Assessment of subclinical atherosclerosis using contrast-enhanced ultrasound

Stijn C.H. van den Oord1,2, Gerrit L. ten Kate3, Zeynettin Akkus2, Guillaume Renaud2, Eric J.G. Sijbrands3, Folkert J. ten Cate1, Aad van der Lugt4, Johan G. Bosch2, Nico de Jong2, Antonius F.W. van der Steen2, and Arend F.L. Schinkel1,3*

1Department of Cardiology, Thoraxcenter Room Ba 304, Erasmus Medical Center, 's-Gravendijkwal 230, 3015 CE Rotterdam, The Netherlands; 2Department of Biomedical Engineering, Thoraxcenter, Erasmus Medical Center, Rotterdam, The Netherlands; 3Division of Pharmacology, Vascular and Metabolic Diseases, Department of Internal Medicine, Erasmus Medical Center, Rotterdam, The Netherlands; and 4Department of Radiology, Erasmus Medical Center, Rotterdam, The Netherlands

Received 14 March 2012; accepted after revision 2 May 2012; online publish-ahead-of-print 29 May 2012

Aims
The sensitivity of standard carotid ultrasound and colour Doppler for the detection of subclinical atherosclerotic plaques is suboptimal. The aim of this study is to assess whether contrast-enhanced ultrasound (CEUS) added to standard carotid ultrasound improves the detection of subclinical atherosclerosis.

Methods and results
Carotid intima–media thickness (CIMT) measurement, standard carotid ultrasound including colour Doppler imaging, and CEUS were performed in 100 asymptomatic patients with one or more risk factors for atherosclerosis. CEUS was performed using intravenous administration of SonoVue™ contrast agent (Bracco S.p.A., Milan, Italy). CIMT, standard ultrasound, colour Doppler, and CEUS were reviewed by two independent observers. Standard ultrasound, colour Doppler, and CEUS were scored for the presence of atherosclerotic plaques. Subclinical atherosclerosis was diagnosed if patients had a CIMT above their age-corrected threshold value or if atherosclerotic plaques were present on standard carotid ultrasound clips or CEUS clips. McNemar’s test was performed to compare between groups. Twenty-one patients (21%) had a thickened CIMT value and were considered to have subclinical atherosclerosis. Standard carotid ultrasound including colour Doppler demonstrated atherosclerotic plaques in 77 patients (77%). The addition of CEUS to the standard ultrasound protocol demonstrated atherosclerotic plaques in 88 patients (88%). The incorporation of CEUS into the standard carotid ultrasound protocol resulted in a significantly improved detection of patients with subclinical atherosclerosis (P < 0.01).

Conclusion
CEUS has an incremental value for the detection of subclinical atherosclerosis in the carotid arteries. Atherosclerotic plaques which were only detected with CEUS and not with standard carotid ultrasound and colour Doppler imaging were predominantly hypoechoic.

Keywords
Atherosclerosis • Carotid ultrasound • Contrast-enhanced ultrasound • Intima–media thickness

Introduction
Atherosclerosis is the leading cause of morbidity and mortality in western countries.¹ It is by far the most frequent underlying cause of coronary artery disease, carotid artery disease, and peripheral arterial disease. Generally, the development of atherosclerosis starts at young age and progresses asymptptomatically through adult life. The progression of atherosclerosis may result in atherosclerotic plaques prone to rupture. The rupture of these atherosclerotic plaques may cause acute thrombosis leading to clinical events.² The detection of atherosclerosis before it leads to clinical events may improve risk-reducing strategies.

Carotid ultrasound is a widely available and relatively inexpensive technique to detect subclinical atherosclerosis. Carotid intima–media thickness (CIMT), as measured by B-mode ultrasound, has frequently been used as a surrogate marker for atherosclerosis.³,⁴ CIMT measurement is accurate, reproducible, and significantly related with clinical outcome. Still, there are indications that a systematic examination of the extracranial carotid arteries, including screening for the presence of plaques, may...
increase the sensitivity of ultrasound for identifying subclinical atherosclerosis. A recent meta-analysis demonstrated that the presence of plaques is a better predictor of clinical events when compared with CIMT measurement. Nevertheless, the sensitivity of standard carotid ultrasound of the carotid arteries to detect atherosclerotic plaques is suboptimal.

