Review

Contrast-Enhanced Ultrasound Feasibility in Assessing Carotid Plaque Vulnerability—Narrative Review

Ewa Kopyto [1], Marcin Czeczelewski [1,*], Eryk Mikos [1], Karol Stępniak [1], Maja Kopyto [1], Małgorzata Matuszek [1], Karolina Nieoczym [1], Adam Czarnecki [1], Maryla Kuczyńska [2], Mateusz Cheda [2], Anna Drelich-Zbroja [2] and Tomasz Jargiełło [2]

[1] Students' Scientific Society, Department of Interventional Radiology and Neuroradiology, Medical University of Lublin, 20-594 Lublin, Poland; ewa.kopyto@gmail.com (E.K.); mikoseryk@gmail.com (E.M.); karol.stepniak3@gmail.com (K.S.); maja.kopyto@gmail.com (M.K.); matuszek.ma@wp.pl (M.M.); karolinanieoczym1@gmail.com (K.N.); adam.czarnecki1234@gmail.com (A.C.)

[2] Department of Interventional Radiology and Neuroradiology, Medical University of Lublin, 20-594 Lublin, Poland; maryla.kuczynska@gmail.com (M.K.); mateusz.cheda@gmail.com (M.C.); zbroanna@interia.pl (A.D.-Z.); tomasz.jargiello@umlub.pl (T.J.)

* Correspondence: marcin.czeczelewski@gmail.com

Abstract: The risk assessment for carotid atherosclerotic lesions involves not only determining the degree of stenosis but also plaque morphology and its composition. Recently, carotid contrast-enhanced ultrasound (CEUS) has gained importance for evaluating vulnerable plaques. This review explores CEUS's utility in detecting carotid plaque surface irregularities and ulcerations as well as intraplaque neovascularization and its alignment with histology. Initial indications suggest that CEUS might have the potential to anticipate cerebrovascular incidents. Nevertheless, there is a need for extensive, multicenter prospective studies that explore the relationships between CEUS observations and patient clinical outcomes in cases of carotid atherosclerotic disease.

Keywords: contrast-enhanced ultrasound; ischemic stroke; vulnerable plaque; atherosclerosis

1. Introduction

Cardiovascular diseases (CVDs) persist as the predominant contributors to morbidity and mortality in industrialized countries [1]. Strokes are the third highest cause of death and a common factor leading to disabilities and cognitive deficits [2]. They affect 15 million people worldwide per year; 1/3 of these people are physically challenged for the rest of their lives and 1/3 die [3]. Atherosclerotic plaques within the carotid arteries are a fundamental cause of strokes and transient ischemic attacks (TIAs) while also serving as an indicator for the existence of systemic atherosclerosis [4].

Atherosclerosis is a chronic inflammatory, metabolic, and multifocal process that affects the intima of medium and large arteries [5,6]. Inside the arteries, the bifurcations are particularly susceptible to the formation of atherosclerotic plaques [7]. In this location, hemodynamic stress and turbulent blood flow lead to prolonged damage to endothelial cells [8].

Endothelial dysfunction, the migration and proliferation of macrophages, smooth muscle cells (SMCs), lymphocytes and neutrophils, localized oxidative stress, lipid accumulation, extracellular matrix synthesis, and neovascularization within atherosclerotic plaques collectively contribute to the development of atherosclerosis [9,10]. The disease has a subclinical phase lasting numerous years, with a gradual formation of fatty streaks within arterial walls that evolve over time into atherosclerotic plaques [11,12]. Atherosclerosis can be confined to the arteries supplying a single organ system, although multiple vascular regions are typically involved [13,14]. The initiation of atherosclerotic plaque formation

commonly occurs early in life and progresses with age [15–17]. Nonetheless, the rate of progression is variable and not entirely predictable among individuals [18].

In the past, evaluating carotid artery stenosis exclusively relied on assessing the extent of narrowing within the artery's passage, which was regarded as the primary indicator for intervention as per global recommendations [19]. However, relying solely on carotid stenosis for a risk assessment has become obsolete [20]. A shift in the paradigm regarding the relationship between atherosclerosis and cerebrovascular accidents has emerged from contemporary research. Certain high-risk plaques possess considerable damage potential such as causing stenosis or occlusions. These plaques can initiate a sequence leading to cerebral microembolisms, potentially causing ischemic strokes and sudden death [21]. These high-risk plaques are termed "unstable" or "vulnerable" due to their potential for adverse outcomes [22,23]. Notably, the 2023 ESVS guidelines indicate that individuals with only a single risk factor, such as carotid stenosis exceeding 80%, are no longer categorized as being at a high risk of a stroke [24].

2. Aim of the Review and Search Strategy

The main objectives of this narrative review were to highlight the current findings related to the application of contrast-enhanced ultrasound in evaluating vulnerable carotid plaques and to relate this method to conventional ultrasound and other modalities. A search of articles related to the topic of this paper was conducted via the use of PubMed, Scopus, and Web of Science databases in June 2023. The first identification aimed to search for and identify the papers utilizing CEUS in the examination of carotid atherosclerotic plaques; the search strategy was (CEUS OR contrast enhanced ultrasound) AND (carotid stenosis OR vulnerable plaque). A second identification of the literature was performed using the following search strategy: (carotid stenosis OR vulnerable plaque) AND (computed tomography OR magnetic resonance OR ultrasound OR imaging). There were no restrictions regarding the year of the publication and we only chose the articles written in English.

3. Vulnerable Plaques

Comprehensive investigations have revealed that while severe carotid stenosis remains one of the causes of strokes, individuals with non-severe carotid stenosis may also develop ischemic symptoms [25]. In fact, the most acute cardiovascular incidents arise from the rupture or erosion of the vulnerable plaque phenotypes [26]. The emergence of the vulnerable plaque concept is a result of advancements in the understanding of the natural progression of atherosclerosis, which has enabled the identification of distinct traits that are associated with an increased risk of strokes. Vulnerable plaques are characterized by numerous histological characteristics, including a thin fibrous cap, a lipid-rich necrotic core, high macrophage counts, intraplaque neovascularization, and intraplaque hemorrhages [27]. Atherosclerotic plaques have the potential to undergo a gradual transformation into vulnerable plaques that are prone to inducing local ruptures and thrombosis, eventually occluding the affected artery [28].

Neovascularization is a central pathophysiological phenomenon involved in the development of vulnerable atherosclerotic plaques [29–31]. It involves the development of new blood vessels within an atheromatous lesion, facilitated by endothelial cells [32]. Hypoxia and inflammatory conditions induce the release of angiogenic and inflammatory agents, which stimulate spontaneous angiogenesis [33]. Studies have indicated that the neovessels primarily originate from a pre-existing vasa vasorum, which constitutes a microvasculature network responsible for supplying arterial walls [34,35]. Neovessels are commonly found in the fibrous cap as well as in the medial and lateral corners of plaques, but their presence at the base is infrequent [36]. The development of new pathological capillaries promotes macrophage infiltration, inflammation, and lipid deposition as well as intraplaque hemorrhages that contribute to a progressive increase in the instability of plaques [37]. A rupture is commonly found in the plaques where the fibrous cap thickness is less than 0.065 mm and

the lipid core volume constitutes 40% of the total plaque volume [38]. Also, an intraplaque hemorrhage is considered to be a risk factor for plaque rupture [39,40].

4. Need for Early Identification

The screening for the presence of vulnerable carotid plaques is less routine compared with screening for carotid stenosis as it requires imaging techniques capable of thoroughly examining the morphology of atherosclerotic plaques [41,42]. Nonetheless, the early identification of individuals with vulnerable plaques allows early therapy and, therefore, may prevent clinical complications such as TIAs and strokes [43]. The European Society for Vascular Surgery recommended taking into account the morphological characteristics of plaques when determining the suitability of patients for revascularization interventions such as carotid endarterectomy or carotid artery stenting [24].

5. Screening for Vulnerable Plaques

The morphological features of unstable carotid plaques can be identified and measured with the use of advanced vascular imaging technologies [44]. Several non-invasive imaging modalities, including ultrasonography (US), computed tomography (CT), high-resolution magnetic resonance imaging (MRI), and nuclear imaging techniques, have been employed to detect these plaque attributes [45]. The goal is to achieve precise risk stratification and offer insights to guide clinical decision-making [46]. The characteristics of ultrasound make it an excellent imaging modality for the screening of patients with carotid atherosclerosis in the pursuit of identifying vulnerable plaques [47,48]. It is cost-efficient, rapid, and widely available, which provides the possibility for frequent re-examination [49]. Moreover, the characteristics of plaque vulnerability, including ulceration and intraplaque neovascularization, can be evaluated with the use of an intravascular ultrasound contrast agent [50].

6. B-Mode US with Doppler

Conventional B-mode US with Doppler is frequently used for first-line examinations of patients with recent cerebrovascular events in order to screen for atherosclerotic disease in peripheral arteries [51,52]. In advanced atherosclerosis, plaques may become visible on an ultrasound examination, allowing for a direct baseline morphological characterization [53,54]. A number of visualized plaques as well as the total plaque area or total plaque volume have been reported to be independent predictors of future cardiovascular mortality and coronary events [55,56].

Plaque vulnerability is a factor that should be at the center of interest for every sonographer because it has a direct impact on the patient's risk of a stroke [57]. The proper assessment of plaque instability should include the thickness of the fibrous cap, the size of the lipid–necrotic nucleus, ascertaining the presence of plaque neovascularization, determining the direction of atherosclerotic plaque remodeling, and ascertaining the presence of ulceration [58,59]. Vulnerability also defines the type of recommended treatment; it should be conservative in the case of stable plaques or interventional when it comes to unstable, ulcerated plaques [60].

