Imaging of the Vulnerable Carotid Plaque: Role of Imaging Techniques and a Research Agenda

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ABSTRACT

Objectives: Atherothrombosis in the carotid arteries is a main cause of ischemic stroke, and may depend on plaque propensity to complicate with rupture or erosion, in turn related to vulnerability features amenable to in vivo imaging. This would provide an opportunity for risk stratification and – potentially – local treatment of more "vulnerable" plaques. We here review current information on this topic.

Methods: We systematically reviewed the literature for concepts derived from pathophysiological, histopathological and clinical studies on imaging techniques attempting at identifying vulnerable carotid lesions.

Results: Ultrasound, magnetic resonance imaging, computed tomography, and nuclear medicine-based techniques, alone or with multimodality approaches, all have a link to pathophysiology, and describe different – potentially complementary – aspects of lesions prone to complications. There is also, however, a true paucity of head-to-head comparisons of such techniques for practical implementation of a thorough and cost-effective diagnostic strategy based on evaluation of outcomes. Especially in asymptomatic patients, major international societies leave wide margins of indecision in the advice to techniques guiding interventions to prevent atherothrombotic stroke.

Conclusions: To improve practical management of such patients – in addition to patient's vulnerability for systemic reasons – a more precise identification of the vulnerable plaque is needed. A better definition of the diagnostic yield of each imaging approach in comparison with the others should be pursued for a cost-effective translation of the single techniques. Practical translation to guide future clinical practice should be based on improved knowledge of the specific pathophysiologic correlates and on a comparative modality approach, linked to subsequent stroke outcomes.

Keywords: vulnerable plaque; stroke; transient ischemic attack; carotid artery stenosis; imaging; risk prediction.

List of Abbreviations in the manuscript (in alphabetical order; in extenso in the text at first mention)

- American Heart Association **(AHA)**
- Carotid Endarterectomy **(CEA)**
- Computed Tomography **(CT)**
- Contrast-Enhanced Ultrasonography **(CEUS)**
- **e-1, e-2. e-3**, etc.: electronic references, reported in the **Online Appendix**
- Fast Spin Echo **(FSE)**
- Fluorodeoxyglucose **(FDG)**
- Magnetic Resonance Imaging **(MRI)**
- Microembolic Signals **(MESs)**
- Positron Emission Tomography **(PET)**
- Transient Ischemic Attack **(TIA)**

Introduction

Stroke is a most relevant health burden [e-1]. Ischemic stroke accounts for 87% of all stroke cases [1]. Silent stroke (defined at magnetic resonance imaging - MRI) (Online Figure 1) [Data available from Dryad (Appendix) https://doi.org/10.5061/dryad.0vt4b8gvf] has a reported incidence of 10-15%, and may be an important cause of cognitive decline and dementia [e-1,e-2]. At least 10 to 20% of ischemic strokes – known as atherothrombotic strokes – are related to thromboembolism deriving from a 50–99% diameter stenosis due to an atherosclerotic plaque of the common or internal carotid arteries [e-1,e-2]. At carotid ultrasound, the prevalence of asymptomatic moderate and severe stenoses has been reported as 2.0% and 0.5% of the general population [e-1,e-3]. Besides the simple evaluation of the stenosis degree, however, recognition of in vivo qualitative features of atherosclerotic carotid plaques prone to complications – "vulnerable plaques" – has an important potential for identifying patients at risk for stroke.

The objective of this review will be, therefore, to summarize basic pathophysiological and histopathological concepts on the vulnerable carotid plaque and to report the state of the art and the perspectives for using various imaging techniques to assess carotid plaque vulnerability in terms of risk prediction and patients' outcomes.

Search Strategy

We performed a comprehensive search of the literature in English, according to the PRISMA statement, as detailed in the Online Supplement and Online Figure 2 [Data available from Dryad (Appendix) https://doi.org/10.5061/dryad.0vt4b8gvf].

Stroke Risk Assessment—Vulnerable Patients and Vulnerable Plaques

Stenosis degree in a carotid artery is the most commonly appreciated predictor of cerebrovascular ischemic events [e-4,e-5]. International guidelines have provided recommendations for treatment largely based on this parameter [e-4,e-5,e-6]. Although carotid revascularization is an established treatment for patients with symptomatic carotid stenosis ≥70%, there are concerns around a specific threshold for treatment simply associated with stenosis severity. This is in part related to the paucity of outcome data able to isolate the impact of the single specific feature of diameter stenosis [e-7], but is also suspected to be related a different propensity of individual atherosclerotic plaque to complicate with a thrombotic event.

Previous strokes or transient ischemic attacks (TIAs) are major risk factors for future ischemic events (annual risk for future ischemic stroke: 3% to 4% [e-8]). Based on data from randomized trials of endarterectomy for symptomatic patients [e-9], demographic and clinical risk factors for stroke recurrence include male sex, age >75 years, hemispheric symptoms, recent symptomatic status and relevant comorbidities. Listed imaging risk factors comprise contra-lateral carotid artery occlusion, the absence of collaterals, degree of stenosis, markers of previous embolic lesions, such as cerebral

lesions at MRI or computed tomography (CT) and micro-embolic signals at transcranial Doppler ultrasonography, as well as, but also – vaguely defined – plaque composition [e-10].

Prediction of a first stroke episode in an asymptomatic subject (primary prevention) is a much greater challenge, and is currently only based on conventional risk factors for atherosclerotic disease, thus predicting the occurrence of atherosclerotic plaques in the carotid arteries as well as in other districts [e-11]. Although conceptually appealing, few data have linked circulating biomarker activity with the risk of late stroke [e-12], and are not implemented in daily routine. These will likely improve overall risk prediction, but will not pinpoint the risk of the individual plaque. Progression of an asymptomatic stenosis identifies a subgroup of patients with about twice the risk of ipsilateral stroke compared with those without progression, but the rate of stenosis progression is reported to be low, so that this feature can only account for a minority of stroke recurrences [e-13,e-14].

