**DRAFT PROTOCOL**

**A**symptomatic **C**arotid **S**tenosis and **R**isk of **S**troke

in Patients who are on **O**ptimal **M**edical **T**herapy (**ACSRS-OMT**)

(International Multicenter Prospective Natural History Study

 under the Auspices of the IUA)

**Co-ordinating Centre**

Cardiovascular Disease Educational and Research Trust (CDERT)

(UK registered charity number 326648)

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**CENTERS INVOLVED IN DATA COLLECTION AND ANALYSIS,**

**AND THEIR CONTRIBUTION**

1. **CDER TRUST (UK) (Prof A Nicolaides and UK team)**

Coordination and record keeping. Creation of master database.

1. **Imperial College, London (UK) (Mr George Geroulakos and team)**

Statistical advice, interim and final analyses; training centre for north Europe.

1. **St George’s London University / University of Nicosia Medical School, Cyprus (A Nicolaides and Cyprus team)**

Image analysis according to ACSRS publications; training center for Middle East.

1. **University of Cyprus, Nicosia, Cyprus (Prof C Pattichis and team)**

Image analysis using SGLDM and AM-FM texture features; development of automation.

1. **University of Patras, Greece (Prof Stavros Kakkos and team)**

Blind duplication of image analysis according to ACSRS publications; analysis using neural networks

1. **University of Thessaly at Larissa (Prof A Giannoukas and team)**

Training center for south Europe

1. **University of New Mexico, USA (Prof M Pattichis and team)**

Plaque motion analysis; automation; USA training center.

1. **Italian Center (to be decided)**

Italian training center

1. **Australian Centre (to be decided)**

Australian training center

**CENTERS INVOLVED IN CONTRIBUTING PATIENTS WITH ACS WORLD WIDE**

List is in preparation.

It is expected that at least 50 centers will participate.

**1. BACKGROUND: THE NEED FOR ACSRS-OMT**

***Introduction***

Two randomized controlled trials, the ACAS in 1995(1) and ACST in 2004(2) reported that in patients with asymptomatic ICA stenosis >60-70% (NASCET) carotid endarterectomy reduced the risk of stroke from 2% to 1% per year (1,2). In these trials carotid endarterectomy was associated with a 2-3% perioperative rate of stroke or death. The implication was that 90 operations were needed to prevent approximately one stroke in one year, which was a heavy financial burden on health care providers. In addition, in the ACAS and ACST studies, medical intervention, which was left to the discretion of the local teams was suboptimal in relation to current practice (2).

A recent review of natural history studies and medical arms of RCTs indicates that the average annual risk of ipsilateral cerebral and any territory stroke among patients with asymptomatic moderate to severe ICA stenosis receiving medical intervention alone has fallen to approximately 1% (3-6). The decreased incidence of stroke has been attributed to modern medical therapy and has made opinion leaders demand a revision of management strategies (4) by either refraining from carotid endarterectomy (7) or by identifying high risk patients (8). Thus, if patient subgroups with sufficiently higher than average risk, despite optimal medical intervention, could be reliably identified, then carotid surgery could be performed in those that are likely to benefit.

Prospective studies in patients with asymptomatic carotid stenosis have demonstrated that three methods are able to identify high risk subgroups for stroke: (a) Transcranial Doppler (TCD), (b) CT-Brain scans and (c) Ultrasonic plaque imaging.

***TCD Embolic Signals***

In the ACES study, which involved 467 asymptomatic patients (mean follow-up 2 years) with ≥70% internal carotid stenosis, the presence of TCD microembolic signals during a recording for one hour could identify a subgroup with a 7.5% annual stroke rate (9). A meta-analysis of 6 prospective studies including the ACES has confirmed these results (9). TCD microembolic signals are found in 15-20% of patients with asymptomatic carotid stenosis and characterize patients that constitute a high risk group. However, this group contains only 57% of the strokes that occur during follow-up. Thus, 43% of the plaques that produced a stroke are missed.

***Silent Infarcts on CT-Brain Scans***

The prevalence of silent infarcts on CT-Brain scans in patients with asymptomatic carotid stenosis varies from 10-24% (10-12). The presence of embolic infarcts (small cortical, discrete subcortical, basal ganglia non-lacunar infarcts) can identify a high risk group with an average annual stroke risk of 3.6% (13). However, this group contains only 30% of the strokes that occur during follow-up. Thus, 70% of the plaques that produce a stroke are missed. This suggests that the majority of carotid plaques may rupture producing strokes without giving off prior emboli that result in silent infarcts.