The 2019 European Society of Cardiology (ESC) guidelines for the diagnosis and management of chronic coronary syndromes recommend that carotid B-mode US can be performed on patients with a suspicion of chronic coronary syndrome [61]. Carotid US has a similar risk prediction as the coronary calcium score and can be used in the screening of cardiovascular disease (CVD) [62].

7. Gray–Weale–Nicolaides Scale

Evaluations of the echogenicity of plaques can add information about their presumed lipid, fibrous, or calcium composition, which is crucial for vulnerability assessments [63]. For standardization purposes, the Gray–Weale–Nicolaides scale is applied. It uses a grading system based on echogenicity that classifies atherosclerotic plaques into five types. Type

1 is uniformly echolucent and mostly composed of lipid and necrotic components; type 2 is predominantly echolucent, with small areas of echogenicity caused by calcifications of up to 25% of the plaque volume; type 3 is predominantly echogenic, with small areas of echolucency and calcifications that consist of up to 50% of the plaque volume; type 4 is uniformly echogenic due to calcification of over 50% of the volume; and type 5 consists of plaques that cannot be classified owing to heavy calcification and acoustic shadows [38]. Hypoechoic plaques (types 1 and 2) (Figure 1) are associated with intraplaque hemorrhages and lipid accumulation, whereas hyperechoic homogeneous plaques are predominantly fibrous or calcified in nature [64]. In type 1 and 2 plaques, the risk of ipsilateral strokes is higher due to a greater tendency of disruption or rupture, whereas types 4 and 5 are associated with asymptomatic sclerotization of carotid arteries due to the stabilizing role of collagen and calcifications. Nevertheless, calcified intraluminal plaques may occasionally cause ischemia when a calcified material obstructs part of the brain microcirculation [65,66].
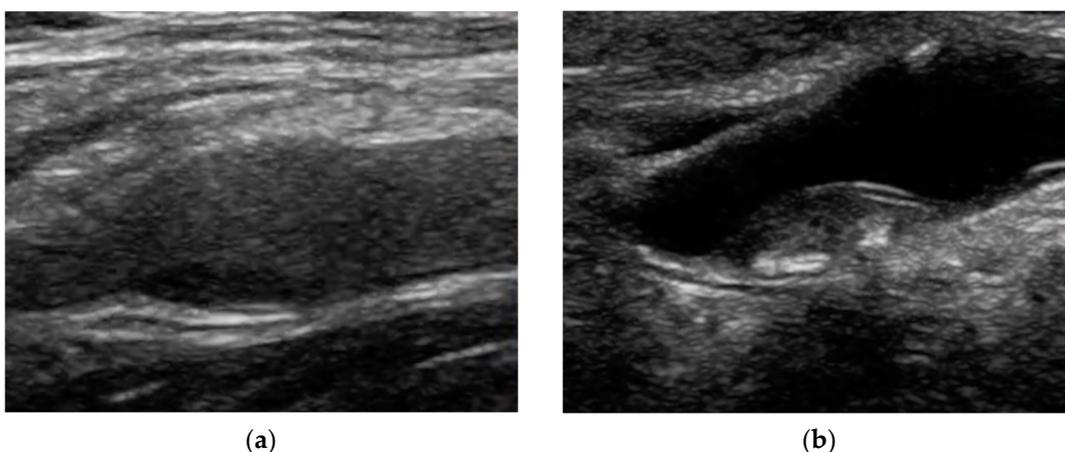

(**a**)   (**b**)

Figure 1. Hypoechoic plaques (types 1 and 2 in Gray–Weale–Nicolaides classification) are associated with intraplaque hemorrhages and lipid accumulation. Type 1 (**a**) is uniformly echolucent and mainly composed of lipid and necrotic components (plaque is marked with an asterisk). Type 2 (**b**) is predominantly echolucent, with small areas of echogenicity caused by calcifications.

8. Intima–Media Thickness

Subclinical atherosclerotic disease can be investigated with the use of a carotid intima–media thickness (IMT) measurement [67,68]. It is the quantitative parameter that is calculated by summing the thickness of the two inner layers within the carotid artery; namely, the intimal and medial layers [49,69]. The normal values for adults range between 650 and 900 μm, with an increase of 0–40 μm every year [49]. A large number of studies have shown a correlation between an increase in the IMT complex value among patients with CVD [70]. However, current guidelines from the ESC do not recommend the use of carotid ultrasound IMT assessments for the screening and risk assessment of asymptomatic cardiovascular patients [71].

The IMT assessment is performed at precisely defined points of individual arteries; specifically, in the proximal, intermediate, and distal CCA and ICA and in the CCA bifurcation [72]. The final IMT value is the average of all calculated measurements [73].

9. Ulceration

Carotid plaque ulceration is one of the plaque vulnerability constituents and has long been considered a major risk factor for strokes [74]. Plaque ulcerations are the cause of embolisms much more often than ruptures (74% and 40% of cases, respectively). It should be noted that plaque ulceration and its complications are considered to be the cause of approximately 20% of sudden deaths in both cerebrovascular and cardiovascular events [75].

The investigation of plaque ulcers is not an easy task. Muraki's criteria are a reference point for a sonographer. They define an ulcer as a cavity on a plaque surface, regardless of its size, where echogenicity at a cavity base should be less than that of an adjacent intima–blood border. Due to a swirling pattern of flow in the ulcer region, the bloodstream forms a "yin-yang" sign that can be observed using a B-mode technique or color Doppler [64]. The characteristic feature of an ulcer is its unusual arrangement in relation to the plaque, which is usually extreme and at the edge of the plaque, with the presence of calcifications on the edge of the ulcer [75].

10. Doppler US

Doppler is a feature that long ago became inseparable from ultrasonographic examinations. It can provide accurate information on blood-flow velocities, which is crucial in stenosis assessments. As the flow volume through the vessel is constant, the velocity of the flow is fastest at the stenotic segment. When it comes to carotid arteries, the consensus from 2003 states that a peak velocity starts to increase to above 125 cm/sec in a situation when stenosis rises to over 50% of the vessel lumen. In a 70% stenosis, the mentioned value is doubled. Thus, blood-flow velocity is a marker of segmental stenosis and systemic atherosclerosis [76].

The limitation of this technique is noticeable when the calcified wall of the carotid artery makes it difficult to obtain a proper sonic window. Stenosis can occur on short fragments; thus, the visualization of a vessel for its full length is crucial [76].

In addition, Doppler US can also investigate arterial stiffness, which is one of the structural changes that represent independent predictors of future cardiovascular diseases and susceptibility of arteries to the atherosclerosis process [77]. Arterial stiffness can be measured through the aortic pulse wave velocity (PWV). It is calculated as the distance traveled by the pulse wave divided by the time taken to travel the distance. An increase in arterial stiffness is associated with an increase in the propagation speed of the pulse wave in the arteries. The PWV is estimated from the common carotid artery to the common femoral artery or brachial–ankle PWV. A number of longitudinal studies have shown that the measurement of the PWV can identify subclinical atherosclerosis as well as predict future cardiovascular events [78].

11. Contrast-Enhanced Ultrasound

Carotid plaques initially identified from standard unenhanced US images and examined with Doppler US may be further investigated in the context of their intraplaque neovascularization and plaque surface by performing a CEUS examination [79]. Physicians performing CEUS need to familiarize themselves with the technique's principles (Table 1). This will enable them to precisely interpret findings and promptly identify any artifacts [80].

Table 1. Carotid contrast-enhanced ultrasound (CEUS) workflow and technical details of the procedure.

Carotid CEUS Workflow	Reference/Setting
Ultrasound machine setup	• Ultrasound machine with pulse-inversion technique • MI < 0.2 • Linear array probe with frequency range of 3–11 MHz
Ultrasonographic contrast agent administration	• 2.4 mL SonoVue® flushed with 5–10 mL of 0.9% saline • Bolus injection from antecubital fossa or central line • Large lumen cannula (<20 gauge) to avoid microbubble damage
Image acquisition	• Contrast agent usually appears in the carotid arteries within 20–30 s • Cine loop of 280–360 s is recorded
Image analysis	• Qualitative assessment of recorded cine loops • Post-processing analysis with quantification

CEUS utilizes an ultrasonographic contrast agent, an ultrasound machine for the emission of ultrasound beam sequences interacting with tissue, and the software necessary for image post-processing and analysis. Numerous contrast agents are on the market with slightly different compositions; however, they all share a common structure of a microbubble consisting of an internal gas encapsulated by an external shell composed of either phospholipids or albumin [81]. SonoVue® (Bracco, Milan, Italy) is the most commonly employed contrast agent for vascular assessments [82]. It has received European approval for various applications, including cerebral arteries, peripheral arteries, and the portal vein [83].

SonoVue® consists of 2.5 μm bubbles of sulfur hexafluoride gas encapsulated in a monolayer of phospholipids. The phospholipid shell properties allow the gas to be kept inside, yet remain elastic enough to enable the oscillation of microbubbles that is essential for the generation of a CEUS signal. The size of the bubbles is adjusted to allow their free movement inside the capillary bed, but restricts them from passing through the endothelium. Therefore, the SonoVue® contrast agent is exclusively intravascular, which is the basis of its vascular applications [84]. Although this contrast agent is intravenously administered, its route of excretion differs from typical CT or MRI contrast agents. The microbubbles decompose and then the phospholipids are metabolized by the liver while non-toxic sulfur hexafluoride is exhaled by the respiratory system. This property allows SonoVue® to be administered to patients with renal failure, which is a contradiction for other contrast agents [85].

