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Sample size calculation for clinical trials using magnetic resonance imaging for the quantitative assessment of carotid atherosclerosis

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Purpose. To provide sample size calculation for the quantitative assessment of carotid atherosclerotic plaque using non-invasive magnetic resonance imaging in multi-center clinical trials. *Methods.* As part of a broader double-blind randomized trial of an experimental pharmaceutical agent, 20 asymptomatic placebo-control subjects were recruited from 5 clinical sites for a multi-center study. Subjects had 4 scans in 13 weeks on GE 1.5 T scanners, using TOF, T1-/PD-/T2- and contrast-enhanced T1-weighted images. Measurement variability was assessed by comparing quantitative data from the index carotid artery over the four time points. The wall/outer wall (W/OW) ratio was calculated as wall volume divided by outer wall volume. The percent lipid-rich/necrotic core (%LR/NC) and calcification (%Ca) were measured as a proportion of the vessel wall. For %LR/NC and %Ca, only those subjects that exhibited LR/NC or Ca components were used in the analysis. *Results.* Measurement error was 5.8% for wall volume, 3.2% for W/OW ratio, 11.1% for %LR/NC volume and 18.6% for %Ca volume. Power analysis based on these values shows that a study with 14 participants in each group could detect a 5% change in W/OW ratio, 10% change in wall volume, and 20% change in %LR/NC volume (power = 80%, $p < .05$). The calculated measurement errors presume any true biological changes were negligible over the 3 months that subjects received placebo. *Conclusion.* In vivo MRI is capable of quantifying plaque volume and plaque composition, such as %lipid-rich/necrotic core and %calcification, in the clinical setting of a multi-center trial with low inter-scan variability. This study provides the basis for sample size calculation of future MRI trials.

Key Words: Carotid arteries; Atherosclerosis; Magnetic resonance imaging

1. Introduction

High-resolution MRI is a noninvasive technique that can identify morphological and compositional features of atherosclerotic plaque in the carotid arteries (1–5), the coronary arteries (6) and the aorta (7, 8). MRI permits highly accurate in vivo measurement of artery wall dimensions in human atherosclerotic plaques (9). MRI also has a unique ability to provide information on plaque composition comparable to that obtained by histology (10–13). Volumetric MRI measurements of the wall and the main plaque components, such as lipid-rich/necrotic core (LR/NC) and calcification (Ca) show good correlation for ex vivo (13) and in vivo (12) MRI scans. These capabilities underscore the potential use of MRI in longitudinal clinical trials that study changes in plaque composition and plaque volume simultaneously.

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Non-invasive methods for investigating changes in plaque composition over time have two specific applications. First, studying the impact of plaque composition on plaque progression and regression will give us new insights into the mechanisms and time course of atherosclerotic disease development and may help to identify plaque features that contribute to plaque stability or plaque vulnerability. Second, studying the effects of new and existing drugs on plaque composition might be a more sensitive way to demonstrate treatment effects than plaque volume measurements alone.

To establish MRI as a viable, alternative tool for use in clinical trials, low variability due to measurement error must be achieved. Furthermore, sample size calculation is needed to establish guidelines for the planning of future MRI trials. Although the variability of lumen and wall measurements from high-resolution MRI were previously reported (14), that study was conducted at a single institution. Furthermore, to the best of our knowledge, the variability for quantitative in vivo MRI measurements of plaque components in the clinical setting of a multi-center trial has not been reported.

The aim of this study was 1) to determine the variability of quantitative MRI measurements of carotid plaques in the setting of a multi-center clinical trial and 2) to provide sample size calculation based on the variability obtained for the planning of future MRI trials.

2. Material and methods

2.1. *Subjects*

As part of a broader double-blind randomized trial of an experimental pharmaceutical agent, 20 asymptomatic placebo-control subjects were recruited either in the cardiac catheter laboratory or in the ultrasound laboratory from 5 clinical sites for a multi-center study. They included 6 from Site 1, 8 from Site 2, 1 from Site 3, 1 from Site 4, and 4 from Site 5. Inclusion criteria by MRI were eccentric plaque formation in at least one carotid artery. Institutional review boards of each site approved the consent forms and study protocols. Only the measurements from this placebo group were used for statistical analysis of variability.

2.2. *MRI scanner and carotid coil*

MRI scans were performed with GE 1.5 T whole-body scanners (GE Healthcare, Milwaukee, USA) with different software and hardware configurations: EchoSpeed Signa 5.8 (Site 1); EchoSpeed Signa 9.0 (Site 2); Echospeed Signa 9.0 and CVi 8.4 (Site 3); EchoSpeed LX 9.1 (Site 4), Horizon Signa 9.0 and 9.1 (Site 5). A standardized, carotid coil apparatus (Pathway MRI Inc., Redmond, WA) was used at all sites. This apparatus includes two phased-array coils (15) for bilateral carotid scans with improved signal-to-noise performance and a head holder to keep subjects in a stable position.

