Contrast Enhanced Ultrasound (CEUS) Is Not Able to Identify Vulnerable Plaques in Asymptomatic Carotid Atherosclerotic Disease

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WHAT THIS PAPER ADDS

Several studies have shown the diagnostic accuracy and efficacy of contrast enhanced ultrasound (CEUS) for detection of intra-plaque neo-angiogenesis (IPN) in carotid atherosclerotic disease. CEUS was studied to identify vulnerable carotid plaques (as assessed by histology) and identify subgroups of patients at high risk of cerebral embolisation. There were two main findings: (a) vulnerable plaques had denser IPN, but did not show more pronounced contrast enhancement at CEUS; (b) the correlation between immunohistochemical analysis and CEUS analysis was significant for both vulnerable and non-vulnerable plaques, but it was direct for the former and inverse for the latter. Thus, whether the CEUS detection of microbubbles within carotid plaques truly represents IPN can be questioned.

Objectives: Contrast enhanced ultrasound (CEUS) has been suggested as an imaging tool for detection of asymptomatic carotid atherosclerotic disease (ACAD) at high risk of cerebral embolisation. The objective of this study was to evaluate CEUS and immunohistochemical (IHC) patterns in ACAD (i.e., without any neurologic symptoms in the last 6 months) and their correlations with histology.

Methods: CEUS analysis was classified on a semiquantitative basis using a three-point classification scale. Plaque morphology was assessed using the American Heart Association (AHA) classification of atherosclerotic plaques, then accordingly assigned as non-vulnerable (AHA Type IV/V) or vulnerable (AHA Type VI). IHC analysis for intra-plaque neo-angiogenesis (IPN) was identified by CD34/VEGF immunostaining and classified on a semiquantitative basis using a four-point classification scale. Both CEUS and IHC analyses were performed and scored by single observers.

Results: Fifty-eight consecutive asymptomatic patients (mean age 73 years, 33 males) undergoing carotid endarterectomy were included in the final analysis. Nineteen had AHA Class IV/V plaques, and the remaining 39 had AHA Class VI plaques. There were two main findings of the study: (a) histologically proven vulnerable plaques compared with histologically proven non-vulnerable plaques had denser IPN ($p = .004$), but did not show more pronounced contrast enhancement; (b) the correlation between IHC analysis and CEUS analysis was significant for both vulnerable and non-vulnerable plaques ($p = .04$ and $p = .01$, respectively), but it was direct for AHA Type IV/V plaques and inverse for AHA Type VI plaques.

Conclusions: The main findings of the study were that histologically proven vulnerable plaques (i.e., AHA Class VI) as compared with histologically proven non-vulnerable plaques (i.e., AHA Class IV/V) had denser neo-vascularisation, but not more pronounced contrast enhancement.

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INTRODUCTION

In patients with asymptomatic carotid atherosclerotic disease (ACAD) the percentage of carotid stenosis is the main parameter for indicating elective revascularisation. However, plaque morphology could play a significant role in the development of neurological symptoms and should therefore be considered in the decision making process.[1,2]

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Histological studies have documented that intra-plaque haemorrhage (IPH) is associated with plaque disruption and is considered to be one of the main characteristics of vulnerable plaques.[3] Since intraplaque neo-angiogenesis (IPN) is reputed to be the most likely precursor of IPH, its in vivo imaging may allow identification of vulnerable plaques. Contrast enhanced ultrasound (CEUS) is a well accepted technique for detection of IPN and has been suggested as an imaging tool for detection of ACAD at high risk of cerebral embolisation.[4]

The objective of this study was to evaluate CEUS and immunohistochemical (IHC) patterns in ACAD (i.e., without any symptoms of stroke/transient ischaemic attack in the last 6 months) and their correlations with histology.

METHODS

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the Helsinki Declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all participants involved in the study.

Study design

CEUS was measured against the neo-vascular density observed within the tissue specimens obtained at the time of carotid endarterectomy (CEA). All patients with ACAD scheduled for elective CEA at a single tertiary institution between January 2014 and December 2016 were enrolled. ACAD, identified by duplex ultrasound (DUS) as first line, was subsequently confirmed by computed tomographic angiography (CTA). Patients with critical carotid stenosis ($\geq$70%), as defined by concordant findings at both DUS and CTA imaging, were referred for elective revascularisation (i.e., CEA). Plaque specimens were excised, then histological and IHC analyses were performed. All data were collected prospectively.

Image acquisition and analysis: carotid DUS

DUS examinations of the carotid arteries were performed in all patients with a latest generation scanner (MyLab, Esaote, Florence, Italy) equipped with a 6—8 MHz 9L4 probe. DUS examinations were performed with the patient lying in a supine position and with the carotid arteries visualised in both the longitudinal and the transverse planes. The entire length of the extrathoracic common carotid artery (CCA), the carotid bifurcation, and the extracranial internal carotid artery (ICA) available for observation were examined. Carotid plaques were identified initially on DUS images in terms of their location, echo, and maximum stenosis prior to performing CEUS examination. If carotid plaques were identified, the view showing the thickest longitudinal section of the plaque was used to measure the maximum stenosis with electronic calipers. In patients with more than one separate plaque, only the thickest one was evaluated and recorded for analysis. All DUS/CEUS examinations were performed by a single experienced investigator (M.F.) with more than 10 years of experience. In this setting, there was no testing for inter-observer reproducibility by a second observer.