Contrast-enhanced ultrasound (CEUS) provides a better delineation of the carotid arterial lumen than standard carotid ultrasound. However, the incremental value of CEUS for the detection of subclinical atherosclerosis in the carotid arteries has not been tested. The purpose of the present study is to assess whether CEUS added to standard carotid ultrasound improves the detection of subclinical atherosclerosis.

Methods

Patient population and study protocol

The study protocol was approved by the local Ethics Committee and all study participants provided informed consent prior to the ultrasound examination. Between April 2010 and February 2011, 100 consecutive patients (45 females) from the outpatient clinics of vascular medicine and cardiology were included in this prospective study. All patients had no known atherosclerosis but had an increased cardiovascular risk profile. Inclusion criteria were: one or more clinical risk factors for atherosclerosis and age ≥ 18 years. Risk factors were dyslipidaemia, hypertension, diabetes mellitus, smoking, and a familial history of cardiovascular disease. Exclusion criteria were contraindications for the use of ultrasound contrast agent, such as unstable angina, acute cardiac failure, acute endocarditis, known right-to-left shunts, and known allergy for microbubble contrast agents. Patient characteristics were registered, and a structured clinical interview and physical examination were performed. All patients underwent CIMT measurement and a standard carotid ultrasound examination in conjunction with CEUS.

Carotid ultrasound acquisition

Standard carotid ultrasound including colour Doppler, and CEUS were performed with a Philips IU-22 ultrasound system (Philips Medical Systems, Bothell, WA, USA), equipped with an L9-3 transducer. Image acquisition was performed by a trained sonographer technician using a standard scanning protocol according to the American Society of Echocardiography consensus statement. In short, both left and right carotid arteries were examined with the patient in a supine position with the head supported at a 45° angle turned to the contralateral side. The left and the right common carotid artery (CCA), carotid bifurcation, internal carotid artery (ICA), external carotid artery (ECA), and vertebral arteries were imaged by B-mode ultrasound, colour Doppler, and pulse-wave Doppler. All anatomical sites were examined from different angles of view. Gain and imaging depth were adjusted per patient to obtain optimal ultrasound images. Each side was extensively evaluated for the presence of carotid plaques (Figure 1A). The degree of stenosis of the CCA, ICA, and ECA was assessed according to the current guidelines on the basis of spectral Doppler velocities.

After standard carotid ultrasound, CEUS was performed using intravenous administration of SonoVue™ contrast agent (Bracco S.p.A., Milan, Italy). The contrast mode of the ultrasound system, using amplitude modulation and a mechanical index of 0.06–0.08, was used to optimize the CEUS examination. CEUS clips were recorded with a dual display mode for simultaneous standard B-mode ultrasound and CEUS view (Figure 1B). Before injection of the ultrasound contrast agent, the intravenous access was flushed with a 5.0 mL NaCl 0.9% solution bolus injection. The ultrasound contrast agent was injected in boluses of 0.5 mL. Each contrast agent bolus was followed by a saline flush using 2.0 mL NaCl 0.9% solution. After administration of contrast agent, high-quality contrast images could be obtained for ~1 min. Contrast administration was repeated when required up to a maximum total dose of 10.0 mL. Both carotid arteries were examined using a standard acquisition protocol. Still frames and cineclips were digitally stored for offline analysis.

Carotid ultrasound analysis

Standard ultrasound and CEUS were reviewed offline by two independent observers unaware of the clinical data. For each patient, the same analysis protocol was followed. First, CIMT was measured in the left CCA, followed by screening for the presence of atherosclerotic plaques in the left carotid artery using the standard ultrasound clips. This analysis was repeated for the right carotid artery. Finally, CEUS clips were analysed. First, the left carotid artery was screened for the presence of atherosclerotic plaques. This analysis was repeated for the right carotid artery. In accordance with previously published studies, the CIMT was measured in the far wall of the distal 1 cm of the CCA. Semi-automated CIMT measurement was performed using Qlab quantification software (Philips Healthcare, Best, The Netherlands). For each side, the CIMT measurement was performed three times on selected still frames on different R-peaks of the ECG signal. The mean value of three measurements was used in further statistical analysis. The threshold for a high CIMT value was calculated for each patient individually according to the formula reported by Jager and Staub (threshold value in mm = decade of life/10 + 0.2 mm). Patients were considered to have subclinical atherosclerosis if their mean CIMT was above this threshold value.