***Ultrasonic Plaque Features***

Using ultrasound imaging of carotid plaques, several features that have been proposed to predict stroke in patients with asymptomatic carotid stenosis and thus contribute to risk stratification, have been confirmed in prospective studies. They include severity of stenosis (14), low gray scale median (GSM) (15,16), plaque area ≥80 mm2, a history of contralateral TIAs or stroke, the presence of a juxtaluminal black plaque area in the plaque image without a visible echogenic cap (JBA) ≥8 mm2 (17) and the presence of discrete white areas (DWA) without acoustic shadowing indicating neovascularization (15) and ulceration on 3D ultrasound (18).

 In the ACSRS study which involved 1121 patients with asymptomatic carotid stenosis ≥ 50% (ECST) and a mean follow-up of 6 months to 8 years (mean 4 years), a GSM < 30 was found in 22% of plaques. It identified a high risk group with an average annual stroke risk of 3.0% (15). However, this group contained only 54% of the strokes that occurred during follow-up. In contrast, the presence of a JBA ≥8 mm sq identified a high risk group with an average annual stroke risk of 4.1% (17). This group contained 71% of the strokes that occurred during follow-up.

***Clinical features***

Of the many clinical features tested such as age, hyperlipidemia, hypertension, diabetes, smoking and elevated creatinine, only the presence of a history of contralateral TIA’s or stroke was an independent predictor of stroke risk (15). However, such a history was present only in 15% of patients. The poor ability of clinical risk factors to predict stroke has been attributed to the fact that risk factors for atherosclerosis are present in practically all patients with asymptomatic carotid stenosis.

***Key message***

A key message that emerged from the evidence discussed above was that there was not a single feature that could identify all the potentially unstable and high risk plaques. This is because there are several mechanisms that result in embolization. Some plaques produce emboli because of a thrombus on their surface, some because they rupture having a large lipid core and a thin fibrous cap eroded by macrophages, while others rupture because of mechanical forces irrespective of their structure. It has been argued that a combination of plaque features should perform better than a single feature alone. Indeed this hypothesis was tested in the ACSRS cohort.

***Combined features***

In the ACSRS study, five features have now emerged as independent predictors of risk: degree of stenosis, history of contralateral TIAs or stroke, GSM < 30, a JBA ≥ 8 mm2 and the presence of DWAs after image normalization. These 5 features, which are independent of ultrasonic equipment, can be used to calculate risk for every patient (range of stroke risk: < 1% to 10% per year) (15). In the ACSRS study the risk was < 1% in 734 patients, 1-1.9% in 94 patients, 2-3.9% in 134 patients, 4-5.9% in 125 patients and 6-10% in 34 patients.

A subsequent analysis of the baseline plaque images of the ACSRS study, using a combination of texture features based on second order statistics, spatial gray level dependence matrices (SGLDM) predicted 89 (82%) of the 108 cases that developed ipsilateral symptoms with a sensitivity of 82% and specificity of 72%. The area under the ROC curve was 0.832 (95% CI 0.808 to 0.853) (19). This approach has the advantage that it can be developed to automatic analysis of images and expression of stroke risk by computer.

An adaptive multiscale spatio-temporal AM-FM (amplitude modulation-frequency modulation) texture analysis system has been developed by members of our team (University of Cyprus and University of New Mexico) (20,21) and has been applied to the baseline images of the ACSRS. In a logistic regression analysis four features were independent predictors of stroke and allowed us to calculate the risk of every patient. The cumulative stroke free survival was 97% or less for the lower three quintiles, 92% for the fourth quintile and 84% for the 5th quintile. The corresponding annual stroke rate was 0.6%, 1.8% and 3.2%. For the full 8 year follow-up, there were 2 strokes in the first quintile, 5 strokes in each of the second and third quintiles, 18 strokes in the fourth quintile and 26 strokes in the 5th quintile (unpublished data). This is another approach that has the advantage that it can be developed as an automatic analysis of images and expression of stroke risk by computer.

***Plaque rupture due to mechanical stresses***

In the ACSRS study 18 (29%) of the 59 ipsilateral ischemic strokes occurred in the low risk groups that did not have any of the ultrasonic features that were independent predictors of stroke risk. This suggests that a number of plaques may rupture because of external forces rather than inherent instability. It has been suggested that during the cardiac cycle parts of a plaque move in the same direction (concordant movement) in most plaques, while parts move in different directions in some plaques (discordant movement) (22,23).

 Special software for plaque motion analysis has been developed and published by our group in collaboration with the team of Prof. Marios Pattichis at the University of New Mexico (24). This software can track pixels in the B-mode video recording of a plaque during a series of cardiac cycles producing vectors indicating the direction of motion and distance (Figs 1A and 2A). In practice, a cine loop of a B-mode image of a carotid plaque is obtained over 10 cardiac cycles. This facility is now available on all modern ultrasound scanning equipment. Video frames are compared at intervals of 0.1 sec and for each comparison a 360 degree histogram is produced. The fun-width of the histogram is plotted against maximum pixel motion. The area under the curve for all the comparisons during the 10 cardiac cycles is a measure of discordant movement. Figure 1 shows the results from a plaque with concordant movement and Figure 2 the results of a plaque showing discordant movement.