In essence, there is a knowledge gap on whether qualitative features of the carotid plaque, which, if any, and how detectable in vivo may truly be clinically helpful in stratifying the risk of future stroke.

Histopathological Features of Vulnerable Carotid Plaques

The "vulnerable atherosclerotic plaque" may be defined in several, varied, aspects, related to the risk of future acute manifestations of vascular disease [e-15]. It can be defined as a plaque with *propensity* to complicate with thrombosis or with surface erosion and subclinical embolic spread. The different nature of atherosclerotic plaques – more or less vulnerable – may explain the large variability in outcomes for plaques with similar impingement on the vascular lumen. Outcomes range from acute stroke or TIA to silent serial lacunar events, ultimately also resulting in cognitive loss and dementia. Of particular importance in this context, only 40% of unstable plaques are associated with >75% luminal narrowing. The relevance of an improved risk stratification based on qualitative features of the plaque is therefore obvious [e-16].

For decades cardiovascular science has pursued the quest to identify vulnerable atherosclerotic plaque in patients, hoping to predict and ultimately prevent acute events (Online Table I) [Data available from Dryad (Appendix) https://doi.org/10.5061/dryad.0vt4b8gvf]. Most concepts and beliefs on "vulnerable" lesions developed for coronary plaques have been simply translated to extracranial carotid arteries, and have been largely inferred from the correlations of plaque morphology with already occurred clinical events ("culprit" plaques). The paucity of prospective studies truly addressing "vulnerable" – as opposed to "culprit" plaques as derived from correlation studies – is a general limitation of this entire area of research. For the purpose of standardization, and mostly derived from analyses of culprit plaques, vulnerable carotid plaques may be histopathologically defined as atherosclerotic lesions with a thin fibrous cap of <200 μm overlying large necrotic/lipid core, often containing intra-plaque hemorrhage and/or calcifications and neovascularization [e-17].

In 1995, the American Heart Association (AHA) developed criteria for the histological classification of atherosclerotic plaques [e-18], mainly derived from evidence in the coronary district. Also in the carotid district, however, ischemic stroke secondary to extracranial carotid artery disease is thought to be due

to progression of Type IV and V plaques to Type VI plaques, with plaque ulceration/erosion and superimposed thrombosis [e-19] (Online Table II) [Data available from Dryad (Appendix) https://doi.org/10.5061/dryad.0vt4b8gvf]. The incidence of variably defined vulnerable coronary artery plaques has been reported to range between 4 and 13% [e-16], but similar data are lacking for the carotid artery. Online Table III [Data available from Dryad (Appendix) https://doi.org/10.5061/dryad.0vt4b8gvf] summarizes the central differential diagnostic aspects between the carotid and the coronary artery districts in terms of plaque pathophysiology. Altered metabolic signatures in high-risk plaques were consistent with a change to increased glycolysis, elevated amino acid utilization and decreased fatty acid oxidation, similar to what is found in activated leucocytes and cancer cells [e-20]. These have been postulated to potentially result in specific imaging patterns associated with clinical events [e-20].

Several processes may account for the development of vulnerability in atherosclerotic plaques in general. These are: the presence of inflammatory cells; intra-plaque hemorrhage and the rapid expansion of an existing necrotic core. These features associate with thin-fibrous caps and the development of surface ulcer(s)/luminal thrombi [e-19]. Multiple healed plaque ruptures and erosions have been described in the carotid arteries, similar to the coronary arteries, and also in the carotids the degree of luminal narrowing may be related to the repeated layering of reparative healed repair sites [e-21]. The proportion of plaque ruptures vs erosions has been described as changed towards a higher representation of erosions vs ruptures in recent years in the carotid arteries [e-22], possibly as the result of more aggressive risk factor management, especially with the larger use of statins [e-23]. There are virtually no data on how the propensity to rupture vs erosion may be predicted with in vivo imaging.

Imaging Techniques for the In Vivo Detection of the Vulnerable Plaque

Current guidelines [e-6] have established the degree of stenosis as the primary means to evaluate stroke risk and to provide indications for intervention [e-24,e-25]. However, there is an overall consensus that qualitative plaque features are potentially more important in determining vulnerability [e-26] (**Tables 1, 2** and **3**). Therefore, carotid imaging modalities have raised considerable interest for their promise of characterizing plaque features in vivo as predictors of future events [e-27]. Recommended tools to predict carotid artery-related risk, based on the literature, include: the presence of silent brain infarctions at brain imaging (in essence, the characterization of "culprit" – not truly "vulnerable" lesions), a large plaque area (>40 mm^2), the presence of an irregular stenosis, the presence of a contralateral occlusion, increasing stenosis severity (>20%) at two separate examinations, the presence of tandem intracranial disease, the failure to recruit intracranial collaterals, a low grayscale median value, the presence of intraplaque hemorrhage documented at MRI, the occurrence of spontaneous embolization at transcranial Doppler, and increased ^{18}F-fluorodeoxyglucose (FDG) uptake in the carotid plaque at positron emission tomography (PET) [e-10]. We will here briefly review features and potentials of the main imaging modalities to detect such plaques.

Ultrasound

Ultrasound is the first-line and most widespread imaging method to assess carotid atherosclerotic disease. With newer modalities, it is now possible to identify several characteristics that correlate with plaque histopathology.

