.74	0.40-1.36	.329					
Antiplatelet therapy		0.87	0.60-1.27	.481	1.72	0.52-5.64	.373
0.99	0.39-2.54	.992					
Lipid lowering therapy		**0.66**	**0.48-0.91**	**.011**	0.74	0.32-1.73	.494
2.52	**1.38-4.02**	**.003**					
Contr. carotid occlusion		0.93	0.57-1.52	.774	2.07	0.80-5.36	.136
1.89	0.66-4.32	.269					
Presence of DWA (>1)		1.22	0.92-1.63	.165	1.19	0.56-2.52	.642
0.52	**0.29-0.96**	**.037**					

***** *Significant risk factors are in bold.*

10.3.5.*Factors linked to occlusion*

From Table 10.2 it is apparent that only elevated creatinine levels, ipsilateral stenosis, low GSM, male gender and contralateral stenosis were associated with increased incidence of occlusion.

Model 2-Carotid occlusion (table 10.3)

In a multivariable Cox proportional hazards model with the significant features from Table 10.2 as covariates, only GSM, ipsilateral stenosis, contralateral stenosis and gender were independent predictors of occurrence of occlusion. On the basis of model 2 the ROC curve constructed and produced had an area under the curve of 0.793 (95% CI 0.719 to 0.867); $P < 0.001$.

10.3.6. Factors associated with regression

The ipsilateral stenosis and lipid lowering therapy with statins were associated with increased incidence of regression whereas age and the presence of discrete white areas (DWA) were associated with a decreased incidence of regression (Table 10.2).

Model 3- Carotid stenosis regression

In the multivariable Cox proportional hazards model 3 with the significant factors from Table 2 as covariates, the presence of DWA, the degree of ipsilateral stenosis, the administration of statin therapy and the patients' age were independent predictors of occurrence of regression (Table 10.3). On the basis of this model 3, the linear predictor scores $x\beta$ of the model were calculated for each patient and the ROC curve that were constructed had an area under the curve of 0.704 (95% CI 0.620 to 0.788); $P < 0.001$.

Table 10.3:Proportional hazards models including significant variables from Table 2 with ipsilateral stenosis progression (model 1) and occlusion (model 2) as the dependent variable.

Variable	β	HR	95% CI	P value
Model 1: Progression of carotid stenosis as dependent variable				
Creatinine (20% increase)	0.191	1.210	1.114-1.315	<.001
Ipsilateral stenosis (10% increase)	- 0.193	0.824	0.742-0.916	<.001
Plaque area $^{1/3}$ (mm^2)	0.280	1.323	1.095-1.599	.004
Male gender	0.357	1.429	1.067-1.914	.017
Lipid lowering therapy	- 0.375	0.687	0.498-0.948	.022
Model 2: Carotid occlusion as the dependent variable				
Log (GSM+40)	-1.296	0.274	0.084-0.894	.032
Ipsilat. stenosis (10% increase)	0.865	2.376	1.565-3.607	<.001
Contralat. stenosis (10% increase)	0.192	1.212	1.039-1.414	.014
Male gender	1.130	3.094	1.271-7.534	.013
Model 3: Carotid stenosis regression as the dependent variable				
Age (10 year increase)	-0.450	0.638	0.450-0.904	.011
Ipsilat. stenosis (10% increase)	0.466	1.594	1.179-2.155	.002
Lipid lowering therapy	0.820	2.272	1.232-4.189	.009
DWA	-0.693	0.500	0.272-0.920	.026

10.4. Discussion

This study showed that the overall incidence of ipsilateral carotid stenosis progression (19.8%) is similar to that found in past studies with a similar follow-up duration [Abbot A, 2009]. On the other hand the incidence of regression was less common (3.8%).

Regarding the association between carotid stenosis progression and other factors, it has been previously shown that coronary artery disease (Liapis C, 2000), age (Sabeti S, 2007) and hypertension (Bertges DJ,2003; Ballotta E,2007) were strongly associated. Although, this study also suggested that the incidence of progression might also be linked to male gender, elevated creatinine and plaque area. In addition, this is one of the first studies to show that a decrease in the incidence of progression was seen in patients with severe stenosis and those with statin therapy. Creatinine levels and their association with carotid stenosis progression was probably expected since serum creatinine is an independent predictor of cardiovascular risk and especially ischemic stroke as it has been shown by previous research. [Wannamethee SG, 1997; Sprengers RW, 2009] Unfortunately, though, these factors that were associated with progression did not improve the stroke-prediction model and thus it is unlikely to have significant clinical usefulness.

In this study statin therapy was linked to an increase in the incidence of regression. The association between lipid lowering therapy and plaque regression in patients with carotid plaque disease has already been demonstrated as we have also shown in a big systematic review of the available literature (Makris GC, 2010; Spence JD, 2010). In this study the regression prediction using risk factors such as statin therapy, age, ipsilateral stenosis and DWA in a Cox proportional hazards model (Model 3; Table 10.3) was moderate as indicated by the area under the ROC curve (AUC: 0.704).

Even if progression of carotid stenosis identifies a high- risk group, only 19 of the strokes occurred in this group whereas 40 strokes occurred in the group with no change in the degree of carotid

stenosis. This finding alone implies that carotid progression is a rather poor indicator of plaque instability. This is also stressed by the fact that carotid plaque progression was not a significant covariate when added to a Cox model, which has already shown to be able to stratify patients according to the risk of stroke.

One of the main limitations of this study was that medical therapy was left to the discretion of the attending physician and that was before the introduction of the today's strict management with lipid lowering medications.

10.5.Conclusion

Regular interval screening for carotid stenosis progression detection in patients with asymptomatic carotid stenosis may improve the stroke-risk prediction based on stenosis alone. If, however, it is compared with stenosis combined with plaque composition analysis then it does not seem to contribute significantly more.

Finally our study emphasized on the fact that regression of carotid stenosis was linked to lipid lowering therapy and that none of the patients with regression developed a stroke.

In the future it would be worthwhile to study the effect of aggressive medical therapy on plaque regression by regular ultrasound scanning. If this favourable effect of plaque regression on the incidence of stroke is definite, then plaque regression may become an imaging marker to monitor the effectiveness of therapy.

Chapter 11

Juxta-luminal black area in asymptomatic carotid plaques and risk of stroke

11.1. Introduction

Studies evaluating histology in extracted carotid samples have shown that symptomatic plaques have a necrotic core, which can be twice as close to the vessel lumen compared to asymptomatic plaques (Bassiouny HS,1997). This observation correlates with the observations made by cross-sectional ultrasound studies, which showed a link between juxta-luminal black (black) area (JBA) and the incidence of cerebrovascular events (Griffin MB,2010; Pedro LM,2002; Sztajzel R,2006). JBA in ultrasonic images of asymptomatic carotid artery plaques has been shown to correspond to a lipid core close to the lumen when histological analysis is performed (Sztajzel R,2005). A previous cross-sectional study of patients with both symptomatic and asymptomatic carotid plaques has demonstrated that a JBA >8mm^2 without a sonographically visible fibrous cap was associated with a increased prevalence of symptomatic carotid plaques (Griffin MB,2010).

However, despite the above evidence, the predictive role of JBA in prospective studies of asymptomatic carotid stenosis and its contribution to stroke-risk stratification remains largely unclear. It has been previously suggested by our group that JBA cut-off points from cross-sectional studies need to be validated in longitudinal studies of asymptomatic patients (Griffin MB,2010)

The aim of our study was to use the ACSRS imaging cohort to test the hypothesis that the presence and size of JBA without an obvious fibrous cap can predict future ipsilateral ischemic events.

11.2.Methods

11.2.1. Patient recruitment and image analysis.

Briefly, inclusion criteria were newly referred patients with 50% to 99% ICA stenosis in relation to the carotid bulb diameter (European Carotid Surgery Trial [ECST] method) without previous ipsilateral cerebral or retinal ischemic (CORI) symptoms and without neurological abnormality; these were recruited to the study after they had provided written informed consent. Inclusion and exclusion criteria and recruitment sources have been previously reported. Carotid duplex scanning was performed on admission to the study as previously described (chapter 5). The image acquisition and analysis of carotid plaques have also been previously described.

11.2.2.JBA measurement

The largest juxta-luminal black area of the selected plaque (ie, area with pixels having a gray-scale value of less than 25) without a visible echogenic fibrous cap (ie, pixels with gray-scale value higher than 25) was outlined using the computer mouse (image 11.1). Then the JBA area was automatically calculated by the software. The value of the larger area was used when there were two plaque components with black areas. The reproducibility of JBA measurements has been reported in previous publication of our group (Griffin MB,2010).

11.2.3.Outcome measures.

The primary outcome measure of the ACSRS study was ipsilateral cerebral ischemic stroke.

11.2.4.Statistical analysis.

Receiver operating characteristic (ROC) curves for JBA was constructed with stroke as the state variable. The area under the curve was also calculated. Several cut-off points were tested to categorise JBA, including a previously suggested cut-off point (8 mm^2) based on a cross-sectional study in order to produce stratified Kaplan-Meier plots. JBA was then entered into a multivariate proportional hazards model with cerebrovascular events or stroke as the dependent variable. Other plaque features known to be associated with increased risk such degree of ECST stenosis, presence of discrete white areas, GSM, plaque area and history of contralateral TIA or stroke were also include in the model.

Statistical analysis was performed with PASW/SPSS Statistics 18 or later (SPSS Inc, Chicago, Ill, USA).

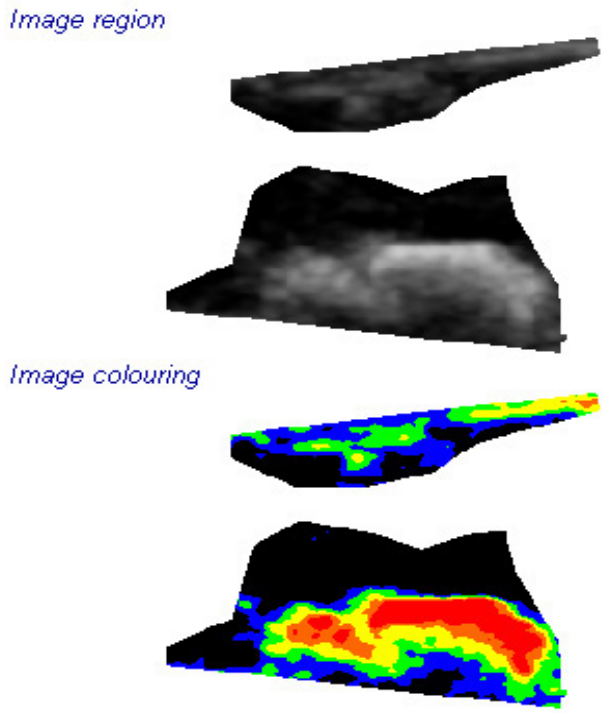


Image 11.1: The segmented gray scale image of the same plaque before and after colour contouring according to the gray level of pixels (Gray scale: 0-25 = black; 26-50 = blue; 51-75 = green; 76-100 = yellow; 101-125 = orange; ≥ 125 = red).

11.3.Results

As previously reported, during follow-up, a total of 130 first ipsilateral cerebrovascular events occurred (59 strokes, 49 TIAs, and 22 amaurosis fugax).

11.3.1.Ipsilateral cerebrovascular events (AF, TIA or stroke)

Using Kaplan Meier curves, the 5-year cerebrovascular event rate was 3% in patients with JBA < 4mm^2. In patients with JBA between 4 and 8 mm^2 was 21% and 36% when JBA was 8-10 mm^2 .If JBA was above 10mm^2 the 5-year cerebrovascular event rate mounted to 43% (P <0.001, Fig. 11.1.A). This translates to corresponding annual rates of 0.6%, 4.2%, 7.2% and 8.6%, respectively.

In multivariate analysis using Cox proportional-hazards regression with ipsilateral cerebrovascular events as the dependent variable and after adjusting for other plaque features, which are known to be associated with increased stroke-risk (Table 11.1, Model 1), the JBA (<4, 4-8, >8, in mm2) was still significant whereas plaque area and GSM were not.

11.3.2.Ipsilateral stroke

For stroke alone and after using Kaplan Meier curves, the corresponding annual rates (mean for 5 years) for JBA: <4, 4-8,8-10 and >10mm^2 were 0.4%, 1.4%, 3.2% and 5%, respectively (P<0.001, Fig. 11.1B).

The ROC area under the curve for stroke using JBA as a continuous variable was 0.816 (95% CI 0.77-0.86, P<0.001). Fatal stroke occurred in 0.2% of patients with a JBA < 8 mm2 and in 4.1% of those with a JBA ≥ 8mm^2 (P<0.001, Fisher's exact test, OR: 18.7, 95% CI 4.1-85.9). After 8 years of follow up, fatal stroke-free survival was 99.5% in those with a JBA < 8mm^2 and 90.1% in patients with a JBA ≥ 8mm^2 and (P<0.001, HR: 19.6, 95% CI 4.3-89.6).

Multivariate analysis was then performed using Cox proportional-hazards regression, with ipsilateral stroke as the dependent variable. JBA as a categorical value (<4, 4-8, >8, in mm2) was still significant after adjusting for other plaque features as previously (Table 11.1, Model 2).

Using only the significant variables from the previous models (stenosis, DWA, JBA and history of contralateral TIA or stroke) in the Cox model (Table 1, Model 3) the five-year stroke free and average annual risk of stroke was calculated for each patient. This way, patients were classified in 5 subgroups of annual stroke risk (<1.0, 1.0-1.9, 2.0-3.9, 4.0-5.9 and ≥6) (Table 11.2 A-C).

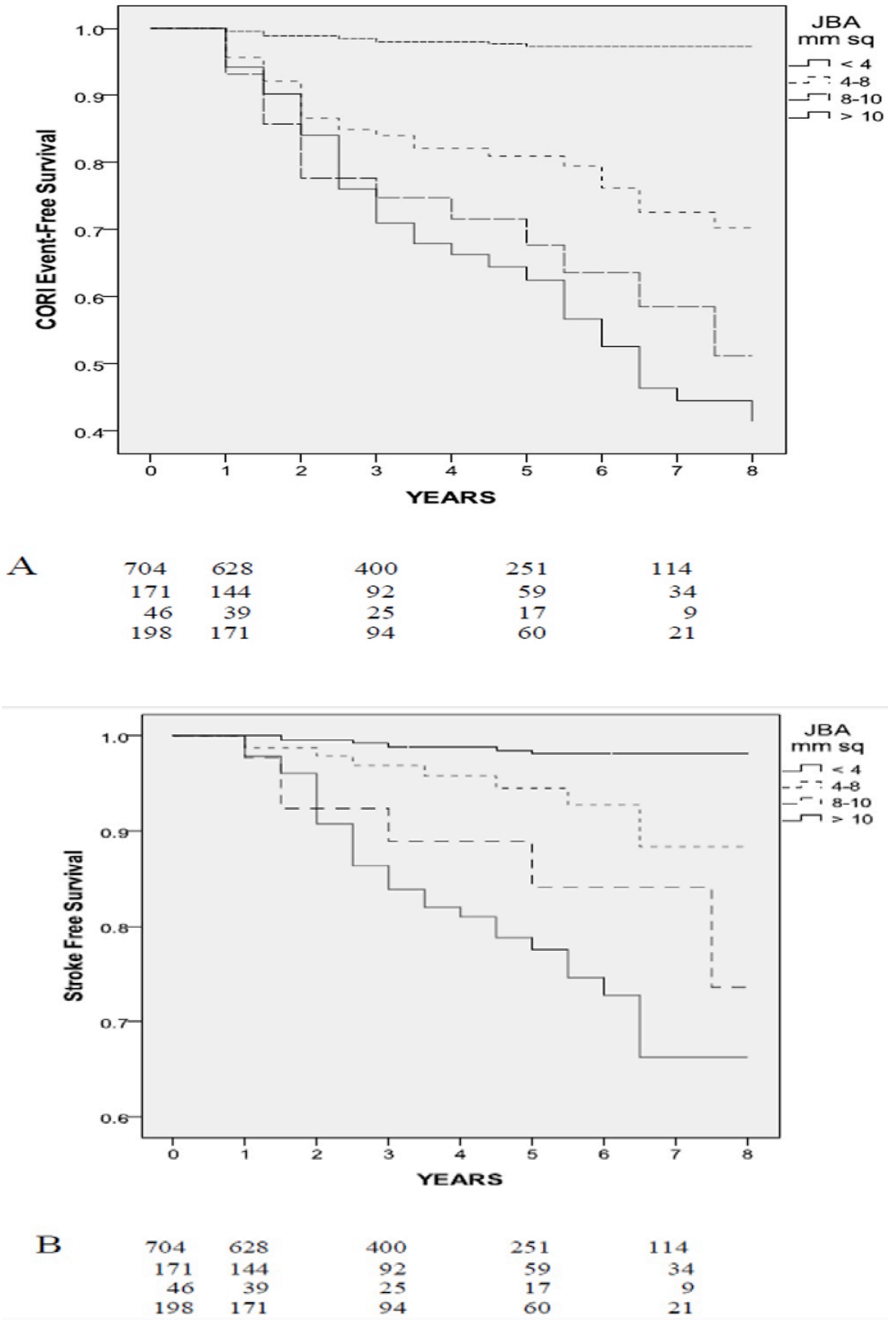


Figure 11.1: Kaplan-Meier plots showing (A) ipsilateral cerebrovascular or retinal ischemic event free survival and (B) stroke free survival, stratified by Juxtalumunal Black Area (JBA). The number of patients at risk at each interval is shown at the bottom of each

Table 11.1: Cox proportional hazards models including juxta-luminal black area and other plaque features known to be associated with increased risk. Ipsilateral cerebrovascular events (stroke, AF,TIAs) was the dependent variable in Model 1 and ipsilateral ischemic stroke as the dependent varuable in Models 2 and 3.

Variable	β	HR	95% CI	P value
Model 1				
JBA (4, 4-8, 8-10, >10)	0.816	2.26	1.84 to 2.78	< 0.001
Stenosis (50-69, 70-89, 90-99)	0.241	1.27	0.98 to 1.66	0.073
DWA (present, absent)	0.883	2.42	1.52 to 3.85	< 0.001
History of contr. TIA or stroke (present, absent)	0.518	1.68	1.14 to 2.47	0.008
GSM (<15, 15-30, >30)	-0.041	0.96	0.72 to 1.27	0.776
Plaque area (<40, 40-80, >80)	0.099	1.04	0.83 to 1.47	0.502
Model 2				
JBA (4, 4-8, 8-10, >10)	0.773	2.16	1.58 to 2.97	< 0.001
Stenosis (50-69, 70-89, 90-99)	0.465	1.59	1.06 to 2.38	0.025
DWA (present, absent)	0.619	1.86	0.98 to 3.51	0.057
History of contr. TIA or stroke (present, absent)	0.793	2.21	1.28 to 3.61	0.004
GSM (<15, 15-30, >30)	0.163	1.18	0.75 to 1.83	0.471
Plaque area (<40, 40-80, >80)	0.051	1.05	0.68 to 1.62	0.818
Model 3				
JBA (4, 4-8, 8-10, >10)	0.853	2.34	1.89 to 2.91	< 0.001
Stenosis (50-69, 70-89, 90-99)	0.462	1.59	1.06 to 2.37	0.023
DWA (present, absent)	0.582	1.90	0.98 to 3.27	0.059
History of contr. TIA or stroke (present, absent)	0.788	2.20	1.27 to 3.79	0.005

Table 11.2.A: Stenosis 90-99% ECST (83-99% NASCET) (n=325) and predicted annual stroke risk

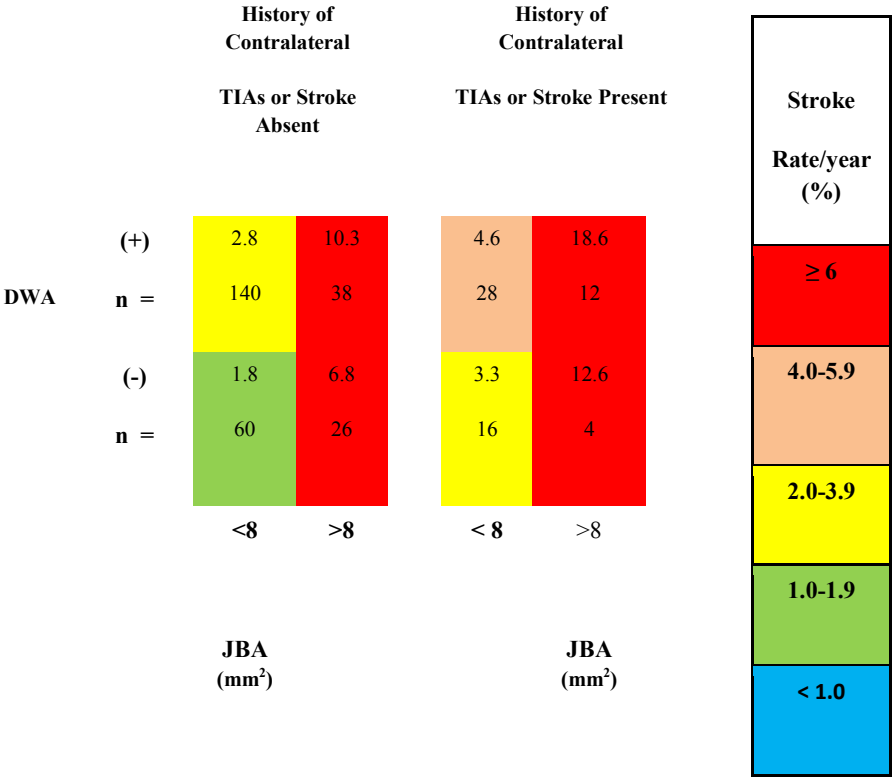


Table 11.2.B: Stenosis 70-89% ECST (50-82% NASCET) (n=598) and predicted annual stroke risk