 In a pilot study, video loops of B-mode ultrasound images of 35 carotid bifurcation plaques were obtained from patients with carotid bifurcation atherosclerosis. Each video loop was edited, so that a series of 8-10 consecutive cardiac cycles were included that did not include any motion artifacts such as carotid movement due to swallowing or neck movement. Plaque motion was classified visually as concordant “stable” or discordant “unstable”. Stable plaques were those that had all their parts move simultaneously in the same direction throughout the cardiac cycle. Unstable plaques were those that at certain parts of the cardiac cycle different parts were seen to move in different directions, especially at peak systole.

 Plaque Motion Analysis (PMA) was studied using the software from the University of New Mexico, USA. For every cine loop a plot of peak motion against fan width was obtained (Fig 1C and 2C). From each plot, the sum of the mean fan widths (MFWs) for pixel movements 5 to 3 (SMFW5-3) were calculated. There was an excellent discrimination between the mechanically stable and unstable plaques (Figure 3).



(A)



(B)



(C)

Figure 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.



(A)



(B)



(C)

Figure 2. (A) Frame 249 at peak systole compared with the frame 253 (interval of 0.1 sec) shows that pixels in different parts of the plaque move in different directions. (B) The data of frame 301 show the histogram of the pixels and the motion amplitude. The wide fan on the circular presentation confirms the three different direction of pixel motion. (C) Scattergram of peak motion against fan width for all the frame comparison. Each square “dot” represents the result of motion for 0.1 sec. There is a characteristic wide fan-width for peak motion of 6 pixels down to 2.



Fig 3. The SMFW5to3 with a cut-off point of 200 provides the best separation between mechanically stable and unstable plaques.

***Justification of the new ACSRS-OMT study***

Similar to the medical arm of the ACST trial (2), in the ACSRS study only 20% of the patients were on statins and only 80% on antiplatelet therapy. In addition, unlike modern medical therapy, statins and antihypertensive drugs were not administered to target i.e. until a level of LDL-cholesterol or BP as predefined by evidence based guidelines was achieved

Recent RCTs which included patients with atherosclerotic arterial disease (25,26) have demonstrated that aggressive reduction of LDL cholesterol have resulted in a 25-30% reduction of stroke and MI. Reduction of LDL to 70mg/dl or lower was associated with coronary artery plaque regression (27,28). In the METEOR primary prevention trial rosuvastatin delayed the progression of carotid atherosclerosis in individuals with subclinical atherosclerosis and moderately elevated cholesterol levels (29).

A review of 17 prospective studies of which 9 were RCTs (30) reported changes in plaque morphology as a result of statin therapy. There was increase in plaque echogenicity (9 studies), decrease in lipid core size (9 studies), plaque regression or slower progression (7 studies) and reduction in TCD microemboli (1 study)

In a Canadian study involving 1686 individuals without previous MI or stroke, statin therapy produced regression of plaque area in 28% and arrested progression in 16% (31). At 5 years, in patients with plaque regression or no progression there was a 50% reduction in the combined endpoint of stroke, MI and cardiovascular death.

In the ACSRS study, only 294 of the 1121 patients were on lipid lowering therapy. The incidence of stroke was zero in the presence of plaque regression; it was 1.1% per year in patients with plaques that did not change and 2.2% per year in the presence of plaques that showed progression (17)

In current practice some surgeons believe that in patients with moderate to severe asymptomatic carotid stenosis OMT alone is adequate because the overall annual risk of stroke is likely to be 1% or less. In the absence of a RCT to demonstrate that OMT is superior to surgery continue to operate if the stenosisis greater than 70-80%. They are reluctant to apply a method of stroke risk stratification such as the presence of embolic signals using TCD or plaque image analysis because these methods are based on studies such as the ACES (5) and ACSRS (15) in which patients were not on OMT. Thus, the successful stroke risk stratification achieved by the ACSRS study needs to be repeated in a subsequent study in which patients are on optimal medical therapy (OMT). **Our hypothesis is that a 25-30% reduction in stroke produced by OMT should still allow the identification of high risk subgroups.**

Our expectation that the methodology of risk stratification based on plaque characterization as developed in the ACSRS study would have been incorporated into the Medical Arm of new RCTs (CREST2 in the USA and ECST2 in Europe) which include patients with asymptomatic carotid stenosis on OMT has not yet been fulfilled. As far as we know both studies have started without the prerequisite training of ultrasonographers on equipment settings and image capture suitable for image normalization and plaque characterization due to lack of funding. A pilot study performed by us on the first 40 patients in the ECST2 recruited from London has demonstrated the feasibility. Funding for this pilot study has been provided by the Cardiovascular Disease Educational and Research Trust (CDERT).

