Does quantification of carotid plaque surface irregularities better detects symptomatic plaques as compared to the subjective classification?

**Manuscript category**: original research

**Running title**: Quantifying carotid plaque surface irregularity

Vasileios Rafailidis MD MSc PhD1, Ioannis Chryssogonidis Asst Prof1, Enrico Grisan Asst Prof2, Chrysostomos Xerras MD MSc3, Grigorios-Aris Cheimariotis MSc4, Thomas Tegos Assoc Prof3, Dimitrios Rafailidis MD PhD5, Paul S Sidhu Prof6,Afroditi Charitanti-Kouridou Prof1

1 Department of Radiology, AHEPA University General Hospital, Aristotle University of Thessaloniki, Thessaloniki, Greece

2 Department of Information Engineering, University of Padova, Padova (Italy), School of Imaging Sciences and Biomedical Engineering, King's College London, London (UK)

3 1st Neurological Department, AHEPA University General Hospital, Aristotle University of Thessaloniki, Thessaloniki, Greece

4 Laboratory of Computing, Medical Informatics and Biomedical Imaging Technologies, School of Medicine, Aristotle University of Thessaloniki, Thessaloniki, Greece

5 Department of Radiology, “G. Gennimatas” General Hospital of Thessaloniki, Thessaloniki, Greece.

6 Department of Radiology, King's College Hospital, Denmark Hill, London, SE5 9RS, UK.

Institution: AHEPA University General Hospital of Thessaloniki

St. Kiriakidis 1, 54636, Thessaloniki, Greece

Corresponding author: Vasileios Rafailidis,

St. Kiriakidis 1, 54636, Thessaloniki, Greece

Tel: 0030 6976161113,

Email: billraf@hotmail.com

**Abstract**

Objectives: To evaluate the inter-observer agreement of colour Doppler ultrasound (CDU) and contrast-enhanced ultrasound (CEUS) for quantification of carotid plaque surface irregularities and correlate objective and subjective measures with stroke occurrence.

Methods: Observational study involving 54 patients with 62 internal carotid artery or carotid bulb plaques (31 symptomatic), undergoing CDU and CEUS between February 2016 and February 2018, with retrospective interpretation of prospectively acquired data. Plaques were included if causing moderate (50-69%) or severe (70-99%) stenosis based on velocity criteria and their surface was classified as smooth, irregular or ulcerated based on CEUS. The surface irregularities were quantified in the form of a surface irregularity index (SII), by two observers, based on CDU and CEUS. The SII was evaluated for inter-observer agreement with CDU and CEUS and correlated with the occurrence of stroke, as was the subjective characterization of plaque surface.

Results: CDU and CEUS showed good inter-observer agreement (Intra-class correlation coefficient: 0.979 and 0.952 respectively). Plaques were characterized as smooth in 30.6% of cases, irregular in 50% and ulcerated in 19.4%. Subjective classification of plaque surface did not correlate with stroke occurrence (p>0.05, chi-square test). SII values were significantly higher for symptomatic plaques, both with CDU and CEUS (p<0.05).

Conclusions: CDU and CEUS can quantify carotid plaque surface irregularities with good inter-observer agreement. The resulting quantitative measure is significantly higher in symptomatic plaques, whereas the subjective characterization of plaque surface failed to differ between symptomatic and asymptomatic plaques.

**Keywords:** carotid, atherosclerosis, stroke, ultrasound, contrast-enhanced ultrasound

**Introduction**

 Carotid disease represents a significant cause of stroke with 10-15% of all cases currently being attributed to thromboembolism from a moderately or severely stenotic internal carotid artery plaque [1]. The percentage of internal carotid artery stenosis along with the presence or absence of neurologic symptoms has long been used as a well-established criterion for determining optimal patient treatment [2, 3]. Nevertheless, it has become evident that factors other than the percentage of stenosis also contribute to the plaque’s potential for stroke; also termed the plaque “vulnerability”. Vulnerable plaques are those associated with greater risk for neurologic symptoms, either in the form of transient ischemic attack (TIA) or stroke. Such factors include the plaque composition and surface characteristics and can be evaluated with current imaging modalities with a varying degree of accuracy [4-7].