In the context of image acquisition with ultrasonographic contrast agents, the key property is the mechanical index (MI). The MI indicates the insonation power of the ultrasound beam and is defined as the ratio of peak negative pressure to the square root of the US frequency. With ultrasound techniques such as B-mode or Doppler, the MI is usually over 1.6. In contrast to enhanced ultrasonography, the MI value is decreased to values ranging from 0.08 to 0.2 for vascular clinical applications. A low-energy ultrasound beam affects the microbubbles present in the vessels, making them contract and expand in a pattern called oscillation. The contrast agent not only reflects the baseline frequency, but also generates harmonic frequencies as the expansion of microbubbles is stronger than the contraction. Eventually, the bubbles are ruptured by the ultrasound beam, but the area of interest can be observed in real time for a period of a few minutes. The appropriate emission of ultrasound pulses allows for the selective visualization of contrast agents and suppresses the returning signal from static tissue. The results of the CEUS exanimation are displayed on the ultrasound machine screen, juxtaposing a contrast-enhanced image with a low-MI grayscale image for improved orientation [86,87].

12. CEUS Assessment of Intraplaque Neovascularization

Intraplaque neovascularization—the accumulation of leaky and fragile capillaries—is involved in the pathogenesis of vulnerable plaques. The pathological vessels destabilize atherosclerotic plaques, making them susceptible to hemorrhaging within the plaque, which can lead to rupture [88]. As the diameter and flexibility of the contrast microbubbles are similar to red blood cells and as they do not leave the lumen of the vessel, the contrast enhancement of the plaque reflects its microvasculature [89]. Later investigations have demonstrated a robust correlation between CEUS enhancement and the histological vascular density of carotid plaques [90–93].

Frequently, intraplaque neovascularization is assessed using a visual-based 3-point grading system (Figure 2) [48,94]. Grade 1 indicates the absence of moving bubbles within the plaque or microbubbles confined to the adventitial layer (no observable microbubbles). Grade 2 denotes a moderate visual presence of bubbles within the plaque at the adventitial side or plaque shoulder (restricted to moderate microbubbles). Grade 3 represents substantial intraplaque neovascularization, characterized by the distinctly visible movement of bubbles towards the plaque core (extensive presence of microbubbles within the plaque).
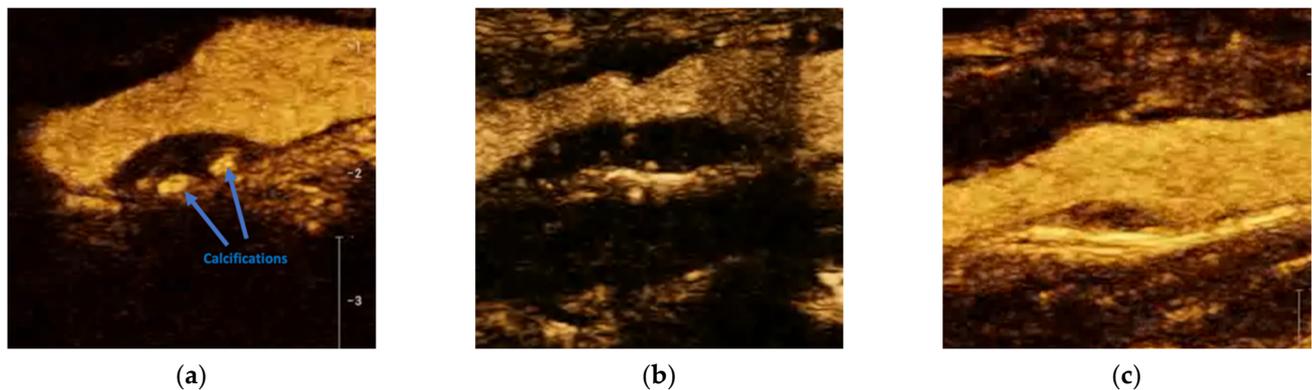
(a)   (b)   (c)

Figure 2. The figure illustrates the qualitative grading system used to assess intraplaque neovascularization within carotid plaques. Grade 1 (**a**) is given to carotid plaques that show no moving microbubbles within the plaque or if there are any, they are limited to the nearby adventitial layer. It is important differentiate artifacts induced by calcification with contrast enhancement (blue arrows). Grade 2 (**b**): there is moderate visibility of moving bubbles within the plaque, mostly at the adventitial side or plaque shoulder. Grade 3 (**c**): there is a clear and visible presence of bubbles moving towards the plaque core or diffused enhancement.

Furthermore, CEUS can not only evaluate intraplaque neovascularization, but also forecast other histological features of plaque vulnerability. Recent studies correlated contrast enhancement with overall plaque immunohistological vulnerability, including the presence of inflammatory cells, fibrous capsule thickness, and size of lipid core [95–97]. The researchers found that CEUS exhibited a sensitivity of 94% and a positive predictive value of 87% in diagnosing histologically vulnerable plaques [93].

13. CEUS Assessment of Plaque Surfaces

When evaluating carotid arteries, CEUS provides valuable information at the macrovascular level. Irregularities and ulceration on carotid plaque surfaces hold substantial clinical importance. Numerous studies have linked them to neurologic symptoms and strokes [21, 51,98]. Although the evaluation of carotid plaque surface morphology is an integral part of atherosclerosis evaluations, the sensitivity of B-mode and Doppler US can be limited [99,100]. The use of a contrast agent allows for the accurate delineation of plaque surfaces that is independent of a slow blood flow or technical artifacts.

Carotid atherosclerotic plaques have varying surface morphologies such as smooth, irregular, or ulcerated (Figure 3). For CEUS, the accepted criteria state:

- Smooth refers to a regular surface with no notable irregularities.
- Irregular plaques have fluctuations between 0.3 and 0.9 mm.
- Ulceration is the most severe irregularity, with a cavity of at least 1×1 mm [101,102].

Plaque ulceration causes severe endothelial damage, leading to the exposure of plaque's necrotic core to the circulation, which is strongly related to subsequent ischemic accidents [103]. In several studies comparing conventional color Doppler techniques with CEUS, the latter achieved significantly higher sensitivity in detecting plaque ulceration, reaching up to $88-94\%$ with computed tomography angiography as a reference [100,104]. Apart from plaque surface irregularities, CEUS may be of benefit to the detection of the intraluminal thrombus [105]. When assessing CEUS images, it is crucial to differentiate the hyperechogenic parts of plaques (calcifications) resembling contrast enchantment by cross-checking with low-MI grayscale images [80].
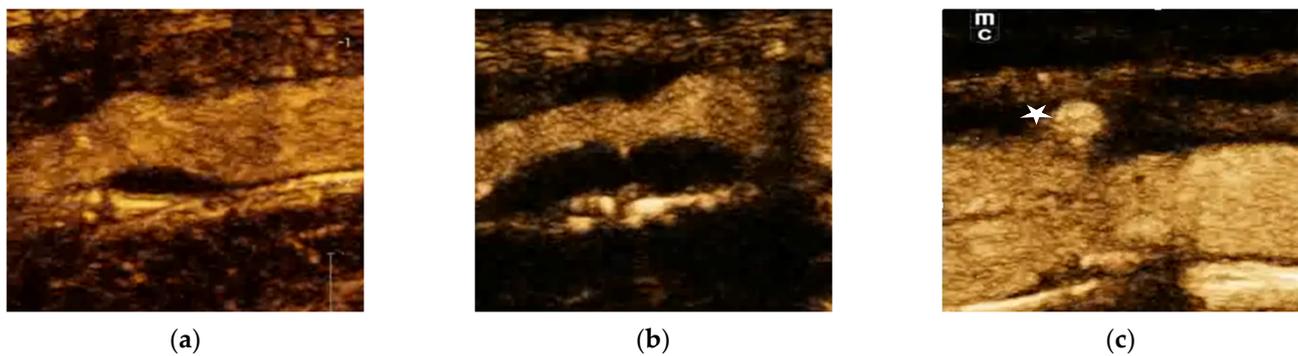
(**a**) (**b**) (**c**)

Figure 3. Carotid atherosclerotic plaques exhibit diverse surface morphologies, including smooth, irregular, or ulcerated. In accordance with CEUS imaging, smooth (**a**) denotes a regular plaque surface devoid of significant irregularities. Irregular plaques (**b**) are characterized by surface fluctuations ranging from 0.3 to 0.9 mm. Ulceration (**c**) represents the most severe irregularity, featuring a cavity measuring at least 1×1 mm in size (marked with an asterisk).

14. Quantification of CEUS

Quantitative analysis is currently a vital trend in radiology. It aims to minimize subjectivity and enhance inter-observer consensus. The majority of CEUS studies utilize a qualitative assessment of carotid plaque properties. However, some approaches aim to quantitatively assess the plaque surface and intraplaque enhancement [106–108].

For the assessment of intraplaque neovascularization, quantification software analyses the cine loops recorded after contrast agent administration. One of the software packages enabling the automated quantification of intraplaque microvessels is VueBox® v. 7 (Bracco, Milan, Italy), developed by the manufacturer of the SonoVue® agent [109]. Following the administration of the contrast, the enhancement within a designated plaque region of interest is compared with the enhancement within the lumen of the carotid artery on a time–intensity curve. The software allows for the quantification of maximal intraplaque intensity and relative perfused area that can be directly compared between patients.

A quantitative evaluation of carotid plaque surfaces has been proposed as an index obtained by dividing the sum of angular deviations of the plaque surface from a straight line by the plaque's length [110]; however, this method is not used in solutions available on the market. Quantifying carotid plaque irregularities through CEUS seems both achievable and a promising approach for the identification of vulnerable carotid plaques.

15. Clinical Applications

It is valuable to investigate whether CEUS can assess plaque vulnerability and forecast cerebrovascular outcomes in individuals with carotid atherosclerotic disease. Numerous studies have provided indications that CEUS enhancement might correlate with prior or ongoing cerebrovascular events.