Capturing the appropriate views of plaque images using ultrasound is now within the capability of every vascular ultrasonographer following one day’s training. Such training had been provided in the past not only to all participants of the ACSRS study, but also of the ICAROS study (33) which involved centers on both sides of the Atlantic. Image normalization and measurement of GSM and JBA size using commercially available software is relatively simple on a laptop. Risk stratification is now provided by a limited number of vascular laboratories, when requested. In the ACSRS2 it will be performed centrally

Plaque motion analysis will be performed on the same images also.

Performance of image analysis will be optional for partner centers. Partner centers will be provided with the software and be trained to do image analysis if they so wish.

**2. OBJECTIVES OF ACSRS2 STUDY**

The plan of the proposed project is to study a group of patients with asymptomatic carotid stenosis greater than 70% in relation to the bulb (ECST) or its equivalent of 50% stenosis in relation to the distal internal carotid diameter (NASCET) on optimal medical therapy (OMT) and follow them up for 5 years.

The primary aim is to identify risk factors (clinical, biochemical and plaque characteristics using ultrasound) that will be independent predictors of ipsilateral cerebrovascular or retinal ischemic (CORI) events and will enable us to provide stroke risk stratification from less than 1% to more than 10% per year.

A secondary aim will be to correlate cerebrovascular events with changes in plaque texture features and magnitude of risk factor modification.

**3. PLAN OF THE INVESTIGATION**

This is a multicenter international study instigated by the co-ordinating centre and other members of the proposed International Concerted Action with input from the scientific committee of the International Union of Angiology (IUA) and advice from members of the International advisory committee. Each one of the teams listed on page 3 has to offer expertise in one particular area needed for the study. Although the teams are geographically apart they have a track record of collaboration and joint scientific publications.

***Eligibility of centers contributing patients***

Participating centres should have an active noninvasive vascular laboratory with a duplex scanner and experience (> 100 carotid scans per year) in the investigation of patients with extracranial cerebrovascular disease, a neurologist and a vascular physician or surgeon. Also, they should be able to provide an average of 30 individuals or patients with asymptomatic atherosclerotic carotid bifurcation disease that can be entered into the study.

***Inclusion Criteria***

Newly referred (<3 months) patients with 50-99% ICA stenosis in relation to the normal distal internal carotid diameter (NASCET method) (70-99% using ECST method) without previous ipsilateral cerebral or retinal ischemic (CORI) symptoms and without neurological abnormality will be recruited to the study after written informed consent. Patients who had had contralateral cerebral hemispheric/retinal or vertebrobasilar symptoms or signs of stroke/TIA will be 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 will be considered ipsilateral (the study artery).

*Exclusion Criteria*

Patients who cannot attend for a 6 monthly neurological assessment, assessment of efficacy of and adherence to OMT and those with a life expectancy of less than two years will be excluded. Also, patients who refuse to be on medical therapy.

*Baseline Clinical and Biochemical Characteristics*

At baseline, all patients will have a history taken and a physical examination by the local neurologist to ensure that they are truly asymptomatic, electrocardiographic (ECG) examination and collection of fasting blood for determination of the following:

 - Age, gender, systolic and diastolic blood pressure, smoking history and accrued pack-years.

 - Medication usage including antiplatelet, anticoagulant, anti-hypertensive and lipid lowering agents.

 - Presence of hypertension (antihypertensive medication or BP≥140 mmHg systolic or ≥90 mm Hg diastolic); coronary artery disease (documented myocardial infarction/angina, coronary artery bypass or stenting); diabetes (antihyperglycemic therapy or fasting blood glucose >120 mg/dL) and previous contralateral stroke/TIA or vertebrobasilar symptoms.

 - ECG evidence of atrial fibrillation, previous myocardial infarction (MI), myocardial ischemia and left ventricular hypertrophy (LVH) on baseline ECG. ECGs will be reported at the coordinating centre by two cardiologists.

 - Fasting lipids (total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides), glucose, serum creatinine, liver function tests (bilirubin,AST, ALT, Gamma Glutamyl Transpeptidase, Alkaline Phospatase) and HbA1c if patient is diabetic.

*Duplex Scanning*

Bilateral carotid duplex scanning will be performed on admission to the study. Ultrasonographers from all centres will be trained at dedicated centers (one day course) in criteria for grading internal carotid stenosis and plaque image capture (15). A B-mode image of the Carotid plaque and a color flow image will be obtained as described below. In addition two AVI cine loops of the plaque will be obtained over at least 10 cardiac cycles, one in B-Mode and one in color. They will be sent to the coordinating centre via drop box.

