ULTRASONOGRAPHIC AND BIOCHEMICAL MARKERS FOR VULNERABLE ATHEROMATOUS PLAQUE

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ULTRASONOGRAPHIC AND BIOCHEMICAL MARKERS FOR VULNERABLE ATHEROMATOUS PLAQUE

1-Introduction:

- Vulnerable plaque.
- Vulnerable blood.
- Vulnerable patient.

2- Biomarkers for Vulnerable blood.

3- Biomarkers for Vulnerable plaque.

-Arterial Stenosis.

-Plaque Area/Volume.

-Plaque Surface.

-Plaque Characterization.

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Models for predicting vascular risk are developed
(Framingham, Sheffield, New Zealand, Canadian,British, European, Dundee, Munster [PROCAM], MONICA).

1-There is a patient group (25-50%) in which we are not able to predict de cardiovascular event.
2-These models doesn´t predict the short term vascular risk (ie CV risk >5% during the first year).

Vulnerable Patient : Prone to atherothrombosis event in the short term.

- Blood Factors: Inflamatory, metabolic, hipercoagulabily.
- Morphologic factors: Vulnerable Plaque .

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A thin fibrous cap, large lipid-rich necrotic core, low amount of collagen, and high inflammatory activity are major determinants of plaque vulnerability

Still, up to now it remains a challenge to predict plaque rupture in individual patients

There is a call for new definitions and risk assessment strategies regarding atherothrombosis. Besides morphology of plaques, vulnerability of blood (e.g. hypercoagulability and inflammation) as contributor to atherothrombosis.

There is a knowledge gap with respect to biomarkers of ongoing plaque destabilization.

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Combining:

1- Morphological characteristics: Vulnerable plaque.

2- Biomarkers for :

2.1 Thrombus instability.

2.2 Plaque instability.

Should result in a pan-arterial approach in which atherothrombotic risk establishment becomes more accurate.

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Vulnerable Patient: Patient at risk of atherothrombosis manifestation.

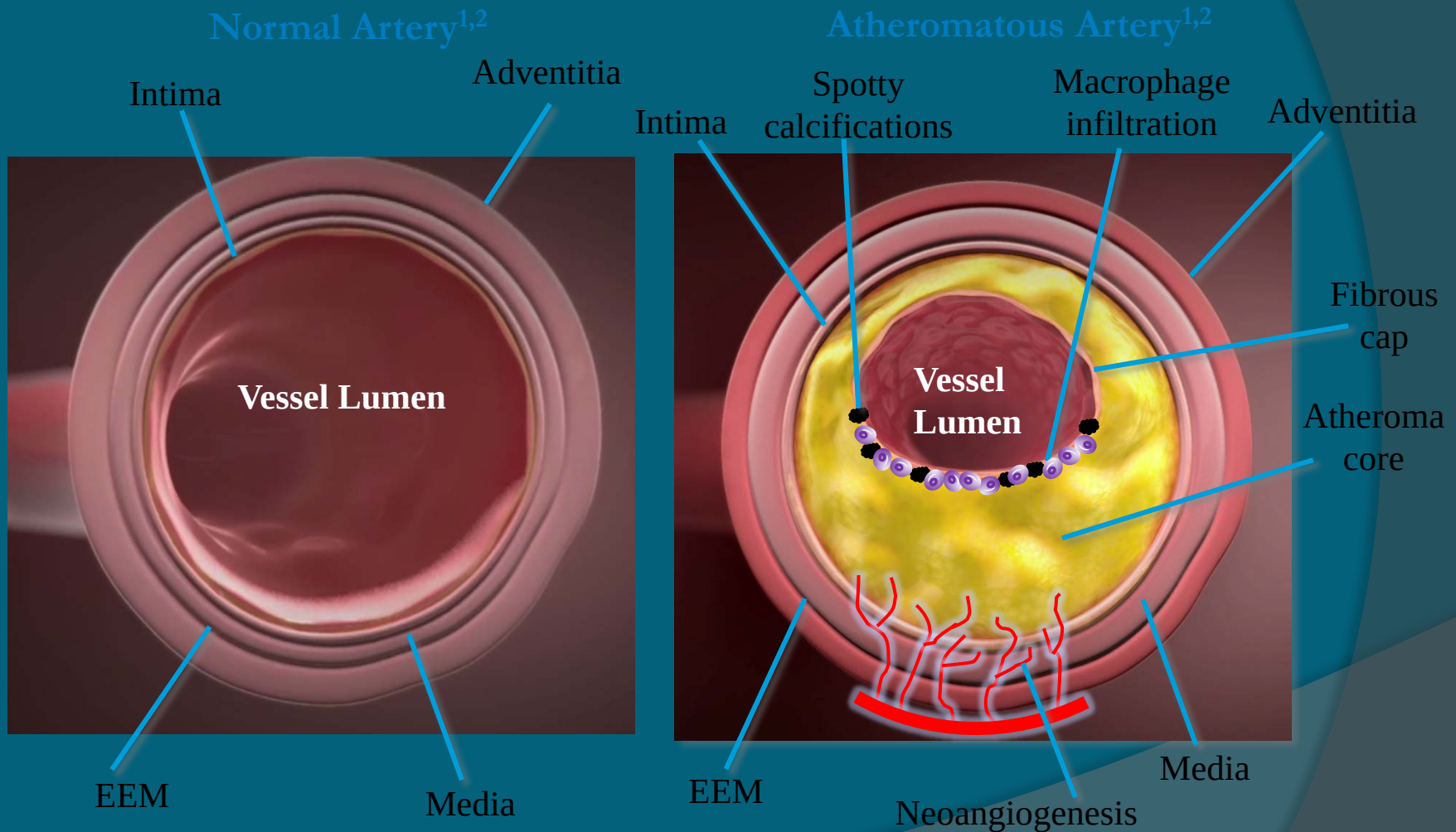
In the short term.

- Vulnerable plaque.
- Vulnerable blood.

Naghavi M et al. From vulnerable plaque to vulnerable patient: a call for new definitions and risk assessment strategies: Part II. Circulation. 2003 Oct 14;108(15):1772-8.

It would permit an indivisualized and a precise strategy for atherothrombosis treatment.

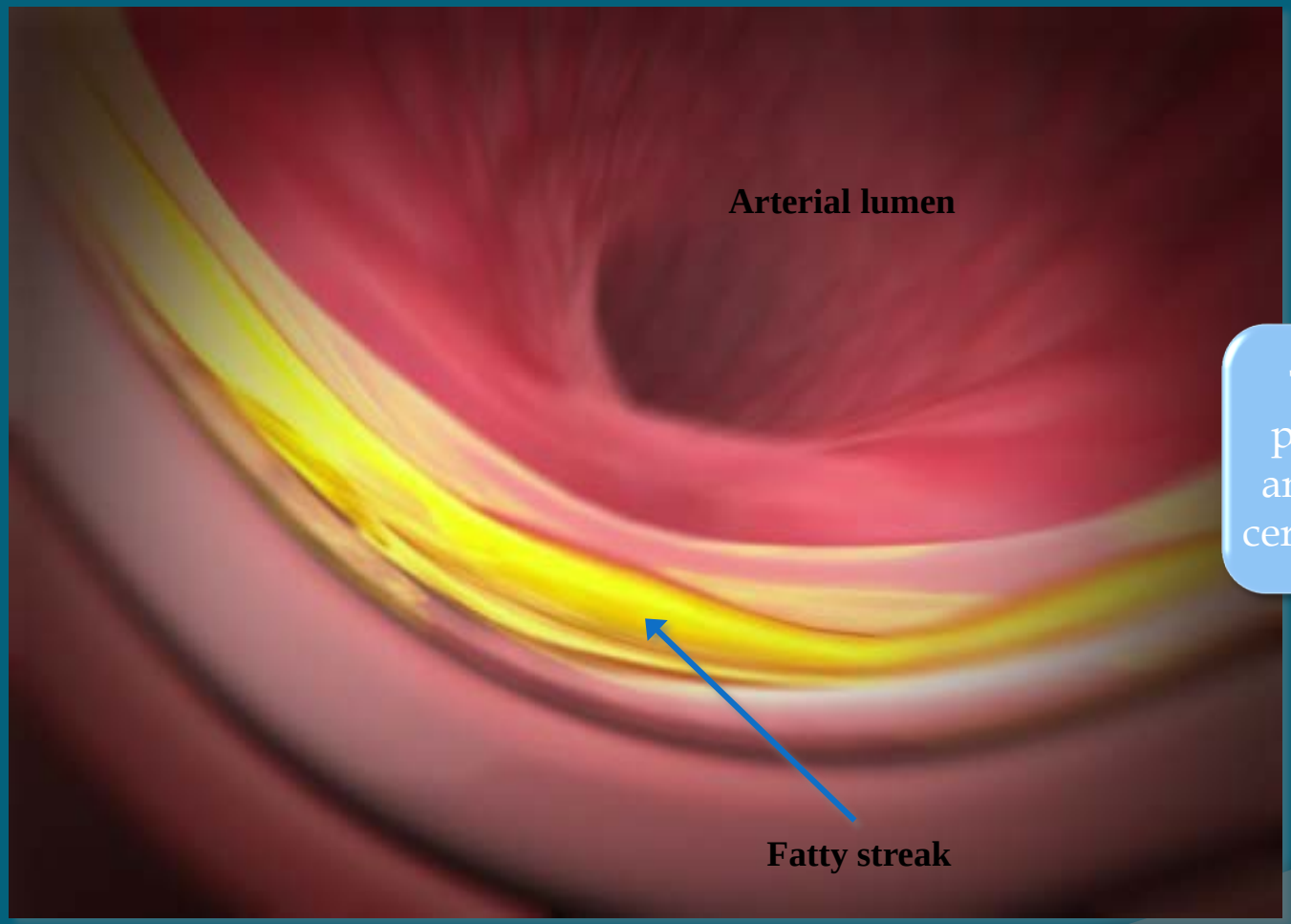
Anatomy of Normal and Atheromatous Artery[1,2]



EEM = external elastic membrane.

1. Vancraeynest D, et al. *J Am Coll Cardiol*. 2011;57:1961–1979. 2.Tardif JC, et al. *Circ Cardiovasc Imaging*. 2011;4:319–333.

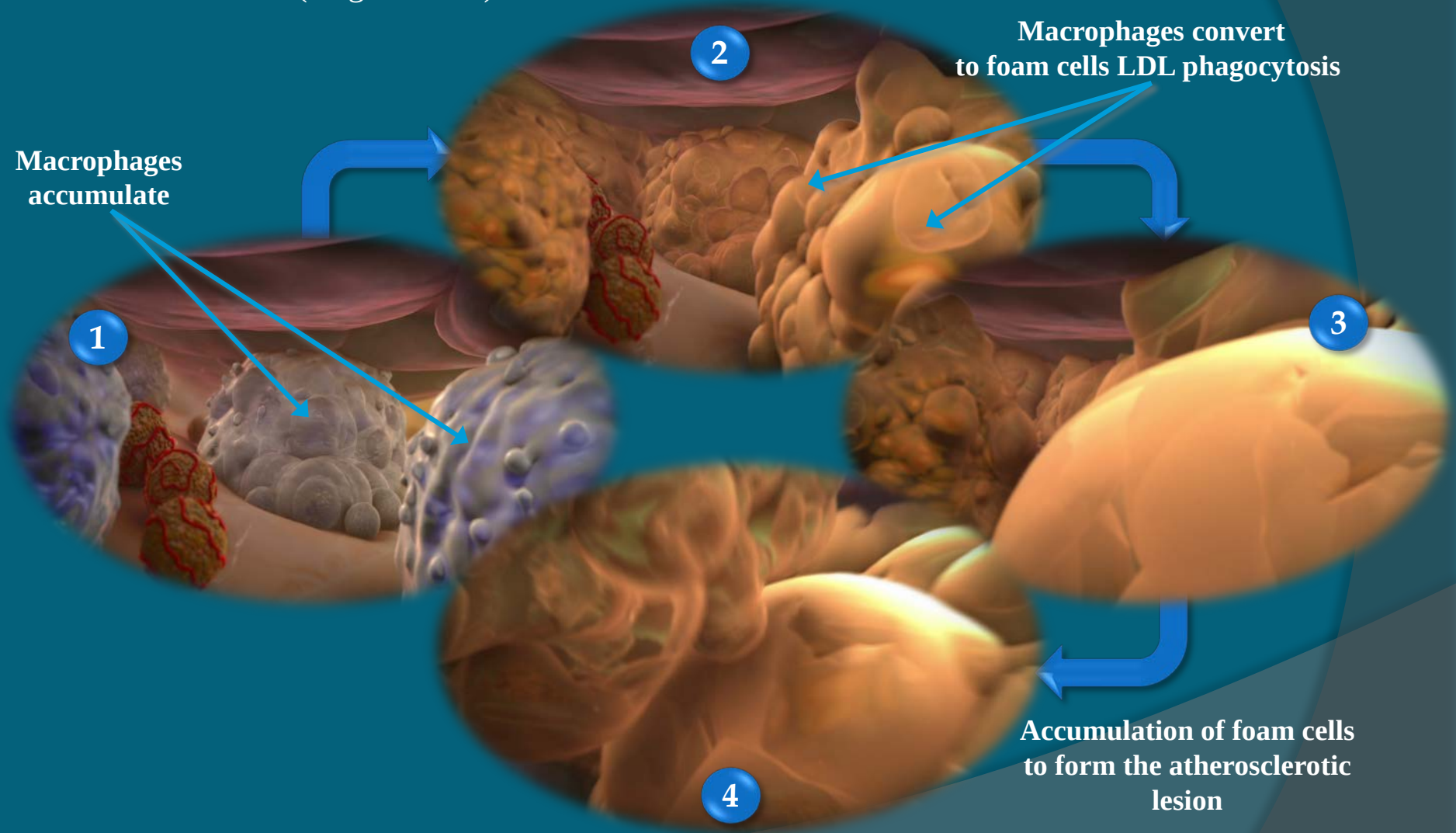
The Initial Accumulation of Foam Cells is Seen as a Fatty Streak[1,2]



The atherosclerotic process may occur in any artery – coronary, cerebral, or peripheral[1,2]

1. Samson S, et al. *Cholesterol*. 2012;2012:571846. 2. Hall JE, et al. In: *Guyton and Hall Textbook of Medical Physiology*. 12th ed. Philadelphia, PA: Saunders Elsevier; 2011:819–830.