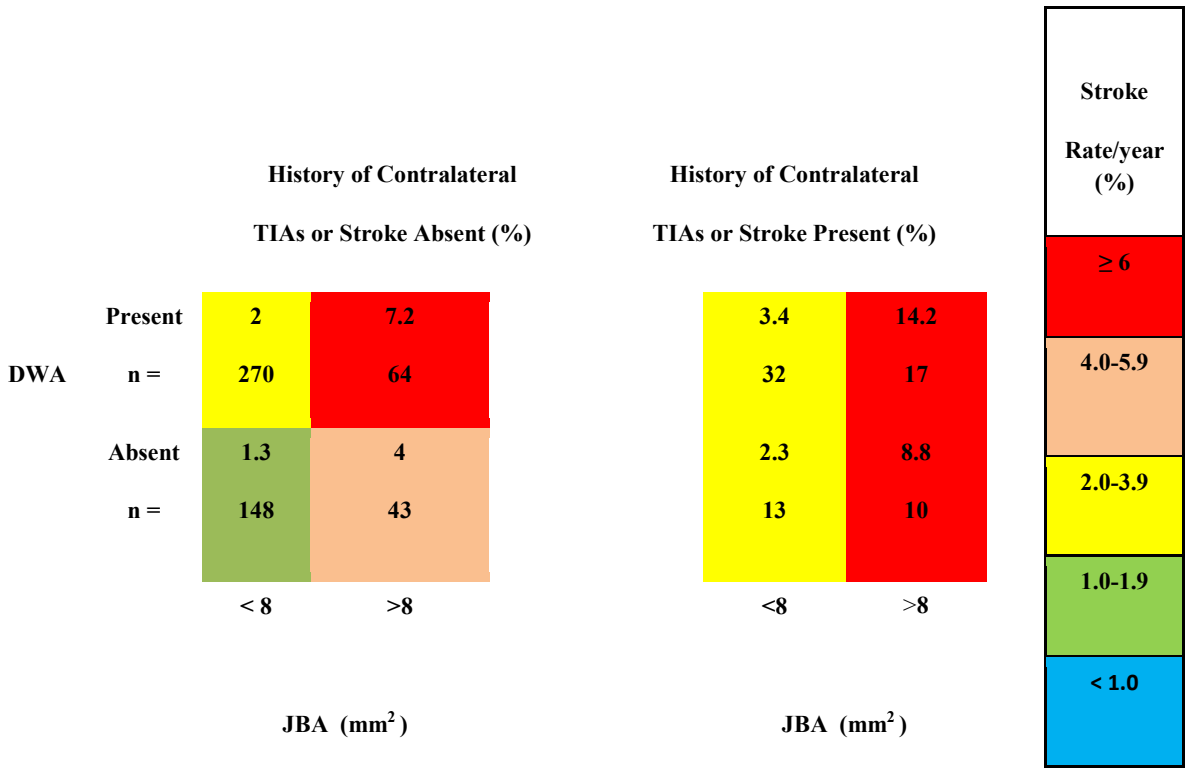
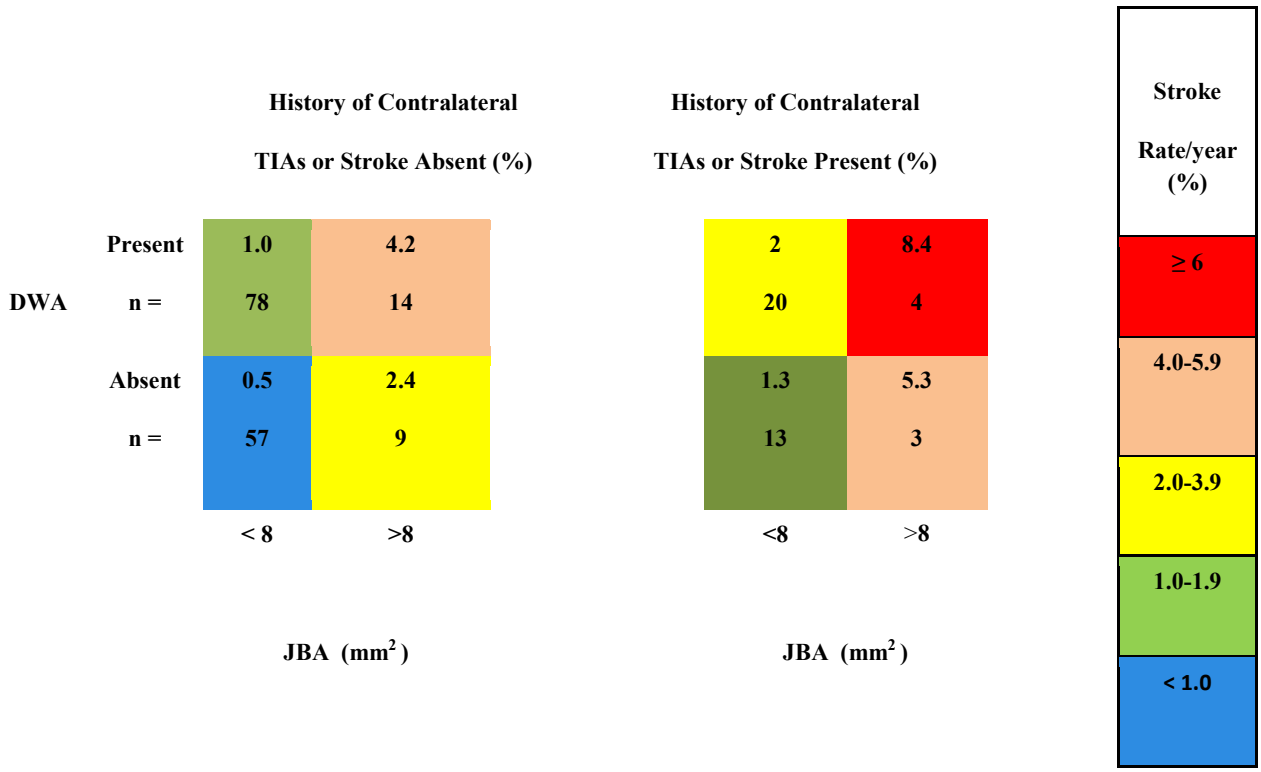


Table 11.2.C: Stenosis 50-69% ECST (< 50% NASCET) (n=198) and predicted annual stroke risk



11.4. Discussion

This is one of the first studies to show that the size of juxta-luminal black carotid plaque area (JBA) on ultrasound can stratify the risk of for patients with asymptomatic carotid artery disease.

Even though JBA was associated with the occurrence of ipsilateral carotid symptoms in two previous studies (Pedro LM, 2002; Kakkos SK, Nicolaides AN,2011) the cross-sectional nature of them precluded the findings from stroke-risk assessment modelling. That is why prospective studies, like the ACSRS were necessary. Indeed, in this study we demonstrated that the presence of a JBA without a visible fibrous cap was linked to ipsilateral neurological events in patients with asymptomatic carotid artery stenosis. This link was still significant after adjusting for other significant factors associated with plaque vulnerability.

Another important finding from our study was that JBA size was a very strong predictor of stroke, with the area under the ROC curve being 0.816. In addition the finding that not only neurological events as a whole, but also ispilateral ischemic stroke, a hard endpoint, is associated with the size of JBA increases the validity of our results.

Finally, another interesting association from our study was that between JBA and fatal stroke. The possible explanation behind this is that a larger JBA is able to produce a larger ulcer on the carotid surface, which can attract a large amount of thrombus, which in turn can cause a large, fatal brain infarct.

The main limitation of this study was that there was no assessment of optimum GSM cut-off points for JBA determination. However, as indicated in the method section the black area was defined as an area with gravy pixel values equal to or less than 25. The theory behind this, based on a previous study by our group (Griffin MB,2010) is that the human eye is unlikely to distinguish shades of grey within the range of 0-25. However, regarding JBA area the $8mm^2$ cut-off value was proved to be optimum.

11.5.Conclusion

The juxta-luminal black area size appears to be linearly related to the risk of stroke and could be useful in risk stratification models. Of course our findings have to be validated in future prospective studies or in the medical arm of randomized trials and in the presence of best medical treatment.

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PART IV

Clinical 3D Ultrasound Studies

Chapter 12

3D ultrasound for the evaluation of carotid plaque disease; a systematic approach to the available evidence.

12.1. Introduction

Two dimensional (2D) ultrasound imaging has been a powerful tool for the diagnosis and management of carotid plaque atherosclerosis. Ultrasound imaging provides the vascular surgeon with a valuable asset due to its non-invasive nature, its low cost and its undoubted effectiveness in identifying and evaluating the carotid anatomy and associated pathologies such as the formation of atherosclerotic plaque [U-King-Im JM,2009]. Even today, in the era of advanced imaging techniques such as angio-MRI and angio-CT , 2D ultrasound remains our first choice imaging for the initial investigation of patients with carotid plaque disease.

Indeed, for a significant number of patients with carotid plaque disease, 2D ultrasound investigation and its subsequent analysis are sufficient to determine the best possible plan of care. Nevertheless, 2D ultrasound can only provide us with a limited number of views (longitudinal and transverse) the quality of which can vary since it is an operator-dependent technique. Moreover, once the 2D slices have been captured, images of other planes can no longer be extracted [Fenster A, Landry A,2004; Fenster A, Downey DB,2001]. This may affect the accuracy of plaque characterization since information is only sampled from one section, which is then assumed to be representative of the entire plaque.

Furthermore, there is accumulating evidence that plaque composition [Grønholdt ML,2001; Kakkos SK, Stevens JM,2007], morphology [Prabhakaran S,2006; Rothwell PM,2000] and size [Spence JD,2002] may significantly impact plaque vulnerability. The 2D approach may not be sufficient to accurately evaluate these features for the entire plaque volumes since a large number of carotid plaques have variable compositions with some of their components being more echolucent than others. Thus, in heterogeneous carotid plaques, 2D ultrasound imaging may not provide with an accurate assessment of the composition or size, with obvious implications in risk stratification.

Traditional 2D ultrasound imaging is an adequate and cost-effective method for a significant proportion of patients with carotid plaque disease, although, there exists a need for a more sensitive means of identifying the subgroup of high-risk asymptomatic patients who may benefit from more radical management [Makris GC, Nicolaides AN,2011]. The development of angio-MRI and angio-CT introduced a new era in volumetric vascular imaging. Following the use of highly sophisticated reconstruction software and the acquisition of images containing volume information in three dimensions, the visualization of vascular structures in different planes from different angles is possible. Thus, a more holistic evaluation of the plaque properties can be achieved. Nevertheless, these modalities have their own limitations including limited accessibility, increased cost, exposure to radiation, a more invasive nature, increased patient inconvenience and contraindication in patients with contrast-allergy or renal impairment.

3D ultrasound may represent an alternative option combining the advantages of volumetric vascular imaging using MRI or CT with those of ultrasound imaging (increased availability, non-invasive nature and decreased cost). Cardiology and obstetrics are among the first medical fields where 3D ultrasound already had significant applications [Lang RM,2006]. The new volumetric acquisition techniques facilitate the storage of multiple, consecutive and equidistant views which are subsequently converted in a matrix of points (voxels) representative of the volume scanned. The navigation and the 3D reconstruction of the underlying structures can then be performed by sophisticated software.

In this chapter we sought to identify and critically review the clinical evidence regarding the reproducibility and effectiveness of 3D ultrasound in carotid plaque characterization.

12.2.Methods

The PubMed, Scopus and Cochrane databases were searched for clinical studies evaluating the reproducibility and effectiveness of 3D ultrasound on carotid plaque characterization in terms of plaque size, volume, morphology or composition. The search terms used were: “3-dimensional”, “ultrasound”, “carotid plaque” AND “volume” OR “composition” OR “morphology” OR “stenosis” OR “flow velocities” in various combinations. Two independent reviewers Dr. G Makris and Dr. A Lavida performed the literature search and data extraction. The selected studies were manually searched for relevant publications out of their reference lists.

All clinical studies which reported results on the reproducibility of 3D ultrasound measurements or its effectiveness in evaluating the carotid plaque progression post medical treatment were retrieved and included in the final analysis. In vitro or animal only studies using 3D ultrasound were excluded from the analysis. Case report studies were also excluded. There was no language or time limit to our search.

12.3.Results

12.3.1.Reproducibility of 3D carotid measurements of volume and stenosis

The sample size in most studies was small and ranged from 10 to 105 carotid plaques in total. The majority of the studies evaluated the total plaque volume (TPV) and only one the vessel wall volume (VWV). (Table 12.1) All studies concluded that there was good intra- and inter-observer reproducibility of 3D ultrasound in carotid plaque volume measurements ranging from 2.8-6.0% and 4.2-7.6%, respectively. The effect of the distance between the studied slices (ISD) as well as the effect of the initial plaque size in the volume reproducibility measurements was assessed by 2 studies. Both studies concluded that volume measurement variability can be smaller for larger plaques, while Fenster et al., suggested a possible effect of the ISD on the observed variability. [Landry A,2004]

The reproducibility of the degree of stenosis using 3D ultrasound was investigated by four studies. In two of the studies 2D and 3D ultrasound were used to assess the degree of stenosis while Yao et al.[Yao J,1998], used carotid angiography and Wessels et al. [Wessels T,2004] used digital subtraction angiography to evaluate stenosis. All studies suggested good agreement between the compared methods (Table 12.1). Lastly, the peak systolic velocities (PSV) were evaluated by Needleman et al., [Forsberg F,2008] who showed good correlation between the measurements obtained by 3D and 2D ultrasound ($r \geq 0.32$; $P < .002$).

12.3.2. Reproducibility of 3D ultrasound for the evaluation of plaque composition

Only 2 studies [Heliopoulos J,2009; Schminke U,2000] were identified investigating the clinical use of 3D ultrasound for the evaluation of carotid plaque morphology. Morphology features such as plaque surface irregularity [Heliopoulos J,2009] or the presence of ulceration [Heliopoulos J,2009; Schminke U,2000] were mainly evaluated. Good intra- and inter-observer reproducibility in the characterization of plaque morphology was demonstrated by those studies ranging from 8.9- 9.4% and 9.7-2.3%, respectively , suggesting that 3D ultrasound can be used for the evaluation of carotid plaque surface. In the study by Heliopoulos et al., [Heliopoulos J,2000] it was also shown that 3D ultrasound was superior to 2D in detecting ulceration in a sample of 62 plaques which were evaluated by both 3 and 2D ultrasound and by 2 independent observers (16.1% and 14.5% of plaques, for observers 1 and 2, respectively, versus 6.5% and 9.7% of plaques, for observers 1 and 2, respectively, p= 0.125 and p= 0.063, for observers 1 and 2, respectively).

Three studies reported reproducibility data and apart from the study by Heliopoulos et al., [Heliopoulos J,2009] which included 214 patients, the remaining two [Denzel C,2009; Seabra JC,2009] included only 19 and 5 patients respectively. Grey scale median (GSM) and mean grey values (MGV) from the image grey scale histogram were used for the evaluation of plaque composition with no reference to other texture features. Fine intra and inter-observer reproducibility was reported with intra-and inter-observer correlation coefficients of >0.935 and >0.89, respectively [Heliopoulos J,2009; Denzel C,2009]. All studies concluded that 3D ultrasound could adequately evaluate plaque echodensity. Particularly Denzel et al., [Denzel C,2009] who studied 24 endarterectomy samples using both 2D and 3D ultrasound, suggested that 3D imaging can be equivalent to 2D in the assessment of plaque echodensity ($p < 0.001$, $r = 0.8$), although, these results were supported by a small sample. (Table 12.2)

12.3.3. Effectiveness of 3D ultrasound in the evaluation of carotid plaque progression

Eleven studies were identified regarding the use of 3D ultrasound for the evaluation of carotid plaque progression with or without treatment (Table 12.2). Two of these studies evaluated the plaque progression without any treatment, five studies evaluated the effect of statins on various plaque characteristics and three studies the effect of other medication such as anti-hypertensives. The study design, as well the follow-up time varied significantly between the studies, ranging from 7 years to 3 months. Only five studies of these studies were randomized control trials. The sample size also varied from a maximum of 165 to 7 patients. (Table 12.1)

The majority of studies used plaque volume or vessel wall volume changes and four of them assessed changes in plaque composition. All studies concluded that 3D ultrasound could be a useful tool for the evaluation of various carotid plaque characteristics and their progression through time, with or without the effect of any particular treatment. In addition two studies showed that 3D ultrasound can be sensitive enough to detect plaque changes in short periods of time or in small sample sizes and thus can be particularly useful for the evaluation of new treatments. Finally it should be noted that no studies were identified evaluating both carotid plaque volume and composition changes (Table 12.2).

A comparison between the sensitivity of 2D and 3D ultrasound measurements as a response to treatment was only performed in 4 studies (Table 12.2). In these studies the change of 3D US volume measurements as a response to treatment was compared to the changes of the intima media thickness (IMT) of the internal carotid artery, which is one-dimensional. All studies concluded that 3D volume measurements are more sensitive than IMT measurement for the evaluation of carotid plaque progression post-treatment. More specifically, while there was significant change in 3D plaque volume during the follow-up period there was no change in IMT. However, no studies

were found comparing the 3D plaque volume or composition (i.e. grey scale median) changes with the 2D plaque area or composition features post-medical treatment.

Table 12.1: Clinical evidence regarding the reproducibility of 3D ultrasound on carotid plaque volume and stenosis/flow measurements

Author /year	Study design and number of patients	3D US specifications	Reproducibility outcomes		Conclusion
			VOLUME	STENOSIS/ FLOW VELOCITIES	
Delcker A, / 1994	Repeated measurements were performed at weekly intervals using three different evaluation procedures/ N= 70	Three newly developed evaluation procedures of plaque volumes (manual tracing, threshold procedure, watershed algorithm) were assessed.	Intraexaminer variability ranged from 2.8% (watershed algorithm) to 4.1% (threshold procedure). Interexaminer variability ranged from 4.2% (manual tracing) to 7.6% (threshold procedure) Follow-up measurements ranged from 5.2% (watershed algorithm) to 9.4% (threshold procedure). Pearson correlation coefficients for interexaminer agreement and for the three measurement procedures were all highly significant ($p<0.01$).	-	3D computer based image reconstruction provides with highly reliable quantification of moderate atherosclerosis.
Griewing B, et al./ 1997	Conventional colour coded Doppler US and 3D US angiography were used to evaluate carotid artery atherosclerosis, PV ranged from 0.053 to 0.685 ml./ N=21	Conventional color-coded Doppler ultrasound and 3D ultrasound angiography Data collection for 3D imaging required five minutes per patient	The intraobserver and interobserver variabilities were 4.16 and 5.87%, respectively ($r = 0.96$, $p < 0.0001$; $r = 0.89$, $p < 0.0001$). 3D Color Doppler and 3D ultrasound angiography (power mode) assessments of plaque volume differed by 8.5%. Plaques were more easily differentiated using 3D US and quantification of plaque volume was less affected by echo shadowing after 3D reconstruction.	-	3D ultrasound angiography offers a more precise quantitative method for the quantification of atherosclerotic lesion than other techniques for prospective, clinical studies.
Palombo C, et al./ 1998 [13]	In vitro quantitive analysis of known test phantoms and in vivo reproducibility of plaque volume (2 observers)/ N=27 phantoms (range, 100 to 600 mm^3) and N=47 fibroadipous plaques (range, 7 to 450 mm^3)	Controlled sequential tomographic data acquisition performed by scanning in a fanlike motion with a linear array of the high-frequency transducer (7.5/10 MHz) +/-ECG synchronization	In vitro: Excellent correlation between the volume of plaque phantoms measured using the 3D data and the true volume *($r=0.99$; $y=0.96x+12.38$, $P<0.01$)* In vivo: High reproducibility was found for measurement of carotid plaque volume (mean difference between two observers: *$0.6\pm11.2\ mm^3$* and correlation (r=0.99, Y=1.02x-2.03; P<0.01).	-	3D US imaging of carotid artery is an accurate and highly reproducible means for measuring atherosclerotic PV.

Yao et al./ 1998	Symptomatic pts with severe carotid artery stenosis (>70%) imaged with 3D US and angiography before endarterectomy, and 12 of the operated carotids were imaged aftewards by two observers/ N=14.	|Commercially available ultrasound system (AU3 Partner, Esaote Biomedica, SpA) incorporated with a 3D prototype probe (linear array transducer operating at dual frequencies of 10/7.5 MHz) +/-ECG synchronization	3D US showed good reproducibility as the intra and inter observer variability was SEE=1.9 r=.99 and SEE=2.3 r=.99 respectively in plague length measurement and SEE=42.9 r=.99 and SEE=54.4 r=.99 respectively in plaque volume measurement..	Good correlation between the percentage of carotid stenosis measured by angiography and 3D US was observed (SEE=12.4%, r=0.76, and mean difference=7.0+/-12.3% with the diameter method; SEE=10.5%, r=0.82, and mean difference=1.8+/-10.5% with the area method)	The quantitative and qualitative analyses possible using 3D US (especially volumetric) may have important clinical implications in following plaque progression or regression and assessing the outcomes of clinical interventions due to its excellent reproducibility.
Keberle M, et al./2000	Prospectively screened pts for CAD using power 3D US and 2D US; the 3D data was analyzed by two independent reviewers/ N=75	Doppler US and 3D power Doppler sonography, utilizing a 7.5-MHz linear-array transducer combined with tissue harmonic imaging.	-	The degree of stenosis determined by the conventional Doppler highly correlated with that determined using 3D US (r = 0.982-0.998). Good correlation of measurements of length of lesion and distance from the bulb using conventional and 3D US (r = 0.986). For 3D US the interobserver variability rate was 3.7% +/- 0.5%.	3D power Doppler US provides with an easy and accurate method for screening atherosclerotic lesions of the carotids and the excellent volume surveys produced may be helpful in planning surgical intervention.
Wessels T et al./2004 [16]	Evaluate agreement of ICA stenosis using 3D US compared with DSA /N=49	Color-coded duplex system using the power mode was applied. 3D system consisted of an electromagnet that induces a low-intensity magnetic field near the patient's head.	-	High agreement between two observers was found in 3D US (weighted kappa coefficient of 0.88). 3D US slightly underestimated the mean stenotic degree (mean 3D CDS 68.47+/-10.5 vs. DSA 71.3+/-10.0). Good agreement between DSA and 3D US Mean sensitivity of 3D CDS= 93%, mean specificity=82.5%, mean positive predictive value=82%, and mean negative predictive value=98%.	3D CDS findings demonstrated good agreement compared with the DSA, yielding higher accuracy than CDS alone.
Landry A, et al./ 2004 [17]	Multiple-observer study investigating observer variability and reliability in the	Mechanical linear 3D scanning system (LIS Inc) with a 3D image transducer (50 mm; L12–5, Philips) while video frames	Intraobserver and interobserver measurement reliabilities were 94% and 93.2%, respectively. PV measurement variability decreased as PV increased	-	3D US provides with an accurate and reliable means of measuring plaque volume which is affected by plaque size and chosen ISD.

	measurement of plaque volume. / N=40 plaques (range, 37.43 to 604.10 mm^3)	from an US machine (ATL HDI 5000, Philips) were digitized and saved to a computer workstation.	(ranging from 27.1% to 2.2%). ISDs between 1.0 and 3.0 mm did not affect PV measurement precision, but PV measurement variability increased with ISD. Repeated 3D US scan measurements gave the same values as single-scan measurements (p=0.867).		
Egger M, et al./ 2007 [18]	3D US images acquired from the right and left ICA of subjects with carotid atherosclerosis scanned twice within a period of two wks (two observers) / N=10	3D US images by ultrasound transducer (L12-5,50 mm, Philips, Bothell, WA, USA) along the neck of the patient every 4.0 cm (ATL HDI 5000, Philips, Bothell, WA, USA)	COV and ICC for VWV measurements (scan COV = 4.6% ICC = 0.95, rescan COV = 3.4%, ICC = 0.96) (COV = 5.7%, ICC = 0.85) and TPV measurements (intraobserver variability scan COV = 22.7% ICC = 0.85, rescan COV = 21.1% ICC = 0.88 and interscan variability, COV = 31.1%, ICC = 0.83), indicated higher intraobserver and interscan reproducibility for VWV.	-	3D US VWV measurements show higher intraobserver and interscan reproducibility than TPV measurements and thus VWV measurements may provide with additional information about the plaques.
Ludwig et al. / 2008 [19]	N=45 plaques used to assess PV intra- and inter- observer reproducibility (three sonographers) and N=60 plaque images used to assess the effects of plaque size and number of slices (5 vs. 10) in manual planimetry	3D US high-resolution Voluson 530 D MT, 2D-/3D CFM-Ultrasound System (Kretz-Technik AG, Zipf, Austria)	ICC for intraobserver variability was 0.985, 0.967, and 0.969 respectively for each of the appointed observers. The interobserver ICC= 0.964, thus both intra- and inter-observer variability were small, AND smaller for larger (PV>60µl) than smaller plaques (PV<60µl). Using 5 or 10 slices did not have a significant effect on the variability (mean CV= 3.4 and 3.1 for 5 and 10 slices respectively). Mean CVs for the larger plaques (40-60µl) were smaller than for smaller plaques (20-40µl) for both the 5 and 10 slide methods	-	3D US can produce highly reliable and reproducible re-quantification of plaque volume In smaller plaques, a 10 slice procedure as opposed to a 5 slice, can prove more reliable.
Forsberg F, et al/2008 [20]	compare PSV and degree of stenosis measurements obtained using a real-time 3D Doppler US scanner and a conventional Doppler US scanner/N=59	3D Doppler US (Encore PV; VueSonix Sensors Inc, Wayne, PA) and conventional Doppler US	-	The methods for measuring stenosis showed good agreement using ICA PSV (ICC= 0.83; P < .0001) and to a lightly lesser extent using ICA/CCA PSV ratio (ICC = 0.65; P < .0001). PSV measurements obtained using conventional US and 3D US correlated if all vessels (r >or= 0.32; P < .002).	The semi-automated 3D Doppler device is comparable to conventional 2D US in the measurement of PSV and thus the assessment of carotid stenosis.

