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**"THE ROLE OF NEUROSONOLOGY IN THE
THERAPEUTIC DECISION OF PATIENTS WITH
ASYMPTOMATIC ATHEROMATOUS DISEASE OF THE
CAROTID BIFURCATION"**

EΥΧΑΡΙΣΤΙΕΣ

Για τη διεκπεραίωση της παρούσας Διπλωματικής Μεταπτυχιακής Εργασίας, θα ήθελα να ευχαριστήσω τον επιβλέποντα μου ακτινολόγο κύριο Λιάση Νικόλαο, καταρχάς για την απόλυτη εμπιστοσύνη που επέδειξε προς το πρόσωπό μου καθώς και για την άριστη συνεργασία και την πολύτιμη συμβολή του στην ολοκλήρωση της. Επιπλέον, θα ήθελα να ευχαριστήσω τον καθηγητή Αγγειοχειρουργικής κύριο Γιαννούκα Αθανάσιο που με ενέπνευσε στην επιλογή του θέματος της παρούσας Διπλωματικής Μεταπτυχιακής Εργασίας. Τέλος ένα μεγάλο ευχαριστώ στον σύζυγο, στον πατέρα μου και στην αδελφή μου για την υπομονή και την στήριξή τους.

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Εισαγωγή

Η καρωτιδική νόσος συνιστά σημαντική αιτία παροδικού και ισχαιμικού αγγειακού εγκεφαλικού επεισοδίου. Η αντιμετώπιση της ασυμπτωματικής καρωτιδικής στένωσης αποτελεί συχνά αντικείμενο διαφωνίας, καθώς η πρόοδος της βέλτιστης φαρμακευτικής θεραπείας έχει αμφισβητήσει την χρησιμότητα της καρωτιδικής ενδοαρθρεκτομής ή του stenting της καρωτίδας. Ωστόσο, οι θεραπείες επαναγγείωσης εξακολουθούν να κρίνονται κατάλληλες για μια μικρή υποομάδα ασθενών «υψηλού κινδύνου». Ο συνδυασμός της υπερηχοτομογραφικής μελέτης των καρωτίδων με το διακρανιακό υπερηχογράφημα των ενδοκράνιων αγγείων, συμβάλλει στον εντοπισμό αυτών των ασθενών και στη διαστρωμάτωση του κινδύνου για εγκεφαλικό αγγειακό επεισόδιο.

Μέθοδοι

Εξετάσαμε την τρέχουσα βιβλιογραφία σχετικά με τη συμβολή της υπερηχοτομογραφίας στην ταυτοποίηση των «υψηλού κινδύνου» ασθενών με ασυμπτωματική σημαντικού βαθμού στένωση της έσω καρωτίδας, οι οποίοι θα μπορούσαν να επωφεληθούν από επεμβατική παρέμβαση.

Αποτελέσματα

Έχει καταστεί πλέον σαφές ό,τι μόνο ο βαθμός της καρωτιδικής στένωσης δεν επαρκεί για τη λήψη της θεραπευτικής απόφασης και σταδιακά έχει αναπτυχθεί η έννοια της «ευάλωτης πλάκας». Ισχυροί υπερηχογραφικοί δείκτες της ευάλωτης πλάκας αποτελούν η υποχηρογένεια της πλάκας (κύρια υποχηρογενής), η μεγάλη περιοχή πλάκας (σε mm²), η μεγάλη παρά τον αυλό μαύρη περιοχή στην ηλεκτρονική ανάλυση της πλάκας, η παρουσία ενδοπλακικής νεοαγγείωσης και εξέλκωσης με τη χρήση ειδικών για τους υπερήχους σκιαγραφικών μέσων καθώς και η ανίχνευση μικροεμβολικών σημάτων στο διακρανιακό υπερηχογράφημα. Παράλληλα, η πρόοδος της στένωσης, η αμφοτερόπλευρη καρωτιδική νόσος καθώς και η ανάδειξη διαταραχής στην εγκεφαλική αυτορρύθμιση με τη συμβολή του διακρανιακού υπερηχογραφήματος, συνιστούν πρόσθετους προγνωστικούς παράγοντες του αγγειακού εγκεφαλικού επεισοδίου.

Συμπέρασμα

Οι υπερηχοτομογραφικές μελέτες χρησιμεύουν ως δείκτες της βαρύτητας της ασυμπτωματικής καρωτιδικής νόσου, βοηθώντας σημαντικά στη διαστρωμάτωση του κινδύνου για μελλοντικά καρδιαγγειακά συμβάματα. Κατά συνέπεια, συνιστάται ιδιαίτερα η ενσωμάτωσή τους στην ανάπτυξη των θεραπευτικών αλγορίθμων για την αναγνώριση ασυμπτωματικών ασθενών «υψηλού κινδύνου».

Λέξεις- Κλειδιά: Ασυμπτωματική καρωτιδική στένωση, υπερηχογράφημα, διακρανιακό υπερηχογράφημα, ευάλωτη πλάκα, αγγειακό εγκεφαλικό επεισόδιο

Abstract

Introduction

Carotid artery disease is a significant cause of ischemic stroke and transient ischemic attack (TIA). Management of asymptomatic carotid stenosis (ACS) constitutes a debate, as the advancement of best medical treatment has questioned carotid endarterectomy or carotid artery stenting. However, selected patients with “high risk” ACS should be identified and offered revascularization procedures. Carotid ultrasound combined with Transcranial Doppler (TCD) have been found to provide valuable information on risk stratification of stroke.

Methods

We reviewed the current literature regarding the contribution of neurosonology to the identification of patients with “high-risk” ACS, who could benefit from invasive intervention.

Results

There has been growing evidence over the last years that moving beyond the degree of luminal stenosis was mandatory and the concept of “plaque vulnerability” has emerged. Strong sonographic markers of vulnerable plaque represent plaque echolucency (predominantly echolucent), large plaque area, large juxta-luminal black area on computerized plaque analysis, intraplaque neovascularization and plaque ulceration by using contrast-enhanced ultrasound studies and detection of microembolic signals on TCD. Stenosis progression, bilateral carotid disease and impaired cerebrovascular reserve and collateralization assessed with TCD constitute additional predictors of stroke and TIA.

Conclusion

Neurosonology examinations serve as markers of carotid plaque burden assisting significantly in risk stratification of cerebrovascular events. Consequently, incorporation of neurosonology in the development of therapeutic algorithms for “high risk” ACS is highly recommended.

Key words: Asymptomatic carotid stenosis, ultrasound, transcranial doppler, vulnerable plaque, stroke

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1. Introduction

Atherosclerosis and its cardiovascular ischemic complications are the most common causes of death and disability worldwide (1). Indeed, the World Health Organization (WHO) reported in 2010 that cardiovascular disease represents around 30% of global deaths and estimated that by 2030 more than 23.3 million persons will die annually from cardiovascular disease (2). More specifically, stroke is the second largest cause of death in Europe and the leading cause of permanent neurological disability (3).

Carotid artery stenosis related to atherosclerosis, is a well-established risk factor for ischemic stroke, contributing to 15-20% of acute ischemic strokes (AIS) or transient ischemic attacks (TIAs) (4). Neurological symptoms due to carotid artery stenosis, are typically caused by distal embolization, but may also occasionally be due to cerebral hypoperfusion caused by severe stenosis of the carotid artery (5).

As for the secondary prevention of symptomatic carotid artery disease, recent guidelines of European Society for Vascular Surgery (ESVS) recommend carotid endarterectomy (CEA) or for some certain indications carotid artery stenting (CAS), within 14 days of symptom onset combined with best medical treatment (BMT), provided the documented procedural death/stroke rate is <6% (6).

Asymptomatic carotid stenosis (ACS) is defined as significant ($\geq 50\%$) narrowing of the carotid artery in patients who have not had ipsilateral neurological symptoms in the previous 6 months. ACS is of great importance, as it is also a marker of systemic atherosclerosis simultaneously affecting other arterial beds. Consequently, people even with minor ACS are at higher risk of myocardial infarction and undoubtedly need treatment (7).

Decades ago, two randomized, multicenter studies, the Asymptomatic Carotid Atherosclerosis Study (ACAS) (8) and the Asymptomatic Carotid Surgery Trial (ACST) (9), provided evidence that CEA offers a 50% risk reduction for ipsilateral stroke in patients with moderate to severe asymptomatic carotid artery stenosis (60-99% stenosis) compared to medical treatment alone. However, at the time when these trials were performed, patients had received a treatment that today would be considered as suboptimal medical treatment. For example, in the ACST statins were used in only 17% of patients for the first years of randomization and in 70% at the last year, while 10% of population was not taking antiplatelet therapy. Furthermore, dietary modification, blood pressure control and statin dosage were not close to those that are currently used in practice. The following years, more scepticism emerged, as it has been suggested that up to 94% of CEA performed on the basis of these trials recommendations, are unnecessary and that to prevent a stroke in asymptomatic patients the number needed to treat (NNT) was 83 (10).

The advancement of BMT has questioned the invasive intervention (CEA or CAS) for asymptomatic patients, as the annual risk of ipsilateral stroke with intensive medical therapy is now 1% (11). Consequently, most patients with ACS (~90%) would be better treated with intensive medical therapy, which includes lifestyle modifications (smoking cessation, a Mediterranean diet, weight reduction, exercise), effective blood pressure control, antiplatelet therapy, diabetes mellitus control and intensive lipid lowering therapy (12).

Nevertheless, for about ~10% of patients, invasive intervention may still be justified. Identification of such “high-risk” patients who could benefit from CEA or CAS in addition to BMT is thus of crucial importance. To that direction, recent ESVS

guidelines recommend that neuroimaging modalities could serve as biomarkers for better stroke risk stratification (6).

In particular, moving beyond luminal stenosis, atherosclerotic plaque vulnerability and cerebrovascular reactivity and reserve in the ipsilateral hemisphere, can both be studied with various imaging strategies. Carotid duplex ultrasonography (CDU), computed tomography angiography (CTA), magnetic resonance angiography (MRA), and rarely digital subtraction angiography (DSA) are the diagnostic modalities that are currently used in the evaluation of carotid steno-occlusive disease. CTA and MRA have the limitations of radiation exposure, intravenous contrast agents and overestimation of the degree of stenosis, respectively. On the other hand, they provide simultaneous visualization of the aortic arch, supraortic vessels, distal internal carotid artery (ICA), and intracranial arteries (13, 14). CDU is a widely available, low-cost, bedside, noninvasive and radiation free tool that is limited by its operator dependency and limitations in visualization of all ICA segments. It can be successfully combined with transcranial doppler (TCD) and may offer complementary information to other imaging modalities (15).

The aim of this narrative review is to summarize the utility of neurosonology in diagnosis and stroke risk stratification of ACS as well as its contribution to therapeutic management of ACS.

2. Methods

This review included all available articles regarding the use of ultrasound in diagnosis of asymptomatic carotid disease and the contribution of neurosonological markers to risk stratification of cerebrovascular events and further therapeutic management of these patients. Data were collected from the online MEDLINE database (all until June of 2018) using PubMed (National Center for Biotechnology Information, US National Library of Medicine, Bethesda, MD). The search was restricted to articles published in English without regard to when they were published and to studies in humans. The search strategy included multiple key words. In particular, search terms included “asymptomatic carotid stenosis”, “atherosclerosis”, “ultrasound”, “transcranial doppler”, “vulnerable plaque”, “plaque echogenicity”, “plaque heterogeneity”, “plaque ulceration”, “contrast-enhanced”, “microembolic signals”, “cerebrovascular reserve”, “stroke”, “transient ischemic attack”, “endarterectomy”, “stenting” and finally “best medical treatment”.

3. Discussion

3.1. Pathophysiology of atherosclerosis and ischemic events

Atherosclerosis has always been a major cause of mortality in developed countries, involving all the vascular system from aorta to coronary arteries. It is a progressive lipid-driven inflammatory disease of vascular intima, characterized by intimal plaques (16, 17). The term is of Greek origin, consisting of two parts; “athero” (accumulation of fat accompanied by several macrophages) and “sclerosis” (fibrosis layer comprising smooth muscle cells, leukocytes and connective tissue) (18, 19).

The first step in the development of atherosclerosis is the exposure of vascular cells to excess lipid (LDLs) with concomitant endothelial activation/dysfunction and the internalization and deposition of lipids in the intima. The continuous exposure to other pathogenic factors, such as hypertension, diabetes, stress and smoking, contributes to endothelial injury. The dysfunctional and permeable endothelium allows LDL particles to further infiltrate and accumulate in the extracellular matrix (ECM), where they become targets for oxidative and enzymatic modifications. Modified LDLs enhance a series of proinflammatory reactions perpetuating the activation, recruitment and transmigration of different innate immune cells (monocytes, mast cells, neutrophils, natural killer cells and dendritic cells), with monocytes playing the most crucial role. Also, acquired immunity, mainly dependent on T cells (T helper 1 and 2) and antibodies, is also critically involved in the progression of atherosclerosis (20, 21) and cytokine IL-18 has been shown to orchestrate the immunological link between innate and adaptive immunity, enhancing atherosclerosis activity and progression (22). Once monocytes transmigrate and reach the subendothelium they differentiate into macrophages. Macrophages depending on the local microenvironment, can assume different phenotypes and functional characteristics (23). Distinct macrophage subtypes (M1 and M2) have been detected depending on the stage of atherosclerosis development. Once differentiated, macrophages exhibit high levels of surface pattern recognition receptors for modified LDLs, become lipid-laden and convert into foam cells (24). Lesion complication occurs when foam cells release growth factors and cytokines which further stimulate vascular smooth muscle cell (VSMC) migration from the media into the intima where they divide and produce ECM components, contributing to the development of the fibrous cap (25). Many of these foam cells undergo apoptosis at early stages of atherosclerosis development and are removed by M2 macrophages with the process of efferocytosis (26). Excessive efferocytosis, has as a consequence the release of lipids, pro-inflammatory/pro-thrombotic mediators (tissue factor) and metalloproteinases (MMPs). MMPs digest the ECM scaffold, including the overlying fibrous cap, favoring plaque susceptibility to rupture. Plaque vulnerability is also determined by fewer VSMCs as well as formation of immature and leaky microvessels within the necrotic core (27).