DUS criteria for defining stenosis thresholds using ICA peak systolic velocity (PSV) and the ICA-PSV/CCA-PSV ratio were based on the NASCET criteria.[5] Critical carotid stenosis was defined as stenosis $\geq$70% according to the aforementioned criteria (ICA-PSV $\geq$ 230 cm/s and ICA-PSV/CCA-PSV ratio $\geq$ 4). The local narrowing at B-mode was then expressed as a percentage diameter reduction borrowed from the European Carotid Surgery (ECST) criteria[6] (Figs. 1A—3A).

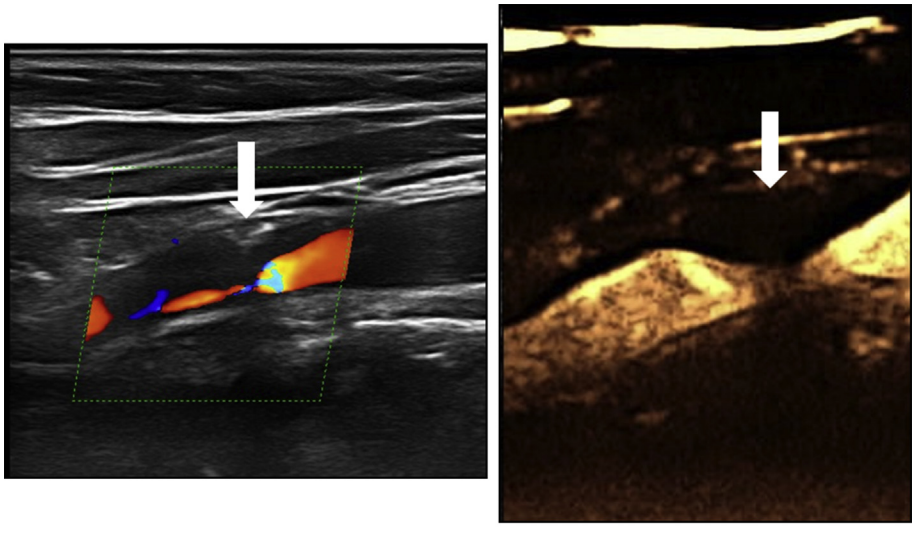


Figure 1. (A) Color Doppler ultrasound examination of severe right ICA stenosis with Type I (uniformly echolucent) plaque. (B) Contrast enhanced ultrasound examination showing no visible microbubbles within the plaque (Grade 0).

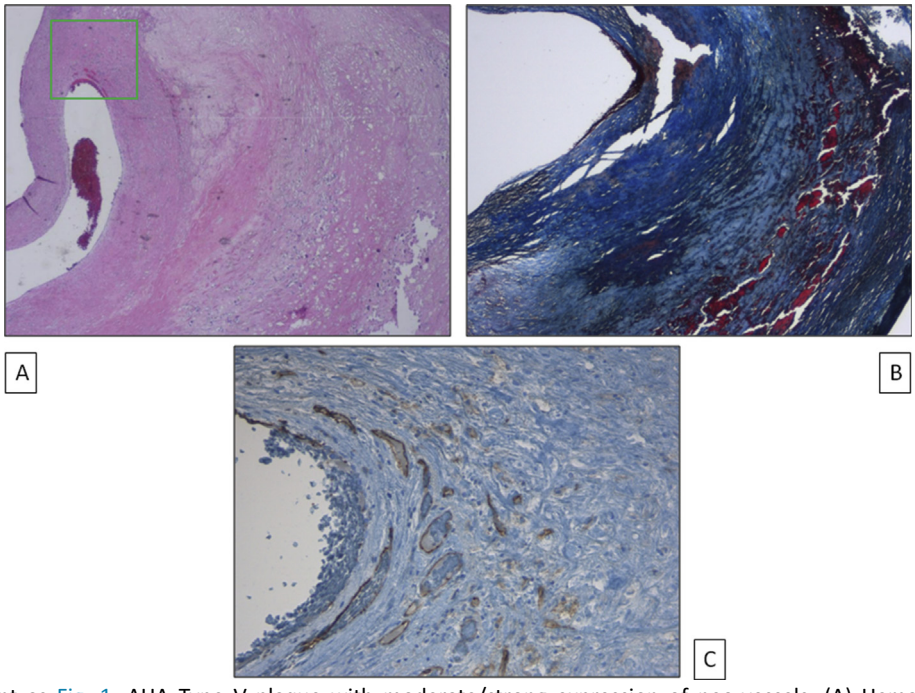
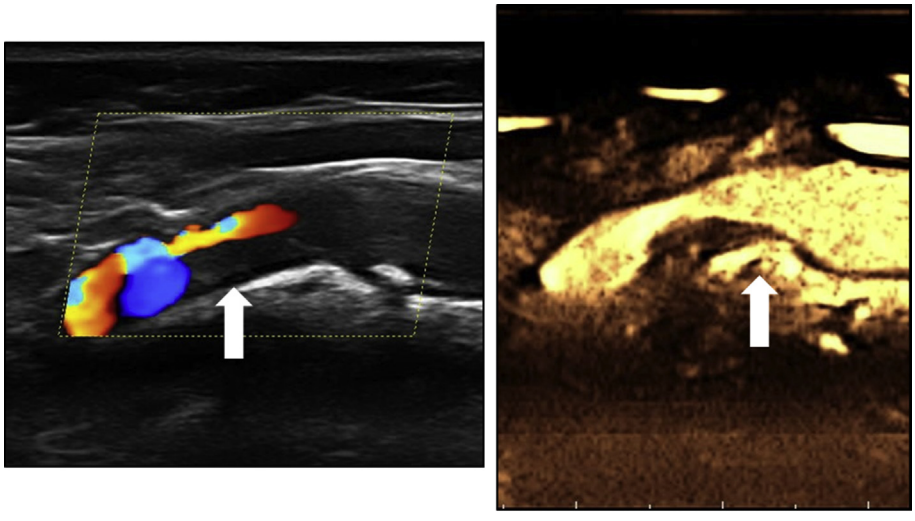