 Carotid plaque surface morphology can be subjectively classified in three types: smooth, irregular or ulcerated and constitutes an important plaque characteristic. It has been shown that surface morphology either in the form of ulceration or surface irregularities, significantly contributes to plaque vulnerability with ulcerated and irregular plaque being more prone to cause neurovascular symptoms [8-10]. This association has been justified on the grounds of higher occurrence of ulcerated plaque among symptomatic patients, increased occurrence of new neurological symptoms in asymptomatic patients with carotid disease or the detection of embolic signals on transcranial Doppler ultrasound (US) in patients with ulcerated carotid plaque [9]. Even plaques characterized by an irregular surface, but not a true ulcer, are associated with increased risk for stroke and the occurrence of neurologic symptoms [9, 11, 12]. US is a well-established imaging modality for screening, grading and monitoring carotid atherosclerosis, with inherent advantages including low cost, good accuracy and repeatability [13].

Beyond grading stenosis, US has also the potential to provide qualitative and quantitative information regarding plaque composition and surface morphology with high spatial and temporal resolution. However, quantitative measures are preferable to subjective interpretation, being independent of operator perception; with previous efforts to quantify carotid plaque surface irregularities using conventional US techniques resulting in varying degrees of success [14; 15]. Recently, contrast-enhanced ultrasound (CEUS) has become a valuable complementary ultrasonographic technique for the evaluation of carotid disease, both in terms of intraplaque neovascularization and improved surface irregularities characterization [16-19].

The aim of this study was to evaluate colour Doppler Ultrasound (CDU) and CEUS in terms of inter-observer agreement for the evaluation of carotid plaque surface irregularities quantification, using computer-based software and investigate the association of resulting quantitative measures and subjective characterization of plaque surface morphology, with the occurrence of stroke in patients with carotid disease.

**Materials and methods**

**Patients and inclusion / exclusion criteria**

This study was approved by the institutional ethics review board. Patients were recruited on a prospective consecutive pattern from the Radiology and Neurology Department of our institution between February 2016 and February 2018, after written informed consent and their data were retrospectively interpreted. Both symptomatic and asymptomatic patients with carotid disease were referred for carotid US either due to the occurrence of transient ischemic attack (TIA) or stroke, or for screening purposes (preoperatively or due to the existence of cardiovascular risk factors). For a patient to be considered symptomatic with stroke, the neurologic symptoms consistent with ischemia should have occurred within the last six-month period, as documented based on the patient’s history, clinical examination or review of radiologic studies (computed tomography or magnetic resonance imaging documenting stroke). Additionally, an internal carotid artery or a carotid bulb atherosclerotic plaque with moderate (50-69%) or severe (70-99%) stenosis should have been documented on US, ipsilateral to the side of stroke to be considered symptomatic or contralateral, to be considered asymptomatic. Exclusion criteria included history of allergy or other contraindication to US contrast agent used, presence of other diseases mimicking stroke symptoms or comorbidities that could cause stroke (including arrhythmias, cardiac anatomic abnormalities, thrombophilia and immunologic diseases like antiphospholipid syndrome) or presence of extensively calcified plaques unsuitable for quantitative analysis due to acoustic shadowing. Such plaques were only excluded if positioned in the near carotid wall, hence causing acoustic shadowing, while they were normally analyzed if affecting the distant wall, with their surface being fully visible.