Normally, macrophages leave through the basement membrane, but when too many LDL particles are present, the macrophages accumulate (diagram: 1). After internalising the oxidised LDL, the macrophages are converted to foam cells (diagram: 2). The accumulation of these foam cells is the hallmark of the atherosclerotic lesion (diagram: 3, 4)



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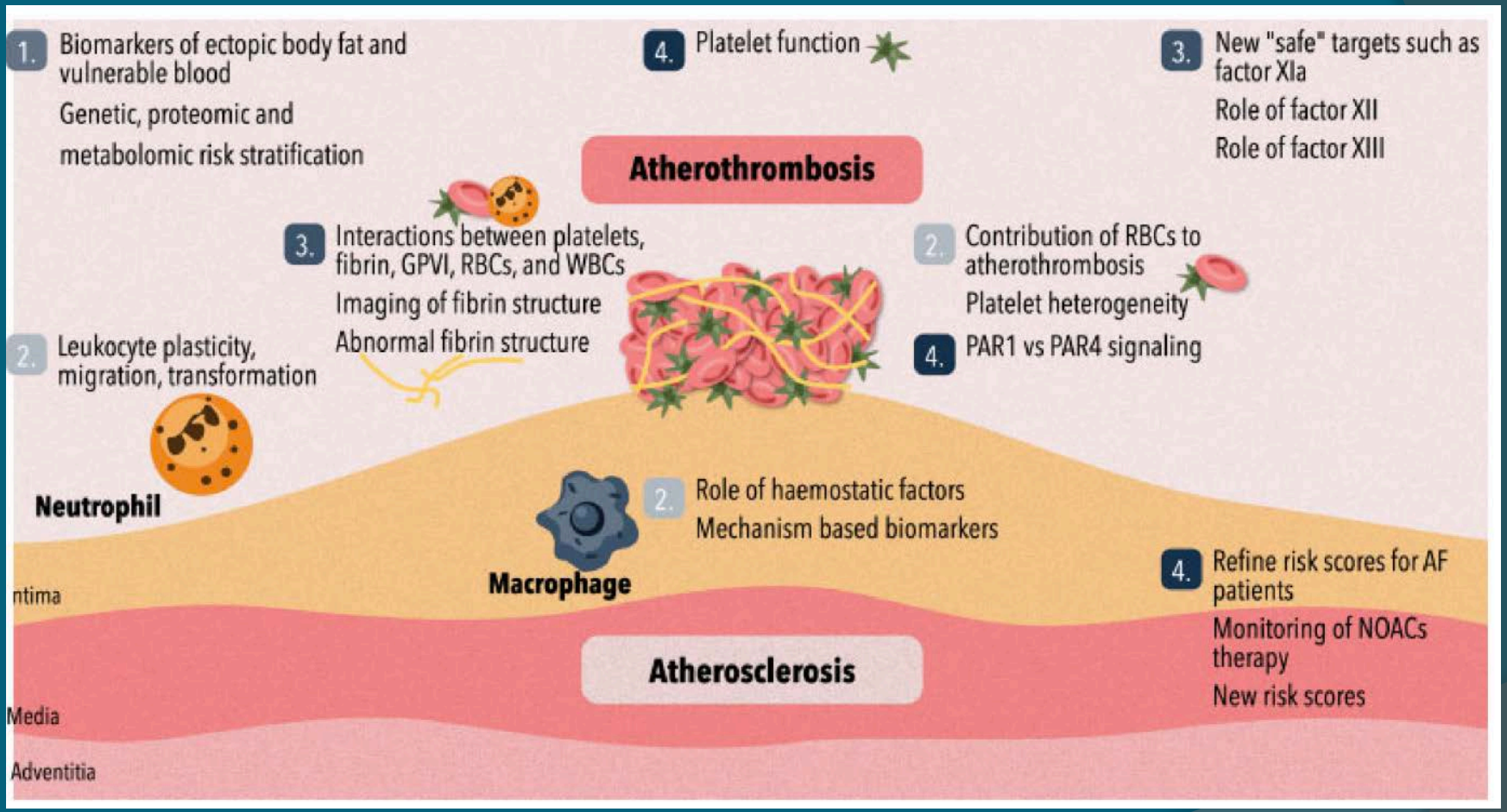

Spronk et al. Thromb Haemost. 2018

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Vulnerable Patient.

Vulnerable Blood:

Inflamatory or hipercoagulable biomarkers.

TABLE 1. Serological Markers of Vulnerability (Reflecting Metabolic and Immune Disorders)

- Abnormal lipoprotein profile (eg, high LDL, low HDL, abnormal LDL and HDL size density, lipoprotein [a], etc)
- Nonspecific markers of inflammation (eg, hsCRP, CD40L, ICAM-1, VCAM-1, P-selectin, leukocytosis, and other serological markers related to the immune system; these markers may not be specific for atherosclerosis or plaque inflammation)
- Serum markers of metabolic syndrome (eg, diabetes or hypertriglyceridemia)
- Specific markers of immune activation (eg, anti-LDL antibody, anti-HSP antibody)
- Markers of lipid peroxidation (eg, ox-LDL and ox-HDL)
- Homocysteine
- PAPP-A
- Circulating apoptosis marker(s) (eg, Fas/Fas ligand, not specific to plaque)
- ADMA/DDAH
- Circulating nonesterified fatty acids (eg, NEFA)

TABLE 2. Blood Markers of Vulnerability (Reflecting Hypercoagulability)

- Markers of blood hypercoagulability (eg, fibrinogen, D-dimer, and factor V Leiden)
- Increased platelet activation and aggregation (eg, gene polymorphisms of platelet glycoproteins IIb/IIIa, Ia/IIa, and Ib/IX)
- Increased coagulation factors (eg, clotting of factors V, VII, and VIII; von Willebrand factor; and factor XIII)
- Decreased anticoagulation factors (eg, proteins S and C, thrombomodulin, and antithrombin III)
- Decreased endogenous fibrinolysis activity (eg, reduced t-PA, increased PAI-1, certain PAI-1 polymorphisms)
- Prothrombin mutation (eg, G20210A)
- Other thrombogenic factors (eg, anticardiolipin antibodies, thrombocytosis, sickle cell disease, polycythemia, diabetes mellitus, hypercholesterolemia, hyperhomocysteinemia)
- Increased viscosity
- Transient hypercoagulability (eg, smoking, dehydration, infection, adrenergic surge, cocaine, estrogens, postprandial, etc)

t-PA indicates tissue plasminogen activator; PAI, type 1 plasminogen activator inhibitor.

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Meta-analyses of prospective studies have established positive associations of circulating levels of **fibrinogen, von Willebrand factor (VWF), fibrin D-dimer, and tissue plasminogen activator** with risk of coronary heart disease (CHD) and stroke.
Lowe G, Thromb Haemost 2014

Given their importance as potential therapeutic targets in CVD, additional biomarkers for vWF, factors VIII, IX, XI, and XII merit further research.

Proteomic and metabolomic data need to be implemented with genomic data in multicentre trials.

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Cellular Biomarkers:

1- Mechanisms of leukocyte plasticity, migration, and transformation

2- Haemostatic factors in macrophages related to inflammation and atherothrombosis.

3- Causal contribution of Red Blood Cells in thrombus formation.

4- Platelet heterogeneity, how this translates into the formation of platelet populations

Spronk et al. Thromb Haemost. 2018

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Vulnerable Plaque Markers.

Plaque

Morphology/Structure

- Plaque cap thickness
- Plaque lipid core size
- Plaque stenosis (luminal narrowing)
- Remodeling (expansive vs constrictive remodeling)
- Color (yellow, glistening yellow, red, etc)
- Collagen content versus lipid content, mechanical stability (stiffness and elasticity)
- Calcification burden and pattern (nodule vs scattered, superficial vs deep, etc)
- Shear stress (flow pattern throughout the coronary artery)

Naghavi M. From vulnerable plaque to vulnerable patient: a call for new definitions and risk assessment strategies: Part I. Circulation. 2003 7;108(14):1664-72.

ULTRASONOGRAPHIC AND BIOCHEMICAL MARKERS FOR VULNERABLE ATHEROMATOUS PLAQUE

Vulnerable Plaque:

TABLE 4. Criteria for Defining Vulnerable Plaque, Based on the Study of Culprit Plaques

Major criteria

- Active inflammation (monocyte/macrophage and sometimes T-cell infiltration)
- Thin cap with large lipid core
- Endothelial denudation with superficial platelet aggregation
- Fissured plaque
- Stenosis >90%

Minor criteria

- Superficial calcified nodule
- Glistening yellow
- Intraplaque hemorrhage
- Endothelial dysfunction
- Outward (positive) remodeling

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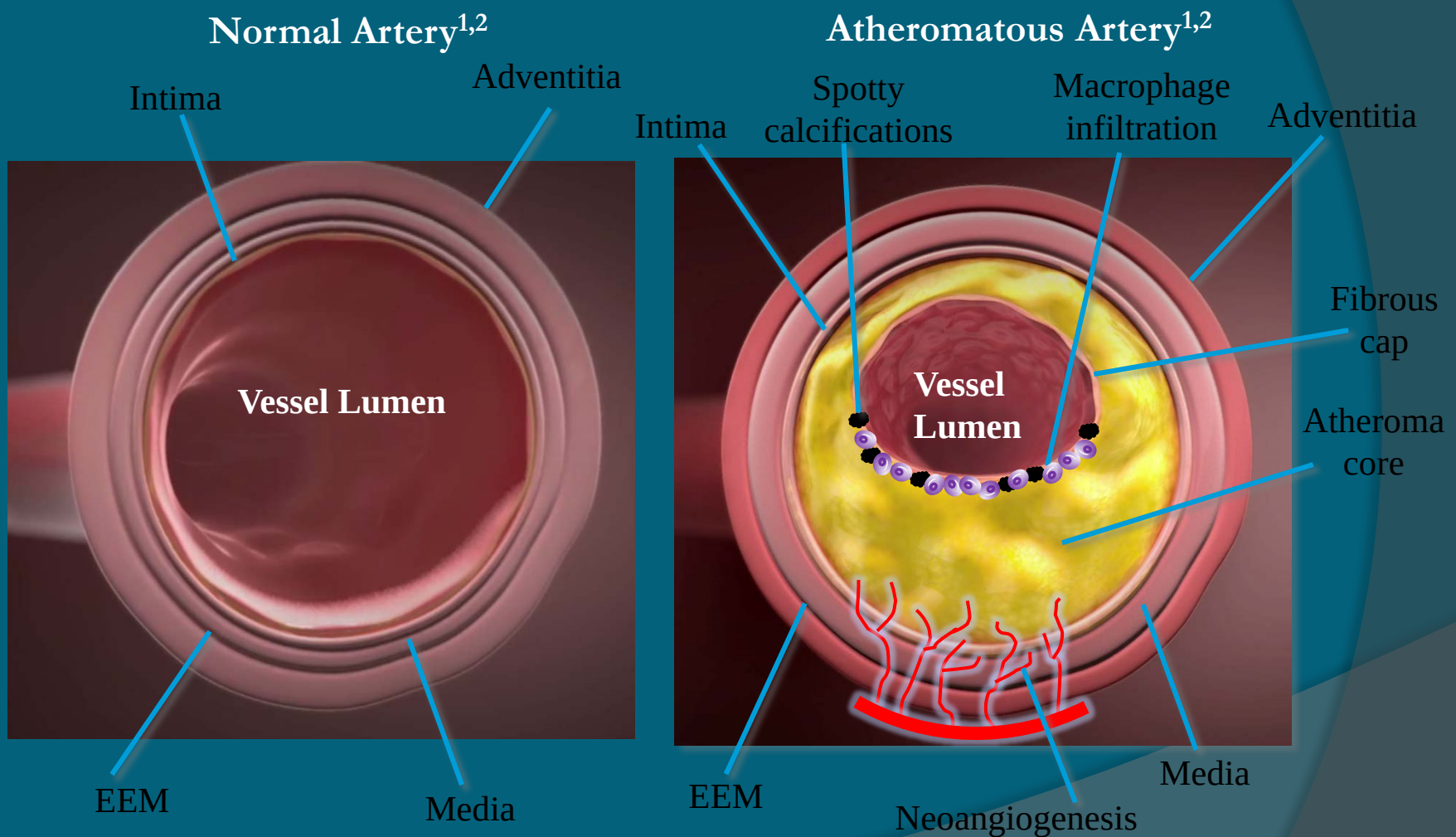
Anatomy of Normal and Atheromatous Artery[1,2]



