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RISK MARKER VARIABILITY IN SUBCLINICAL CAROTID PLAQUES BASED ON ULTRASOUND IS INFLUENCED BY CARDIAC PHASE, ECHOGENICITY AND SIZE

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Abstract—Identification of risk markers based on quantitative ultrasound texture analysis of carotid plaques has the ability to define vulnerable components that correlate with increased cardiovascular risk. However, data describing factors with the potential to influence the measurement variability of risk markers are limited. The aim of this study was to evaluate the influence of electrocardiogram-guided image selection, plaque echogenicity and area on carotid plaque risk markers and their variability in asymptomatic carotid plaques. Plaque risk markers were measured in 57 plaques during three consecutive heartbeats at two cardiac cycle time instants corresponding to the electrocardiogram R-wave (end diastole) and end of T-wave (end systole), resulting in six measurements for each plaque. Risk marker variability was quantified by computing the coefficient of variation (CV) across the three heartbeats. The CV was significantly higher for small plaques (area <15 mm², 10%) than for large plaques (area >15 mm², 6%) (p < 0.001) in measurements of area, and the CV for measurements of gray-scale median were higher for echolucent plaques (<40, 15%) than for echogenic plaques (>40, 9%) (p < 0.001). No significant differences were found between systole and diastole for the mean of any risk marker or the corresponding CV value. However, in a sub-analysis, the echolucent plaques were found to have a higher CV during systole compared with diastole. The variability also caused plaque type reclassification in 16% to 25% of the plaques depending on cutoff value. The results of this study indicate that echolucent and small plaques each contribute to increased risk marker variability. Based on these results, we recommend that measurements in diastole are preferred to reduce variation, although we found that it may not be possible to characterize small plaques accurately using contemporary applied risk markers. (E-mail: emma.nyman@umu.se) © 2018 The Author(s). Published by Elsevier Inc. on behalf of World Federation for Ultrasound in Medicine & Biology. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Key Words: Carotid plaque, Risk markers, Cardiac cycle, Echogenicity, Size, Classification.

INTRODUCTION

The majority of individuals with cardiovascular disease (CVD) are estimated to be at low to intermediate risk on the basis of traditional risk factors such as family history, hypertension, hypercholesterolemia and smoking; and for many, sudden death can be the first manifestation of the disease (Conroy et al. 2003; Dahlof 2010). Stroke is one of the leading causes of death worldwide, and around 20% of all ischemic strokes are caused by atherosclerosis in the carotid artery (Kolominsky-Rabas et al. 2001; Mozaffarian et al. 2015). Thickening of, or fatty streaks in, the carotid artery wall can start during youth, and asymptomatic plaque formation may develop over many decades. Carotid B-mode ultrasound has been reported to have potential value for detection of carotid plaques and risk stratification (Inaba et al. 2012; Lorenz et al. 2007; Naqvi and Lee 2014; Naqvi et al. 2010; Spence 2006; Stein et al. 2008).

Carotid plaque risk markers based on quantitative ultrasound texture analysis have been found able to identify vulnerable plaque components (Mathiesen et al. 2001; Nicolaides et al. 2010; Topkian et al. 2011). Rupture-prone or vulnerable plaques are defined histologically by a lipid-rich necrotic core with a thin fibrotic cap, intraplaque hemorrhage and high degree of inflammatory components (Mughal et al. 2011; Redgrave et al. 2006). The ultrasound gray-scale median (GSM) is an...
ultrasound-based risk marker that measures plaque echogenicity; lipids and hemorrhage appear echolucent, whereas stable components, such as calcification and fibrous tissue, appear echogenic (Christodoulou et al. 2003; Doonan et al. 2016; Johri et al. 2017; Mitchell et al. 2017). Population-based studies have indicated that homogeneous, echolucent plaques and large plaque areas correlate with increased risk for cerebrovascular events (Mathiesen et al. 2001, 2011). In the literature, a vulnerable plaque is described as having a GSM range from 25 to 40 (Biasi et al. 2004; Christodoulou et al. 2003; Kyriacou et al. 2010; Ruiz-Ares et al. 2014). However, no consensus on carotid plaque risk markers has been published, and the method has not been adopted clinically.