2-D Echo

Besides being able to assess stenosis area, 2-D ultrasound and color-Doppler are routinely used to assess features related to plaque vulnerability. However, although a statistically relevant correlation has been demonstrated between ultrasonographic and histopathological features of carotid plaques using 2-D ultrasound, this technique has only moderate sensitivity in identifying plaque characteristics (e.g., ulceration, Figure 1, upper panel) of the plaque surface[3]. Plaques with complex features, particularly those with prominent echolucency, neovascularization, ulceration and intra-plaque motion, have been found to be associated with ischemic symptoms[4]. In particular, symptomatic compared to asymptomatic carotid artery plaques have been described to feature plaque characteristics with a higher degree of neovascularization, tissue texture complexity, ulceration, echolucency, and intra-plaque motion. Plaque echolucency is the sonographic equivalent of the presence of a lipid-rich necrotic core, and is reported in up to 50% of recently symptomatic plaques compared with less than 5% of asymptomatic plaques[5]. Regardless of the degree of stenosis, the reported 3-years risk of stroke among patients with echolucent plaques, regardless of the degree of stenosis, is up to 13%, which is higher than the risk of stroke among patients with high-grade stenosis. A recent meta-analysis also showed an association between echolucent carotid plaques and future cardiovascular events in asymptomatic patients [6].

The size of a juxtaluminal black (hypoechoic) area in ultrasound images of asymptomatic carotid artery plaques was able to predict future ipsilateral ischemic stroke, and resulted useful when implemented in risk stratification models [7].

An important limitation in 2-D echo used in the characterization of carotid plaques is, however, the lack of consistent inter- and intra-observer agreement [e-25].

Plaque Texture analysis

Ultrasound image-editing programs can be used to analyze the grayscale histogram of isolated plaques, allowing a simple, reproducible method for the determination of the grayscale median of the plaque. It is thus possible to quantify echogenicity and potentially determine a cut-off value for high-risk plaque. In one of the largest studies in this context, echolucent plaques were more likely to result in embolism in association with angioplasty and stenting during or after the procedure [8].

The potential limitation of grayscale median analysis—not taking into account plaque heterogeneity—may be overcome by texture analysis [9]. Integrated backscatter analysis is a quantitative method of

plaque echogenicity characterization that directly measures radiofrequency signals and relies on the scattering of acoustic waves in all spatial directions when they encounter a structure [10]. Decibel values of vulnerable plaques are approximately 10-fold less than in fibrous plaques [11].

Contrast-enhanced ultrasound

Contrast-enhanced ultrasound (CEUS) [12] (Figure 1, lower panel) can be used both to detect plaque enhancement and neovascularization (using intravenous microbubbles as purely intravascular contrast agent) and for the molecular targeting of plaque inflammation [13]. A recent meta-analysis of 7 studies comparing the contrast echo diagnosis of intraplaque neovascularization with histologic specimens and/or the clinical diagnosis found a significant predictive value for quantitative CEUS [14]. The standardization of the technique, however, remains debated [e-25].

3-D Echography

The introduction of 3-D imaging with carotid ultrasound to assess plaque vulnerability has advantages in the recognition of plaque morphology, an improved ability to evaluate plaque surface (ulceration); and a better evaluation of plaque texture [15]. In particular, a redefinition of plaque ulceration is a major advance of 3-D technology [16]. Subjects with a global ulcer volume ≥5 mm^3, assessed with 3-D methods, have considerably greater risk of acute cerebrovascular events than subjects with lower values [17].

Finally, detection of subclinical atherosclerosis has been shown to improve risk prediction beyond cardiovascular risk factors only, and risk scores plus the quantification of plaque burden with 3-D vascular ultrasound has been shown to improve it further [18].

Transcranial Doppler Ultrasonography

Transcranial Doppler is a complement to other techniques of carotid imaging for the evaluation of cerebral microembolic signals (MESs), and is one of the best validated method for the identification of high-risk patients with asymptomatic carotid stenosis [19]. In particular, the long-term clinical significance of microembolic events is in its contribution in terms of cognitive decline and dementia [20].

Being an evaluation of the existence of “culprit” lesions – an evolution of plaque vulnerability – and not – strictly speaking – an imaging technique for one specific plaque itself, it will not be here addressed further.

Newer emerging ultrasound-based techniques

Analysis of raw radiofrequency data have a strong potential to improve the assessment of plaque vulnerability. A recently validated ultrasound-derived vulnerability index showed significant associations with the inflammatory and metabolomic profile of carotid plaques [e-20]. Investigation of the role of biomechanical forces [21] also appears promising, with "soft" plaques exhibiting a higher spontaneous deformation assessed measuring carotid distension through parallel ultrasound lines [22]. Carotid wall shear rate, an additional factor affecting wall physiology and plaque vulnerability, can now also be studied with new ultrasound platforms [23].

Magnetic Resonance Imaging

MRI is the best-established not-invasive imaging modality for plaque characterization [24]. Studies comparing MRI findings with histopathology have demonstrated that MRI can accurately distinguish plaque components [25] (Figure 2, upper panel). MRI imaging allows a detailed characterization of plaque composition, including detection of a lipid-rich necrotic core. Pulse sequences including fast spin echo and gradient echo are available for plaque characterization [27], while the black-blood technique allows for a quick assessment of intra-plaque hemorrhage [28]. Contrast-enhanced images potentially differentiate various plaque components. Gadolinium (Gd)-based contrast imaging can be used to evaluate plaque neo-vascularity and differentiate between a necrotic core and fibrous tissue. By this technique, intra-plaque hemorrhage without rupture of the fibrous cap is apparently not associated with clinical symptoms, whereas juxtaluminal hemorrhage and a thrombus indicate erosion, ulceration, or rupture, each of which is recognized as a marker of plaque complications [29].

Carotid plaque composition assessed by MRI has been associated with cardiovascular events including stroke, and appeared to improve the reclassification of baseline cardiovascular risk based on risk factors, while carotid artery evaluation of intima-media thickness did not [30]. Studies of both asymptomatic and symptomatic patients with moderate (50-70%) carotid stenosis have reported that MRI findings of intra-plaque hemorrhage are associated with a high risk of future ipsilateral ischemic events [31].