Sites with low or oscillatory endothelial shear stress, located near branch points and along inner curvatures, are most susceptible for atherosclerosis development. Abdominal aorta, coronary arteries, iliofemoral arteries and carotid bifurcations are typically the most affected (28).

A simple histological classification of atherosclerotic lesions, which emphasizes the link between lesion morphology and clinical disease (29) includes:

A) *Adaptive intimal thickening*: Characterized by smooth muscle cells accumulation within the intima, in the absence of lipids and inflammatory cells.

B) *Intimal xanthoma* (“*fatty streak*”): Characterized by accumulation of foam cells into the intima. These types of lesions could be fully reversible if the stimuli that caused them disappear.

C) *Pathological intimal thickening* or “intermediate lesion”: True necrosis is not present. The fibrous cap is rich in smooth muscle cells and proteoglycans, while macrophages and lymphocytes are usually sparse.

D) *Fibroatheroma*: Corresponds to the formation of necrotic core. Foam cells, macrophages and smooth muscle cells accumulated in the intima over time could undergo apoptosis, while neovascularization and intraplaque hemorrhage are often present.

E) *Fibrocalcific plaque*: Calcification is common in progressive atherosclerosis. Apoptotic cells, ECM and necrotic core are plaque components that become calcified. Sometimes necrotic core can completely calcify.

The most dramatic complication of atherosclerotic plaque progression is thrombosis, which may be caused by three different mechanisms: a) plaque rupture, b) plaque erosion or c) calcified nodule (30).

Plaque rupture is the most common, and is defined as a structural defect—a gap—in the fibrous cap which exposes the highly thrombogenic core to the blood. Ruptured lesions typically have a large necrotic core and a disrupted fibrous cap infiltrated by macrophages and lymphocytes, while smooth muscle cell content within the fibrous cap at the rupture site may be quite sparse. Plaque rupture is followed by a cascade of events; exposure of thrombogenic parts of plaques, activation of the coagulation cascade, platelet adhesion, activation and aggregation leading finally to thrombosis and hemodynamic compromise (31). In carotid artery, artery-to- artery embolization is the most common mechanism causing ischemic stroke (5).

Plaque erosion is identified when serial sectioning of a thrombosed arterial segment fails to reveal fibrous cap rupture (absence of communication between blood contents and the necrotic core). Typically, the endothelium is absent at the erosion site. The exposed intima consists predominantly of smooth muscle cells and proteoglycans, with minimal inflammation (31).

The least frequent morphology associated with luminal thrombosis, represent calcified nodules, that are characterized by protrusion of the eruptive dense calcified bodies into the luminal space (32).

3.2. Ultrasound risk stratification of Asymptomatic Carotid Stenosis

3.2.a. Degree of carotid stenosis

Sonographically, carotid plaque is defined as a focal structure that encroaches into the arterial lumen of at least 0.5 mm or 50% of the surrounding intima-media thickness or demonstrated a thickness of $\geq 1,5$ mm (33).

Traditionally, luminal stenosis measurements determined by DSA, were used to select patients for the first two major treatment trials of symptomatic carotid stenosis, including the North American Symptomatic Carotid Endarterectomy Trial (NASCET) (34) and the European Carotid Surgery Trial (ECST) (35). Using NASCET criteria, degree of stenosis is calculated using the formula $1 - (R/D)/100$, where R represents the diameter of the residual lumen and D the luminal diameter at a visible, disease-free point, upstream the stenosis. Using ECST criteria, degree of stenosis is calculated with the formula $1 - (R/S)/100$, where S stands for the diameter of vessel at the most stenotic site and R represents the diameter of the residual lumen. Each method provides different measures of stenosis severity and can be corrected using the formula: $NASCET = (ECST - 40)/0,6$ (36).

CDU combined with color flow, has been shown to be accurately comparable with DSA and today it is the most widely used method for evaluating carotid stenosis (37). Doppler flow velocity measurements include ICA peak systolic velocity (PSV) and end diastolic velocity (EDV), the ICA/ Common carotid artery (CCA) PSV ratio and ratio of the ICA-PSV and the distal CCA-EDV (38). The Society of Radiologists in Ultrasound Consensus Conference recommends velocity criteria for grading the stenosis, that are widely accepted as seen in detail in *Table 1* (39). Hemodynamically significant stenosis is defined when the residual lumen is $<40\%$ to $<20\%$ of the initial diameter (40).

% Stenosis (NASCET)	PSV ICA (cm/s)	PSV ICA/PSV CCA	PSV ICA/ EDV CCA
< 50%	< 125	< 2	< 8
50-69%	≥ 125	2.0 - 4	8 - 10
70-79%	≥ 230	≥ 4	14 - 21
80-89%			22 - 29
>90% but not near occlusion	≥ 400	≥ 5	≥ 30
Near occlusion	High, low-string flow	Variable	Variable
Occlusion	No flow	-	-

Table 1. Diagnostic velocity criteria for NASCET- based carotid stenosis.

The relationship between stenosis severity and future ischemic events, was widely studied, with conflicting evidence. The largest prospective, non-randomized study of

1.121 patients with ACS undergoing medical intervention alone, Asymptomatic Carotid Stenosis and Risk of Stroke (ACSRS) study (41), determined the risk of ipsilateral ischemic neurological events in relation to the degree of ACS. More specifically, the average annual stroke rate was proportionally increased with the increase in the degree of stenosis from 0,8% for 50-69% stenosis, to 1,4% for 70-89% stenosis and 2,4% for 90-99% stenosis. Nevertheless, the other two large randomized studies for ACS, ACAS (8) and ACST (9) had not found any evidence that increasing stenosis severity is a predictive of increased risk of stroke in the medically treated asymptomatic patients.

Patients with bilateral carotid disease are at higher risk for stroke due to hypoperfusion, that can no longer be compensated by the collateral flow of the circle of Willis. The degree of contralateral ICA stands as an independent predictor of preoperative ipsilateral stroke, which allows an enhanced risk stratification of these patients beyond ipsilateral high-grade carotid stenosis (42).

3.2.b. Progression of the severity of stenosis

Most would agree that the progression of the severity of ACS in successive ultrasound examinations despite the implementation of BMT is not a good sign.

ACSRS study (41), demonstrated quite clearly that progression in the severity of ACS was a predictor of future stroke. More specifically, the 8-year cumulative ipsilateral ischemic stroke rate was 0% in patients with regression of stenosis, 9% if the stenosis was unchanged and 16% if there was progression of stenosis. In the subgroup with unchanged stenosis, the 8-year cumulative ipsilateral cerebral ischemic stroke rate for patients with baseline stenosis of 50%–69%, 70%–89% and 90%–99% was 4%, 8% and 13%, respectively. In contrast, in the presence of progression, the stroke rate was 8%, 15% and 25%, respectively. These results were also verified by other prospective studies some years later (43, 44).

Despite suggestions that stenosis progression was associated with doubling annual rate of stroke, in ACSRS study during the follow-up, approximately 70% of the occurring ischemic strokes affected patients with no evidence of stenosis progression. Thus, implications of a low accuracy of the method for identifying high-risk patients occur (45).

3.2.c. Identifying the “vulnerable/unstable” plaque: From Histology to Ultrasound

Over the last decade, there has been a paradigm shift on the imaging-based risk stratification of carotid disease from static measurements of the degree of carotid artery stenosis to characterization of the dynamic biological processes occurring within carotid plaques. Clinical experience highlights the necessity to move beyond luminal stenosis. Firstly, we have to mention cryptogenic stroke, in other words, stroke with no definite cause despite extensive workup, which occurs in up to 30% of patients with ischemic stroke. One-third of them, have nonstenotic (mild or minimal stenosis) carotid atherosclerotic plaques ipsilateral to the stroke, but on Magnetic Resonance Imaging (MRI), these plaques often demonstrate variable characteristics of

vulnerability (46). Thus, it is likely that a high proportion of cryptogenic strokes are due to rupture or erosion of these nonstenotic, high-risk plaques. Furthermore, very often patients with a high degree of stenosis have stable plaques that are thought to be at low risk of rupture (47).

In general, plaques that have been associated with increased risk of ischemic events are defined as unstable or vulnerable. Histologically, vulnerable plaques typically exhibit a thin, highly inflamed (mostly concentration of macrophages and monocytes), fibrous cap overlaying a large lipid pool, a large necrotic core, neovascularization, intraplaque hemorrhage, medial and adventitial changes with positive vessel wall remodeling and speckled or fragmented micro-calcification (48). *Figure 1* depicts schematically the histopathological features of vulnerable plaque.

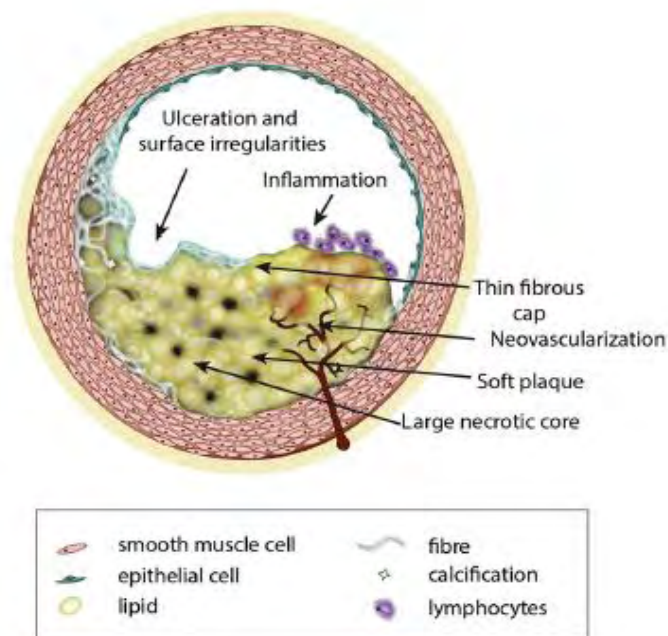


Figure 1. Histological features of vulnerable plaque

(Johri et al. Novel Ultrasound Methods to Investigate Carotid Artery Plaque Vulnerability. J Am Soc Echocardiogr. 2017;30(2):139-48)

3.2.d. Ultrasound markers of plaque vulnerability

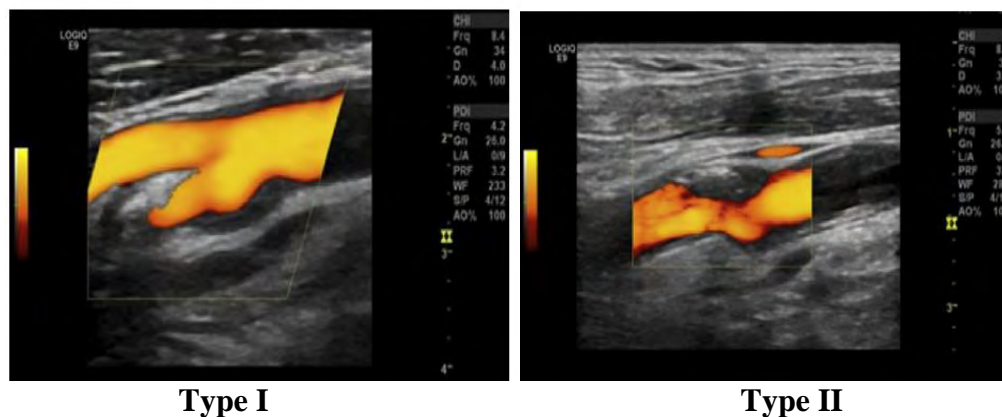
Sonographic characteristics of vulnerable carotid plaques constitute an important field of interest of many studies, for better cardiovascular risk stratification.

3.2.d. A. Plaque echogenicity and heterogeneity

Traditionally, morphology of carotid plaques, as assessed by ultrasound imaging, has been done by visual (qualitative) grading of the plaque's echo-reflection (echogenicity), and its echo pattern (heterogeneous, homogeneous). Echogenicity describes the overall distribution of gray tones (overall brightness). Plaques with areas consisting of lipid, necrotic core or blood can be determined as "echolucent" (anechoic, hypoechoic), corresponding to dark/ black plaques on sonograms. On the contrary, plaques composed of calcified or fibrous tissue can be designated as "echogenic" (hyperechoic), corresponding to bright/ white plaques (49).

Based on the spatial variation of gray tones of the plaque in the image, plaques are defined as either "homogeneous," correlated with a uniform echo pattern or "heterogeneous," correlated with a non-uniform echo pattern (plaques with mixed black and white areas) (49). More specifically, heterogeneity, is defined by >20% of the plaque area being occupied by two or more echogenicity grades that differ from the rest of the plaque (50).

Gray-Weale criteria, introduced in 1988, using visual assessment of echogenicity and heterogeneity, describe five types of plaques (51): type I is uniformly echolucent, type II is predominantly echolucent (>50% echolucent), type III is predominantly echogenic (<50% echolucent), type IV is uniformly echogenic, and type V represents a highly calcified plaque and is considered unclassifiable due to acoustic shadowing. Several studies have reported an association between visually assessed carotid plaque echolucency and cerebrovascular disease, independent of the degree of stenosis (52). *Figure 2* depicts sonograms of carotid plaques near to bifurcation, classified according to Gray-Weale criteria. However, visual evaluation of plaque echogenicity has some limitations because it is subjective and also plaque echogenicity could be affected by ultrasound machine settings during the patient's examination (53).