Earlier results indicated that the quantification of plaque echogenicity lacks sensitivity and specificity for risk prediction (Christodoulou et al. 2003), and this, in combination with poor reproducibility, emphasizes the importance of methodological improvements (Fosse et al. 2006; Kyriacou et al. 2010; Ostling et al. 2013). Because of the pressure changes during the cardiac cycle, dilation and reduction cause natural oscillations of artery diameter, which in turn affect the plaque measurements. Although some work has been done to further understand how these oscillations influence risk marker estimation, it has not yet been determined whether the measurements should be electrocardiogram (ECG) time point guided (Kanber et al. 2013; Soulis et al. 2016). Plaques develop from small to large and may change their composition with the progress of atherosclerotic disease, meaning that area and echogenicity are important risk markers. There is limited information on how area influences risk marker variation, and to our knowledge no study has evaluated the impact of echogenicity on risk marker estimation variation.

The aim of this study was to evaluate the influence of ECG-guided image selection, plaque echogenicity and size on risk markers in participants with sub-clinical carotid plaques. Furthermore, we assessed the potential clinical relevance of our findings by the impact of this variability on previously suggested vulnerability classifications.

**METHODS**

**Patients**

We retrospectively selected patients from the population-based cohort study VIPVIZA (Visualization of Asymptomatic Atherosclerotic Disease for Optimum Cardiovascular Prevention—a randomized controlled study within the Västerbotten Intervention Programme [ClinicalTrials.gov Identifier: NCT01849575], recruiting 40-, 50- and 60-year-olds). Participants selected for the study described here included those referred for an expanded ultrasound examination during the years 2013–2016 in accordance with the VIPVIZA study protocol (Fig. 1) who were found to have subclinical atherosclerosis and plaques visible on ultrasound images.

Written informed consent was obtained from the participants, and the regional ethical review board at Umeå University approved the study (DNR 2011-445-31 M, 2012-463-32 M, 2013-373-32 M).

**Carotid ultrasound examination and image selection**

Ultrasound examinations were performed at the Department of Clinical Physiology, Heart Centre, Umeå University Hospital, Umeå, Sweden. The examination consisted of bilateral carotid artery scanning using a Philips iU22 (Andover, MA, USA) ultrasound machine with a linear 9- to 3-MHz probe. All examinations were performed by experienced vascular ultrasonographers using standard clinical settings to optimize B-mode image quality with an examination frame rate standard of 28–33 Hz and harmonic imaging. Color Doppler images were also acquired to assist initial plaque screening. A carotid plaque was defined by Mannheim consensus as a focal structure that encroaches into the lumen by at least 0.5 mm or 50% of the surrounding intima media thickness value or that has a thickness >1.5 mm as measured from the media–adventitia interface to the intima–lumen interface (Touboul et al. 2012). Participants with B-mode cine loops of plaques that presented distinctly in longitudinal view, with attached three-lead ECG and without external motions and shadows, were selected for analysis. Selected cine loops were exported in DICOM format for offline analysis of plaque risk markers. All offline analyses were conducted by a single investigator (E.N.).
Risk marker calculation

The principle underlying plaque detection and image analysis is illustrated in Figure 2a-e. Plaque risk markers were calculated using a plaque texture analysis software (PLAQ, Department of Biomedical Engineering R&D, Västerbotten County Council, Umeå, Sweden). The software has been described and used in earlier publications by this research group (Ibrahimi et al. 2014, 2015). The analysis consisted of the following steps: Image normalization was carried out by linear scaling because of subjective selection of the darkest region in the lumen, where the regions of interest (ROIs) were manually outlined and the darkest pixels within that area rescaled to 0 (Fig. 2c). The same procedure was carried out for the subjectively selected brightest region of the adventitia, which was mapped to 190 (Fig. 2c). Plaques were manually outlined (Fig. 2e). If multiple plaques were present (near and far wall of the artery or in a different location, including common, external or internal carotid artery and the bifurcation), the plaques were outlined individually in a separate analysis. The risk markers were measured at the end of systole and diastole for three consecutive heartbeats. The end-diastolic time was defined by the R-wave on ECG, and end systole, by the end of the T-wave (Fig. 2b).