Computed Tomography

The two main CT techniques for plaque characterization are multi-detector-row CT and dual-source CT [32,e-25]. Multi-detector-row CT, in particular, allows for a characterization of plaque calcification, ulcerations, fibrous plaque thickness, intra-plaque hemorrhage, and the presence of lipid-rich necrotic cores (**Figure 2, lower panel**) [e-25]. The lower the density, the more likely is the probability for the plaque to be vulnerable; clinically symptomatic plaques have a lower degree of calcification than asymptomatic plaques. Multi-detector-row CT findings are strongly correlated with patient symptoms as well. A significant positive relationship was found for the presence of a soft plaque, plaque

ulceration and increased common carotid artery wall thickness with cerebrovascular ischemia, whilst an inverse relationship was found between calcified plaques and ipsilateral ischemia [33].

Dual-source computed tomography facilitates the use of two different X-ray sources, allowing the simultaneous use of two different X-ray energies to derive different Hounsfield Units density estimates in the tissue, for potential tissue differentiation and advanced post-processing [34]. In several reports, this technique, applied to carotid arteries, resulted feasible and accurate in the evaluation of plaque composition [35].

Plaque enhancement following contrast injection is also an extremely promising imaging parameter: symptomatic plaques have a significantly higher degree of plaque enhancement following contrast administration than asymptomatic plaques, indicating a greater degree of vascularization [36].

Nuclear and Molecular Imaging

Cellular/molecular imaging attempts at visualizing specific biological processes occurring within the plaque in vivo, and holds the promise of earlier, as well as more specific diagnoses [37, 38].

Early attempts, including our own [37], focused on the possibility of detecting thrombus deposition on carotid artery plaques with single-photon emission computed tomography (SPECT)-based ^{111}Indium-labeled platelets, as markers of plaque thrombogenicity **(**Online Figure 3) [Data available from Dryad (Appendix) https://doi.org/10.5061/dryad.0vt4b8gvf]**.** The technique is, however, not suitable for widespread clinical use**.** Most recent research has recently focused on PET tracers (Figure 3 and Table 4). Metabolic processes amenable to detection by PET include macrophage-mediated inflammation, microcalcification, and hypoxia, generating a mix of triggers that increase the risk of plaque rupture and may be ideal targets for already validated or emerging PET tracers [39]. Hypoxia in plaques prone to rupture occurs due to increased oxygen demand from foam cells, exacerbated by an increased size of the necrotic core, plaque thickness and distance from the luminal wall, while microcalcifications within the plaque fibrous cap result in mechanical destabilization and increased plaque stress, predisposing to rupture [40]. Inflammation correlates with a higher uptake of FDG, now become the main tracer used to evaluate carotid plaques with the use of PET, alone or in combination with CT (Figure 3) [41]. Carotid maximal standardized uptake value (SUV-max) at 180 minutes was strongly associated with the 10-year risk for fatal cardiovascular disease [42]. However, several confounding factors may enhance the FDG signal, including the activity of smooth muscle cells, challenging the specificity of the signal for vulnerable plaques [43].

Multi-modality Imaging

An updated understanding of the complex pathophysiology beneath carotid plaque vulnerability has led to renewed and multi-parametric (i.e., multi-imaging) approaches [44], with the aim of targeting different aspects of the disease in order to reinforce the strength of each technique and also to limit, to some extent, its potential side effects (such as radiation exposure). In particular, many attempts have

been made in combining MRI and nuclear techniques, combining spatial, textural and functional data [45, 46]. Although promising, these approaches still lack of prospective evaluation and have been so far conducted on limited populations.

Clinical Insight

From a clinical standpoint, we have already now a multitude of techniques able to detect plaque features that correlate with the histological construct of "plaque vulnerability". Yet, we lack a systematic, univocal, possibly sequential, and clinically validated approach to the imaging evaluation of vulnerable plaques. Clinicians should be aware of potential advantages and findings derived from every single methodology (Tables 1 and 2), as well as of their costs and potential side effects, but should also be aware of the largely anecdotal correlation of such disparate techniques with clinical events. Most of the proposed approaches are limited to single-center experience or expertise and to the center preferences in the use of available information for practical decision-making. From a head-to-head comparison of the specific features of different techniques, as reported in Tables 1 and 2, we can foresee that ultrasound-based techniques, especially when improved with better quantification and reproducibility, have the potential to gather information on most of the physical, biologic and histopathological characteristics of plaque vulnerability, while PET could be reserved to very selected cases where metabolic characterization of the lesion would affect patient management. An additional obvious advantage of ultrasound-based techniques as compared to CT, PET and MRI, due to radiation exposure of CT and PET and the costs of all three, is their repeatability, in a clinical setting where serial examinations are a very frequent need to plan interventions. Yet, we currently do not know whether the additional information from second-tier techniques may be truly useful clinically.

Research Directions and Unmet Needs

The most recent American [e-4] and European guidelines [e-6,e-5] highlight important weaknesses in previous recommendations to interventions based only on lumen diameter reduction. In summary, ultrasound imaging (as first-line technique), CT and/or MRI are the commonly recommended techniques for evaluating the extent and severity of extracranial carotid stenosis (I B). In "average surgical risk" patients with an asymptomatic 60–99% stenosis, the presence of multiple imaging characteristics (**Table 3**) associated with an increased risk of late ipsilateral stroke may influence, together with clinical predictors, the indication to endarterectomy or carotid stenting (IIa/b B). It is easy to remark the lack of commitment as to the ideal diagnostic work-up in the single patient. The AHA [47] had also repeatedly advised that only "*highly selected*" asymptomatic patients should undergo carotid endarterectomy (CEA), but did not define what "*highly selected*" means. Indications to intervention are also based on evidence accrued without our currently recommended "optimal medical therapy" (mostly including statins, anti-hypertensive and antithrombotic treatments according to today's standards). For many individuals, medical therapy may provide excellent risk reduction without the periprocedural risk of endarterectomy or stenting. Considering the extremely limited benefit of CEA in asymptomatic subjects, the declining annual risk of stroke [now reported to be, on

