Current Strategies and Possible Perspectives of Ultrasonic Risk Stratification of Ischemic Stroke in Internal Carotid Artery Disease

Ultraschalldiagnostik der A. carotis interna zur Risikostratifizierung des ischämischen Schlaganfalls – eine Standortbestimmung mit Perspektive

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Zusammenfassung
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Häufige Ursache eines akuten ischämischen Schlaganfalls ist eine atherosklerotische Veränderung der Halsschlagader. Es ist bisher nicht eindeutig geklärt, welche Veränderungen das Risiko eines Schlaganfalls definieren. Mit der stetig verbesserten Qualität von Ultraschalluntersuchungen gelingt es zunehmend, unterschiedliche Veränderungen in atherosklerotischen Plaques darstellen zu können. Sollte auf diesem Wege eine bessere diagnostische Identifizierung von „Risikoplaques" gelingen, so könnte die therapeutische Entscheidung, ob und insbesondere wie eine Karotisstenose behandelt werden muss, in erheblich rationalerer Art erfolgen. In dieser Übersicht werden aktuelle Entwicklungen in der Ultraschalldiagnostik atherosklerotischer Gefäßwandveränderungen insbesondere der Arteria carotis interna beschrieben. Stattgehabte technische Entwicklungen sowie die Rationale der kontrastmittelgestützten Darstellung der Plaque-Neovaskularisation werden vor dem Hintergrund methodischer Limitationen und möglicher weiterer Entwicklungen erläutert.

Abstract
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Atherosclerotic alterations of the internal carotid artery frequently result in ischemic stroke. However, it remains unclear which specific factor mainly causes an increased risk of stroke. Constant improvements of the diagnostic possibilities of ultrasonic examinations provide increasingly profound insight into plaque alterations. If "risk plaque" could be reliably identified, the therapeutic decision for either medical or surgical treatment could be made more rationally. In this review, we summarize current developments in the ultrasound imaging of atherosclerotic changes in carotid artery disease with special emphasis on technical aspects and the rationale of contrast imaging of plaque vascularization. Methodological limitations and possible future applications are discussed.

Background
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In Germany, about 200 – 300000 patients suffer from ischemic stroke every year. Besides general cardiovascular and neoplastic diseases, stroke is the third most frequent cause of death worldwide. One out of ten Western Europeans dies due to long-term consequences of stroke, which is also the most common reason for invalidity. The most common reason for stroke is a sudden arterial obstruction. Other than in ischemic heart disease, brain cells are extremely sensitive to ischemia and rapidly undergo cell death after discontinuation of the oxygen supply.

The internal carotid artery (ICA) is of special interest with respect to both the causation and early diagnosis of ischemic stroke. Due to the superficial location of the vessel, the ICA is best suited for the early detection of changes in the context of the systemic disease atherosclerosis. Furthermore, these changes may directly expose the underlying cause of the ischemic incident. It is considered that about 30000 strokes in Germany are directly attributable to atherosclerotic changes of the ICA [1]. In this context, ischemic stroke can be caused by two different underlying etiologies: first, the embolic obstruction of a cerebral vessel with the embolus originating from

a disrupted thrombus on top of a severe stenosis of ICA (arterio-arterial embolic cause) and second, less frequent, due to insufficient local blood supply distal to high grade ICA stenosis (hemodynamic cause). Since the risk of ischemic stroke evidently increases with increasing obstruction of the ICA, the grade of stenosis is one of the most important factors when making decisions about surgical or interventional procedures for the elimination of vessel obstruction on top of best medical treatment [2]. However, there is growing doubt as to whether the grade of stenosis alone sufficiently defines the risk of upcoming (embolic) stroke. For example, degradation or ulcerations of the vessel wall will result in the activation of platelet aggregation in order to cover up wall lesions. This, however, may lead to potentially instable clot formation, which covers the lesion but may also be disrupted by the blood stream causing embolic stroke as described above.

It is a matter of current discussion if screening examinations of previously asymptomatic people can reduce the rate of cardiovascular events such as stroke, heart attack or vascular death by the early detection and possible eradication of atherosclerotic lesions [3]. There are four potential examination modalities for the estimation of ICA alterations: 1) digital subtraction angiography DSA; 2) CT angiography CTA; 3) MRI angiography MRA; 4) B-mode and color-coded duplex sonography CCDS. All methods are validated with respect to sensitivity and specificity for the grading of vessel obstructions. However, ultrasound examination by itself is advantageous in that no radiation occurs, no contrast agent has to be employed, and the examination is relatively cost-effective and can be performed repeatedly at the bedside [4]. Last but not least, B-mode imaging can provide morphological evidence regarding the atherosclerotic process better than angiographic techniques which only display the narrowing of the lumen.