Figure 2. Same patient as Fig. 1. AHA Type V plaque with moderate/strong expression of neo-vessels. (A) Hematoxylin/Eosin staining (×2.5). (**B**) Azan/Mallory staining (×10). (**C**) CD34 staining (×20).

Plaque echo was classified on a semiquantitative basis using a five point classification scale according to the modified Gray—Weale scale: Type 1, uniformly echolucent; Type 2, predominantly echolucent; Type 3, predominantly echogenic; Type 4, uniformly echogenic; Type 5, unclassifiable because of heavy calcification and/or acoustic shadow.[7,8] In patients having heavy calcification with acoustic shadow (i.e., Type five carotid plaque according to the modified Gray—Weale scale) the technical difficulty was overcome by a multiplanar scanning of the plaque calculating the average maximum stenosis in three different projections on the longitudinal axis. For clinical simplicity, plaque echo was classified as either echolucent (Type 1 and Type 2), echogenic (Type 3 and Type 4), or unclassifiable (Type 5).

Image acquisition and analysis: carotid CEUS

All carotid plaques underwent CEUS examination, which was performed according to a protocol adopted in previously described studies.[9—11] SonoVue (Bracco, Italy), a second generation ultrasound contrast agent consisting of phospholipidic stabilised microbubbles of sulfur hexafluoride was used for CEUS examination.[12] The contrast agent was administered intravenously as a 4 mL bolus dissolved in .9% saline solution. Each dose was injected through the antecubital vein with an 18—20G cannula within 2—3 s, with each injection followed by flushing with a 5 mL bolus injection of .9% saline solution. CEUS examination, focused at the level of the previously identified maximum stenosis, was performed at low acoustic pressure using longitudinal acquisition scans. The preset real time CEUS modality with coded pulse inversion technique was switched on, and image settings were adjusted to maximise visualisation of the contrast signal. To reduce microbubble destruction a mechanical index of .13 was used; the frame rate and image depth were adjusted respectively to 12 per second and to 3—5 cm according to the size of the carotid artery, and the focus position was set at the level of the carotid artery (below the region of interest to reduce the intensity of the ultrasonographic beam in the zone of microbubble flow). Time gain compensation was adjusted to achieve a homogeneous signal intensity of the carotid artery while reducing noise from the wall of the carotid artery and the plaque. All these settings were kept constant throughout each examination. The appearance of the contrast effect was observed inside the lumen of the carotid artery within 15—30 s after contrast injection. Real time contrast enhanced carotid artery cine loops were obtained following contrast injection, including images obtained at least 3 s before and 5 min after the appearance of the contrast effect in the lumen of the carotid artery. The clips were recorded in real time with a dual display mode for simultaneous standard and contrast enhanced view, then digitally stored as DICOM (Digital Imaging and Communication in Medicine) files. The evaluation of plaque contrast enhancement was performed online first, thereafter a second offline analysis was performed in a blinded fashion. For disagreement, the results of offline analysis were chosen for the definitive report. A previously published semi-quantitative visual approach was used to evaluate and quantify contrast enhancement.[11,13] Plaque contrast enhancement was classified on a semi-quantitative basis using a three point classification scale as follows: Grade 0, no visible microbubbles within the plaque; Grade 1, microbubbles confined to the shoulder and/or adventitial side of the plaque; Grade 2, microbubbles reaching plaque core and/or extensive contrast agent enhancement



A B

Figure 3. (**A**) Color Doppler ultrasound examination of severe left ICA stenosis with Type I (uniformly echolucent) plaque. (**B**) Contrast enhanced ultrasound examination showing microbubbles confined to the shoulder and/or adventitial side of the plaque (Grade 1).

throughout the plaque (Figs. 1B—3B). For clinical simplicity, plaque contrast enhancement was classified on a two level scale as either negative (Grade 0) or positive (Grade 1 and Grade 2). Intra-observer variability for two level CEUS analysis revealed substantial agreement, with a proportion in agreement of .89 and a *k* value of .78 (95% CI 0.67—.90; $p < .001$).

Carotid endarterectomy

Conventional CEA (cCEA) was performed according to the following procedure: short ($\leq$50 mm in length) longitudinal incision of common and ICAs on the anterior wall with meticulous dissection of the plaque leaving the luminal surface of the atheroma up to the medial arterial layer, so that the plaque as a whole could be examined. The arteriotomy was closed either by direct (dCEA) or a patched closure (pCEA). All surgical procedures were performed under general anaesthesia with neurological monitoring achieved through continuous electroencephalographic (EEG) monitoring, with selective shunt placement in cases of acute EEG wave deterioration compared with preoperative baseline measurement.