The risk markers chosen for variability analysis were measurements of echogenicity, heterogeneity, size and plaque type. Echogenicity was measured by the GSM, calculated as the median of all pixel values in the plaque region after image normalization (Nicolaides et al. 2010). Plaque heterogeneity was measured by the coarseness variable, calculated using the neighborhood gray-tone difference matrix, where low values are associated with heterogeneous composition (Amadasun and King 1989; Christodoulou et al. 2003). Plaque type was determined by modified Geroulakos plaque-type classifications 1–4 (Kanber et al. 2013; Nicolaides et al. 2010), which were automatically generated by the software, based on modified Geroulakos classification (Geroulakos et al. 1993): type 1 = uniformly echolucent, <15% of pixels in the plaque area with GSM values >25; type 2 = mainly echolucent, 15%–50% of pixels in the plaque with GSM values >25;
type 3 = mainly echogenic, 50%–85% of pixels in the plaque with GSM values >25; and type 4 = uniformly echogenic, >85% of the pixels in the plaque with GSM values >25.

In addition, an un-normalized GSM was calculated to analyze echogenicity change in plaque without the influence of the normalization procedure and echogenicity changes in blood or adventitia (Kanber et al. 2013).

**Statistics**

Statistical analyses were carried out using SPSS Statistics Version 22 (IBM, Armonk, NY, USA).

Variability was quantified for each risk marker using the coefficient of variation (CV), calculated as standard deviations divided by the sample mean. Risk marker variability was computed from the CV for the six measurements on each plaque, three in systole and three in diastole. In comparisons of cardiac phases, CV was calculated from the three measurements in systole and diastole, respectively.

The effects of plaque size and echogenicity were assessed using dichotomized groups, divided at the median based on area and GSM, respectively. The paired parametric t-test was used to investigate whether the mean of the risk markers and the CV differed significantly between the systolic and diastolic cardiac phases. An unpaired, non-parametric Mann–Whitney U-test was used to assess whether the CV of the risk markers differed between the dichotomized groups. The effect on vulnerability reclassification was assessed by counting the number of plaques that progressed from plaque type ≤2 to ≥3 or crossed the threshold levels mentioned in the literature during the three consecutive heartbeats analyzed for each plaque. Two cutoff values for GSM values <32 and <24 were chosen for analysis of the effect of variations on reclassification based on the range (Christodoulou et al. 2003; El-Barghouty et al. 1995). For analysis of plaque type, the cutoff value was ≤2 (Langsfeld et al. 1989). Reclassification accuracy was estimated by the standard deviation of random sampling with replacement. The p value was set to 0.003 by Bonferroni correction (0.05/16) because of multiple comparisons.

**Inter- and intra-rater reliability**

Two operators assessed inter-rater reliability using 23 randomly selected plaque image sequences. Their analyses were carried out independently and included image normalization and plaque segmentation. Reliability was evaluated with the intra-class correlation coefficient (ICC) with a 95% confidence interval (CI).

Intra-rater reliability, defined by the ICC, was assessed by one operator using 30 randomly selected plaque image sequences, with a 6-mo period between the two analyses.

**RESULTS**

Characteristics of the 28 participants are summarized in Table 1. The risk markers from 57 carotid plaques selected from these participants are listed in Table 2.

Plaque area ranged from 4.3 to 54.0 mm², GSM from 5.9 to 99.3, un-normalized GSM from 7.2 to 129.8 and coarseness from 6.3 to 35.1. The variabilities for area, GSM, un-normalized GSM and coarseness were, on average, 8.2%, 11.4%, 10.5% and 9.2% respectively.

**Influence of cardiac phase**

Figure 3 illustrates an example of the beat-to-beat variation in the measured risk markers over three consecutive heartbeats of measurements made in the cardiac phases from one analyzed plaque.

No significant differences were found between systole and diastole for any risk markers’ mean or corresponding CV value (Table 2). However, there was a trend for un-normalized GSM and coarseness to be slightly higher in systole than in diastole (p = 0.025 and p = 0.034, respectively).

**Influence of echogenicity and size**

The coefficients of variation in measurements of area were significantly higher for small plaques (<15 mm², 10%) than for large plaques (>15 mm², 6%) (p =< 0.001), and measurements of GSM CVs were higher for echolucent plaques (GSM <40, 15%) than for echogenic plaques (GSM >40, 9%) (p =< 0.001). Similar results were found for un-normalized GSMs, although there was no significant difference for coarseness (Table 2).