**Ultrasound scan technique**

The ultrasound examinations were performed by a radiologist with 10 years of experience using a GE Logiq S8 (GE Healthcare) with XDclear technology device and a linear transducer (type 9L) with a bandwidth of 3-10 MHz. B-mode and colour Doppler technique were used for the initial part of the examination. Routine examination included grading of stenosis based on velocity criteria [20] in combination with direct diametric measurements on axial CDU images and examination of plaque characteristics. During US examination, the patient lied in the supine position, with his head in extension and with the face turned slightly away of the side examined. One single focus was used and located at the level of arterial lumen examined. Default settings for carotid examination provided by the US machine’s manufacturer (GE Healthcare) including edge enhancement and real-time spatial compound technique (CrossBeam) were active and used in all examinations performed. If a plaque was detected and the patient met the inclusion criteria, a CEUS examination was performed within one week following the conventional US. CEUS was performed after the bolus intravenous administration of 2.4 mL of SonoVue (Bracco SpA) followed by a flush of 10 mL of normal saline and using the low-Mechanical Index (MI) ultrasound contrast-specific mode of the device in order to prevent microbubbles destruction. A bolus injection technique was opted for the purpose of this study, achieving adequate arterial lumen opacification in approximately 20 to 30 seconds, depending on the patient’s circulatory status. Adequate arterial lumen opacification was achieved for approximately three to four minutes. The Mechanical Index (MI) was kept to less than 0.1 in all examinations and the gain was properly adjusted in order to achieve optimal microbubbles visualization.

**Image analysis**

Video clips of the affected internal carotid artery or carotid bulb in long axis were recorded by the radiologist performing the examination, both with CDU and CEUS technique. The clips were then given to two different radiologists – observers (A and B with 18 and 15 years of experience respectively), blinded to the patient’s history, who classified each plaque as smooth, irregular or ulcerated based on CEUS images (reaching a consensus). As with previously reported criteria, a plaque was defined as ulcerated if a focal cavity of at least 1 x 1 mm was detected, filled with blood flow signals on CDU and microbubbles in CEUS [21]. The two observers chose a frame of CDU and CEUS video clip which optimally visualized carotid plaque surface morphology. Subsequently, the two observers used the surface irregularities software developed for the purpose of this study to quantify the surface irregularities, while being blinded to each other’s results.

The software used in this study was developed using MATLAB (Mathworks) and aims to quantify an atherosclerotic plaque’s surface irregularities. The physician using this software has the possibility to manually place points on the plaque surface and the outer blood vessel wall, for both the upper and lower vessel wall, depending on the plaque’s distribution. As previously described by Kanber et al, a plaque surface irregularity index (SII) is calculated by the computational summation of angular deviations of the luminal plaque surface from the straight line, divided by the physical length of the plaque surface [14, 22]. The plaque’s surface is delineated by colour Doppler signals in CDU and the border of microbubble column in CEUS. Given that the US images used by the software are in DICOM format, pixel spacing and length information are included within the image file. As a result, the software can measure angles using the cosine rule for the consecutive triangles formed. The sum SII of both carotid walls (upper and lower) was recorded for analysis.

**Statistical analysis**

IBM SPSS Statistics version 23.0 was used for statistical analyses. Descriptive statistics included mean and standard deviation (SD) of normally distributed variables and median and inter-quartile range (IQR) for non-normally distributed variables. The Kolmogorov-Smirnov test was used to test the normal distribution of variables. Inter-observer agreement was investigated by calculating the mean and SD of difference between two measurements, limits of agreement and intra-class correlation coefficient (ICC) for compared variables. Mann-Whitney U test and t-test were used to compare means between groups. Statistical significance level was set at 0.05.

**Results**

**Patients**

Fifty-four patients (39 males, 15 females) were recruited prospectively, having a median age of 61 years (IQR: 17 years). In total, 62 plaques (31 symptomatic and 31 asymptomatic) met the inclusion criteria and were included in this study, while 6 plaques were excluded due to extensive calcification with acoustic shadowing hindering quantitative analysis. The mean degree of stenosis was 68.9% with a SD of 12.8%. The plaque surface was subjectively classified as smooth in 30.6% of cases (n=19), irregular in 50.0% (n=31) and ulcerated in 19.4% (n=12). Median and IQR values of SII calculated by observer A and B were as follow, respectively: 13.4 (7.6) for CDU, 16.8 (12.2) for CEUS, 15.6 (11.5) for CDU and a mean value of 18.8 (SD: 10.8) for CEUS. No adverse reaction to the US contrast agent was observed in this study.

**Inter-observer agreement for SII with CDU and CEUS**

 The difference between the SII values measured by each observer for the same plaque and using the same technique was calculated in order to evaluate the inter-observer agreement of CDU and CEUS for calculation of SII. The mean, SD, limits of agreement and ICC can be found in table 1. Good inter-observer agreement in the calculation of SII was found between the two observers using both the CDU and CEUS technique as demonstrated by the high ICC (0.979 and 0.952 respectively) and the low values of mean difference calculated (-0,8 for SII-CDU and -0.7 for SII-CEUS).