*Grading of internal carotid stenosis*

Velocities will be obtained at the point of maximum stenosis in the internal carotid artery and 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 (e.g. in the presence of cardiac arrhythmia) or overestimate stenosis (e.g. in the presence of severe contralateral disease), ultrasonographers at each center will be trained to use a combination of absolute velocity measurements and velocity ratios (Table 1). In plaques that are not calcified, anatomical criteria using color flow or power Doppler imaging of the artery in transverse section (percent diameter stenosis from measurements of vessel and residual lumen diameter at the site of maximum stenosis) will be used to supplement velocity criteria. Contralateral ICA occlusion will be noted. Bilateral vertebral artery flow will be reported as cephalad, reversed or not visualised.

*Recording of plaque images*

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

1. Maximum dynamic range will be used in order to ensure the greatest possible display of gray scale values.

2. Persistence will be set on low and frame rate on high, the latter ensuring good temporal scale values

3. The time gain compensation curve (TGC) will be sloping through the tissues but will be positioned vertically through the lumen of the vessel because there will be little attenuation of the ultrasound beam as it passes through blood. This will ensure that the brightness of the adventitia of the anterior and posterior walls will be similar.

4. The overall gain will be adjusted to give optimum image quality. This will be achieved by adjusting of the gain control to minimize but not abolish noise in the vessel lumen.

5. The most linear post-processing available curve will be used.

6. The ultrasound beam will be at 900 to the arterial wall.

7. 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, which will be performed at the coordinating center. Ultrasonographers from participating centers will attend for a day’s training at the coordinating center or other regional dedicated centers. They will be trained not only on equipment settings and method of image recording but also on the method of image normalization and plaque texture analysis. Although they will not be expected to perform image analysis, knowledge of how it is 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 will be provided so that they will be able to train other ultrasonographers on their team.

A B-mode image of the Carotid plaque and a color flow image will be obtained. In addition, two cine loops of at least 10 cardiac cycles, one in B-mode and one in color (or power Doppler) will be captured with the patient holding their breath to avoid motion artifacts. This method will enable the person doing the image analysis to select the most appropriate color frame ensuring adequate outlining of a hypoechoic (black) plaque.

In addition, plaque motion analysis will be performed on the B-mode cine loop. The cine loops (in AVI format) will be sent to the co-ordinating center via DropBox.

*Image normalization, segmentation and analysis*

The optimum color frame and corresponding B-mode image will be selected from the video loops at the coordinating center by two members of the team who will be experienced in carotid scanning. Image normalization, standardization and analysis will be performed by the same members of the team.

 The “Plaque Texture Analysis software” version 4.5.1 (LifeQ Medical; [www.lifeqmedical.com](http://www.lifeqmedical.com)) (21) which is a dedicated research software package will be used. Normalisation will be performed with the “Image Normalization” module using linear scaling with blood (gray scale value assigned: 0) and adventitia (gray scale value assigned: 190) as reference points will be performed as previously described (15,17). First, a sample of “blood” will be selected from the vessel lumen avoiding areas of “noise”. Next, using the zoom facility the brightest part of adventitia adjacent to the plaque will be magnified at least four times and the middle two fourths were selected. The normalized image will appear automatically in the window next to the original image and will be saved as a separate file with letter n added as the last letter to the original name. The normalized image will then be standardised to a pixel density of 20 pixels per mm using the bicubic method available in the “Pixel Density Normalisation” module and saved in the database as a separate file. The “Image Scaling” module will subsequently be used to register the scale distance (usually 10 mm) as shown on the side of the image. The “Image Crop” module will then be used. This has two windows, one for the normalized black and white image and the other for the color flow image for guidance. Visualization of the color flow or power Doppler images as recorded by the ultrasonographer will enable the operator to identify the dark areas of plaque adjacent to the lumen (absence of color) and the areas of ulceration (presence of color). The “Log image” facility that allows a temporary logarithmic transformation of the image producing a better definition of the plaque outline will subsequently be used. The plaque outline will then be traced with the mouse and the plaque area within this outline will be saved as a separate plaque image file with the same name and extension “.plq”. Care will be taken not to include adventitia. Both components of a plaque (anterior and posterior wall) will be selected. For plaques with a calcified cap, both the calcified area and the area of the plaque adjacent to the calcification outside the acoustic shadow will be included.

 Using the “Feature Extraction” module a variety of texture features based on first order such as GSM and plaque area, and second order statistics (SGLDM) will be extracted and automatically calculated. These features will automatically be saved in a text database that could be opened by “Microsoft Office Excel” (Microsoft Inc. Redmond, Washington, USA) or SPSS for subsequent statistical analysis. Plaque images will be automatically contoured and color-coded. Pixels with gray values 0-24 will be colored black, 25-49 in blue, 50-74 in green, 75-99 in yellow, 100-124 in orange and pixels greater than 124 in red (Fig. ??). The size of the juxtaluminal plaque area wiil be outlined by the operator and measured by the software in mm2.