Histological analysis

At the time of CEA, the carotid plaque was immediately fixed in 10% buffered formalin and decalcified (if necessary) with ethylenediaminetetraacetic acid. The segment with the greatest plaque area was defined as the culprit lesion and transversely sectioned at 2 mm intervals. Each interval section was embedded in a paraffin block, from which 2 µm sections were collected. Sections were then stained (hematoxylin and eosin and Masson's trichrome) and examined at different magnifications. All histological/IHC examinations were performed by a single experienced investigator (R.B.) with more than 10 years of experience. In this setting, there was no testing for inter-observer reproducibility by a second observer. The pathologist was blinded to the DUS/CEUS images when performing the histological sample preparation and analysis. Plaque morphology was assessed using the American Heart Association (AHA) classification of atherosclerotic plaques[14] (Figs. 2A—4A). Carotid plaques were accordingly assigned as non-vulnerable (AHA Type IV/V) or vulnerable (AHA Type VI). The latter type included carotid plaques with one or more of ulcer, intra-plaque haemorrhage/thrombus or large lipid rich necrotic core.

IHC analysis

Tissue sections were deparaffinised (with xylene) and rehydrated (with ethanol and distilled water), then immersed in citrate tampon pH 6, heated for 15 min at 100 °C, followed by incubation in peroxidase block 3% for 15 min to block endogenous peroxidase activity. Thereafter they were incubated overnight at 4 °C with different dilutions of monoclonal antibodies, then washed three times for 10 min each in buffer containing 1% polymerised bovine albumin (BSA), and finally mounted on gelatin coated slides and examined with Olympus BX61.

Intra-plaque microvessels were identified by CD34 positive immunostaining, which was performed an all specimens. Each section was examined using a grid to manually measure the number of neovessels and ensure coverage of the entire area of the section. The number of positively stained cells was expressed as a percentage of the total number of stained and unstained cells at a magnification 40× and averaged for all slices. Furthermore, to enhance confidence in the validity of the observations, a double CD34/VEGF (vascular endothelial growth factor) staining was performed in 20 randomly selected patients participating in the study and no visual differences were observed between the single CD34 staining and the double CD34/

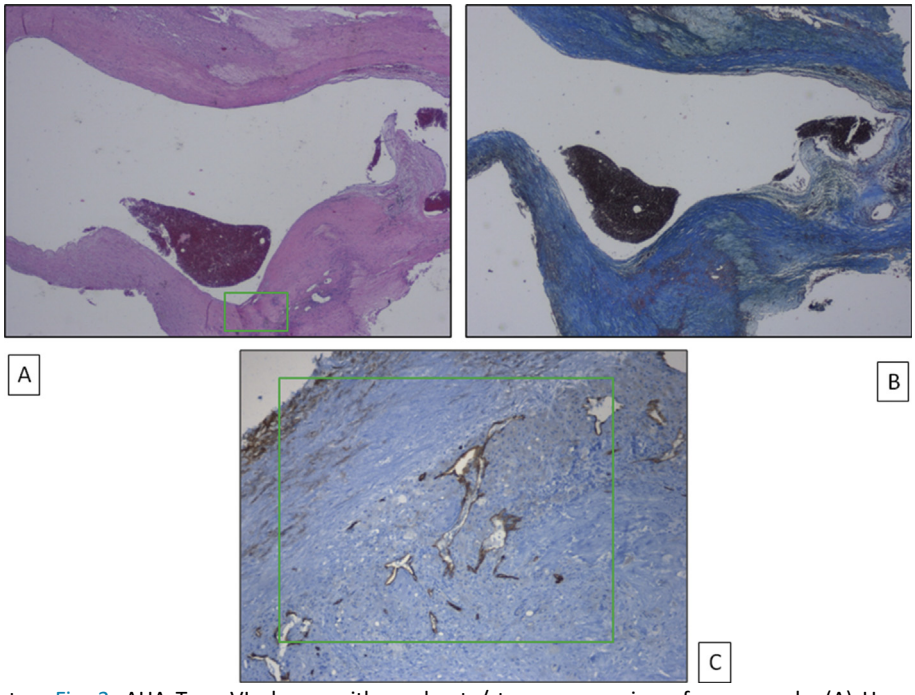


Figure 4. Same patient as Fig. 3; AHA Type VI plaque with moderate/strong expression of neo-vessels. (A) Hematoxylin/eosin staining (×2.5). (B) Azan/Mallory staining (×2.5). (C) CD34 staining (×10).

VEGF staining (Supplementary material). CD34/VEGF expression was classified on a semi-quantitative basis using a four point classification scale as follows: Grade 0, absence of any CD34/VEGF staining; Grade 1, weak CD34/VEGF staining (<20% of total cells positively stained); Grade 2, moderate CD34/VEGF staining (20—50% of total cells positively stained); Grade 3: strong CD34/VEGF staining (>50% of total cells positively stained).[10,11] For clinical simplicity, CD34/VEGF expression was classified on a two level scale as either absent/weak (Grade 0 and Grade 1) or moderate/strong (Grade 2 and Grade 3) (Figs. 2B—4B). Intra-observer variability for two level IHC analysis revealed almost perfect agreement, with a proportion in agreement of .93 and a *k* value of .88 (95% CI 0.83—.93; $p < .001$).

Statistical analysis

Continuous variables are presented as mean and standard deviation. Dichotomic/categorical variables are presented as absolute numbers and percentages. The two sided Fisher exact test was used to assess differences in dichotomic/categorical variables. The Student *t*-test was used to assess differences in continuous variables. A p value $< .05$ was considered to be statistically significant. The analysis was carried out on SPSS 21.0 (IBM Corp, Armonk, NY, USA).