 **Comparison of subjective surface classification and SII between symptomatic and asymptomatic patients**

 In the group of plaques with smooth surface, 8 were symptomatic and 11 asymptomatic. The respective numbers for irregular plaques were 15 and 16, while for ulcerated 8 and 4 respectively. Subjective classification was thus not significantly associated with the occurrence of stroke, based on chi-square test (p<0.05), showing that the subjective classification may not be sufficient to adequately characterize plaque surface morphology.

The SII values were compared between symptomatic and asymptomatic plaques to test whether symptomatic plaques are more irregular than asymptomatic. Mann-Whitney U test and t-test (non-normally and normally distributed variables respectively) were used for that purpose and the results can be found in table 2. The respective boxplots can be found in figure 1. Both observers and techniques (CDU and CEUS) found significantly higher values of SII for symptomatic plaques, compared to asymptomatic. Greater differences and lower levels of significance were detected with CEUS, compared to CDU.

 Examples of quantitative analysis of carotid plaque surface morphology can be found in figure 2.

**Discussion**

 This study showed that both CDU and CEUS are characterized by good inter-observer agreement, when two different observers evaluate the same plaque, as indicated by the high values of ICC calculated (0.979 and 0.952 respectively). When it comes to the clinical significance of the SII index, it was found that there is significant difference between symptomatic and asymptomatic plaques, when the SII is calculated using both CDU and CEUS, although greater differences and significance were noted with CEUS. Nevertheless, the conventional subjective classification of a plaque surface into smooth, irregular and ulcerated failed to correlate with the occurrence of stroke. As a result, CDU and CEUS both appear to accurately delineate carotid plaques, although the latter appears to provide more clinically significant information. Consequently, some form of surface irregularities quantification, such as SII calculation, could be potentially incorporated into the CEUS examination protocol performed for quantification of intraplaque neovascularization, in order to increase the information provided regarding the vulnerability of the plaque. This could be easily done as both neovascularization and surface irregularities can be quantified using a video clip and one still image of this clip, respectively.

 It is recognized that carotid plaque surface morphology plays an important role in determining the plaque’s vulnerability. The arterio-arterial embolization of thrombotic material formed within ulceration and into the intracranial circulation represents an essential mechanism for occurrence of stroke in patients with ulcerated carotid plaques [8, 10, 23, 24]. Ulceration has been correlated with a 2.32 to >3 times risk for neurologic symptoms [25, 26]. Even a simply irregular carotid plaque on CDU may increase the risk for stroke up to 7.7 times [12, 27]. A different study concluded that smooth plaques are associated with a 3% risk for stroke, while irregular plaques carry a risk of 8.5% [28]. Consequently, careful examination of the plaque’s surface morphology should be included in current imaging protocols with every imaging modality, including US, the first-line imaging modality for the evaluation of carotid disease. The subjective classification of a plaque into smooth, irregular or ulcerated is essential but quantitative measures would be more beneficial as they are independent of physician’s subjectivity and more repeatable.

 The quantification of carotid plaque surface irregularities has been previously attempted, although with varying degrees of success, depending on technique of quantification. Tegos et al. have evaluated the value of bending energy; a quantitative variable of surface irregularities representing the energy needed to bend a rod in the shape of the plaque’s surface. This study failed to detect significant difference in the bending energy values between symptomatic and asymptomatic plaques, while the former were characterized by a higher mean value of bending energy [15]. Kanber et al. proposed a different quantitative index, termed the SII, which was found to be significantly different between symptomatic and asymptomatic plaques with the former exhibiting higher values of this index. The same study showed that subjective characterization of plaques as smooth or irregular did not correlate significantly with the presence of neurologic symptoms, showing the superiority of quantitative measures [14]. In comparison with this study, we used the same principle to quantify the surface irregularities, but there are some differences in the software developed: in the present study plaque delineation was done manually by the performing physician, based on static images of CDU and CEUS technique, while in the study by Kanber the surface was semi-automatically delineated by the software based on B-mode images; with CDU being used for delineation of hypoechoic plaques. In keeping with this study, significantly higher SII values were calculated for symptomatic plaques, based both on CDU and CEUS images, although greater differences were found for the latter. Also in agreement with this study, our findings showed that the subjective classification may not be correlated with the occurrence of stroke. CEUS is well-known to outperform conventional CDU in terms of carotid plaque delineation with high spatial and temporal resolution and good contrast of microbubbles with static tissues. Moreover, CEUS is independent of artifacts applicable to CDU such as Doppler angle dependence, overwriting artifacts and low sensitivity to slow flow, all of which could hinder accurate plaque delineation with CDU [16, 17, 29-32].