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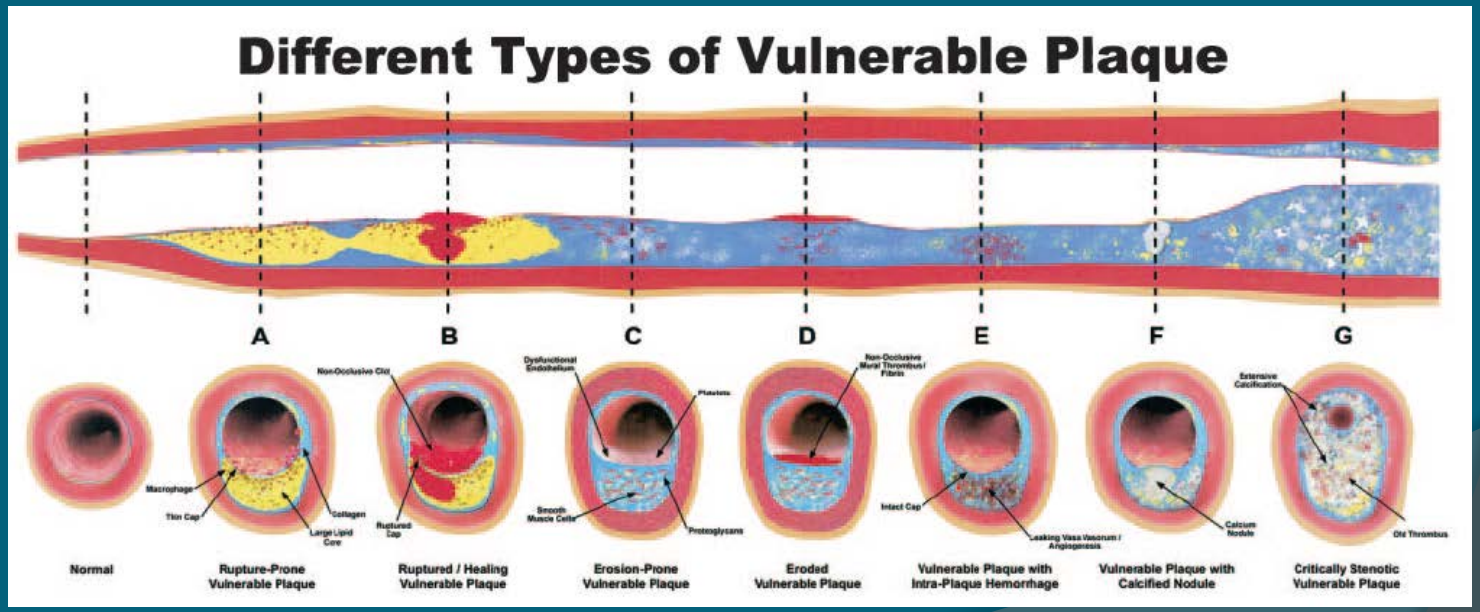
Vulnerable Plaque: At risk of atherothrombotic complication in the short term. . (AHA tipo IV).



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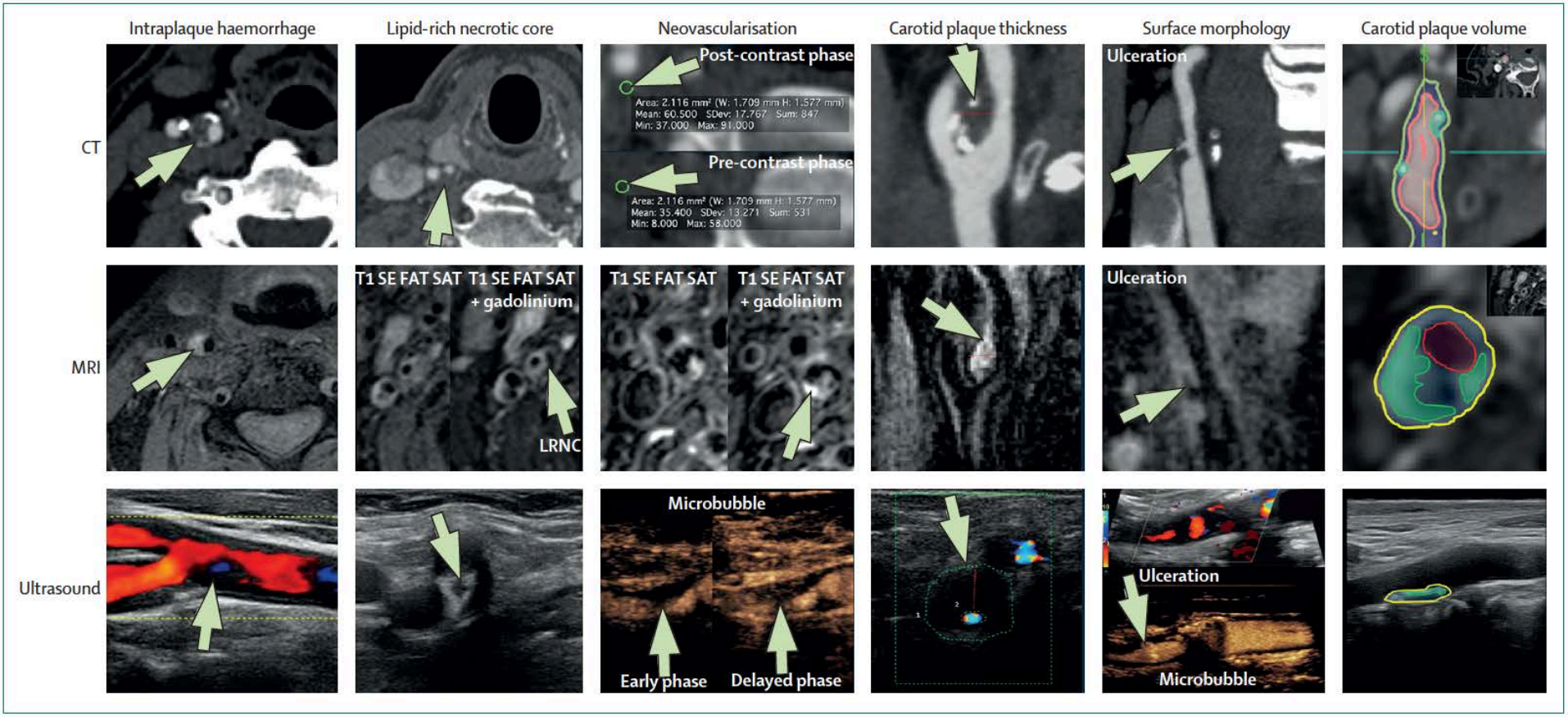

Imaging biomarkers of vulnerable carotid plaques for stroke risk prediction and their potential clinical implications

Luca Saba, Tobias Saam, H Rolf Jäger, Chun Yuan, Thomas S Hatsukami, David Saloner, Bruce A Wasserman, Leo H Bonati, Max Wintermark

Lancet Neurol 2019; 18: 559–72

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Biomarkers for Vulnerable plaque.

1-Arterial Stenosis.

2 -Plaque Area/Volume.

3 -Plaque Surface.

4 -Plaque Characterization.

ULTRASONOGRAPHIC AND BIOCHEMICAL MARKERS FOR VULNERABLE ATHEROMATOUS PLAQUE

	Validation studies (imaging method *vs* histopathology)	Reproducibility studies	Comments and limitations
Quantitative measurements: lumen and vessel wall			
MRI	N >10; Pearson's R 0·84 for wall, 0·81 for lumen area[22]	N >5; intra-reader, ICC 0·99 for lumen, ICC 0·98 for wall, CV 3·2–4·1% for lumen, CV 3·4–5·1% for wall;[32] inter-reader, ICC 0·98–0·99 for lumen, ICC 0·84–0·90 for wall, CV 5·3% for lumen, CV 7·9% for wall;[32] scan-rescan, ICC 0·99 for lumen, ICC 0·97 for wall, CV 4·3% for lumen, CV 5·8% for wall[67]	Highly accurate imaging method with excellent reproducibility; wall and lumen area measurements by MRI are ideally suited for cross-sectional and longitudinal studies; measurement errors can be used for power calculation for clinical trials[67]
CT	N >10; Pearson's R 0·85 for wall[24]	N >5: intrareader, CV 3% for lumen, CV 8% for wall;[24] inter-reader, CV 4% for lumen, CV 19% for wall[24]	Calcification can lead to overestimation of wall areas; variability of wall area measurements substantial because of difficulties to delineate the vessel wall from surrounding soft tissue with similar densities
Ultrasound	N >5; Pearson's R 0·76 for wall[53]	N >100; 2D measurements, ICC 0·65–0·9, CV 5–20%; data vary wildly;[96] 3D measurements, intra-reader, CV 2·8–6·0% for wall;[68] 3D measurements, inter-reader, CV 4·2–7·6% for wall[68]	Widely available, accurate, and reproducible imaging method for CIMT and plaque measurements; manual measurements are more observer-dependent than semiautomatic systems; 3D ultrasound can help to improve accuracy and reliability; calcification can lead to acoustic shadowing

Lancet Neurol 2019; 18: 559–72

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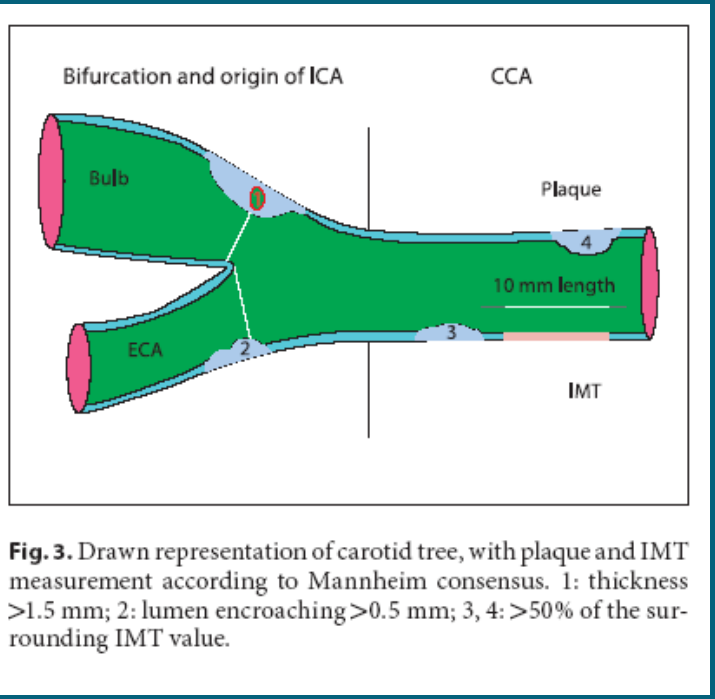


Fig. 3. Drawn representation of carotid tree, with plaque and IMT measurement according to Mannheim consensus. 1: thickness >1.5 mm; 2: lumen encroaching >0.5 mm; 3, 4: >50% of the surrounding IMT value.

Mannheim Carotid Intima-Media Thickness Consensus (2004–2006)

Cerebrovasc Dis 2007;23:75–80

ULTRASONOGRAPHIC AND BIOCHEMICAL MARKERS FOR VULNERABLE ATHEROMATOUS PLAQUE

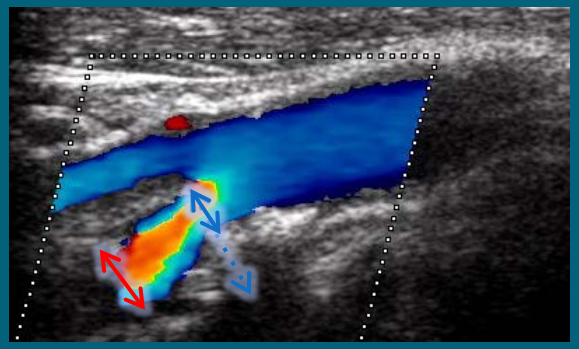
Asymptomatic Carotid Artery Atherothrombotic Stenosis risk for ipsilateral stroke :

CASANOVA, MACE, Veterans Affairs Cooperative Study, ACAS, ACST.

ULTRASONOGRAPHIC AND BIOCHEMICAL MARKERS FOR VULNERABLE ATHEROMATOUS PLAQUE

Asymptomatic Carotid Artery Atherothrombotic Stenosis : (ACRS Nicolaides AN 2005) Risk per year ipsilateral stroke (7 years).

ECST	50-69%	70-90%	90-99%
NASCET	<50%	50-80%	80-99%
Ipsilateral Stroke	1.6%	4.6%	6.5%



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ACRS Nicolaides AN Vascular 2005.