RESULTS

Patients and carotid plaques

Fifty-eight consecutive asymptomatic patients (mean age 72.8 ± 6.6 years, 33 males and 25 females) underwent cCEA during the study period and were included in the final analysis. Comprehensive baseline clinical characteristics, risk factors, and prior medication are presented in Table 1. As shown in Table 2, 27 were echolucent plaques and 26 were echogenic plaques, while five were unclassifiable plaques (i.e., Type V plaques according to the modified

Table 1. Baseline population characteristics.

	Total	Type IV/V plaques	Type VI plaques	*p*
Patient total (m/f), *n*	58 (33/25)	19 (14/5)	39 (19/20)	.09
Age, years (mean ± SD)	72.8 ± 6.6	74.4 ± 7.5	72.1 ± 6.1	.75
Endarterectomy; right, *n* (%)	32 (55)	10 (52)	22 (56)	.78
Hypertension, *n* (%)	46 (80)	16 (84)	30 (79)	.73
Diabetes mellitus, *n* (%)	24 (42)	9 (47)	15 (39)	.59
Dyslipidaemia, *n* (%)	45 (79)	14 (74)	31 (82)	.51
Smoking, *n* (%)	27 (47)	12 (63)	15 (39)	.10
Coronary artery disease, *n* (%)	23 (40)	8 (42)	15 (39)	1.00
Chronic obstructive pulmonary disease, *n* (%)	7 (12)	2 (10)	5 (13)	1.00
Chronic kidney disease, *n* (%)	4 (7)	2 (10)	2 (5)	.68
Peripheral arterial disease, *n* (%)	11 (19)	5 (26)	6 (16)	.48
Antiplatelet drugs, *n* (%)	53 (92)	18 (95)	35 (92)	1.00
Statins, *n* (%)	49 (85)	16 (84)	33 (86)	1.00

Table 2. Immunohistochemical analysis and contrast enhanced ultrasound analysis for total population, American Heart Association (AHA) Type IV/V plaques and AHA Type VI plaques.

	Total	AHA Type IV/V plaques	AHA Type VI plaques	*p*	Difference (95% CI)
CD34/VEGF expression				.004	40% (1—78)
Absent/weak, *n* (%)	21 (36)	12 (63)	9 (23)		
Moderate/strong, *n* (%)	37 (64)	7 (37)	30 (77)		
Plaque echogenicity				.76	
Echolucent, *n* (%)	27 (47)	10 (52)	17 (44)		
Echogenic, *n* (%)	26 (45)	9 (48)	17 (44)		
Unclassifiable	5 (8)	0	5 (12)		
Plaque contrast enhancement				.15	
Negative, *n* (%)	36 (62)	9 (47)	27 (69)		
Positive, *n* (%)	22 (38)	10 (53)	12 (31)		

Gray—Weale scale). All patients underwent successful cCEA (32 right and 26 left, 40 dCEA and 18 pCEA) with a perioperative stroke/death rate of 0%.

All surgical specimens were evaluated. Overall, 19 plaques (32.75%) were classified as non-vulnerable (2 AHA Type IV, 17 AHA Type V), while 39 plaques (67.25%) were classified as vulnerable (AHA Type VI). Vulnerable plaques were present more frequently in males ($p = .09$) and in active smokers ($p = .1$). For the rest, baseline population characteristics were similar between groups (Table 1).

IHC: total population, AHA Type IV/V plaques and AHA Type VI plaques

IHC analysis showed that in the overall population 37 (64%) carotid plaques had moderate/strong CD34/VEGF expression and 21 (36%) had absent/weak CD34/VEGF expression.

The number of intra-plaque microvessels was significantly greater ($p = .004$) in AHA Type VI plaques than in AHA Type IV/V plaques. In the vulnerable plaque group the staining for CD34/VEGF was moderate/strong in 77% ($n = 30$) and absent/weak in 23% ($n = 9$), while in the non-vulnerable plaque group the staining for CD34/VEGF was moderate/strong in 37% ($n = 7$) and absent/weak in 63% ($n = 12$).

CEUS analysis: total population, AHA Type IV/V plaques and AHA Type VI plaques

CEUS analysis showed that in the overall population 36 (62%) carotid plaques had negative (Grade 0) enhancement and 21 (38%) had positive enhancement (4 Grade 1 plaques, 18 Grade 2 plaques).

AHA Type VI plaques did not exhibit a significantly higher CEUS positivity than AHA Type IV/V plaques. In the vulnerable plaque group, CEUS was positive in 31% ($n = 12$; 2 Grade 1 plaques, 10 Grade 2 plaques) and negative in 69% ($n = 27$), while in the non-vulnerable plaque group CEUS was positive in 53% ($n = 10$: 2 plaques Grade 1, 8 plaques Grade 2) and negative in 47% ($n = 9$).

Correlations: IHC analysis and CEUS analysis

Correlations between CD34/VEGF expression and plaque contrast enhancement were calculated for the total population, for AHA Type IV/V plaques, and for AHA Type VI plaques:

1. For the total population, no significant correlation between CD34/VEGF expression and plaque contrast enhancement was found;
2. For AHA Type IV/V plaques, a significant direct correlation ($p = .04$) between CD34/VEGF expression and plaque contrast enhancement was found;
3. For AHA Type VI plaques, a significant inverse correlation ($p = .01$) between CD34/VEGF expression and plaque contrast enhancement was found.