Lately, some important technological developments concerning MRI and ultrasound have to be taken into consideration. They all have in common that they target a more detailed depiction of morphological plaque alteration. It has become common sense that not only the grading of the obstruction defines the stability of the plaque. For example, the integrity of the fibrous cap, neovascularization of the plaque, and composition of the plaque have been identified as major risk factors for the formation of appositional thrombus and embolic events ("unstable plaque") [5]. Therefore, ambitions for improved imaging are mainly concentrated on these phenomena [6]. Up to now, contrast-enhanced high resolution MRI techniques have seemed to be best suited for the identification of plaque composition [7].

There is an ongoing debate regarding possible treatment strategies [8]. If best medical treatment alone is not sufficient, two invasive procedures are available. Conventional thrombendarterectomy (TEA) is an open surgical operation, commonly combining the removal of the plaque with subsequent patch closure of the vessel. Percutaneous transluminal angioplasty (PTA) is an interventional procedure, combining widening of the vessel obstruction with stent insertion. After several comparative studies, it is now common sense that both procedures can be safely performed [60]. However, long-term data strongly suggest an advantage of TEA due to superior clinical outcome data and lower rates of re-stenosis [9]. Ultrasound imaging may be especially helpful to identify patients at risk for peri-interventional complications in PTA due to flaking of thrombotic debris by the catheter causing an iatrogenic embolism [10].

Different Modalities of Ultrasound Imaging
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B-mode imaging for displaying plaque morphology

Newer findings have led to the identification of different factors which are able to act as markers of plaque instability (● Fig. 1). These include several forms of inflammatory reactions, as well as apoptosis, angiogenesis, level of calcification, intraplaque hemorrhage, a thin or ruptured fibrous cap with consecutive thrombus deposits as well as a large lipid core [11]. Hence a crucial aspect of sophisticated plaque evaluation is the depiction of morphological changes within the plaque, which are typically visualized by means of B-mode ultrasound and have formerly led to the definition of the Gray-Weale classification [12, 13]. Advancements in B-mode sonography (broadband arrays) and the introduction of harmonic imaging techniques have led to an improved display of the intima-media thickness (IMT) as an early marker of atherosclerotic changes in the common carotid artery [14, 15]. However, with progressive understanding of the processes that lead to the progression of atherosclerosis, the need for improved tissue imaging modalities has become evident. There is increasing understanding of the fact that tissue changes within the plaque and thereby its characteristics in the B-mode image are an important marker for plaque stability and its embolic risk, potentially more important than the grade of stenosis alone [5]. Tissue characteristics that are important for the evaluation of the embolic risk of plaques include the grayscale, the integrity of the plaque surface (i.e., the presence of ulcers on the plaque surface) as well as the homogeneity of the plaque [16–18] (● Fig. 2, 3). Recent studies have shown that the grayscale values of symptomatic versus asymptomatic plaques differ significantly [19].

Visual scores, however, have the disadvantage of relatively high subjectivity, which is illustrated by a rather poor interrater and intrarater correlation [20]. To achieve a more consistent evaluation of the plaque tissue, different automated algorithms have been presented allowing qualitative assessment of the plaque morphology in terms of the Gray-Weale classification [21]. The GSM scale (grayscale median) describes the distribution of grayscale values on a scale from 0–255. By means of digital processing of stored ultrasound pictures, it is relatively simple to perform normalization based on reference tissue like adventitia (GSM 185–195) or blood (GSM 0–5), thereby achieving better reproducibility of the data [22]. It
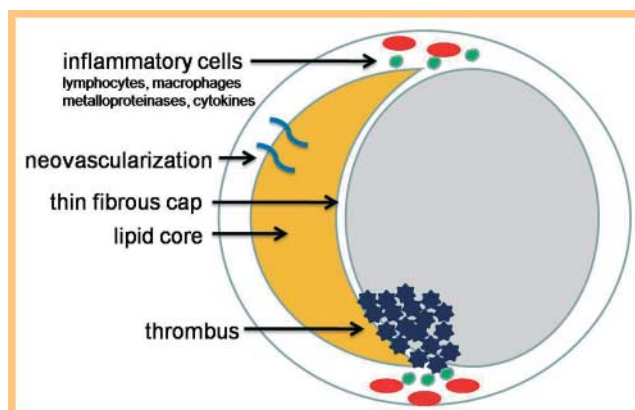

Fig. 1   Factors known to promote "unstable plaque".