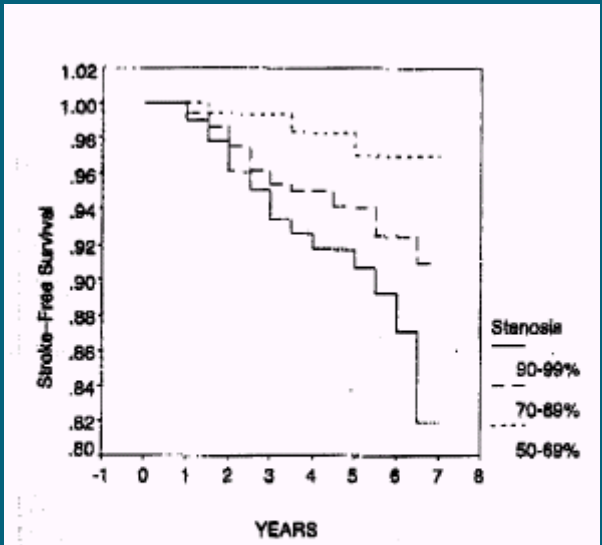

Figure 1. Kaplan-Meier estimates of the ipsilateral stroke-free survival rate in patients with mild (50–69%), moderate (70–89%), and severe (90–99%) stenosis (percent stenosis in relation to the bulb: European Carotid Stenosis Trial [ECST] method).

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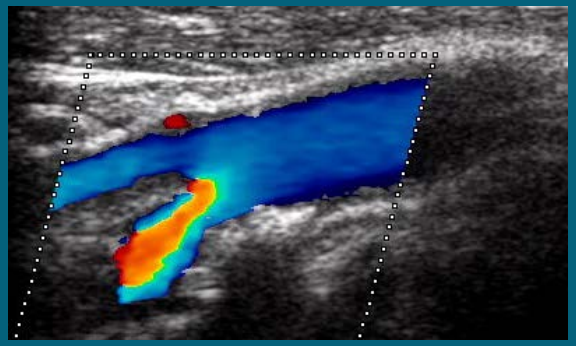
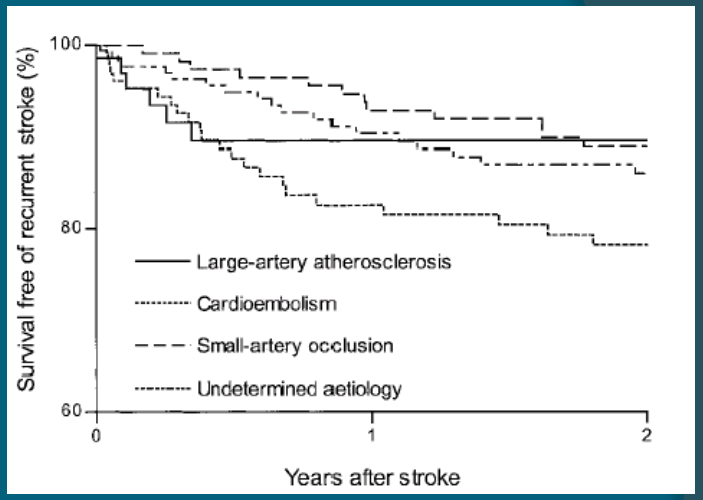
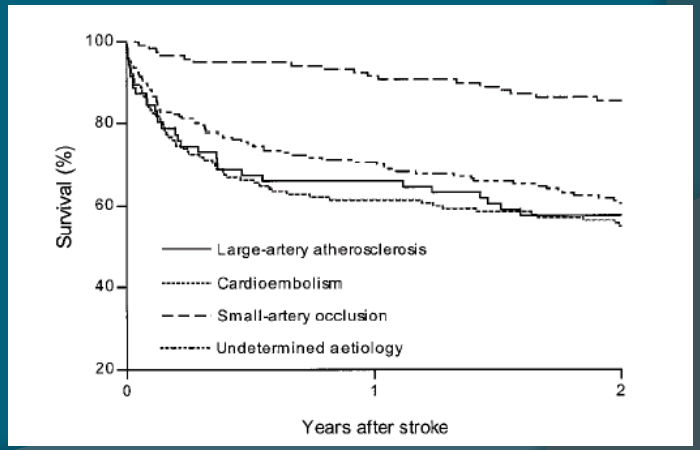
Symptomatic Atherothrombotic Carotid Artery Stenosis :

Kolominsky-Rabas PL

Stroke 2001







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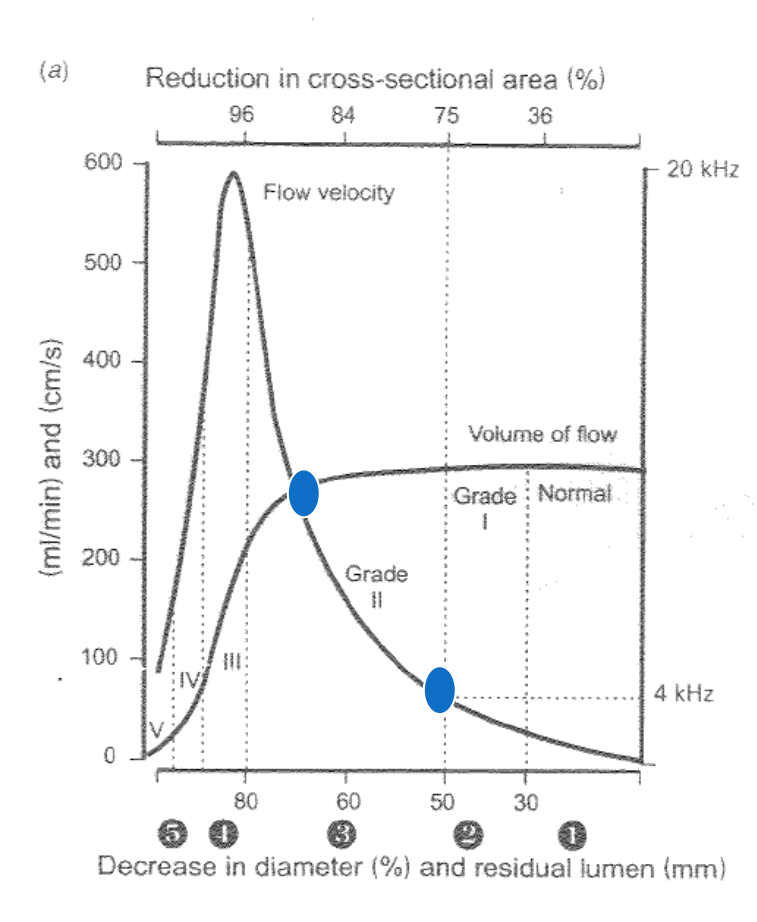

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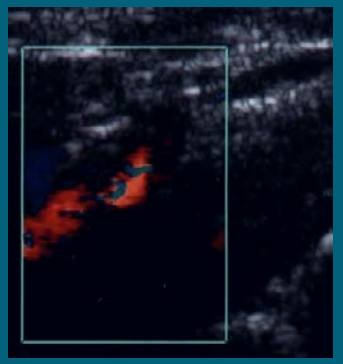
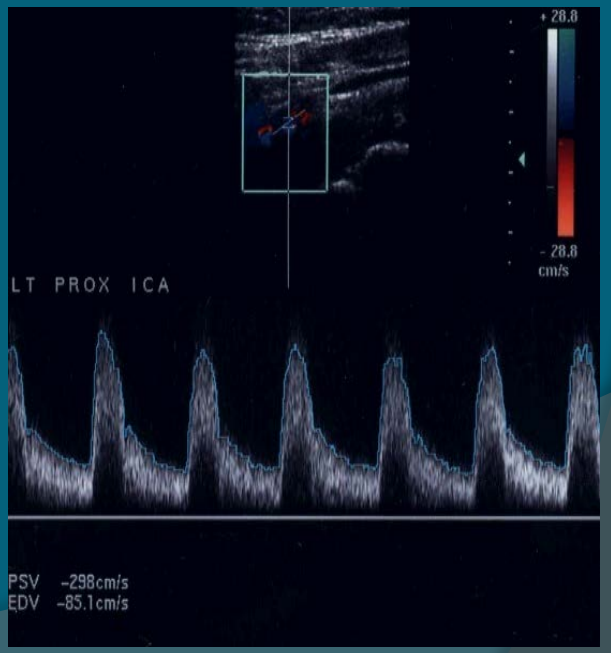
Symptomatic Atherothrombotic Carotid Artery Stenosis ≥ 70%: Systolic velocity 230 cm/seg.

Huston J 3rd et al.Redefined duplex ultrasonographic criteria for diagnosis of carotid artery stenosis. Mayo Clin Proc. 2000 Nov;75(11):1133-40.





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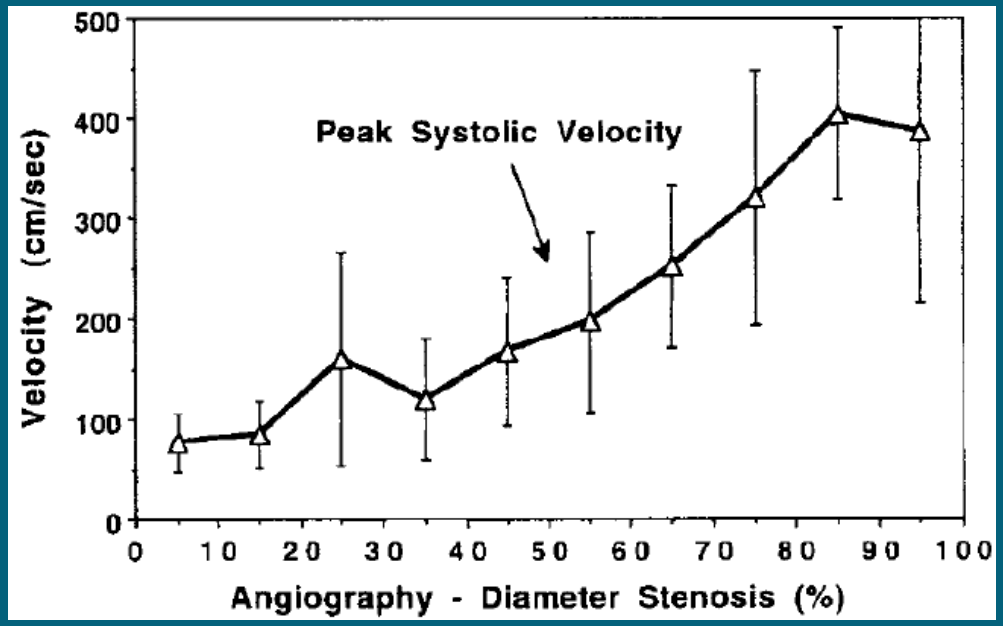

Grant EG, Duerinckx AJ, El Saden SM, et al. Ability to use duplex US to quantify internal carotid arterial stenoses: fact or fiction? Radiology 2000; 214:247–252.

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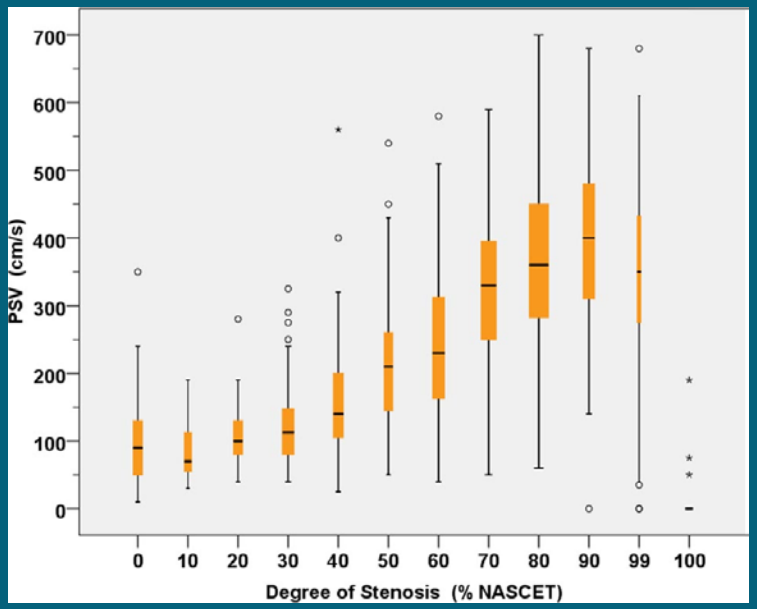

Fillinger MF, Baker RJ Jr, Zwolak RM, Musson A, Lenz JE, Mott J, et al. Carotid duplex criteria for a 60% or greater angiographic ste- nosis: variation according to equipment. *J Vasc Surg*. 1996;24: 856 – 864.

Hunink MG, Polak JF, Barlan MM, O'Leary DH. Detection and quanti- fication of carotid artery stenosis: efficacy of various Doppler velocity parameters. *AJR Am J Roentgenol*. 1993;160:619–625.

Koga M, Kimura K, Minematsu K, Yamaguchi T. Diagnosis of internal carotid artery stenosis greater than 70% with power Doppler duplex sonography. *AJNR Am J Neuroradiol*. 2001;22:413–417.

Moneta GL, Edwards JM, Papanicolaou G, Hatsukami T, Taylor LM Jr, Strandness DE Jr, et al. Screening for asymptomatic internal carotid artery stenosis: duplex criteria for discriminating 60% to 99% stenosis. *J Vasc Surg*. 1995;21:989 –994.

Neschis DG, Lexa FJ, Davis JT, Carpenter JP. Duplex criteria for deter- mination of 50% or greater carotid stenosis. *J Ultrasound Med*. 2001;20: 207–215.