Abbreviations :3D*: three-dimensional;* ***US****: ultrasound;* ***PV****:* plaque volume; **ml**: millilitres; ***mm***^{3} *millimetres;* ***MHz****: Megahertz;* ***ECG****: echocardiogram* ***pts****: patients;* ***CAD:*** *carotid artery disease;* ***2D****: two-dimensional;* ***LIS*****:** life imaging Systems Inc,; **ATL**: Advance Technology Laboratories; **HDI**: high definition imaging; ***ISD:*** *interscile distance;* ***ICA:*** *internal carotid artery;* ***DSA****: digital subtraction angiography;* ***vs.:*** *versus;* **CDS:** color Doppler sonography; **wks:** weeks; **cm:** centimetre; **WA:** Washington; **USA:** United States of America; ***COV:*** *coefficients of variation;* **ICC:** intraclass correlation coefficient; **VWV:** Vessel Wall Volume; **TPV:** Total Plaque Volume; **CFM:** color flow mode; **CV:** coefficient of variation; **PSV:** peak systolic velocities; **PA:** Pennsylvania; **CCA:** common carotid artery

Table 12.2. Evaluation of carotid plaque progression using 3D ultrasound with or without treatment

Author/Year	Number of patients/Study Design/ Type of treatment	Evaluated plaque characteristics/ Follow up	Study Outcome	Comments
Hennerici M, et al./1991 [29]	N=7/ four flat and 17 soft carotid plaques were investigated during, and after heparin-induced extracorporeal LDL precipitation (HELP).	Plaque volume progression and regresion/ Average f/u was 17 months (minimal=7; maximal=24 months)	During treatment, plaque regression was seen in all subjects within 6 (n=6) or 12 (n=l) months of f/u. After this, during continuous HELP treatment the vessel wall changes persisted in all patients spare one who experienced plaque progression	Specially designed quantitative 3D US analysis, provides with a useful way to evaluate significant plaque volume reduction in all subjects.
Schminke et al./ 2000 [30]	N=32/ Carotid artery plaques were examined using 3D reconstruction of parallel 2D gray scale b-mode US with power mode./No treatment was given,	3D power Doppler used to measure PV and its progression/regression at baseline and after an average of 18.9 months	Carotid plaque progression was observed in 15% of cases, while plaque volume stayed constant (a change less than 20%) in 85%. Progressive plaques were usually hypoechoic (3/5), while those which were progressive and hyperechoic had an ulcerated surface. There was a significant increase in plaque volume in symptomatic patients ($p<0.05$) and patients with carotid stenosis of >50% ($p<0.05$). Drug therapy and other risk factors showed no correlation to plaque progression, probably due to the small number of plaques with progression	3D reconstruction of atherosclerotic carotid artery plaques enables the reproducible quantification of plaque volume thus making it an excellent technique for longitudinal trials assessing progression or regression of plaques.
Kessler et al./ 2002 [31]	N=23 / Patients (mean age = 61.7 +/- 7.5 years) with hypercholesterolemia were treated with statins and the progression of 31 carotid artery plaques was assessed	Quantitative measurements of carotid artery PV using 3D reconstruction of exactly parallel transverse duplex ultrasound scans (slice distance = 0.1 mm) over 15.1 +/- 4.5 months.	Plaques significantly less frequently progressive if hypoechoic (11%, n = 9 vs. 64%, n = 14; P = .016) or if the baseline serum cholesterol levels were above 8.0 mmol/L (9%, n = 11 vs. 75%, n = 12; P = .002).	3D US allows the measurement of the volume of an entire atherosclerotic plaque rather than the arterial wall thickness and plaque morphology and configuration may be analyzed.
Pollex RL, et al./2005 [32]	N=98/ case-control study included 49 Oji-Cree adults with diabetes or impaired glucose tolerance, aged 21-69, and 49 sex- and age-matched normoglycaemic subjects	Carotid arterial IMT and total PV were assessed at baseline and 7 years later.	Total PV (P = 0.037), though not IMT were higher in subjects with diabetes/impaired glucose tolerance vs. normoglycaemic controls at f/u. Correlation between IMT and total PV was moderate (r = 0.524) To achieve power >0.7 using IMT measurements for diabetic vs. non diabetic subjects, thousands of subjects are required, while only	Total PV appears to capture the atherosclerotic disease burden more effectively in subjects with type 2 diabetes than IMT, and thus would be an appropriate outcome measure for studies aimed at changing assessing these patients.

			hundreds are needed when using total PV. For total PV, intra- and inter-observer reliability were 0.94 [n = 40] and 0.93 [n = 40], respectively (both P < 0.01).	
Ainsworth et al./ 2005	N=38/ Patients with carotid stenosis >60%, age ±SD: 69.42±7.87 years randomly assigned in a double-blind fashion to 80 mg atorvastatin daily (n=17) vs. placebo (n=21)	3D plaque volume at baseline and after 3 months of treatment	Plaque regression of -90.25± 85.12 mm^3 seen in patients taking atorvastatin vs. plaque progression of 16.81±74.10 mm^3 in those taking placebo (P<0.0001).	3D PV measurements can be used to demonstrate the large effects of therapy on atherosclerosis in 3 months in sample sizes of 20 patients per group and over a short period of time (3-6 months).
Stumpe et al./ 2007	N=165/MORE RCT/ patients randomized to olmesartan (20-40 mg/day) or atenolol (50-100 mg/day) and were investigated using 2D and 3D US in order to compare the two treatments	Change from baseline (Delta) in CC-IMT assessed by 2D US. Secondary outcomes included Delta PV assessed by 3D US and blood pressure (BP)/ US performed at baseline and 28, 52 and 104 wks	The two treatments produced similar significant reductions in CCA IMT. Mean Delta PV (SEM) was -4.4 (2.3) microl and 0.1 (1.5) microl in the olmesartan and atenolol treated subjects, respectively, without significant between-treatment differences. In patients with baseline PV > or = median (33.7 microl), there were significant between-treatment differences in Delta PV (p = 0.023), as PV regressed significantly with olmesartan (Delta PV: -11.5 (4.4) microl) but not with atenolol (Delta PV: 0.6 (2.5) microl	While carotid IMT and BP decreased similarly with olmesartan and atenolol, only olmesartan reduced the volume of larger atherosclerotic plaques.
Mallett et al./ 2009	N=77/ Longitudinal, placebo controlled study where patients were randomized to receiving vitamin B9, B6 and cobalamin (group A) vs. Placebo (group B).	Analysed the sensitivity of three US phenotypes, B mode derived IMT and TPA and 3D US derived $VWV_{CCA+ICA}$./ subjects blindly scanned at baseline and 2.3+/- 1 year later (range: 0.5 to 4.5 y)	The VWV rate of change for CCA and ICA, was significantly greater than 0 for group B (53 +/- 110 mm^3/y) (p=0.008), but not significantly different than 0 for group A (-12 +/- 137 mm^3/y) (p=0.6). A significant difference between the VWV rates of change between treatment groups was seen (p=0.034). There was a significant relationship between deltaVWV and deltaIMT. In group A they were positively correlated (r=0.44, p<0.05) and in group B negatively correlated (r=-0.44, p<0.01).	3D US measured VWV seems to be more sensitive to temporal changes in carotid atherosclerosis of pts with diabetic nephropathy than IMT of TPA. 3D US VWV is adequate for longitudinal studies of atherosclerosis providing the necessary sensitivity and specificity
Yamada K, et al./ 2009	N=40/ consecutive non- or slightly hypercholesterolemic pts with moderate carotid artery stenosis randomly assigned to diet group (n = 20) or atorvastatin group (n = 20)	IMT and 3D IBS values measured at baseline and after 6 months.	Relative lipid volume of carotid plaques significantly decreased from 58.4 +/- 25.6 to 47.8 +/- 23.5% in the statin group (p < 0.01), whereas no significant decrease was seen in the diet group. IBS values and relative lipid volume between baseline and 6 months were correlated with the change in LDL (r = 0.31, p < 0.05, and r = 0.34, p < 0.05, respectively)	Quantitative assessment of carotid plaques using 3D IBS analysis was a clinically useful method for monitoring atherosclerotic lesion progression

Krasinski A, et al./ 2009	N=35/ RCT/pts with carotid stenosis >60% were randomized to receive either 80 mg atorvastatin (16 subjects, nine male, mean age 68+/-8.6 y) or placebo (19 subjects, 15 male, mean age 70+/-9.4 y) daily	3D US VWV was manually segmented by a single observer. Individual lumen and wall segmentation contours used to generate carotid atherosclerosis thickness difference maps/ Pts evaluated at baseline and 3m	VWV increased by 70+/-140 mm^3 (+4.9+/-10.3%) in the placebo group and decreased by 30+/-110 mm^3 (-1.4+/-7.7%) in the atorvastatin group ($p<0.05$).	3D US VWV provides with a quantitative measure of atherosclerosis burden including the IMT and plaque, and has sensitivity to detect changes over short periods of time. VWV thickness difference maps can provide visual evidence of the spatial and temporal dynamics of carotid artery changes.
Awad J, et al./ 2010	N=53 with ICA stenosis>60% were enrolled and 38 subjects completed the study (17 subjects on atorvastatin and 21 on placebo)/ cross-sectional study	Using 3D US images 270 texture features from seven texture techniques were extracted from manually segmented carotid arteries based on the IMT boundary / 3 months	Texture features improved the detection of statin-related changes using DBC to 0.5199, using WRS to 0.002, and ACC to 63.87%, respectively. The texture techniques that most differentiated between atorvastatin and placebo classes were Fourier power spectrum and Laws texture energy measures. The use of specific texture features resulted in the AUC value of 0.9988 +/- 0.0069 compared to 0.617 +/- 0.15 using carotid VWV.	Texture features derived from 3D US can be more sensitive in detecting statin-related changes in carotid atherosclerosis than VWV.

Abbreviations: LDL: *low density lipoprotein;* ***f/u****:follow-up;* ***3D****:3-dimensional,* ***US:*** *ultrasound,* ***2D****: two dimensional;* ***PV:*** *plaque volume;* ***mm:*** *millimetre;* ***vs.:*** *versus;* ***mmol/L****: millimol per litre;* ***IMT****: intima-media thickness;* ***SD:*** *standard deviation;* ***mg:*** *milligram;* ***mm:*** *millimetre;* ***MORE:*** *Multicentre Olmesartan atherosclerosis Regression Evaluation;* ***RCT:*** *randomized control trial;* ***CCA:*** *Common Carotid;* ***SEM:*** *standard error of the mean;* ***BP:*** *blood pressure;* ***TPA:*** *total plaque area;* ***VWV:*** *vessel wall volume;* **ICA:** Internal carotid artery; **y:** year; ***Delta****: change from baseline;* ***pts:*** *patients;* ***IBS:*** integrated backscatter analysis., ***DBC:*** *distance between classes;* ***WRS:*** *Wilcoxon rank sum;* ***ACC:*** *median accuracy measures;* ***AUC****: Area under the* receiver-operator characteristic curve

12.3.4. 3D ultrasound for carotid plaque disease, in patients with or without stroke

Only one study [Heliopoulos J,2008] was identified investigating the potential differences in 3D ultrasound features between patients with symptomatic or asymptomatic carotid plaque disease. The investigators evaluated the cases of 110 symptomatic and 104 asymptomatic patients using 3D ultrasound. They assessed the echodensity of the plaques by means of 3D imaging using the mean grey values of the whole plaque without any information regarding plaque volume or morphology changes. They concluded that, especially, at moderate degrees of ICA stenosis (less than 60-70%), lower mean grey values can be an important factor, indicating plaque vulnerability and increased risk for a stroke. Finally, there were no studies regarding the use of 3D ultrasound for the evaluation of carotid-restenosis after stenting, while there was only one regarding carotid artery changes post-endarterectomy [Yao J,1998].

12.4.Discussion

The purpose of this chapter was to systematically review the available clinical evidence regarding the reproducibility and effectiveness of 3D ultrasound in the evaluation of carotid plaque disease. A number of studies were identified evaluating various plaque characteristics such as volume, morphology (i.e. presence of ulceration), composition or degree of stenosis. All studies concluded in favour of the good reproducibility of 3D ultrasound. The majority of them (Table 12.1) focused on the reproducibility of plaque volume measurements (>90% for both inter- and intra observer variability), although there were also studies suggesting good reproducibility in the assessment of plaque morphology or composition. There were studies suggesting equal [Forsberg F,2008] or sometimes better results than 2D ultrasound [Heliopoulos J,2009; Pollex RL,2005] regarding plaque characterization within small samples or short follow-up periods [Ainsworth CD,2005]. However, there was one study that failed to show any additional benefit from the use of 3D ultrasound for the evaluation of carotid plaque echodensity when compared to 2D [Denzel C, 2009].

Only a small number of studies evaluated the reproducibility and effectiveness of 3D ultrasound on the evaluation of carotid plaque composition and morphology. Ulceration [Fisher M,2005] or the presence of a heterogenous, echolucent plaque [Grønholdt ML,2001; Kakkos SK, Stevens JM,2007] with increased lipid component has been recently associated with increased risk of cerebrovascular events. All the studies in this review suggested that 3D ultrasound could be a reliable method for the evaluation of both plaque morphology and composition in terms of presence of ulceration or grey scale levels, respectively. However, despite the encouraging data, due to the small number of the available studies more research is warranted in order to define the exact potential of 3D ultrasound on the evaluation of plaque composition and morphology.

Total plaque volume (TPV) was the most commonly evaluated characteristic for the assessment of alterations in plaque burden and evaluation of treatment effects (Table 12.2). While TPV is a useful

measurement for the quantitative assessment of plaque burden, it does not provide information regarding the location of the plaque in the artery, where the volume changes occur [Landry A,2002; Zahalka A,2001]. Moreover, measurement of total plaque volume from 3D images can be more technically challenging, since it requires trained observers who are able to distinguish plaque boundaries from vessel wall, although it must be pointed out that good reproducibility was reported by all studies, which evaluated total plaque volume (table 12.1). Vessel wall volume (VWV) is a measurement of vessel wall thickness and plaque within the internal and common carotid. It is considered to be a more reproducible method of volume evaluation since it only requires the segmentation of the media-adventitia and lumen-intima (plaque) boundaries in the 3D images. These boundaries can be more regular and identifiable by an automated or semi-automated segmentation method. Nevertheless the VWV measurement was less reported [Egger M,2007] but was also well reproducible.

In addition it should be noted, that despite the preliminary data which suggest that 3D plaque volume measurements are more sensitive than one-dimensional IMT measurements in identifying plaque changes post treatment [Pollex RL,2005; Mallett C,2009], this has to be interpreted with caution. The IMT is a vessel-related parameter and is not the best marker to assess plaque changes since the intima media complex and the carotid plaque are two biologically distinct structures [43]. In addition the mean increase of IMT in untreated individuals is 0.015 mm per year [Bots ML,2003] when the resolution of the ultrasound is ~0.3 mm. In contrast, carotid plaque volume has been shown to increase by 50 mm^3/year and plaque area by 5 mm^2/year and, thus, potential change can be more easily detected [Spence JD,2005]. Two-dimensional measurements of plaque area and GSM have shown significant potential in identifying vulnerable plaques [Nicolaides AN, Kakkos SK,2010] and thus their predictive ability should be compared with that of 3D volume or composition measurement. Unfortunately no such studies were identified.

Moreover, there was only one study comparing the 3D plaque composition findings between two groups with or without cerebrovascular symptoms. The study by Heliopoulos et al., showed that at moderate degrees of stenosis (between 60-70%), lower total plaque echogenicity might be a significant risk factor for the development of cerebrovascular events [Heliopoulos J,2008]. Lower grey scale levels can be attributed to echolucent material within the plaque such as lipids, haemorrhage or thrombus. This finding may have significant implications in the re-stratification of asymptomatic patients with moderate degree of stenosis. According to the current guidelines these patients are not suitable for any intervention, apart from optimization of their medical management [Liapis CD,2010]. Nevertheless, more studies are needed in order to clarify if 3D composition or morphology evaluation is more sensitive than 2D in the identification of the high-risk individuals within the asymptomatic group. In addition the use of novel texture features, such as second order statistics [Kakkos SK, Stevens JM,2007] for 3D plaque- characterization of both echodensity and heterogeneity, was only explored in one cross-sectional study [Awad J,2010]. Fenster and his colleagues suggested in this study that these features could be more sensitive in the detection of pharmacological changes to plaque than volume measurements. Some of these features have been previously associated with ipsilateral embolic brain infraction [Kakkos SK, Stevens JM,2007]. More data is also needed to clarify the potential of these novel features when used in combination with 3D imaging.

A recent systematic review of the literature also showed that other novel diagnostic approaches for the identification of vulnerable carotid plaques, such as stress wall analysis of the plaque's biomechanical properties, may have promising clinical potential [Makris GC,2010]. Currently, 2D ultrasound and MRI imaging can be combined with fluid–structure interaction (FSI) techniques to create maps of the stress variation upon the plaque's surface, with increased cost and complexity. 3D ultrasound could potentially simplify this process since it combines the increased availability of ultrasound with optimized volumetric information for the whole plaque similar to that provided by MRI [Augst AD,2003].In their study, Gao and Long et al., using 3D ultrasound and FSI simulation-

showed that areas of increased stress on the fibrous cap of the plaque can be identified [Gao H,2008]. However, no studies were identified comparing the volumetric information obtained by 3D ultrasound and that by MRI. Further research on this issue is also warranted.

The main limitation of this systematic review of the available evidence is the heterogeneity between the evaluated studies, which made the meta-analysis of the data unfeasible. Various techniques of 3D image acquisition and analysis were utilized as a result of the technological advances in 3D technology occurring between the publication of the first 3D studies and the latest available reports in the last 20 years. The development of 3D ultrasound probes and the introduction of sophisticated image- analysis software have simplified the 3D imaging process providing us with the opportunity to study more plaque characteristics at the same time and thus to obtain more information for the identification of vulnerable plaques.

Another limitation is the small sample size in most studies and the heterogeneity in outcome measures and follow-up. Most studies evaluated 3D plaque volume whereas only a small number evaluated plaque composition. No studies evaluating both plaque composition and volume were identified. In addition, there was only one study comparing 3D composition between symptomatic and asymptomatic patients, without any reference to plaque volume changes. Finally the possibility of publication bias cannot be excluded.

Despite the above limitations 3D ultrasound may have a significant clinical potential for the identification of vulnerable carotid plaques. The fine reproducibility results that were presented above (Table 12.1) show that despite the current complexity of the involved image analysis methodology, measurements can be easily reproduced for most plaque features. However, there are still areas that need further investigation in order to evaluate the effectiveness of this novel approach. The accuracy of 3D measurements regarding internal carotid artery stenosis was excellent when compared with

traditional 2D ultrasound or even angiography, though similar data regarding the accuracy in plaque, composition or morphology evaluations was rather limited. More studies are currently warranted evaluating the accuracy of 3D ultrasound in total plaque volume, morphology or composition measurements. In addition, more studies are needed comparing the differences of the above mentioned ultrasound features between 2D and 3D ultrasound measurements in patients with asymptomatic and symptomatic carotid plaque disease in order to identify their prognostic ability in the identification of vulnerable plaques. The potential effectiveness of this ultrasound modality for further stroke-risk stratification in symptomatic patients with less than 70% internal carotid stenosis should also be investigated in the future.

12.5.Conclusion

The current guidelines for the management of carotid plaque disease are based on the degree of stenosis and the presence of cerebrovascular symptoms. However, if patients with asymptomatic disease but higher risk could be identified, invasive treatment could only target patients at an increased risk. The good reproducibility of 3D ultrasound in the assessment of various plaque features, as well as the preliminary evidence which suggests increased sensitivity to identify plaque changes post-treatment indicate a significant clinical potential. However, the exact clinical applications of this approach remain undetermined since the existing evidence fails to provide solid data regarding the superiority of 3D ultrasound in comparison to the traditional 2D approach. More research is needed to demonstrate the cost-effectiveness and the exact sensitivity of this promising approach in the identification of the vulnerable plaque and the re-stratification of asymptomatic patients with carotid artery stenosis.

Chapter 13

3D ultrasound features versus stenosis for the identification of the vulnerable plaque

13.1.Introduction

Two dimensional (2D) ultrasound imaging and the degree of carotid stenosis have been for decades a powerful tool for the diagnosis and management of carotid plaque disease. Ultrasound imaging became soon after its first introduction a valuable asset for the vascular surgeon due to its non-invasive nature, the low cost and its undoubted effectiveness to identify and evaluate the carotid anatomy and the associated pathologies such as the formation of an atherosclerotic plaque. Even today in the era of high-tech imaging techniques such as angio-MRI and angio-CT the 2D ultrasound is still surviving and represents our first choice for the initial investigation of a patient with peripheral arterial disease.

Indeed, for a significant number of patients with carotid plaque disease the 2D ultrasound and the subsequent analysis of the information that is provided are sufficient in order to determine the best possible plan of patient care. However, 2D ultrasound can only provide us with a limited number of views (longitudinal and transverse) and certainly their quality and information that they contain can vary since it is an operator-dependant technique. In addition once the slices (2D) have been captured it is not possible to extract images of other planes from them, which prevent us from having the complete picture with regard to this plaque structure.

The 2D ultrasound provides us with the same information as if we were taking an imaging “biopsy” of a certain part of the plaque, which then we consider as representative of the whole plaque. The reason behind this is that atherosclerotic plaques “grow” in a 3-dimensional environment in all directions both in the longitudinal and transverse axis of the artery. In addition, in the case of heterogeneous plaques the single depiction of a 2D ultrasound slice of the carotid plaque may be not really representative in terms of composition (as this can be evaluated by GSM and possible other texture features). Of course, as it was mentioned before, even this approach may be sufficient and cost-effective for a significant number of patients, however, the detrimental effects of cerebrovascular

events urge for the investigation of every potential improvement in the diagnosis and management of the vulnerable carotid plaque.

The development of angio-MRI and angio-CT has introduced a new era in the volumetric vascular imaging. Using highly sophisticated reconstruction software and following the acquisition of images containing volume information in three dimensions (in real time) we can visualize vascular structures in different planes from different angles acquiring more information about them. Nevertheless, these modalities have their own limitation such as: limited accessibility, increased cost, radiation, more invasive nature, increased patient inconvenience and of course they can be contraindicated when contrast has to be used in patient with allergic reaction to that or if renal impairment is present.

Three-dimensional ultrasound could represent the solution in order to combine the advantages of volumetric vascular imaging with MRI or CT with those of ultrasound imaging (increased availability, non-invasive nature and decreased cost). The new volumetric acquisition techniques facilitate the storage of multiple, consecutive and equidistant views which are subsequently converted in a matrix of points which are representative of the volume scanned. The navigation and the 3D reconstruction of the underlying structures can be then performed by sophisticated software such as VOCAL® or Q-Lab®.

The application of three-dimensional ultrasound for the evaluation of carotid plaques is not new as it was demonstrated in the previous chapter (chapter 12). So far the majority of studies focused on the use of novel 3D ultrasound systems for the evaluation of carotid plaque volume and its association with cerebrovascular events. On the other hand there is not enough data regarding the use of 3D ultrasound for the evaluation of carotid plaque composition and texture. Plaque volume is only one of the features that characterise the carotid plaque but there are texture features such as the grey scale median (GSM) that have shown promise in the identification of the vulnerable plaque.