 Limitations of this study include the potentially small number of plaques examined. Secondly, manual segmentation of plaques was chosen in this study so that the delineation was directly completed by the performing physician. Although this may introduce some operator-dependency, it was still able to detect significant differences. A two-dimensional technique was used in this study, while a three-dimensional ultrasonographic technique would likely be able to evaluate carotid plaques more thoroughly, but such technology is not yet widely available. Further studies are needed to investigate the role of surface irregularities quantitative measures in everyday diagnostic work-up of carotid disease and its implications for patient treatment.

**Conclusion**

Both CDU and CEUS are characterized by good inter-observer agreement for the quantification of carotid plaque surface irregularities. The values of SII derived from CDU and CEUS significantly differed between symptomatic and asymptomatic plaques, being higher in the former, although the subjective characterization of plaque surface morphology failed to correlate with the occurrence of stroke. Quantification of surface irregularities is thus superior to subjective surface characterization and could be incorporated in a carotid CEUS examination performed for assessment of intraplaque neovascularization, in order to increase the information provided regarding plaque vulnerability and potentially the accuracy for the detection of symptomatic carotid plaque.

**Acknowledgements**

V.R. has received a scholarship for his PhD studies on «Imaging of the carotid vulnerable plaque with contrast-enhanced ultrasound and multi-detector computed tomography angiography» from the Alexander S. Onassis Public Benefit Foundation [grant number G ZJ 050-2/2015-2016].

Author P.S. has received lecture fees from Bracco, Siemens, Samsung, Philips, and Hitachi.

The rest of the authors declared no potential conflict of interest regarding the publication of this manuscript.

This study is derived from a patient cohort recruited for the purpose of the PhD studies of V.R. This patient cohort was also used for a different study published in European Radiology and is entitled: “A comparative study of color Doppler imaging and contrast-enhanced ultrasound for the detection of ulceration in patients with carotid atherosclerotic disease”. The present study is also included in an oral presentation which was accepted for presentation in European Congress of Radiology 2019, which is entitled: “Imaging carotid artery-vulnerable plaque with ultrasound and contrast-enhanced ultrasound: correlation of cerebrovascular symptoms with quantitative and multi-parametric indexes”.

**References**

1 Aboyans V, Ricco JB, Bartelink MEL et al. 2017 ESC Guidelines on the Diagnosis and Treatment of Peripheral Arterial Diseases, in collaboration with the European Society for Vascular Surgery (ESVS): Document covering atherosclerotic disease of extracranial carotid and vertebral, mesenteric, renal, upper and lower extremity arteriesEndorsed by: the European Stroke Organization (ESO)The Task Force for the Diagnosis and Treatment of Peripheral Arterial Diseases of the European Society of Cardiology (ESC) and of the European Society for Vascular Surgery (ESVS). Eur Heart J 2018; 39:763-816.

2 MRC European Carotid Surgery Trial: interim results for symptomatic patients with severe (70-99%) or with mild (0-29%) carotid stenosis. European Carotid Surgery Trialists' Collaborative Group. Lancet 1991; 337:1235-1243.

3 North American Symptomatic Carotid Endarterectomy Trial Collaborators, Barnett HJM, Taylor DW, et al. Beneficial effect of carotid endarterectomy in symptomatic patients with high-grade carotid stenosis. N Engl J Med 1991; 325:445-453.