ULTRASONOGRAPHIC AND BIOCHEMICAL MARKERS FOR VULNERABLE ATHEROMATOUS PLAQUE

Symptomatic Atherothrombotic Carotid Artery Stenosis : ≥ 50% y ≥ 70%

NASCET	<50%	50-69%	70-99%
ACI/ACC (SV)*	<2	2-4	≥ 4
ACIp/ACId**	<3	3-5	≥ 5
B-mode+FColor***	<50%	≥ 50%	≥ 70%

*Polak JF, Dobkin GR, O'Leary DH, Wang AM, Cutler SS. Internal carotid artery stenosis: accuracy and reproducibility of color-Doppler-assisted duplex imaging. Radiology. 1989 Dec;173(3):793-8.
**Ranke C, Creutzig A, Becker H, Trappe HJ. Standardization of carotid ultrasound: a hemodynamic method to normalize for interindividual and interequipment variability. Stroke. 1999 Feb;30(2):402-6.
***Sitzer M, Furst G, Fischer H, Siebler M, Fehlings T, Kleinschmidt A, Kahn T, Steinmetz H. Between-method correlation in quantifying internal carotid stenosis. Stroke. 1993 Oct;24(10):1513-8.

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Symptomatic Atherothrombotic Carotid Artery Stenosis :

DIRECT AND INDIRECT SIGNS Consensus:

Velocities Measures in the stenotic segment.

Carotid ICA/CCA indices.

MCA Hemodynamic parameters.

Primary and secondary collateralization.

Grading Carotid Stenosis Using Ultrasonic Methods Gerhard-Michael von Reutern, MD, PhD; Michael-Wolfgang Goertler, MD, PhD; Natan M Bornstein, MD; Massimo Del Sette, MD; David H. Evans, PhD, DSc; Andreas Hetzel, MD, PhD; Manfred Kaps, MD, PhD; Fabienne Perren, MD, PhD; Alexander Razumovky, PhD; Toshiyuki Shiogai, MD, PhD;Ekaterina Titianova, MD, PhD, DSc; Pavel Traubner, MD, PhD; Narayanaswamy Venketasubramanian, MD; Lawrence K.S. Wong, MD; Masahiro Yasaka, MD, PhD; on behalf of the Neurosonology Research Group of the World Federation of Neurology ***Stroke*. 2012;43:916-921**

ULTRASONOGRAPHIC AND BIOCHEMICAL MARKERS FOR VULNERABLE ATHEROMATOUS PLAQUE

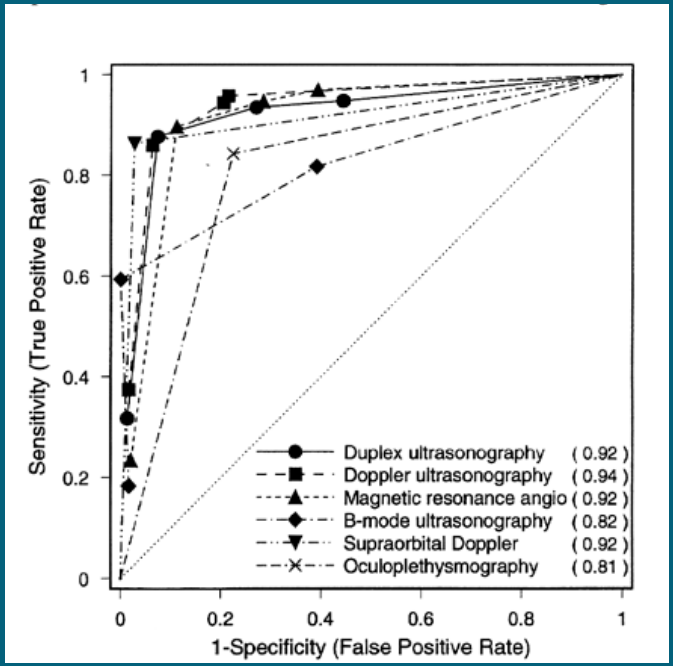
Degree of Stenosis as Defined by NASCET (%)	Grading of Internal Carotid Stenosis						
	10–40	50	60	70	80	90	Occlusion
Main criteria							
1. B-mode image, diameter	Applicable	Possibly applicable					Imaging of occluded artery
2. Color Doppler image	Plaque delineation	Flow	Flow	Flow	Flow	Flow	Absence of flow
3. PSV threshold (cm/s)		125		230		NA	NA
4a. PSV average (cm/s)	≤160	210	240	330	370	Variable	NA
4b. PSV poststenotic (cm/s)				≥50	<50	<30	NA
5. Collateral flow (periorbital arteries or circle of Willis)				Possible	Present	Present	Present
Additional criteria							
6. Prestenotic flow (diastole) (CCA)				Possibly reduced	Reduced	Reduced	Reduced
7. Poststenotic flow disturbances (severity and length)		Moderate	Pronounced	Pronounced	Pronounced	Variable	NA
8. End-diastolic flow velocity in the stenosis (cm/s)			<100	>100		Variable	NA
9. Carotid ratio ICA/CCA	<2	≥2	≥2	>4	>4	Variable	NA

Grading Carotid Stenosis Using Ultrasonic Methods Gerhard-Michael von Reutern, MD, PhD; Michael-Wolfgang Goertler, MD, PhD; Natan M Bornstein, MD; Massimo Del Sette, MD; David H. Evans, PhD, DSc; Andreas Hetzel, MD, PhD; Manfred Kaps, MD, PhD; Fabienne Perren, MD, PhD; Alexander Razumovky, PhD; Toshiyuki Shiogai, MD, PhD;Ekaterina Titianova, MD, PhD, DSc; Pavel Traubner, MD, PhD; Narayanaswamy Venketasubramanian, MD; Lawrence K.S. Wong, MD; Masahiro Yasaka, MD, PhD; on behalf of the Neurosonology Research Group of the World Federation of Neurology ***Stroke*. 2012;43:916-921**

ULTRASONOGRAPHIC AND BIOCHEMICAL MARKERS FOR VULNERABLE ATHEROMATOUS PLAQUE

Symptomatic Atherothrombotic Carotid Artery Stenosis ≥ 70%

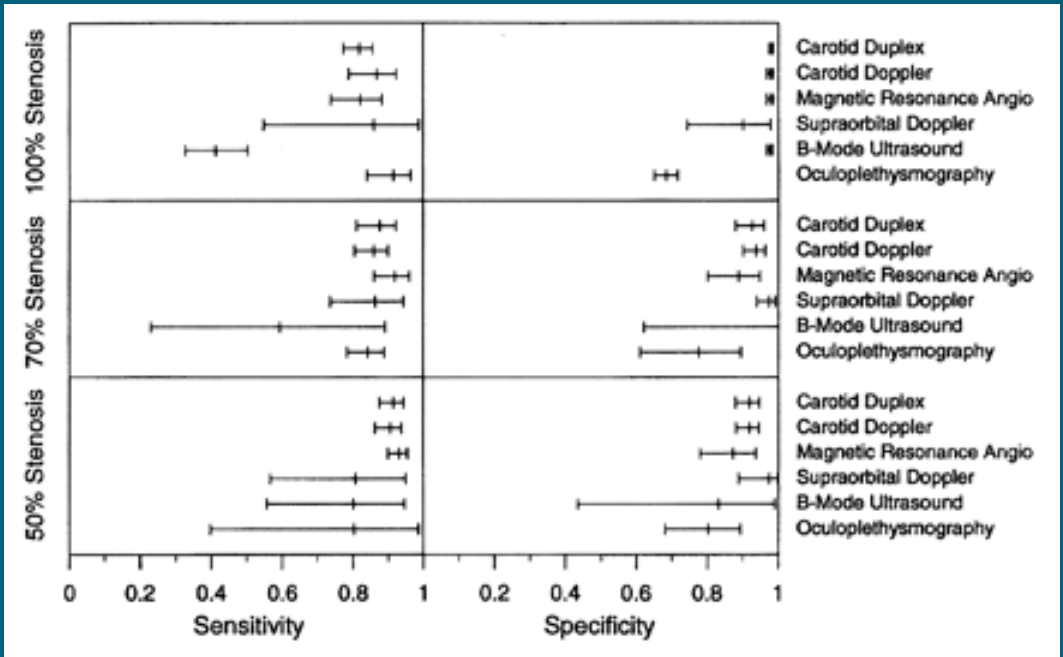
ROC curve:



Blakeley DD, Oddone EZ, Hasselblad V, Simel DL, Matchar DB.Noninvasive carotid artery testing. A meta-analytic review.Ann Intern Med. 1995 Mar 1;122(5):360-7.

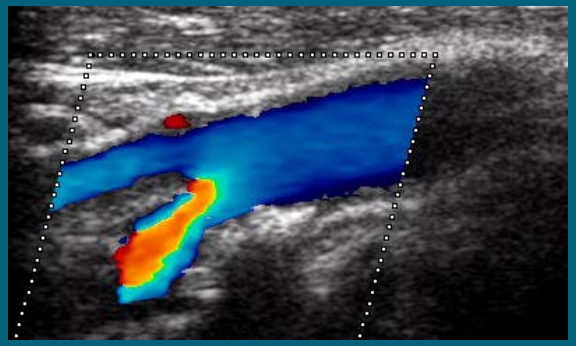
ULTRASONOGRAPHIC AND BIOCHEMICAL MARKERS FOR VULNERABLE ATHEROMATOUS PLAQUE

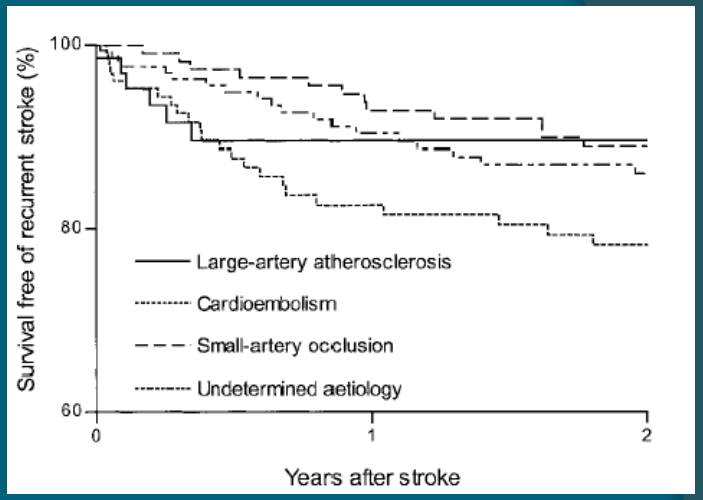
Symptomatic Atherothrombotic Carotid Artery Stenosis ≥ 70%
Sensitivity y Especificity:

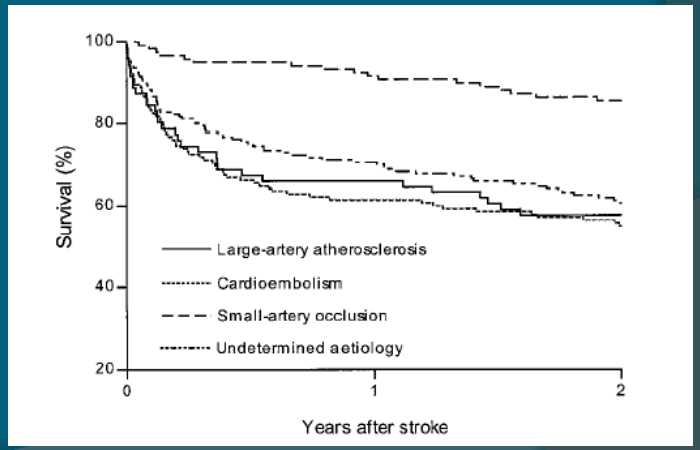


Blakeley DD, Oddone EZ, Hasselblad V, Simel DL, Matchar DB. Noninvasive carotid artery testing. A meta-analytic review.Ann Intern Med. 1995 Mar 1;122(5):360-7.

Kolominsky-Rabas PL
Stroke 2001







ULTRASONOGRAPHIC AND BIOCHEMICAL MARKERS FOR VULNERABLE ATHEROMATOUS PLAQUE

Biomarkers for Vulnerable plaque.

1-Arterial Stenosis.

2 -Plaque Area/Volume.

3 -Plaque Surface.

4 -Plaque Characterization.

