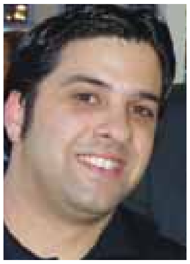

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DETECTION OF ADVENTITIAL VASA VASORUM AND INTRAPLAQUE NEOVASCULARIZATION IN CAROTID ATHEROSCLEROTIC LESIONS WITH CONTRAST-ENHANCED ULTRASOUND AND THEIR ROLE IN ATHEROSCLEROSIS

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Introduction

The development of new surrogate markers of atherosclerosis is a crucial goal in the clinical setting, as they may allow physicians to recognize unstable lesions early and identify individuals with vulnerable or unstable lesions who bear an increased risk of future cardio- and cerebrovascular complications.[1, 2] These surrogate markers should be capable of being measured noninvasively using safe and reliable methods.[3] Recently, Nambi et al. demonstrated how the combined measurement of carotid intima media thickness (CIMT) and occurrence of carotid plaques improves the risk prediction of cardiovascular outcomes.[4] These results confirm the value of carotid ultrasound (US) when combined with traditional Framingham risk factors in assessing a patient's risk for atherosclerotic disease.

Contrast-enhanced ultrasound (CEUS) is a new imaging approach that is evolving and may become a standard clinical tool for further atherosclerotic risk stratification in the future.[5] The contrast agents used in US imaging are safe, commercially available, and approved for use in echocardiography by the FDA.[6] Thus, CEUS is technically feasible with existing approved commercial equipment and can be performed at the bedside or in an outpatient setting. However, US contrast agents are not yet approved by the FDA for visualization and assessment of the carotid artery and its associated pathologies. The cost effectiveness of CEUS has been shown in the context of gastrointestinal imaging,[7] and CEUS of carotid atherosclerotic lesions is emerging as an approach to complement unenhanced US imaging.[8] By providing the direct visualization of adventitial vasa vasorum (VV) and intraplaque neovascularization, CEUS is capable of depicting two new surrogate markers of atherosclerosis — namely, adventitial VV and intraplaque neovascularization.[5, 9]

The Biological Rationale Behind Adventitial Vasa Vasorum and Intraplaque Neovascularization

Adventitial VV are a network of small blood vessels that grow to supply nutrients to the arterial wall. They are induced by vessel wall ischemia and inflammation, which lead to the release of pro-angiogenic growth factors (like vascular endothelial growth factor and hypoxia inducible factor) as part of the atherosclerotic process.[10] These factors trigger the growth of the adventitial VV and, in later phases of atherosclerosis, also promote the development of an intraplaque neovasculature (Figure 1). Finally, the vulnerable atherosclerotic lesion and its surrounding arterial wall are embedded in a rich microvessel network.[11] These newly formed vessels are immature, with a thin wall and no surrounding pericytes, which further contributes to the instability of the lesion due to vascular leakage and inflammatory cell recruitment.[9, 12] The resulting leaky vessels are associated with intraplaque hemorrhage and ulcerations, predisposing the plaque to rupture and thrombosis.[9]

Data from Moreno et al. demonstrated the involvement of adventitial VV in the origin of atherosclerosis.[13] Growth of adventitial VV was detected in the earliest phases of lesion formation, even preceding the increase in CIMT, a well-known and clinically established marker of early atherosclerosis.[13, 14] In later

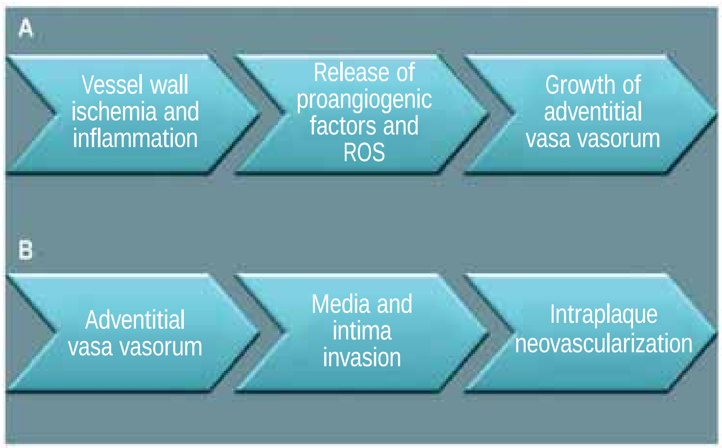

Figure 1. The biology rationale behind the microvessel system in the atherosclerotic arterial wall. Stepwise approach for development of (A) adventitial vasa vasorum and (B) intraplaque neovascularization. ROS: reactive oxygen species.