Our hypothesis was that certain 3D volume and texture measurements would distinguish the symptomatic from the asymptomatic group, when used in a small sample sizes, better than the degree of stenosis alone.

13.2.Methods

13.2.1Study design

A cross-sectional, case -control study evaluating the diagnostic effect of 3D volume –texture ultrasound on symptomatic and asymptomatic patients with carotid plaque disease was designed. Eligible patients attended one visit during which they had both the traditional 2D ultrasound imaging as well as the 3D ultrasound test (Figure 13.1).

13.2.2Eligibility criteria

i. Asymptomatic patients with no reported cerebrovascular events and internal carotid stenosis (ICA) >20% (ECST)

ii. Patient with history of stroke, transient ischemic attack or amaurosis fugax within the previous 6 months and ICA stenosis>20%

iii. Patients presenting at the Vascular Non Invasive Diagnostic Centre (Weymouth 30, London, UK), Ealing Hospital, NHS Trust, London, UK , Charing Cross Hospital, NHS Trust, London, and University College of London Hospitals, UK for investigation of their carotid plaque disease.

iv. Patients able to understand and sign the provided consent form.

13.2.3.Exclusion criteria

i. Patients with ICA stenosis <20%

ii. Patients with extensive carotid plaque calcification and acoustic shadowing (not included in the analysis)

iii. Patients with high carotid bifurcation (unable to scan due to the size of the 3D probe)

13.2.4. Study Sites

The recruitment of the study-subjects, the brief physical examination, the signing of the inform consent as well as the ultrasound examinations (2 and 3D) were performed within the premises of the Vascular Non-invasive Screening and Diagnostic Centre (CDER Trust, 30 Weymouth Street, London, W1G 7BS).

The ultrasound scanning was blindly performed by Dr Maura Griffin who is an experienced clinical scientist without her being aware of the clinical status of the patient.

Patients were also identified at the following centres:

i. Outpatient clinic of Mr Geroulakos who is a consultant vascular surgeon at Ealing Hospital, NHS Trust, London, UK.

ii. Stroke unit of Charing Cross Hospital, NHS Trust with the help of Dr P Bentley who is a consultant neurologist.

iii. Vascular surgery department (Mr T Richards -consultant vascular surgeon at University College of London Hospital, FRCS), the Hyper Acute Stroke Unit and the neurology department (Professor M Brown) of the University College London Hospitals.

13.2.5.Scanning technique and image analysis

This was discussed at the Methods section of the thesis at Chapter 6. For the image analysis in this study we used thick slices of 2.5mm to assess the feasibility of performing volumetric measurement and assessment of plaque's composition and ultrasound texture. Plaques with excessive acoustic shadowing or image degradation due to motion or other artefacts were excluded from the final analysis. The image analysis was performed blindly with regard to the symptoms of the patient.

13.2.6.Statistical analysis

The variables were explored regarding their pattern of distribution and when normal distribution was demonstrated then a t-test for dependant variables was used to compare the means between the symptomatic and asympomatic groups. To compare the means between 3 groups (i.e asymptomatics vs. TIA group vs. Stroke group) the ANOVA statistical test was used. When normal distribution was not the case then the variables where transformed using appropriate transformations. Finally the receiver operator curves were used to plot sensitivity and specificity of the various imaging features according

to the presence of symptoms or not. The area under the curve was also calculated. The level of statistical significance was set to $p<0.05$. The SPSS statistical package was used.

13.2.7. Ethics

Ethical approval for this was granted by the West London research ethics committee, UK (11/LO/0299).

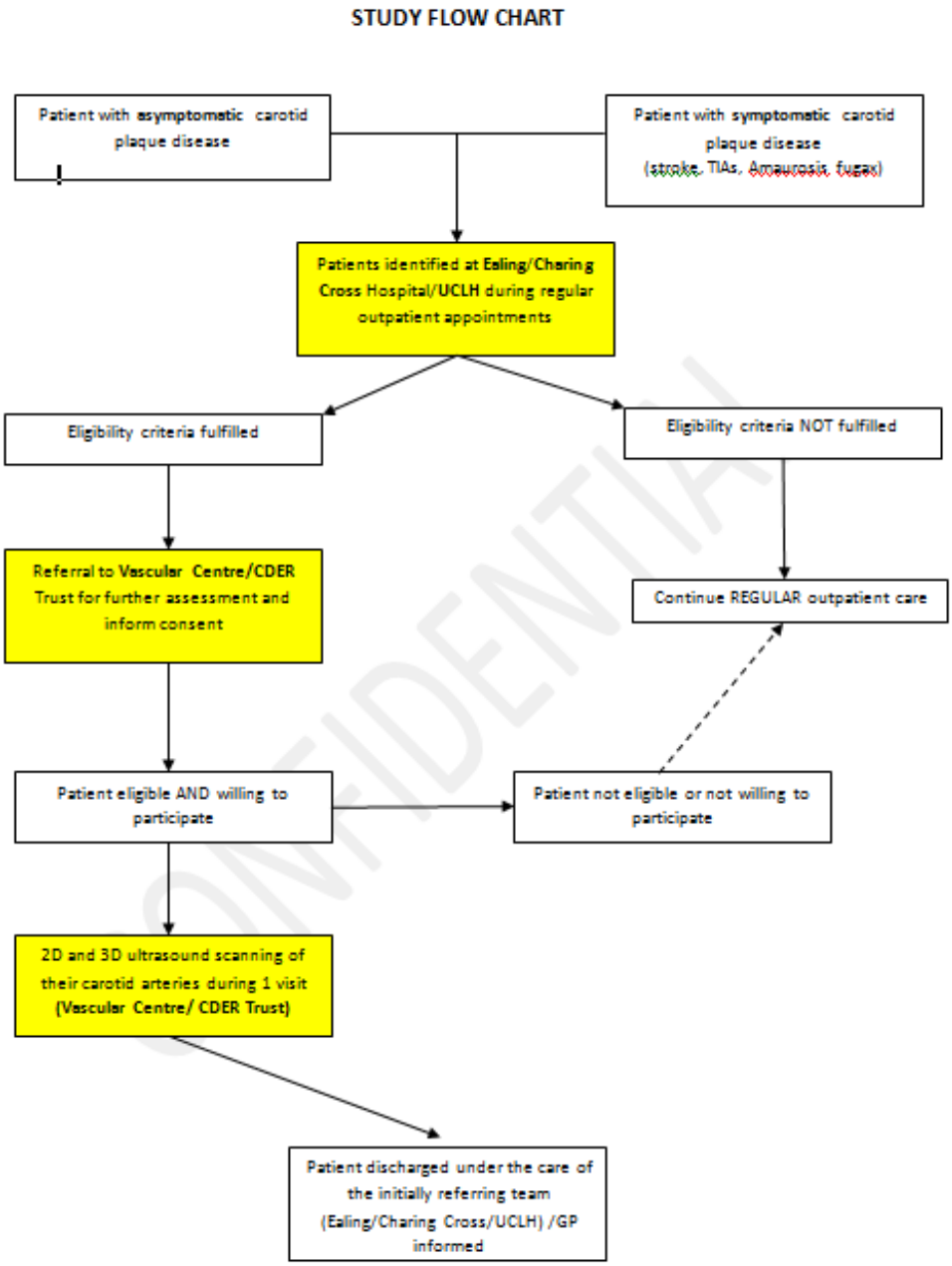


Figure 13.1: Flow chart diagram of the patient recruitment process.

13.3.Results

13.3.1. Patient Demographics

In total 90 patients were identified and signed the informed consent form. They were all scanned with the new 3D probe as previously described. The carotid plaque images from 21 of them were not suitable for further image analysis. The main reasons for exclusion were plaque calcification, high carotid bifurcation and motion artefact. As a result a number of 87 carotid plaques entered the final analysis. Males were the vast majority (80.2%) with 57 asymptomatic and 20 symptomatic cases (1:3 ratio). The mean ECST carotid stenosis was 62% (SD: 19.52).

13.3.2. Volumetric analysis using 3D

The volumetric analysis consisted of measurements regarding the total plaque volume (mm^3), the average plaque area (average of cross-section plane-mm^2) and the total plaque area (mm^2) that was imaged. Using 2.5mm sections for the image analysis of the plaques, we retrieved 62 complete measurements. When the volume and plaque area measurements were compared between the asymptomatic and the symptomatic group (both TIA/AF and stroke), respectively, the results were as follow:

- Mean Plaque Volume(mm^3): **238.3** (SD:107) vs. **200.9** (SD:125) , p=0.568
- Plaque Area average (mm^2): **16.3** (SD:6) vs. **14.7** (SD:5.9), p=0.809
- Mean Plaque Area total (mm^2): **95.4** (SD:42) vs. **81.8**(48.4), p=0.648

No statistical significance between the symptomatic and the asymptomatic group was identified for the three variables.

In subgroup analysis we separated the group of symptomatics into those who had TIA/AF and those who had strokes only. By using the ANOVA statistical test we were able to compare the volumetric and plaque area means between the asymptomatic vs. TIA/AF group vs. stroke group as it is shown below.

- Mean Plaque Volume(mm^3): **238** (SD:107) vs. **260** (SD:130) vs. **148** (SD:101), *p=0.081*

- Plaque Area average (mm^2): **16** (SD:6) vs. **17** (SD:7) vs. **12** (SD:2), *p=0.112*

- Mean Plaque Area total (mm^2): **95**(SD:42) vs. **104**(SD:52) vs. **62** (SD: 37), *p=0.106*

In the subgroup analysis no statistical significance between the symptomatic and the asymptomatic group was identified for the three variables.

Finally, ROC curves were used to compare the predictive ability of models that use as discriminating factor plaque volume vs. Plaque area vs. Stenosis (Figure 13.2). The percentage of ECST degree of stenosis was also used, as it is currently the gold standard for the surgical planning. The area under the curve was used to compare the predictive ability with regard to Stenosis vs. Plaque volume vs. Average plaque area (mm2) vs. Total plaque area, respectively:

- **0.604** (SE:0.082) vs. **0.372** (SE:0.087) vs.**0.397** (SE:0.086) vs **0.373** (SE:0.086), *p>0.05*

The volumetric parameters failed to improve the diagnostic ability when compared to stenosis.(Figure 13.2)

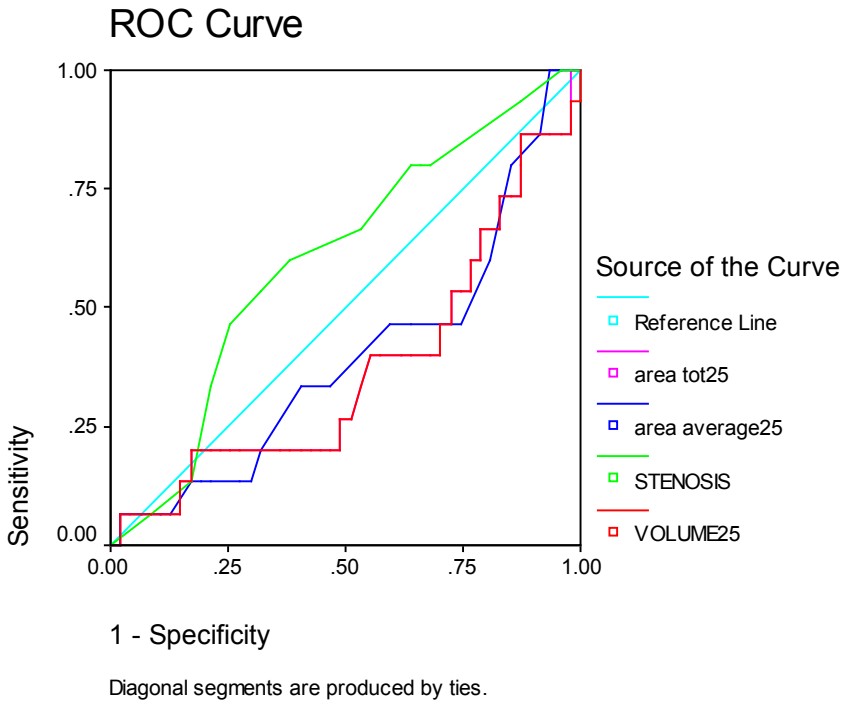


Figure13.2: ROC curves comparing stenosis vs. Plaque volume vs. Average plaque area vs. Total plaque area. Volumetric parameters failed to improve the diagnostic ability when compared to stenosis.

(**Abbreviations:** *area tot25*: total plaque area measured with 2.5mm slices in 3D; *area average 25*: plaque area measured with 2.5mm slices in 3D; *stenosis*: measured in ECST degrees and in 2D; *volume*: plaque volume measured with 2.5mm slices in 3D)

13.3.3.Composition analysis using 3D

The composition analysis consisted of measurements of the grey scale median (GSM) of the entire plaque and the juxtaluminal black area (JBA) using the 3 dimensional observations. Sections of 2.5mm thickness were obtained from 66 carotid plaques. After comparing the GSM and the JBA for entire plaque volume between the asymptomatic and the symptomatic group (both TIA/AF and stroke), respectively, the results were as follow:

- GSM (entire plaque): **35.2** (SD: 14) vs. **33.5** (SD: 15.9), p=0.9
- JBA (entire plaque-mm2): **3.2** (SD: 3.4) vs. **4.1** (SD: 3.5), p=0.35

No statistically significant difference between the groups was identified for the GSM and JBA. In subgroup analysis, as previously, we separated the group of symptomatics into those who had TIA/AF and those who had strokes only. By using the ANOVA statistical test again we were able to compare the composition features (GSM and JBA) between the asymptomatic vs. TIA/AF group vs. Stroke group, respectively, as it shown below.

- GSM (entire plaque): **35.2** (SD:14) vs**. 39.5** (SD:19) vs. **28.3** (SD:11), *p=0.305*
- JBA (entire plaque-mm2): **3.2** (SD:3) vs. **3.9**(SD:4.1) vs. **4.1** (SD:3.1), *p=0.722*

In the subgroup analysis no statistical significant difference between the groups was identified for the two variables. Finally, ROC curves were used to compare the predictive ability of models that use as discriminating factor GSM vs. JBA vs. Stenosis (Figure 13.3) . The percentage of ECST stenosis was again used, as it is currently the gold standard for the surgical planning. The area under the curve was used to compare the predictive ability with regard to Stenosis vs. GSM vs. JBA (mm2) , respectively:

- **0.582**(SE:0.081) vs. **0.446**(SE:0.087) vs.**0.562** (SE:0.086) *p>0.05*

The plaque composition parameters failed to improve the diagnostic ability when compared to degree of stenosis. (Figure 13.3)

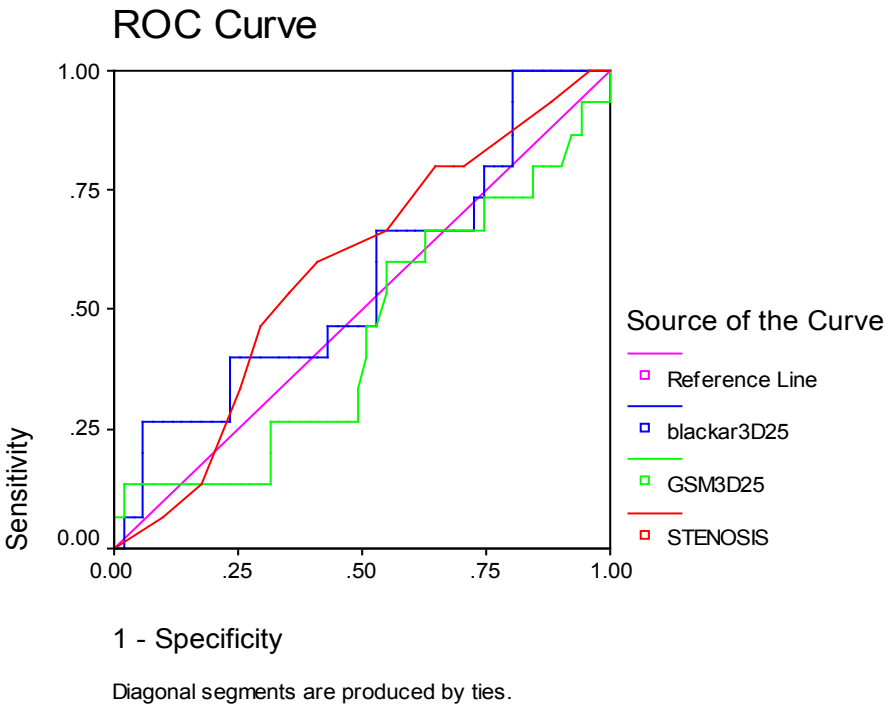


Figure 13.3: ROC curves were used to compare the predictive ability of models that use as discriminating factor GSM vs. JBA vs. Stenosis

(**Abbreviations:** black *ar 3D 25*: Juxtaluminal black area measured with 2.5mm slices in 3D; *GSM 25*: Grey scale median with 2.5mm slices in 3D; *stenosis*: measured in ECST degrees and in 2D)

13.4.Discussion

This is the first study to assess the 3D songraphic changes between symptomatic and asymptomatic carotid plaques in terms of both plaque composition and volume. Two-dimensional ultrasound cannot provide us with volumetric information unless very laborious reconstructions are undertaken. In addition the composition information that we receive when we analyse 2D carotid images can be limited in cases of heterogeneous plaques since we only have an average measurement from a specific imaging angle. In theory this is the advantage of this new 3D technique; that we can relatively easily evaluate the entire plaque with one scan and simultaneously assess both the plaque volume and composition.

Only one study was identified, as previously discussed, assessing the possible differences in 3D ultrasound features between patients with asymptomatic or symptomatic carotid disease. [Heliopoulos J,2009] The studies investigated 110 cases of symptomatic and 104 asymptomatic patients using 3D ultrasound. They assessed the echotexture of the plaques by means of 3D imaging using the mean grey scale value of the whole plaque without any information regarding plaque volume or morphology changes. They concluded that, especially, at moderate degrees of ICA stenosis (less than 60-70%), lower mean grey values could be an important factor indicating plaque vulnerability and increased risk for a stroke.

In this study we managed to obtain a number of 3D carotid images that were successfully analysed providing us with both compositional and volume information for the entire plaque volume. For this study thick slices of 2.5mm were used to minimise the necessary time for computer processing and analysis of the sections. Features such as the plaque volume, plaque area, grey scale median and juxtaluminal black area (JBA) were measured and compared within the two groups. Despite the fact that there seemed to be some differences between the volume or texture features of symptomatic versus asymptomatic plaques, these differences did not reach statistical significance. In addition they

also did not seem to improve on the diagnostic ability currently achieved using the simple measurement of the degree of stenosis.

The main limitations of this study is the small number of patients especially at the symptomatic group which is mainly due to the difficulty in accessing those patients before they have their operation which usually happens within the first 48 hours after the event. In the future we have already applied for an extension of our ethics approval in order to include more patients especially in the symptomatic group. Another possible limitation is the selection of slice thickness that has been shown to affect the accuracy of measurements. It is possible that the 2.5mm slice thickness was relatively thick to allow improvement on the prediction ability in comparison to the degree of stenosis or other traditional 2D ultrasound features.

13.5 Conclusion

The analysis of 3D carotid images using thick cross sectional slices did not significantly improve our ability to differentiate between symptomatic and asymptomatic plaque when compared to traditional 2D ultrasound measurements.

Chapter 14

3D ultrasound slice thickness and its effect on stroke prediction

14.1. Introduction

In the previous chapter we assessed the effectiveness of certain 3D ultrasound features in distinguishing between symptomatic and asymptomatic plaques. For the purposes of that analysis we used 2.5mm inter-slice distances (ISD) for the sectioning of the plaque. Unfortunately this approach did not result in the identification of more sensitive imaging markers for the identification of the vulnerable plaque. One of the possible limitations was the use of thick slices that may not accurately represent the entire plaque.

Indeed, the effect of the ISD as well as the effect of the initial plaque size in the volume reproducibility measurements has already been assessed by 2 studies [Landry A,2004; Ludwig M,2008]. Both of them concluded that ISD might have an effect on measurement variability. More specifically they showed that volume measurement variability can be smaller for larger plaques, while Fenster et al., suggested a possible effect of the ISD on the observed variability. [Landry A,2004] There were no studies though exploring the effect of ISD on the identification of the vulnerable plaque and stroke prediction.

The purpose of this study was to assess if thinner 3D ultrasound slices will improve the predictive ability of the 3D measurements in terms of differentiating between symptomatic and asymptomatic plaques.

14.2.Methods

14.2.1.Study population and design

The study population characteristics and the study design were discussed in the Methods section of Chapter 13.

14.2.3.Image analysis

All images from the 3-dimensional dataset from the eligible patients, that were analysed previously, ware cropped again using thinner sections of 1.5mm. Again, the image analysis and cropping were performed blindly in relation to the symptoms of the patient and by the same operator (GCM).

14.2.4.Statistical analysis

The variables were explored regarding their pattern of distribution and when normal distribution was demonstrated then a t-test for dependant variables was used to compare the means between the symptomatic and asympomatic groups. To compare the means between 3 groups (i.e asymptomatics vs. TIA group vs. Stroke group) the ANOVA statistical test was used. When normal distribution was not the case then the variables where transformed using appropriate transformations. Finally the receiver operator curves were used to plot sensitivity and specificity of the various imaging features according to the presence of symptoms or not. The area under the curve was also calculated. The level of statistical significance was set to $p<0.05$. The SPSS statistical package was used.

14.3.Results

A number of 67 carotid plaques were available for analysis using thinner slices. Using 1.5mm thickness slices we produced on average of 9.5 (95CI: 8.7-10.2) slices vs. 5.4 (95% CI: 5.07-5.92) that were produced by using 2.5mm inter-slice distance.

14.3.1.Differences between 2.5mm and 1.5mm slice thickness

We assessed the differences in the observed values in composition and volume features and compared them between the thinner (1.5mm) and thicker (2.5mm) slices protocols. As it can be seen on table 14.1 there are statistically significant differences in all variables between the thin and thick slices group.

14.3.2.Volumetric imaging with thinner slices vs. stenosis

The volumetric analysis consisted of measurements regarding the total plaque volume (mm^3), the average plaque area (average of cross-section plane-mm^2) and the total plaque area (mm^2) that was imaged. Using 1.5mm sections for the image analysis of the plaques we retrieved 76 complete measurements for 76 carotid plaques. By comparing the volume and plaque area between the asymptomatic and the symptomatic group (both TIA/AF and stroke), respectively, the results were as follow:

- Mean Plaque Volume (mm^3): **342.42** (SD:168) vs. **318.4** (SD:198), p=0.795
- Plaque Area average (mm^2): **22.3** (SD:8.72) vs. **23.47** (SD:8.14), p=0.752
- Mean Plaque Area total (mm^2): **229.33** (SD: 124) vs. **208.37** (SD:134), p=0.623

No statistical significance between the groups was identified with the thinner slices for the three variables.

Table 14.1: Differences in the observed values in composition and volume features when compared between the thinner (1.5mm) and thicker (2.5mm) slices

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Pair 1	VOLUME15 - VOLUME25	114.25	132.19	16.15	82.01	146.50	7.075	66	.000
Pair 2	GSM3D15 - GSM3D25	2.197	9.116	1.060	8.522E-02	4.309	2.073	73	.042
Pair 3	area average15 - area average25	7.13	7.79	.95	5.23	9.03	7.500	66	.000
Pair 4	area average15 - area average25	7.13	7.79	.95	5.23	9.03	7.500	66	.000
Pair 5	blackar3D15 - blackar3D25	1.2071	3.8622	.4490	.3124	2.1019	2.689	73	.009

Abbreviations*:* *GSM3D15*: Grey scale media with 1.5mm slices in 3D; *GSM3D25:* Grey scale media with 1.5mm slices in 3D; *blackar3D25:* juxtaluminal black are with 2.5mm slices in 3D; *blackar3D15:* juxtaluminal black are with 1.5mm slices in 3D.