Carotid plaque screening was performed using the standard carotid ultrasound images and colour Doppler clips. Atherosclerotic plaque was defined as a focal structure encroaching into the lumen of at least 0.5 mm or 50% of the surrounding CIMT or demonstrates a thickness of >1.5 mm as measured from the media–adventitia interface to the intima–lumen interface. The presence of plaques was recorded for each side.

Subsequently, carotid plaque screening was performed using the CEUS images. In the CEUS images, the carotid lumen is enhanced by the presence of contrast agent, resulting in enhanced visualization of plaque and luminal morphology. Typically, the lumen appears hyperechoic, plaques and intima–media complex are hypoechoic, and the adventitial layer is hypechoic. The definition of atherosclerotic plaques was identical to that used for standard ultrasound. The presence of plaques was recorded for each side.

If a new atherosclerotic plaque was found on CEUS, both standard carotid ultrasound and CEUS clips were reviewed in order to investigate why this plaque had been initially missed on standard carotid ultrasound alone. A systematic analysis of the standard carotid ultrasound clips of the region of the missed plaque was performed using the dual-display mode with simultaneous standard carotid ultrasound and CEUS. First, the image quality of the region of interest was scored based on a three-point scale as good-, moderate-, or poor-quality depending on artefacts or ultrasound shadowing. Secondly, echolucency was determined in the region of the missed plaque using the Gray-Weale score as follows: 1, predominantly echoluent plaque; 2, substantially echoluent lesions with small areas of echogenicity; 3, predominantly echogenic lesions with small areas of echolucency; and 4, uniformly echogenic lesions.
Statistical analyses

Statistical analysis was performed using SPSS PASW software for Windows (Version 17.0.2, SPSS Inc., Chicago, IL, USA). Continuous data were expressed as mean ± standard deviation, and categorical variables were expressed as counts and percentages and/or median value. McNemar’s test was used to compare between groups. Intra- and inter-observer reproducibility for the assessment of CIMT and atherosclerotic plaque was calculated using Pearson’s correlation and \( \kappa \)-statistics. A value of \( P < 0.05 \) was considered statistically significant.

Results

Patient characteristics

Clinical characteristics of the 100 patients (mean age 56 ± 9 years, 45% females) are summarized in Table 1. A total of 27 patients had one clinical risk factor for atherosclerosis, 34 patients had two clinical risk factors, 33 patients had three clinical risk factors, and 6 patients had four clinical risk factors. The standard carotid ultrasound and CEUS examinations were performed without adverse reactions in all patients.

Analysis of carotid arteries

A total of 200 carotid arteries were evaluated. The mean CIMT was 0.69 ± 0.16 mm. The standard ultrasound examination, including colour Doppler, revealed atherosclerotic plaques in 129 carotid arteries (65%), and in the remaining 71 carotid arteries (36%), no atherosclerotic plaques were detected. There were no haemodynamically significant carotid stenoses detected. The addition of CEUS to the standard ultrasound protocol demonstrated atherosclerotic plaques in 155 carotid arteries (78%). In 26 carotid arteries (13%), standard ultrasound demonstrated no atherosclerosis, whereas CEUS demonstrated atherosclerotic...
plaques (Figures 1 and 2). In another 26 carotid arteries (13%), atherosclerotic plaques were detected on standard carotid ultrasound, and additional atherosclerotic plaques were detected using CEUS. The incorporation of CEUS into the standard carotid ultrasound protocol resulted in a significantly improved detection of subclinical atherosclerotic plaques \( (P < 0.001) \).

The atherosclerotic plaques which were only detected with CEUS and had been missed using the standard carotid ultrasound examination including colour Doppler were predominantly hypoechoic, with a median Gray-Weale score of 1. A total of 39 plaque regions (70%) had Gray-Weale score 1; 13 plaque regions (23%) had Gray-Weale score 2; 4 plaque regions (7%) had Gray-Weale score 3; and 0 plaque region (0%) had Gray-Weale score 4. The image quality of standard carotid ultrasound in the regions with plaques that were detected using CEUS but missed using standard carotid ultrasound only was good in 32 regions (57%), moderate in 17 regions (30%), and poor in 7 regions (13%).

