Carotid plaque neovascularization detected with Superb Micro-vascular Imaging (SMI) ultrasound without using contrast media

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Introduction

Ischemic stroke caused by thromboembolism from an unstable carotid artery plaque can be effectively prevented by carotid endarterectomy surgery (CEA)[1, 2]. In current clinical practice the selection of patients for CEA is based on the degree of carotid artery stenosis and presence or absence of cerebral ischemic symptoms. It has in recent years, however, become increasingly clear that the degree of carotid artery stenosis alone is not the best predictor of stroke risk. This has led to the concept of the "unstable plaque" for the description of carotid plaques that carry high risk of stroke irrespective of the degree of artery stenosis. Research has therefore increasingly focused on identifying factors other than degree of artery stenosis that are important for plaque destabilization. One of these factors is intraplaque neovascularization (IPN) which has been documented to play a key role in plaque instability[3-7]. With increasing degrees of vascularization, plaques become more prone to hemorrhage and rupture, making them unstable and increasing the risk of thromboembolic stroke.

Ultrasound visualization of the vasa vasorum (VV) and quantification of IPN has a potential important role in cerebrovascular risk assessment and may be a potential surrogate marker of carotid plaque instability and stroke risk.

Conventional Doppler based ultrasound, however, applies a filter to remove wall motion artifacts and clutter which also excludes the detection of low velocity blood flow signals from IPN[8, 9]. Both contrast-enhanced ultrasound (CEUS) and Superb Micro-vascular Imaging (SMI) have shown promise in visualizing this neovascularization[10-16]. Indeed, several studies have found that IPN, assessed with CEUS, correlates well with micro-vessel density on histological assessments of excised plaques[10, 12, 14]. CEUS requires, however, an intra-venous injection of a contrast agent with an associated risk which limits its use in clinical practice.

SMI is a new ultrasound imaging technique, developed to overcome the limitations of conventional Doppler ultrasound which enables the visualization of neo-vessels without the use of intravenous contrast. This technique applies an exclusive algorithm to differentiate true microvascular flow signals from wall motion artifacts and clutter, thereby allowing the visualization of intraplaque microvascular flow (IMVF) signals.

Recent studies comparing the detection of IPN using CEUS and comparing results with SMI demonstrated a good agreement between the two methods[16-18]. To secure validity of these results, IPN detection using SMI ultrasound should also be verified by histological assessments which were not carried out in most previous reports. We aimed therefore to assess the ability of SMI to detect carotid plaque IPN, compare the level of agreement between SMI and CEUS assessment of plaque IPN and correlate findings with histological analyses[19].

Methods

Patients with internal carotid stenosis ≥50% visiting our outpatient clinic from 2016 to 2018 were consecutively included in the study. Patients underwent conventional Doppler ultrasound, SMI and CEUS of the carotid arteries prior to carotid endarterectomy or at a routine ultrasonographic control for patients without cerebrovascular symptoms. Plaques from operated patients were assessed histologically.

Ultrasonography

Imaging was performed with Canon's Aplio ultrasound system using a 7.5 MHz linear probe on both carotid arteries for standard Doppler ultrasound, CEUS and SMI. The course of common carotid artery, carotid bifurcation and internal carotid arteries were examined in longitudinal and transverse planes in standard ultrasound. The degree of carotid artery stenosis was determined based on peak systolic and end diastolic velocities[20]. Plaque echogenicity was classified visually in high-resolution B-mode grey-scale pictures as follows: 1. Uniformly hypoechoic, 2. Predominantly hypoechoic (hypoechoic with small hyperechoic regions), 3. Predominantly hyperechoic (hyperechoic with small <25% hypoechoic regions) or 4. Uniformly hyperechoic[21, 22].

SMI and CEUS

For SMI assessments, plaques were firstly observed in transverse plane thereafter in longitudinal plane for two minutes and the video images were stored in the ultrasound scanner's hard drive. Static enhancements were excluded and moving enhancements were classified as IMVF.

CEUS was performed using a contrast-specific imaging mode with a low mechanical index (MI -0.12) to prevent destruction of contrast microbubbles (sized 1-11 μm). Patients received an intravenous bolus injection of 2.5 mL contrast microbubbles (Sono Vue; Bracco, Milan, Italy). Digital recordings were started when the contrast microbubbles arrived at the carotid artery bifurcation and continued for up to seven minutes. Native raw data was stored in the scanners hard drive for later off-line assessments. A time-intensity curve (TIC) was plotted using built-in quantification software. A ROI was manually drawn around the plaque and a second circular ROI was placed in the lumen of the artery as reference. Curve fitting was applied to the TIC and TIC derived peak intensity (PI) values were obtained. PI is the maximum intensity of the TIC compared to that for local blood flow in the ROI in the artery. This gives a quantitative measure of plaque enhancement.