phases, adventitial VV extend further until they reach the media and intima of the lesion, supporting the concepts that VV extension correlates with atherosclerotic severity and that intraplaque neovascularization is derived from adventitial VV.[1, 15, 16] In an autopsy study, extensive adventitial VV and ectopic intraplaque neovascularization were identified in patients with symptomatic atherosclerotic disease and in those who died from cardiovascular events.[14] Hence, intraplaque neovascularization plays a key role in the initiation, progression and rupture of carotid atherosclerotic lesions.[13, 14, 17, 18] A recent study confirmed noticeable intraplaque neovascularization in carotid atherosclerotic histological specimens as an independent predictor of future cardiovascular events including stroke, myocardial infarction, and vascular death.[19] Moreover, it triggers the development of complex lesions and is a predictor of the vulnerability of the lesions, representing a marker of inflammation throughout the systemic vascular bed.[5, 14, 20-22]

Contrast-Enhanced Ultrasound for Visualization of the Microvessel Systems in Carotid Atherosclerotic Plaques

CEUS is capable of assessing in real-time the dynamic spatial and temporal heterogeneity of tissue microvessel systems.[1] The contrast agents used in CEUS are gas-filled microspheres that have a diameter of 1–4 µm and thus are smaller than red blood cells.[9] They serve as true intravascular tracers and make direct visualization of adventitial VV and intraplaque neovascularization in carotid atherosclerotic lesions feasible by passing through these microvessels.[23-28]

Studies have evaluated the use of various microsphere systems as acoustic reflectors for detection of these two microvessel systems in the carotid artery, including the approved perflutren type-A microspheres (Optison™, GE Healthcare, Pasadena, CA) and perflutren lipid microspheres (Definity®, Bristol-Myers Squibb, Plainsboro, NJ). The detection of adventitial VV and intraplaque neovascularization as atherosclerotic surrogate markers with CEUS provides information about the status of vessel wall biology and inflammation.[6] The visualization of these two microvessel networks represents a new imaging approach for analyzing the

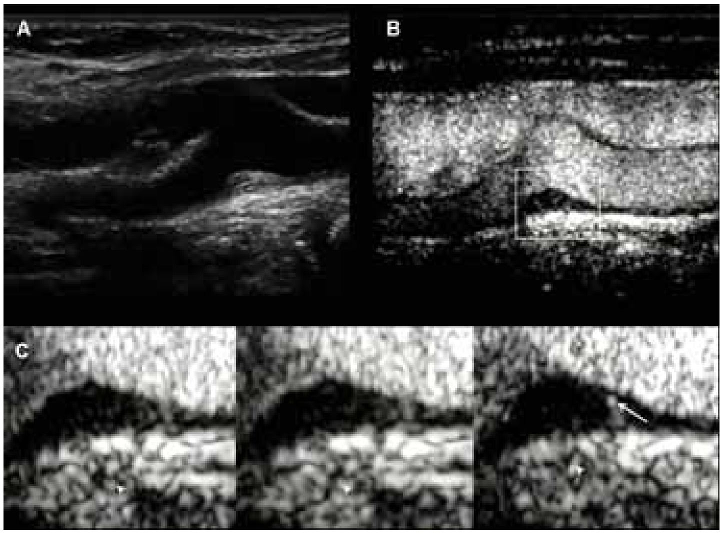

Figure 2. Carotid artery with vasa vasorum on CEUS. (A) Plaque on B-mode US imaging at the origin of the internal carotid artery. (B) Corresponding presentation on CEUS imaging. (C) Zoom of consecutive frames of this lesion with visible microbubbles in the periadventitial tissue (adventitial vasa vasorum, depicted by small arrow) and within the plaque (intraplaque neovascularization, depicted by long arrow).