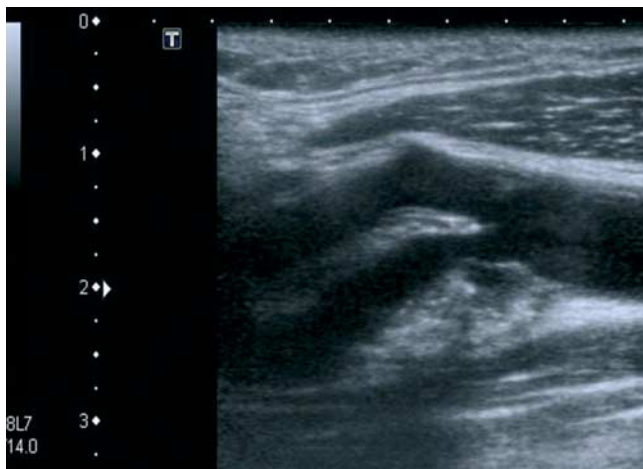Abb. 1   Verschiedene Faktoren in der Entstehung des „instabilen Plaques".

Fig. 2 Morphology of inhomogeneous plaque displayed in B-mode imaging.

Abb. 2 B-Bild-Darstellung der morphologischen Veränderungen eines inhomogenen Plaques.

Fig. 4 Combined Doppler and Duplex sonography of more than 70% ICA stenosis.

Abb. 4 Kombinierte Doppler- und Duplex-Sonografie einer über 70 %igen ACI-Stenose.

Fig. 3 ADF® (Advanced Dynamic Flow) image of an ulcerated plaque.

Abb. 3 ADF®(Advanced Dynamic Flow)-Darstellung eines ulzerierten Plaques.

has been demonstrated that the "normalized" GSM values acquired in this way consistently correlate with clinical events, i.e., they describe the risk of stroke connected to the morphological changes of the plaque [23, 24]. More advanced recent approaches attempt to not only display the mean grayscale values, but also to provide a visual impression of their distribution [25]. The first industry manufactured tools for this purpose are becoming available. However, it remains to be seen if these solutions can contribute to a more substantial evaluation of plaque morphology.

The automated plaque characterization described above does not include the spatial distribution of the different grayscale values within the plaque, so that there might be a lack of information concerning plaque "homogeneity". Furthermore, it was shown that the elastin and calcium content of a plaque have a greater influence on the grayscale median than the lipid content, but it is this lipid content that is known to play an important role in the pathogenesis of a complicated, unstable plaque [26]. In summary, there are a number of studies

offering different results and also variable cut-off values for the GSM, thereby underlining the need for a more differentiated approach to assessing plaque stability than the grayscale values alone. One such approach might be the quantitative display of the distribution of the plaque echogenicity pattern, as it was recently described by Hashimoto et al. [27].

Color-coded duplex sonography for graduation of stenosis

The European and North American Symptomatic Carotid Endarterectomy Trials ECST [28] and NASCET [29] have established the need for exact graduation of carotid stenosis. It is currently indisputable that the accurate grading of internal carotid stenosis can be reliably done by means of ultrasound. There are several studies in which ultrasound has reached excellent sensitivity and specificity values for this purpose as compared to other methods [30]. For exact stenosis quantification, a combination of B-mode imaging, the superimposed color-coded duplex analysis and analysis of the derived Doppler spectrum is used [31] (◗ **Fig. 4**). The evaluation therefore contains information regarding stenosis morphology as well as information regarding the flow velocity measured in cm per second, the peak systolic velocity (PSV) and the end diastolic velocity (EDV) being the most prominent parameters. According to the current literature, a PSV of > 130 cm/sec will be found in more than 50% of cases of ICA stenosis, whereas a PSV of > 250 cm/sec and end diastolic velocity of > 120 cm/sec describe more than 70% of cases of stenosis [61]. Since color-coded duplex ultrasound and spectrum analysis are particularly dependent on the experience of the examiner and the angle of the ultrasound probe, there is a trend toward using alternative methods for stenosis grading in the clinical routine. For example, a recent study compared B-flow Imaging (BFI) with color-coded duplex sonography, CTA and MRA in a group of 21 patients with symptomatic carotid stenosis and found BFI to display the best correlation with conventional angiography, which served as the gold standard [32]. BFI (GE Healthcare) is a digital ultrasound technique based on B-mode images. It uses digital processing of these ultrasound frames to provide a velocity-independent display of blood flow without the need for the superimposition of images [33]. This provides display of