Xiong et al. studied the link between CEUS enhancement and a history of transient ischemic attacks (TIAs) or cerebrovascular ischemic strokes. While plaque thickness and ulceration did not significantly differ in symptomatic and asymptomatic patients, statistically significant differences emerged in the enhancement pattern, intensity, and plaque-to-lumen enhancement ratio on CEUS imaging [111]. Likewise, Staub et al. identified a correlation between higher-grade neovascularization in CEUS and increased cardiovascular event frequency [112]. In the same vein, Xu et al. detected significant contrast enhancement differences in cerebral infarction patients [113]. Li et al. reported noteworthy contrast enhancement distinctions in acute ischemic infarction patients [91]. A semi-quantitative CEUS study by Huang et al. revealed stroke-rate disparities between the highest and lowest enhancement grades [114]. Faggioli et al. found heightened CEUS peak enhancement intensity in patients with neurological symptoms [115].

16. CEUS versus Other Imaging Methods

Neovascularization and hemorrhaging are histopathological characteristics associated with vulnerable plaques that can result in cerebrovascular accidents [116]. These associations have prompted advancements in imaging tools for the detection of vulnerable carotid plaques (Table 2). CEUS is a recognized and effective imaging technique to assess vascularization [117]. However, it is essential to keep in mind that CEUS shares the limitations of conventional ultrasound. It relies on the operator's skill and may not provide sufficient information in certain scenarios such as when dealing with large and calcified plaques [118]. Moreover, traditional US imaging is constrained by its ability to assess intravascular events beyond a specific threshold of molecular expression.

Table 2. Application scenarios, advantages, and limitations of the various non-invasive techniques used for plaque vulnerability examinations.

Imaging Technique	Application Scenarios	Advantages	Limitations
CEUS	• Contrast agent indicates regions with high neovascularization • Good visualization of plaque surface and ulceration	• Non-invasive • Radiation-free • Good availability • Limited cost	• Operator dependency • Variability • Resolution
CT	• High resolution allows for examination of plaque density and ulceration • Most effective technique for detection of calcification	• Non-invasive • High resolution • Reproducibility	• Radiation • Contrast agents • Artifacts present due to calcification
MRI	• Good differentiation between fibrous cap and necrotic lumen • Intraplaque hemorrhage detection	• Non-invasive • Radiation-free • High resolution • Reproducibility	• Gadolinium contrast is often needed • Costs • Time • Availability

High-resolution, multicontrast MRI of the carotid artery also stands out as a tool to identify and quantify different components of atherosclerotic plaques [119]. By employing a combination of diverse MRI sequences such as pre-and post-contrast T1-weighted turbo-spin echo imaging, it becomes possible to discern hemorrhagic regions, calcium deposits, lipid accumulations, and fibrous tissue within carotid plaques [120]. MRI is currently acknowledged as the most valuable imaging method to quantify the carotid plaque burden and non-invasively assess plaque compositions [121]. Its validation, reproducibility, and ability to predict strokes and evaluate treatment outcomes are well-established [121,122].

Furthermore, MRI can be employed to measure plaque inflammation using ultra-small superparamagnetic iron oxide particles (ferumoxtran-10) due to their uptake by macrophages within the plaque [123,124]. While magnetic resonance angiography (MRA) has the advantage of being radiation-free, it comes with limitations such as low spatial and temporal resolutions, resulting in extended scan durations. Additionally, MRA is a complex, costly, and less readily accessible imaging modality when compared with other options [125,126].

CT angiography (CTA) offers the advantage of assessing both extra- and intracranial blood vessels spanning from the aortic arch to the circle of Willis as well as cerebral tissue, all within a single session. In the detection of carotid artery stenosis and irregularities in plaque surfaces, CTA demonstrates sensitivity and specificity on a par with digital subtraction angiography (DSA). It additionally provides insights into the vascular wall and valuable information about plaque compositions [127].

Modern CTA, when combined with specialized segmentation software analyses, can identify the soft-tissue subcomponents of plaques and quantify intraplaque neovascularization [128]. The degree of contrast enhancement appears to correlate with the extent of neovascularization [129]. Studies have shown that multidetector CT angiography out-

performs DSA and ultrasound in detecting ulcerations with higher sensitivity and specificity [99]. Nonetheless, a certain level of controversy exists in the literature regarding CTA's reliability in characterizing soft-tissue components. Some authors have reported lower sensitivity in distinguishing lipid cores and intraplaque hemorrhages when dealing with small lesions [130].

17. Conclusions

In conclusion, CEUS is a significant tool when evaluating the sonographic indicators of carotid plaque vulnerability, portraying surface irregularities, ulceration, intraplaque neovascularization, and adventitial vasa vasorum development (Table 3). CEUS-detected intraplaque neovascularization closely aligns with histological capillary density, indicating vulnerability. Preliminary evidence suggests CEUS could directly predict the high-risk plaque histologic grade. CEUS correlates with past cerebrovascular events and shows potential for forecasting future cerebrovascular and cardiovascular incidents. Despite progress, CEUS studies often lack a prospective design. Larger multicenter studies are needed to further establish CEUS's role in carotid atherosclerotic disease assessments.

Table 3. Indicators of carotid plaque vulnerability detected by carotid contrast-enhanced ultrasound (CEUS).

Marker	Classification	Details
Plaque surface	Visual-based qualitative system	Smooth plaque surface Plaque surface irregularities (0.3 and 0.9 mm) Plaque ulceration ($>1 \times 1$ mm)
	Surface irregularity index	Index obtained by dividing the sum of angular deviations of the plaque surface from the straight line by the length of the plaque
Intraplaque neovascularization	Visual-based qualitative system	Grade 1: no vascularization Grade 2: moderate vascularization Grade 3: extensive vascularization
	Quantitative assessment with dedicated software, e.g., VueBox	Plaque region of interest compared with the lumen of the carotid artery

References

1. Stanhewicz, A.E.; Wenner, M.M.; Stachenfeld, N.S. Sex differences in endothelial function important to vascular health and overall cardiovascular disease risk across the lifespan. *Am. J. Physiol. Circ. Physiol.* **2018**, *315*, H1569–H1588. [CrossRef] [PubMed]
2. Murphy, S.J.; Werring, D.J. Stroke: Causes and clinical features. *Medicine* **2020**, *48*, 561–566. [CrossRef]