DISCUSSION

To date, the prediction of cerebrovascular events risk in patients with ACAD predominantly depends on the degree of stenosis, but imaging features of carotid plaque morphology and composition may further aid in predicting the embolic potential of atherosclerotic lesions.[15,16] The proliferation of vasa vasorum plays a central role in the process of plaque progression/instability, and the relationship between carotid IPN with plaque rupture and neurological symptoms has been identified.[1,2] Even if no single imaging modality can reliably identify the vulnerable plaque in relation to development of future stroke, CEUS remains one of the most widely examined techniques for in vivo quantification of IPN.[13,17—23]

The were two main findings of the study: (a) histologically proven vulnerable plaques (i.e., AHA Class VI) compared with histologically proven non-vulnerable plaques (i.e., AHA Class IV/V) had denser IPN as assessed by CD34/VEGF expression ($p = .004$), but did not show more pronounced contrast enhancement with CEUS (Table 2); (b) the correlation between IHC analysis and CEUS analysis was significant for both vulnerable and non-vulnerable plaques ($p = .04$ and $p = .01$, respectively), but it was direct for AHA Type IV/V plaques and inverse for AHA Type VI plaques (Table 3). Indeed, AHA Type IV/V plaques with moderate/strong CD34 expression had positive CEUS enhancement in 86% of cases, but AHA Type VI plaques with moderate/strong CD34/VEGF expression had negative CEUS enhancement in 80% of cases. All of the results were obtained after single observer scoring with low intra-observer variability (a second observer was not available so inter-observer variability was relevant).

The results, confirming those of a recent study by Vavuranakis et al.,[10] might be explained by the fact that in

Table 3. Correlations of plaque contrast enhancement with CD34/VEGF expression and with plaque echo.

	Plaque contrast enhancement		*p*	Difference (95% CI)
	Negative	Positive		
CD34/VEGF expression (total population), *n* (%)			.27	
Absent/weak	11 (52)	10 (48)		
Moderate/strong	25 (68)	12 (32)		
CD34/VEGF expression (AHA type IV/V plaques), *n* (%)			.04	53% (0—95)
Absent/weak	8 (67)	4 (33)		
Moderate/strong	1 (14)	6 (86)		
CD34/VEGF expression (AHA Type VI plaques), *n* (%)			.01	47% (0—95)
Absent/weak	3 (33)	6 (67)		
Moderate/strong	24 (80)	6 (20)		
Plaque echogenicity (total population), *n* (%)			.95	
Echolucent	16 (59)	11 (41)		
Echogenic	16 (61)	10 (39)		
Plaque echogenicity (AHA Type IV/V plaques), *n* (%)			.95	
Echolucent	4 (40)	6 (60)		
Echogenic	4 (44)	5 (56)		
Plaque echogenicity (AHA Type VI plaques), *n* (%)			.72	
Echolucent	13 (76)	4 (24)		
Echogenic	11 (64)	6 (36)		

histologically proven non-vulnerable plaques the microbubbles were actually transferred into the plaque through the network of microvessels (which penetrate the medial layer via the adventitial layer). Conversely, an alternative mechanism beyond IPN may be involved in the process of plaque enhancement for histologically proven vulnerable plaques (e.g., direct microbubble penetration through plaque fissures or macrophage mediated transport). Thus, whether the detection of microbubbles by CEUS within carotid plaques truly represents IPN may be questioned.

Qualitative evaluated echolucent plaques on DUS examination have been associated with a higher ipsilateral risk of stroke.[24,25] Even if previous studies had correlated the Gray—Weale scale with the CEUS detected IPN extension[13,26] no correlation was found between plaque echo and plaque contrast enhancement (Table 3), nor that more echolucent lesions presented a significantly higher degree of neo-vascularisation than more echogenic ones (Table 4). These findings are similar to those of recent studies[27,28] and

Table 4. Correlations of plaque echo with CD34/VEGF expression.

	Plaque echo		*p*	Difference (95% CI)
	Echolucent	Echogenic		
CD34/VEGF expression (total population), *n* (%)			.55	
Absent/weak	8 (40)	12 (60)		
Moderate/strong	19 (57)	14 (43)		
CD34/VEGF expression (AHA Type IV/V plaques), *n* (%)			.30	
Absent/weak	7 (70)	3 (30)		
Moderate/strong	3 (33)	6 (67)		
CD34/VEGF expression (AHA Type VI plaques), *n* (%)			.32	
Absent/weak	2 (20)	8 (80)		
Moderate/strong	15 (62)	9 (38)		

one should be aware that the so called vulnerable atherosclerotic plaque is constituted by a multifaceted morphological and functional system, which DUS and CEUS may only partially identify.

Neo-angiogenesis is considered to be an important biological process in atherosclerotic disease progression, and among the various factors contributing to its development vascular endothelial growth factor (VEGF) is a leading candidate.[29] In the present study, no visual differences were observed between the single CD34 staining and the double CD34/VEGF staining. These findings seem to confirm the concept that the environment of vulnerable plaques (likely to be the hypoxic stimulus) directly promotes neovascularisation, which in turn may increase plaque instability.

Inflammatory infiltration is another well recognised feature of vulnerable plaques, but previous research on the correlation between CEUS enhancement and histological markers of inflammation has been discordant.[27,30,31] A recent study has found that inflammation and neovascularisation are not systematically associated in carotid plaques, suggesting a temporal separation between them.[32] However, CEUS does not display vascular anatomy but only represents flow though a vascular bed; thus, contrast enhancement does not directly reflect the inflammatory process, but may only indirectly represent the consequences of inflammation such as neo-vascularity.