4 Saba L, Anzidei M, Marincola BC et al. Imaging of the carotid artery vulnerable plaque. Cardiovasc Intervent Radiol 2014; 37:572-585.

5 Brinjikji W, Huston J, Rabinstein AA, Kim GM, Lerman A, Lanzino G. Contemporary carotid imaging: from degree of stenosis to plaque vulnerability. J Neurosurg 2016; 124:27-42.

6 Saba L, Yuan C, Hatsukami TS 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. AJNR Am J Neuroradiol 2018; 39:E9-e31.

7 Naylor AR. Time to rethink management strategies in asymptomatic carotid artery disease. Nat Rev Cardiol 2012; 9:116-124.

8 Fisher M, Paganini-Hill A, Martin A et al. Carotid plaque pathology: thrombosis, ulceration, and stroke pathogenesis. Stroke 2005; 36:253-257.

9 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.

10 Bonati LH, Nederkoorn PJ. Clinical Perspective of Carotid Plaque Imaging. Neuroimaging Clin N Am 2016; 26:175-182.

11 Hokari M, Kuroda S, Yasuda H et al. Lumen morphology in mild-to-moderate internal carotid artery stenosis correlates with neurological symptoms. J Neuroimaging 2011; 21:348-354.

12 Rothwell PM, Gibson R, Warlow CP. Interrelation between plaque surface morphology and degree of stenosis on carotid angiograms and the risk of ischemic stroke in patients with symptomatic carotid stenosis. On behalf of the European Carotid Surgery Trialists' Collaborative Group. Stroke 2000; 31:615-621.

13 Tahmasebpour HR, Buckley AR, Cooperberg PL, Fix CH. Sonographic examination of the carotid arteries. Radiographics 2005; 25:1561-1575.

14 Kanber B, Hartshorne TC, Horsfield MA, Naylor AR, Robinson TG, Ramnarine KV. Quantitative assessment of carotid plaque surface irregularities and correlation to cerebrovascular symptoms. Cardiovasc Ultrasound 2013; 11:38.

15 Tegos TJ, Kalomiris KJ, Sabetai MM, Kalodiki E, Nicolaides AN. Significance of sonographic tissue and surface characteristics of carotid plaques. AJNR Am J Neuroradiol 2001; 22:1605-1612.

16 Rafailidis V, Charitanti A, Tegos T, Destanis E, Chryssogonidis I. Contrast-enhanced ultrasound of the carotid system: a review of the current literature. J Ultrasound 2017; 20:97-109.

17 Piscaglia F, Nolsoe C, Dietrich CF et al. The EFSUMB Guidelines and Recommendations on the Clinical Practice of Contrast Enhanced Ultrasound (CEUS): update 2011 on non-hepatic applications. Ultraschall Med 2012; 33:33-59.

18 Sidhu PS, Cantisani V, Dietrich CF 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 2018; 39:e2-e44.

19 Rafailidis V, Chryssogonidis I, Xerras C et al. A comparative study of color Doppler imaging and contrast-enhanced ultrasound for the detection of ulceration in patients with carotid atherosclerotic disease [published online ahead of print October 22, 2018]. Eur Radiol. Doi: 10.1007/s00330-018-5773-8.

20 Grant EG, Benson CB, Moneta GL et al. Carotid artery stenosis: gray-scale and Doppler US diagnosis--Society of Radiologists in Ultrasound Consensus Conference. Radiology 2003; 229:340-346.

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

22 Kanber B, Hartshorne TC, Horsfield MA, Naylor AR, Robinson TG, Ramnarine KV. A Novel Ultrasound-Based Carotid Plaque Risk Index Associated with the Presence of Cerebrovascular Symptoms. Ultraschall Med 2015; 36:480-486.

23 Wechsler LR. Ulceration and carotid artery disease. Stroke 1988; 19:650-653.

24 Kessler C, von Maravic M, Bruckmann H, Kompf D. Ultrasound for the assessment of the embolic risk of carotid plaques. Acta Neurol Scand 1995; 92:231-234.

25 Eliasziw M, Streifler JY, Fox AJ, Hachinski VC, Ferguson GG, Barnett HJ. Significance of plaque ulceration in symptomatic patients with high-grade carotid stenosis. North American Symptomatic Carotid Endarterectomy Trial. Stroke 1994; 25:304-308.