medical treatment, 0.34% (95% CI, 0.01 to 1.87) for any ipsilateral ischemic stroke], with the low rate attributed both to the changing prevalence of cardiovascular risk factors and to the increasing use of preventive therapies – mostly antithrombotic, anti-hypertensive and lipid-lowering agents [48] – there is a strong need to develop scientifically proven clinical/imaging algorithms to specifically identify small cohorts of patients at higher-risk for stroke to whom CEA/carotid artery stenting might be targeted. Here multi-parametric imaging in prospective studies comparing the yield of complementary, but also inevitably expensive, techniques variously proposed in the literature, needs to be undertaken, possibly including recent advances from machine learning [49]. In this respect, artificial intelligence has an important potential role, after advances in this area have opened-up avenues for creating novel modeling and predictive methods for clinical use. Deep learning, by unbiased creation of risk models that incorporate multiple imaging features from different techniques without a priori selection of those features, might provide the ability to identify patterns of imaging information that improve risk stratification [50]. Considering also the emerging long-term clinical implications of subclinical strokes (Online Figure 1) [Data available from Dryad (Appendix) https://doi.org/10.5061/dryad.0vt4b8gvf], these – rather than the much less prevalent clinical strokes – might here be a most relevant end point [51], allowing reduced sample size despite the disadvantage of performing imaging in all patients during follow-up.

Such studies would ideally link the vulnerable carotid plaque to the "vulnerable brain" and the "vulnerable patient", in an accurate, broad and comprehensive approach to carotid artery disease (Figure 4). Such studies should also clarify whether the accurate assessment of the atherosclerotic plaque burden outperforms any evaluation of the single plaque features to render the quest for single-plaque vulnerability futile [52]. An important aspect of these much-needed prospective studies would be to avoid the unnecessary use of redundant techniques leading to an unnecessary and avoidable increase in health expenditures. Such studies are admittedly not easy to perform, require multiple approaches in parallel in the same patient and a reasonably long follow-up to capture subsequent events in a statistically robust fashion. They are, however, a current imperative to translate biologic knowledge and claims from isolated reports into pragmatic diagnostic recommendations. Most of current claims indeed focus on the use of approaches only selected because of their local availability. A better ability to predict the development of stroke by one or several imaging techniques in patients with carotid artery plaques would eventually allow a scientifically proven diagnostic path, and possibly more focused systemic (drugs) or local therapeutic approaches. These latter might include not only mechanical treatments (stenting, endarterectomy), but also – potentially – site directed drugs).

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favoring large comprehensive reviews. The Authors apologize for injustice made to many extremely valuable original contributions.

Appendix 1: Authors

Name	Location	Contribution
Iacopo Fabiani, MD	University of Pisa, Pisa	Design and conceptualized study; reviewed and tabulated the literature; drafted the manuscript
Carlo Palombo, MD	University of Pisa, Pisa	Drafted and reviewed the ultrasound section
Davide Caramella, MD	University of Pisa, Pisa	Reviewed the manuscript for accuracy as the sections dedicated to radiological techniques
Jan Nilsson, MD	University of Lund, Sweden	Reviewed the general section of plaque vulnerability. Critically reviewed the manuscript.
Raffaele De Caterina, MD, PhD	University of Pisa, Pisa	Conceived the manuscript structure and scope. Wrote and validated the final manuscript.

References

1. Barrett KM, Brott TG. Stroke Caused by Extracranial Disease. Circ Res 2017;120:496-501.

2. Rafailidis V, Chryssogonidis I, Tegos T, et al. Imaging of the ulcerated carotid atherosclerotic plaque: a review of the literature. *Insights Imaging*. 2017;8:213-225.

3. Nakamura T, Tsutsumi Y, Shimizu Y, Uchiyama S. Ulcerated carotid plaques with ultrasonic echolucency are causatively associated with thromboembolic cerebrovascular events. J Stroke Cerebrovasc Dis 2013;22:93-99.

4. Singh AS, Atam V, Jain N, Yathish BE, Patil MR, Das L. Association of carotid plaque echogenicity with recurrence of ischemic stroke. N Am J Med Sci 2013;5:371-376.

5. Mathiesen EB, Bonaa KH, Joakimsen O. Echolucent plaques are associated with high risk of ischemic cerebrovascular events in carotid stenosis: the Tromsø study. Circulation 2001;103:2171-2175.

6. Gupta A, Kesavabhotla K, Baradaran H, et al. Plaque echolucency and stroke risk in asymptomatic carotid stenosis: a systematic review and meta-analysis. Stroke 2015;46:91-97.

7. Kakkos SK, Griffin MB, Nicolaides AN, et al. The size of juxtaluminal hypoechoic area in ultrasound images of asymptomatic carotid plaques predicts the occurrence of stroke. J Vasc Surg 2013;57:609-618 e601; discussion 617-608.

8. Biasi GM, Froio A, Deleo G, Piazzoni C, Camesasca V. What have we learned from the Imaging in Carotid Angioplasty and Risk of Stroke (ICAROS) study? Vascular 2004;12:62-68.

9. Reiter M, Effenberger I, Sabeti S, et al. Increasing carotid plaque echolucency is predictive of cardiovascular events in high-risk patients. Radiology 2008;248:1050-1055.

10. Karjalainen JP, Toyras 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 Med Biol 2009;35:1376-1384.

11. Bando M, Yamada H, Kusunose K, et al. Noninvasive Quantitative Tissue Characterization of Carotid Plaques Using Color-Coded Mapping Based on Ultrasound Integrated Backscatter. JACC Cardiovasc Imaging 2016;9:625-627.

12. ten Kate GL, van Dijk AC, van den Oord SC, et al. Usefulness of contrast-enhanced ultrasound for detection of carotid plaque ulceration in patients with symptomatic carotid atherosclerosis. Am J Cardiol 2013;112:292-298.