The use of magnetic resonance imaging (MRI) for IPH detection has been reviewed recently.[33,34] In particular, MRI studies have documented that IPH is associated with plaque enlargement, whereas without it plaques do not progress.[35,36] In the Plaque At RISK (PARISK) Study, the presence of IPH in patients with mild to moderate carotid artery stenosis was associated with plaque surface disruption but whether it actually increased the risk of plaque rupture and ischaemic stroke was not definitively proven.[37,38] However, a common viewpoint is that small leaky neo-vessels are

responsible for the occurrence of IPH and subsequent development of plaque growth and instability.[29,39–41] Thus, identification of IPN on CEUS may provide a surrogate marker for eventual progression to IPH. Furthermore, the association between carotid plaque enhancement on CEUS and plaque vulnerability evaluated by MRI has been reported recently, which warrants further investigations on CEUS.[42]

Whether identification of vulnerable plaque features may actually translate into a clinical benefit for patients remains a matter of debate. In the coronary setting, the most common cause of lumen thrombosis is plaque rupture, with the thin cap fibroatheroma (TCFA) being postulated as its precursor lesion.[43] More recently, the concept of a vulnerable patient is growing over the concept of a vulnerable plaque, moving the focus from the features of individual plaques to the atherosclerotic disease burden.[44] Plaque rupture is frequently clinically silent (with subsequent healing eventually leading to progressive lumen obstruction) and even if it is indeed responsible for most culprit lesions in patients with acute events, because the frequency of subclinical plaque disruption is vastly underestimated, it is exceedingly difficult to predict adverse outcomes associated with particular lesions.[44,45]

In the present study there was a higher proportion of vulnerable plaques than that expected on the basis of previous studies.[46] A possible explanation could be that with the use of modern medical therapy, even those plaques with morphological features traditionally associated with symptoms may actually remain silent. There is overwhelming evidence that antiplatelet (AP) and lipid lowering (LL) drugs are of benefit in patients at increased vascular risk,[47–49] and the vast majority of patients in this study received AP and LL therapy (Table 1). However, ex vivo histopathology still remains the accepted gold standard technique to identify and quantify the presence of high risk characteristics[50] and it must be acknowledged that vulnerable plaque features have been defined post hoc for symptomatic patients, but it is not known which asymptomatic plaques will actually become symptomatic. As such, there is a need for conducting prospective studies using repetitive imaging in asymptomatic patients to truly answer this question.

Current practice guidelines for the management of atherosclerotic carotid disease from the European Society for Vascular Surgery (ESVS) do not include CEUS among the imaging strategies to identify high risk ACAD.[51] To date, no published studies have evaluated the effect of contrast uptake in carotid plaques as a way to predict future ipsilateral cerebrovascular events and the clinical usefulness of CEUS in ACAD is uncertain.[52] Nevertheless, the need to develop a validated algorithm for identifying "high risk of stroke" asymptomatic patients still represents an unresolved issue and an uncritical recommendation to selectively revascularise high risk patients without defining who these patients might be cannot be justified and warrants further research.[53] In particular, future CEUS studies should focus on the use of targeted microbubbles (to detect and quantify critical molecular processes[30,54,55]) and application of 3D/4D Doppler techniques to CEUS for a global spatial and temporal evaluation of the carotid plaque.[17,56]

Study limitations

There are several limitations to this study that must be acknowledged. First is the small sample size; however, the study population is similar to that of other published studies.[11,13,30,57]

Second, is the operator dependency and lack of reproducibility of CEUS analysis, since a single observer semi-quantitative approach was used for the evaluation of carotid plaque enhancement. Even if standardised and reproducible techniques to measure ultrasound contrast uptake are described,[57] a recent systematic review with meta-analysis has concluded that the use of semi-automatic software for quantitative assessment of carotid plaque enhancement is currently of limited value in clinical practice.[58] With regards to CEUS protocol, the one used in the present study was chosen on the basis of a recent study which has underlined that dynamic CEUS with high dose (i.e., 4 mL) SonoVue offers a greater diagnostic performance for detection of histologically proven vulnerable carotid plaques than late phase CEUS or low dose (i.e., 2 mL) SonoVue.[11]

Third, is the use of two level classification for reporting of CEUS and IHC results. The choice to dichotomise the results of CEUS analysis as either positive or negative was also taken according to previously published studies showing that two level and multiple level classification systems were both robustly correlated with the density of intra-plaque neo-vessels, so that the reliability and reproducibility of the results were not diminished.[59] Even for IHC analysis, the choice of adopting a two level classification for CD34/VEGF expression is concordant with the methods adopted in previously published studies.[10] However, it is acknowledged that the same methodological shortcomings as reported for CEUS analysis (i.e., single observer, semi-quantitative approach) apply to IHC analysis.

Fourth, the exact topography of the evaluated plaques may well act as a confounding factor. In particular, when plaques located in the posterior arterial wall are evaluated, microbubbles may produce acoustic shadowing that alters grayscale intensity and therefore image interpretation, a phenomenon known as far wall pseudo-enhancement.[60] Furthermore, limited detection of contrast uptake in calcific plaques with associated acoustic shadowing may interfere with positive findings. However, the percentage of plaques with unfavourable characteristics for CEUS examination (i.e., Type V plaques according to the modified Gray–Weale scale) in the present study was only 8% ($n = 5$); in all of these cases a multiplanar scanning technique was adopted to overcome the technical difficulty and avoid misidentification of carotid plaque enhancement.