flow characteristics within a blood vessel with reduced artifacts [34]. Alternative methods for improved discrimination of blood flow and the luminal vessel outline like the Advanced Dynamic Flow (ADF, Toshiba [35]) are still in need of studies evaluating their role in the depiction and grading of carotid stenosis.

plaque Neovascularization on Contrast-enhanced ultrasound imaging

The present second generation of ultrasound contrast agents (SonoVue® Bracco, Optison™ GE Healthcare, Definity® Lantheus) consists of microbubbles of high-molecular-weight gas encapsulated by an albumin or lipid shell [36] and is utilized according to international guidelines and recommendations for good clinical practice [37]. The size of microbubbles ranges from 1 to 5 µm, thus permitting uninhibited transit throughout the capillary system including the pulmonary microcirculation. The microbubbles are injected into the bloodstream intravenously and serve as intravascular reflectors of ultrasound waves. The ultrasound characteristics of microbubbles are distinctly different from those of the surrounding blood cells and tissue. Therefore, in conjunction with contrast-specific imaging techniques that are based on the nonlinear scattering properties of microbubbles (oscillation, resonance, higher harmonics), and based on the small size of microbubbles, in contrast to other intravenous contrast agents for DSA, CTA, or MRA, ultrasound contrast agents are true intravascular tracers, used to enhance vascular structures of the macrovasculature and more importantly, to produce high resolution real-time images of the microvasculature including the quantification of tissue perfusion [38].

The vasa vasorum-derived angiogenesis within the atherosclerotic plaque is known to be an important feature in plaque development [39]. Therefore, imaging of the intraplaque neovasculature has recently captured world-wide attention [40]. Several studies have shown that this ectopic neovascularization (angiogenesis) is inherently linked with plaque vulnerability and plaque rupture, initiated and triggered by vascular cell leakage, inflammatory cell recruitment, and intraplaque

hemorrhage [11, 41]. Different reports using real-time contrast-enhanced ultrasound imaging techniques to directly visualize the microvasculature of the adventitial and intraplaque angiogenesis of the carotid artery in humans have recently been published [19, 42, 43]. Normally, contrast-enhanced ultrasound examinations in carotid vascular clinical studies are performed using a high-end ultrasound system equipped with a linear array, vascular probe with transmission frequencies ranging from 4 to 10 MHz, a low mechanical index, and dedicated contrast imaging software utilizing pulse inversion or harmonic techniques. Therefore, when using a reduced mechanical index for imaging, the plaques and corresponding intima-media complex appear hypoechoic while the adventitial layer is observed as echogenic (❂ Fig. 5). The dynamic flow patterns of the adventitial and intraplaque microvasculature is represented by the presence of intravascular tracers (acoustic microspheres) which pass unhindered through the adventitial and the intraplaque vasa vasorum (❂ Fig. 5). The automated visualization and quantification of angiogenesis within the vascular system remains a major issue confronting the future development and implementation of contrast-enhanced ultrasound as a clinically useful imaging technique. Currently, all the studies which use contrast-enhanced ultrasound for the detection and quantification of vasa vasorum employ visual-based semi-quantitative approaches [44–46].