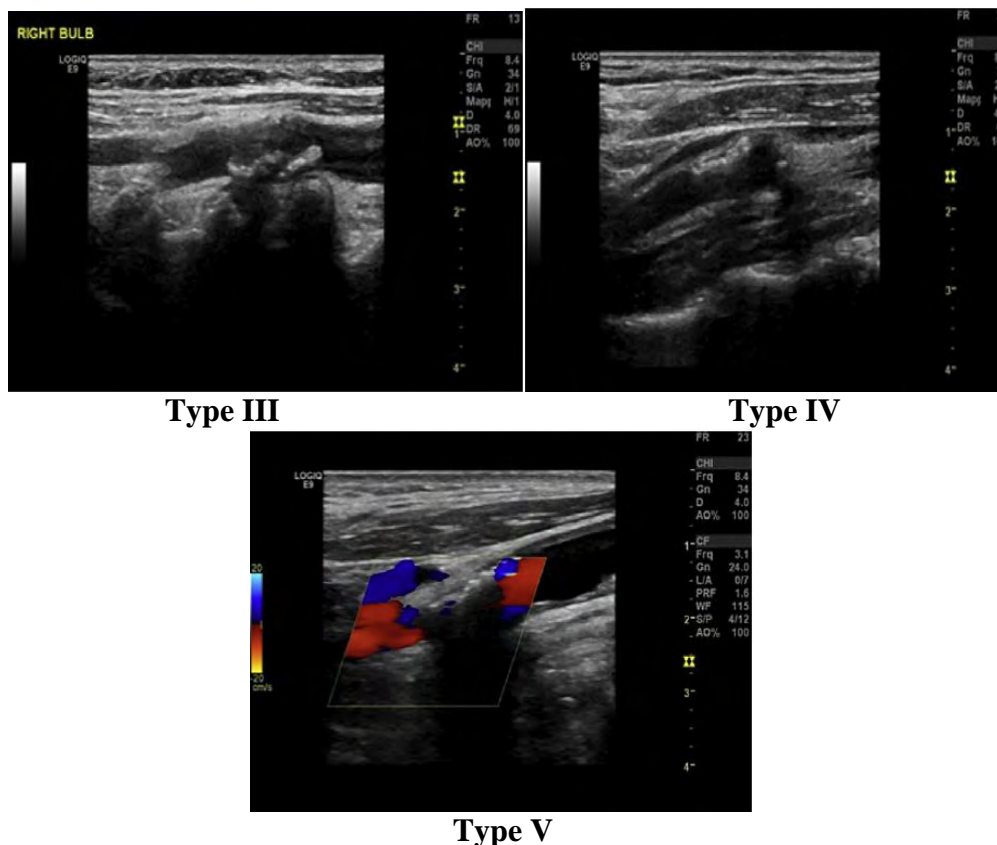


Figure 2. Gray-Weale classification of carotid plaque

(Casadei A. et al. Sonographic characteristics of carotid artery plaques: Implications for follow-up planning? Journal of Ultrasound.2012;15,151e157)

One decade later, ACSRS study, had undertaken a number of computerized and thus quantitative plaque analysis studies, that constituted Gray Scale Median (GSM), (Nicolaides et al, 2010) (54). More specifically, GSM is a measurement of the “gray” values of plaque pixels after image normalization. Image normalization involves an area of blood being scaled to zero, while the brightest area of the adventitia is normalized to a gray scale of 190. Following image normalization, the lower the GSM the more echolucent is the plaque, while echogenic plaques seem to have a higher GSM. In the ACSRS study, asymptomatic plaques with a GSM >30 had a very low annual rate of stroke (0,6%), compared to 1,6% in patients with a GSM between 15-30 and to 3,6% in patients with a GSM <15 (54).

In addition to GSM evaluation, in ACSRS study, plaques could be objectively classified into four groups according to the percentage of echolucent pixels in the normalized images of the carotid plaques, based on Gray-Weale criteria modified by Geroulakos. Plaque type I was defined with <15% of pixels with gray-scale values >25 in the plaque area; in plaque type II pixels with gray-scale values >25 occupied 15-50% of the plaque, in type III 50-85% of the plaque and finally, type IV occupied >85% of the plaque (55). Patients with type IV plaques had a 0,4% annual risk of stroke, compared with 0,8% in type III plaques, while in patients with type I and II plaques the annual risk of stroke was 3,0% (54). Plaque area and juxta-luminal black

area (JBA) also emerged from ACSRS study, and can be used in risk stratification models (54).

For the calculation of plaque area (mm^2), the imaging software had used the distance scale on the side of the image frame for calibration, and the plaque area was outlined by the operator. Plaque area $<40 \text{ mm}^2$ was correlated with a low annual rate of stroke (1,0%), which increased to 1,4% in patients with a plaque area of 40-80 mm^2 . In patients with a plaque area $>80 \text{ mm}^2$, was observed the highest annual rate of stroke (4,6%) (54).

JBA (mm^2), represent softer components of the plaque adjacent to the vessel lumen (histologically corresponding to necrotic core, lipid, hemorrhage, thrombus). JBA is defined as the area of the plaque components having grey-scale <25 , without a visible echogenic cap, after image normalization. In ACSRS study, JBA had a linear association with future stroke rate. More specifically, JBA $<4 \text{ mm}^2$ was accompanied by a 0,4% annual risk of stroke, which increased to 1,4% with a JBA between 4-8 mm^2 , up to 3,2% for a JBA of 8-10 mm^2 and 5,0% for a JBA $>10 \text{ mm}^2$ (54). Additionally, presence of noncalcified (absence of acoustic shadow) discrete white areas (DWAs) within black areas are thought to indicate plaque heterogeneity and to correspond to plaque neovascularization. The ACSRS study demonstrated that DWAs, defined as areas with pixels having grayscale values >124 , constituted a significant risk factor for cerebrovascular or retinal ischemic event (hazard ratio 2,32, p-value $<0,05$) (54).

From ACSRS study, derived an algorithm for predicting the possibility of annual rate of stroke on individual basis, based upon a multivariate analysis of their ultrasound-based parameters. For instance, a patient with asymptomatic stenosis of 80-99%, with no history of contralateral TIA, with plaque area $<40 \text{ mm}^2$ and GSM >30 is predicted to have an annual stroke rate of 0,5%. On the contrary, a patient with an 80-99% stenosis, with a prior history of contralateral TIA, with plaque area $>80 \text{ mm}^2$ and GSM <25 , would be predicted to have a 10% annual rate of stroke, and would be the ideal candidate for interventional therapy (CEA/CAS) (54).

Recently, in 2016, was published a systematic review and meta- analysis for the role of carotid plaque echogenicity in predicting the development of cardiovascular (CV) events in patients with asymptomatic and symptomatic carotid stenosis (56). Studies that were included, were cohort prospective studies, with ≥ 30 subjects, that explored the association of plaque echogenicity with symptoms (stroke, TIA, amaurosis fugax) and with US characterization of carotid artery plaque either visually based on Gray-Weale criteria or using computer-assisted analysis [Geroulakos classification or grey scale median (GSM) cut-off]. Finally, eight studies with 7.937 asymptomatic patients and three studies with 499 symptomatic patients were meta-analyzed. Four studies (57-60) analyzed visually plaque echogenicity and six studies had performed computer assisted analysis (61-66). Two studies defined plaque echolucency based on a cut-off value of GSM of <69 and <75 (61, 63) and eight studies defined plaque type (PT) I and II as echolucent and PT III and IV as echogenic. All studies performed longitudinal section images to measure plaque echogenicity. Patients clinical outcome was defined as stroke, TIA or amaurosis fugax in seven studies (57-60, 62-64) and as recurrent CV accidents in two studies (65,66). One study (61) separately evaluated the risk of stroke in asymptomatic patients and recurrent CV accidents in symptomatic patients. Meta-analysis confirmed that echolucent carotid plaques predicted future CV accidents in asymptomatic patients with a pooled relative risk (RR) of 2,72 and recurrent events in symptomatic patients with a pooled RR of 2,97. A clear association between echolucent plaques and future CV symptoms was shown for all degrees of stenosis, and indeed was higher among patients with severe stenosis. Also,

studies that included US data collected after 2000 showed higher RR (4,65) than those before year 2000 (RR 1,97), implying that more advanced US devices and computer-assisted analysis of plaque echogenicity seem to help for better recognition of plaque echolucency and as a consequence, accurate prediction of future ischemic events.

However, from a clinical perspective, even though carotid plaque echogenicity quantification is a technique with great potential in daily clinical practice for better patient stratification, still is not a real-life analysis; since it needs special software and expertise, as it is an operator-dependent technique with some potential technical difficulties in some patients.

Another limitation of GSM analysis is that if a plaque contains both soft (black) and hard (bright) components, by analyzing the median gray value of the plaque as a whole, it may overlook significant heterogeneity within the plaque and misrepresent vulnerability. As an alternative technique, is proposed texture analysis, which takes heterogeneity and spatial variations in pixel intensity into consideration, giving information about structure or composition of the plaque (67). Several different computer-assisted techniques have been developed for measuring plaque texture (50), however, there is no standard method. Probably, the combination of GSM and texture analysis is the key for the identification of echolucent sites of a plaque and localization of specific vulnerable regions in a plaque (68).

Finally, another computer-assisted technique for objective plaque echogenicity evaluation is integrated backscatter (IBS) analysis. It is based on measurement of radiofrequency signals and relies on the scattering of acoustic waves in all spatial directions when they encounter a structure. Scattered signals of different intensities are received by the transducer and translated into the 2D image (69). Higher IB values correspond to echogenic structures, whereas lower IB values correspond to echolucent structures (for example lipids) (70). As far the carotid arteries, it has been found that echolucent carotid plaques with low IBS values predict the presence of complex coronary plaques and the development of future coronary events, in patients with stable coronary disease, suggesting that IBS is clinically useful for the assessment of coronary plaque vulnerability (71). Also, IBS analysis evaluated the effects of statin therapy on carotid plaque morphology and found increased plaque IBS, suggesting that statin therapy stabilizes plaque morphology (72).

3.2.d.B. Surface Plaque Morphology

Plaque surface morphology, and especially carotid plaque ulceration is an important feature of vulnerability and a strong predictor of cerebrovascular events. Two large studies, in healthy populations, in Japanese population (1.358 subjects) (73) and the North Manhattan Stroke Study (1.939 subjects) (74) evaluated the association of carotid plaque surface irregularity and the risk of ischemic stroke and confirmed plaque surface irregularities as prognostic factor for stroke.

From a histologic point of view, ulceration describes an endothelial defect of at least 1.000 μm in width, resulting in the exposure of the plaque's necrotic core to blood circulation (75). In terms of imaging, carotid plaques are typically classified into smooth, irregular or ulcerated (76). Smooth surface is characterized by a regular luminal morphology, which indicates plaque's stability. Irregular morphology corresponds to plaques whose surface fluctuates from 0,3 mm to 0,9 mm, while ulceration corresponds to cavities measuring at least 1 mm (77) or 2 mm (78) according to different studies.

Ulcerations more often affect fatty plaques, less often fibrous and rarely calcified ones and are strongly associated with plaque hemorrhage (75). On the basis of ulceration location, studies demonstrated that the majority of cases affect the site proximal to maximum stenosis, as a consequence of the applied higher shear stress (75). The degree of stenosis is associated with ulceration frequency; thus, the incidence is higher in greater stenosis (75). Nevertheless, ulceration is also significant even in plaques with mild stenosis <50% (79).

US is the first line modality for evaluation of carotid plaque ulceration, but with some limitations. The case seems easy as far as large plaque ulcerations is concerned, which are easily identified as obvious craters within the plaque with reversed or stagnated flow. During recent years, the image quality and resolution power of ultrasound machines have been markedly improved by the introduction of higher-frequency transducers and digital technologies, providing improved diagnostic accuracy (80).

Several different criteria have been used to define sonographic features of ulceration. The most widely used criteria were proposed in 1997 by de Bray et al; according to them plaque ulcer requires the size of the concavity to be at least 2 mm in depth and 2 mm in length, with a well-demarcated posterior wall at its base on B-mode sonography and an area of reversed flow within the recess on Doppler color-flow imaging (81). De Bray criteria lack in sensitivity (35,7%), though demonstrate a specificity of 75%. In 2012, newer criteria proposed that simplified the sonographic ulceration diagnosis, increasing both sensitivity and specificity (85,7% and 81,3% respectively) (81). According to them, any concavity with an echogenic line at the plaque base compared to the nearby endothelium corresponds to a plaque ulcer, irrespective of size or the findings obtained from Doppler color-flow imaging. A limitation that still exists even with the new criteria, is the acoustic shadow caused by calcified plaques. Modern studies proposed indirect findings of ulceration, such as a fine trembling motion of echogenic structures inside the plaque (sensitivity 93%, specificity 60%) (82).

Contrast enhanced US (CEUS) and 3D reconstructions were found to detect more ulcers than conventional US and may play a prominent additive role in risk stratifying patients (83, 84). On CEUS the ulcer definition requires the interruption of the plaque-lumen border for at least $1 \times 1 \text{ mm}$ (77), and for 3D US, the volume criterion of a cavity measuring at least 1 mm^3 has been used (85).

3.2.d.C. Neovascularization and intraplaque hemorrhage

Normally, adventitia of the middle and large size arteries presents with a vasculature network (vasa vasorum) that supplies the outermost part of the artery with oxygen and nutrients, while intima is supplied with oxygen from the lumen through diffusion. In atherosclerosis, oxygen cannot achieve the deeper parts of the plaque, resulting in an imbalance between oxygen supply and oxygen demand. Hypoxia, as a consequence, activates transcription factors and microvessels proliferate from the adventitia towards the intima, known as neovessels (86). These microvessels are formed by simple endothelial cells, with no surrounding connective tissue, basilar membrane, or receptors for blood flow or blood pressure. Their wall is thus immature, very fragile and leaky and therefore they are prone to rupture and intraplaque hemorrhage. Additionally, due to wider gap junctions they serve as a port of entry for other inflammatory cells, lipids, which contribute to plaque growth. In particular, macrophages produce MMPs, like MMP-9 and other collagenases that destroy the connective fibrous tissue, thus stimulating the neovessels growth (87).

This hyperplastic network of vasa vasorum characterize vulnerable patients suffering from symptomatic atherosclerosis, with diabetes, and affects different arterial beds, supporting the concept of symptomatic atherosclerosis as a panarterial disease. Ectopic microvessels in the intima and media are associated with advanced atherosclerotic lesions (88).

CEUS represents one of the major breakthroughs in the field of diagnostic ultrasound. In the setting of carotid stenosis, CEUS can be used to distinguish between total carotid occlusion and high-grade stenosis (89), identify plaque ulceration (83), and evaluate carotid plaque neovascularization (90).