CONCLUSIONS

In conclusion, there were two main findings: (a) histologically proven vulnerable plaques (i.e., AHA Class VI)

compared with histologically proven non-vulnerable plaques (i.e., AHA Class IV/V) had denser neo-vascularisation, but did not show more pronounced contrast enhancement; (b) the correlation between neo-vascularisation and contrast enhancement was direct for non-vulnerable plaques and inverse for vulnerable plaques. The results may indicate the need for a more sceptical interpretation of CEUS findings in the setting of ACAD reflecting the current difficulty of stratifying carotid plaques vulnerability with CEUS.

CONFLICTS OF INTEREST

None.

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COUP D'OEIL

Subclavian Vein Aneurysm

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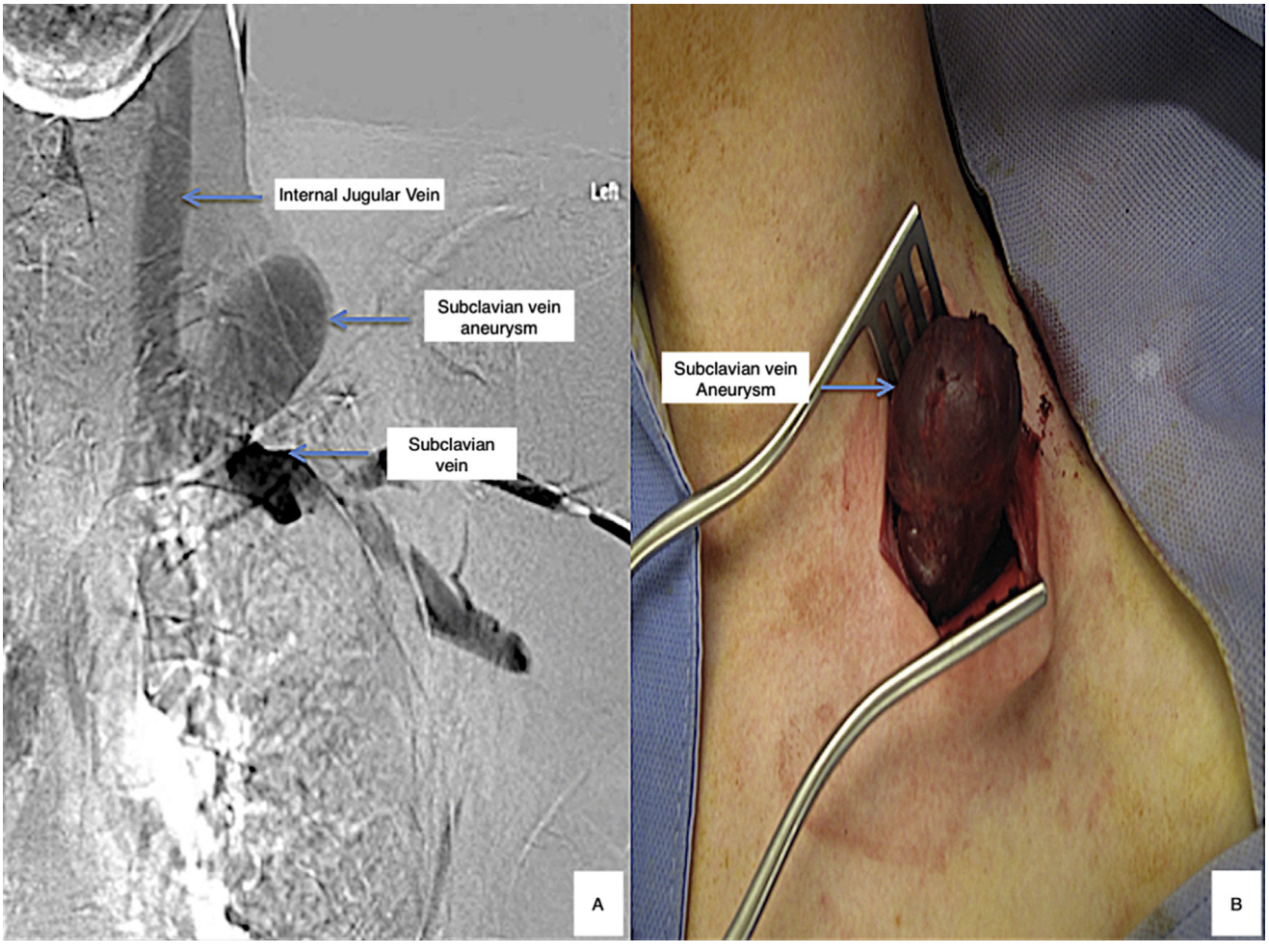

A 19 year old boy presented with gradual onset of left supraclavicular swelling, increasing in size during exercise, coughing or straining. On examination, he had a soft supraclavicular mass that was readily compressible. The mass increased in size with a Valsalva manoeuvre. An ultrasound scan suggested a 31 × 28 mm left subclavian vein aneurysm, which was confirmed by venography (A). A transverse incision was made in the left supraclavicular fossa. The aneurysm neck (which had a diameter of 10 mm) was clamped tangential to the subclavian vein and the aneurysm then resected (B). Histological examination confirmed the diagnosis of venous aneurysm.

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