13. Ten Kate GL, van den Oord SC, Sijbrands EJ, et al. Current status and future developments of contrast-enhanced ultrasound of carotid atherosclerosis. J Vasc Surg 2013;57:539-546.

14. Huang R, Abdelmoneim SS, Ball CA, et al. Detection of Carotid Atherosclerotic Plaque Neovascularization Using Contrast Enhanced Ultrasound: A Systematic Review and Meta-Analysis of Diagnostic Accuracy Studies. J Am Soc Echocardiogr 2016;29:491-502.

15. Johri AM, Herr JE, Li TY, Yau O, Nambi V. Novel Ultrasound Methods to Investigate Carotid Artery Plaque Vulnerability. J Am Soc Echocardiogr 2017;30:139-148.

16. Schminke U, Motsch L, Hilker L, Kessler C. Three-dimensional ultrasound observation of carotid artery plaque ulceration. Stroke 2000;31:1651-1655.

17. 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;45:1437-1441.

18. Lopez-Melgar B, Fernandez-Friera L, Oliva B, et al. Subclinical Atherosclerosis Burden by 3D Ultrasound in Mid-Life: The PESA Study. J Am Coll Cardiol 2017;70:301-313.

19. Markus HS, King A, Shipley M, et al. Asymptomatic embolisation for prediction of stroke in the Asymptomatic Carotid Emboli Study (ACES): a prospective observational study. Lancet Neurol 2010;9:663-671.

20. Goldberg I, Auriel E, Russell D, Korczyn AD. Microembolism, silent brain infarcts and dementia. J Neurol Sci 2012;322:250-253.

21. Brown AJ, Teng Z, Evans PC, Gillard JH, Samady H, Bennett MR. Role of biomechanical forces in the natural history of coronary atherosclerosis. Nat Rev Cardiol 2016;13:210-220.

22. Paini A, Boutouyrie P, Calvet D, Zidi M, Agabiti-Rosei E, Laurent S. Multiaxial mechanical characteristics of carotid plaque: analysis by multiarray echotracking system. Stroke 2007;38:117-123.

23. Ricci S, Ramalli A, Bassi L, Boni E, Tortoli P. Real-Time Blood Velocity Vector Measurement Over a 2-D Region. IEEE Trans Ultrason Ferroelectr Freq Control 2018;65:201-209.

24. Esposito-Bauer L, Saam T, Ghodrati I, et al. MRI plaque imaging detects carotid plaques with a high risk for future cerebrovascular events in asymptomatic patients. PLoS One 2013;8:e67927.

25. den Hartog AG, Bovens SM, Koning W, et al. Current status of clinical magnetic resonance imaging for plaque characterisation in patients with carotid artery stenosis. Eur J Vasc Endovasc Surg 2013;45:7-21.

26. Cosottini M, Michelassi MC, Lazzarotti G. Vessels of the neck. In: *MRI of the Heart and Vessels*. Springer, Milano; 2005:121-143.

27. Qiao Y, Etesami M, Astor BC, Zeiler SR, Trout HH, 3rd, Wasserman BA. Carotid plaque neovascularization and hemorrhage detected by MR imaging are associated with recent cerebrovascular ischemic events. Am J Neuroradiol 2012;33:755-760.

28. Saam T, Hatsukami TS, Takaya N, et al. The vulnerable, or high-risk, atherosclerotic plaque: noninvasive MR imaging for characterization and assessment. Radiology 2007;244:64-77.

29. Gaens ME, Backes WH, Rozel S, et al. Dynamic contrast-enhanced MR imaging of carotid atherosclerotic plaque: model selection, reproducibility, and validation. Radiology 2013;266:271-279.

30. Zhang Y, Guallar E, Malhotra S, et al. Carotid Artery Wall Thickness and Incident Cardiovascular Events: A Comparison between US and MRI in the Multi-Ethnic Study of Atherosclerosis (MESA). Radiology 2018;289:649-657.

31. Singh N, Moody AR, Gladstone DJ, et al. Moderate carotid artery stenosis: MR imaging-depicted intraplaque hemorrhage predicts risk of cerebrovascular ischemic events in asymptomatic men. Radiology 2009;252:502-508.

32. Saba L, Tamponi E, Raz E, et al. Correlation between fissured fibrous cap and contrast enhancement: preliminary results with the use of CTA and histologic validation. Am J Neuroradiol 2014;35:754-759.

33. Baradaran H, Al-Dasuqi K, Knight-Greenfield A, et al. Association between Carotid Plaque Features on CTA and Cerebrovascular Ischemia: A Systematic Review and Meta-Analysis. Am J Neuroradiol 2017;38:2321-2326.

34. Leschka S, Scheffel H, Desbiolles L, et al. Image quality and reconstruction intervals of dual-source CT coronary angiography: recommendations for ECG-pulsing windowing. Invest Radiol 2007;42:543-549.

35. Tognolini A, Arellano CS, Marfori W, et al. Comprehensive low-dose imaging of carotid and coronary arteries with a single-injection dual-source CT angiography protocol. Clin Radiol 2014;69:246-253.

36. Wintermark M, Jawadi SS, Rapp JH, et al. High-resolution CT imaging of carotid artery atherosclerotic plaques. Am J Neuroradiol 2008;29:875-882.

37. Manca G, Parenti G, Bellina R, et al. 111In platelet scintigraphy for the noninvasive detection of carotid plaque thrombosis. Stroke 2001;32:719-727.

38. Evans NR, Tarkin JM, Buscombe JR, Markus HS, Rudd JHF, Warburton EA. PET imaging of the neurovascular interface in cerebrovascular disease. Nat Rev Neurol 2018;14:313.

39. Vesey AT, Jenkins WS, Irkle A, et al. (18)F-Fluoride and (18)F-Fluorodeoxyglucose Positron Emission Tomography After Transient Ischemic Attack or Minor Ischemic Stroke: Case-Control Study. Circ Cardiovasc Imaging 2017;10.