Shah et al. used a semi-quantitative grading scale (grade 0 = no neovascularization, grade 1 = limited neovascularization, grade 2 = moderate neovascularization, grade 3 = pulsating, arterial vessel) to provide a distinction between the variations observed in intraplaque neovascularization [44]. Using a similar, dichotomous grading system, various recent studies categorized contrast-enhanced ultrasound enhancement as no contrast effect (grade 1) or high (grade 2) based on the visual detection of the contrast effect within the plaque [46]. Systematic histopathological validation revealed a direct positive correlation between contrast enhancement using this semi-quantitative grading scale and histology [44, 45]. Furthermore, wholly consistent with the concept that more vulner-
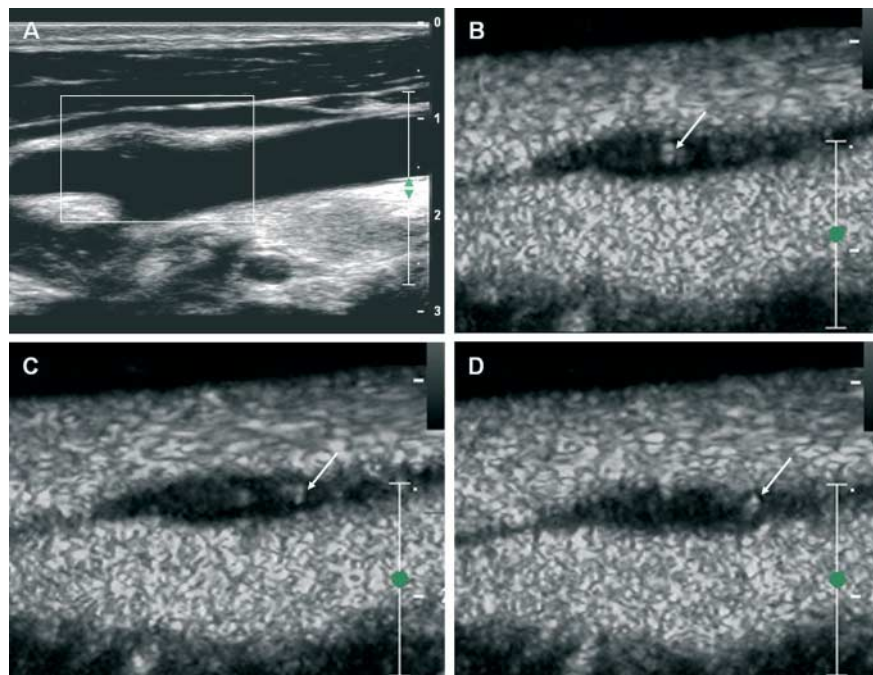

Fig. 5 Carotid artery with plaque neovascularization on contrast-enhanced ultrasound imaging. **A** Unenhanced B-mode imaging of a hypoechoic plaque at the origin of the internal carotid artery. **B–D** Corresponding consecutive frames on contrast-enhanced ultrasound imaging with visible microbubbles within the plaque (also see online video). ▶ **Video 1**

Abb. 5 Bifurkation der Arteria carotis communis mit Darstellung von Neovaskularisation nach Ultraschallkontrastmittelgabe. **A** B-Bild-Darstellung eines hypoechogenen Plaques im Abgang der ACI vor Kontrastmittelgabe. **B–D** entsprechende Darstellung von konsekutiven Bildsequenzen nach Kontrastmittelgabe, die Pfeile markieren sichtbare Mikrobläschen in der Plaque (siehe auch Online-Video). ▶ **Video 1**

able plaque contains a higher degree of neovascularization, recent retrospective studies revealed the association between plaque enhancement on contrast-enhanced ultrasound imaging and clinical symptoms including cerebrovascular events in patients with carotid atherosclerosis [19, 46, 47]. Therefore, contrast-enhanced ultrasound examination of the carotid artery may provide a novel, noninvasive clinical tool to identify and quantify the presence and the extent of arterial plaque neovascularization in patients at risk for developing symptomatic atherosclerosis, thus permitting more reliable assessment of cardiovascular risk.