**Effect on reclassification**

Figure 4 illustrates the GSM range in the cine loop for each plaque and the vulnerability classification cutoff values at 24 and 32. At the cutoff value of 32 (n = 9), 16% of the analyzed plaques were reclassified. At the cutoff value of 24 (n = 14), 25% were reclassified.

For plaque type, 19% (11/57) of the plaques changed plaque type during the six analyses made of the cine loop.

| Table 1. Baseline characteristics of the 28 participants |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Sex, female (%) | 16 (57%)        | Age             | 60 y | 25 (89%) | 50 y | 3 (11%) |
| Systolic blood pressure (mm Hg) | 131 (±14) | Diastolic blood pressure (mm Hg) | 78 (±10) |
| Low-density lipoprotein cholesterol (mmol/L) | 3.2 (±1.0) | Diabetes, yes | 2 (7 %) |
| Overweight (body mass index ≥25.0) | 21 (75%) | Family history of cardiovascular disease, yes | 11 (39%) |
| Current smoker, yes | 5 (18%) |

Data are expressed as the number (%) and mean ± standard deviation.
Table 2. Measurements of plaques: Overall results and comparison based on cardiac cycle time points end diastole and end systole, echogenicity and size

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Overall (n = 57)</th>
<th>Cardiac cycle time point (n = 57)</th>
<th>Echogenicity (GSM)</th>
<th>Size (area)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>General</td>
<td>Systole</td>
<td>Diastole</td>
<td>(n = 26)</td>
</tr>
<tr>
<td>Plaque area (mm&lt;sup&gt;2&lt;/sup&gt;)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>18.7</td>
<td>18.7</td>
<td>18.7</td>
<td>0.855</td>
</tr>
<tr>
<td>SD</td>
<td>±11.9</td>
<td>±11.9</td>
<td>±11.8</td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>4.3–54.0</td>
<td>4.7–53.8</td>
<td>3.8–54.3</td>
<td></td>
</tr>
<tr>
<td>CV</td>
<td>8.2</td>
<td>7.9</td>
<td>7.5</td>
<td>0.469</td>
</tr>
<tr>
<td>SD</td>
<td>±3.5</td>
<td>±4.9</td>
<td>±4.3</td>
<td></td>
</tr>
<tr>
<td>GSM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>45.3</td>
<td>45.6</td>
<td>44.9</td>
<td>0.226</td>
</tr>
<tr>
<td>SD</td>
<td>±22.0</td>
<td>±21.6</td>
<td>±22.6</td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>5.9–99.3</td>
<td>6.9–98.3</td>
<td>4.8–101.9</td>
<td></td>
</tr>
<tr>
<td>CV</td>
<td>11.4</td>
<td>11.0</td>
<td>9.4</td>
<td>0.119</td>
</tr>
<tr>
<td>SD</td>
<td>±7.5</td>
<td>±9.4</td>
<td>±7.9</td>
<td></td>
</tr>
<tr>
<td>Un-normalized GSM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>51.8</td>
<td>52.4</td>
<td>51.4</td>
<td>0.025</td>
</tr>
<tr>
<td>SD</td>
<td>±27.9</td>
<td>±27.4</td>
<td>±28.5</td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>7.2–129.8</td>
<td>8.2–127.3</td>
<td>6.3–132.4</td>
<td></td>
</tr>
<tr>
<td>CV</td>
<td>10.5</td>
<td>10.4</td>
<td>8.6</td>
<td>0.236</td>
</tr>
<tr>
<td>SD</td>
<td>±6.5</td>
<td>±8.1</td>
<td>±7.1</td>
<td></td>
</tr>
<tr>
<td>Coarseness</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>20.8</td>
<td>21.1</td>
<td>20.5</td>
<td>0.034</td>
</tr>
<tr>
<td>SD</td>
<td>±6.0</td>
<td>±6.3</td>
<td>±6.0</td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>6.3–35.1</td>
<td>6.6–35.6</td>
<td>5.4–34.5</td>
<td></td>
</tr>
<tr>
<td>CV</td>
<td>9.2</td>
<td>7.6</td>
<td>8.5</td>
<td>0.327</td>
</tr>
<tr>
<td>SD</td>
<td>±6.1</td>
<td>±6.4</td>
<td>±7.2</td>
<td></td>
</tr>
</tbody>
</table>

SD = standard deviation; GSM = gray-scale median; CV = coefficient of variation (σ/μ).
* Paired sample t-test.
† A p value < 0.005 was considered to indicate significance because of Bonferroni correction.
‡ Mann–Whitney U-test.