40. Cocker MS, Spence JD, Hammond R, et al. [(18)F]-NaF PET/CT Identifies Active Calcification in Carotid Plaque. JACC Cardiovasc Imaging 2017;10:486-488.

41. Chowdhury MM, Tarkin JM, Evans NR, et al. (18)F-FDG Uptake on PET/CT in Symptomatic versus Asymptomatic Carotid Disease: a Meta-Analysis. Eur J Vasc Endovasc Surg 2018;56:172-179.

42. Blomberg BA, Thomassen A, Takx RA, et al. Delayed (1)(8)F-fluorodeoxyglucose PET/CT imaging improves quantitation of atherosclerotic plaque inflammation: results from the CAMONA study. J Nucl Cardiol 2014;21:588-597.

43. Libby P, Bhatt DL, Di Carli M. Fluorodeoxyglucose Uptake in Atheroma: Not So Simple. J Am Coll Cardiol 2019;74:1233-1236.

44. Kwee RM, Teule GJ, van Oostenbrugge RJ, et al. Multimodality imaging of carotid artery plaques: 18F-fluoro-2-deoxyglucose positron emission tomography, computed tomography, and magnetic resonance imaging. Stroke 2009;40:3718-3724.

45. Truijman MT, Kwee RM, van Hoof RH, et al. Combined 18F-FDG PET-CT and DCE-MRI to assess inflammation and microvascularization in atherosclerotic plaques. Stroke 2013;44:3568-3570.

46. Fernández-Friera L, Fuster V, López-Melgar B, et al. Vascular Inflammation in Subclinical Atherosclerosis Detected by Hybrid PET/MRI. Journal of the American College of Cardiology 2019;73:1371-1382.

47. Meschia JF, Bushnell C, Boden-Albala B, et al. Guidelines for the primary prevention of stroke: a statement for healthcare professionals from the American Heart Association/American Stroke Association. Stroke 2014;45:3754-3832.

48. Marquardt L, Geraghty OC, Mehta Z, Rothwell PM. Low risk of ipsilateral stroke in patients with asymptomatic carotid stenosis on best medical treatment: a prospective, population-based study. Stroke 2010;41:e11-17.

49. Sharma AM, Gupta A, Kumar PK, et al. A Review on Carotid Ultrasound Atherosclerotic Tissue Characterization and Stroke Risk Stratification in Machine Learning Framework. Curr Atheroscler Rep 2015;17:55.

50. Roy-Cardinal MH, Destrempes F, Soulez G, Cloutier G. Assessment of Carotid Artery Plaque Components With Machine Learning Classification Using Homodyned-K Parametric Maps and Elastograms. IEEE Trans Ultrason Ferroelectr Freq Control 2019;66:493-504.

51. Knopman DS, Griswold ME, Lirette ST, et al. Vascular imaging abnormalities and cognition: mediation by cortical volume in nondemented individuals: atherosclerosis risk in communities-neurocognitive study. Stroke 2015;46:433-440.

52. Arbab-Zadeh A, Fuster V. The myth of the "vulnerable plaque": transitioning from a focus on individual lesions to atherosclerotic disease burden for coronary artery disease risk assessment. J Am Coll Cardiol 2015;65:846-855.

Figures

Figure 1: Upper panels (a, b): Echographic appearance of a symptomatic complicated plaque from a 68-years-old diabetic, dyslipidemic male patient with a recent hospitalization for a transient ischemic attack. The 2D-echo (panel a) shows a large complicated plaque with a relevant crater-like lesion, surrounded by a thin homogeneous cap and a fibrous shoulder. In panel b, the color-Doppler images from the same patient in the same projection, show an area of turbulence (red arrow; stenosis estimate: 60%) and the slow flow inside the crater.like lesion (red circle). **Lower panels (c, d, e, f): Echographic evaluation of the right internal carotid artery in a patient with a recent transient ischemic attack, and the use of contrast enhancement.** 2-D echography shows a non-calcified, homogeneous, hypo-echogenic plaque (panel c: long axis; panel d: short axis). The same plaque was then evaluated after a single 2.4 mL bolus injection of a contrast agent (Sonovue, Bracco), in contrast mode, with a low mechanical index (panel e), then analyzed for a quantification of plaque neovascularization (Vuebox, Bracco) with a dedicated off-line software (panel f, showing the "Region-of-Interest" (ROI) for plaque analysis. Panels A and B were originally published in figure 6, panels A and B, in "Imaging of the ulcerated carotid atherosclerotic plaque: a review of the literature" by V. Rafailidis et al.[2]

Figure 2: Upper panels (a, b, c): A severe stenosis in the left internal carotid artery (in the red boxes) evaluatied with magnetic resonance imaging (MRI) techniques. Panel a: MRI-angiography with gadolinium. Panel b: comparison with digital subtraction angiography. Panel c. a cross-sectional fast spin echo (FSE) T2-weighted sequence, demonstrating a homogeneous plaque with a prevalent lipid core and calcified spots). These images refer to a patient evaluated after a recent transient ischemic attack before any interventional procedure. **Lower panels (d, e, f): Carotid plaque assessment with multi-detector computed tomography (CT).** In red boxes, details of a dense calcified plaque with high density values (a); a mixed plaque (b) and an ulcerated plaque (c), showing multiple areas of irregularity and decreased density suggestive of a lipid core. Plaque (d) belonged to a 68 years-old male patient with echographic finding of a significant carotid stenosis. Plaques (e) and (f) were identified in patients recently become symptomatic for amaurosis fugax, after plaque identification during an echographic evaluation in the Emergency department. Panels A and C were originally published as figure 6.8 of chapter 6 in *MRI of the Heart and Vessels* by Springer.[26] Plaques D–F provided courtesy of Dr. Lorenzo Faggioni (Pisa University Hospital, Italy).