Staub D, et al. Vasa vasorum and plaque neovascularization on contrast-enhanced carotid ultrasound imaging correlates with cardiovascular disease and past cardiovascular events. Stroke. 2010;41(1):41-7.[27]

course and severity of atherosclerosis and for better assessing carotid plaque vulnerability and the risk of future cardio- and cerebrovascular events.[1] The concept of risk estimation by these two surrogate markers has already been confirmed in different histological studies.[14, 19, 20, 22] Furthermore, this technique has the potential for diagnosis and risk stratification of preclinical atherosclerotic disease and early plaque progression, hence facilitating therapy initiation towards more aggressive approaches such as surgical or endovascular interventions in at-risk patients.[5, 29]

Adventitial Vasa Vasorum

In a CEUS study, more pronounced detection of adventitial VV occurred in a patient group with carotid stenosis compared to the control group without carotid artery plaques. This study also showed that adventitial VV are correlated with CIMT.[24] Another CEUS study in this field demonstrated that subjects with the presence of adventitial VV in carotid atherosclerotic lesions had more cardiovascular diseases (Figure 2).[27] Adventitial VV depicted by CEUS may be a valuable surrogate marker of atherosclerosis in its earliest stage, indicating a vulnerable patient.[14]

Intraplaque Neovascularization

The correlation of intraplaque neovascularization visualized using CEUS with information obtained from histological specimens after carotid endarterectomy has been confirmed in several studies.[23, 25, 30, 31] It is well known that echolucent carotid lesions on standard US correspond to soft atheromatous material in tissue probes; this material typically consists of a high lipid content and macrophage density as well as cell debris, necrotic residuals, and hemorrhagic areas.[32, 33] Ipsilateral cerebral infarction on computed tomography is associated with more echolucent carotid atherosclerotic plaques.[34] Thus, these lesions are associated with a higher risk of rupture leading to cerebrovascular events.[33, 35-37] Several studies have shown more pronounced carotid intraplaque neovascularization detected by CEUS in more echolucent lesions on standard US (Figure 3).[23, 27, 28, 38] Moreover, intraplaque neovascularization in carotid lesions assessed by CEUS was observed in patients with symptomatic atherosclerotic disease.[30, 38] These results are in line with histological studies in which more immature microvessels could be detected in carotid lesions of symptomatic individuals after endarterectomy.[12, 21, 34] One recent study showed that the presence of intraplaque neovascularization visualized with CEUS was significantly higher in patients with a history of prior cardiovascular events, namely myocardial infarction, transient ischemic attack, or stroke.[27] These studies reveal intraplaque neovascularization detected by CEUS to be

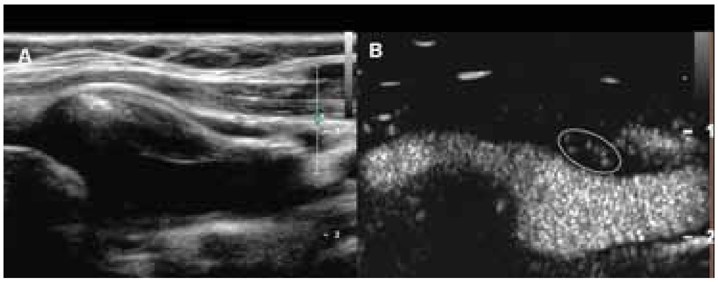

Figure 3. Hypoechogenic carotid artery lesion on standard US image, with intraplaque neovascularization on CEUS image in 71-year-old woman who had symptomatic cerebrovascular disease at presentation. (A) Non-enhanced US image shows a predominantly echolucent lesion at the origin of the internal carotid artery. (B) Corresponding CEUS image shows microbubbles moving to the plaque core (intraplaque neovascularization in the circled region).[26]

a proven hallmark of carotid lesion vulnerability and therefore useful for identifying the patient at risk for future atherosclerotic diseases and their associated complications.

Limitations of Contrast-Enhanced US in Carotid Atherosclerotic Lesions

Nearly all published CEUS studies for detecting microvessel systems in carotid lesions employed semiquantitative approaches by visual assessment using dichotomous[23] or more detailed grading systems.[25] However, a true quantifiable analysis of the CEUS signal remains elusive. Few authors have investigated the quantification of CEUS signals on an experimental basis. One case report quantified intraplaque neovascularization by grayscale level and entropy measurements within the plaque region.[39] Another group performed time-signal intensity curve analysis after setting the region of interest within the carotid plaque for quantification of intraplaque neovascularization. They measured higher signal intensity in symptomatic patients compared to asymptomatic patients.[38] Another study investigated the automatic quantification of intraplaque neovascularization with an algorithm that integrated several imaging processing methods. It demonstrated a good correlation of this quantification approach with the detection of intraplaque neovascularization on histological specimens and the amount of inflammatory cells in the same specimens.[31] Periadventitial flow signal thickness by contrast-enhanced B-flow imaging was used in one study as a quantitative approach for measuring adventitial VV.[24] Refining these quantification systems and determining how to best quantify the CEUS signal in carotid lesions will be necessary before this imaging technique is routinely used in the clinical setting. This refining should also include the development of volumetric quantitative analysis regarding adventitial VV and intraplaque neovascularization with real-time 3D ultrasound systems.[8]