IPN on both SMI and CEUS was assessed by two methods: A) IMVF was visually (semi-quantitively) graded as follows: Grade 0: no IMVF within the plaque or IMVF confined to the adjacent adventitia, Grade 1: moving IMVF confined to the adventitial side, Grade 2: moving IMVF at the plaque shoulder, Grade 3: IMVF moving to the plaque core, Figure 1(B), Figure 2 and Grade 4: extensive IMVF. B) quantitively; for SMI a visual count of the IMVF signals was carried out and the number of neo-vessels observed in a two-minute SMI video clip was counted. For CEUS the TIC was plotted using built-in quantification software in order to obtain TIC derived PI values.

Histological assessments

Carotid plaques removed en bloc (intact) at endarterectomy were examined histologically and in each plaque section the number and diameter of vessels with a lumen diameter of 0.01 mm or greater were recorded. The plaques were also ranked based on the size of the measured areas of 1. Granulation tissue, 2. Lipid, 3. Inflammation and then given a total rank score by combining all three components. The IPN findings assessed using SMI and CEUS were compared and correlated to histological findings.

Results

Carotid plaque neovascularization detected using SMI

A total of 31 patients were included in the study. Ultrasound examinations of plaques using SMI visualized IMVF signals in 21 of the 31 plaques. A comparison of the 5-level visual classifications of IMVF using SMI and CEUS showed that patients with higher degrees of IMVF on SMI also had higher degrees of enhancement on CEUS (Grade 3) Figure 1. Patients with high neo-vessel counts on SMI also had higher grading of plaque enhancement on semi-quantitative CEUS and higher PI values on quantitative CEUS.

Carotid plaque enhancement and correlation with echogenicity on ultrasound

Hypoechoic plaques on B-mode ultrasound had significantly higher grades of IMVF signals, higher neo-vessel counts on SMI and enhancement on CEUS.

Intraplaque neovascularization SMI correlated with histology

Higher grades of IMVF on SMI were positively correlated to increasing number of observed neo-vessels on histological assessments. Increasing number of neo-vessels counted on SMI was also positively correlated to increased area of inflammation and the total plaque rank score assessed on histology.