 The definitions and reproducibility of texture features used in the ACSRS study are given below.

*1. Gray Scale Median (GSM).*

This is the median of the gray values of all the pixels in the plaque image (21-24). In a reproducibility study of 35 plaques measured by two observers the interobserver mean difference of GSM was 3.6, the within-subject standard deviation was 13.6 and the intra-class correlation coefficient was 0.93 (21).

*2. Plaque area*

This is calculated by the software using the distance scale on the side of the image frame for calibration and the plaque area as outlined by the operator. It is expressed in mm2 (21,27). In a reproducibility study involving 50 plaques the interobserver intraclass correlation coefficient was 0.73

*3. Discrete White Areas (DWA).* The presence of DWA defined as areas with pixels having gray scale values >124 (colored red by the software for easy visual identification) not producing acoustic shadowing in plaque types 1-3 was noted (Fig. 2 b and c). A reproducibility study involving 80 plaques classified visually after image normalization by two observers for presence or absence of DWA had a kappa statistic of 0.83 (P < 0.01).

***4. Juxtaluminal Black Area (JBA).*** The largest juxtaluminal black area of the contoured image (i.e., area with pixels having a gray-scale value of less than 25) without a visible echogenic cap (i.e., pixels with gray-scale value higher than 25) will be outlined using the mouse. JBA area will be automatically calculated by the software and expressed as mm2, (14) (Fig. 1). The larger value will be used in cases where there are two plaque components with black areas. When the JBA (range 0-110 mm2) of all the plaques was measured by two observers using the “Plaque Texture Analysis” software the interobserver mean difference between repeat measurements was 2.5 mm2, the within-subject standard deviation was 6.9 mm2 and the intra-class correlation coefficient was 0.94 (14).

***5. Additional texture features.*** Beyond the standard texture features that have already been used in the ACSRS study, we propose to use (a) the SGLDM texture features described above (b) the adaptive multi-scale spatio-temporal AM-FM (amplitude modulation-frequency modulation) texture analysis system also described above (University of Cyprus) and (c) extract features from plaque motion analysis (University of New Mexico). The latter should identify plaques that are likely to rupture as a result of external forces rather than internal structural weaknesses. All three systems are amenable to automation so that an integrated intelligent system for the stratification of stroke risk combining different clinical and imaging risk factors but based primarily on the morphology and motion characteristics of the ultrasound video images of carotid bifurcation plaques producing moderate to severe stenosis will be developed. Thus, a library of algorithms (software) will be developed that will enable real-time video image analysis that will be made available to the community. (For details of methodology please see Appendix).

**Optimal Medical Therapy**

Patients with asymptomatic carotid stenosis >70% have a high 10 year cardiovascular risk (> 30%) and will be treated with aggressive risk factor modification as recommended in recent guidelines (36) for such patients and those used in the SPARKL study (37). Success at achieving risk factor control and treatment targets will be monitored at the 6 monthly visits.

***Antiplatelet therapy*** according to clinical practice at the centre, e.g. aspirin 75-81 mg/day or aspirin and dipyridamole combined or clopidogrel monotherapy.

***Lipid lowering therapy.*** The most appropriate drug will be selected depending on patient tolerability and renal function and the dose adjusted to maintain a target total cholesterol 4.0mmol/L (154 mg/dL), and an LDL cholesterol target level of 1.8 mmol/L (70 mg/dL) together with low cholesterol diet. Statin dose should be increased until target is achieved or until side effects develop. If statin monotherapy is not enough to reach the target, ezetimibe or colesevelam may have to be added (38). CoQ10 supplements may be added if part of the local policy on management of hyperlipidemia. Liver enzymes should be checked prior to initiating treatment with a statin, at 3 months and then at each six monthly visit.

***Blood pressure lowering therapy.*** Treatment should be adjusted to maintain a target BP of 130/80 mmHg or less. A higher threshold may be used in patients with contralateral severe carotid stenosis or occlusion or those who develop side effects at target values. Hypertensive patients will be referred to the local clinic.

***Smoking cessation***. Patients smoking at the time of admission to the study will be encouraged to join a smoking cessation and support program and documentary evidence of this returned to the trial office.

***Control of diabetes.*** Patients with diabetes mellitus will be referred to the local diabetic clinic for optimization of glycaemic control. HbA1c will be reported at the 6 monthly visits and will be used as a guide to therapeutic efficacy.

***Dietary control.*** The Panayiotacos quantitative diet questionnaire will be administered at baseline. This is a validated short (11 questions) questionnaire that assesses how close the patient’s diet is to the ideal Mediterranean diet (34,35) (see Appendix). The patient will be referred to the local dietician for advice and diet optimization. The diet questionnaire will be repeated every 6 months.

***Exercise.*** A record of the patient’s activity and exercise will be obtained at baseline. Advice to exercise whenever possible will be given.