One methodological limitation of the currently published CEUS studies is their retrospective nature. A lack of prospective studies exists, and large prospective studies are required to analyze the prognostic value and natural course of adventitial VV and intraplaque neovascularization prior to establishing this imaging technique as a diagnostic tool for atherosclerotic disease. After resolving these two main drawbacks, international standardization of acquisition techniques and methods of image analysis are necessary to integrate CEUS of carotid atherosclerotic lesions into practice guidelines.[40]

Future Directions

By identifying early signs of atherosclerosis through CEUS detection of adventitial VV and intraplaque neovascularization, therapeutic approaches can be quickly tailored to the needs of patients with the aim of reducing cardio- and cerebrovascular events.[6] This imaging procedure may have a significant impact on the treatment of asymptomatic patients with carotid lesions and patients with a lower degree of carotid stenosis who suffer from plaque rupture with consecutive embolism and stroke due to unknown mechanisms.[9] Among these groups, CEUS may identify a subgroup at risk for atherosclerotic complications that may benefit from interventions.[40]

Future applications of CEUS in the carotid artery may include monitoring the success of specific therapies aimed at remodeling the carotid plaque and follow-up investigations regarding the atherosclerotic course. In one study, for example, the adventitial VV in the carotid artery of a diabetic patient showed regression after 8 months of statin therapy.[6] However, further investigations are urgently needed to assess the role of CEUS in monitoring therapy for carotid atherosclerotic lesions.

Conclusion

CEUS imaging of the carotid atherosclerotic lesion is a novel, evolving, noninvasive clinical tool to directly visualize microvessel networks within the arterial wall (adventitial VV) and within carotid plaques (intraplaque neovascularization). This imaging approach has the potential to identify those patients at risk for developing symptomatic atherosclerotic diseases and their associated complications, including localized complications such as transient ischemic attack and stroke as well as generalized complications such as coronary artery disease and peripheral arterial disease. Hence, this technique can provide valuable information in clinical settings. In the future, CEUS of carotid atherosclerotic lesions should improve risk stratification of the disease and facilitate treatment decisions.

References

1. Feinstein SB. The powerful microbubble: from bench to bedside, from intravascular indicator to therapeutic delivery system, and beyond. Am J Physiol Heart Circ Physiol. 2004 Aug;287(2):H450-57.
2. Naghavi M, Libby P, Falk E, Casscells SW, Litovsky S, Rumberger J, et al. From vulnerable plaque to vulnerable patient: a call for new definitions and risk assessment strategies: Part I. Circulation. 2003 Oct 14;108(14):1664-72.
3. Naghavi M, Falk E, Hecht HS, Jamieson MJ, Kaul S, Berman D, et al. From vulnerable plaque to vulnerable patient--Part III: Executive summary of the Screening for Heart Attack Prevention and Education (SHAPE) Task Force report. Am J Cardiol. 2006 Jul 17;98(2A):2H-15H.
4. Nambi V, Chambless L, Folsom AR, He M, Hu Y, Mosley T, et al. Carotid intima-media thickness and presence or absence of plaque improves prediction of coronary heart disease risk: the ARIC (Atherosclerosis Risk In Communities) study. J Am Coll Cardiol. 2010 April 13;55(15):1600-07.
5. Granada JF, Feinstein SB. Imaging of the vasa vasorum. Nat Clin Pract Cardiovasc Med. 2008 Aug;5 Suppl 2:S18-25.
6. Feinstein SB. Contrast ultrasound imaging of the carotid artery vasa vasorum and atherosclerotic plaque neovascularization. J Am Coll Cardiol. 2006 Jul 18;48(2):236-43.
7. Wilson SR, Greenbaum LD, Goldberg BB. Contrast-enhanced ultrasound: what is the evidence and what are the obstacles? AJR Am J Roentgenol. 2009 Jul;193(1):55-60.
8. Feinstein SB, Coll B, Staub D, Adam D, Schinkel AF, ten Cate FJ, et al. Contrast enhanced ultrasound imaging. J Nucl Cardiol. 2010 Jan-Feb;17(1):106-15.
9. Vicenzini E, Giannoni MF, Benedetti-Valentini F, Lenzi GL. Imaging of carotid plaque angiogenesis. Cerebrovasc Dis. 2009;27 Suppl 2:48-54.
10. Sluimer JC, Daemen MJ. Novel concepts in atherogenesis: angiogenesis and hypoxia in atherosclerosis. J Pathol. 2009 May;218(1):7-29.
11. Carlier S, Kakadiaris IA, Dib N, Vavuranakis M, O’Malley SM, Gul K, et al. Vasa vasorum imaging: a new window to the clinical detection of vulnerable atherosclerotic plaques. Curr Atheroscler Rep. 2005 Mar;7(2):164-9.
12. Dunmore BJ, McCarthy MJ, Naylor AR, Brindle NP. Carotid plaque instability and ischemic symptoms are linked to immaturity of microvessels within plaques. J Vasc Surg. 2007 Jan;45(1):155-9.
13. Moreno PR, Purushothaman KR, Sirol M, Levy AP, Fuster V. Neovascularization in human atherosclerosis. Circulation. 2006 May 9;113(18):2245-52.