ULTRASONOGRAPHIC AND BIOCHEMICAL MARKERS FOR VULNERABLE ATHEROMATOUS PLAQUE

	Validation studies (imaging method *vs* histopathology)	Reproducibility studies	Comments and limitations
Quantitative measurements: lumen and vessel wall			
MRI	N >10; Pearson's R 0·84 for wall, 0·81 for lumen area[22]	N >5; intra-reader, ICC 0·99 for lumen, ICC 0·98 for wall, CV 3·2–4·1% for lumen, CV 3·4–5·1% for wall;[32] inter-reader, ICC 0·98–0·99 for lumen, ICC 0·84–0·90 for wall, CV 5·3% for lumen, CV 7·9% for wall;[32] scan-rescan, ICC 0·99 for lumen, ICC 0·97 for wall, CV 4·3% for lumen, CV 5·8% for wall[67]	Highly accurate imaging method with excellent reproducibility; wall and lumen area measurements by MRI are ideally suited for cross-sectional and longitudinal studies; measurement errors can be used for power calculation for clinical trials[67]
CT	N >10; Pearson's R 0·85 for wall[24]	N >5: intrareader, CV 3% for lumen, CV 8% for wall;[24] inter-reader, CV 4% for lumen, CV 19% for wall[24]	Calcification can lead to overestimation of wall areas; variability of wall area measurements substantial because of difficulties to delineate the vessel wall from surrounding soft tissue with similar densities
Ultrasound	N >5; Pearson's R 0·76 for wall[53]	N >100; 2D measurements, ICC 0·65–0·9, CV 5–20%; data vary wildly;[96] 3D measurements, intra-reader, CV 2·8–6·0% for wall;[68] 3D measurements, inter-reader, CV 4·2–7·6% for wall[68]	Widely available, accurate, and reproducible imaging method for CIMT and plaque measurements; manual measurements are more observer-dependent than semiautomatic systems; 3D ultrasound can help to improve accuracy and reliability; calcification can lead to acoustic shadowing

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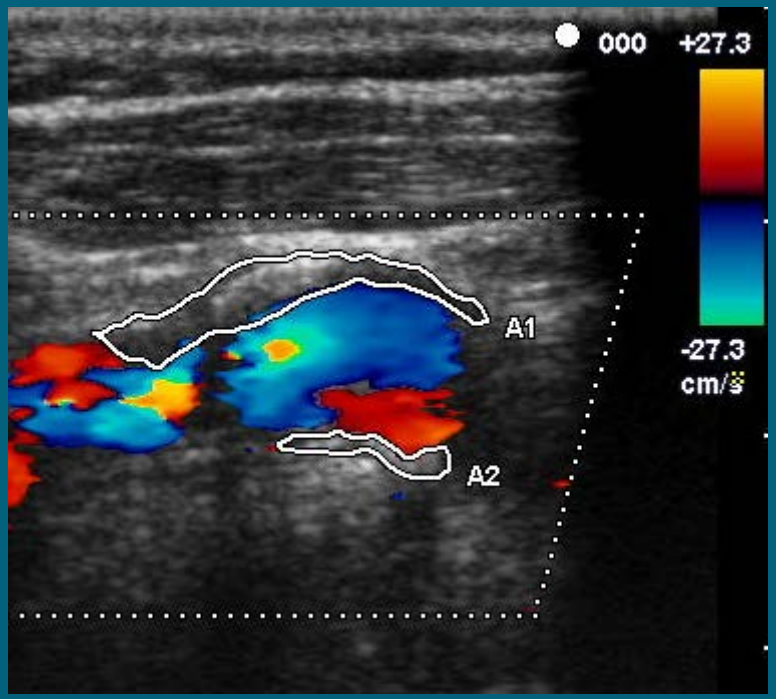
ULTRASONOGRAPHIC AND BIOCHEMICAL MARKERS FOR VULNERABLE ATHEROMATOUS PLAQUE

Plaque Area.



Spence JD Stroke 2002

ULTRASONOGRAPHIC AND BIOCHEMICAL MARKERS FOR VULNERABLE ATHEROMATOUS PLAQUE

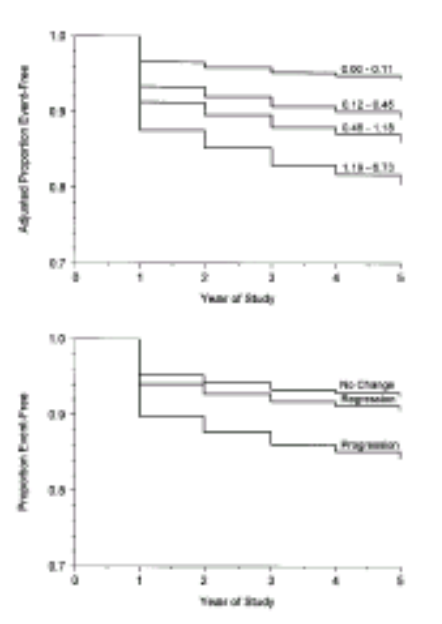
TABLE 2. Unadjusted and Adjusted 5-Year Risks and Relative Risks of Combined Outcome of Stroke, Myocardial Infarction, and Vascular Death by Quartile of Carotid Plaque Area (cm²)

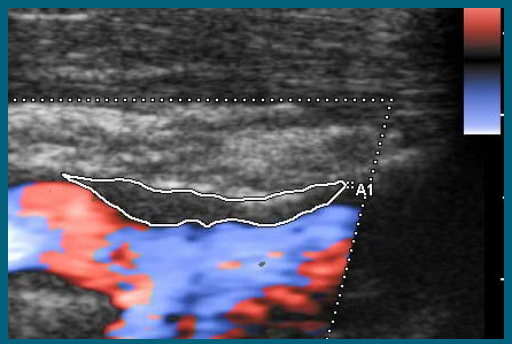
Plaque Area	5-Year Risk (%)	Relative Risk (95% CI)	P Value
Unadjusted			
0.00–0.11	6.3	1.0*	
0.12–0.45	11.3	1.8 (1.1 to 3.0)	0.02
0.46–1.18	13.2	2.1 (1.2 to 3.5)	0.004
1.19–6.73	19.1	3.0 (1.8 to 4.9)	<0.001
Adjusted†			
0.00–0.11	5.6	1.0*	
0.12–0.45	10.7	1.9 (1.1 to 3.3)	0.02
0.46–1.18	13.9	2.5 (1.4 to 4.4)	0.001
1.19–6.73	19.5	3.5 (1.8 to 6.7)	<0.001

*Reference category.
†Adjusted for all baseline patient characteristics listed in Table 1.

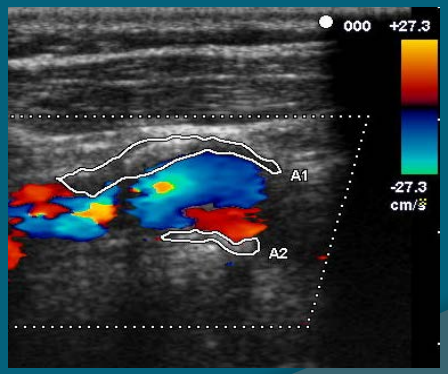
The plaque area and its progression predicts the vascular atherothrombotic event.

Spence JD Stroke 2002.





Area Plaque
0.24 cm2



Area Plaque
0.50 cm2

ULTRASONOGRAPHIC AND BIOCHEMICAL MARKERS FOR VULNERABLE ATHEROMATOUS PLAQUE

Biomarkers for Vulnerable plaque.

1-Arterial Stenosis.

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3 -Plaque Surface.

4 -Plaque Characterization.

ULTRASONOGRAPHIC AND BIOCHEMICAL MARKERS FOR VULNERABLE ATHEROMATOUS PLAQUE

	Validation studies (imaging method *vs* histopathology)	Reproducibility studies	Comments and limitations
Ulceration			
MRA	N >10; sensitivity 80%; specificity 82%[23]	Good reliability	Good for ulcer detection; contrast-enhanced MRA superior to non-contrast-enhanced MRA
CTA	N >10; Cohen's kappa 0·86 for ulcer detection[25]	Good reliability	Excellent for ulcer detection; superior to contrast-enhanced MRA because of better spatial resolution
Ultrasound	N >10; sensitivity 33–75%; specificity 33–92%[101]	N >10; large variability; operator-dependent	Ultrasound is not the imaging method of choice for ulcer detection; detection can be improved with contrast-enhanced ultrasound and 3D methods

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ULTRASONOGRAPHIC AND BIOCHEMICAL MARKERS FOR VULNERABLE ATHEROMATOUS PLAQUE

	Validation studies (imaging method *vs* histopathology)	Reproducibility studies	Comments and limitations
Fibrous cap			
MRI	Identification of fibrous cap: N >5; Cohen's kappa 0·74–0·85 for intact *vs* ruptured fibrous cap;[23] quantification of fibrous cap: N >2; Pearson´s R 0·8 for area measurements[31]	N >5; intra-reader, Cohen's kappa 0·33–0·96;[29,30] inter-reader, Cohen´s kappa 0·26–0·78;[29,30] N>1; intrareader, ICC 0·72 for fibrous cap area;[31] inter-reader, ICC 0·78 for fibrous cap area[31]	MRI can identify and quantify the fibrous cap with good correlation to histopathology; contrast-enhanced T1-weighted sequences improves delineation of fibrous cap; reproducibility varies wildly; the best sequence to detect the fibrous cap is uncertain
CT	Identification and quantification of fibrous cap not feasible	Not applicable	The fibrous cap cannot be differentiated from soft plaque component
Ultrasound	N >5; sensitivity 73%, specificity 67%[101]	N >10; large variability, operator-dependent	Not the imaging modality of choice to assess the fibrous cap

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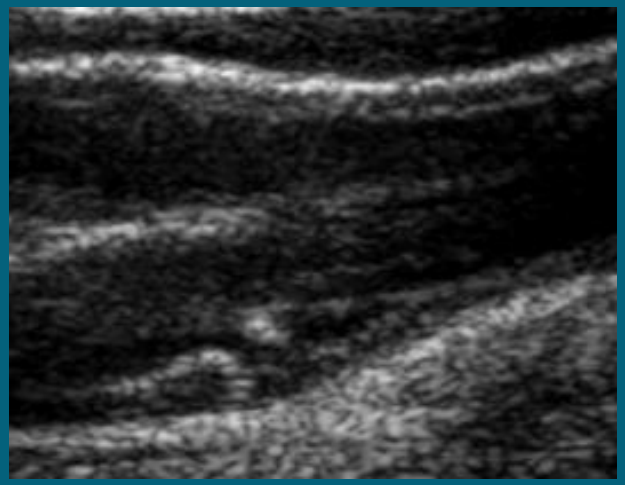
ULTRASONOGRAPHIC AND BIOCHEMICAL MARKERS FOR VULNERABLE ATHEROMATOUS PLAQUE

Plaque surface

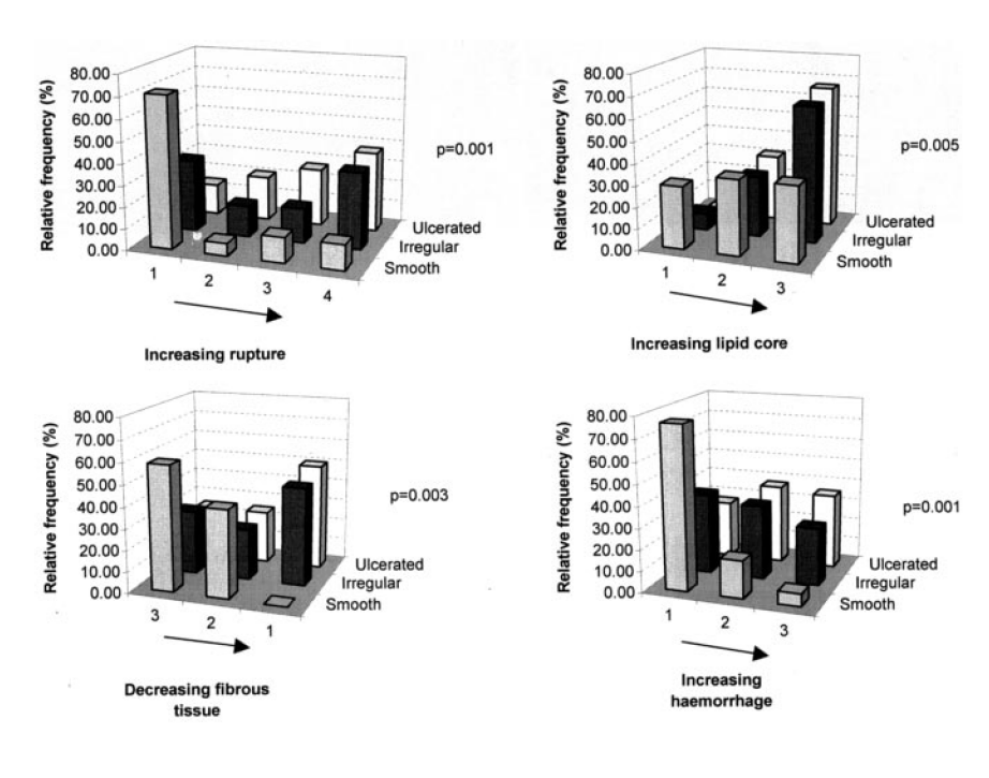


Irregular surface : 0,4-2 mm

Plaque ulceration : >2 mm

Kern R. Stroke 2004;35;870-875

ULTRASONOGRAPHIC AND BIOCHEMICAL MARKERS FOR VULNERABLE ATHEROMATOUS PLAQUE



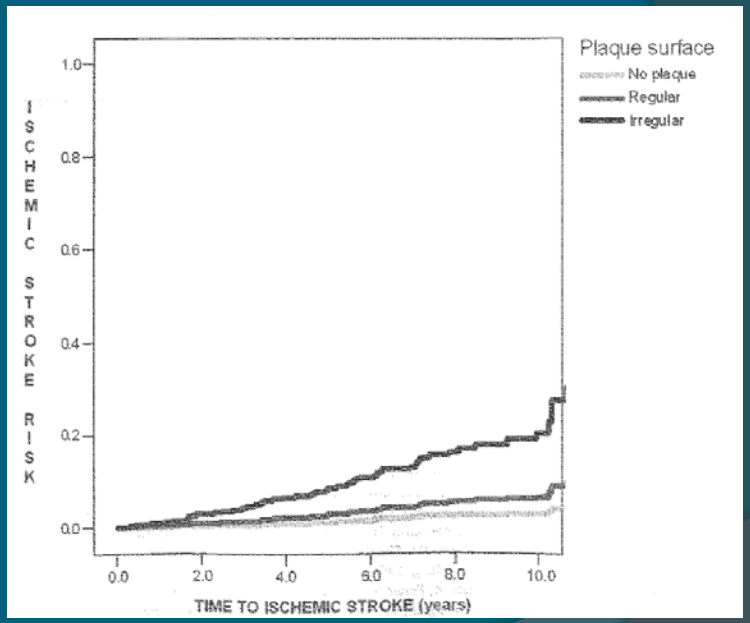
Lovett JK, Gallagher PJ, Hands LJ, Walton J, Rothwell PM. Histological correlates of carotid plaque surface morphology on lumen contrast imaging. Circulation. 2004 Oct 12;110(15):2190-7.