Inter- and intra-rater reliability

The intra-rater reliability ICCs for area, GSM and coarseness were 0.95 (95% CI: 0.89–0.97), 0.95 (95% CI: 0.90–0.98) and 0.87 (95% CI: 0.75–0.94), respectively. The inter-rater reliability ICCs for area, GSM and coarseness were 0.73 (95% CI: 0.47–0.88), 0.75 (95% CI: 0.50–0.89) and 0.86 (95% CI: 0.69–0.94), respectively.

DISCUSSION

We investigated carotid plaque risk marker measurements in asymptomatic patients with atherosclerotic carotid plaques. The variability in the measures was found to range from 8% to 11% and caused reclassification of plaque vulnerability category in approximately 20% of the plaques based on cutoff values reported in the literature.

Influence of cardiac phase

Cyclic variations of GSM and area within the cardiac cycle length were clear in the majority of measurements (example seen in Fig. 3); these cyclic variations have been described in earlier studies of risk markers (Kanber et al. 2013; Soulis et al. 2016).

A slight difference was found in un-normalized GSM and coarseness between end-systole and end-diastole. This finding can be explained by the increased pressure during the systolic phase, compressing the plaque or causing out-of-plane movement. Although a difference was seen only in un-normalized GSM and not in normalized GSM, this finding is likely caused by the principle of propagating errors occurring in imaging-based atherosclerotic plaque characterization. The errors are, in general, propagating with all processing steps during image formation and, in the present specific case, the plaque characterization and image normalization procedure (Athanasiou et al. 2015). Recent studies have indicated that texture features vary significantly between systole and diastole, with significantly higher GSM values reported in systole (Loizou et al. 2017; Soulis et al. 2016). However, these studies had longer sequences and excluded plaque types 1 and 4, which are likely to have generated more stable measurements.

Influence of size

Smaller plaques had a higher variability in plaque area, which contrasts with previous studies that failed to find any size-dependent associations (Kanber et al. 2013). This...
result may be explained by differences in the plaques used for analysis. In our study, analysis was based on subclinical atherosclerotic plaques with a mean area of 19 mm² (range: 4–54 mm²), as compared with the analysis carried out by Kanber et al. (2013), who used symptomatic plaques with a larger mean area of 30 mm² (range: 7–90 mm²). Our result indicates that plaque size affects the variability of carotid plaque risk markers, where variation decreased as plaque area increased (Fig. 5).

The increased variability may have been caused either by the greater sensitivity of small plaques to precise delineation or by small plaque out-of-plane motion. As the variability of coarseness also increased for the small plaques, this finding supports the hypothesis that out-of-plane movement is causing the changes in heterogeneity of the texture. In addition, coarseness was slightly lower in smaller plaques compared with large plaques, indicating that smaller plaques had a more heterogeneous composition.

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**Fig. 3.** Example of risk marker measurement for one carotid plaque during three consecutive heartbeats for (a) area, (b) gray-scale median, (c) coarseness and (d) plaque type classification. GSM = gray scale median; D = end diastole; S = end-systole.

**Fig. 4.** Illustration of reclassification based on maximum (○) and minimum (♦) values for measured gray-scale median on 57 carotid plaques. Reclassification is defined by a crossing cutoff value of 24 (solid line) (Christodoulou et al. 2003) or 32 (dotted line) (El-Barghouty et al. 1995). GSM = gray-scale median.

**Fig. 5.** How coefficients of variation for gray-scale median (▼), coarseness (○) and area (♦) are affected by plaque size.
In the present study, the degree of variability in small plaques suggested that they were less suitable for further risk marker analysis. Other research groups (Ostling et al. 2007) have considered only plaques >10 mm² as large enough for risk marker analysis, and this cutoff has been set empirically. Our results support that including plaques larger than a certain area would result in more stable estimates of the risk markers. However, if subclinical atherosclerosis were being studied (small plaques present), such exclusion criteria would cause a significant bias of the included plaques. Therefore, our results suggest that there is a need to find other plaque risk markers to characterize small plaques.