The method involves the injection of highly echogenic gas-filled microbubbles (diameter of 1-10 μ m) with a phospholipid or albumin coating, containing a low solubility perfluorinated gas, into the circulation. Owing to their small size, these bubbles do not diffuse into surrounding tissues like other contrast agents; as a consequence, all signals from CEUS examinations are intravascular, which allows for accurate assessment of vessel lumen and neovascularity within the carotid plaque (91). These microbubbles stay in the vascular system for few minutes and then are eliminated by the respiratory tract. Among advantages of ultrasound contrasts, are not nephrotoxic, do not require radiation, and complications such as severe anaphylactic reactions are rare (<1 in 100,000). The more common side effects are minor and include headache, injection site pain, burning, or paresthesias. Contraindications include patients with known right-to-left shunts, severe pulmonary hypertension, uncontrolled systemic hypertension, acute heart failure, endocarditis, unstable angina and patients with adult respiratory distress syndrome (92).

CEUS has the ability to directly visualize the spatial and temporal heterogeneity of intraplaque and adventitial neovascularization within the vessel wall. The adventitial vasa vasorum appears as echogenic bubbles within the adventitial or periadventitial layer, while intraplaque neovascularization may be identified by the presence of microbubbles within the plaque moving from the adventitial side or plaque shoulder toward the plaque core (91).

Importantly, intraplaque enhancement in contrast-enhanced ultrasound studies correlates well with histologically verified intraplaque neovascularization and hemorrhage, as well as macrophage infiltration (93, 94).

Plaque enhancement has been studied both in asymptomatic and symptomatic patients, and demonstrated that the enhancement is markedly increased in patients with symptomatic plaque and is associated with a higher rate of cardiovascular events

in general (91), (95, 96). After the administration of ultrasound contrast, some carotid plaques remain enhanced for a long period of time; even up to 30 minutes post-injection. This observation has been described as “late phase enhancement” and can be identified objectively by imaging the plaque six minutes after contrast administration, and has been attributed to macrophages that phagocytose the contrast agent (97). Studies that supported this observation found that plaques with higher late-phase CEUS signals have significantly higher levels of chemical markers of angiogenesis and inflammation, predisposing the plaque to early rupture (98, 99). Additionally, many studies concluded that enhancement on CEUS and thus histological neovascularization is higher within hypoechoic or mixed type plaques, compared to calcified ones, a parameter that concur with the overall greater vulnerability of hypoechoic carotid plaques (93, 95), (100).

Both visual grading (qualitative) and semi-quantitative techniques have been used for the analysis of the images obtained with CEUS. Intraplaque neovascularization can be classified by visual interpretation into three grades: mild when moving microbubbles are seen only at the outer part of the plaque near the adventitia; moderate when microbubbles are present at the plaque shoulder and inside the plaque but not at the plaque’s apex, and severe when microbubbles are present throughout the plaque including its apex (83). This visual technique allows a direct interpretation of the CEUS examination as is easy applicable, however, it is highly operator dependent and prone to high interobserver variability (101). Schematically, *Figure 3* (A-D), depicts unenhanced B-mode imaging of a hypoechoic plaque at the origin of the ICA (A), followed of corresponding consecutive frames on contrast enhanced ultrasound imaging with visible microbubbles within the plaque (arrows) (B-D).

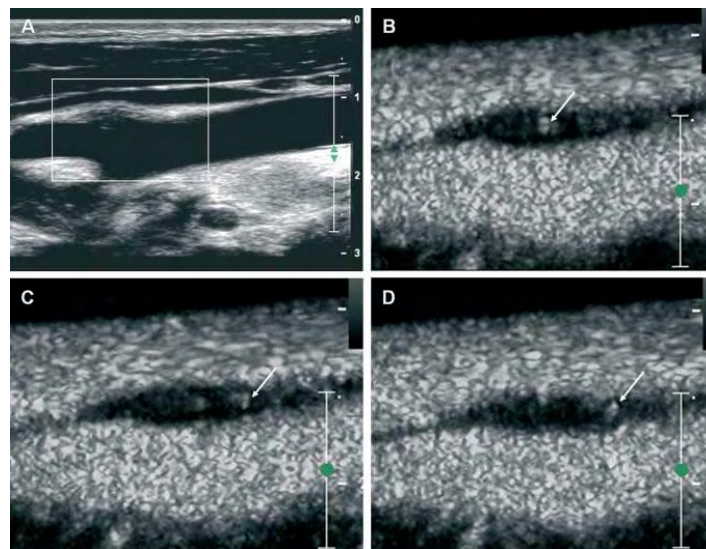


Figure 3. Carotid artery with plaque neovascularization on contrast-enhanced ultrasound imaging.

(Eyding et al. Current strategies and possible perspectives of ultrasonic risk stratification of ischemic stroke in internal carotid artery disease. *Ultraschall Med.* 2011 Jun;32(3):267-73.)

Alternatively, semi-automated methods developed, using specialized software. They analyze the variation of enhancement intensity over time in a region of interest, and therefore CEUS is often described on the basis of location of microbubbles and the extent of enhancement. Although variations of this grading system exist, a typical quantitative classification is of Huang et al. in four grades (*Figure 4, A-D corresponds to Grade I-IV respectively*). Grade I indicates no plaque enhancement, grade II indicates enhancement only of the arterial wall vasa vasorum, grade III indicates enhancement of intraplaque neovascularization at the adventitial side or shoulder of the plaque, and grade IV indicates extensive enhancement within the plaque core arterial wall vasa vasorum and also plaque shoulder enhancement (102). The rate of ischemic stroke in patients with grade IV or III plaques was significantly higher than that in patients with grade I or II plaques (102).

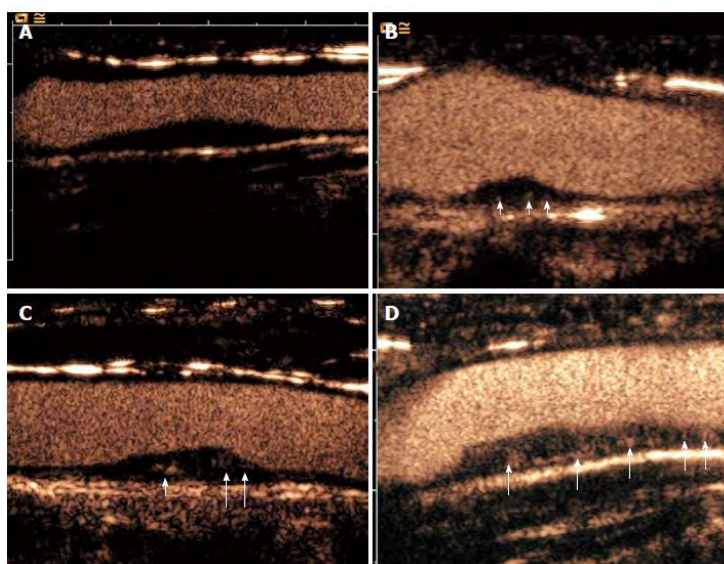


Figure 4. Grades of plaque enhancement.

Recently, in 2016, was published a meta- analysis and a systematic review of studies evaluating the accuracy of quantitative and qualitative analysis of CEUS intensity for the diagnosis of intraplaque neovascularization compared to histologic findings and/or symptomatic carotid disease (103). Finally, 20 studies met the inclusion criteria for systematic review and seven for meta- analysis. 461 patients were included, 188 of whom were symptomatic. Reference tests were histology in three studies (104-106) and clinical diagnosis of symptomatic plaque in four studies (95), (102), (107), (108). Four studies (95), (102), (105), (107), reported quantitative evaluations of signal intensity after echocardiographic contrast agent infusion, using different quantification methods of signal enhancement. As a final outcome, both qualitative and quantitative methods had good diagnostic accuracy, but qualitative assessment had a higher diagnostic odds ratio than quantitative methods (15,54 versus 7,06

respectively). Undoubtedly, this meta-analysis presented some important limitations. Firstly, a priori, included studies were heterogeneous and a random-effects model was used for all pooled analysis. Also, the reference test was either histology or symptoms, revealing a sample selection bias. Firstly, sample selection bias was due to the difference between the histologic sample plane and the ultrasound image plane, and the difference between microvessel anatomy and perfusion that may reduce the correlation between tissue specimens and CEUS. Secondly, histologic sample as reference test, implies generally high-grade stenotic plaques that are subject to carotid endarterectomy. However, vascularization is also detectable in plaques without significant stenosis. Among other general limitations of CEUS, we have to mention that CEUS is prone to an artifact known as pseudoenhancement, in which ultrasound waves are propagated through the contrast agent leading to increased signal in the vessel wall furthest from the probe, thus leading to overinterpretation of vessel wall enhancement, leading to false positive results (109). Furthermore, quantitative analysis can be difficult to achieve because the contrast material does not enter the vasa vasorum as a bolus but rather as individual bubbles that pass through the intraplaque microvessels a few at a time; as a result, the analysis of multiple frames can be difficult because of movement of the plaque with arterial pulsation. Efforts have been done to overcome these difficulties with new software, but it has not been validated with histology (110).

From the data collected in literature definitely, CEUS is a promising noninvasive diagnostic modality for detecting intraplaque neovascularization and further define the cerebrovascular risk stratification. Therefore, it becomes mandatory to create a generally shared operative protocol for the interpretation of CEUS results.

3.2.d.D. Microembolic signals in Transcranial Doppler Ultrasound

Detection of microembolic signals (MES) on transcranial Doppler (TCD), constitutes another important factor characterizing plaque instability in asymptomatic patients, since it is argued that microemboli detection is perhaps the best-validated factor for the identification of plaque vulnerability (111).

TCD is a portable and noninvasive technique used for the imaging of intracranial vasculature. The principle role of TCD in carotid imaging is the detection of circulating emboli on monitoring of the ipsilateral middle cerebral artery (MCA), demonstrating high sensitivity and specificity in vivo (112) and in vitro (113). Criteria for the definition of MES, are established since 1995 and according to them, these emboli appear as transient signals with short duration (of less than 300 milliseconds), with high-intensive amplitude (of at least 3 decibels greater than the spectral background), unidirectional and accompanied by a characteristic chirping sound (114). *Figure 5* demonstrates microembolic signal on TCD (the arrow points to an image of a microembolus).

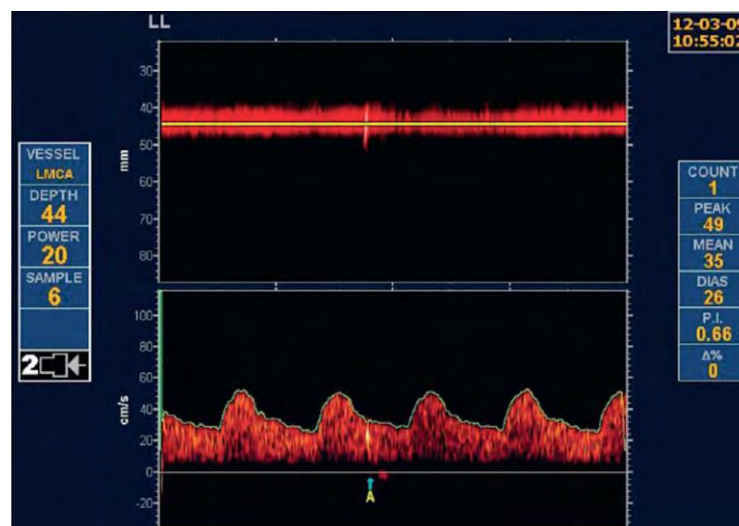


Figure 5. Microembolic signal on transcranial Doppler

(Spence JD. Transcranial Doppler monitoring for microemboli: a marker of a high-risk carotid plaque. *Seminars in vascular surgery*. 2017;30(1):62-6.)

A number of studies have suggested that MES are a strong marker of unstable plaque. In 2005, Spence et al, observed that patients with asymptomatic stenosis with two or more microemboli during an hour of TCD monitoring, had a 15% 1-year risk of stroke, compared with only a 1% risk among patients without microemboli (115). The same year, Markus et al. studied symptomatic patients with >50% carotid stenosis with TCD. The absence of MES identified a group at low risk for stroke, implying better therapeutic decisions for the future (116). In 2008, a systematic review was

conducted by Ritter et al. and found approximately that 40% of patients with symptomatic carotid stenosis had MES on TCD compared with just 10% of asymptomatic patients. Furthermore, presence of just 1 MES was associated with higher odds of future symptomatic event (7,5 times in symptomatic patients and 13,4 times in asymptomatic respectively) (117).

Subsequently, in 2010, was published the first prospective observational multicenter Asymptomatic Carotid Emboli Study (ACES), in 482 asymptomatic patients with a stenosis at least of 70% (118). Its aim was the predictive value of the detection of embolic signals in a 2 year follow up. More specifically, patients with MES on TCD demonstrated an absolute annual risk of ipsilateral stroke of 3,6%, in contrast with the 0,7% of patients without MES.

In 2016, a meta- analysis about the role of TCD in detection of MES, as a predictor of cerebral events in patients with symptomatic and asymptomatic carotid disease was published (119). 28 studies were included, of whom 22 papers reported data on stroke and TIA as an outcome, 19 on stroke alone, and 8 on stroke and TIA with increased positivity threshold, 3 studies used two microemboli as positivity rather than one and 1 study had TCD recordings for 30 min rather than an hour. As far the stroke outcome alone, the sensitivity and specificity were 73,14 and 70,27 respectively. At the median pre-test probability of stroke of 3,0%, the post-test probabilities of a positive and negative TCD were 7,1% and 1,2% respectively. The same analysis was performed separately for pre-operative patients and peri and post-operative patients. For the pre-operative group, the sensitivity and specificity were 71,27 and 83,72 respectively. At the median pre-test probability of 3,0%, the post-test probabilities of a positive and negative test were 12% and 1,1% respectively. For the peri- and post-operative group the sensitivity and specificity were 78,86 and 47,43 respectively. At the median pretest probability of 3,0%, the post-test probabilities of a positive and negative test were 4,5% and 1,4% respectively. The differences between subgroups were found to be significant. Consequently, TCD has a different ability to predict stroke or TIA in pre-operative patients and peri- or post-operative patients. MES counts tend to be very high during surgery and in the immediate post-operative period because of the physical stress exerted on the plaque, as well as iatrogenic sources of emboli. In this period the baseline number of microemboli that indicates high risk of plaque rupture should be raised. From this meta-analysis, certain limitations were raised, as the general quality of the evidence was low and there were methodological issues with patient selection because of the observational nature of the data, making mandatory the design of large-scale randomized control trials in order to incorporate TCD into assessment of patients with carotid disease.