ULTRASONOGRAPHIC AND BIOCHEMICAL MARKERS FOR VULNERABLE ATHEROMATOUS PLAQUE

Prabhakaram S. Stroke 2006;37:2696-2701. Plaque surface.

Prospective study accumulated 5 years stroke risk 1.3% (without plaque), 3% (regular plaque) , 8.5% (irregular plaque).

The stroke risk increment is independent from vascular risk factors, stenosis and plaque diameter .
RR 3.1 (IC 95% 1.1-8.5).



ULTRASONOGRAPHIC AND BIOCHEMICAL MARKERS FOR VULNERABLE ATHEROMATOUS PLAQUE

Biomarkers for Vulnerable plaque.

1-Arterial Stenosis.

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3 -Plaque Surface.

4 -Plaque Characterization.

ULTRASONOGRAPHIC AND BIOCHEMICAL MARKERS FOR VULNERABLE ATHEROMATOUS PLAQUE

Atheroma plaque characterizacion :

Homogeneus vs heterogeneous plaque and ipsilateral stroke risk. :

Several studies failed to demonstrate it:.
Bassiouny HS 1989.
Leen EJ 1990.
Lennihan L 1987.

ULTRASONOGRAPHIC AND BIOCHEMICAL MARKERS FOR VULNERABLE ATHEROMATOUS PLAQUE

	Validation studies (imaging method *vs* histopathology)	Reproducibility studies	Comments and limitations
Identification of plaque components (present *vs* absent)			
MRI	N >100; Cohen's kappa 0·52–0·95 for IPH, 0·73–0·98 for LRNC, 0·65–0·75 for calcification;[23] sensitivity 77–100% for IPH, 82–100% for LRNC;[23] specificity 74–100% for IPH, 65–100% for LRNC[23]	N>10; intra-reader, Cohen's kappa 0·82–0·90 for IPH, 0·69 for LRNC, 0·8 for calcification;[30] inter-reader, Cohen's kappa 0·62–0·75 for IPH, 0·58 for LRNC, 0·74 for calcification[23,30]	Best imaging method for detection of IPH and LRNC; good reproducibility; extensively validated
CT	N >10; excellent identification of calcification, debated for all other components	N>3; results and reproducibility vary wildly, small studies only	Best imaging method for detection of calcification; overlap of tissue densities for LRNC, IPH, and fibrous tissue
Ultrasound	N >10; overlap of echolucency between LRNC, fibrous tissue, and IPH[100]	N>10; no consistent data available, results vary wildly	Can distinguish between echolucent and echorich plaques but is unable to differentiate between the main plaque components (eg, IPH and LRNC)

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ULTRASONOGRAPHIC AND BIOCHEMICAL MARKERS FOR VULNERABLE ATHEROMATOUS PLAQUE

Geroulakos G Br J Surg 1993.

Tipo I Uniformly hypoechoic.

Tipo II Fundamentally hypoechoic

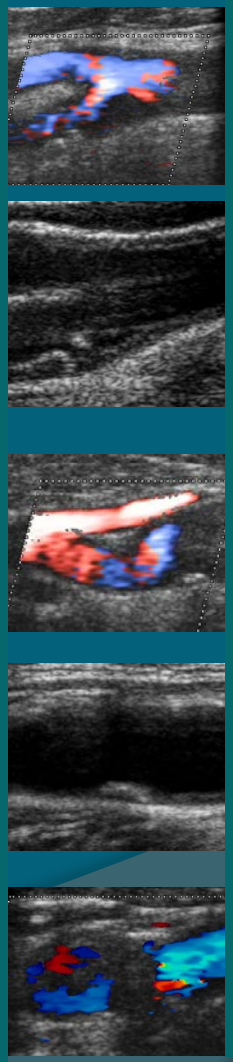
(>50% area hypoecoic)

Tipo III Fundamentally Isoechogenic

(>50% area is hyper-isoecogenic).

Tipo IV Uniformly hyper-isoecogenic.

Tipo V No clasificable: Calcificada.



ULTRASONOGRAPHIC AND BIOCHEMICAL MARKERS FOR VULNERABLE ATHEROMATOUS PLAQUE

Geroulakos G Br J Surg 1993. Topakian ACES Neurology 2011

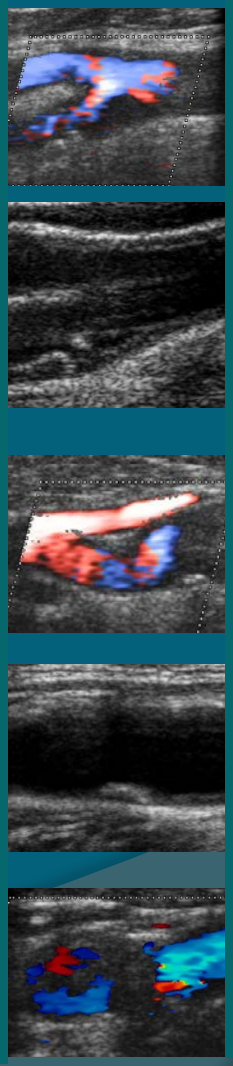
Tipo I Uniformly hypoechoic.

Tipo II Fundamentally hypoechoic (>50% area hypoecoic)

Tipo III Fundamentally Isoechogenic (>50% area is hyper-isoecogenic).

Tipo IV Uniformly hyper-isoecogenic.

Tipo V No clasificable: Calcificada.



ULTRASONOGRAPHIC AND BIOCHEMICAL MARKERS FOR VULNERABLE ATHEROMATOUS PLAQUE

Geroulakos G Br J Surg 1993. Topakian ACES Neurology 2011

Tipo I Uniformemente hipoecoica

Tipo II Fundamentalmente hipoecoica

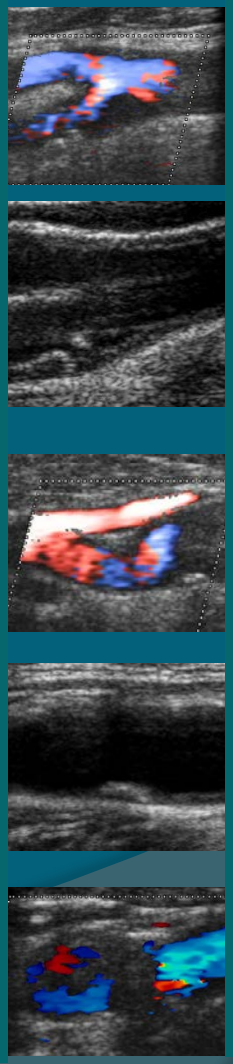
(>50% del área es hipoecogénica)

Tipo III Fundamentalmente ecogénica

(>50% del área es hiper-isoecogénica)

Tipo IV Homogénea

Tipo V No clasificable: Calcificada.



ULTRASONOGRAPHIC AND BIOCHEMICAL MARKERS FOR VULNERABLE ATHEROMATOUS PLAQUE

	Validation studies (imaging method *vs* histopathology)	Reproducibility studies	Comments and limitations
Quantitative measurements: plaque components			
MRI	N >10; Pearson's R 0·75 for LRNC, 0·74 for calcification, 0·66 for IPH[22]	N >5: intra-reader, ICC 0·89–0·99 for LRNC,[22,32] ICC 0·9 for calcification,[22] ICC 0·74 for haemorrhage,[22] CV 8·7% for LRNC;[67] inter-reader, ICC 0·89–0·93 for LRNC,[22,32] ICC 0·9 for calcification,[22] ICC 0·74 (95% CI 0·45–0·89) for haemorrhage,[22] CV 17·6% for LRNC;[67] scan-rescan, ICC 0·99 for LRNC, ICC 0·95 for calcification, CV 11·1% for LRNC, CV 30·8% for calcification[67]	Optimum reproducibility for plaque components; contrast-enhanced T1 sequences improve delineation of LRNC; plaque component measurements by MRI are ideally suited for cross-sectional and longitudinal studies; measurement errors can be used for power calculation for clinical trials[67]
CT	N >5; Pearson's R 0·86 for calcification, 0·48 for LRNC; data for IPH not available	N >5: intrareader, CV 15% for LRNC, 8% for calcification; inter-reader, CV 40% for LRNC, 8% for calcification[24]	Only tissue component that can be reliably identified is calcification; accurate and reliable quantification of IPH and LRNC not feasible; automated segmentation might improve performance
Ultrasound	N >5; accurate quantification of plaque components not feasible	N >5; reliable quantification of plaque components not feasible	Not useful for quantification of LRNC, IPH, and calcification

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ULTRASONOGRAPHIC AND BIOCHEMICAL MARKERS FOR VULNERABLE ATHEROMATOUS PLAQUE

ACRS Nicolaides AN Vascular 2005

Grey Scale Measurement Normalization GSM

Table 3. Agreement between Plaque Classification before and after Image Normalization (kappa = 0.22)

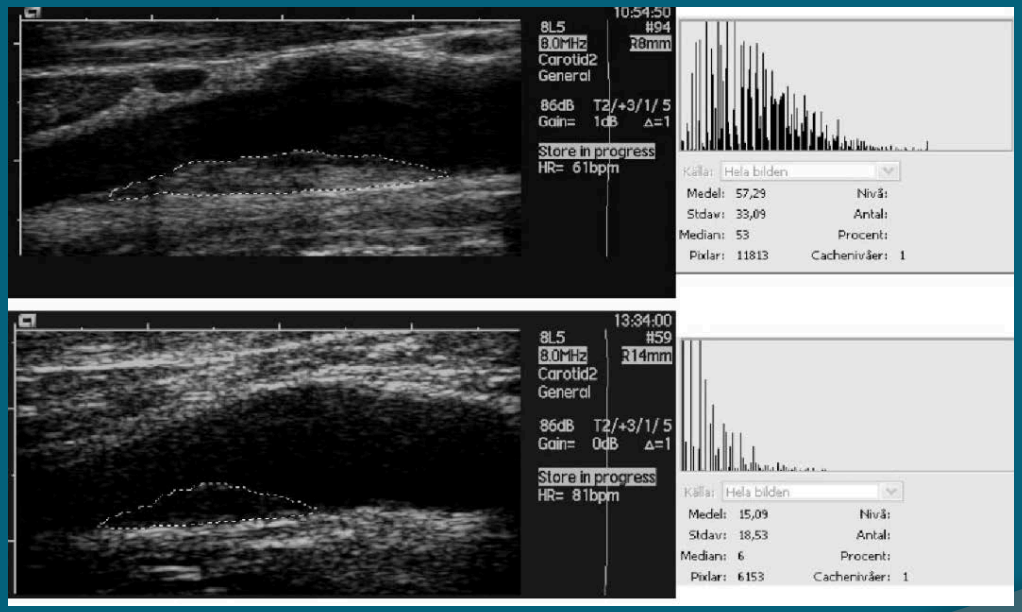
	Plaque Type after Image Normalization, *n* (%)					
	1	2	3	4	5	Total
Plaque type before image normalization						
1	44 (34)	54 (41)	22 (17)	11 (7)	0	131 (100)
2	23 (8)	148 (51)	97 (34)	16 (6)	4 (1.4)	288 (100)
3	10 (3)	68 (21)	173 (54)	54 (17)	14 (4)	319 (100)
4	0	35 (21)	62 (37)	57 (34)	12 (7)	166 (100)
5	0	27 (19)	96 (51)	47 (25)	18 (10)	188 (100)
Total	77 (7)	332 (31)	450 (41)	185 (17)	48 (6)	1,092 (100)

ULTRASONOGRAPHIC AND BIOCHEMICAL MARKERS FOR VULNERABLE ATHEROMATOUS PLAQUE

ACRS Nicolaides AN Vascular 2005

GSM Carotid Plaque Normalization:

Ipsilateral stroke risk

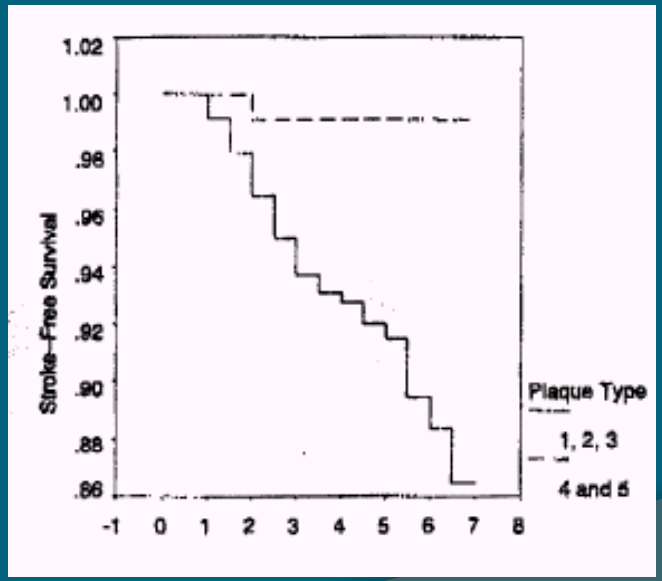


ULTRASONOGRAPHIC AND BIOCHEMICAL MARKERS FOR VULNERABLE ATHEROMATOUS PLAQUE

ACRS Nicolaides AN Vascular 2005

GSM Carotid Plaque Normalization:

Ipsilateral stroke risk



ULTRASONOGRAPHIC AND BIOCHEMICAL MARKERS FOR VULNERABLE ATHEROMATOUS PLAQUE

	Validation studies (imaging method *vs* histopathology)	Reproducibility studies	Comments and limitations
Plaque inflammation and neovascularisation			
DCE-MRI	N >10; Pearson's R 0·75 for k-trans *vs* macrophage content, 0·68 for v(p) *vs* neovasculature[40]	N >3; no sufficient data; reported reproducibility varies wildly; dependent on pharmacokinetic model and on type of contrast agent	Quantification of inflammation and neovessel density feasible; no consensus on best technique; results are not comparable across centres; only for research studies
CT	N <3; Pearson´s R 0·53 for carotid plaque enhancement *vs* microvessel density[49]	N <3; no significant difference between observers[49]	Requires precontrast and post-contrast scan (increased radiation); only for research
Contrast-enhanced ultrasound	N >10; Pearson's R* 0·88 for neovascularisation, 0·78 for inflammation[46]	N=5; no reliable and consistent data available	Use of microbubbles allows detection and quantification of neovascularisation and inflammation; no clear consensus on assessment; method operator-dependent
^{18}F-FDG PET and CT	N >10; Pearson's R 0·70 for FDG uptake *vs* macrophage content, 0·85 for FDG uptake (mean tissue to background ratio) *vs* CD68 as marker of inflammation[38]	N >10; intra-reader, ICC 0·93–0·98;[37] inter-reader, ICC 0·71–0·92;[37] N >1; scan-rescan, ICC 0·79–0·92[37]	Best imaging method for accurate and reliable detection of plaque inflammation; main disadvantage is the high radiation dose; has the same limitation for other plaque components as CT alone

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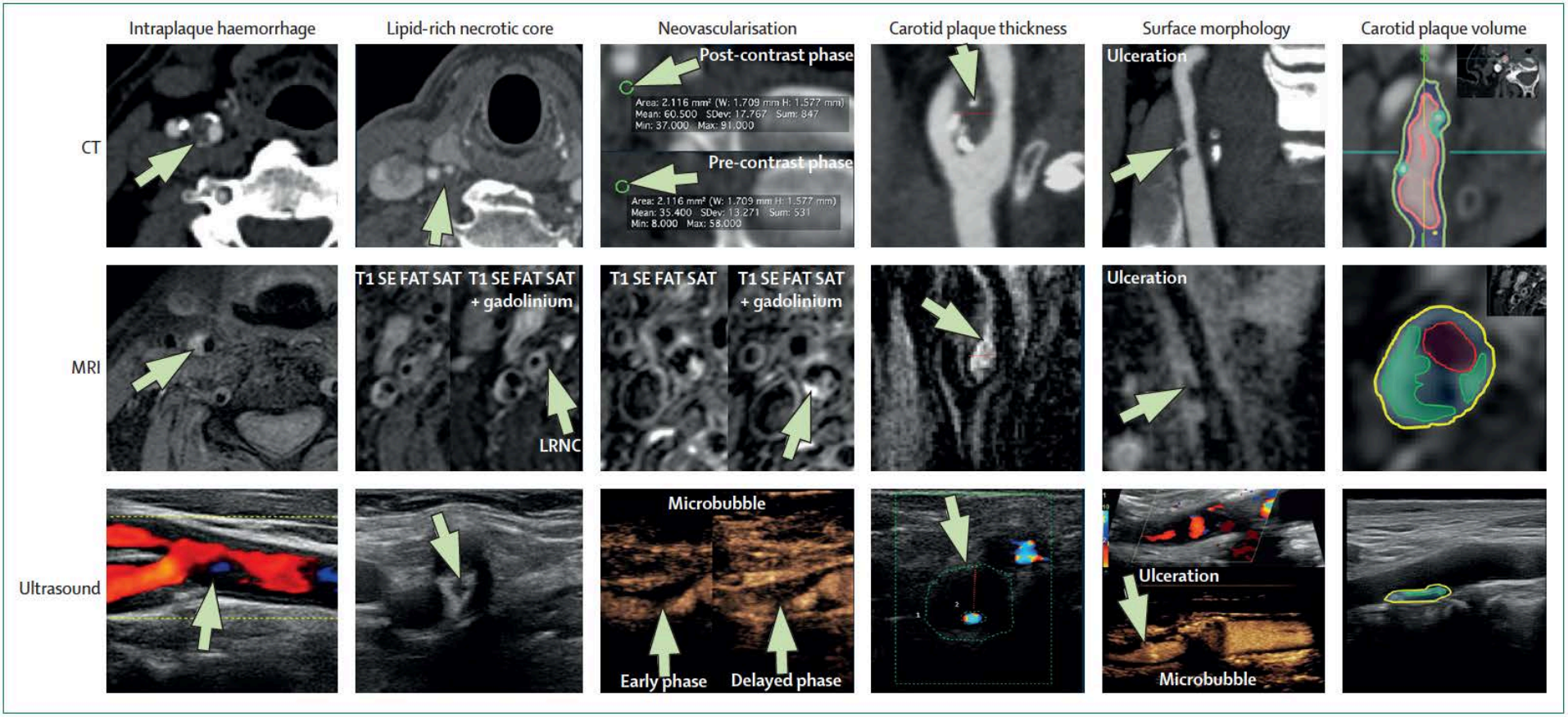
ULTRASONOGRAPHIC AND BIOCHEMICAL MARKERS FOR VULNERABLE ATHEROMATOUS PLAQUE



Imaging biomarkers of vulnerable carotid plaques for stroke risk prediction and their potential clinical implications

Luca Saba, Tobias Saam, H Rolf Jäger, Chun Yuan, Thomas S Hatsukami, David Saloner, Bruce A Wasserman, Leo H Bonati, Max Wintermark

Lancet Neurol 2019; 18: 559–72

ULTRASONOGRAPHIC AND BIOCHEMICAL MARKERS FOR VULNERABLE ATHEROMATOUS PLAQUE

CONCLUSIONS I:

Meta-analyses of prospective studies have established positive associations of circulating levels of **fibrinogen, von Willebrand factor (VWF), fibrin D-dimer, and tissue plasminogen activator** with risk of coronary heart disease (CHD) and stroke.

Lowe G, Thromb Haemost 2014

Proteomic and metabolomic data need to be implemented with genomic data in multicentre trials.

ULTRASONOGRAPHIC AND BIOCHEMICAL MARKERS FOR VULNERABLE ATHEROMATOUS PLAQUE

Conclusions II:

The morphologic markers for plaque vulnerability identified in prospective studies are:

- Arterial stenosis. Grade of arterial stenosis.
- Plaque measurement:
  Area/volume. Diameter in aortic arch.
- Plaque surface.
- Hypoechoic component.

ULTRASONOGRAPHIC AND BIOCHEMICAL MARKERS FOR VULNERABLE ATHEROMATOUS PLAQUE

	Imaging methods used	Study design	Primary endpoint	Participants enrolled (n)	Completion year*	Recruitment status
PARISK (Plaque at Risk; NCT01208025)	MRI	Prospective cohort	The main objective is to show whether imaging characteristics assessed at baseline can predict clinical events in patients with 30–69% (moderate) symptomatic carotid stenosis	244	2017	Completed
CAPIAS (CArotid Plaque Imaging in Acute Stroke; NCT01284933)	MRI	Prospective cohort	To determine the frequency, characteristics, and outcomes of vulnerable carotid artery plaques ipsilateral to an acute ischaemic stroke or transient ischaemic attack in the territory of the internal carotid artery	300	2019	Recruiting
CAIN (MRI Characterization of Carotid Plaque and Prediction of End-organ and Clinical Outcomes; NCT01440296)	MRI	Prospective cohort	To accurately characterise carotid plaque morphology in non-surgical patients with mild-to-moderate (50–70%) carotid disease and assessment of ischaemic brain disease	500	2018	Recruiting
SCAPIS (Swedish CArdioPulmonary bioImage Study; NCT0304920)	Ultrasound, CT, MRI	Prospective cohort	To use advanced imaging methods to examine atherosclerosis in the coronary and carotid arteries together with information obtained by proteomics, metabolomics, or genomics technologies to improve risk prediction for cardiovascular disease	30 000	2018	Recruiting
SRSP (Smart Risk Stroke Prediction by MRI; NCT00860184)	MRI	Prospective cohort	To determine whether the magnetic resonance SmartRisk module is effective at stratifying risk of a carotid-related cerebrovascular event in patients with asymptomatic 50–79% (moderate) carotid stenosis	300	2018	Recruiting
ROTTERDAM Scan Study	MRI	Prospective cohort	To determine how carotid plaque components and which cardiovascular risk factors are associated with the development of cerebrovascular events	3392	Not specified	Recruiting
ACTRIS (Endarterectomy combined with OMT *vs* OMT alone in patients with asymptomatic severe atherosclerotic carotid artery stenosis at higher-than-average risk of ipsilateral stroke; NCT02841098)	MRI	Randomised controlled trial	To determine whether carotid surgery combined with OMT improves long-term survival free of ipsilateral stroke in patients with asymptomatic carotid stenosis at higher-than-average risk of ipsilateral stroke when compared with OMT alone	700	2024	Not yet recruiting
ECST-2 (European Carotid Surgery Trial 2; ISRCTN97744893)	MRI	Randomised controlled trial	To determine whether in patients with carotid stenosis with low and intermediate risk for stroke, OMT alone is as effective in the long-term prevention of cerebral infarction and myocardial infarction as is revascularisation and OMT combined	200	2022	Not yet recruiting

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ULTRASONOGRAPHIC AND BIOCHEMICAL MARKERS FOR VULNERABLE ATHEROMATOUS PLAQUE

	Imaging methods used	Study design	Primary endpoint	Participants enrolled (n)	Completion year*	Recruitment status
PARISK (Plaque at Risk; NCT01208025)	MRI	Prospective cohort	The main objective is to show whether imaging characteristics assessed at baseline can predict clinical events in patients with 30–69% (moderate) symptomatic carotid stenosis	244	2017	Completed
CAPIAS (CArotid Plaque Imaging in Acute Stroke; NCT01284933)	MRI	Prospective cohort	To determine the frequency, characteristics, and outcomes of vulnerable carotid artery plaques ipsilateral to an acute ischaemic stroke or transient ischaemic attack in the territory of the internal carotid artery	300	2019	Recruiting
CAIN (MRI Characterization of Carotid Plaque and Prediction of End-organ and Clinical Outcomes; NCT01440296)	MRI	Prospective cohort	To accurately characterise carotid plaque morphology in non-surgical patients with mild-to-moderate (50–70%) carotid disease and assessment of ischaemic brain disease	500	2018	Recruiting
SCAPIS (Swedish CArdioPulmonary bioImage Study; NCT0304920)	Ultrasound, CT, MRI	Prospective cohort	To use advanced imaging methods to examine atherosclerosis in the coronary and carotid arteries together with information obtained by proteomics, metabolomics, or genomics technologies to improve risk prediction for cardiovascular disease	30 000	2018	Recruiting
SRSP (Smart Risk Stroke Prediction by MRI; NCT00860184)	MRI	Prospective cohort	To determine whether the magnetic resonance SmartRisk module is effective at stratifying risk of a carotid-related cerebrovascular event in patients with asymptomatic 50–79% (moderate) carotid stenosis	300	2018	Recruiting
ROTTERDAM Scan Study	MRI	Prospective cohort	To determine how carotid plaque components and which cardiovascular risk factors are associated with the development of cerebrovascular events	3392	Not specified	Recruiting
ACTRIS (Endarterectomy combined with OMT *vs* OMT alone in patients with asymptomatic severe atherosclerotic carotid artery stenosis at higher-than-average risk of ipsilateral stroke; NCT02841098)	MRI	Randomised controlled trial	To determine whether carotid surgery combined with OMT improves long-term survival free of ipsilateral stroke in patients with asymptomatic carotid stenosis at higher-than-average risk of ipsilateral stroke when compared with OMT alone	700	2024	Not yet recruiting
ECST-2 (European Carotid Surgery Trial 2; ISRCTN97744893)	MRI	Randomised controlled trial	To determine whether in patients with carotid stenosis with low and intermediate risk for stroke, OMT alone is as effective in the long-term prevention of cerebral infarction and myocardial infarction as is revascularisation and OMT combined	200	2022	Not yet recruiting

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