Influence of echogenicity

Hypo-echoic plaques had higher variability in GSM than echogenic plaques. This could be explained by difficulties in manual delineation of the echolucent plaques. Nevertheless, it was expected that this would have been accompanied by a higher variability in plaque area; however, our results indicated no such difference between the groups (Table 2). In addition, coarseness was lower for the hypo-echoic plaques, indicating that their composition was more heterogeneous. Thus, it appears that the variation in plaque echogenicity was real, and it was not the manual outlining of the plaque that caused the increased variability.

Echolucent plaques have been found to consist of more fatty components, which renders them more elastic and vulnerable to deformation caused by variations in blood pressure over the cardiac cycle, compared with echogenic plaques composed of fibrotic and calcified tissues (Mitchell et al. 2017; Shi et al. 2008). Such deformation or strain of the plaque would likely cause a change in the acoustic impedance, resulting in higher ultrasonic reflection during systole. To investigate if this effect could be observed, we carried out a sub-analysis of the difference between GSM at systole and GSM at diastole for the hypo-echoic plaques (GSM <40). The comparison revealed that echolucent plaques had both higher GSM (28.2 vs. 26.7, \( p = 0.043 \)) and higher variability during systole than during diastole, whereas the CV for echogenic plaques (GSM >40) was the same during systole and diastole (Fig. 6). Taken together, these results indicate that vulnerable plaques (low GSM) are more influenced by the cardiac cycle and have higher variability than stable plaques. In addition, the diastolic phase has less variability, suggesting that this phase should be selected for risk marker assessment.

Impact on vulnerability classification

The variability in GSM caused reclassification in 16% to 20% of plaques, depending on the cutoff value chosen. This variation is directly related to calculations of sensitivity and specificity in receiver operating characteristic analysis. Previous work has reported an area under the curve of 0.74 to discriminate asymptomatic from symptomatic plaques (Christodoulou et al. 2003). The reclassification results indicate concerns about the stability of measurements of the carotid plaque, which should be considered particularly in longitudinal studies.

Limitations

Ultrasound is operator dependent; complex anatomy and artifacts, such as plaque shadows, can complicate imaging in carotid examination. In our study, all plaques affected by shadows were excluded, which may have influenced selection and presented a potential bias. In the early phase of the study, the ECG attachment was not used consistently by all sonographers, resulting in relatively large exclusion because of the lack of an ECG (Fig. 1).

In addition, quantitative risk marker assessment of carotid plaques in ultrasound images is affected by the propagation of errors during the many steps included (Athanasiou et al. 2015). Analysis of the un-normalized GSM is a way to minimize the sources of error. Frame selection is also a potential source of error, and in this study we tried to analyze its impact by employing ECG guidance. In the present study, 2-D images were used, which enabled only one projection area of the plaque volume. A consequence of this is that the images are influenced by out-of-plane motion, which could be generated by the sonographer, the study subject or natural mechanical movements in the artery. The use of 3-D ultrasound and volume-based measurements may improve carotid plaque

![Fig. 6. Comparison of coefficients of variation between systole and diastole for echolucent and echogenic plaques. Bars represent 95% confidence intervals. \( p \) Values were obtained with the Wilcoxon signed rank test. CV = coefficient of variation; GSM = gray-scale median.](image)
visualization and risk marker analysis, as the whole plaque volume could be assessed (Kalashyan et al. 2014).

Manual region-of-interest selection may have influenced our results; however, measurements exhibited excellent intra-rater reliability (ICC = 0.95). This indicates that the manual steps in the method had a low impact on our results. Inter-rater reliability was lower than intra-rater reliability, but similar to inter-rater reliability reported on our results. Inter-rater reliability was lower than intra-

cates that the manual steps in the method had a low impact excellent intra-rater reliability (ICC

rater reliability of risk markers. The clinical relevance of this variability was indicated by a 16% to 25% rate of reclassification of vulnerability based on the cutoff value chosen. It also is new evidence that echoluent and small plaques each contribute to increased risk marker variability. Based on these results, we recommend that measurements under diastole are preferable to reduce variation and that small plaques may have to be ignored, as it may not be possible to characterize small plaques accurately with the current risk markers employed.

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REFERENCES


Dahllof B. Cardiovascular disease risk factors: Epidemiology and risk assessment. Am J Cardiol 2010;105:3A–9A.