In subgroup analysis we separated the group of symptomatic patients into those who had TIA/AF and those who had strokes only. By using the ANOVA statistical test we were able to compare the volumetric and plaque area means between the symptomatic vs. TIA/AF group vs. Stroke group as it is shown below.

- Mean Plaque Volume (mm^3): **342.41** (SD: 168) vs. **359.30**(SD: 219) vs. **273** (SD:173), p=0.503
- Plaque Area average (mm^2): **22.3** (SD:8) vs**. 25.9** (SD:8) vs. **20.78** (SD:6.67), p=0.385
- Mean Plaque Area total (mm^2): **229.3** (SD:124) vs. **236.9** (SD:146) vs. **176.6** (SD:119), *p=0.492*

In the subgroup analysis no statistical significance between the groups was identified for the three variables.

Finally, ROC curves were used to compare the predictive ability of models that use as discriminating factor plaque volume vs. Plaque area vs. Stenosis (Figure 14.1). The percentage of ECST degree of stenosis was used, as it is currently the gold standard for the surgical planning. The area under the curve was used to compare the predictive ability with regard to Stenosis vs. Plaque volume vs. Average plaque area (mm2) vs. Total plaque area, respectively:

- **0.646** (SE:0.073) vs. **0.426** (SE: 0.076) vs**. 0.543** (SE: 0.076) vs. **0.426** (SE:0.076)

The volumetric parameters does not seem to improve the diagnostic ability when compared to stenosis even when thinner slices were used.

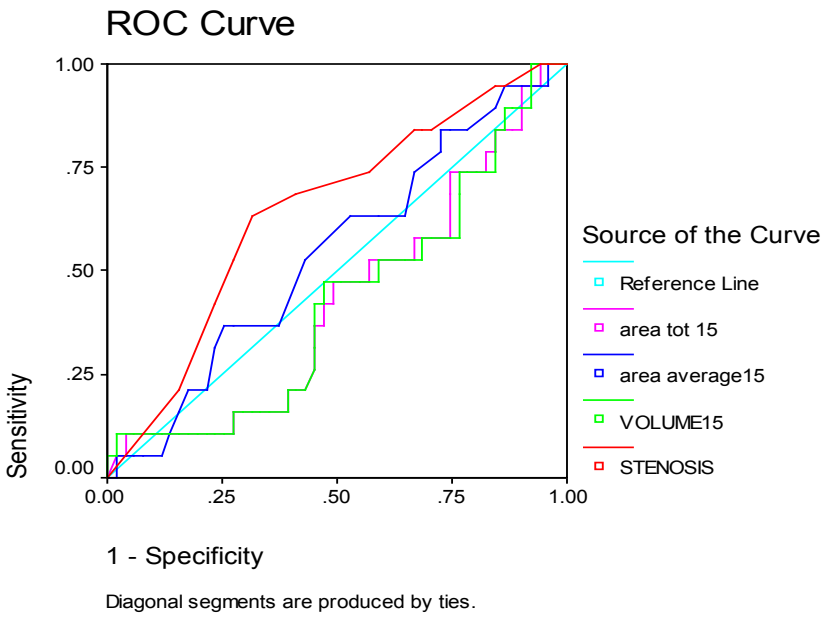


Figure 14.1: ROC curves comparing plaque volume vs. Plaque area vs. Stenosis regarding their use as tools for stroke risk prediction.

14.3.3.Composition measurements with thinner slices vs. Stenosis

The composition analysis consisted of measurements of the grey scale median (GSM) of the entire plaque and the juxtaluminal black area using the 3 dimensional observations. Sections of 1.5mm thickness were obtained from 66 carotid plaques. After comparing the GSM and the JBA for entire plaque volume between the asymptomatic and the symptomatic group (both TIA/AF and stroke), respectively, the results were as follow:

- GSM (entire plaque): **36.25** (SD:13.8) vs. **35.2** (SD:18.2), *p=0.116*
- JBA (entire plaque-mm2): **4.5** (SD: 4) vs. **6.03** (SD:5.2), *p=0.099*

No statistical significance in the GSM and JBA values between the groups was identified.

In subgroup analysis, as previously, we separated the group of symptomatics into those who had TIA/AF and those who had strokes only. By using the ANOVA statistical test we were able to compare the composition features (GSM and JBA) between the symptomatic vs. TIA/AF group vs. Stroke group, respectively, as it shown below.

- GSM (entire plaque): **36.2** (SD:13) vs. **35.6** (SD:18) vs. **34.8** (SD: 18),p>0.05
- JBA (entire plaque-mm2): **4.5** (SD:4) vs. **6.8** (SD: 5.5) vs. **5** (SD: 5), p>0.05

In the subgroup analysis no statistical significance between the groups was identified for the two variables. Finally, ROC curves were used to compare the predictive ability of models that use as discriminating factors the GSM vs. JBA vs. Stenosis for the thinner slices (Figure 14.2). The percentage of ECST stenosis was again used, as it is currently the gold standard for the surgical planning. The area under the curve was used to compare the predictive ability with regard to stenosis vs. GSM vs. JBA (mm2) , respectively:

- **0.464**(SE:0.8,p=0.068) vs. **0.464**(SE:0.083, p=0.636) vs. **0.574** (SE:0.08, p=0.334)

The composition features also failed to improve the diagnostic ability when compared to stenosis.

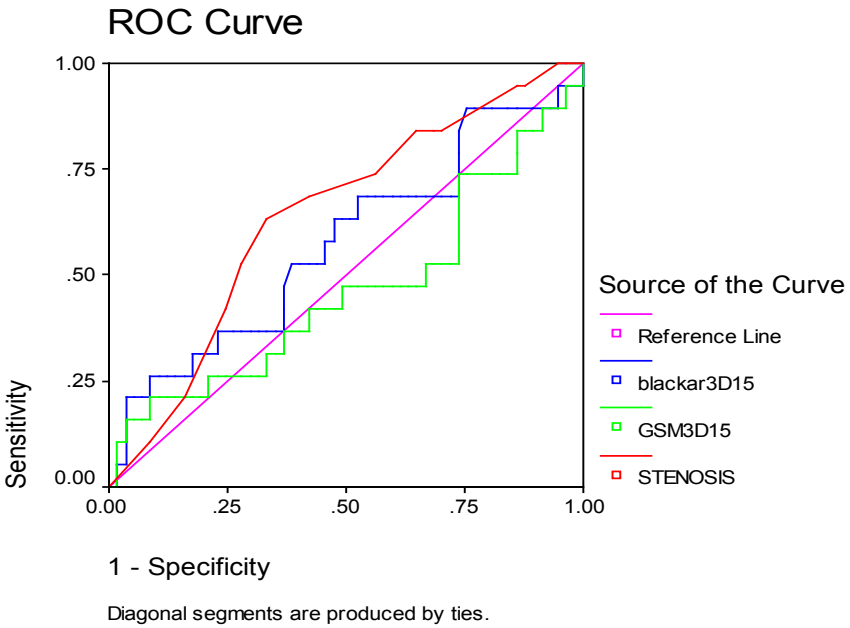


Figure 14.2: ROC curves were used to compare the predictive ability of models that use as discriminating factors the GSM vs. JBA vs. Stenosis for the thinner slices (1.5mm).

(Abbreviations: *Blackare3D15:* juxtaluminal black area with 1.5mm sections in 3D; *GSM3D15*: Grey scale median with 1.5mm slices in 3D)

14.4.Discussion

This study investigates for the first time the effect of thin 3D ultrasound slices on the identification of vulnerable carotid plaque plaques. After evaluating the effectiveness of 2.5mm in slices in (chapter 13) we repeated the entire process using thinner transverse sections (1.5mm) to see if we can improve the predictive ability of this approach. Indeed, the overall measurements from the thinner and thicker slices were significantly different, which may have significant implications for future applications of the 3D ultrasound. Especially with regard to the selected interslice distance (ISD) it can alter the characterization of the plaque according to how heterogeneous the plaque is.

The analysis of the thinner slices was obviously more time-consuming than then thicker ones since more transverse plaque sections were produced and which had to be manually cropped and analysed. The main question was if that extra time and effort would actually translate in diagnostic benefit in terms of identifying more vulnerable plaques when compared to the thicker slices. Unfortunately that did not proved to be the case. The volume and composition features of the thinner slices again did not seem to perform better than the degree of stenosis in terms of stroke prediction.

The effect of the inter-slice distance as well as the effect of the initial plaque size in the volume reproducibility measurements has been assessed in the past by only two studies [Landry A,2004; Ludwig M,2008]. Both studies concluded that volume measurement variability can be smaller for larger plaques, while Fenster et al., [Landry A,2004] suggested a possible effect of the ISD on the observed variability. More specifically Fenster et al., showed that carotid plaque volume measurement variability decreased with increasing plaque volume whereas it increases with ISD. In another study by Stumpe et al., it was also shown that both intra- and inter-reader variability of the measurement of carotid plaque volume were lower for larger than for smaller plaques. [Ludwig M,2008].

14.5 Conclusion

The results from previous studies as well as from the current one indicate that the plaque volume and the inter-slice distance are important factors and have to be taken into account when analysing 3D images.

Our study showed that the thickness of the analysed slice did not really affect the stroke risk predictive ability but this has to be verified by other studies with large sample sizes.

Chapter 15

3D versus 2D ultrasound for carotid plaque assessment and risk for stroke

15.1.Introduction

Two-dimensional ultrasound has been for decades a powerful tool for the assessment of carotid atherosclerosis. The recent technological advances though, have provided us with opportunities to assess carotid plaques in all their 3 dimensions. Currently there are no studies comparing the traditional 2D versus the novel 3D ultrasound probes, in terms of their effectiveness in characterising carotid plaques. Given the significantly higher price for a 3D probe, a cost effectiveness analysis would be reasonable to support or reject the wide implementation and switch of healthcare practitioners to the more expensive 3D probes.

The main theoretical benefits from the use of 3D ultrasound versus the 2D are:

- Automatic acquisition of the entire plaque volume, which may prove beneficial especially for the characterization of heterogeneous plaques
- Possibilities for semiautomatic segmentation in different plains and orientation and off-line analysis with the possibility of various reconstructions.

The main disadvantages on the other hand are:

- Increased cost
- Increased scanning time
- Bulkier probes that make handling more demanding

This is one of the first studies to investigate the effect of 3D ultrasound measurements in carotid plaque characterisation and stroke prediction versus the use of traditional 2D ultrasound. Our main objective was to determine if 3D measurements of traditional and novel features have better predictive ability compared to the 2D ones with regard to stroke risk assessment.

15.2.Methods

15.2.1.Study population and study design

As discussed in the methods section of Chapter 15.

15.2.2.Image analysis

All eligible patients apart from a 3D ultrasound scan, also had a traditional 2D scan of the corresponding carotid plaque. The corresponding images to the 3D dataset were separately processed, cropped and analysed as it has been described in Part II, Chapter 6. The analysis of the 2D carotid views was performed at the longitudinal view of the carotid plaque in order to summarise the entire plaque. As before, images with extensive acoustic shadowing, calcification or motion artefact were excluded.

We also calculated the values for all available novel texture features (second order statistics) that were discussed in chapter 4 from the 3D dataset. All the novel textures features were calculated both for the thin (1.5mm) and thick slices (2.5mm). The features from the 2D dataset were also extracted as previously described (chapter 4).

15.2.3.Statistical analysis

Evaluation of the stroke predictive ability of 3D vs. 2D ultrasound features

The predictive ability of traditional features such as degree of stenosis, grey scale median, and JBA were compared between the 2D and 3D measurements (thin and thick slices) using ROC curves and the area under the curve. Odds ratio for traditional texture features as well as novel ultrasound features (i.e second order statistics-APPENDIX I) were determined using unadjusted logistic regression for each variable separately. Continuous risk factors were transformed to an unskewed distribution where possible. Risk factors which were significant at $p < 0.05$ in these unadjusted models were then considered in multivariable logistic regression model. The ability of each feature to predict a cerebrovascular event was tested by using the ROC curve and the area under the curve The IBM SPSS statistical package (version 20, SPSS Inc, Chicago, Ill) was used for statistical analysis and construction of graphs

15.3. Results

15.3.1.Evaluation of the stroke-predictive ability of volumetric imaging: 2D vs. 3D

There was no improvement in the stroke risk predictive ability between the 2D and 3D volume and plaque area measurements. The degree of stenosis appears superior in identifying the plaques with increased risk for stroke. The area under the curve for every variable can be seen on table 15.1.

15.3.2. Evaluation of the stroke predictive ability of Composition measurements: 2D vs.3D

There was no improvement in the stroke risk predictive ability between the 2D and 3D plaque texture analysis measurements either. No improvement over the percentage of stenosis alone was noted .The area under the curve for every variable can be seen on table 15.1.

Table 15.1: Area under the curve for Composition and volume measurements in 2 and 3 dimensions for thin and thick slices

Test Result Variable(s)	Area	Std. Error[a]	Asymptotic Sig.[b]	Asymptotic 95% Confidence Interval	
				Lower Bound	Upper Bound
STENOSIS	.594	.083	.280	.432	.756
AREA_MM2	.433	.081	.444	.274	.592
area tot 15	.398	.087	.240	.227	.569
area tot25	.373	.088	.144	.200	.545
GSM3D15	.481	.090	.824	.305	.657
GSM3D25	.446	.087	.530	.276	.617
GSM	.565	.080	.449	.407	.722
VOLUME25	.371	.088	.139	.199	.544
VOLUME15	.400	.088	.251	.228	.572
blackar3D15	.562	.086	.467	.394	.730
blackar3D25	.562	.086	.467	.393	.731

Abbreviations: AREA_MM2: plaque area measured in 2D**, AREA TOT 15**: plaque area measured in 3D with 1.5mm slice thickness**, AREA TOT 25**: plaque area measured in 3D with 2.5mm slice thickness, **GSM**: grey scale media in 2D, **GSM3D15**: GSM measured in 3D with 1.5mm slices, **GSM3D25**: GSM measured in 3d with 2.5mm slices,**VOLUME25**: plaque volume measured in 3D with the 2.5mm slice thickness**, VOLUME15**: plaque volume measured in 3D with 1.5mm slice thickness, **Blackar3D15**: juxtaluminal black area measured in 3D with 1.5mm slices, **Blackar3D25**: juxtaluminal black area measured in 3D with 2.5mm slices

15.3.3.Evaluation of 2D and 3D novel texture features association with cerebrovascular events

As we shown before (chapter 8) certain novel texture features can improve the identification of the vulnerable plaque. We sought to assess if that is case when using the 3D ultrasound measurements and if there would be any improvement over the corresponding 2D values of the same variables.

The second order statistics (Appendix II) SGLD-VAR (1.5mm), SGLD_SVA (1.5mm), SGLD-DEN (2.5mm), SGLD_DVA (2.5mm), SGLD_DVA (2D), SGLD_DEN(2D), were the only significant factors at the univariate logistic regression analysis which were associated with the occurrence or cerebrovascular events (table 15.2)

However, in the multivariable logistic regression model with all the significant features from Table 15.2 as covariates, only SGDL-VAR (1.5mm) and SGLD-SVA (1.5mm) were independent predictors of cerebrovascular events (Table 15.3).

The ROC curves from the resulting features SGDL-VAR (1.5mm) and SGLD-SVA (1.5mm) had an area under the curve of 0.498 (SE:0. 086, p=0.98) and 0.5 (SE:0.086, p=0.98) respectively, but without any improvement when compared to the degree of stenosis (figure 15.1)

Table 15.2.Univariate logistic regression of all novel texture features and risk of stroke

	B	S.E.	Wald	df	Sig.	OR
GSM3D15	.048	.030	2.520	1	.112	1.049
VOLUME15	.005	.010	.223	1	.637	1.005
AREAVR15	.080	.059	1.850	1	.174	1.083
AREATOT1	-.014	.015	.829	1	.362	.986
BLAKAR15	.171	.110	2.404	1	.121	1.186
VOLUME25	.076	.169	.201	1	.654	1.079
GSM3D25	.022	.031	.510	1	.475	1.023
ARAVER25	-.053	.087	.374	1	.541	.948
AREATO25	-.192	.423	.207	1	.649	.825
BLAKAR25	.163	.135	1.454	1	.228	1.177
STENOSIS	***.035***	***.018***	***3.907***	***1***	***.048***	***1.036***
GSM	.016	.016	.989	1	.320	1.016
AREA_MM2	-.008	.010	.601	1	.438	.992
AGECOD	-.007	.023	.080	1	.777	.993
FPSFANG	.000	.001	.002	1	.969	1.000
FSFANG15	.002	.002	1.884	1	.170	1.002
FSFANG25	-.001	.002	.536	1	.464	.999
FPSFRAD	.000	.001	.015	1	.901	1.000
FSFRAD15	-.001	.001	1.602	1	.206	.999
FSFRAD2	.001	.002	.400	1	.527	1.001
GLDCON15	-.032	.034	.925	1	.336	.968
GLDCON25	.006	.026	.047	1	.829	1.006
GLDMENE	-36.828	80.1	.207	1	.649	.000
GDMENE15	-58.426	65.5	.794	1	.373	.000
GLDMENE25	1.601	1.397	1.312	1	.252	4.957
GLDMENT	-.767	8.2	.009	1	.926	.464
GDMENT15	-.402	5.689	.005	1	.944	.669
GLDENT25	-14.810	10.8	1.848	1	.174	.000
SGLDEN25	***-13***	***9.769***	***1.807***	***1***	***.029***	***.930***
GLDHHOM	-1.212	3.331	.133	1	.716	.297
GLMHOM15	-2.954	29.38	.010	1	.920	.052
GLDHOM25	-.634	23.8	.001	1	.979	.530
GLDMMEA	.009	.090	.010	1	.919	1.009
GDMMEA15	-2.783	2.739	1.032	1	.310	.062
GLDMEA25	-.269	.419	.412	1	.521	.764
RUNLGLD	-.002	.004	.256	1	.613	.998
RUNGLD15	-1.228	.801	2.351	1	.125	.293
SGLDDEN	***-.8***	***1.948***	***.188***	***1***	***.045***	***.430***
RUNGLD25	.303	.519	.340	1	.560	1.354
RUNLLRE	.077	.178	.188	1	.665	1.080
RUNLRE15	1.033	3.052	.114	1	.735	2.808
RUNLRE25	-1.732	3.191	.295	1	.587	0.177
RUNLRLD	.000	.000	.115	1	.734	1.000
SGLDVA25	***.02***	***.049***	***.238***	***1***	***.035***	***1.024***
RUNRLD15	-.001	.003	.169	1	.681	.999
RUNRLD25	-.003	.004	.855	1	.355	.997
RUNRP15	.664	2.023	.108	1	.743	1.942
RUNRP25	2.526	2.931	.743	1	.389	12.506
RUNSRE15	1.8	15.656	.014	1	.907	6.181
RUNSRE25	-5.3	15.715	.114	1	.735	.005

	B	S.E.	Wald	df	Sig.	OR
SGDCON15	-.05	.037	2.241	1	.134	.946
SGLDDVA	***.14***	***.088***	***2.584***	***1***	***.018***	***1.151***
SGLCON25	.033	.023	1.947	1	.163	1.033
SGLDCON	.024	.025	.886	1	.346	1.024
SGLSVA25	-.05	.024	2.094	1	.148	.966
SGDVAR15	***-.23***	***.143***	***3.128***	***1***	***.041***	***.777***
SGLVAR25	.136	.096	2.016	1	.156	1.145
SGDSVA15	.06	.036	3.221	1	.053	1.067
SGLSVA25	-.03	.024	2.094	1	.148	.966
SGDSVA15	***.1***	***.056***	***3.998***	***1***	***.046***	***1.118***
SGLVAR25	.13	.096	2.016	1	.156	1.145

Abbreviations: Please see Appendix II

Table 15.3: Multivariate logistic regression of significant features from table 15.2 and risk of stroke.

	B	S.E.	Wald	df	Sig.	Exp(B)
SGDVAR15	***-.4***	***.223***	***3.964***	***1***	***.046***	***.642***
STENOSIS	.03	.022	1.062	1	.303	1.023
SGDSVA15	***.11***	***.056***	***3.998***	***1***	***.046***	***1.118***
SGLDEN25	-13	9.769	1.807	1	.179	.000
SGLDVA25	.02	.049	.238	1	.625	1.024
SGLDDVA	.14	.088	2.584	1	.108	1.151
SGLDDEN	-.8	1.948	.188	1	.665	.430

Abbreviations: Please see Appendix II.

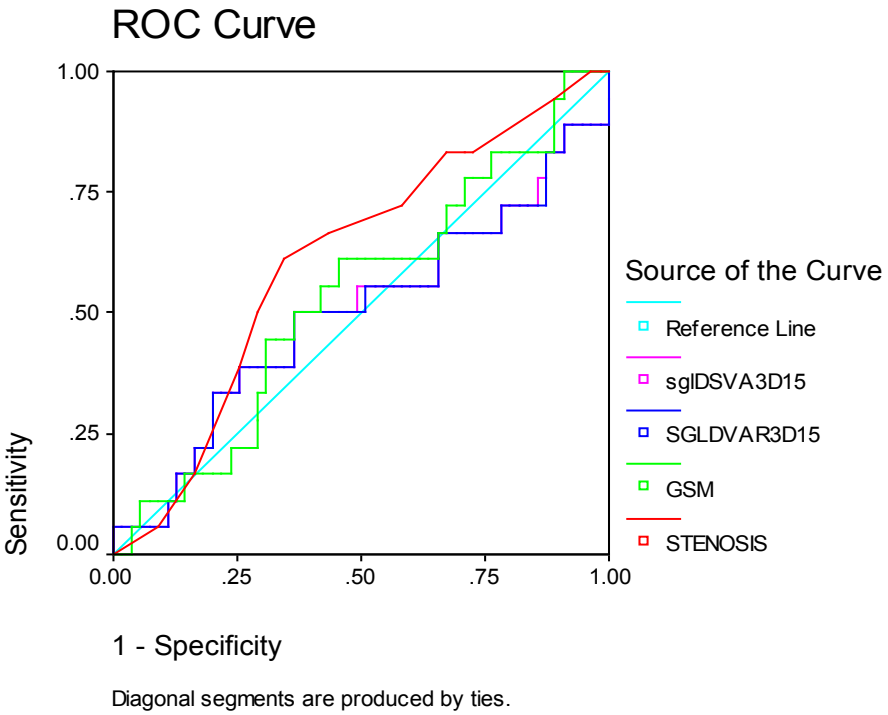


Figure 15.1. ROC curves for the selected variables from the multivariate regression model of table 15.3.

(Abbreviations: *SGLDSVA3D15:* SGLD_SVA measurement with 1.5mm slices in 3D; *SGLDVAR3D15*: SGLD_VAR measurement with 1.5mm slices in 3D; *GSM*: Grey scale median measured in 2D.)

15.4.Discussion

The grey scale median (GSM) has been for decades one of the few texture features that has been used for the characterization of carotid plaque lesions with promising results [ref]. However, it remains more of a research tool rather than a feature used in the daily clinical practice. The plaque volume is a similar example with reasonable support in the literature but also very few actual clinical applications. This study is one of the first studies to simultaneously assess these features in a study where a symptomatic and asymptomatic group are compared using measurements both from a 2D and a 3D ultrasound probe. Another novelty of our study is the evaluation of other texture features apart from GSM, which has been shown to be useful for the identification of the vulnerable plaque (chapter 8).