26 Gao P, Chen ZQ, Jiao LQ, Ling F. The correlation of carotid plaque pathohistologic features and neurological symptoms: a meta-analysis of observational studies. Neurol India 2007; 55:122-129.

27 Kitamura A, Iso H, Imano H et al. Carotid intima-media thickness and plaque characteristics as a risk factor for stroke in Japanese elderly men. Stroke 2004; 35:2788-2794.

28 Prabhakaran S, Rundek T, Ramas R et al. Carotid plaque surface irregularity predicts ischemic stroke: the northern Manhattan study. Stroke 2006; 37:2696-2701.

29 Staub D, Partovi S, Imfeld S et al. Novel applications of contrast-enhanced ultrasound imaging in vascular medicine. Vasa 2013; 42:17-31.

30 Schinkel AF, Kaspar M, Staub D. Contrast-enhanced ultrasound: clinical applications in patients with atherosclerosis. Int J Cardiovasc Imaging 2016; 32:35-48.

31 Sirlin CB, Lee YZ, Girard MS et al. Contrast-enhanced B-mode US angiography in the assessment of experimental in vivo and in vitro atherosclerotic disease. Acad Radiol 2001; 8:162-172.

32 Kono Y, Pinnell SP, Sirlin CB et al. Carotid arteries: contrast-enhanced US angiography--preliminary clinical experience. Radiology 2004; 230:561-568.

**Tables**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Agreement examined | MeanDifference | SD ofDifference | Lower limit of agreement | Upper limit of agreement | ICC | Lower 95% CI | Upper 95% CI |
| SII-CDU | -0.8 | 4.7 | -10.1 | 8.5 | 0.979 | 0.964 | 0.987 |
| SII-CEUS | -0.7 | 4.4 | -9.5 | 8.0 | 0.952 | 0.92 | 0.971 |
| SD: standard deviation, ICC: Intra-class correlation coefficient, CI: Confidence Interval |

Table 1. Statistics of the CDU and CEUS inter-observer agreement between the two observers and the inter-observer agreement between CDU and CEUS.

|  |  |
| --- | --- |
| **Variable** | **Level of significance (Mann-Whitney U Test / t-test)** |
| SII CDU Obs. Α | 0.035\* |
| SII CDU Obs. B | 0.042\* |
| SII CEUS Obs. Α | 0.01\* |
| SII CEUS Obs. B | 0.022\* |
|  | Median | IQR |
| SII CDU Obs. Α | Asymptomatic | 12.2 | 6.3 |
|  | Symptomatic | 13.8 | 7.8 |
| SII CDU Obs. B | Asymptomatic | 11.0 | 9.6 |
|  | Symptomatic | 15.9 | 9.0 |
| SII CEUS Obs. Α | Asymptomatic | 13.3 | 11.6 |
|  | Symptomatic | 19.2 | 12.2 |
| SII CEUS Obs. B\*\* | Asymptomatic | 15.7 | 8.2 |
|  | Symptomatic | 21.9 | 12.3 |
| Obs. Α: observer Α, Obs. Β: observer Β\* Statistically significant result (difference) \*\* Mean and SD are presented |

Table 2. Comparison of SII between symptomatic and asymptomatic plaques.

**Figures / legends**

Figure 1. Boxplots of SII comparison between symptomatic and asymptomatic plaques. Results recorded by observer A are presented in images a and b, while results recorded by observer B are presented in images c and d. CDI stands for Colour Doppler Imaging and CEUS for contrast-enhanced ultrasound.

Figure 2. Subjective classification and quantification of carotid plaque surface irregularities. CDU (A) and CEUS (B) image of a smooth carotid plaque being used for quantitative analysis of surface irregularities. Similar analysis of CDU (C) and CEUS (D) images of an irregular carotid plaque. Similar analysis of CDU (E) and CEUS (F) images of an ulcerated carotid plaque. Note the better plaque surface delineation provided by CEUS, particularly in the case of the ulcerated plaque. Magnification of images was performed within the software used for SII calculation.