3. Jagannathan, R.; Patel, S.A.; Ali, M.K.; Narayan, K.M.V. Global Updates on Cardiovascular Disease Mortality Trends and Attribution of Traditional Risk Factors. *Curr. Diabetes Rep.* **2019**, *19*, 44. [CrossRef] [PubMed]
4. Wang, Z.; Korantzopoulos, P.; Liu, T. Correction to: Carotid Atherosclerosis in Patients with Atrial Fibrillation. *Curr. Atheroscler. Rep.* **2019**, *21*, 1. [CrossRef] [PubMed]
5. Syed, M.B.; Fletcher, A.J.; Forsythe, O.R.; Kaczynski, J.; Newby, E.D.; Dweck, M.R.; van Beek, E.J. Emerging techniques in atherosclerosis imaging. *Br. J. Radiol.* **2019**, *92*, 20180309. [CrossRef]
6. Camaré, C.; Pucelle, M.; Nègre-Salvayre, A.; Salvayre, R. Angiogenesis in the atherosclerotic plaque. *Redox Biol.* **2017**, *12*, 18–34. [CrossRef]
7. Avgerinos, N.A.; Neofytou, P. Mathematical Modelling and Simulation of Atherosclerosis Formation and Progress: A Review. *Ann. Biomed. Eng.* **2019**, *47*, 1764–1785. [CrossRef]
8. Kotlyarov, S. Diversity of Lipid Function in Atherogenesis: A Focus on Endothelial Mechanobiology. *Int. J. Mol. Sci.* **2021**, *22*, 11545. [CrossRef]
9. Deng, W.; Tang, T.; Hou, Y.; Zeng, Q.; Wang, Y.; Fan, W.; Qu, S. Extracellular vesicles in atherosclerosis. *Clin. Chim. Acta* **2019**, *495*, 109–117. [CrossRef]
10. Singh, R.B.; Mengi, A.S.; Xu, Y.-J.; Arneja, A.S.; Dhalla, N.S. Pathogenesis of atherosclerosis: A multifactorial process. *Exp. Clin. Cardiol.* **2002**, *7*, 40–53.
11. Logan, J.K.; Ayers, M.P. Noninvasive Imaging for the Asymptomatic Patient. *Med. Clin. N. Am.* **2022**, *106*, 377–388. [CrossRef] [PubMed]
12. Björkegren, J.L.; Lusis, A.J. Atherosclerosis: Recent developments. *Cell* **2022**, *185*, 1630–1645. [CrossRef] [PubMed]
13. Xu, Y.; Kovacic, J.C. Endothelial to Mesenchymal Transition in Health and Disease. *Annu. Rev. Physiol.* **2023**, *85*, 245–267. [CrossRef]
14. Keeter, W.C.; Ma, S.; Stahr, N.; Moriarty, A.K.; Galkina, E.V. Atherosclerosis and multi-organ-associated pathologies. *Semin. Immunopathol.* **2022**, *44*, 363–374. [CrossRef] [PubMed]
15. Raitakari, O.; Pahkala, K.; Magnussen, C.G. Prevention of atherosclerosis from childhood. *Nat. Rev. Cardiol.* **2022**, *19*, 543–554. [CrossRef] [PubMed]
16. da Luz, P.L.; Chagas, A.C.P.; Dourado, P.M.M.; Laurindo, F.R. Endothelium in Atherosclerosis: Plaque Formation and Its Complications. In *Endothelium and Cardiovascular Diseases: Vascular Biology and Clinical Syndromes*; Academic Press: Cambridge, MA, USA, 2018; pp. 493–512. [CrossRef]
17. Campbell, B.C.V.; De Silva, D.A.; Macleod, M.R.; Coutts, S.B.; Schwamm, L.H.; Davis, S.M.; Donnan, G.A. Ischaemic stroke. *Nat. Rev. Dis. Prim.* **2019**, *5*, 70. [CrossRef]
18. Kronenberg, F.; Mora, S.; Stroes, E.S.G.; Ference, A.B.; Arsenault, B.J.; Berglund, L.; Dweck, M.R.; Koschinsky, M.; Lambert, G.; Mach, F.; et al. Lipoprotein(a) in atherosclerotic cardiovascular disease and aortic stenosis: A European Atherosclerosis Society consensus statement. *Eur. Heart J.* **2022**, *43*, 3925–3946. [CrossRef] [PubMed]
19. Leiner, T.; Bogaert, J.; Friedrich, M.G.; Mohiaddin, R.; Muthurangu, V.; Myerson, S.; Powell, A.J.; Raman, S.V.; Pennell, D.J. SCMR Position Paper (2020) on clinical indications for cardiovascular magnetic resonance. *J. Cardiovasc. Magn. Reson.* **2020**, *22*, 76. [CrossRef]
20. Kim, H.W.; Regenhardt, R.W.; D'Amato, A.S.; Nahhas, M.I.; Dmytriw, A.A.; Hirsch, A.J.; Silverman, S.B.; Martinez-Gutierrez, J.C. Asymptomatic carotid artery stenosis: A summary of current state of evidence for revascularization and emerging high-risk features. *J. NeuroInterventional Surg.* **2022**, *15*, 717–722. [CrossRef]
21. Brinjikji, W.; Rabinstein, A.A.; Lanzino, G.; Murad, M.H.; Williamson, E.E.; DeMarco, J.K.; Iii, J.H. Ultrasound Characteristics of Symptomatic Carotid Plaques: A Systematic Review and Meta-Analysis. *Cerebrovasc. Dis.* **2015**, *40*, 165–174. [CrossRef]
22. Casscells, W.; Naghavi, M.; Willerson, J.T. Vulnerable Atherosclerotic Plaque. *Circulation* **2003**, *107*, 2072–2075. [CrossRef]
23. Naghavi, M.; Libby, P.; Falk, E.; Casscells, S.; Litovsky, S.; Rumberger, J.; Badimon, J.J.; Stefanadis, C.; Moreno, P.; Pasterkamp, G.; et al. From Vulnerable Plaque to Vulnerable Patient: A call for new definitions and risk assessment strategies: Part I. *Circulation* **2003**, *108*, 1664–1672. [CrossRef] [PubMed]
24. Naylor, R.; Rantner, B.; Ancetti, S.; de Borst, G.J.; De Carlo, M.; Halliday, A.; Kakkos, S.K.; Markus, H.S.; McCabe, D.J.; Sillesen, H.; et al. Editor's Choice–European Society for Vascular Surgery (ESVS) 2023 Clinical Practice Guidelines on the Management of Atherosclerotic Carotid and Vertebral Artery Disease. *Eur. J. Vasc. Endovasc. Surg.* **2022**, *65*, 7–111. [CrossRef] [PubMed]
25. Heck, D.; Jost, A. Carotid stenosis, stroke, and carotid artery revascularization. *Prog. Cardiovasc. Dis.* **2021**, *65*, 49–54. [CrossRef] [PubMed]
26. Libby, P.; Pasterkamp, G.; Crea, F.; Jang, I.-K. Reassessing the Mechanisms of Acute Coronary Syndromes. *Circ. Res.* **2019**, *124*, 150–160. [CrossRef]
27. Mury, P.; Chirico, E.N.; Mura, M.; Millon, A.; Canet-Soulas, E.; Pialoux, V. Oxidative Stress and Inflammation, Key Targets of Ather-osclerotic Plaque Progression and Vulnerability: Potential Impact of Physical Activity. *Sports Med.* **2018**, *48*, 2725–2741. [CrossRef]
28. Nielsen, S.H.; Jonasson, L.; Kalogeropoulos, K.; Karsdal, M.A.; Reese-Petersen, A.L.; Keller, U.A.D.; Genovese, F.; Nilsson, J.; Goncalves, I. Exploring the role of extracellular matrix proteins to develop biomarkers of plaque vulnerability and outcome. *J. Intern. Med.* **2020**, *287*, 493–513. [CrossRef]

29. Chiorescu, R.M.; Mocan, M.; Inceu, A.I.; Buda, A.P.; Blendea, D.; Vlaicu, S.I. Vulnerable Atherosclerotic Plaque: Is There a Molecular Signature? *Int. J. Mol. Sci.* **2022**, *23*, 13638. [CrossRef]

30. Cai, Y.; Pan, J.; Li, Z. Mathematical modeling of intraplaque neovascularization and hemorrhage in a carotid atherosclerotic plaque. *Biomed. Eng. Online* **2021**, *20*, 42. [CrossRef]

31. Liberale, L. The Role of Vascular Aging in Atherosclerotic Plaque Development and Vulnerability. *Curr. Pharm. Des.* **2019**, *25*, 3098–3111. [CrossRef]

32. Li, J.; Ma, Y.; Miao, X.-H.; Guo, J.-D.; Li, D.-W. Neovascularization and tissue regeneration by endothelial progenitor cells in ischemic stroke. *Neurol. Sci.* **2021**, *42*, 3585–3593. [CrossRef] [PubMed]

33. Sedding, D.G.; Boyle, E.C.; Demandt, J.A.F.; Sluimer, J.C.; Dutzmann, J.; Haverich, A.; Bauersachs, J. Vasa Vasorum Angiogenesis: Key Player in the Initiation and Progression of Atherosclerosis and Potential Target for the Treatment of Cardiovascular Disease. *Front. Immunol.* **2018**, *9*, 706. [CrossRef] [PubMed]

34. Wu, W.; Li, X.; Zuo, G.; Pu, J.; Wu, X.; Chen, S. The Role of Angiogenesis in Coronary Artery Disease: A Double-Edged Sword: Intraplaque Angiogenesis in Physiopathology and Therapeutic Angiogenesis for Treatment. *Curr. Pharm. Des.* **2018**, *24*, 451–464. [CrossRef] [PubMed]

35. Wong, N.K.P.; Solly, E.L.; Bursill, C.A.; Tan, J.T.M.; Ng, M.K.C. Pathophysiology of Angiogenesis and Its Role in Vascular Disease. In *Mechanisms of Vascular Disease: A Textbook for Vascular Specialists*; Springer: Berlin/Heidelberg, Germany, 2020; pp. 89–116. [CrossRef]

36. Bonafiglia, Q.A.; Bendeck, M.; Gotlieb, A.I. Vascular Pathobiology: Atherosclerosis and Large Vessel Disease. In *Cardiovascular Pathology*; Academic Press: Cambridge, MA, USA, 2022; pp. 265–306. [CrossRef]

37. Mushenkova, N.V.; Nikiforov, N.G.; Melnichenko, A.A.; Kalmykov, V.; Shakhpazyan, N.K.; Orekhova, V.A.; Orekhov, A.N. Functional Phenotypes of Intraplaque Macrophages and Their Distinct Roles in Atherosclerosis Development and Atheroinflammation. *Biomedicines* **2022**, *10*, 452. [CrossRef] [PubMed]

38. Singh, A.; Nasir, U.; Segal, J.; Waheed, T.A.; Ameen, M.; Hafeez, H. The utility of ultrasound and computed tomography in the as-sessment of carotid artery plaque vulnerability–A mini review. *Front. Cardiovasc. Med.* **2022**, *9*, 1023562. [CrossRef] [PubMed]

39. Mura, M.; Della Schiava, N.; Long, A.; Chirico, E.N.; Pialoux, V.; Millon, A. Carotid intraplaque haemorrhage: Pathogenesis, histological classification, imaging methods and clinical value. *Ann. Transl. Med.* **2020**, *8*, 1273. [CrossRef]

40. Porcu, M.; Mannelli, L.; Melis, M.; Suri, J.S.; Gerosa, C.; Cerrone, G.; Defazio, G.; Faa, G.; Saba, L. Carotid plaque imaging profiling in subjects with risk factors (diabetes and hypertension). *Cardiovasc. Diagn. Ther.* **2020**, *10*, 1005–1018. [CrossRef]

41. Zhu, G.; Hom, J.; Li, Y.; Jiang, B.; Rodriguez, F.; Fleischmann, D.; Saloner, D.; Porcu, M.; Zhang, Y.; Saba, L.; et al. Carotid plaque imaging and the risk of atherosclerotic cardiovascular disease. *Cardiovasc. Diagn. Ther.* **2020**, *10*, 1048–1067. [CrossRef]

42. Zamani, M.; Skagen, K.; Scott, H.; Russell, D.; Skjelland, M. Advanced ultrasound methods in assessment of carotid plaque instability: A prospective multimodal study. *BMC Neurol.* **2020**, *20*, 39. [CrossRef]

43. Saba, L.; Antignani, P.L.; Gupta, A.; Cau, R.; Paraskevas, K.I.; Poredos, P.; Wasserman, B.A.; Kamel, H.; Avgerinos, E.D.; Salgado, R.; et al. International Union of Angiology (IUA) consensus paper on imaging strategies in atherosclerotic carotid artery imaging: From basic strategies to advanced approaches. *Atherosclerosis* **2022**, *354*, 23–40. [CrossRef]

44. Golemati, S.; Cokkinos, D.D. Recent advances in vascular ultrasound imaging technology and their clinical implications. *Ultrasonics* **2021**, *119*, 106599. [CrossRef] [PubMed]