In the first part of this study we assessed the risk of stroke predictive ability of traditional features such as GSM, plaque area and plaque volume, to evaluate if there is any additional benefit from the use of these features with 3D ultrasound imaging. Then we compare the finding with those from 2D ultrasound imaging. Our study showed that there was not significant improvement from the use of the 3D measurements of the above variables in terms of stroke prediction. The 2D ultrasound imaging and the assessment of texture and plaque area appeared to be equivalent to the more expensive and time consuming 3D imaging, however, without offering the possibility for volume measurements.

At the second part we attempted to reassess the previous studied, novel second order statistics for plaque analysis (chapter5) but this time using the measurements obtained by the 3D ultrasound probe. Fenster and his colleagues have previously suggested [Fenster A, 2004] that these features can be more sensitive in the detection of plaque-related pharmacological changes than volume measurements. Some of these features have been previously associated with ipsilateral embolic brain infraction [Kakkos SK, 2010]. We managed to identify novel features that appeared to be associated with the presence of symptoms. However, again they did not appear to improve on the predictive ability achieved by the degree of stenosis alone or the GSM values from the 2D ultrasound.

15.5 Conclusion

There was no additional benefit from the use of 3D ultrasound in risk of stroke stratification when compared to traditional and novel 2D ultrasound measurements. However, due to the relative small sample size of our cohort, larger studies are needed in order to confirm the above statement.

PART V

Introduction to carotid plaque motion analysis

Chapter 16

Introduction to carotid plaque motion analysis

16.1. Introduction

Plaques develop in a dynamic environment and may rupture not only as a result of inherent instability, but also due to the excessive mechanical forces during the cardiac cycle. In a study by Meairs et al., it was suggested that plaque surface motion analysis maybe able to distinguish between high and low risk plaques. [Meairs S,1999] The approach was based on reconstructions of plaque motions using temporal 3-D ultrasound. This study found significant differences in the maximal discrepant surface velocity motion between patients with and without symptoms. Similarly, Murillo et al., utilised a multi-scale plaque motion approach to estimate 2D plaque motion to help differentiate between symptomatic and asymptomatic plaques. [Murillo S, 2012]

In this chapter will test the image analysis methodology for quantifying discordant plaque motion that was previously described in Part II of this thesis. Discordant motion is the phenomenon of different parts of the plaque moving in different directions with different velocities during the cardiac cycle. On the other hand, concordant plaque motion is the phenomenon of all carotid plaque parts moving in the same direction and with the same velocity. Naturally, discordant motion is believed to be associated with higher strain whereas concordant motion is linked to lower strain values.

The main objective of this chapter is to assess if it is feasible to classify and quantify the carotid plaque motions in order to help clinicians differentiate between plaques that are likely to rupture.

16.2.Methods

16.2.1. Ultrasound video acquisition and visual classification

Video loops of ultrasound images of 35 carotid bifurcation plaques were obtained from 4 symptomatic and 31 asymptomatic patients with carotid bifurcation atherosclerosis. Ethics Committee approval had been obtained by the local research ethics committee. These images were collected for the purposed of the 3D study (Part IV of this thesis). The video loops were anonymised first and studied without knowledge of the presence or not of symptoms. The ultrasound videos were of size 568x448 pixels at a frame rate of 40 frames per second (fps).

From each video loop, we extracted a series of 8-10 consecutive cardiac cycles that did not include any motion artefacts such as carotid movement due to neck movement or swallowing. Video loops were classified visually as mechanically concordant or discordant.

16.2.3. Video motion estimation and relevant parameter selection

This has been previously discussed in detail in chapter 7 of this thesis.

16.2.4.Plaque motion visualization

Statistical analysis of the estimated motion vectors is performed over the region of the plaque. A limitation of the current version of the software comes from the fact that we are not tracking the motion of the plaque boundary throughout the cardiac cycle.

To visualize the motion, the user can select the spacing between the estimated motion vectors ($Motion\ Vectors\ Spacing$) and can also magnify the display of the motion vectors ($Motion\ Vectors\ Magnification$).

16.2.5. *Statistical Analysis*

The discrimination between concordant and discordant motions is based on estimating the motion velocity spread over each video frame, over the entire video. To determine the motion spread we used the orientation-histogram to determine the angular spread that reaches 50% of the peak orientation (half-peak spread). For each video frame, we have an estimate of the angular spread ($width$) and the $median\_value$ summarized as a single point: ($width, median\_value$). A collection of all of the points over the entire video is used to generate a scatterplot.

The maximum fan-width for each median value was defined using the following:

$$MAXFW(mval)=\max median\_value=mval width. \quad (1)$$

The maximum fan-width measures the maximum angular spread (in degrees) for a fixed motion magnitude. For this study we ignore the motion magnitudes for integer-valued motions $mval$ = 2, 3, 4, and 5 pixels. The sum of the maxima over several integer motions is defined using

$$SMAXFW(mval=i,\ldots,j)=\Sigma MAXFW(mval)mval=jmval=i. \quad (2)$$

To differentiate between concordant and non-concordant motions, the sum of the motion spreads as expressed by (2) was studied. For this study the basic idea was that larger spreads would characterise non-concordant motions. [G. Farnebäck,2002]

16.3.Results

To differentiate between concordant and disconcordant motions, we collected ultrasound videos from 35 patients as previously described. These videos were visually classified into concordant p (n=22) and discordant (n=13) plaques.

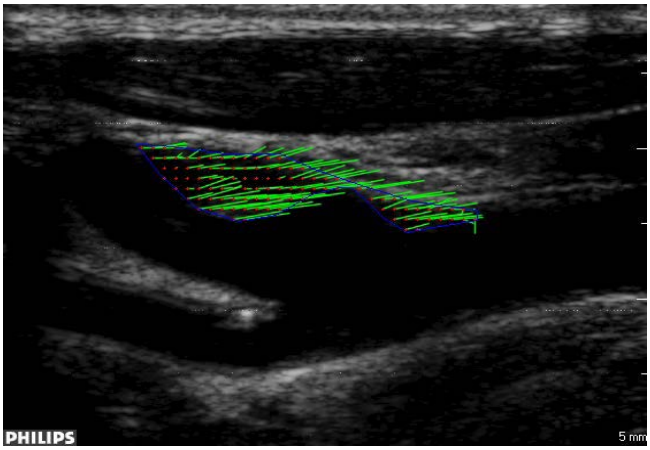
Figure 16.1 is a comparative example between a stable and an unstable carotid plaque. The orientation histogram of the visually classified as concordant plaque of Fig. 16(a), shows a narrow fan width of 6^{o} degrees at the peak of the cardiac systole. Then figure 16(b) shows the orientation histogram for the visually classified as discordant plaque. In this case, the orientation spread is approximately 84^{o} degrees at the peak systole.

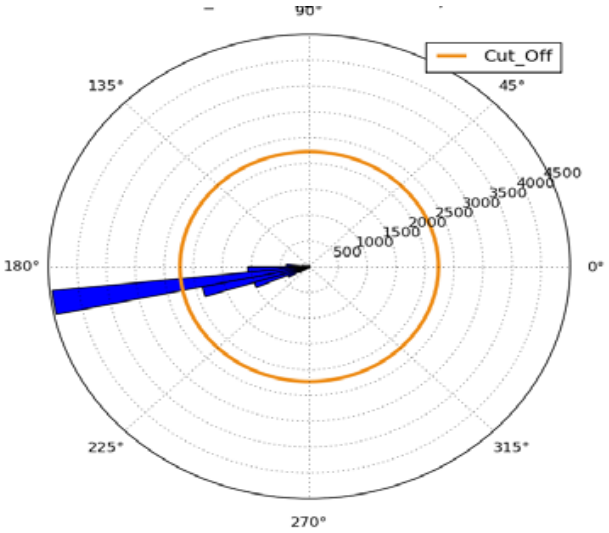
We compared the scatterplots for all cases. In Figure 16.2 the MAXFW and SMAXFW for the different cases described in (1) and (2) are presented. From the boxplots, we can see that SMAXFW(i=3,4,5) provides the best separation between stable and unstable plaques.

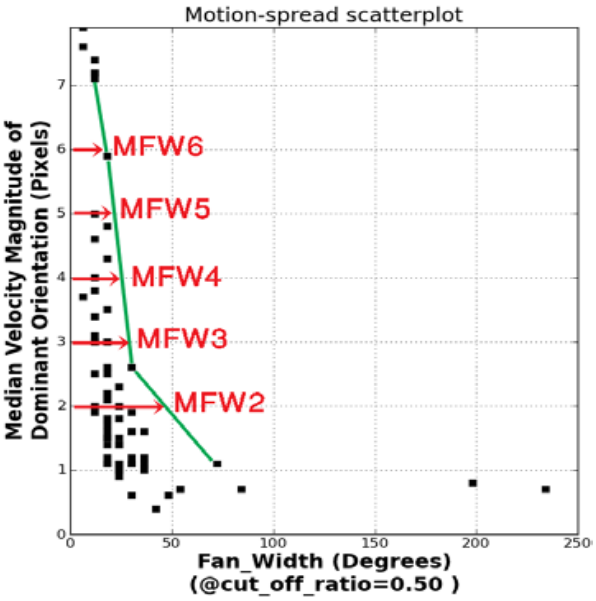
The sum of maximum fan widths for the median pixel motions 5 to 3 (SMFW5to3) had a median value of 100 degrees and inter-quartile range (IQR) of (80, 110) degrees for the concordant plaques and 270, (230, 430) for the discordant plaques ($P < 0.001$).

Thus, a possible new tool for objective differentiation between concordant and discordant plaques has been produced.

(a)


PHILIPS
5 mm


Cut_Off
90°
135°
45°
180°
0°
225°
315°
270°
500
1000
1500
2000
2500
3000
3500
4000
4500


Motion-spread scatterplot
Median Velocity Magnitude of Dominant Orientation (Pixels)
MFW6
MFW5
MFW4
MFW3
MFW2
Fan_Width (Degrees)
(@cut_off_ratio=0.50)

(b)

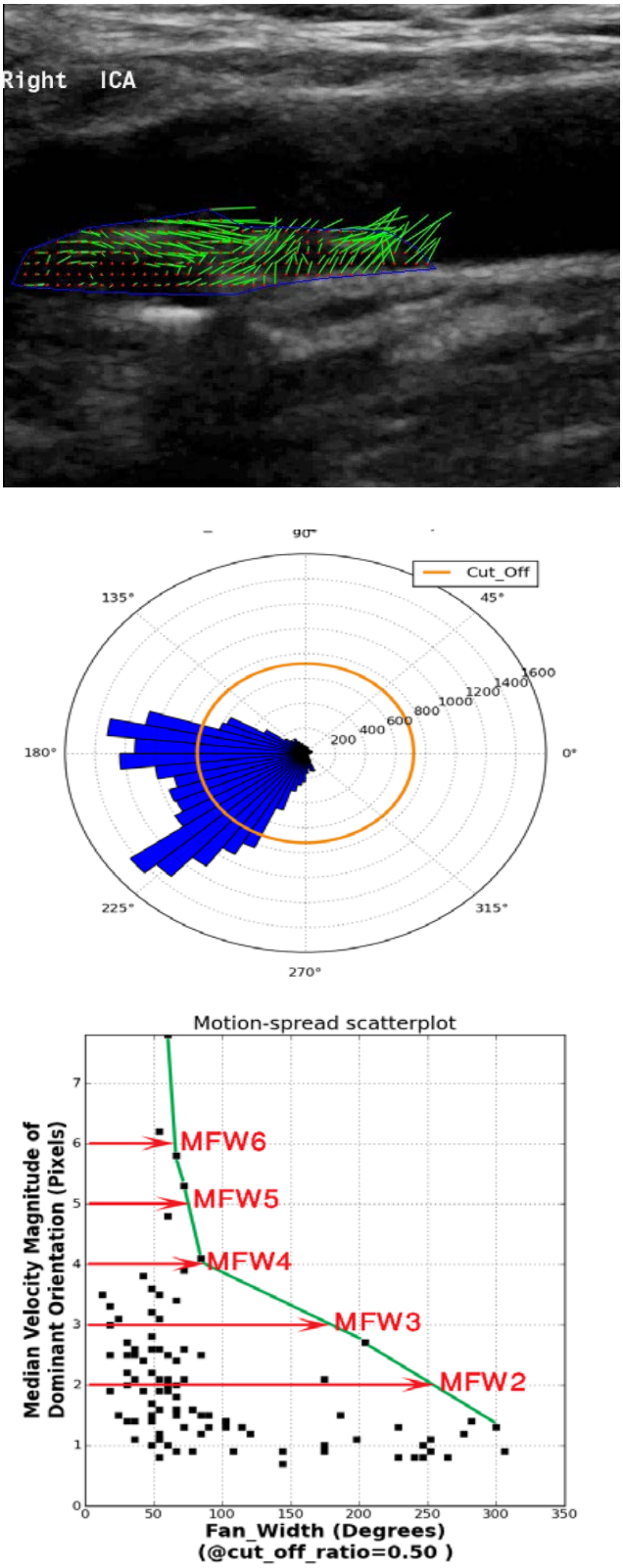


Figure 16.1: Carotid bifurcation plaque motion analysis. **(a)** Plaque with concordant motion at peak systole. The histogram plot shows the magnitude and orientation. The motion-spread scatterplot for is characteristic of a concordant plaque. **(b)** Plaque with discordant motion at peak systole. The histogram plot shows the magnitude and orientation. The motion-spread scatterplot for is characteristic of a non concordant plaque. *(Reproduced with permission from Pattichis etl. IEEE 2012)*

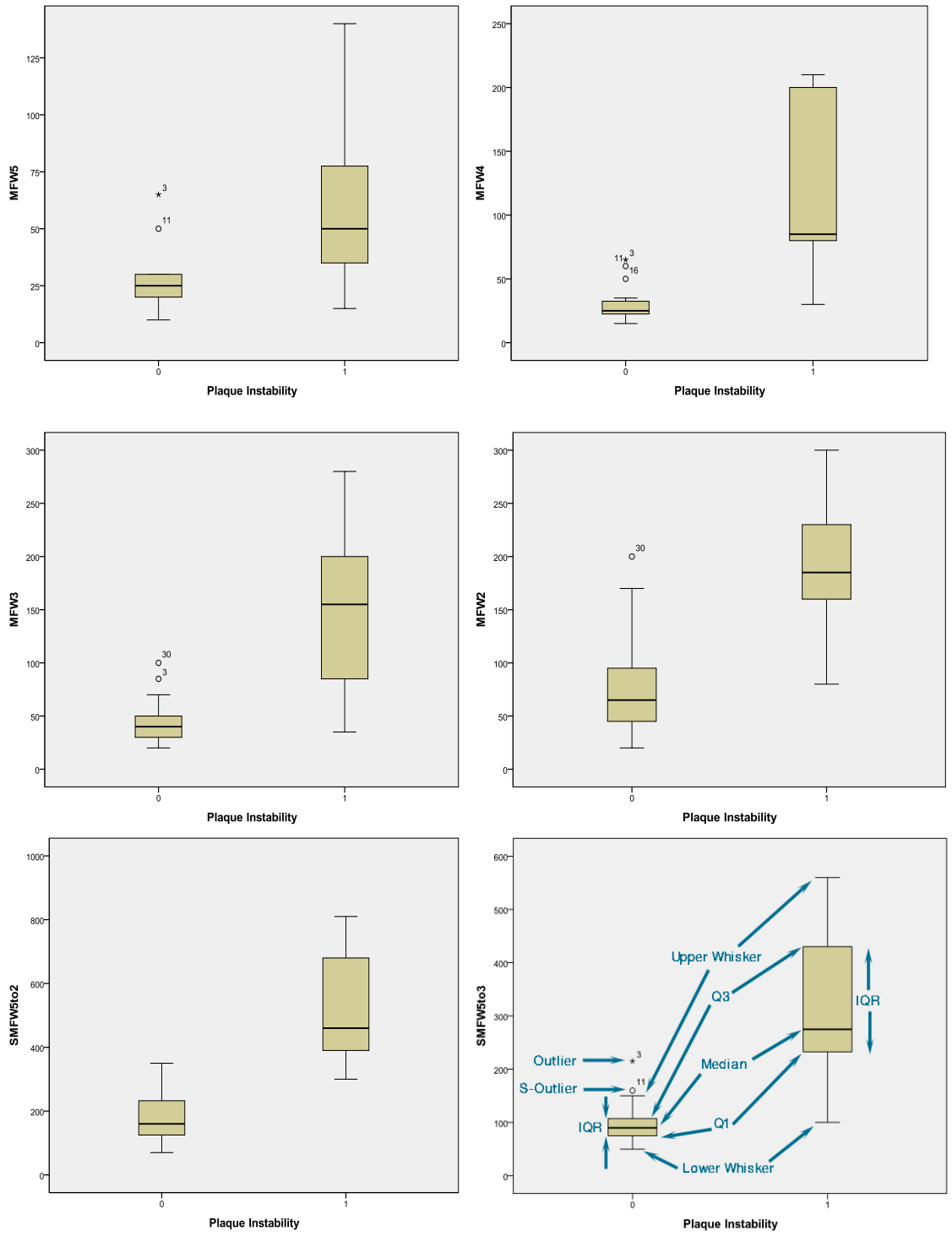


Figure 16.2. Boxplots of the maximum fan-width (*MAXFW* in (8)) and the sum of the maximum fan widths (*SMAXFW* in (9)) for different, integer pixel motions. Here, "0" refers to stable plaques and "1" refers to "unstable" plaques. **(a)** Boxplot for *MAXFW*(5). **(b)** Boxplot for *MAXFW*(4). **(c)** Boxplot for *MAXFW*(3). **(d)** Boxplot for *MAXFW*(2). **(e)** Boxplot for *SMAXFW*(*i*=2,3,4,5). **(f)** Boxplot for *SMAXFW*(i=3,4,5). Boxplot Legend. Q1/Q3: 1st/3rd quartile or 25th/75th percentile shown as the lower/upper hinge of the box. M: median shown as a line segment across the box.
IQR: interquartile range is the box height from Q1 to Q3. Lower/Upper Whisker: The smallest/largest observed value within 1.5×IQR below/above the Q1/Q3.
S-Outlier: Suspected outliers shown by unfilled circles are between 1.5×IQR to 3×IQR below/above Q1/Q3. Outlier: Outliers shown by 5-pointed stars are 3×IQR or more below/above the Q1/Q3. The outliers are labelled by the identifying numbers. *(Reproduced with permission from Pattichis etl. IEEE 2012)*

16.4. Discussion

We developed a real-time motion analysis system that can estimate velocities between consecutive video frames. The spread of the motion orientation around the dominant orientation over each video frame was measured. The spreads of the motion orientations for different motion magnitudes were calculated for each video. These motion-spread measurements were also useful in quantifying discordant movement.

Our study showed that it is possible to objectively quantify and study carotid plaque motion using plain 2D ultrasound and customised software. There was no extra cost and no inconvenience for the patient. All the analysis was performed with a personal computer at a later stage. There were no special requirements for the patients and obtaining a video loop proved to be a relatively simple task, which was not adding to the duration of examination.

This can be used in the future to assess if plaque motion can be used to differentiate between stable and unstable plaques adding more dynamic information on carotid plaque characterizations. At the moment we are at the process of analysing all the available video loops obtained during the course of the 3D study and we will report on the results soon.

The evaluation of carotid plaque has so far been limited to static measurements of the volume, composition or degree of stenosis. It is possible that the combination of these measurements with the dynamic information obtained by our plaque motion software may improve the identification of the vulnerable carotid plaque. Obviously, further clinical research is warranted to standardise the technique and provide solid evidence regarding the cost effectiveness of this approach.

Finally it is possible that this approach can have important applications in the calculation of biomechanical stresses on the plaque surface simplifying the otherwise very complicated process of plaque surface strain analysis. As shown by a recent review of the literature by our group [Makris et al., 2010] there is preliminary evidence suggesting that the combined effect of the various types of

stress acting on the carotid plaque may help us to distinguish between high and low risk plaque. However, the current methodologies are based on complicated simulations that make this type of analysis clinically impractical. The use of our software might be able to simplify this process by providing real time data for the stress wall analysis models but again further research is warranted.

16.5. Conclusion

Using customised software and B-Mode video loops it is possible to objectively quantify carotid plaque motion. Further research is currently in progress to determine if this approach can provide us with useful information for the improvement of carotid plaque characterisation and the reliable identification of the high risk for stroke individuals.

PART VI

Chapter 17

Discussion

17.1. Background

In 2008, cardiovascular disease (CVD) was the primary cause of 811,940 of all 2,471,984 deaths in the United States [Roger VL, 2012]. The prevalence of CVD among American adults is greater than 1 in 3. The total direct medical costs of CVD are estimated to triple from $273 billion to $818 billion within the next twenty years and this is despite the fact that the death rate by CVD has declined to 30.6% within the past decade. About half of this decrease is attributed to the increased use of best medical therapy and 44% to environmental and life style changes. In 2008, there were 795,000 cases of new or recurrent stroke which accounted for 1 of every 18 deaths in United States. Among the survivors of ischemic stroke who were older than 65 years, many experience long-term disabilities [Roger VL, 2012]. Carotid plaque associated stroke is, thus, a source of significant mortality and morbidity. For years now the identification of that patient group that will benefit more from surgical interventions to prevent a stroke has become the “Holy Grail” in carotid vascular surgery. As it was pointed out in the introduction of this thesis, if we only use the traditional approach, which depends almost exclusively on the degree of stenosis, we need 20 CEA operations to prevent one stroke.

For more than decade now research groups from around the world have been focusing on new imaging techniques that would make the selection process more sensitive. In other words, techniques that we would enable us to identify those patients that despite the currently available best medical treatment are more likely to suffer a stroke. This problem has been proven to be more complicated than we originally imagined. Carotid plaques are dynamic structures growing in a complicated and constantly changing environment with many possible parameters affecting their structural abilities to resist fatigue and rupture.

Two-dimensional ultrasound has been for years the method of choice for the screening of patients with cerebrovascular events. It is cheap, relatively easy to use and can provide us with a usually straightforward answer. However, it has become evident that ultrasound and especially the use of the degree of stenosis alone as a sole indicator of disease severity is not enough. [Nicolaides AN, 2010] For many researchers this did not come as a surprise since there are many other factors that appear to have an effect on carotid plaque stability. Factors such as the presence of fibrous cap, the presence and the size of the lipid core, the overall composition of the carotid plaque, the presence of neovessels within the plaque and the amount of biomechanical forces that apply on the plaque surface due to the blood flow, can affect the carotid plaque stability and risk of stroke as it is becoming increasingly evident by emerging data [Abbot A, 2009].

In this thesis we tried to identify novel ultrasound features to differentiate between stable and unstable plaques. Firstly, we used the previously developed methodology by our group to objectively analyse carotid plaque images from the large ACSRS cohort study. This was in order to identify novel 2D ultrasound features of plaque composition, such as features of plaque texture heterogeneity, which were not previously studied during the course of that study. In addition, and recognizing the possible limitations from two dimensional ultrasound imaging, we developed a unique methodology for 3D ultrasound image acquisition, composition and volume analysis that we subsequently applied to a prospective, cross-sectional study that we designed and run. Finally, in collaboration with the University of New Mexico we developed software for the objective quantification of carotid plaque motion that can be useful for the further characterization of this dynamic structure.

17.2. New methodologies for carotid plaque characterization

The use of 3-dimensional ultrasound has been so far limited to the plaque volume measurements. Our group aimed to investigate if it is possible to use the 3-dimensional ultrasound in order to obtain information regarding the composition of the entire plaque as well as its volume. In order to achieve this we combined two different types of software. One for the semi-automated sectioning of the plaque volume (Q-lab) and another for the texture analysis of every provided slice (Incosoft Plaque analysis software). This way and after using special formulas we showed that it is feasible to evaluate specific texture features such as GSM for the entire plaque and at the same time calculate the plaque volume. This can have significant implications for future studies and for the way we assess carotid plaque characteristics.