**Follow-up.**

This will be done every 6 months to perform a repeat Duplex examination, as described above, to be seen by a neurologist, record clinical events (see below) if any, check BP, repeat fasting lipid profile, creatinine, liver enzymes and fasting blood glucose (HbA1c if diabetic) and check compliance to OMT. Changes in diet will be assessed by the quantitative diet questionnaire of Panayiotakos. The family doctor and a close relative will be asked to let the local team know if any event occurs prior to a planned follow-up visit.

Outcome Measures

*Primary outcome measures* will be (a) ipsilateral CORI events i.e. cerebral or retinal ischemic events which include stroke and (b) ipsilateral cerebral ischemic stroke (fatal or non-fatal). Stroke and TIA are defined as cerebral deficits of most likely vascular origin lasting >24 hours or <24 hours, respectively. When a stroke will be reported, details recorded by the local neurologist, a 6-month modified Rankin score (36) and CT or MRI brain scan results will be requested. Two coordinating centre members including a neurologist, will make the final classification of ipsilateral strokes. The diagnosis of TIAs and amaurosis fugax will be made by the local team..

*Secondary outcome measures* will be all other strokes and TIAs, contralateral retinal vascular events and all other deaths. Cause of death will be determined by local team members, using death certificates, hospital records and family doctor information.

Study Exit Points

Follow-up will cease with the first occurrence of any of the following: the first primary outcome measure, death from causes other than ipsilateral stroke or loss to follow-up.

3 STATISTICAL ANALYSIS

Initially, Kaplan-Meier curves will be used for the whole cohort of patients to determine overall ipsilateral CORI event and stroke free survival over time. Stratified Kaplan-Meier curves will also be constructed for % stenosis, history of contralateral TIAs or stroke and plaque texture features. Continuous variables will be categorised for stratified Kaplan-Meier plots. For example, stenosis will be categorized as moderate (70-89% ECST/50-82 NASCET) or severe (90-99% ECST/ 83-99% NASCET).

 Subsequently, hazard ratios for clinical, biochemical and ultrasonic features for ipsilateral CORI events and stroke will be determined using an unadjusted Cox model for each variable. Continuous risk factors will be transformed to an un-skewed distribution where possible.

Risk factors which will be significant at *p*<0.05 in unadjusted models for CORI events or stroke will be considered in multivariable proportional hazards models. Flexible parametric models of Royston & Parmar(32) will be used because the baseline hazard function at 5 years is erratic in Cox models. Hazard ratios from these models will be compared to equivalent Cox models.

 On the basis of the best model, ipsilateral cerebral ischemic stroke free survival curves will be produced for different combinations of risk factor subgroups from which 5-year stroke rates will be calculated. The assumption of proportional hazards will be tested using the Schoenfeld residuals.

The covariates included in a model will be used to calculate the linear predictor score, ***βx*** (the sum of the product of mean-centred covariate values and corresponding parameter estimates) for each patient. ROC curves will be constructed for ***βx*** against observed 5-year CORI event rates (in the same set of patients). For the final model, internal calibration will be assessed by comparing predicted risk of stroke at 5 years to the observed proportion experiencing stroke by 5 years.

On the basis of the final model, tables for moderate and severe stenosis will be produced using catecorized significant risk factors and plaque texture features in which the risk of stroke will be indicated.

**4. POWER CALCULATIONS**

In the ACSRS there were 1121 patients with 50-99% stenosis in relation to the bulb.

There were 59 strokes during the 8 year follow-up of which 52 occurred in the first 5 years. The number of patients, strokes and average annual stroke rate are shown in the table below.

ECST stenosis N Number of strokes Average annual

 stroke rate

Severe 90-99% 289 21 2.4%

Moderate 70-89% 643 32 1.4%

Mild 50-69% 189 6 0.8%

Overall average mortality was 5% per year. There were a total of 184 deaths of which 111(60%) from cardiovascular causes, mainly MI.

In the ACSRS study the 903 patients with 70-99% stenosis were stratified by the significant risk factors (plaque texture features, stenosis and history of contralateral TIAs or Stroke) into the following stroke risk groups:

Average annual No stroke Stroke Total

stroke rate

< 1% 571 5 (0.86%) 576

1-1.9% 69 4 (5.4%) 73

2-2.9% 96 14 (12.7%) 110

3-3.9% 24 5 (17.3%) 29

4-5.9% 50 7 (12.2%) 57

>6% 40 18 (31.6%) 58

Total 850 53 903

By taking 2% annual stroke rate as the cut-off point for decision to operate the table above becomes:

Average annual No stroke Stroke Total

stroke rate

< 2% 640 9 (1.4%) 649

≥ 2% 210 44 (17.3%) 254

Total 850 53 903

P < 0.0001 OR 7.93 (95% CI 4.02 to 15.6)

In the ACSRS 20% of the patients were on statins and 80% on antiplatelet drugs at baseline. By the end of the study (year 8) 80% were on statins, but not necessarily on maximum dose that could be tolerated by the patient.