TCD is especially helpful when combined with other imaging modalities. In ACES study, studied in 435 patients the predictive value of a score based on echolucent plaque morphology on carotid ultrasound and MES in the ipsilateral MCA on TCD. It was demonstrated that the combination of plaque echolucency on B-mode US and MES on TCD was associated with a 10 times higher risk of stroke in patients with asymptomatic carotid stenosis. These patients had an annual stroke risk of 8% compared with < 1% in the low-risk cohort (64). Additionally, MES detection on TCD could be combined with CEUS as surrogate markers of future vascular events (120).

Finally, the fact that MES constitute an important marker of plaque instability is further supported by the observation that MES rapidly diminish following CEA (121) and intensive medical treatment, implying that their reduction increases plaque stability (122, 123).

3.2.e. Evaluation of cerebrovascular reserve capacity

Hemodynamic factors may play an important role in the pathogenesis of ischemic stroke for patients with extracranial or intracranial artery stenosis. Assessment of cerebrovascular reserve with TCD may further allow identification of patients with carotid artery stenosis who are at higher risk for stroke, and thus, are better candidates for revascularization procedures (124).

A hemodynamically severe stenosis of extracranial or intracranial artery can result to a decrease in cerebral perfusion pressure (CPP), in other words in cerebral hypoperfusion. Brain, in order to compensate and preserve normal regional cerebral blood flow (rCBF), activates collateral circulation (125) and autoregulatory vascular mechanisms (124). In particular, cerebral autoregulation or cerebrovascular reserve (CVR) refers to the capacity of the brain to increase cerebral blood volume (CBV) and to maintain a constant rCBF, when CPP is decreased. CVR is mediated through complex mechanisms (myogenic, chemical, neuronal and metabolic). As far as the chemical mechanisms are concerned, hypoxia and hypercapnia cause cerebral vasodilation. More specifically, on the basis of a CPP reduction, local hemodynamic changes include firstly (Stage 1) autoregulatory vasodilation of arterioles to maintain a constant rCBF, with increase in CBV and mean transit time, while oxygen extraction fraction (OEF) remains normal. Subsequently, Stage 2 is characterized by hemodynamic failure, as maximally dilated arterioles are unable to maintain normal rCBF and OEF increases. Finally, ischemia results when cerebral metabolic rate of oxygen decreases (126).

TCD constitutes the easiest, fastest, harmless and less expensive study in order to assess CVR capacity. The method is based to the fact that changes in CO₂ concentration induce a vasomotor response that changes rCBF in parallel with velocity changes. In particular, TCD measurements of vasomotor reactivity (VMR), involve mean flow velocities (MFVs) from both M1-MCA segment, both before and after a vasodilatory stimulus (127). Various vasodilatory stimuli have been reported in the literature; they include increasing levels of CO₂, such as with breath-holding or inhalation of gas mixtures (128) and pharmacological challenge with acetazolamide (129). Voluntary breath-holding maybe represents the simplest method of VMR assessment (130). Baseline MFVs are obtained during inhalation of room air, followed by a 30 seconds of apnea, followed by a 4 seconds recording of the highest MFV. Breath holding index (BHI) is defined as the ratio of the percent MFV increase during hypercapnia over the time of breath-holding (in seconds). In other words, BHI is given by the formula [MFV at the end of breath-holding minus MFV at rest divided by MFV at rest] multiplied by 100, divided by seconds of breath- holding (131). Normally, BHI is usually greater than 1 (124), while values less than 0,69 are generally considered abnormal (131). Additionally, significant asymmetry of BHI between the two MCAs should always been taken into consideration. In *Figure 6* is demonstrated the normal vasomotor reactivity during voluntary breath holding of 30 seconds.

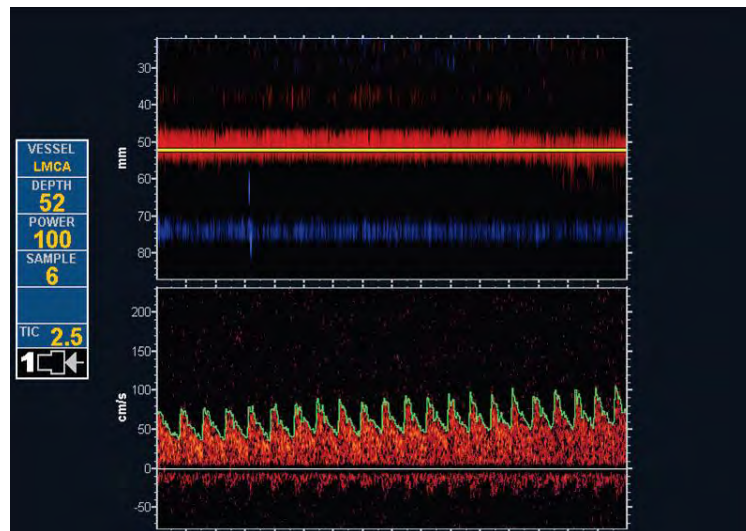


Figure 6

TCD of the left MCA shows gradual increase of MFV (from a baseline of 46 cm/sec to 61 cm/sec) (Tsivgoulis G, Alexandrov AV. Cerebral hemodynamics in acute stroke: pathophysiology and clinical implications. Journal of vascular and interventional neurology. 2008;1(3):65-9.)

Impaired cerebrovascular reactivity is proposed as an independent predictor of stroke and TIA.

Indicatively, in 2000 in a prospective cohort of 94 patients with asymptomatic carotid stenosis $\geq 70\%$ who underwent TCD CVR testing with a medium follow-up of approximately 2 years, the annual risk of ischemic events was 4% in those with normal CVR, contrary to 14% in patients with an abnormal CVR (BHI < 0.69) (131).

In 2012, a systematic review and a meta-analysis were published about the association of CVR impairment and risk of stroke (132). More specifically, 13 studies were included with a total of 991 patients with a carotid stenosis of at least 70%, in whom CVR testing was performed via TCD [8 studies], (131), (133), (134), (135), (136), (137), (138), (139) or nuclear medicine flow studies [5 studies], (140), (141), (142), (143), (144). Among the studies, 5 included only asymptomatic patients (131, 133-136), 4 studies only symptomatic (140-143) and other 4 studies both asymptomatic and symptomatic patients (137-139, 144). Additionally, different vasodilatory stimuli were administrated. With a mean follow-up of 3 years after baseline CVR testing, the presence of impaired cerebrovascular reserve was associated with an approximately 4-fold increased risk of future stroke compared with patients with similar stenosis severity or occlusion but with a normal cerebrovascular reserve. A similar risk of future stroke was demonstrated in the studies with asymptomatic carotid stenosis alone, suggesting a significant positive relationship between impairment of CVR and development of stroke for both symptomatic and asymptomatic carotid disease. This meta-analysis was limited by combining different methodologies of estimating CBF (TCD vs nuclear medicine methods), different vasodilatory stimuli (CO_2 vs acetazolamide), by heterogeneous patient risk profiles (symptomatic vs asymptomatic

disease), cutoff values, and the definition of study end-points (stroke or TIA). Also, in the majority of the studies the investigators were not blinded to the CVR results.

Two years later, in 2014 a meta-analysis was published, based on individual patient data focused exclusively on TCD CVR testing with a vasodilatory CO₂ challenge (145). The meta-analysis included individual data from 9 studies (131, 133, 136, 138, 139), (146-149) from 754 asymptomatic or symptomatic patients with carotid stenosis of at least 70% with a follow-up of approximately 2 years. Impaired CVR was found to be independently associated with increased risk of ipsilateral stroke in carotid disease (hazard ratio 3,69). Also, in the specific subgroup of asymptomatic patients, was found a highly predictive effect of impaired CO₂ reactivity (hazard ratio 2,90). Undoubtedly, this study had some limitations, including no information on changes in the degree of stenosis and treatment details during the follow-up period (medication, carotid revascularization) and some specific details about ischemic event (severity, border zone or embolic pattern in neuroimaging).

It has been suggested that combining CVR testing with the detection of MES with TCD, may further improve risk stratification. The combination reflects the two distinct pathophysiologic mechanisms of stroke in carotid artery disease: embolization due to unstable plaque and poor hemodynamic compensation leading to impaired washout of emboli or cerebral hypoperfusion (150). In a preplanned substudy of ACES, 106 patients, were recruited with $\geq 70\%$ asymptomatic carotid stenosis and underwent TCD testing of CVR with a vasodilatory challenge of CO₂ or acetazolamide. A relation between lower CVR and increased number of MES was found, but the low recurrent event rate meant that it was underpowered (133).

Recently, in 2016 a study was published with 60 patients, with symptomatic (38 individuals) or asymptomatic (22 individuals) atherosclerotic ICA stenosis $\geq 50\%$ (151). The vasoreactivity parameters that were calculated were vasomotor reactivity reserve (VMRr) and BHI. VMRr test was calculated as the percentage change of mean velocity in the MCA measured during hypocapnia obtained through hyperventilation (duration of 2 minutes) in relation to hypercapnia caused by breath holding (for 30 seconds). These parameters were found significantly lower in the group of patients with stenosis $\geq 70\%$ and in patients with ulcerations on the plaque surface. Also, patients who showed MES had significantly lower values of VMRr and mean velocity in the MCA than the patients without MES. The BHI was also lower, in the MES subgroup, but the difference did not reach statistical significance, probably because of the small number of the studied group and the methodological differences (VMRr test is based on the measurement of mean velocity during hypo- and hypercapnia while in the BHI only hypercapnic phase is included).

A suggestion for the future could be the design of a prospective study of CVR measurement in patients with asymptomatic carotid disease receiving current optimal medical therapy, that would evaluate whether treatment strategies in ACS based on CVR testing could improve patient outcomes.

3.3. Treatment Strategies For Asymptomatic Carotid Disease: Ongoing Trials

For decades ago, three landmark randomized controlled trials, the Veterans Affairs trial (VACS) (152), the ACAS (8) and the ACST-1 (9), demonstrated that CEA offered a 50% reduction in the 5-year stroke risk compared with BMT alone. As a result, CEA was considered as the treatment-of-choice for patients with ACS for a lot of years. However, in the early and mid-2000s, this began to change, because the concept of BMT was far from the current guidelines of aggressive medical therapy (antiplatelets, antilipidemic agents, hypertension and diabetes mellitus control) combined with lifestyle modifications (Mediterranean diet, smoking cessation, exercise). Due to improvements in BMT, the annual risk of ipsilateral stroke with intensive medical therapy is now 1% (11). This fact has led to the need for contemporary RCTs that would clarify treatment strategies in asymptomatic patients by including an additional limb for BMT.

Firstly, in 2009, the Stent-protected Angioplasty in Asymptomatic Carotid Artery Stenosis versus Endarterectomy trial (SPACE-2) was planned as a three-armed RCT (BMT alone versus CEA plus BMT versus CAS plus BMT) (153). The goal was to randomize more than 3.000 patients over a 5-year period. Because of slow patient recruitment, the three-arm study design was amended in July 2013 to become two parallel randomized studies (BMT alone versus CEA plus BMT and BMT alone versus CAS plus BMT). However, again because of slow recruitment, the trial was ceased after randomization of 513 patients over a 5-year period.

The second Carotid Revascularization versus Stenting Trial (CREST-2) (154) and the second Asymptomatic Carotid Surgery Trial (ACST-2) (155) are currently running to provide level A of evidence in regards of treatment strategy for asymptomatic carotid artery disease.

More specifically, CREST-2 will clarify whether revascularization interventions provide long-term benefit to patients treated by current best-available medical therapy and consists of two parallel RCTs. The first trial will compare BMT to CEA plus BMT and the parallel trial will compare BMT to CAS plus BMT. An estimated 2.480 participants will be enrolled in CREST-2 at approximately 120 sites in the United States and in several Canadian sites. A total of 574 patients have been recruited as of April 13, 2017, and recruitment and follow-up are ongoing.

ACST-2 is a large international RCT comparing CEA versus CAS in patients with asymptomatic carotid stenosis. ACST-2 is currently recruiting patients from over 112 centers in over 20 countries worldwide. The trial is on track to recruit 3.600 patients by 2019.

Finally, the French randomized trial Asymptomatic Severe Atherosclerotic Carotid Artery Stenosis at Higher than average Risk of Ipsilateral Stroke (ACTRIS) has not yet started, but will compare BMT and CEA/CAS in asymptomatic patients who exhibit one or more features suggestive of them being at higher risk of suffering a late ipsilateral stroke (156).

Conclusion

Traditionally, risk stratification and patient management with carotid disease was based on the presence or absence of symptoms and the degree of stenosis. For asymptomatic carotid disease, the therapeutic options include the optimal medical treatment either alone or combined with revascularization procedures (CEA or CAS). However, it is estimated that the benefit of CEA in asymptomatic patients remains small, as the number needed to treat based on existing guidelines is rather high. Moreover, medical therapy has advanced and nowadays the optimal medical treatment is superior to that tested in the 3 landmark RCTs in the decades of 1980s and 1990s, fact that has questioned further the invasive intervention. Consequently, in order to select the appropriate therapeutic strategy and finally improve stroke prevention, the search for a better risk stratification of patients with asymptomatic carotid artery stenosis is obligatory.