45. Weng, S.-T.; Lai, Q.-L.; Cai, M.-T.; Wang, J.-J.; Zhuang, L.-Y.; Cheng, L.; Mo, Y.-J.; Liu, L.; Zang, Y.-X.; Qiao, S. Detecting vulnerable carotid plaque and its component characteristics: Progress in related imaging techniques. *Front. Neurol.* **2022**, *13*, 982147. [CrossRef] [PubMed]

46. Schlesinger, D.E.; Stultz, C.M. Deep Learning for Cardiovascular Risk Stratification. *Curr. Treat. Options Cardiovasc. Med.* **2020**, *22*, 15. [CrossRef]

47. Fabiani, I.; Palombo, C.; Caramella, D.; Nilsson, J.; De Caterina, R. Imaging of the vulnerable carotid plaque. *Neurology* **2020**, *94*, 922–932. [CrossRef]

48. Schinkel, A.F.; Bosch, J.G.; Staub, D.; Adam, D.; Feinstein, S.B. Contrast-Enhanced Ultrasound to Assess Carotid Intraplaque Neovascularization. *Ultrasound Med. Biol.* **2019**, *46*, 466–478. [CrossRef]

49. Cismaru, G.; Serban, T.; Tirpe, A. Ultrasound Methods in the Evaluation of Atherosclerosis: From Pathophysiology to Clinic. *Biomedicines* **2021**, *9*, 418. [CrossRef]

50. Zhang, L.; Li, X.; Lyu, Q.; Shi, G. Imaging diagnosis and research progress of carotid plaque vulnerability. *J. Clin. Ultrasound* **2022**, *50*, 905–912. [CrossRef]

51. Rafailidis, V.; Charitanti, A.; Tegos, T.; Rafailidis, D.; Chryssogonidis, I. Swirling of microbubbles: Demonstration of a new finding of carotid plaque ulceration on contrast-enhanced ultrasound explaining the arterio-arterial embolism mechanism. *Clin. Hemorheol. Microcirc.* **2016**, *64*, 245–250. [CrossRef]

52. Vlachopoulos, C.; Georgakopoulos, C.; Koutagiar, I.; Tousoulis, D. Diagnostic modalities in peripheral artery disease. *Curr. Opin. Pharmacol.* **2018**, *39*, 68–76. [CrossRef]

53. Yonetsu, T.; Jang, I.-K. Advances in Intravascular Imaging: New Insights into the Vulnerable Plaque from Imaging Studies. *Korean Circ. J.* **2018**, *48*, 1–15. [CrossRef]

54. Mushenkova, N.V.; Summerhill, V.I.; Zhang, D.; Romanenko, E.B.; Grechko, A.V.; Orekhov, A.N. Current Advances in the Diagnostic Imaging of Atherosclerosis: Insights into the Pathophysiology of Vulnerable Plaque. *Int. J. Mol. Sci.* **2020**, *21*, 2992. [CrossRef] [PubMed]

55. Andrews, J.P.; Fayad, Z.A.; Dweck, M.R. New methods to image unstable atherosclerotic plaques. *Atherosclerosis* **2018**, *272*, 118–128. [CrossRef] [PubMed]

56. Brandt, V.; Bekeredjian, R.; Schoepf, U.J.; Varga-Szemes, A.; Emrich, T.; Aquino, G.J.; Decker, J.; Bayer, R.R.; Ellis, L.; Ebersberger, U.; et al. Prognostic value of epicardial adipose tissue volume in combination with coronary plaque and flow assessment for the prediction of major adverse cardiac events. *Eur. J. Radiol.* **2022**, *148*, 110157. [CrossRef] [PubMed]

57. Rafailidis, V.; Li, X.; Sidhu, P.S.; Partovi, S.; Staub, D. Contrast imaging ultrasound for the detection and characterization of carotid vulnerable plaque. *Cardiovasc. Diagn. Ther.* **2020**, *10*, 965–981. [CrossRef] [PubMed]

58. Liu, S.; Li, L.; Wang, H.; Tan, J.; Wei, L.; Weng, Y.; Chen, J. Recent Advances: From Cell Biology to Cell Therapy in Atherosclerosis Plaque via Stent Implantation. *Curr. Med. Chem.* **2022**, *30*, 3582–3613. [CrossRef]

59. Shishikura, D.; Octavia, Y.; Hayat, U.; Thondapu, V.; Barlis, P. Atherogenesis and Inflammation. *Eur. Heart J.* **1993**, *14*, 2–6. [CrossRef]

60. Pandey, R.; Kumar, M.; Majdoubi, J.; Rahimi-Gorji, M.; Srivastav, V.K. A review study on blood in human coronary artery: Numerical approach. *Comput. Methods Programs Biomed.* **2019**, *187*, 105243. [CrossRef]

61. Knuuti, J.; Wijns, W.; Saraste, A.; Capodanno, D.; Barbato, E.; Funck-Brentano, C.; Prescott, E.; Storey, R.F.; Deaton, C.; Cuisset, T.; et al. 2019 ESC Guidelines for the diagnosis and management of chronic coronary syndromes. *Eur. Heart J.* **2020**, *41*, 407–477. [CrossRef]

62. Greenland, P.; Blaha, M.J.; Budoff, M.J.; Erbel, R.; Watson, K.E. Coronary Calcium Score and Cardiovascular Risk. *J. Am. Coll. Cardiol.* **2018**, *72*, 434–447. [CrossRef]

63. Saito, Y.; Kobayashi, Y.; Fujii, K.; Sonoda, S.; Tsujita, K.; Hibi, K.; Morino, Y.; Okura, H.; Ikari, Y.; Honye, J. Clinical expert consensus document on standards for measurements and assessment of intravascular ultrasound from the Japanese Association of Cardiovascular Intervention and Therapeutics. *Cardiovasc. Interv. Ther.* **2020**, *35*, 1–12. [CrossRef]

64. Alexandratou, M.; Papachristodoulou, A.; Li, X.; Partovi, S.; Davidhi, A.; Rafailidis, V.; Prassopoulos, P.; Kamperidis, V.; Koutroulou, I.; Tsivgoulis, G.; et al. Advances in Noninvasive Carotid Wall Imaging with Ultrasound: A Narrative Review. *J. Clin. Med.* **2022**, *11*, 6196. [CrossRef] [PubMed]

65. Michel, J.-B. Phylogenic Determinants of Cardiovascular Frailty, Focus on Hemodynamics and Arterial Smooth Muscle Cells. *Physiol. Rev.* **2020**, *100*, 1779–1837. [CrossRef]

66. Syed, M.B.; Doris, M.; Dweck, M.; Forsythe, R.; Newby, D.E. Imaging vascular calcification: Where are we headed. In *Coronary Calcium: A Comprehensive Understanding of Its Biology, Use in Screening, and Interventional Management*; Academic Press: Cambridge, MA, USA, 2019; pp. 203–246. [CrossRef]

67. Fernández-Alvarez, V.; Linares Sánchez, M.; López Alvarez, F.; Suárez Nieto, C.; Mäkitie, A.A.; Olsen, K.D.; Ferlito, A. Evaluation of Intima-Media Thickness and Arterial Stiffness as Early Ultrasound Biomarkers of Carotid Artery Atherosclerosis. *Cardiol. Ther.* **2022**, *11*, 231–247. [CrossRef] [PubMed]

68. Yang, C.-W.; Guo, Y.-C.; Li, C.-I.; Liu, C.-S.; Lin, C.-H.; Liu, C.-H.; Wang, M.-C.; Yang, S.-Y.; Li, T.-C.; Lin, C.-C. Subclinical Atherosclerosis Markers of Carotid Intima-Media Thickness, Carotid Plaques, Carotid Stenosis, and Mortality in Community-Dwelling Adults. *Int. J. Environ. Res. Public Health* **2020**, *17*, 4745. [CrossRef]

69. Johri, A.M.; Nambi, V.; Naqvi, T.Z.; Feinstein, S.B.; Kim, E.S.; Park, M.M.; Becher, H.; Sillesen, H. Recommendations for the Assessment of Carotid Arterial Plaque by Ultrasound for the Characterization of Atherosclerosis and Evaluation of Cardiovascular Risk: From the American Society of Echocardiography. *J. Am. Soc. Echocardiogr.* **2020**, *33*, 917–933. [CrossRef]

70. Kim, H.-L.; Kim, S.-H. Pulse Wave Velocity in Atherosclerosis. *Front. Cardiovasc. Med.* **2019**, *6*, 41. [CrossRef] [PubMed]

71. Saraste, A.; Barbato, E.; Capodanno, D.; Edvardsen, T.; Prescott, E.; Achenbach, S.; Bax, J.J.; Wijns, W.; Knuuti, J. Imaging in ESC clinical guidelines: Chronic coronary syndromes. *Eur. Heart J. Cardiovasc. Imaging* **2019**, *20*, 1187–1197. [CrossRef] [PubMed]

72. Murray, C.S.G.; Nahar, T.; Kalashyan, H.; Becher, H.; Nanda, N.C. Ultrasound assessment of carotid arteries: Current concepts, methodologies, diagnostic criteria, and technological advancements. *Echocardiography* **2018**, *35*, 2079–2091. [CrossRef] [PubMed]

73. Katakami, N.; Matsuoka, T.; Shimomura, I. Clinical utility of carotid ultrasonography: Application for the management of patients with diabetes. *J. Diabetes Investig.* **2019**, *10*, 883–898. [CrossRef] [PubMed]

74. Geiger, M.A.; Flumignan, R.L.G.; Sobreira, M.L.; Avelar, W.M.; Fingerhut, C.; Stein, S.; Guillaumon, A.T. Carotid Plaque Composition and the Im-portance of Non-Invasive in Imaging Stroke Prevention. *Front. Cardiovasc. Med.* **2022**, *9*, 885483. [CrossRef]