*Patients to be included in ASSRS-OMT study*

In view of the low stroke rate, patients with less than 70% stenosis (ECST) will not be included in the proposed new study. Only patients with 70-99% ECST stenosis (50-99% NASCET) will be included. (NOTE: The 70-89% ECST stenosis group (50-83% NASCET) will be included because 32 of the 59 strokes occurred in this group (N=643).

*Assumptions*

1. The ratio of severe to moderate stenosis will be at least the same or even higher. At the time the ACSRS study was performed (1998-2006) surgeons tended to operate on patients with ≥ 90% stenosis and were reluctant to admit them to the study. They are more likely to admit them now.

2. The effect of Optimal Medical Therapy (see definition in this protocol) will reduce stroke by 30% (The reduction of ischemic stroke (either side) or cardiovascular mortality in RCTs on the effect of medical therapy in patients with atherosclerotic disease varied from 24% to 27%).

3. The effect of Optimal Medical Therapy will also reduce cardiovascular mortality by 30%. Thus, overall average annual mortality will be 4%. For the same number of patients as in the ACSRS there will be 33 more patients alive by the end of the study.

*Number of patients needed*

Assuming a 25% reduction in ipsilateral ischemic strokes the table from above will become:

Average annual No stroke Stroke Total

stroke rate

< 2% 642 7 (1.1%) 649

≥ 2% 221 33 (13%) 254

Total 863 40 903

Now, to get the same total number of strokes to 53 we need to increase the numbers by 32.5%

Average annual No stroke Stroke Total

stroke rate

< 2% 850 9 (1%) 859

≥ 2% 292 44 (13.1%) 336

Total 1143 53 1195

Based on the above assumptions the number of patients with 70-99% stenosis needed at the end of the study to maintain the same statistical power and the same absolute number of strokes will be 1195. By having 33 more patients surviving the number needed to enter the study becomes 1162. In order to allow for loss of 50 patients to follow-up the number becomes 1212.

**5. ETHICS COMMITTEE APPROVAL**

Ethics approval will be sought from the National Research Ethics Service in the UK. Individual centres will be required to have local R&D approval before admitting patients to the study.

**6. EXPECTED OUTCOME**

At the end of the study the following should become available.

1. A method or methods of stroke risk stratification for patients with ACS on OMT
2. It is expected that only 20% will have a stroke risk greater than 2% per year and thus be considered for carotid endarterectomy.
3. A semi-automated method of risk calculation that can be incorporated into the software of a duplex scanner
4. The current indications for carotid endarterectomy in patients with asymptomatic carotid stenosis will be refined

The added value is that it has the potential of (a) sparing many patients from an unnecessary operation with a saving health care providers the sum in excess of 1.5 billion Euro in Europe and a similar sum in the USA, and (b) carotid endarterectomy will be performed only in those asymptomatic patients that are likely to benefit.

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**8. PLAN OF ACTION PRIOR TO COMMENCING THE STUDY**

This draft protocol will be sent to the statisticians for confirmation of the power calculations; also to all centers who took part in the ACSRS study and those that have expressed interest in the last few months.

It will be presented at several vascular and angiology meetings in the next few months as well as the IUA World Congress in Sydney and the Veith meeting in New York.

Application will be made to the Ethics Committee when the list of partner centers is compiled

In the meantime we will also approach several Institutions for Funding.

**For expression of interest to join the study please complete the form on the next page and email it to Prof. Andrew Nicolaides**

**A**symptomatic **C**arotid **S**tenosis and **R**isk of **S**troke

in Patients on **O**ptimal **M**edical **T**herapy **(ACSTS-OMT)**

An international multicenter natural history sudy

Under the Auspices of the IUA

The plan of the proposed project is to study a group of patients with asymptomatic carotid stenosis greater than 70% in relation to the bulb (ECST) or its equivalent of 50% stenosis in relation to the distal internal carotid diameter (NASCET) on optimal medical therapy (OMT) and follow them up for 5 years.

The primary aim is to identify risk factors (clinical, biochemical and plaque characteristics using ultrasound) that will be independent predictors of ipsilateral cerebrovascular or retinal ischemic (CORI) events and will enable us to provide stroke risk stratification from less than 1% to more than 10% per year similar to the ACSRS study.

A secondary aim will be to correlate neurological events and changes in plaque texture features with magnitude of risk factor modification.

**Contact details for researchers and centers interested in participation:**

Title/Name

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Affiliation/Department/Address

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Contact email

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Telephone contact numbers

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Please email this form to Prof A Nicolaides anicolaides1@gmail.com