To that direction, there is growing evidence over the last years that moving beyond the degree of luminal stenosis is mandatory, as it is commonly observed that many patients with high-grade carotid stenosis remain asymptomatic for many years, while others with moderate stenosis develop neurologic symptoms sooner. Additionally, the inadequacy of degree of stenosis is illustrated by the entity of cryptogenic stroke, in which one-third of the patients have carotid atherosclerotic plaques ipsilateral to the stroke that cause only mild or minimal stenosis. Therefore, the concept of plaque vulnerability has emerged, and the sonographic characteristics of vulnerable carotid plaque constitute an important marker that may help for better risk stratification in asymptomatic patients, identifying a subgroup of “high risk” patients that will benefit from revascularization procedures.

While awaiting data from CREST-2, ACST-2, and ACTRIS aiming to shed light into the optimal management of asymptomatic carotid stenosis, the new guidelines by the European Society for Vascular Surgery in 2017 recommend that in average surgical risk patients with a 60%–99% ACS, CEA (Class IIa; Level of Evidence: B) or CAS (Class IIb; Level of Evidence: B), should be considered for intervention in the presence of one or more imaging characteristics that may be associated with an increased risk of late ipsilateral stroke, provided that documented perioperative stroke/ death rates are <3% and the patient’s life expectancy exceeds 5 years. More specifically, the incorporation of various neurosonological diagnostic tools in clinical practice based on current literature, seem to provide valuable information on risk stratification of asymptomatic carotid disease. Neurosonology examinations (CDU and TCD) represent the extension of the clinical examination, as are safe, may be easily repeated as needed, are inexpensive and demonstrate excellent sensitivity and specificity.

Several neurosonological methods have been proposed as reliable predictors for the identification of ACS individuals at high risk of stroke. Firstly, stenosis progression and also bilateral carotid disease with the degree of contralateral carotid artery to represent an independent risk factor. Additionally, evidence of plaque vulnerability represents strong predictor of ischemic stroke. In particular, plaque echolucency (predominantly echolucent plaque), large plaque area, large juxta-luminal black area on computerized plaque analysis, intraplaque neovascularization and plaque ulceration by using contrast-enhanced ultrasound studies and detection of microembolic signals on TCD constitute markers of vulnerable plaque. Moreover,

impaired cerebrovascular reserve and collateralization with TCD, represent an additional independent risk factor of stroke and TIA.

Further research on the above (and other possible) imaging markers of carotid plaque burden is essential in order to develop validated algorithms for appropriately selecting a subgroup of patients of “high risk” for stroke, who could benefit from revascularization procedures.

References

1. **Herrington W, Lacey B, Sherliker P, Armitage J, Lewington S.** Epidemiology of Atherosclerosis and the Potential to Reduce the Global Burden of Atherothrombotic Disease. *Circulation research*. 2016;118(4):535-46.
2. **Lozano R, Naghavi M, Foreman K, Lim S, Shibuya K, Aboyans V, et al.** Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010. *Lancet*. 2012;380(9859):2095-128.
3. **Feigin VL, Forouzanfar MH, Krishnamurthi R, Mensah GA, Connor M, Bennett DA, et al.** Global and regional burden of stroke during 1990-2010: findings from the Global Burden of Disease Study 2010. *Lancet*. 2014;383(9913):245-54.
4. **Donnan GA, Fisher M, Macleod M, Davis SM.** Stroke. *Lancet*. 2008;371(9624):1612-23.
5. **Malhotra K, Goyal N, Tsivgoulis G.** Internal Carotid Artery Occlusion: Pathophysiology, Diagnosis, and Management. *Current atherosclerosis reports*. 2017;19(10):41.
6. **Naylor AR, Ricco JB, de Borst GJ, Debus S, de Haro J, Halliday A, et al.** Editor's Choice - Management of Atherosclerotic Carotid and Vertebral Artery Disease: 2017 Clinical Practice Guidelines of the European Society for Vascular Surgery (ESVS). *European journal of vascular and endovascular surgery : the official journal of the European Society for Vascular Surgery*. 2018;55(1):3-81.
7. **Berger JS, Hochman J, Lobach I, Adelman MA, Riles TS, Rockman CB.** Modifiable risk factor burden and the prevalence of peripheral artery disease in different vascular territories. *Journal of vascular surgery*. 2013;58(3):673-81.e1.
8. **Endarterectomy for asymptomatic carotid artery stenosis.** Executive Committee for the Asymptomatic Carotid Atherosclerosis Study. *Jama*. 1995;273(18):1421-8.
9. **Halliday A, Mansfield A, Marro J, Peto C, Peto R, Potter J, et al.** Prevention of disabling and fatal strokes by successful carotid endarterectomy in patients without recent neurological symptoms: randomised controlled trial. *Lancet*. 2004;363(9420):1491-502.
10. **Barnett HJ, Meldrum HE, Eliasziw M.** The appropriate use of carotid endarterectomy. *CMAJ : Canadian Medical Association journal*. 2002;166(9):1169-79.
11. **Naylor AR.** Time to rethink management strategies in asymptomatic carotid artery disease. *Nature reviews Cardiology*. 2011;9(2):116-24.
12. **Paraskevas KI, Mikhailidis DP, Veith FJ, Spence JD.** Definition of Best Medical Treatment in Asymptomatic and Symptomatic Carotid Artery Stenosis. *Angiology*. 2016;67(5):411-9.
13. **Koelemay MJ, Nederkoorn PJ, Reitsma JB, Majoie CB.** Systematic review of computed tomographic angiography for assessment of carotid artery disease. *Stroke*. 2004;35(10):2306-12.
14. **Townsend TC, Saloner D, Pan XM, Rapp JH.** Contrast material-enhanced MRA overestimates severity of carotid stenosis, compared with 3D time-of-flight MRA. *Journal of vascular surgery*. 2003;38(1):36-40.
15. **Brinjikji W, Huston J, 3rd, Rabinstein AA, Kim GM, Lerman A, Lanzino G.** Contemporary carotid imaging: from degree of stenosis to plaque vulnerability. *Journal of neurosurgery*. 2016;124(1):27-42.

16. **Hennekens CH, Gaziano JM.** Antioxidants and heart disease: epidemiology and clinical evidence. *Clinical cardiology*. 1993;16(4 Suppl 1):I10-3; discussion I3-5.
17. **Baradaran A.** Lipoprotein(a), type 2 diabetes and nephropathy; the mystery continues. *Journal of nephropathology*. 2012;1(3):126-9.
18. **Ross R.** Atherosclerosis--an inflammatory disease. *The New England journal of medicine*. 1999;340(2):115-26.
19. **Rahimi Z.** ACE insertion/deletion (I/D) polymorphism and diabetic nephropathy. *Journal of nephropathology*. 2012;1(3):143-51.
20. **Hansson GK, Libby P, Schonbeck U, Yan ZQ.** Innate and adaptive immunity in the pathogenesis of atherosclerosis. *Circulation research*. 2002;91(4):281-91.
21. **McLeod O, Silveira A, Fredrikson GN, Gertow K, Baldassarre D, Veglia F, et al.** Plasma autoantibodies against apolipoprotein B-100 peptide 210 in subclinical atherosclerosis. *Atherosclerosis*. 2014;232(1):242-8.
22. **Badimon L.** Interleukin-18: a potent pro-inflammatory cytokine in atherosclerosis. *Cardiovascular research*. 2012;96(2):172-5; discussion 6-80.
23. **Libby P, Nahrendorf M, Swirski FK.** Monocyte heterogeneity in cardiovascular disease. *Seminars in immunopathology*. 2013;35(5):553-62.
24. **Badimon L, Storey RF, Vilahur G.** Update on lipids, inflammation and atherothrombosis. *Thrombosis and haemostasis*. 2011;105 Suppl 1:S34-42.
25. **Koga J, Aikawa M.** Crosstalk between macrophages and smooth muscle cells in atherosclerotic vascular diseases. *Vascular pharmacology*. 2012;57(1):24-8.
26. **Tabas I.** Macrophage death and defective inflammation resolution in atherosclerosis. *Nature reviews Immunology*. 2010;10(1):36-46.
27. **Virmani R, Kolodgie FD, Burke AP, Finn AV, Gold HK, Tulenko TN, et al.** Atherosclerotic plaque progression and vulnerability to rupture: angiogenesis as a source of intraplaque hemorrhage. *Arteriosclerosis, thrombosis, and vascular biology*. 2005;25(10):2054-61.
28. **Wentzel JJ, Chatzizisis YS, Gijzen FJ, Giannoglou GD, Feldman CL, Stone PH.** Endothelial shear stress in the evolution of coronary atherosclerotic plaque and vascular remodelling: current understanding and remaining questions. *Cardiovascular research*. 2012;96(2):234-43.
29. **Virmani R, Kolodgie FD, Burke AP, Farb A, Schwartz SM.** Lessons from sudden coronary death: a comprehensive morphological classification scheme for atherosclerotic lesions. *Arteriosclerosis, thrombosis, and vascular biology*. 2000;20(5):1262-75.
30. **Bentzon JF, Otsuka F, Virmani R, Falk E.** Mechanisms of plaque formation and rupture. *Circulation research*. 2014;114(12):1852-66.
31. **Albers GW, Amarenco P, Easton JD, Sacco RL, Teal P.** Antithrombotic and thrombolytic therapy for ischemic stroke. *Chest*. 2001;119(1 Suppl):300s-20s.
32. **Badimon L, Vilahur G.** Thrombosis formation on atherosclerotic lesions and plaque rupture. *Journal of internal medicine*. 2014;276(6):618-32.
33. **Stein JH, Korcarz CE, Hurst RT, Lonn E, Kendall CB, Mohler ER, et al.** Use of carotid ultrasound to identify subclinical vascular disease and evaluate cardiovascular disease

risk: a consensus statement from the American Society of Echocardiography Carotid Intima-Media Thickness Task Force. Endorsed by the Society for Vascular Medicine. *Journal of the American Society of Echocardiography*. 2008;21(2):93-111; quiz 89-90.

34. **Barnett HJM, Taylor DW, Haynes RB, Sackett DL, Peerless SJ, Ferguson GG, et al.** Beneficial effect of carotid endarterectomy in symptomatic patients with high-grade carotid stenosis. *The New England journal of medicine*. 1991;325(7):445-53.

35. **MRC European Carotid Surgery Trial:** interim results for symptomatic patients with severe (70-99%) or with mild (0-29%) carotid stenosis. European Carotid Surgery Trialists' Collaborative Group. *Lancet*. 1991;337(8752):1235-43.

36. **Rothwell PM, Eliasziw M, Gutnikov SA, Fox AJ, Taylor DW, Mayberg MR, et al.** Analysis of pooled data from the randomised controlled trials of endarterectomy for symptomatic carotid stenosis. *Lancet*. 2003;361(9352):107-16.

37. **Jahromi AS, Cina CS, Liu Y, Clase CM.** Sensitivity and specificity of color duplex ultrasound measurement in the estimation of internal carotid artery stenosis: a systematic review and meta-analysis. *Journal of vascular surgery*. 2005;41(6):962-72.

38. **Tsivgoulis G, Alexandrov AV.** Ultrasound in Neurology. *Continuum* (Minneapolis, Minn). 2016;22(5, Neuroimaging):1655-77.

39. **Oates CP, Naylor AR, Hartshorne T, Charles SM, Fail T, Humphries K, et al.** Joint recommendations for reporting carotid ultrasound investigations in the United Kingdom. *European journal of vascular and endovascular surgery : the official journal of the European Society for Vascular Surgery*. 2009;37(3):251-61.

40. **Geroulakos G, Hobson RW, Nicolaides A.** Ultrasonographic carotid plaque morphology in predicting stroke risk. *The British journal of surgery*. 1996;83(5):582-7.

41. **Nicolaides AN, Kakkos SK, Griffin M, Sabetai M, Dhanjil S, Tegos T, et al.** Severity of asymptomatic carotid stenosis and risk of ipsilateral hemispheric ischaemic events: results from the ACSRS study. *European journal of vascular and endovascular surgery : the official journal of the European Society for Vascular Surgery*. 2005;30(3):275-84.

42. **Basic J, Assadian A, Strassegger J, Senekowitsch C, Wickenhauser G, Koulas S, et al.** Degree of contralateral carotid stenosis improves preoperative risk stratification of patients with asymptomatic ipsilateral carotid stenosis. *Journal of vascular surgery*. 2016;63(1):82-8.e2.

43. **Conrad MF, Boulom V, Mukhopadhyay S, Garg A, Patel VI, Cambria RP.** Progression of asymptomatic carotid stenosis despite optimal medical therapy. *Journal of vascular surgery*. 2013;58(1):128-35.e1.

44. **Sabeti S, Schlager O, Exner M, Mlekusch W, Amighi J, Dick P, et al.** Progression of carotid stenosis detected by duplex ultrasonography predicts adverse outcomes in cardiovascular high-risk patients. *Stroke*. 2007;38(11):2887-94.

45. **Kakkos SK, Nicolaides AN, Charalambous I, Thomas D, Giannopoulos A, Naylor AR, et al.** Predictors and clinical significance of progression or regression of asymptomatic carotid stenosis. *Journal of vascular surgery*. 2014;59(4):956-67.e1.

46. **Freilinger TM, Schindler A, Schmidt C, Grimm J, Cyran C, Schwarz F, et al.** Prevalence of nonstenosing, complicated atherosclerotic plaques in cryptogenic stroke. *JACC Cardiovascular imaging*. 2012;5(4):397-405.