75. Fedak, A.; Ciuk, K.; Urbanik, A. Ultrasonography of vulnerable atherosclerotic plaque in the carotid arteries: B-mode imaging. *J. Ultrason.* **2020**, *20*, e135–e145. [CrossRef]

76. Lee, W. General principles of carotid Doppler ultrasonography. *Ultrasonography* **2013**, *33*, 11–17. [CrossRef] [PubMed]

77. Thijssen, D.H.J.; Carter, S.E.; Green, D.J. Arterial structure and function in vascular ageing: Are you as old as your arteries? *J. Physiol.* **2015**, *594*, 2275–2284. [CrossRef]

78. Chiha, J.; Mitchell, P.; Gopinath, B.; Burlutsky, G.; Plant, A.; Kovoor, P.; Thiagalingam, A. Prediction of Coronary Artery Disease Extent and Severity Using Pulse Wave Velocity. *PLoS ONE* **2016**, *11*, e0168598. [CrossRef]

79. Staub, D.; Schinkel, A.F.; Coll, B.; Coli, S.; van der Steen, A.F.; Reed, J.D.; Krueger, C.; Thomenius, K.E.; Adam, D.; Sijbrands, E.J.; et al. Contrast-Enhanced Ultrasound Imaging of the Vasa Vasorum: From Early Atherosclerosis to the Identification of Unstable Plaques. *JACC Cardiovasc. Imaging* **2010**, *3*, 761–771. [CrossRef] [PubMed]

80. Fetzer, D.T.; Rafailidis, V.; Peterson, C.; Grant, E.G.; Sidhu, P.; Barr, R.G. Artifacts in contrast-enhanced ultrasound: A pictorial essay. *Abdom. Imaging* **2017**, *43*, 977–997. [CrossRef] [PubMed]

81. Averkiou, M.A.; Bruce, M.F.; Powers, J.E.; Sheeran, P.S.; Burns, P.N. Imaging Methods for Ultrasound Contrast Agents. *Ultrasound Med. Biol.* **2019**, *46*, 498–517. [CrossRef] [PubMed]

82. Greis, C. Technology overview: SonoVue (Bracco, Milan). *Eur. Radiol. Suppl.* **2004**, *14* (Suppl. 8), P11–P15. [CrossRef]

83. Sidhu, P.S.; Cantisani, V.; Dietrich, C.F.; Gilja, O.H.; Saftoiu, A.; Bartels, E.; Bertolotto, M.; Calliada, F.; Clevert, D.-A.; Cosgrove, D.; et al. The EFSUMB Guidelines and Recommendations for the Clinical Practice of Contrast-Enhanced Ultrasound (CEUS) in Non-Hepatic Applications: Update 2017 (Long Version). *Ultraschall Med. Eur. J. Ultrasound* **2018**, *39*, e2–e44. [CrossRef]

84. Morel, D.R.; Schwieger, I.; Hohn, L.; Terrettaz, J.; Llull, J.B.; Cornioley, Y.A.; Schneider, M. Human Pharmacokinetics and Safety Evaluation of SonoVue™, a New Contrast Agent for Ultrasound Imaging. *Investig. Radiol.* **2000**, *35*, 80–85. [CrossRef]

85. Hu, C.; Feng, Y.; Huang, P.; Jin, J. Adverse reactions after the use of SonoVue contrast agent: Characteristics and nursing care experience. *Medicine* **2019**, *98*, e17745. [CrossRef]

86. Dietrich, C.F.; Averkiou, M.; Nielsen, M.B.; Barr, R.G.; Burns, P.N.; Calliada, F.; Cantisani, V.; Choi, B.; Chammas, M.C.; Clevert, D.-A.; et al. How to perform Contrast-Enhanced Ultrasound (CEUS). *Ultrasound Int. Open* **2018**, *04*, E2–E15. [CrossRef] [PubMed]

87. Greis, C. Technical aspects of contrast-enhanced ultrasound (CEUS) examinations: Tips and tricks. *Clin. Hemorheol. Microcirc.* **2014**, *58*, 89–95. [CrossRef] [PubMed]

88. Chistiakov, D.A.; Orekhov, A.N.; Bobryshev, Y.V. Contribution of neovascularization and intraplaque haemorrhage to atherosclerotic plaque progression and instability. *Acta Physiol.* **2015**, *213*, 539–553. [CrossRef] [PubMed]

89. Greis, C. Quantitative evaluation of microvascular blood flow by contrast-enhanced ultrasound (CEUS). *Clin. Hemorheol. Microcirc.* **2011**, *49*, 137–149. [CrossRef] [PubMed]

90. Amamoto, T.; Sakata, N.; Ogata, T.; Shimada, H.; Inoue, T. Intra-Plaque Vessels on Contrast-Enhanced Ultrasound Sonography Predict Carotid Plaque Histology. *Cerebrovasc. Dis.* **2018**, *46*, 265–269. [CrossRef] [PubMed]

91. Li, C.; He, W.; Guo, D.; Chen, L.; Jin, X.; Huang, B.; Wang, W. Quantification of Carotid Plaque Neovascularization Using Contrast-Enhanced Ultrasound with Histopathologic Validation. *Ultrasound Med. Biol.* **2014**, *40*, 1827–1833. [CrossRef]

92. Vavuranakis, M.; Sigala, F.; Vrachatis, D.A.; Papaioannou, T.G.; Filis, K.; Kavantzas, N.; Kalogeras, K.I.; Massoura, C.; Toufektzian, L.; Kariori, M.G.; et al. Quantitative analysis of carotid plaque vasa vasorum by CEUS and correlation with histology after endarterectomy. *Vasa* **2013**, *42*, 184–195. [CrossRef]

93. Iezzi, R.; Petrone, G.; Ferrante, A.; Lauriola, L.; Vincenzoni, C.; la Torre, M.F.; Snider, F.; Rindi, G.; Bonomo, L. The role of contrast-enhanced ultrasound (CEUS) in visualizing atherosclerotic carotid plaque vulnerability: Which injection protocol? Which scanning technique? *Eur. J. Radiol.* **2015**, *84*, 865–871. [CrossRef]

94. Staub, D.; Partovi, S.; Schinkel, A.F.L.; Coll, B.; Uthoff, H.; Aschwanden, M.; Jaeger, K.A.; Feinstein, S.B. Correlation of Carotid Artery Atherosclerotic Lesion Echogenicity and Severity at Standard US with Intraplaque Neovascularization Detected at Contrast-enhanced US. *Radiology* **2011**, *258*, 618–626. [CrossRef]

95. Fleiner, M.; Kummer, M.; Mirlacher, M.; Sauter, G.; Cathomas, G.; Krapf, R.; Biedermann, B.C. Arterial Neovascularization and Inflammation in Vulnerable Patients. *Circulation* **2004**, *110*, 2843–2850. [CrossRef] [PubMed]

96. Michel, J.-B.; Martin-Ventura, J.L.; Nicoletti, A.; Ho-Tin-Noé, B. Pathology of human plaque vulnerability: Mechanisms and consequences of intraplaque haemorrhages. *Atherosclerosis* **2014**, *234*, 311–319. [CrossRef] [PubMed]

97. Giannoni, M.; Vicenzini, E.; Citone, M.; Ricciardi, M.; Irace, L.; Laurito, A.; Scucchi, L.; Di Piero, V.; Gossetti, B.; Mauriello, A.; et al. Contrast Carotid Ultrasound for the Detection of Unstable Plaques with Neoangiogenesis: A Pilot Study. *Eur. J. Vasc. Endovasc. Surg.* **2009**, *37*, 722–727. [CrossRef] [PubMed]

98. Oord, S.C.v.D.; Akkus, Z.; Renaud, G.; Bosch, J.G.; van der Steen, A.F.; Sijbrands, E.J.; Schinkel, A.F. Assessment of carotid atherosclerosis, intraplaque neovascularization, and plaque ulceration using quantitative contrast-enhanced ultrasound in asymptomatic patients with diabetes mellitus. *Eur. Heart J. Cardiovasc. Imaging* **2014**, *15*, 1213–1218. [CrossRef]

99. Rafailidis, V.; Chryssogonidis, I.; Tegos, T.; Kouskouras, K.; Charitanti-Kouridou, A. Imaging of the ulcerated carotid atherosclerotic plaque: A review of the literature. *Insights Imaging* **2017**, *8*, 213–225. [CrossRef]

100. Rafailidis, V.; Chryssogonidis, I.; Xerras, C.; Nikolaou, I.; Tegos, T.; Kouskouras, K.; Rafailidis, D.; Charitanti-Kouridou, A. A comparative study of color Doppler imaging and contrast-enhanced ultrasound for the detection of ulceration in patients with carotid atherosclerotic disease. *Eur. Radiol.* **2018**, *29*, 2137–2145. [CrossRef]

101. Saba, L.; Yuan, C.; Hatsukami, T.; Balu, N.; Qiao, Y.; DeMarco, J.; Saam, T.; Moody, A.; Li, D.; Matouk, C.; et al. Carotid Artery Wall Imaging: Perspective and Guidelines from the ASNR Vessel Wall Imaging Study Group and Expert Consensus Recommendations of the American Society of Neuroradiology. *Am. J. Neuroradiol.* **2018**, *39*, E9–E31. [CrossRef]

102. Piscaglia, F.; Nolsøe, C.; Dietrich, C.; Cosgrove, D.; Gilja, O.; Nielsen, M.B.; Albrecht, T.; Barozzi, L.; Bertolotto, M.; Catalano, O.; et al. The EFSUMB Guidelines and Recommendations on the Clinical Practice of Contrast Enhanced Ultrasound (CEUS): Update 2011 on non-hepatic applications. *Ultraschall Med. Eur. J. Ultrasound* **2011**, *33*, 33–59. [CrossRef]