Another challenging problem was to develop a way of assessing carotid plaque motion in an objective way. It has been suggested that the carotid plaque motion during the cardiac cycle may have implications on its structural properties due to the tensile and shear forces that are being applied. With the invaluable help of the team from the University of New Mexico, we showed that it is possible, using ultrasound and dedicate software, to objectively quantify plaque motion and measure its possible effect on the carotid plaque. To our knowledge this is one of the first studies to present such a methodology that does not required complicated reconstructions or MR imaging.

17.3. Novel 2D ultrasound features and the ACSRS study

17.3.1.Novel texture features for plaque composition analysis

The current ultrasound evaluation of carotid plaque composition is based on the image analysis of specific texture-features and especially grey scale median. Each plaque is digitally represented as a block of black and white pixels in various combinations, according to the proportion of the contained lipid or fiber tissue. A pixel is a square, small element with standardized dimensions that occupies a certain 2-dimensional space. Each pixel is characterized by specific properties such as intensity of the signal (brightness), which is the amplitude of the received echo and determines the black or white contour. The ratio of the black and white pixels depends on the type of plaque. [Geroulakos G, 1993] An objective approach for the evaluation of the plaque texture -features can be achieved using digital image analysis and specialized software [Kakkos SK, Stevens JM,2007]. Certain measures such as the first and second order statistics may provide us with a description of the digital properties of the corresponding image (Appendix I).

The first order statistics (FOS) describe the distribution of the grey levels of pixels without taking into consideration the spatial dependence between the pixels. They are based on the histogram of the frequency of grey tones distribution (x-axis) as a function of the pixel count (y-axis). The grey scale median (GSM) belongs to this group of characteristics and describes the middle gray level above and below which 50% of the overall observations are spread. Despite the descriptive information provided by FOS the potential spatial, within matrix dependence is ignored [Kakkos SK, Nicolaides AN,2006; Wilhjelm JE,1998].

Second order statistics (SOS) address the pixel distribution considering at the same time the spatial dependence and they are indicative of image homogeneity. In other words SOS like Spatial Gray Level Dependence Matrices (SGLDM), Gray level difference statistics (GLDS), Gray level run length statistics (RUNL) and the Fourier power spectrum (FPS) (Appendix I) describe both the distribution of the pixels'

grey level as well as their relative positioning with respect to each other [Haralick RM,1973; Kadah YM,1996; Wilhjelm JE,1998]. So far carotid plaque composition was mainly assessed using means of FOS and especially GSM which is representative of how echolucent or how echogenic a carotid plaque is. Indeed, the correlation of GSM and cerebrovascular events has been significant and it was the beginning of a new era for carotid plaque characterization analysis [Kakkos SK, Stevens JM,2007]. However the importance of image homogeneity as this can be quantified by objective means such as the SOS measures was largely underreported.

Our study showed that the combination of such features for computerized characterization of asymptomatic carotid plaques might enable us to identify a low and high risk of stroke group of patients who can benefit from different treatment strategies. This may suggest that if such a group of patients with asymptomatic carotid plaque disease but with a higher risk of stroke can be identified then maybe carotid endarterectomy can still be justified.

Finally, the size of the lipid core without obvious thin fibrous cap and its effect on risk of stroke was evaluated using the same cohort. Histology studies have shown that the symptomatic plaques were more often associated with a juxta-luminal location of the lipid core compared to the asymptomatic one [Bassiouny HS,1997] In clinical studies JBA in carotid plaque was imaged by ultrasound and was more often found in symptomatic than asymptomatic plaques [Pedro LM,2002] In addition a thin, ruptured or absent fibrous cap has been more frequently noted in carotid plaques causing cerebrovascular symptoms (Pedro LM,2000; Demarco JK,2010). Obviously in those initial stages the determination of the JBA or the echogenic cap was subjective because monitor brightness can alter the results of the visual assessment (Kakkos SK, Nicolaides AN, 2011). Our group developed image normalization of ultrasound images and colour mapping. This technique was employed in JBA evaluation (Sztajzel R, 2005) so that echo-poor areas near the plaque vessel lumen could be well delineated with reproducible measurements of their area, without the subjectivity of the previously mentioned visual assessment (Kakkos SK, Nicolaides AN, 2011).

In chapter 11 of this thesis we actually demonstrated that the presence of a JBA without a visible fibrous cap was linked to ipsilateral neurological events in patients with asymptomatic carotid artery stenosis. This link was still significant after adjusting for other significant factors associated with plaque vulnerability. This is an important finding and in combination with other emerging imaging features can be a significant aid towards the more accurate characterization of the carotid plaque.

Further support to our hypothesis is also provided by previous studies, which have shown a good agreement between carotid ultrasound and plaque histology (Snow M,2007; Hatsukami TS,1994). The presence of a thin or ruptured fibrous cap on carotid MRI in patients with moderate asymptomatic stenosis was a strong predictor of future cerebrovascular events (Takaya N,2006). Moreover, in patients with TIAs and carotid plaques causing mild to moderate stenosis, the presence of juxtaluminal hemorrhage/thrombus on MR has been shown to be a strong predictor of TIA recurrence (Teng Z,2011). Whether risk index or stratified GSM are better than JBA size determination in predicting future stroke in patients with asymptomatic carotid stenosis remains to be determined.

The use of the dedicated “Incosoft Plaque analysis” software has made the objective analysis of carotid plaques more straightforward thanks to its friendly user interface and the many provided measured variables including first and second order statistics as well as total plaque area and juxtaluminal black area. At the moments this is the only commercially available software for carotid plaque evaluation which provides automatic plaque classification according to the “Geroulakos Classification” and which has been tested in a large prospective study such as the ACSRS one. (Geroulakos G, 1993)

17.3.2. Carotid plaque progression and its value for stroke risk stratification

The evaluation of the change of certain plaque features during ultrasound follow-up is another promising approach, especially for the asymptomatic patients and in order to identify those at a high-risk for stroke. Patients with asymptomatic carotid disease are more likely to undergo more than one carotid ultrasound scans during the progress of their disease. However, during these scans its usually only the degree of stenosis that is being assessed with usually no attention paid to any possible composition or morphology changes. As we know carotid plaques develop in a dynamic environment and so far there is limited evidence regarding their change in time with regard to their composition and volume. It is also largely unknown if this change has any effect on the risk of stroke and if it can be used to identify a high risk for stroke groups of patients.

To answer these questions we used the ACSRS cohort and retrospectively evaluate the changes in plaque composition, when the degree of stenosis is constant, in a subgroup of the ACSRS participants. We also evaluated the change in the degree of stenosis through time in the entire ACSRS cohort aiming to assess the predictive ability of the change in the degree of stenosis. In both studies it was shown that useful information could be obtained by the recording of the follow-up measurements of certain plaque texture features as well as of the degree of stenosis, which can be used to identify the high-risk individuals. It was also suggested, that these changes in the composition and size of the plaque can be used as outcome measures to evaluate response in various treatments.

Previous studies have demonstrated links between echolucent plaques [Liapis C, 2000] contralateral stenosis or ipsilateral stenosis and ipsilateral carotid stenosis progression [Sabeti S, 2007]. In our study elevated levels of creatinine, hypoechoic plaques, male gender, sever ipsilateral or contralateral stenosis were associated with progression to occlusion. On the other hand the age of the patient and the presence of discrete white areas were associated with a decreased incidence of regression. It was difficult to interpret these findings since older patients may have more calcified plaques or plaques with more collagen, which are obviously less likely to regress. Finally, the presence of discrete white

areas, possibly indicates neovascularisation within the plaque as shown by carotid plaque perfusion studies [Shah F,2007], which can also be resistant to regression. This may have implications for the need of more aggressive medical therapy in patients with DWA-rich plaques but further research is warranted.

17.4. Current data on 3D ultrasound for the investigation of carotid plaque disease

Our recent systematic review of the literature supports the good reproducibility of 3DUS on the evaluation of carotid plaque volume, however, with high heterogeneity between studies [Makris et al, 2011]. In contrast to the relatively large number of studies evaluating the use of 3DUS to assess plaque volume in this review, there was a limited number of studies evaluating the composition of the entire plaque by grey scale means. Heliopoulos et al. showed that lower plaque echogenicity is more likely to be observed in symptomatic than in asymptomatic patients with moderate degree of carotid stenosis, indicating that it is a significant factor for the production of cerebrovascular symptoms [Heliopoulos J,2008]. During the review of the literature no studies were identified evaluating the use of 3DUS to assess both the composition and the volume of the entire plaque.

In part IV of this thesis we presented for the first time a simple, semi-automated methodology based on commercially available computer software and 3D ultrasound that enable us to obtain simultaneous information on volume and composition for the entire plaque. We tested this methodology on a prospective study in more than 80 patients with both symptomatic and asymptomatic disease. This study showed that 3D measurements of certain plaque features, such as GSM for the entire plaque, do not improve the stroke predictive ability when compared to the traditional measure of degree of stenosis. This did not change even when we analysed an even larger number of sections of the same plaque by using thinner slices. In addition, when the 3D plaque measurements were compared to the corresponding 2D ones there was no statistically significant difference in the risk of stroke predictive ability.

Currently there is no evidence from our study to support the use of 3D ultrasound probes for the evaluation of carotid plaque disease since they are more expensive, more difficult to use (bulkier) and without any apparent clinical benefit. However, we should keep in mind that further improvements in 3D ultrasound technology are already on their way and they are more likely to improve the resolution

and lower the cost of these probes. We secured approval for the continuation of the 3D study and we will expand the sample size in order to increase the power of it.

Finally, even if a diagnostic benefit was not established by the use of 3D ultrasound in our study, the developed methodology may have significant applications in other areas of carotid plaque analysis. In collaboration with the Bioengineering department of Imperial College and Professor Y Xu we have started a joined project where the images from the 3D imaging will be used to produce biomechanical stress maps of the carotid plaque surface. The preliminary data from this collaboration suggests that the obtained 3D ultrasound reconstructions of the plaque can simplify the process of carotid stress map creation making further analysis feasible. [Wang Z., 2012] If 3D ultrasound can provide us with reliable data as a basis for such complicated biomechanical reconstructions then another potential application of it may have just been revealed. This research is still on going.

17.5. Biomarkers and contrast enhanced ultrasound for the further characterisation of carotid plaque disease.

High-sensitivity C-reactive protein (CRP) is one of the most common biomarkers mostly used in research studies and it has been shown potential in stroke risk prediction. It is still unclear if CRP is directly causal to the plaque rupture, however the association with clinical events appears to be clear. [Staub D., 2013] Apart from CRP there are many other systematic biomarkers that have shown promise but have not been thoroughly tested. Some of them include myeloperoxidase, secretory type II phospholipase A2 , oxidized low-density lipoprotein , pregnancy-associated plasma protein A and lipoprotein-associated phospholipase A2. In addition there are several common genetic variations that have been reproducibly correlated to with cerebrovascular event risk. Some characteristics examples include, each copy of the risk allele at 9p21, which seems to increase CAD risk by more than 25%. Recently, a multistage genome study identified a number of genetic variants associated with early-onset myocardial infarction and stroke. [Staub D, 2013] Three of these are newly identified (21q22, 6p24, and 2q33) whereas 6 (9p21, 1p13, 10q11, 1q41, 19p13, and 1p32) are replicated from previous observations. To evaluate the cumulative effect of these single nucleotide polymorphisms on the risk of cardiovascular events, a genotype score from 9 single-nucleotide polymorphisms was generated. Patients with scores in the top quintile had a twice as many chances of suffering a cardio/cerebrovascular event when compared with those in the last quintile [Verhoeven EL,2011]

An important potential use of biomarkers is to guide the use of non-invasive and/or invasive imaging procedures. In 1,004 asymptomatic Korean patients who underwent coronary CTA imaging as part of a health screening, individuals with C-reactive protein ≥2 mg/l had a much higher prevalence of coronary plaque and mixed calcific plaque compared with those with lower C-reactive protein levels [Villanueva FS,2008]

Carotid enhanced ultrasound (CEUS) is a relatively novel invasive approach to image atherosclerotic plaques and it is currently the subject of rigorous research. CEUS has shown to precisely demonstrate

carotid stenosis of more than 70 %. [Fleg JL., 2012] The degree of carotid luminal narrowing on CEUS imaging correlated strongly with magnetic resonance imaging studies and conventional angiography. In addition certain studies have shown that CEUS imaging maybe useful to detect occlusion and distinguish it from tight stenosis. CEUS has also shown promise for the assessment of restenosis post interventional carotid artery stenting. [Garcia-Garcia HM.,2009]

In a study evaluating the use of CEUS for follow-up after carotid stenting showed that CEUS provides a reduction in intra-stenotic flow artefacts providing better visualization and detection of the full length of the stenosis when compared with colored Doppler. [Rogers IS., 2010]

Carotid CEUS may also enhance the visualization of wall irregularities such as dissections, echolucent plaques and , surface ulcers. [Kugiyama K., 1999]. In a study of 100 asymptomatic vasculopaths, standard carotid ultrasound demonstrated atherosclerotic plaques 77 % when the CEUS detected atherosclerotic plaques in 88 % with a statistically significant difference. [Elesber AA.,2006]. The vulnerable, hypoechoic atherosclerotic plaques were more likely to be detected by CEUS and not with standard carotid ultrasound, which shows the potential of CEUS in high-risk symptomatic or asymptomatic patients.

17.6. Future studies

The work started in this thesis will be continued in the future with the aim to improve even further the predictive ability of ultrasound based imaging in carotid plaque disease.

The 3D ultrasound study will be expanded and more patients will be included, as ethical approval to do so has been granted. In addition, the correlation of the 3D ultrasound findings with plaque histology that has started with this thesis (analysis not finished) will be completed providing us with further insight regarding the correlation of 3D ultrasound findings with the actual plaque sample. It is also planned to correlate 3D imaging findings with plaque motion data in order to establish possible links between plaque composition and certain plaque motion patterns that may predict plaque rupture.

Finally a prospective study to evaluate the effect of combination treatment with omega 3 fatty acids and statins has been designed and ethical approval has also been granted. The hypothesis is that the addition of pharmacological doses of fish oil to standard statin therapy will cause regression of carotid plaque characteristics associated with the development of ischemic stroke. The use of 3D ultrasound for measurements of plaque volume and texture in this study has advantages over older methods such as intima-medial thickness (IMT). Firstly the biological correlates are different, IMT represents mainly hypertensive changes in the arterial wall, whereas carotid plaque volume is biologically more relevant to the disease process of ischemic stroke; secondly plaques progress along vessel walls more quickly than they thicken so that methods measuring change in plaque volume are more sensitive to change in plaque biology over time. The full details of the protocol can been found in Appendix III.

PART VII

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PART VIII

APPENDIX

Appendix I

Texture features of carotid plaque echogenicity and heterogeneity:

A. First order gray level parameters

The features mean gray level, variance, median (GSM), mode (expresses the most frequent value), kurtosis, skewness, energy and entropy belong to this category. In this category the parameters are derived directly from the gray level histogram. They describe the gray level distribution without considering spatial independence; as a result they can only describe echogenicity of texture and the overall variation characteristics within the region of interest (ROI).

B.1. Second order statistics- The Spatial Gray Level Dependence Matrices (SGLDM)

The features that belong to this category are : Angular Second Moment (ASM), Contrast, Correlation, Variance (Sum of squares), Inverse Difference Moment (IDM) – Homogeneity, Sum Average, Sum Variance, Sum Entropy, Entropy, Difference Variance, Difference Entropy, Information Measure of Correlation-1 (IMC-1) and Information Measure of Correlation-2 (IMC-2).

The SGLDM algorithm is based on the assumption that texture properties of an image are contained in the overall or ‘‘average’’ spatial relationship between the gray levels in the image. SGLDM is based on the estimation of the second order conditional probability density f(i,j;d,q). Each value f(i,j;d,q) represents the probability that two different resolution cells which are in the direction specified by an angle q and have distance d, will have gray level values i and j respectively.

ASM is a measure of homogeneity of the image. Homogeneous images have very few dominant gray-tone transitions, which result into higher readings. Contrast is a measure of the contrast or the amount of local variations present in an image. Images with large neighbouring gray level differences

are associated with high contrast. Correlation is a measure of gray-tone linear-dependencies in the image and heterogeneity. Heterogeneous images have higher correlation values.

B.2. Gray level difference statistics (GLDS)

The GLDS algorithm is based on the assumption that useful texture information can be extracted using first order statistics of an image. The algorithm is based on the estimation of the probability density pd of image pixel pairs at a given distance d ¼ (Dc,Dy), having a certain absolute gray level difference value. Coarse texture images, result in low gray level difference values, whereas, fine texture images result interpixel gray level differences with great variances. The parameters used from this category are: Entropy, Contrast, Mean, Angular Second Moment e Homogeneity, Energy.

B.3. Gray level run length statistics

This technique is based on grey level run length of the image. If we examine the points that lie along some given direction (run lengths), we will occasionally find runs of consecutive points that all have the same grey level. In a coarse texture relatively long runs occur more often, whereas, fine texture contains primarily short runs. In a directional texture, the run lengths that occur along a given line should depend on the direction of the line. The parameters used from this category are: Short Run Emphasis (SRE), Gray Level Distribution (GLD), Run length distribution (RLD), Long Run Emphasis (LRE), Run Percentage (RP).

C. The Fourier power spectrum (FPS)

The estimation of the power spectrum of an image using Fourier transform provides useful information about texture. Coarse texture will produce results concentrated near the transformation origin, while in images with smooth texture values it will be more spread out.

APPENDIX II

1.1. Investigating for multi-collineatirity among the 51 ultrasound texture features

Multi-collinearity within the data was reduced to the minimum using the technique, which was described, in the methods' section of Chapter 5. As a result the following 22 texture features were included in the following analysis (table 1).

1.2 Linear transformation of the variables

The non-normally distributed variables were linearly transformed using appropriate logarithmic or other arithmetic transformations and approximate normal distribution in most of them apart from the variables col_9 and Kurtosis. The resulting variables are sqcol6, sqcl_bel20, sqcl_bel30, lgskew, lgsgldasm, lnsgldcon, lglgsgldcor, lglgsgldMlnM2, lglgrunl_sre, sqsgldvar, lggldmhom, lggldmcon, sqfpsfrad, lnrunlgld, sqrunlrld, bel_30, col_2, sgldlnM1, lggsm40, sqareamm where the initials ln, lg and sq stand for logarithmic and square root transformations, respectively. (Table 2)

1.3. Factor Analysis

Factor, principal component analysis with Varimax orthogonal rotation was used in order to eliminate redundant variables. The correlation R matrix determinant value was 7.456E-19 and the KMO and Bartlett's test of Sphericity were .794 and 46282.827 (df: 231, $p<0.0001$) respectively (Table 3). Principal component analysis of the transformed textural features extracted 5 factors with eigenvalues of more than 1 (appendix Figure 1 and appendix Table 4). Only the variables with loading factors above 0.8 were considered:

-Factor 1 accounted for the 29.5% of the total variability. The features: bel_30, lggsm40 and lgsgasm (logarithmic form of GSM and SGLD_ASM respectively) correlated strongly with factor 1 (loading factor 0.908, -.0902 and 0.883).

-Factor 2 accounted for the 20.5% of the total variability. The features: lnsgldcon, lggldmcon (transformed forms of SGLD_CON and GLDM_CON) correlated with factor 2 with loading factors of 0.846 and 0.845 respectively.

-Factor 3 accounted for 13.3% of the total variability. Two features strongly correlated with this factor and these were: sqrunlrld (LF: 0.954) and sqareamm (LM: 0.950). These are the transformed foms of RUNL_RLD and plaque area.

-Factor 4 accounted for almost 12% of the total variability. Only Sqclbel3 correlated strongly with this factor with a LF: 0.961 which is the transformed version of CL_bel30.

-Finally the last factor was responsible for the 11.3 % of the total variation and in combination with the previous 4 factors was explaining a cumulative 87.2% of the cumulative variation. The L2gsgcor and Sgldinm1 features correlated strongly with the last factor (LF: 0.852 and 0.876 respectively). These variables represent the transformed versions of the features SGLD_COR and SGLDINM1.

The remaining features had loading factors of less than 0.8 and thus were decided not to be included in the final analyisis.

Descriptive Statistics

	Mean	Std. Deviation	Analysis N
BEL_30	48.217963	25.816679	1118
COL2	23.144310	10.097955	1118
COL6	2.907897	3.192944	1118
COL9	.469694	1.029530	1118
CL_BEL20	12.428970	6.853990	1118
CL_BEL30	11.163337	5.261138	1118
KURT	2.205323	1.255096	1118
MEDIAN	35.870149	24.421165	1118
SKEW	1.439813	.979354	1118
AREA_MM2	46.6891	28.6515	1118
SGLD_ASM	2.78E-02	8.11840E-02	1118
SGLD_CON	77.793478	93.999539	1118
SGLD_COR	.968418	3.10628E-02	1118
SGLD_VAR	1360.347	943.341117	1118
SGLDINM1	-.397157	4.98302E-02	1118
SGLDMINM	.976563	2.85545E-02	1118
GLDM_HOM	.317691	.138471	1118
GLDM_CON	72.336547	76.797083	1118
FPS_FRAD	3170.804	1650.724426	1118
RUNL_SRE	.912515	3.88612E-02	1118
RUNL_GLD	209.0777	166.848069	1118
RUNL_RLD	11883.13	7231.274280	1118

Table 1: The resulting 22 texture feature after multi-collinearity reduction

Descriptive Statistics

	Mean	Std. Deviation	Analysis N
SQCOL6	1.4068	.9641	1118
SQSGLVAR	34.5101	13.0213	1118
SQCLBEL2	3.3688	1.0397	1118
SQFPFRAD	54.4140	14.4951	1118
SQRUNRLD	104.2250	31.9560	1118
SQAREAMM	6.5397	1.9813	1118
SQCLBEL3	3.2354	.8344	1118
LGSKEW	1.0570	3.396E-02	1118
LGSGASM	-2.4376	.8206	1118
LGGLCON	1.6823	.4067	1118
LGGLHOM	-.5345	.1758	1118
LGGSM40	1.8583	.1376	1118
LNSGCON	3.8937	.9686	1118
LNRNGLD	5.0591	.7876	1118
L2GSGCOR	-1.9768	.2762	1118
LLGSGLNM	-2.1239	.2921	1118
LLRUNSRE	-1.4758	.1702	1118
BEL_30	48.217963	25.816679	1118
COL2	23.144310	10.097955	1118
COL9	.469694	1.029530	1118
KURT	2.205323	1.255096	1118
SGLDINM1	-.397157	4.98302E-02	1118

Table 2: Descriptive statistics after the linear transformation of the texture variables

KMO and Bartlett's Test

Kaiser-Meyer-Olkin Measure of Sampling Adequacy.		.794
Bartlett's Test of Sphericity	Approx. Chi-Square	46282.827
	df	231
	Sig.	.000

Table 3: KMO and Bartlett's test from of the resulting factor analysis and PCA

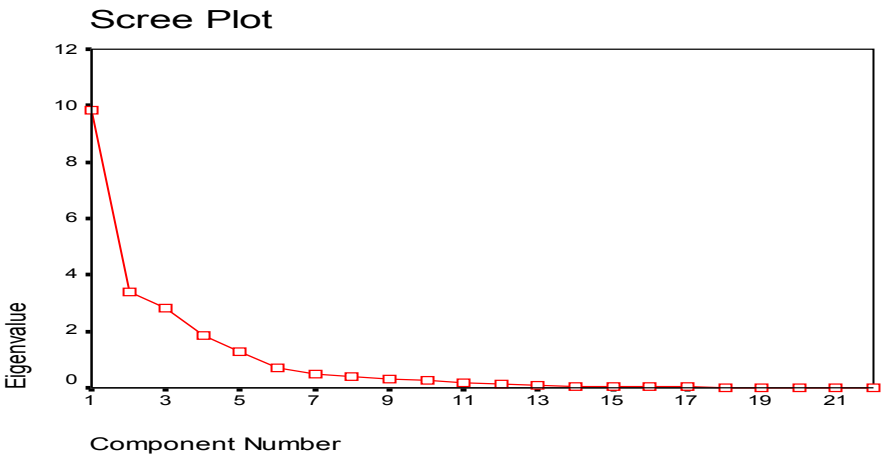

Figure 1: Scree plot- Factors with eigenvalues>1 were selected

Rotated Component Matrix[a]

	Component				
	1	2	3	4	5
SQCOL6					
SQSGLVAR					
SQCLBEL2					
SQFPFRAD					
SQRUNRLD			.954		
SQAREAMM			.950		
SQCLBEL3				.961	
LGSKEW					
LGSGASM	.883				
LGGLCON		.846			
LGGLHOM					
LGGSM40	-.902				
LNSGCON		.845			
LNRNGLD					
L2GSGCOR					.852
LLGSGLNM					
LLRUNSRE					
BEL_30	.908				
COL2					
COL9					
KURT					
SGLDINM1					.876

Extraction Method: Principal Component Analysis.
Rotation Method: Varimax with Kaiser Normalization.

a. Rotation converged in 6 iterations.