### Analysis of patients
A total of 21 patients (21%) had a CIMT value above their age-corrected threshold and were considered to have subclinical atherosclerosis. Standard carotid ultrasound demonstrated atherosclerotic plaques in a total of 77 patients (77%). All 21 patients with a CIMT value above their age-corrected threshold had atherosclerotic plaques on standard carotid ultrasound. Using standard carotid ultrasound, no atherosclerotic plaques were detected in the remaining 23 patients (23%). The addition of CEUS to the standard ultrasound protocol demonstrated atherosclerotic plaques in 88 patients (88%). In 11 patients (11%), standard carotid ultrasound demonstrated no atherosclerosis, but CEUS revealed atherosclerotic plaques. In 43 patients (43%), standard carotid ultrasound demonstrated atherosclerotic plaques and additional atherosclerotic plaques were detected using CEUS. The incorporation of CEUS into the standard carotid ultrasound protocol resulted in a significantly improved detection of patients with subclinical atherosclerosis \( (P < 0.01; \text{Figure } 3) \).

### Reproducibility
Reproducibility of ultrasound-clip analysis was assessed using the complete ultrasound examinations of all 100 patients. The mean difference between inter-observer measurements of the CIMT was 0.02 ± 0.03 mm. The correlation coefficient for inter-observer measurements of the CIMT was good \( (r = 0.97) \). The \( \kappa \)-statistics for the inter-observer agreement of atherosclerotic plaque detection with standard carotid ultrasound was 0.68. For combined standard carotid ultrasound and CEUS, the \( \kappa \)-statistics for inter-observer agreement of atherosclerotic plaque detection was 0.65, which suggests a substantial agreement.

---

**Table 1** Clinical characteristics of the study population \( (N = 100) \)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Data</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>56 ± 9</td>
</tr>
<tr>
<td>Female gender</td>
<td>45 (45)</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>174 ± 10</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>83 ± 17</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27 ± 5</td>
</tr>
<tr>
<td>Cardiovascular risk factors</td>
<td></td>
</tr>
<tr>
<td>Diabetes</td>
<td>22 (22)</td>
</tr>
<tr>
<td>Dyslipidaemia</td>
<td>88 (88)</td>
</tr>
<tr>
<td>Familial history of CVD</td>
<td>51 (51)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>33 (33)</td>
</tr>
<tr>
<td>Smoker (current)</td>
<td>24 (24)</td>
</tr>
<tr>
<td>Smoker (former)</td>
<td>23 (23)</td>
</tr>
</tbody>
</table>

BMI, body mass index; CVD, cardiovascular disease. Data are presented as the numbers of patients (percentages) or as mean ± standard deviation.

---

**Figure 2** Detection of an atherosclerotic plaque using CEUS. Combined B-mode and CEUS still frame of a right carotid artery. An atherosclerotic plaque is visible on the contrast enhanced still frame. It is located in the bulbus and proximal ECA (white arrow).
imaging settings. Kono et al, CEUS enabled an improved depiction of wall abnormal-
Sirlin et al evaluation of subclinical atherosclerosis. predominantly hypoechoic. The combination of standard carotid
CEUS and not with standard ultrasound. These plaques were
of the patients, atherosclerotic plaques were only detected by
CEUS to the standard ultrasound protocol demonstrated
atherosclerotic plaques in 77% of the patients. The addition of
CEUS to the standard ultrasound protocol demonstrated
atherosclerotic plaques in 88% of the study population. In 11%
of the patients, atherosclerotic plaques were only detected by
CEUS and not with standard ultrasound. These plaques were
predominantly hypoechoic. The combination of standard carotid
ultrasound was combined with CEUS, significantly more patients
with subclinical atherosclerosis were detected than with standard
carotid ultrasound only.

Discussion

The main finding of the current study is that CEUS has incremental
value for the detection of subclinical atherosclerosis. According to
a previously suggested age-specific threshold formula for CIMT,12
21% of the patients had subclinical atherosclerosis. Standard
carotid ultrasound including colour Doppler images demonstrated
atherosclerotic plaques in 77% of the patients. The addition of
CEUS to the standard ultrasound protocol demonstrated
atherosclerotic plaques in 88% of the study population. In 11%
of the patients, atherosclerotic plaques were only detected by
CEUS and not with standard ultrasound. These plaques were
predominantly hypoechoic. The combination of standard carotid
ultrasound and CEUS may thus be the preferred strategy for
evaluation of subclinical atherosclerosis.