103. Imbesi, S.; Kerber, C. An Experimental and Angiographic Explanation of Why Ulcerated Carotid Bulbs Embolize. *Interv. Neuroradiol.* **1999**, *5*, 11–18. [CrossRef]

104. Kate, G.L.T.; van Dijk, A.C.; Oord, S.C.v.D.; Hussain, B.; Verhagen, H.J.; Sijbrands, E.J.; van der Steen, A.F.; van der Lugt, A.; Schinkel, A.F. Usefulness of Contrast-Enhanced Ultrasound for Detection of Carotid Plaque Ulceration in Patients with Symptomatic Carotid Atherosclerosis. *Am. J. Cardiol.* **2013**, *112*, 292–298. [CrossRef]

105. Menon, B.K.; Singh, J.; Al-Khataami, A.; Demchuk, A.M.; Goyal, M.; Calgary CTA Study Group. The donut sign on CT angiography: An indicator of reversible intraluminal carotid thrombus? *Neuroradiology* **2010**, *52*, 1055–1056. [CrossRef]

106. Hoogi, A.; Adam, D.; Hoffman, A.; Kerner, H.; Reisner, S.; Gaitini, D. Carotid Plaque Vulnerability: Quantification of Neovascularization on Contrast-Enhanced Ultrasound with Histopathologic Correlation. *Am. J. Roentgenol.* **2011**, *196*, 431–436. [CrossRef] [PubMed]

107. Akkus, Z.; Hoogi, A.; Renaud, G.; Oord, S.C.v.D.; Kate, G.L.T.; Schinkel, A.F.; Adam, D.; de Jong, N.; van der Steen, A.F.; Bosch, J.G. New Quantification Methods for Carotid Intra-plaque Neovascularization Using Contrast-Enhanced Ultrasound. *Ultrasound Med. Biol.* **2014**, *40*, 25–36. [CrossRef] [PubMed]

108. Oord, S.C.H.v.D.; Akkus, Z.; Bosch, J.G.; Hoogi, A.; Kate, G.L.T.; Renaud, G.; Sijbrands, E.J.G.; Verhagen, H.J.; van der Lugt, A.; Adam, D.; et al. Quantitative Contrast-Enhanced Ultrasound of Intraplaque Neovascularization in Patients with Carotid Atherosclerosis. *Ultraschall Med. Eur. J. Ultrasound* **2014**, *36*, 154–161. [CrossRef]

109. Wiesinger, I.; Jung, F.; Jung, E.M. Contrast-enhanced ultrasound (CEUS) and perfusion imaging using VueBox®. *Clin. Hemorheol. Microcirc.* **2021**, *78*, 29–40. [CrossRef] [PubMed]

110. Kanber, B.; Hartshorne, T.C.; Horsfield, M.A.; Naylor, A.R.; Robinson, T.G.; Ramnarine, K.V. A Novel Ultrasound-Based Carotid Plaque Risk Index Associated with the Presence of Cerebrovascular Symptoms. *Ultraschall Med. Eur. J. Ultrasound* **2014**, *36*, 480–486. [CrossRef] [PubMed]

111. Xiong, L.; Deng, Y.-B.; Zhu, Y.; Liu, Y.-N.; Bi, X.-J. Correlation of Carotid Plaque Neovascularization Detected by Using Contrast-enhanced US with Clinical Symptoms. *Radiology* **2009**, *251*, 583–589. [CrossRef]

112. Staub, D.; Patel, M.B.; Tibrewala, A.; Ludden, D.; Johnson, M.; Espinosa, P.; Coll, B.; Jaeger, K.A.; Feinstein, S.B. Vasa Vasorum and Plaque Neovascularization on Contrast-Enhanced Carotid Ultrasound Imaging Correlates with Cardiovascular Disease and Past Cardiovascular Events. *Stroke* **2010**, *41*, 41–47. [CrossRef]

113. Xu, R.; Yin, X.; Xu, W.; Jin, L.; Lu, M.; Wang, Y. Assessment of carotid plaque neovascularization by contrast-enhanced ultrasound and high sensitivity C-reactive protein test in patients with acute cerebral infarction: A comparative study. *Neurol. Sci.* **2016**, *37*, 1107–1112. [CrossRef]

114. Huang, R.; Abdelmoneim, S.S.; Ball, C.A.; Nhola, L.F.; Farrell, A.M.; Feinstein, S.; Mulvagh, S.L. 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. [CrossRef]

115. Faggioli, G.; Pini, R.; Mauro, R.; Pasquinelli, G.; Fittipaldi, S.; Freyrie, A.; Serra, C.; Stella, A. Identification of Carotid 'Vulnerable Plaque' by Contrast-enhanced Ultrasonography: Correlation with Plaque Histology, Symptoms and Cerebral Computed Tomography. *Eur. J. Vasc. Endovasc. Surg.* **2011**, *41*, 238–248. [CrossRef] [PubMed]

116. Van der Veken, B.; De Meyer, G.R.; Martinet, W. Intraplaque neovascularization as a novel therapeutic target in advanced atherosclerosis. *Expert. Opin. Ther. Targets.* **2016**, *20*, 1247–1257. [CrossRef] [PubMed]

117. Cantisani, V.; Grazhdani, H.; Clevert, D.A.; Iezzi, R.; Aiani, L.; Martegani, A.; Fanelli, F.; Di Marzo, L.; Wlderk, A.; Cirelli, C.; et al. EVAR: Benefits of CEUS for monitoring stent-graft status. *Eur. J. Radiol.* **2015**, *84*, 1658–1665. [CrossRef] [PubMed]

118. David, E.; Martinelli, O.; Pacini, P.; Di Serafino, M.; Huang, P.; Dolcetti, V.; Cantisani, V. New Technologies in the Assessment of Carotid Stenosis: Beyond the Color-Doppler Ultrasound—High Frame Rate Vector-Flow and 3D Arterial Analysis Ultrasound. *Diagnostics* **2023**, *13*, 1478. [CrossRef] [PubMed]

119. Kwee, R.M.; Van Oostenbrugge, R.J.; Hofstra, L.; Teule, G.J.; van Engelshoven, J.M.A.; Mess, W.H.; Kooi, M.E. Identifying vulnerable carotid plaques by noninvasive imaging. *Neurology* **2008**, *70*, 2401–2409. [CrossRef]

120. Benson, J.C.; Cheek, H.; Aubry, M.C.; Lanzino, G.; Huston, J., III; Rabinstein, A.; Brinjikji, W. Cervical carotid plaque MRI: Review of atherosclerosis imaging features and their histologic underpinnings. *Clin. Neuroradiol.* **2021**, *31*, 295–306. [CrossRef]

121. Den Hartog, A.G.; Bovens, S.M.; Koning, W.; Hendrikse, J.; Luijten, P.R.; Moll, F.L.; De Borst, G.J. 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. [CrossRef]

122. Saam, T.; Ferguson, M.S.; Yarnykh, V.L.; Takaya, N.; Xu, D.; Polissar, N.L.; Yuan, C. Quantitative evaluation of carotid plaque composition by in vivo MRI. *Arterioscler. Thromb. Vasc. Biol.* **2005**, *25*, 234–239. [CrossRef]

123. Trivedi, R.; U-King-Im, J.; Gillard, J. Accumulation of Ultrasmall Superparamagnetic Particles of Iron Oxide in Human Atherosclerotic Plaques. *Circulation* **2003**, *108*, e140. [CrossRef]

124. Kooi, M.E.; Cappendijk, V.C.; Cleutjens, K.B.J.M.; Kessels, A.G.H.; Kitslaar, P.J.E.H.M.; Borgers, M.; Van Engelshoven, J.M.A. Accumulation of ultrasmall superparamagnetic particles of iron oxide in human atherosclerotic plaques can be detected by in vivo magnetic resonance imaging. *Circulation* **2003**, *107*, 2453–2458. [CrossRef]

125. Qiao, Y.; Etesami, M.; Malhotra, S.; Astor, B.C.; Virmani, R.; Kolodgie, F.D.; Wasserman, B.A. Identification of intraplaque hemorrhage on MR angiography images: A comparison of contrast-enhanced mask and time-of-flight techniques. *Am. J. Neuroradiol.* **2011**, *32*, 454–459. [CrossRef] [PubMed]

126. Chu, B.; Ferguson, M.S.; Chen, H.; Hippe, D.S.; Kerwin, W.S.; Canton, G.; Hatsukami, T.S. Magnetic Resonance Features of the Disruption-Prone and the Disrupted Carotid Plaque. *J. Am. Coll. Cardiol. Imaging* **2009**, *2*, 883–896. [CrossRef] [PubMed]

127. Hermus, L.; Lefrandt, J.D.; Tio, R.A.; Breek, J.C.; Zeebregts, C.J. Carotid plaque formation and serum biomarkers. *Atherosclerosis* **2010**, *213*, 21–29. [CrossRef] [PubMed]

128. Murgia, A.; Erta, M.; Suri, J.S.; Gupta, A.; Wintermark, M.; Saba, L. CT imaging features of carotid artery plaque vulnerability. *Ann. Transl. Med.* **2020**, *8*, 1261. [CrossRef] [PubMed]

129. DeMarco, J.K.; Huston, J. Imaging of high-risk carotid artery plaques: Current status and future directions. *Neurosurg. Focus* **2014**, *36*, E1. [CrossRef] [PubMed]

130. Zhu, G.; Li, Y.; Ding, V.; Jiang, B.; Ball, R.L.; Rodriguez, F.; Wintermark, M. Semiautomated characterization of carotid artery plaque features from computed tomography angiography to predict atherosclerotic cardiovascular disease risk score. *J. Comput. Assist. Tomogr.* **2019**, *43*, 452. [CrossRef]