Table 4: Principal Component Analysis of the 22 texture features. The Varimax rotation method was used with Kaiser Normalization and 5 factor components were retrieved. Only the variables with loading factors above 0.8 were included in the final analysis.

Appendix III

Study protocol

The efficacy of omega-3 fatty acids plus statin therapy vs. statin therapy alone in carotid plaque stabilization and reduction of circulating inflammatory markers. A Pilot trial combining 2 and 3 dimensional ultrasound. (OMEGA 3 TRIAL)

Introduction

Pooled data from two large meta-analyses provide significant evidence illustrating the beneficial effect of statin-treatment. [1, 2] The relative risk reduction rate for stroke was 21% [(OR) 0.79 (0.73-0.85)] with no heterogeneity between studies [2] while the 5-year incidence of major coronary events, coronary revascularization and stroke was reduced by about one-fifth per mmol/l reduction in LDL cholesterol [1]. In addition in the most recent meta-analysis –of statins combined with other preventive strategies for stroke prevention which included 165 792 individuals, there was a reduction in relative risk for stroke of 21.1% (95% CI 6.3-33.5, p=0.009) for every 1 mmol/L decrease (39 mg/dl) in LDL cholesterol. [3]

Reduction rates of 16% and 26% for fatal/ non-fatal strokes and total vascular events respectively were observed after intensive treatment with atorvastatin by the only randomized trial (SPARCL- Stroke Prevention by Aggressive Reduction in Cholesterol Levels) which included patients who had suffered stroke or transient ischemic attacks (TIA) but without manifestations of heart disease and a median follow-up of 4.9 years. [4]

The role of statins in peripheral arterial disease (PAD)

The effect of statins on peripheral artery disease and particularly on carotid plaque disease has been a matter of increasing importance due to the socioeconomic burden which arises from the development of stroke or TIAs. Two systematic reviews of the available literature indicate that there is clinical

evidence supporting the beneficial effect of statins on the development of PAD, though the quality of the evidence was insufficient as most of the studies were non-randomized. [5,6] In parallel, preliminary evidence is documented with respect to the potential reductions of perioperative morbidity and mortality in patients undergoing non-cardiac vascular surgery though further appropriately designed trials are warranted in order to establish the true effect and define the proper duration and dosage of statin treatment needed to achieve maximum benefit. [6]

Plaque progression and vulnerability are invaluable prognostic markers of cerebrovascular events irrespectively of the studied vascular bed. Nevertheless the majority of studies are currently focused on the coronary system. The REVERSAL (Reversal of Atherosclerosis with Aggressive Lipid Lowering) [7] and ASTEROID (A Study to Evaluate the Effect of ROsuvastatin on Intravascular Ultrasound Derived Coronary Atheroma Burden) trials [8] are only two of several randomized studies which concluded that the atheroma volume within the coronaries can be significantly reduced by statin therapy.

The effect of other lipid-lowering agents in atherosclerotic plaque disease

Nevertheless, apart from controlling LDL levels with statins, it has been suggested that targeting higher HDL levels and/or lower triglyceride levels could prove beneficial in terms of stroke prevention - as it was indicated by two large systematic reviews. [9, 10] This effect could be partly anticipated since the atherosclerotic process is not only associated with LDL levels but also with the function and levels of HDL and triglycerides which are also considered major determinants of cardiovascular risk. [11, 12] Niacin, fibrates and omega-3 fatty acids are the main agents that could be considered for combination treatment with statins in order to establish better total lipidaemic control -not just of LDL levels. There is accumulating evidence that indeed combined lipidaemic treatment with niacin [13], fibrates [14] and/or omega-3 fatty acids –which is a well-known triglyceride –lowering agent [15,16] -may result in even more stable plaques thus potentially reducing the rates of stroke. Currently, there is a small number of studies investigating the direct effect of combined treatment with statin and niacin/or fibrates [17, 18] on the carotid plaque composition, although, no studies could be identified with

regard to the combined effect of omega-3 fatty acids and statins in terms of carotid plaque morphology.

There is preliminary evidence regarding the beneficial effect of omega-3 fatty acids to the cardiovascular system. They are associated with reduced the risk of cardiovascular related death, reduced nonfatal coronary events and suppression of cardiac arrhythmias [19]. Moreover higher intake of fish oil has been associated with a decreased risk of embolic infarction, primarily among women who are not on aspirin regularly [15] and elderly individuals [20].,Omega-3 fatty acids can be incorporated in the atherosclerotic plaques inducing structural changes towards potentially more stable forms [21, 22]. Most trials in secondary prevention of casdiovascular event showed significant reduction in stroke rates whereas the evidence from primary prevention trials evaluating the effect of fish oil on stroke was inconsistent- as it was shown in a recent systematic review of the literature-with only minor adverse events [23]. However, so far the exact role of combined therapy with statins and omega-3 fatty acids on the carotid plaque composition and size remains basically unknown.

Hypothesis

The combined effect of statins and omega-3fatty acids (fish oil) will improve the overall plaque stability and decrease the atherosclerosis-related inflammation in men.

Rationale for the proposed research

1. Lack of evidence regarding the combined effect of statins and omega-3 fatty acids (fish oil) to carotid plaque morphology and stability.[24]

2. Limited number of studies with regard to the application of advanced 2D and 3D ultrasound imaging and analysis in carotid plaque morphology. [25]

3. Limited evidence regarding the effect of combined lipid-lowering treatment on the wall stress patterns of the carotid plaque. [26]

4. Novel, proposed serum, inflammatory markers to be measured and correlated with the potentially, observed changes in plaque morphology.

Methods

Study design

This will be a pilot, randomized, double-blind, placebo-control, study. The patients will be screened at their initial visit for the presence of asymptomatic or symptomatic carotid regardless of the degree of the stenosis. Those with the symptomatic disease will only be eligible if they do not require any surgical intervention and providing they are not too disabled to be able to consent or attend scanning centre (see Screening Criteria). After their initiation visit and randomization the patients will be followed up for a total of 12 months. The number of follow-up visits will be 3; every four months post-randomization. The total duration of the patients' involvement to the study will be 1 year.

Study population

Screening Criteria

The following patient groups attending appointments at Ealing and Charing Cross Hospital's outpatient's arterial clinic will be screened. Patients with:

- Peripheral Artery Disease (without indication for revascularization)
- Coronary Artery Disease
- Stroke or Transient ischemic attacks (without indication for revascularization)
- Diabetes Mellitus II (newly diagnosed or in chronic treatment)
- Hypercholesterolemics (newly diagnosed or in chronic treatment)

and generally patients on statin therapy or statin-naïve patients of the above groups.

In addition patients from the Stroke Units of both Ealing and Charing Cross Hospital will be screened for eligibility

Inclusion Criteria:

- Patients with any degree of asymptomatic carotid plaque disease (plaque type I-III according to Geroulakos Classification)
- Patients with any degree of symptomatic carotid plaque disease (plaque type I-III according to Geroulakos Classification) who had recently suffered from Transient Ischaemic Attack (TIA), or mild-to-moderate strokes (NIHSS score < 10) who are able to consent and practically participate in the trial.
- Patients on simvastatin or statin naive patients who are suitable for simvastatin administration.
- Patients with adequate mobility.
- Patients who are able to understand and sign the informed consent form.

Exclusion Criteria:

- Intolerance to statins (for statin naives)
- Intolerance to fish ingredients such as omega-3 fatty acids
- Previous treatment with omega-3 fatty acids products
- Haemorrhagic disorders
- Hepatic impairment
- Pregnancy and breast-feeding
- Chronic or current acute bacterial/viral infections (within the previous two weeks)
- Active rheumatologic disease
- Patients with cancer disease or in chemotherapy
- Immunosuppressed patients
- Physical, geographical or social factors that may preclude appropriate compliance to study requirements

- Receipt of any investigational treatment within the previous 30 days

Total expected number of patients: 100

Investigation Location

The recruitment of the study-subjects, consent, physical examination, some of the ultrasound test and the blood sampling will be performed within the premises of Ealing and Charing Cross Hospital NHS Trust, London, UK. Additional ultrasound tests will be performed at the Vascular Non-invasive Screening and Diagnostic Centre (28 Weymouth Street, London, W1G 7BZ).

The laboratory analysis for the serum inflammatory profile of the included patients will be performed at the Loyola Medical University, Chicago, USA.

Research Network

1. Imperial College, University of London, UK

-Dep't of Vascular Surgery, Ealing Hospital (Mr Geroulakos): Will be responsible for the project design and setup, recruitment of the patients and will have the full co-ordination of the study.

- Imperial College Cerebrovascular Research Unit, Charing Cross Hospital (Dr P Bentley): Screening and recruitment of patients with symptomatic carotid plaque disease.

-Dep't of Chemical engineering (Dr Xu): Will perform the stress-wall analysis.

2. Vascular Centre, CDRES trust, UK. (Prof Nicolaides): Within its premises the majority of the 2-dimensional (2D) and 3-dimensional (3D) ultrasound scanning will be performed. In addition the analysis of the acquired, anonymized images will be also performed by experienced associates of the Trust.

3. Loyola University, Chicago, USA (Prof Fareed). It is there where the analysis of the inflammatory profile will take place in their highly specialized laboratories.

4. Institute of Science and Technology, Cyprus (Prof Patichis). The friendly-user computerized algorithm and software for plaque analysis will be further developed and provided by this group for the purposes of the trial. Plaque motion analysis will be also part of their contribution.

5. Pronova Company, Norway (Omacor); Financial Support (pending) and omacor supplier.

Study Objectives

The primary endpoint will be:

To evaluate the effect on carotid plaque ultrasound characteristics (volume and composition) between the two treatment groups in the acute (after 4 months of treatment) and chronic phase (after 12 months of treatment).

The secondary endpoints will be:

a. The effect on the inflammatory status as evaluated by changes in the levels of the following markers: endothelin, selectin p and e, tissue factor, NO, ADMA, hsCRP, CRP, MCP1, CD40L,vWF, microparticles and serum amyloid A.

b. Correlations between 2D and 3D ultrasound characteristics of the analyzed plaques.

c. The changes in biomechanical stress- patterns/and plaque motion patterns between the two groups during the study period and how this can be affected by the combined lipid-lowering treatment.

d. The correlation between the changes in specific inflammatory markers and plaque characteristics between the two groups.

Research treatment

Treatment Arms

[(statin treatment) and (placebo)] versus [(statin treatment) and (fish oil)]

Route of administration

Both regimens will be administered orally

Dosage and type of regimen

-For statins:

The type of statin will be Simvastatin according to NICE guidelines.

-For fish oil:

The proposed form is omega-3 acid ethylesters in the form of Omacor® (Pronova): 1 g of omega-3-acid ethyl esters 90 contains eicosapentaenoic acid 460 mg and decosahexaenoic acid 380 mg. The proposed dosage scheme is 4 capsules per day (4g/day) with food.

Randomisation and allocation to treatment

Each patient will be allocated to a number which will be produced by dedicated computer software. This number will be the number of the allocated treatment box. Both placebo and actual fish oil capsules will be of the same shape, colour and taste (as much as possible) and will be delivered in randomly numbered white boxes. The real content of each group of boxes will be in a separate sealed envelope which will be opened at the end of the study or in case of serious adverse events which will require the discontinuation of the participation of the particular subject in the trial.

Safety considerations

There is clinical evidence confirming that a significant number of patients do not achieve the LDL targeted levels with intitial statin monotherapy. [27, 28] The recently completed COMBOS study (Combination of Prescription Omega-3 with Simvastatin) confirmed that combined prescription of simvastatin and omega-3 fatty acids succeeded in significantly improving a range of other lipid

indicators apart from the LDL primary target, including lipoprotein particle size, non-high density lipoprotein cholesterol and triglycerides [29].

Omega-3 f.a (OM3FA) alone have been associated with significant reductions in triglycerides and significant increase in HDL and LDL cholesterol [30].Clinical data with OM3FA suggests that their administration is tolerated with minor adverse events such as fishy odour and aftertaste in the mouth, nausea, and gastrointestinal discomfort with belching and bloating, diarrhea, and flatulence while it was not correlated with hepatic or renal impairment, rhabdomyolysis, hyperglycemia, or bleeding disorders [19, 31]. In addition even when OM3FA where co-administered with a statin no undesirable effects on glycemic control or LDL cholesterol levels were observed. [19, 32] The COMBOS study – which so far is the largest trial of this kind – also showed no statistically significant differences in adverse events or LDL control [29]. Finally there is recent evidence from pharmacological studies that the co-administration of statins with omega-3 fatty acids does not affect the pharmocokinetics of simvastatin and that the combination appears well tolerated [33].

Imaging modalities

a. Two dimensional (2D) ultrasound imaging (Commercial model applied)

b. Three dimensional (3D) ultrasound imaging by Phillips (Experimental probe) will be provided by Vascular Non-invasive Screening and Diagnostic Centre (CDER Trust)

Features for analysis:

- 2D U/S: plaque type, Grey Scale Median, Juxtaluminal black area and total plaque area.
- 3D U/S: plaque volume and volume texture

Software for image analysis

The latest version of the “Plaque Texture Analysis Software” (Incosoft International Ltd,PO BOX 172, Greenford, London UB69ZN,UK) which is a dedicated research software package will be applied.

Software and methodology for wall stress analysis:

They will be provided by department of Chemical Engineering of Imperial College, London which will be in close collaboration during the whole duration of the project. The –anonymized- ultrasound images acquired during the treatment and follow-up period will be gathered and sent for blind analysis in order to create maps of biomechanical stress distribution on the carotid.

Methodology for plaque motion analysis

As described above the digitalized copies of the collected images will be sent –anonymized first- to the digital laboratories of the Institute of Science and Technology in Cyprus where after proper blind image analysis, maps of plaque motions distribution will be created

Image Capture

The following technical ultrasound settings will be observed for 2D and 3D to ensure optimum image quality for texture analysis. Maximum dynamic range will be used to ensure the greatest display of gray scale values. Persistence will be set on low and frame rate on high ensuring good temporal scale values. The time gain compensation curve (TGC) will be sloping through the tissues but vertical through the lumen of the vessel so that the brightness of the anterior and posterior wall will be similar. The overall gain will be adjusted to minimize but not abolish noise in the blood. The ultrasound beam will be at 900 to the arterial wall. The minimum depth will be used so that the plaque will occupy a large part of the image. The above settings are essential prerequisites for plaque texture analysis, Images (both with color Doppler or power Doppler and gray scale) will be saved on DVD in TIFF and DICOM format.

Image analysis

The “Plaque Texture Analysis Software” version 3.2 (Iconsoft International Ltd, POBox 172, Greenford, London UB6 9ZN, UK) will be used. Image normalization will be performed using linear scaling with blood (gray scale value assigned: 0) and adventitia (gray scale value assigned: 190) as reference points as previously published (20). The normalized image will then be standardized to a pixel density of 20 pixels per mm using the bicubic method provided by the software. Plaque segmentation will be performed by outlining the plaque in comparison to the corresponding color image and saving it as a separate file. Using the “Feature extraction” module of the software texture features will be automatically extracted by the software. They include gray scale median (GSM), GLD_SEN, RUNL_RP, Entropy and SGLD_DEN and plaque area. The presence and size of a juxtaluminal black area will be measured manually. These features used in combination have been shown to discriminate between symptomatic and asymptomatic carotid plaques in cross-sectional studies (82% correct classification) and to be highly predictive of stroke in a prospective follow-up study of 1121 patients with asymptomatic carotid stenosis (Odds ratio for different features was 3-8). All texture features are automatically saved in a database for easy access and statistical analysis.

3D Image analysis

The 3D plaque images will be initially analysed by “QLAB” the software provided by Philips. Color and gray scale images will be sectioned transversely at 1 mm intervals. Images will be saved in TIFF and DICOM format. Each pair of transverse images (Colour and gray scale) will be analysed as described above for 2D images using the “Plaque Texture Analysis Software”. Thus, instead of plaque area in mm2 we shall measure volume in mm3 and texture features will be averaged for all the transverse sections and will become volume texture measurements.

Serum marker evaluation

At each follow-up visit –apart from the 3rd - two blood samples will be taken. One sample will be kept for analysis at Ealing Hospital (Full blood count, lipid, liver and renal profile AND coagulation profile) and the other one will be temporarily stored at the pathology department of Ealing Hospital until they are shipped to the laboratories of Loyola University.

The more specialized analysis of the inflammatory and coagulation profile the frozen, anonymized blood samples will be shipped –every 4 months- on dry ice, in a container in batches to the following address: Hemostasis &Thrombosis Laboratories of Loyola University Medical Centre where they will be properly analyzed for the indicated inflammatory markers (endothelin, selectin p and e, tissue factor, NO, ADMA, hsCRP, CRP, MCP1, CD40L, vWF, microparticles and serum amyloid A). The samples will be appropriately destroyed after the analysis.

Follow up assessment

After their initiation visit and randomization the patients will be followed up for a total of 12 months. During this period 3 follow-up visits are anticipated every 4 months. Each follow-up visit will consist of 1 hour at the Hospital for physical examination, assessment of concordance, dispensing of study medication and blood sampling and 1 ½ hour at the CDER Trust for the 2 and 3D ultrasound scanning (apart from the 3rd visit during which only physical examination will be performed at Ealing Hospital). The ultrasonographer will be blinded to the allocated treatment. These two visits can take place on the same day or separately, but within one week. The patient's travel expenses will be reimbursed at the end of each visit.

At the end of the study patients are free to return to their normal treatment without the omega-3 fatty acids (apart from those who are currently indicated from NICE guidelines.The reason for all premature departures will be recorded as per CONSORT guidelines [34].

Statistical considerations

Population analyzed: All efficacy analysis will be based on all randomized patients, irrespective of whether the patient actually received omega-3 FA or the placebo capsule.

Sample size estimation: This is the first study investigating the potential effect of combined lipid-lowering treatment with statins and omega-3 FA. Previous studies have investigated the effect of statin therapy alone versus placebo on carotid plaque morphology –using 2D ultrasound characteristics and various serum inflammatory markers [35-37]. For 3D ultrasound characterization of carotid plaques there is evidence from a previous study that plaque volume measurement can show the effects of treatment on atherosclerosis even after only 3 months of treatment [25]. A major advantage of using 3D ultrasound to measure carotid plaque volume is that this technique is more sensitive to change compared to using 2D ultrasound. This increases the sensitivity of the study to establish a treatment effect.

Change in plaque volume: A previous study [25] using 3D ultrasound showed a decrease in plaque volume of 90 mm3 in subjects treated with atorvastatin only compared to an increase of 33 mm3 in placebo group (treatment difference 123 mm3) after 3 months. Assuming that the proposed intervention strategy is only 50% as effective over the full study period of one year, this will give a mean treatment difference of 55 mm3, SD 80, with a study power of 90% (p=0.05) requires 45 subjects in each arm (total subjects =90).

Plaque echogenicity using 2D ultrasound and grey scale median: A previous study using this technique has shown a treatment difference of 4 (SD 6) in plaque echogenicity between statin-treated and placebo subjects. A study power of 90% (p=0.05) requires 48 subjects in each arm (total subjects = 96).

The acquired data will be analysed using both parametric and non-parametric statistics and SPSS software. During the analysis, variables such as age, sex, diabetic and smoking status will be taken into consideration. Because of the small number of patients, this will be performed by allocating subjects

to groups using a process called minimisation, which attempts to balance the groups against measured characteristics. This method is considered more suitable than multivariate analysis when small sample sizes are studied.

Ethics, data and safety

This protocol was approved by the Ealing & West London Research Ethics Committee. Consenting participants will be denied the choice of which treatment they will receive. Patients will only be known by their study number. Information linking their name to their study number will be held on a secure hospital network drive accessible only from the members of the research team and will remain strictly confidential. All the research files will be kept in the Josef Pflug vascular laboratory at Ealing Hospital. All blood samples sent to the laboratory in the USA will be fully anonymized.

MHRA approval: Following communication with this agency it was decided that our study will not be considered as a Clinical Trial of an Investigational Medicinal Product (IMP) as defined by the EU Directive 2001/20/EC

Project management

Every 6 months internal review meetings will be performed in order to evaluate the progress of the project and ensure that milestones are achieved in a timely manner. All members of the research team and the R&D manager of the hospital will participate to these meetings to provide feedback for the research progress. In addition, an advisory committee will be formed by Professor Farreed (Hemostasis & Thrombosis Laboratories of Loyola University Medical Centre, USA), Dr Bentley (Senior Lecturer Imperial College and consultant neurologist, Charing Cross Hospital) and Professor Xu (Bioengineering dept, Imperial College, London, UK). This committee has already provided the research team with useful feedback with regard various issues during the design of the project and they will also participate in the review meetings. Mr Geroulakos and Professor Nicolaides will be the Project managers and will be responsible for the smooth running of the trial and the delegation of

duties during the trial. This multidisciplinary approach will enable us to resolve any potential future issues and improve the interpretation of the results.

Patient and public involvement

The R&D manager and a variety of people from various administrative posts (non-physicians) at Ealing Hospital NHS Trust have been consulted during one-off meetings. They provided the research team with useful feedback regarding different aspects of the proposed design and management of this research project. We plan to continue this collaboration though "Clinical Governance" meetings, which are regularly arranged, at Ealing Hospital for educational purposes.

However, we do realize the importance of further public involvement in every research effort and thus motivated individuals will be selected through local support groups for people with cardiovascular disease and/or with the help of related charities such as the Stroke association and the British Heart Foundation. These individuals will help us in the production of the patient's information packs and consent forms in order to make the information -related with our study- accessible for everyone.

In addition during and at the end of the study a brief report of our progress will be send to the participants of our study in order to keep them updated. In addition we plan to organize regular meetings with the public -end especially with the local community in collaboration with the above mentioned support groups and charities-in order to provide information about our research effort and answer questions regarding research methodology, carotid plaque disease and stroke. It is possible that we will also involve available patients and member of the public to present some of the findings or even share the experience of collaborating with us so we can also further improve as a team.

Appendix IV

Permissions

For the use of published material originating from our own group, permission was obtained when necessary:

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Atherosclerosis

Three-dimensional ultrasound imaging for the evaluation of carotid atherosclerosis

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The size of juxtaluminal hypoechoic area in ultrasound images of asymptomatic carotid plaques predicts the occurrence of stroke

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Atherosclerosis

The effect of statins on carotid plaque morphology: A LDL- associated action or one more pleiotropic effect of statins?