These results are in line with previous experimental studies.
Sirlin et al.8 reported that CEUS provides more accurate delin-
eation of the arterial lumen than standard ultrasound. In an
ex vivo human carotid artery model and an in vivo rabbit aorta
model, CEUS enabled an improved depiction of wall abnormal-
ities, while this study was performed without contrast-specific
imaging settings. Kono et al.7 demonstrated that phase-inversion
harmonic imaging further increases the sensitivity of ultrasound
to contrast agent, leading to an improved visualization of the
vascular lumen and tissues. To the best of our knowledge, the
current study is the first to demonstrate that CEUS has an add-
tional value for the detection of subclinical atherosclerosis.

The atherosclerotic plaques that were only detected using
CEUS and not with the standard carotid ultrasound examination
were predominantly hypoechoic. A number of studies have
demonstrated that hypoechoic plaques contain a large lipid pool,
are related with intraplaque haemorrhage, and an increased inci-
dence of cardiovascular events.15–17 Large lipid pool and intrapla-
que haemorrhage are histopathological features of the vulnerable
atherosclerotic plaque, which is at risk to rupture and may cause
cardiovascular events.2 The hypoechoic atherosclerotic plaques
detected with CEUS may be of the vulnerable plaque type. A
follow-up study is required to investigate whether the newly
found hypoechoic atherosclerotic plaques are associated with an
increased incidence of cardiovascular events.

The present study demonstrates that CEUS provides an
incremental value for the detection of subclinical atherosclerosis,
irrespective of the image quality of the standard carotid ultrasound
images. The image quality of standard carotid ultrasound in the
regions with plaques that were detected by CEUS but missed
using standard ultrasound was good in 57% and impaired
(moderate-or-poor image quality) in 43%.

The current findings may have implications for cardiovascular
risk assessment. Carotid ultrasound has been extensively used
for the non-invasive detection of subclinical atherosclerosis.
Large prospective studies have demonstrated that an increased
CIMT is associated with clinical events, including myocardial
infarction and stroke.18–20 CIMT measurement is an excellent
research tool and numerous clinical trials have studied the
effect of medical therapy on CIMT instead of hard cardiovascular
endpoints. Still, an increased CIMT is associated with but not ne-
necessarily synonymous with atherosclerosis. Moreover, individual
risk prediction using CIMT is challenging because well-established
threshold values for CIMT do not exist. Carotid plaque screening
has therefore been suggested as a better surrogate outcome for
coronary artery disease.21 Systematic examination of the extra-
cranial carotid arteries, including screening for the presence of
plaques, may increase the sensitivity of ultrasound for identifying
subclinical atherosclerosis and improve cardiovascular risk strati-
fication.5,22,23 A recent meta-analysis demonstrated that carotid
ultrasound screening for the presence of plaques is a better pre-
dictor of hard cardiovascular events when compared with CIMT
measurement.5

A limitation of this study is that well-established threshold
values for CIMT do not exist. The formula used to calculate
the threshold for abnormal CIMT only adjusts for age.12 Previous
studies have demonstrated that multiple factors including gender
and ethnicity may influence CIMT. Therefore, in this study, CIMT
measurement may have over- or underestimated the presence of
subclinical atherosclerosis. Furthermore, the predictive value of
the newly detected atherosclerotic plaques for cardiovascular
risk assessment has not been investigated yet. Follow-up
studies are required to determine the additional predictive
value of the absence or presence of subclinical atherosclerosis
detected with CEUS.

In conclusion, CEUS has an additional value for the detection
of subclinical atherosclerosis in the carotid arteries. The athero-
sclerotic plaques that were detected by CEUS but missed using
standard carotid ultrasound including colour Doppler were pre-
dominantly hypoechoic. These results suggest that the combina-
tion of standard carotid ultrasound and CEUS may thus be
the preferred strategy for the evaluation of subclinical
atherosclerosis.
Acknowledgements
The authors thank Lourus van Haveren and Wim Vletter for their valuable contributions to the study.

Conflict of interest: none declared.

Funding
This research was supported by the Center for Translational Molecular Medicine and the Netherlands Heart Foundation (PARISK). There are no relationships with industry.

References