14. Fleiner M, Kummer M, Mirlacher M, Sauter G, Cathomas G, Krapf R, et al. Arterial neovascularization and inflammation in vulnerable patients: early and late signs of symptomatic atherosclerosis. Circulation. 2004 Nov 2;110(18):2843-50.
15. Kolodgie FD, Gold HK, Burke AP, Fowler DR, Kruth HS, Weber DK, et al. Intraplaque hemorrhage and progression of coronary atheroma. N Engl J Med. 2003 Dec 11;349(24):2316-25.
16. Staub D, Schinkel AF, Coll B, Coli S, van der Steen AF, Reed JD, et al. Contrast-enhanced ultrasound imaging of the vasa vasorum: from early atherosclerosis to the identification of unstable plaques. JACC Cardiovasc Imaging. 2010 Jul;3(7): 761-71.
17. de Boer OJ, van der Wal AC, Teeling P, Becker AE. Leucocyte recruitment in rupture prone regions of lipid-rich plaques: a prominent role for neovascularization? Cardiovasc Res. 1999 Feb;41(2):443-9.
18. Moreno PR, Purushothaman KR, Fuster V, Echeverri D, Truszczynska H, Sharma SK, et al. Plaque neovascularization is increased in ruptured atherosclerotic lesions of human aorta: implications for plaque vulnerability. Circulation. 2004 Oct 5;110(14):2032-8. Epub 2004 Sep 27.
19. Hellings WE, Peeters W, Moll FL, Piers SR, van Setten J, Van der Spek PJ, et al. Composition of carotid atherosclerotic plaque is associated with cardiovascular outcome: a prognostic study. Circulation. 2010 May 4;121(17):1941-50. Epub 2010 Apr 19.
20. Fryer JA, Myers PC, Appleberg M. Carotid intraplaque hemorrhage: the significance of neovascularity. J Vasc Surg. 1987 Oct;6(4):341-9.
21. McCarthy MJ, Loftus IM, Thompson MM, Jones L, London NJ, Bell PR, et al. Angiogenesis and the atherosclerotic carotid plaque: an association between symptomatology and plaque morphology. J Vasc Surg. 1999 Aug;30(2):261-8.
22. Mofidi R, Crotty TB, McCarthy P, Sheehan SJ, Mehigan D, Keaveny TV. Association between plaque instability, angiogenesis and symptomatic carotid occlusive disease. Br J Surg. 2001 Jul;88(7):945-50.
23. Coli S, Magnoni M, Sangiorgi G, Marrocco-Trischitta MM, Melisurgo G, Mauriello A, et al. Contrast-enhanced ultrasound imaging of intraplaque neovascularization in carotid arteries: correlation with histology and plaque echogenicity. J Am Coll Cardiol. 2008 Jul 15;52(3):223-30.
24. Magnoni M, Coli S, Marrocco-Trischitta MM, Melisurgo G, De Dominicis D, Cianflone D, et al. Contrast-enhanced ultrasound imaging of periadventitial vasa vasorum in human carotid arteries. Eur J Echocardiogr. 2009 Mar;10(2):260-4. Epub 2008 Aug 29.
25. Shah F, Balan P, Weinberg M, Reddy V, Neems R, Feinstein M, et al. Contrast-enhanced ultrasound imaging of atherosclerotic carotid plaque neovascularization: a new surrogate marker of atherosclerosis? Vasc Med. 2007 Nov;12(4):291-7.
26. Staub D, Partovi S, Schinkel AF, Coll B, Uthoff H, Aschwanden M, et al. Correlation of carotid artery atherosclerotic lesion echogenicity and severity at standard US with intraplaque neovascularization detected at contrast-enhanced US. Radiology. 2011 Feb;258(2):618-26. Epub 2010 Oct 22.