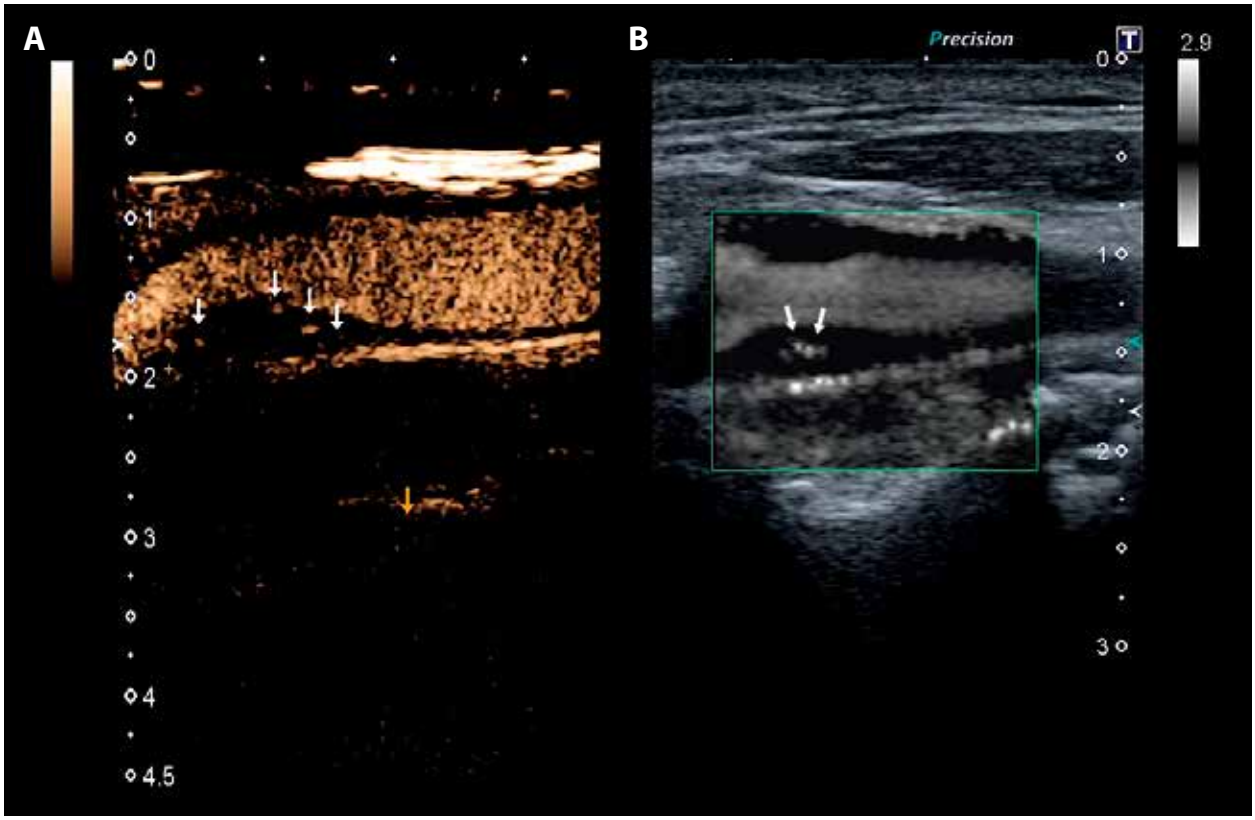

Figure 1 Contrast-enhanced ultrasound (CEUS) (A) and Superb Micro-vascular Imaging (SMI) (B). More than 70% carotid stenosis, predominantly hypoechoic plaque located at the far wall of the right internal carotid artery in an asymptomatic patient. (A, left) CEUS examination of the plaque. Contrast material influx is yellow in color, arrow points at contrast microbubble enhancement moving toward the plaque core and lumen, Grade 3. (B, right) SMI examination of the plaque. The SMI region of interest is positioned to show the entire plaque, arrow points at intraplaque microvascular flow (IMVF) signals moving towards the plaque core and lumen, Grade 3.