Figure 3: [^{18}F] - NaF Positron emission tomography (PET)/Computed Tomography (CT) views of carotid plaques. A: transaxial views; B: sagittal views; from left to right: CT, PET and superimposed PET/CT images in a patient with a 70% left internal carotid stenosis, showing increased radiotracer uptake (green arrows). Carotid plaque views provided courtesy of Prof. Paola Erba (Pisa University Hospital, Italy).

Figure 4: The weight of systemic atherosclerotic burden and carotid plaque vulnerability in determining the risk of stroke. Imaging and bio-humoral correlates of plaque vulnerability (with a direct link with histology and pathophysiological processes) should be addressed in conjunction with the systemic atherosclerotic burden to evaluate the probability of patient outcomes, with silent stroke and microembolization as possible prodromes of clinical symptoms.

Table 1: Characteristics of the vulnerable plaques with the various imaging techniques

Histology	PET	CT	2D-Echography	CEUS	T1-MRI	T2-MRI	Gd-MRI
Intra-plaque hemorrage	/	average 100 Hus	echolucent	echolucent	hypertintense	variable	hypertintense
Lipid rich necrotic core	/	average 30 Hus	echolucent	echolucent	iso/hyperintense	variable	iso/hyperintense
Neo-vascularization	/	enhance	/	enhance	/	/	enhance
Inflammation	increase	enhance	/	enhance	/	/	enhance
Ulceration	/	irregularity	irregularity	irregularity	irregularity	irregularity	irregularity
Calcification	/	average 250 Hus	hyperechoic; shadowing	hyperechoic; shadowing	hypointense	hypointense	hypointense

CEUS, contrast enhanced ultrasound; CT, computed tomography; Gd, gadolinium; HUs, Hounsfield Units; IPH, intra-plaque hemorrhage; LRNC, lipid-rich necrotic core; MRI, magnetic resonance imaging; PET, positron emission tomography

Table 2: Relative advantages and disadvantages of the various imaging techniques

Imaging Modality	Advantages	Disadvantages
MRI	Not invasive; radiation-free; high resolution; sensitivity and specificity for haemorrage, ulceration, necrosis, inflammation, and neovascularity; reproducibility	Time; costs; Gadolinium
CT	Not invasive; resolution; reproducibility; accuracy for calcification, ulceration and neovascularity	Radiation; calcifications; contrast agents
PET	Not-invasive; reproducible; detection of inflammation	Resolution; radiation; time
2D-Echography	Not-invasive; no radiation; wide availability; costs	Resolution; operator dependency; variability
CEUS	Not-invasive; no radiation; good availability; limited costs; good for detection of neovascularization and ulceration	Resolution; operator dependency; variability

CEUS, contrast enhanced ultrasound; CT, computed tomography; MRI, magnetic resonance imaging; PET, positron emisson tomography

Table 3: Clinical/imaging features associated with an increased risk of late stroke in patients with 50%–99% asymptomatic carotid stenosis treated medically

Imaging/clinical parameter	OR/HR (95% CI); p value
Spontaneous embolization on TCD	7.46 (2.24 to 24.89); p=0.001
Plaque echolucency on Duplex US	2.61 (1.47 to 4.63); p=0.001
Spontaneous embolization on TCD + echolucency	10.61 (2.98 to 37.82); p=0.0003
Stenosis progression (50%–99% stenoses)	1.92 (1.14 to 3.25); p=0.05
Stenosis progression (70%–99% stenoses)	4.7 (2.3 to 9.6); p=0.05
Silent infarction on CT (60%–99% stenoses)	3.0 (1.46 to 6.29); p=0.002
Impaired cerebrovascular reserve (70%–99% stenoses)	6.14 (2.77 to 4.95); p<0.01
Juxtaluminal black area on computerized analysis	Trend p<0.001
Intraplaque hemorrhage on MRI	3.66 (2.77 to 4.95); p<0.01
Contralateral stroke/TIA	3.0 (1.9 to 4.73); p=0.0001

CT, computed tomography; MRI, magnetic resonance imaging; TCD, transcranial Doppler; TIA, transient ischemic attack; US, ultrasound.

Adapted from e-28.

Table 4: Positron Emission Tomography (PET) radiotracers in carotid atherosclerotic disease

Abbreviated name	Chemical name	Molecular target	Cellular or physiological target
^{18}F-FDG	Fluorodeoxyglucose	---------	Increased metabolic rate (inflammation), hypoxia,…
^{68}Ga-DOTATATE	[1,4,7,10-tetraazacyclododecane-N,N′,N′′,N′′′-tetraacetic acid]-D-Phe1,Tyr3-octreotate	Somatostatin receptor type 2	Macrophages
^{18}F-NaF	Sodium fluoride	Hydroxyapatite	Microcalcification
^{18}F-FMISO	Fluoromisonidazole	Selective reduction in hypoxia	Hypoxia
^{11}C-PK11195	N-Methyl-N-[1-methylpropyl]-1-[2-chlorophenyl]-isoquinoline-3-carboxamide	TSPO	Macrophages and microglia
^{11}C-PBR28	N-acetyl-N-(2-[11C]-methoxybenzyl)-2-phenoxy-5-pyridinamine	TSPO	Macrophages and microglia
^{18}F-DPA-714	18F-N,N-diethyl-2-(2-(4-(2-fluoroethoxy)phenyl)-5,7-dimethylpyrazolo[1,5-α]pyrimidin-3-yl)acetamide	TSPO	Macrophages and microglia

^{11}C-vinpocetine	(3α,16α)-Eburnamenine-14-carboxylic acid ethyl ester	TSPO	Macrophages and microglia
^{18}F-GE-180	• Flutriciclamide • (4S)-N,N-diethyl-9-[2-[18F]-fluoroethyl]-5-methoxy-2,3,4,9-tetrahydro- ^{1}H-carbazole-4-carboxamide	TSPO	Macrophages and microglia

TSPO, PK11195 targets translocator protein