47. **Horie N, Morikawa M, Ishizaka S, Takeshita T, So G, Hayashi K, et al.** Assessment of carotid plaque stability based on the dynamic enhancement pattern in plaque components with multidetector CT angiography. *Stroke*. 2012;43(2):393-8.
48. **Feeley TM, Leen EJ, Colgan MP, Moore DJ, Hourihane DO, Shanik GD.** Histologic characteristics of carotid artery plaque. *Journal of vascular surgery*. 1991;13(5):719-24.
49. **Bluth EI, Kay D, Merritt CR, Sullivan M, Farr G, Mills NL, et al.** Sonographic characterization of carotid plaque: detection of hemorrhage. *AJR American journal of roentgenology*. 1986;146(5):1061-5.
50. **Acharya UR, Sree SV, Krishnan MM, Molinari F, Saba L, Ho SY, et al.** Atherosclerotic risk stratification strategy for carotid arteries using texture-based features. *Ultrasound in medicine & biology*. 2012;38(6):899-915.
51. **Gray-Weale AC, Graham JC, Burnett JR, Byrne K, Lusby RJ.** Carotid artery atheroma: comparison of preoperative B-mode ultrasound appearance with carotid endarterectomy specimen pathology. *The Journal of cardiovascular surgery*. 1988;29(6):676-81.
52. **Aldemir E, Apaydin M, Varer M, Uluc E.** Echolucency of carotid plaques and cerebrovascular events. *Journal of clinical ultrasound : JCU*. 2012;40(7):399-404.
53. **Fosse E, Johnsen SH, Stensland-Bugge E, Joakimsen O, Mathiesen EB, Arnesen E, et al.** Repeated visual and computer-assisted carotid plaque characterization in a longitudinal population-based ultrasound study: the Tromso study. *Ultrasound in medicine & biology*. 2006;32(1):3-11.
54. **Nicolaides AN, Kakkos SK, Kyriacou E, Griffin M, Sabetai M, Thomas DJ, et al.** Asymptomatic internal carotid artery stenosis and cerebrovascular risk stratification. *Journal of vascular surgery*. 2010;52(6):1486-96.e1-5.
55. **Geroulakos G, Ramaswami G, Nicolaides A, James K, Labropoulos N, Belcaro G, et al.** Characterization of symptomatic and asymptomatic carotid plaques using high-resolution real-time ultrasonography. *The British journal of surgery*. 1993;80(10):1274-7.
56. **Jashari F, Ibrahimi P, Bajraktari G, Gronlund C, Wester P, Henein MY.** Carotid plaque echogenicity predicts cerebrovascular symptoms: a systematic review and meta-analysis. *European journal of neurology*. 2016;23(7):1241-7.
57. **Polak JF, Shemanski L, O'Leary DH, Lefkowitz D, Price TR, Savage PJ, et al.** Hypoechoic plaque at US of the carotid artery: an independent risk factor for incident stroke in adults aged 65 years or older. Cardiovascular Health Study. *Radiology*. 1998;208(3):649-54.
58. **Mathiesen EB, Bonna KH, Joakimsen O.** Echolucent plaques are associated with high risk of ischemic cerebrovascular events in carotid stenosis: the tromso study. *Circulation*. 2001;103(17):2171-5.
59. **Liapis CD, Kakisis JD, Kostakis AG.** Carotid stenosis: factors affecting symptomatology. *Stroke*. 2001;32(12):2782-6.
60. **Silvestrini M, Altamura C, Cerqua R, Pasqualetti P, Viticchi G, Provinciali L, et al.** Ultrasonographic markers of vascular risk in patients with asymptomatic carotid stenosis. *Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism*. 2013;33(4):619-24.

61. **Gronholdt ML, Nordestgaard BG, Schroeder TV, Vorstrup S, Sillesen H.** Ultrasonic echolucent carotid plaques predict future strokes. *Circulation*. 2001;104(1):68-73.
62. **Nicolaides AN, Kakkos SK, Griffin M, Sabetai M, Dhanjil S, Thomas DJ, et al.** Effect of image normalization on carotid plaque classification and the risk of ipsilateral hemispheric ischemic events: results from the asymptomatic carotid stenosis and risk of stroke study. *Vascular*. 2005;13(4):211-21.
63. **Hashimoto H, Tagaya M, Niki H, Etani H.** Computer-assisted analysis of heterogeneity on B-mode imaging predicts instability of asymptomatic carotid plaque. *Cerebrovascular diseases* (Basel, Switzerland). 2009;28(4):357-64.
64. **Topakian R, King A, Kwon SU, Schaafsma A, Shipley M, Markus HS.** Ultrasonic plaque echolucency and emboli signals predict stroke in asymptomatic carotid stenosis. *Neurology*. 2011;77(8):751-8.
65. **Salem MK, Sayers RD, Bown MJ, West K, Moore D, Nicolaides A, et al.** Patients with recurrent ischaemic events from carotid artery disease have a large lipid core and low GSM. *European journal of vascular and endovascular surgery : the official journal of the European Society for Vascular Surgery*. 2012;43(2):147-53.
66. **Singh AS, Atam V, Jain N, Yathish BE, Patil MR, Das L.** Association of carotid plaque echogenicity with recurrence of ischemic stroke. *North American journal of medical sciences*. 2013;5(6):371-6.
67. **Kyriacou EC, Petroudi S, Pattichis CS, Pattichis MS, Griffin M, Kakkos S, et al.** Prediction of high-risk asymptomatic carotid plaques based on ultrasonic image features. *IEEE transactions on information technology in biomedicine : a publication of the IEEE Engineering in Medicine and Biology Society*. 2012;16(5):966-73.
68. **Seabra JC, Pedro LM, e Fernandes JF, Sanches JM.** A 3-D ultrasound-based framework to characterize the echo morphology of carotid plaques. *IEEE transactions on bio-medical engineering*. 2009;56(5):1442-53.
69. **Karjalainen JP, Toyra J, Riekkinen O, Hakulinen M, Jurvelin JS.** Ultrasound backscatter imaging provides frequency-dependent information on structure, composition and mechanical properties of human trabecular bone. *Ultrasound in medicine & biology*. 2009;35(8):1376-84.
70. **Barzilai B, Saffitz JE, Miller JG, Sobel BE.** Quantitative ultrasonic characterization of the nature of atherosclerotic plaques in human aorta. *Circulation research*. 1987;60(3):459-63.
71. **Honda O, Sugiyama S, Kugiyama K, Fukushima H, Nakamura S, Koide S, et al.** Echolucent carotid plaques predict future coronary events in patients with coronary artery disease. *Journal of the American College of Cardiology*. 2004;43(7):1177-84.
72. **Yamagami H, Sakaguchi M, Furukado S, Hoshi T, Abe Y, Hougaku H, et al.** Statin therapy increases carotid plaque echogenicity in hypercholesterolemic patients. *Ultrasound in medicine & biology*. 2008;34(9):1353-9.
73. **Kitamura A, Iso H, Imano H, Ohira T, Okada T, Sato S, et al.** Carotid intima-media thickness and plaque characteristics as a risk factor for stroke in Japanese elderly men. *Stroke*. 2004;35(12):2788-94.
74. **Prabhakaran S, Rundek T, Ramas R, Elkind MS, Paik MC, Boden-Albala B, et al.** Carotid plaque surface irregularity predicts ischemic stroke: the northern Manhattan study. *Stroke*. 2006;37(11):2696-701.

75. **Saba L, Anzidei M, Sanfilippo R, Montisci R, Lucatelli P, Catalano C, et al.** Imaging of the carotid artery. *Atherosclerosis*. 2012;220(2):294-309.
76. **Saba L, Anzidei M, Marincola BC, Piga M, Raz E, Bassareo PP, et al.** Imaging of the carotid artery vulnerable plaque. *Cardiovascular and interventional radiology*. 2014;37(3):572-85.
77. **Ten Kate GL, van Dijk AC, van den Oord SC, Hussain B, Verhagen HJ, Sijbrands EJ, et al.** Usefulness of contrast-enhanced ultrasound for detection of carotid plaque ulceration in patients with symptomatic carotid atherosclerosis. *The American journal of cardiology*. 2013;112(2):292-8.
78. **Brinjikji W, Rabinstein AA, Lanzino G, Murad MH, Williamson EE, DeMarco JK, et al.** Ultrasound Characteristics of Symptomatic Carotid Plaques: A Systematic Review and Meta-Analysis. *Cerebrovascular diseases* (Basel, Switzerland). 2015;40(3-4):165-74.
79. **Homburg PJ, Rozie S, van Gils MJ, van den Bouwhuijsen QJ, Niessen WJ, Dippel DW, et al.** Association between carotid artery plaque ulceration and plaque composition evaluated with multidetector CT angiography. *Stroke*. 2011;42(2):367-72.
80. **Hu CH, Xu XC, Cannata JM, Yen JT, Shung KK.** Development of a real-time, high-frequency ultrasound digital beamformer for high-frequency linear array transducers. *IEEE transactions on ultrasonics, ferroelectrics, and frequency control*. 2006;53(2):317-23.
81. **Muraki M, Mikami T, Yoshimoto T, Fujimoto S, Tokuda K, Kaneko S, et al.** New criteria for the sonographic diagnosis of a plaque ulcer in the extracranial carotid artery. *AJR American journal of roentgenology*. 2012;198(5):1161-6.
82. **Muraki M, Mikami T, Yoshimoto T, Fujimoto S, Kitaguchi M, Kaga S, et al.** Sonographic Detection of Abnormal Plaque Motion of the Carotid Artery: Its Usefulness in Diagnosing High-Risk Lesions Ranging from Plaque Rupture to Ulcer Formation. *Ultrasound in medicine & biology*. 2016;42(2):358-64.
83. **Saha SA, Gourineni V, Feinstein SB.** The Use of Contrast-enhanced Ultrasonography for Imaging of Carotid Atherosclerotic Plaques: Current Evidence, Future Directions. *Neuroimaging clinics of North America*. 2016;26(1):81-96.
84. **Heliopoulos J, Vadikolias K, Piperidou C, Mitsias P.** Detection of carotid artery plaque ulceration using 3-dimensional ultrasound. *Journal of neuroimaging : official journal of the American Society of Neuroimaging*. 2011;21(2):126-31.
85. **Kuk M, Wannarong T, Beletsky V, Parraga G, Fenster A, Spence JD.** Volume of carotid artery ulceration as a predictor of cardiovascular events. *Stroke*. 2014;45(5):1437-41.
86. **Falk E.** Pathogenesis of atherosclerosis. *Journal of the American College of Cardiology*. 2006;47(8 Suppl):C7-12.
87. **Nagase H, Visse R, Murphy G.** Structure and function of matrix metalloproteinases and TIMPs. *Cardiovascular research*. 2006;69(3):562-73.
88. **Fleiner M, Kummer M, Mirlacher M, Sauter G, Cathomas G, Krapf R, et al.** Arterial neovascularization and inflammation in vulnerable patients: early and late signs of symptomatic atherosclerosis. *Circulation*. 2004;110(18):2843-50.
89. **Ferrer JM, Samso JJ, Serrando JR, Valenzuela VF, Montoya SB, Docampo MM.** Use of ultrasound contrast in the diagnosis of carotid artery occlusion. *Journal of vascular surgery*. 2000;31(4):736-41.

90. **Staub D, Partovi S, Imfeld S, Uthoff H, Baldi T, Aschwanden M, et al.** Novel applications of contrast-enhanced ultrasound imaging in vascular medicine. *VASA Zeitschrift fur Gefasskrankheiten*. 2013;42(1):17-31.
91. **Staub D, Patel MB, Tibrewala A, Ludden D, Johnson M, Espinosa P, et al.** Vasa vasorum and plaque neovascularization on contrast-enhanced carotid ultrasound imaging correlates with cardiovascular disease and past cardiovascular events. *Stroke*. 2010;41(1):41-7.
92. **Ten Kate GL, van den Oord SC, Sijbrands EJ, van der Lugt A, de Jong N, Bosch JG, et al.** Current status and future developments of contrast-enhanced ultrasound of carotid atherosclerosis. *Journal of vascular surgery*. 2013;57(2):539-46.
93. **Coli S, Magnoni M, Sangiorgi G, Marrocco-Trischitta MM, Melisurgo G, Mauriello A, et al.** Contrast-enhanced ultrasound imaging of intraplaque neovascularization in carotid arteries: correlation with histology and plaque echogenicity. *Journal of the American College of Cardiology*. 2008;52(3):223-30.
94. **Vavuranakis M, Sigala F, Vrachatis DA, Papaioannou TG, Filis K, Kavantzias N, et al.** Quantitative analysis of carotid plaque vasa vasorum by CEUS and correlation with histology after endarterectomy. *VASA Zeitschrift fur Gefasskrankheiten*. 2013;42(3):184-95.
95. **Xiong L, Deng YB, Zhu Y, Liu YN, Bi XJ.** Correlation of carotid plaque neovascularization detected by using contrast-enhanced US with clinical symptoms. *Radiology*. 2009;251(2):583-9.
96. **Zhu Y, Deng YB, Liu YN, Bi XJ, Sun J, Tang QY, et al.** Use of carotid plaque neovascularization at contrast-enhanced US to predict coronary events in patients with coronary artery disease. *Radiology*. 2013;268(1):54-60.
97. **Lindner JR, Dayton PA, Coggins MP, Ley K, Song J, Ferrara K, et al.** Noninvasive imaging of inflammation by ultrasound detection of phagocytosed microbubbles. *Circulation*. 2000;102(5):531-8.
98. **Yanagisawa K, Moriyasu F, Miyahara T, Yuki M, Iijima H.** Phagocytosis of ultrasound contrast agent microbubbles by Kupffer cells. *Ultrasound in medicine & biology*. 2007;33(2):318-25.
99. **Shalhoub J, Monaco C, Owen DR, Gauthier T, Thapar A, Leen EL, et al.** Late-phase contrast-enhanced ultrasound reflects biological features of instability in human carotid atherosclerosis. *Stroke*. 2011;42(12):3634-6.
100. **Vicenzini E, Giannoni MF, Puccinelli F, Ricciardi MC, Altieri M, Di Piero V, et al.** Detection of carotid adventitial vasa vasorum and plaque vascularization with ultrasound cadence contrast pulse sequencing technique and echo-contrast agent. *Stroke*. 2007;38(10):2841-3.
101. **Varetto G, Gibello L, Bergamasco L, Sapino A, Castellano I, Garneri P, et al.** Contrast enhanced ultrasound in atherosclerotic carotid artery disease. *International angiology : a journal of the International Union of Angiology*. 2012;31(6):565-71.
102. **Huang PT, Chen CC, Aronow WS, Wang XT, Nair CK, Xue NY, et al.** Assessment of neovascularization within carotid plaques in patients with ischemic stroke. *World journal of cardiology*. 2010;2(4):89-97.
103. **Huang R, Abdelmoneim SS, Ball CA, Nhola LF, Farrell AM, Feinstein S, et al.** Detection of Carotid Atherosclerotic Plaque Neovascularization Using Contrast Enhanced

Ultrasound: A Systematic Review and Meta-Analysis of Diagnostic Accuracy Studies. *Journal of the American Society of Echocardiography : official publication of the American Society of Echocardiography*. 2016;29(6):491-502.