Perspectives of Ultrasonography
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Besides the advances in the field of contrast-enhanced ultrasound imaging as presented above, another challenging aspect is the field of ultrasonic molecular imaging. In general, there are two different approaches to be considered. In "passive" molecular imaging, a specific lipid is incorporated into the shell of a contrast agent, which, e.g., can promote the activation and consecutive adhesion of complement to the shell resulting in the binding of activated leucocytes. As such, this technique conforms to functional imaging [53]. In "active" molecular imaging, highly specific ligands ("targeted molecules") such as monoclonal antibodies, glycoproteins, or peptides are attached to the shell. Consequently, the targeted contrast agent can bind to the appropriate target structure. Once all of the contrast agent in the blood pool has been washed out, specifically bound microbubbles remain detectable at the site of the target structure. Initially very promising results have demonstrated successful in vitro targeted imaging of human clots with abciximab immunobubbles [54]. A possible clinical application is the positive detection of thrombotic material either at the ICA plaque or in an occluded cerebral artery. Future developments could also generate additional targeted bubbles, e. g., for the detection of inflammation or angiogenesis. It has been speculated that the binding of targeted microbubbles to the thrombotic material could reduce possible side-effects of sonothrombolytic therapy [54]. Irrespective of this, another possible future application is the binding of therapeutic agents, e.g., thrombolytics, to the bubbles which could then be released by insonification at the site of vascular obstruction hence reducing systemic side-effects of the agent (ultrasound mediated drug delivery). However, these methods have to be regarded as experimental as long as no in vivo examinations have been performed. Therefore, the method is not yet regarded as clinically relevant in humans [55–57]. Irrespective of this, passive phagocytosis of microbubbles into macrophages has recently been demonstrated with the interesting finding that the rate of phagocytosis varied considerably between different contrast agents [58]. However, it is still unclear whether there is any clinical indication for the imaging of atherosclerosis. However, the recent observation that microbubbles can be detected approximately 6 minutes after application (late phase CEUS) significantly more often in symptomatic plaques than in asymptomatic plaques may serve as a marker of inflammation and therefore "active" atherosclerosis in this context [62].

Restrictions of Ultrasonography
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The internal carotid artery is usually easily depicted due to its location close to the surface. In the case of calcified plaque, substantial shadowing may occur distal to the plaque, thus hampering plaque and flow evaluation. Routinely, the exam of the extracranial arteries is carried out as a hand-held 2D examination. Initial efforts regarding automated 3D examinations have been made [50, 51]. However, the technical complexity is such that routine application has not yet been accomplished. In Europe, mainly the sulphurhexafluoride dispersion called SonoVue® (Bracco, Milano, Italy) is currently used for contrast-enhanced ultrasonography. The physical effects on the applied microbubbles are diverse and include destruction at higher energies. Theoretically, this may cause damage to the capillary bed, inducing a secondary inflammatory reaction and neovascularization. However, since the energy in carotid imaging is comparatively low, this problem may be disregarded [52]. Furthermore, some side effects are reported after the application of contrast agents, of which the most frequent are paraesthesia at the site of application and headache after application (below 2%). After the occurrence of sudden deaths in a possible but not proven connection with the application of SonoVue®, approval was restricted as follows: acute coronary syndrome or instable angina, acute heart failure, heart insufficiency grades III/IV or arrhythmia, known right-to-left shunt, severe pulmonary hypertension, uncontrolled systemic hypertension and acute respiratory insufficiency. Although different aspects of atherosclerotic disease can be highlighted by ultrasound techniques as described above, it has not yet been possible to establish a comprehensive synopsis integrating the different information. Since it has become evident that aspects like grade of stenosis, plaque morphology or neovascularization do not contribute to the risk of clinical events solely by themselves, there is a need for a tool that summarizes and evaluates clinical information as well as all the evidence from the ultrasound examination. Such a possible tool may be the PLAC-RISK score, which is proposed and presented as an addendum to this manuscript.

Summary and Outlook
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According to current insights, it is not sufficient to assess the risk of stroke of a carotid plaque solely based on the degree of stenosis and the presence of neurological symptoms. It has become clear after the Asymptomatic Carotid Surgery Trial (ACST) at the latest, that there is a need to identify a subgroup of patients with asymptomatic carotid plaques who would benefit from surgical treatment of their carotid stenosis [59]. Ultrasound takes a prominent place amongst the diagnostic tools available for this purpose, simply because of its methodological advantages. With the further development of B-mode imaging as well as the contrast-enhanced sonographic assessment of plaque morphology, a diagnostic package has become available which has big potential not only for the risk stratification of carotid plaques but also for the follow-up of the effects of plaque-stabilizing drug therapy. Positive clinical case series have demonstrated the value of the different approaches for displaying plaque morphology as well as its surface characteristics and its neovascularization. In order to define the status of the new techniques in comparison to the

established diagnostic methods for plaque evaluation, a prospective, randomized multi-center trial with a large number of patients will be necessary. Within this trial it should be attempted to acquire reproducible und comparable data using a simple and standardized tool for carotid plaque assessment. For this purpose, the authors propose the first draft of the PLAC-RISK score as outlined in the addendum of this manuscript. This score claims to be easily applied in daily practice and should help neurologists, angiologists and vascular surgeons to assess the risk of atherosclerotic carotid plaques and guide their therapeutic decisions. However, this score will have to be validated in prospective studies.

Addendum
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