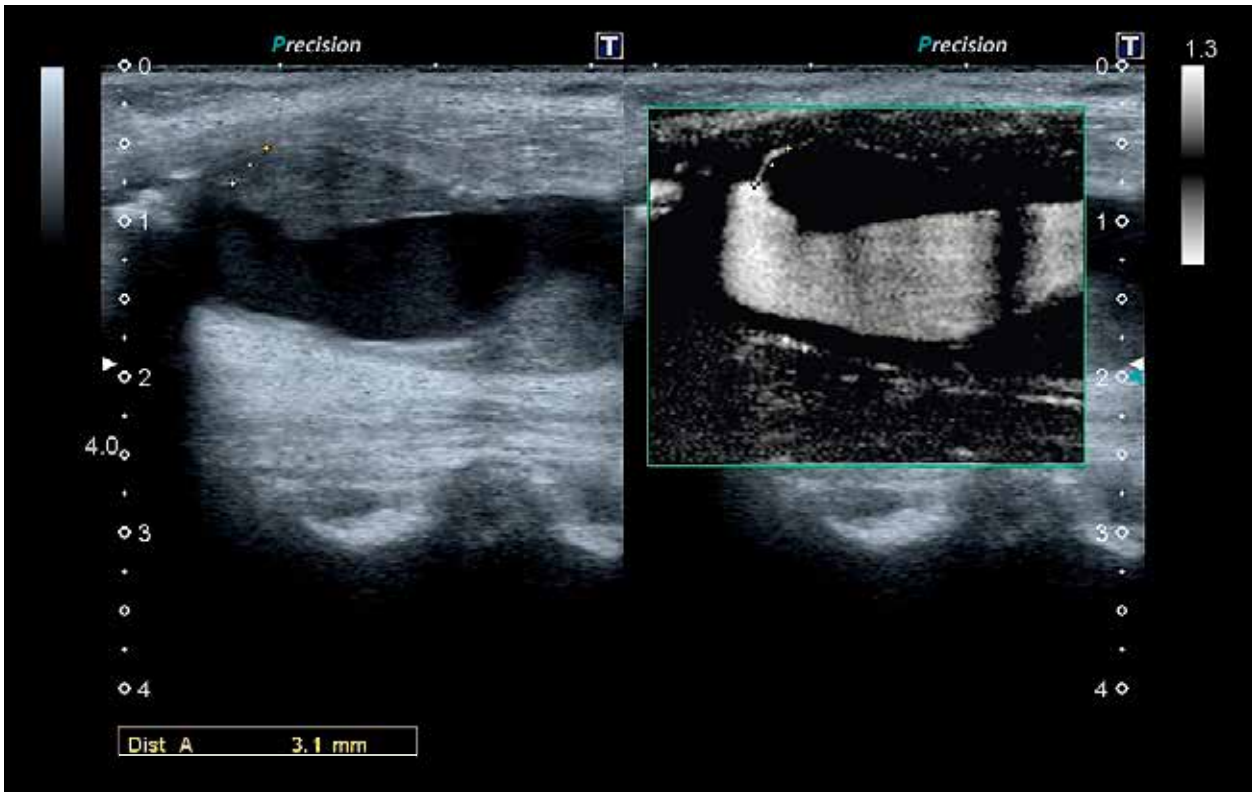

Figure 2 Conventional B-mode ultrasound with and without SMI in TwinView layout. More than 70% carotid stenosis, uniformly hypoechoic plaque located at right carotid artery bifurcation. Left, conventional B-mode ultrasound examination of the plaque. Right, duplicate of the B-mode as shown in the left picture subtracting the background information to focus only on vasculature with SMI and showing intraplaque neovascularization (IPN) in the plaque. Plus signs show the distance measurement of the IPN detected by SMI moving towards the plaque core, Grade 3.

Discussion

The main findings of this study were, firstly, with increasing degrees of IMVF on SMI ultrasound, the degree of enhancement on CEUS also increased. Secondly, increasing IMVF grades on SMI were significantly correlated to increasing number of neo-vessels on histology.

The finding in the current study of good correlation between SMI and CEUS is in keeping with three previous published reports which documented good consistency between SMI and CEUS in the evaluation of IPN[16-18]. However, histological analysis of neovascularization which is important to validate the finding of IPN was only included in one of these[17].

In this current study, we applied the 5-level visual classification of IPN used by Chaolun Li[12], for the semi-quantitative CEUS assessments to our SMI analysis, as this represents in our view the simplest and most intuitive comparison between the two methods. For quantitative assessment of SMI the number of observed neo-vessels within the plaque was counted from a two-minute SMI video clip. This count was then compared with CEUS quantitative assessments (PI values) to which it was significantly, positively correlated. This current study is, to our knowledge, the first to carry out a comparison of both visual and quantitative findings with SMI and CEUS. This provides a more robust comparison of the two methods.

Previous studies on excised carotid plaques have reported a good correlation between micro-vessel density on histology and degree of enhancement on CEUS[10, 23]. The majority of these studies used immunological markers of vascularization such as CD31 staining[10, 14]. In this current study, micro-vessels were defined histologically by direct observations of the vessel endothelial cells allowing for the number of micro-vessels in each plaque to be counted. With this assessment, we found that plaques with higher IMVF SMI counts had higher numbers of neo-vessels and also larger areas of inflammation. This is in agreement with previous studies[14, 17], where areas of inflammation on histology were defined as the direct observation of macrophages. Higher percentages of macrophage infiltration were found in plaques from patients with higher grades of IPN on CEUS[14]. Inflammation plays a key role in atherosclerosis with macrophages and T-lymphocytes recruited during all stages of the disease leading to formation of a necrotic core and a destabilization of the plaque. In addition inflammation is also triggering fibrosis which stabilizes the plaque[24]. This fibrosis starts with formation of granulation tissue which is rich in micro-vessels that are prone to leakage and rupture. We observed this phenomenon on histological assessments as accumulation of red blood cells around micro-vessels in granulation tissue and a lack of surrounding erythrocytes around micro-vessels in fibrotic tissue area. We interpreted the observation of extravasated erythrocytes around micro-vessels in granulation tissue as intraplaque hemorrhage (IPH) secondary to the vessel disruption owing to loss of endothelial integrity. The absence of such erythrocytes around micro-vessels found in fibrotic tissue was interpreted as representative of more maturely developed and stable vessels. In the current study, plaques were therefore histologically assessed using a total plaque rank score which was based on these three tissue components (inflammation, granulation tissue and lipids) known to play a key role in IPN formation. We found that plaques with high total rank scores had higher neo-vessel counts on SMI and higher peak intensity enhancements on quantitative CEUS. This is in keeping with increasing scientific evidence supporting a key role for inflammation in plaque destabilization and thromboembolic stroke risk.

Low echogenicity on B-mode ultrasound is documented to be correlated with both histopathological features of plaque instability[10] as well as an increased risk of stroke. A recent study done by Zhang et al[17] reported an increase in CEUS enhancement levels in low echogenic plaques. We found that hypoechoic plaques on B-mode ultrasound had both higher degrees of IMVF on SMI and higher degrees of enhancement on CEUS.

Conclusion

Our results provide promising evidence that SMI may be a useful tool in bed-side assessment of IPN with a significant advantage over CEUS of not requiring an intravenous contrast injection, allowing for easier use in routine clinical practice.

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