104. **Shah F, Balan P, Weinberg M, Reddy V, Neems R, Feinstein M, et al.** Contrast-enhanced ultrasound imaging of atherosclerotic carotid plaque neovascularization: a new surrogate marker of atherosclerosis? *Vascular medicine* (London, England). 2007;12(4):291-7.

105. **Iezzi R, Petrone G, Ferrante A, Lauriola L, Vincenzoni C, la Torre MF, et al.** The role of contrast-enhanced ultrasound (CEUS) in visualizing atherosclerotic carotid plaque vulnerability: which injection protocol? Which scanning technique? *European journal of radiology*. 2015;84(5):865-71.

106. **Giannoni MF, Vincenzini E, Citone M, Ricciardi MC, Irace L, Laurito A, et al.** Contrast carotid ultrasound for the detection of unstable plaques with neoangiogenesis: a pilot study. *European journal of vascular and endovascular surgery : the official journal of the European Society for Vascular Surgery*. 2009;37(6):722-7.

107. **Owen DR, Shalhoub J, Miller S, Gauthier T, Doryforou O, Davies AH, et al.** Inflammation within carotid atherosclerotic plaque: assessment with late-phase contrast-enhanced US. *Radiology*. 2010;255(2):638-44.

108. **Zhou Y, Xing Y, Li Y, Bai Y, Chen Y, Sun X, et al.** An assessment of the vulnerability of carotid plaques: a comparative study between intraplaque neovascularization and plaque echogenicity. *BMC medical imaging*. 2013;13:13.

109. **Jaipersad AS, Shantsila A, Silverman S, Lip GY, Shantsila E.** Evaluation of carotid plaque neovascularization using contrast ultrasound. *Angiology*. 2013;64(6):447-50.

110. **Akkus Z, Hoogi A, Renaud G, van den Oord SC, Ten Kate GL, Schinkel AF, et al.** New quantification methods for carotid intra-plaque neovascularization using contrast-enhanced ultrasound. *Ultrasound in medicine & biology*. 2014;40(1):25-36.

111. **Spence JD.** Transcranial Doppler monitoring for microemboli: a marker of a high-risk carotid plaque. *Seminars in vascular surgery*. 2017;30(1):62-6.

112. **Russell D, Madden KP, Clark WM, Sandset PM, Zivin JA.** Detection of arterial emboli using Doppler ultrasound in rabbits. *Stroke*. 1991;22(2):253-8.

113. **Markus HS, Brown MM.** Differentiation between different pathological cerebral embolic materials using transcranial Doppler in an in vitro model. *Stroke*. 1993;24(1):1-5.

114. Basic identification criteria of Doppler microembolic signals. **Consensus Committee of the Ninth International Cerebral Hemodynamic Symposium.** *Stroke*. 1995;26(6):1123.

115. **Spence JD, Tamayo A, Lownie SP, Ng WP, Ferguson GG.** Absence of microemboli on transcranial Doppler identifies low-risk patients with asymptomatic carotid stenosis. *Stroke*. 2005;36(11):2373-8.

116. **Markus HS, MacKinnon A.** Asymptomatic embolization detected by Doppler ultrasound predicts stroke risk in symptomatic carotid artery stenosis. *Stroke*. 2005;36(5):971-5.

117. **Ritter MA, Dittrich R, Thoenissen N, Ringelstein EB, Nabavi DG.** Prevalence and prognostic impact of microembolic signals in arterial sources of embolism. A systematic review of the literature. *Journal of neurology*. 2008;255(7):953-61.

118. **Markus HS, King A, Shipley M, Topakian R, Cullinane M, Reihill S, et al.** Asymptomatic embolisation for prediction of stroke in the Asymptomatic Carotid Emboli Study (ACES): a prospective observational study. *The Lancet Neurology*. 2010;9(7):663-71.
119. **Best LM, Webb AC, Gurusamy KS, Cheng SF, Richards T.** Transcranial Doppler Ultrasound Detection of Microemboli as a Predictor of Cerebral Events in Patients with Symptomatic and Asymptomatic Carotid Disease: A Systematic Review and Meta-Analysis. *European journal of vascular and endovascular surgery : the official journal of the European Society for Vascular Surgery*. 2016;52(5):565-80.
120. **Ritter MA, Theismann K, Schmiedel M, Ringelstein EB, Dittrich R.** Vascularization of carotid plaque in recently symptomatic patients is associated with the occurrence of transcranial microembolic signals. *European journal of neurology*. 2013;20(8):1218-21.
121. **van Zuilen EV, Moll FL, Vermeulen FE, Mauser HW, van Gijn J, Ackerstaff RG.** Detection of cerebral microemboli by means of transcranial Doppler monitoring before and after carotid endarterectomy. *Stroke*. 1995;26(2):210-3.
122. **Safouris A, Krogias C, Sharma VK, Katsanos AH, Faissner S, Roussopoulou A, et al.** Statin Pretreatment and Microembolic Signals in Large Artery Atherosclerosis. *Arteriosclerosis, thrombosis, and vascular biology*. 2017;37(7):1415-22.
123. **Spence JD, Coates V, Li H, Tamayo A, Munoz C, Hackam DG, et al.** Effects of intensive medical therapy on microemboli and cardiovascular risk in asymptomatic carotid stenosis. *Archives of neurology*. 2010;67(2):180-6.
124. **Tsivgoulis G, Alexandrov AV.** Cerebral hemodynamics in acute stroke: pathophysiology and clinical implications. *Journal of vascular and interventional neurology*. 2008;1(3):65-9.
125. **Donahue J, Sumer S, Wintermark M.** Assessment of collateral flow in patients with cerebrovascular disorders. *Journal of neuroradiology*. 2014;41(4):234-42.
126. **Powers WJ.** Cerebral hemodynamics in ischemic cerebrovascular disease. *Annals of neurology*. 1991;29(3):231-40.
127. **Sharma VK, Tsivgoulis G, Lao AY, Alexandrov AV.** Role of transcranial Doppler ultrasonography in evaluation of patients with cerebrovascular disease. *Current neurology and neuroscience reports*. 2007;7(1):8-20.
128. **Tancredi FB, Hoge RD.** Comparison of cerebral vascular reactivity measures obtained using breath-holding and CO₂ inhalation. *Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism*. 2013;33(7):1066-74.
129. **Vagal AS, Leach JL, Fernandez-Ulloa M, Zuccarello M.** The acetazolamide challenge: techniques and applications in the evaluation of chronic cerebral ischemia. *AJNR American journal of neuroradiology*. 2009;30(5):876-84.
130. **Markus HS, Harrison MJ.** Estimation of cerebrovascular reactivity using transcranial Doppler, including the use of breath-holding as the vasodilatory stimulus. *Stroke*. 1992;23(5):668-73.
131. **Silvestrini M, Vernieri F, Pasqualetti P, Matteis M, Passarelli F, Troisi E, et al.** Impaired cerebral vasoreactivity and risk of stroke in patients with asymptomatic carotid artery stenosis. *Jama*. 2000;283(16):2122-7.

132. **Gupta A, Chazen JL, Hartman M, Delgado D, Anumula N, Shao H, et al.** Cerebrovascular reserve and stroke risk in patients with carotid stenosis or occlusion: a systematic review and meta-analysis. *Stroke*. 2012;43(11):2884-91.
133. **King A, Serena J, Bornstein NM, Markus HS.** Does impaired cerebrovascular reactivity predict stroke risk in asymptomatic carotid stenosis? A prospective substudy of the asymptomatic carotid emboli study. *Stroke*. 2011;42(6):1550-5.
134. **Gur AY, Bova I, Bornstein NM.** Is impaired cerebral vasomotor reactivity a predictive factor of stroke in asymptomatic patients? *Stroke*. 1996;27(12):2188-90.
135. **Kimiagar I, Bass A, Rabey JM, Bornstein NM, Gur AY.** Long-term follow-up of patients with asymptomatic occlusion of the internal carotid artery with good and impaired cerebral vasomotor reactivity. *European journal of neurology*. 2010;17(10):1285-90.
136. **Markus H, Cullinane M.** Severely impaired cerebrovascular reactivity predicts stroke and TIA risk in patients with carotid artery stenosis and occlusion. *Brain*. 2001;124(Pt 3):457-67.
137. **Kleiser B, Widder B.** Course of carotid artery occlusions with impaired cerebrovascular reactivity. *Stroke*. 1992;23(2):171-4.
138. **Reinhard M, Gerds TA, Grabiak D, Zimmermann PR, Roth M, Guschlbauer B, et al.** Cerebral dysautoregulation and the risk of ischemic events in occlusive carotid artery disease. *Journal of neurology*. 2008;255(8):1182-9.
139. **Vernieri F, Pasqualetti P, Passarelli F, Rossini PM, Silvestrini M.** Outcome of carotid artery occlusion is predicted by cerebrovascular reactivity. *Stroke*. 1999;30(3):593-8.
140. **Isozaki M, Arai Y, Kudo T, Kiyono Y, Kobayashi M, Kubota T, et al.** Clinical implication and prognosis of normal baseline cerebral blood flow with impaired vascular reserve in patients with major cerebral artery occlusive disease. *Annals of nuclear medicine*. 2010;24(5):371-7.
141. **Kuroda S, Houkin K, Kamiyama H, Mitsumori K, Iwasaki Y, Abe H.** Long-term prognosis of medically treated patients with internal carotid or middle cerebral artery occlusion: can acetazolamide test predict it? *Stroke*. 2001;32(9):2110-6.
142. **Ogasawara K, Ogawa A, Terasaki K, Shimizu H, Tominaga T, Yoshimoto T.** Use of cerebrovascular reactivity in patients with symptomatic major cerebral artery occlusion to predict 5-year outcome: comparison of xenon-133 and iodine-123-IMP single-photon emission computed tomography. *Journal of cerebral blood flow and metabolism*. 2002;22(9):1142-8.
143. **Webster MW, Makaroun MS, Steed DL, Smith HA, Johnson DW, Yonas H.** Compromised cerebral blood flow reactivity is a predictor of stroke in patients with symptomatic carotid artery occlusive disease. *Journal of vascular surgery*. 1995;21(2):338-44; discussion 44-5.
144. **Yamamoto KK, Miyata T, Momose T, Nagayoshi M, Akagi D, Hosaka A, et al.** Reduced vascular reserve measured by stressed single photon emission computed tomography carries a high risk for stroke in patients with carotid stenosis. *International angiology : a journal of the International Union of Angiology*. 2006;25(4):385-8.
145. **Reinhard M, Schwarzer G, Briel M, Altamura C, Palazzo P, King A, et al.** Cerebrovascular reactivity predicts stroke in high-grade carotid artery disease. *Neurology*. 2014;83(16):1424-31.

146. **Klijn CJ, Kappelle LJ, van Huffelen AC, Visser GH, Algra A, Tulleken CA, et al.** Recurrent ischemia in symptomatic carotid occlusion: prognostic value of hemodynamic factors. *Neurology*. 2000;55(12):1806-12.
147. **Vernieri F, Pasqualetti P, Matteis M, Passarelli F, Troisi E, Rossini PM, et al.** Effect of collateral blood flow and cerebral vasomotor reactivity on the outcome of carotid artery occlusion. *Stroke*. 2001;32(7):1552-8.
148. **Marshall RS, Rundek T, Sproule DM, Fitzsimmons BF, Schwartz S, Lazar RM.** Monitoring of cerebral vasodilatory capacity with transcranial Doppler carbon dioxide inhalation in patients with severe carotid artery disease. *Stroke*. 2003;34(4):945-9.
149. **Palazzo P, Tibuzzi F, Pasqualetti P, Altamura C, Silvestrini M, Passarelli F, et al.** Is there a role of near-infrared spectroscopy in predicting the outcome of patients with carotid artery occlusion? *Journal of the neurological sciences*. 2010;292(1-2):36-9.
150. **Momjian-Mayor I, Baron JC.** The pathophysiology of watershed infarction in internal carotid artery disease: review of cerebral perfusion studies. *Stroke*. 2005;36(3):567-77.
151. **Puz P, Lasek-Bal A, Urbanek T, Kazibutowska Z.** Assessment of cerebral embolism and vascular reserve parameters in patients with carotid artery stenosis. *Neurologia i neurochirurgia polska*. 2016;50(5):356-62.
152. **Hobson RW, 2nd, Weiss DG, Fields WS, Goldstone J, Moore WS, Towne JB, et al.** Efficacy of carotid endarterectomy for asymptomatic carotid stenosis. The Veterans Affairs Cooperative Study Group. *The New England journal of medicine*. 1993;328(4):221-7.
153. **Eckstein HH, Reiff T, Ringleb P, Jansen O, Mansmann U, Hacke W.** SPACE-2: A Missed Opportunity to Compare Carotid Endarterectomy, Carotid Stenting, and Best Medical Treatment in Patients with Asymptomatic Carotid Stenoses. *European journal of vascular and endovascular surgery : the official journal of the European Society for Vascular Surgery*. 2016;51(6):761-5.
154. **Lal BK, Meschia JF, Brott TG.** Clinical need, design, and goals for the Carotid Revascularization and Medical Management for Asymptomatic Carotid Stenosis trial. *Seminars in vascular surgery*. 2017;30(1):2-7.
155. **Bulbulia R, Halliday A.** The Asymptomatic Carotid Surgery Trial-2 (ACST-2): an ongoing randomised controlled trial comparing carotid endarterectomy with carotid artery stenting to prevent stroke. *Health technology assessment* (Winchester, England). 2017;21(57):1-40.
156. **Endarterectomy Combined With Optimal Medical Therapy (OMT) vs OMT Alone in Patients With Asymptomatic Severe Atherosclerotic Carotid Artery Stenosis at Higher-than-average Risk of Ipsilateral Stroke (ACTRIS).** www.clinicaltrials.gov/NCT02841098. [Accessed 16 April 2017].