27. Staub D, Patel MB, Tibrewala A, Ludden D, Johnson M, Espinosa P, et al. Vasa vasorum and plaque neovascularization on contrast-enhanced carotid ultrasound imaging correlates with cardiovascular disease and past cardiovascular events. Stroke. 2010 Jan;41(1):41-7. Epub 2009 Nov 12.
28. Vicenzini E, Giannoni MF, Puccinelli F, Ricciardi MC, Altieri M, Di Piero V, et al. Detection of carotid adventitial vasa vasorum and plaque vascularization with ultrasound cadence contrast pulse sequencing technique and echo-contrast agent. Stroke. 2007 Oct;38(10):2841-3. Epub 2007 Aug 30.
29. Rothwell PM, Eliasziw M, Gutnikov SA, Fox AJ, Taylor DW, Mayberg MR, et al. Analysis of pooled data from the randomised controlled trials of endarterectomy for symptomatic carotid stenosis. Lancet. 2003 Jan 11;361(9352):107-16.
30. Giannoni MF, Vicenzini E, Citone M, Ricciardi MC, Irace L, Laurito A, et al. Contrast carotid ultrasound for the detection of unstable plaques with neoangiogenesis: a pilot study. Eur J Vasc Endovasc Surg. 2009 Jun;37(6):722-7. Epub 2009 Mar 27.
31. Hoogi A, Adam D, Hoffman A, Kerner H, Reisner S, Gaitini D. Carotid plaque vulnerability: quantification of neovascularization on contrast-enhanced ultrasound with histopathologic correlation. AJR Am J Roentgenol. 2011 Feb;196(2):431-6.
32. Gronholdt ML, Nordestgaard BG, Bentzon J, Wiebe BM, Zhou J, Falk E, et al. Macrophages are associated with lipid-rich carotid artery plaques, echolucency on B-mode imaging, and elevated plasma lipid levels. J Vasc Surg. 2002 Jan;35(1): 137-45.
33. Gronholdt ML, Nordestgaard BG, Schroeder TV, Vorstrup S, Sillesen H. Ultrasonic echolucent carotid plaques predict future strokes. Circulation. 2001 Jul 3;104(1):68-73.
34. el-Barghouty N, Nicolaides A, Bahal V, Geroulakos G, Androulakis A. The identification of the high risk carotid plaque. Eur J Vasc Endovasc Surg. 1996 May;11(4):470-8.
35. Honda O, Sugiyama S, Kugiyama K, Fukushima H, Nakamura S, Koide S, et al. Echolucent carotid plaques predict future coronary events in patients with coronary artery disease. J Am Coll Cardiol. 2004 Apr 7;43(7):1177-84.
36. Mathiesen EB, Bønaa KH, Joakimsen O. Echolucent plaques are associated with high risk of ischemic cerebrovascular events in carotid stenosis: the tromsø study. Circulation. 2001 May 1;103(17):2171-5.
37. Polak JF, Shemanski L, O'Leary DH, Lefkowitz D, Price TR, Savage PJ, et al. Hypoechoic plaque at US of the carotid artery: an independent risk factor for incident stroke in adults aged 65 years or older. Cardiovascular Health Study. Radiology. 1998 Sep;208(3):649-54.
38. Xiong L, Deng YB, Zhu Y, Liu YN, Bi XJ. Correlation of carotid plaque neovascularization detected by using contrast-enhanced US with clinical symptoms. Radiology. 2009 May;251(2):583-9. Epub 2009 Mar 20.
39. Papaioannou TG, Vavuranakis M, Androulakis A, Lazaros G, Kakadiaris I, Vlaseros I, et al. In-vivo imaging of carotid plaque neoangiogenesis with contrast-enhanced harmonic ultrasound. Int J Cardiol. 2009 May 29;134(3):e110-2. Epub 2008 May 20.
40. Shalhoub J, Owen DR, Gauthier T, Monaco C, Leen EL, Davies AH. The use of contrast enhanced ultrasound in carotid arterial disease. Eur J Vasc Endovasc Surg. 2010 Apr;39(4):381-7. Epub 2010 Jan 8.