2.3. *MRI protocol*

A standardized protocol (Table 1) was used to obtain five contrast-weightings of bilateral carotid arteries in the transverse plane: pre- and post-contrast enhanced (CE) T1weighted (T1W), proton-density-weighted (PDW), T2-weighted (T2W), 3-dimensional time-of-flight (3D-TOF) MR angiography. CE-T1W was performed approximately 5 minutes after the intravenous injection of 0.1 mmol/kg (0.2 mL/kg) Gadolinium-DTPA-BMA (Omniscan, GE Healthcare, Milwaukee, USA) at a rate of 2 mL/sec. Fat suppression was used for pre- and post-CE-T1W, PDW, and T2W images to reduce signals from subcutaneous fat. Each scan covered 24 mm (2 mm thickness × 12 matched images among 5 weightings).

Table 1. MRI parameters used in protocol

	T1W (Pre- and post CE)	PDW	T2W	3D-TOF
Technique	2D-FSE, QIR	2D-FSE, MDIR	2D-FSE, MDIR	3D-GRE
TR (ms)	800	2400	3000	23
Effective TE (ms)	11	8.9	50	3
ETL	10	12	12	—
TI (ms)	500	260	230	—
NEX	2	2	2	2
FOV (mm)	160 × 120	160 × 120	160 × 120	160 × 120
Matrix	256 × 256	256 × 256	256 × 256	256 × 256
Slices	12	16	12	56
Slice thickness (mm)	2	2	2	2
Interslice spacing (mm)	0	0	0	−1
Coverage (mm)	24	32	24	56
Scan time (min)	8	3	4	3

T1W = T1-weighted; CE = contrast enhanced; PDW = proton-density-weighted; T2W = T2-weighted; 3D-TOF = Three-dimensional time-of-flight; FSE = fast spin echo; GRE = gradient recalled echo; QIR = quadruple inversion-recovery; MDIR = multi-slice double inversion-recovery; TR = repetition time; TE = echo time; ETL = echo train length; TI = inversion time; NEX = number of excitations; FOV = field-of-view; − 1 = 1 mm overlapping between adjacent slices.

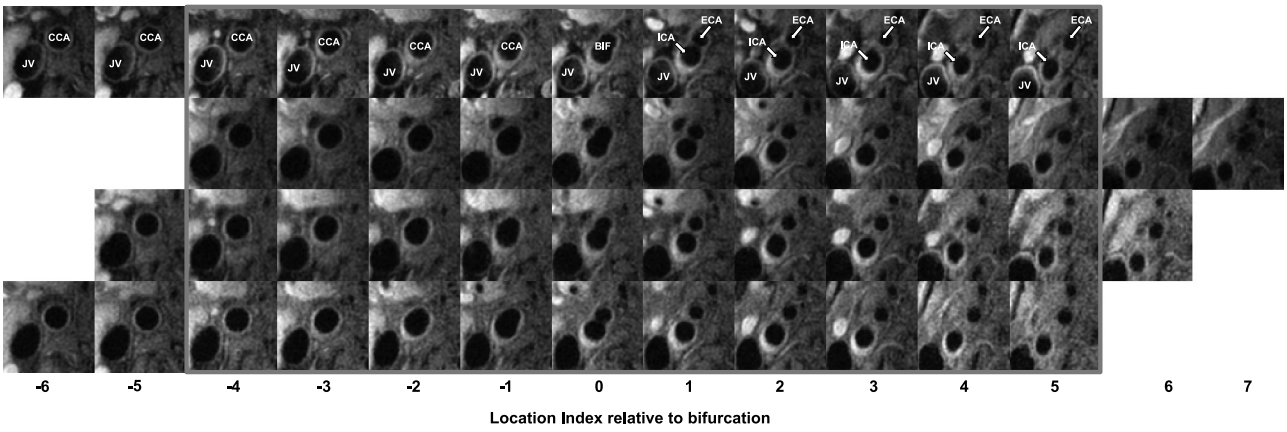

Figure 1. Inter-scan matching: The bifurcation was assigned to the location at or just below the point where the common carotid artery (CCA) separates into the internal (ICA) and external carotid artery (ECA). The bifurcation is labeled with the location index 0; locations in the CCA have negative values, ranging from −6 to −1, and locations in the ICA have positive values, ranging from 1 to 7. Scan 1 (1st row) has a coverage of −6 to 5; scan 2 (2nd row) coverage is −4 to 7; scan 3 (3rd row) is −5 to 6; and scan 4 (4th row) is −6 to 5. The highlighted locations from −4 to 5 represent the coverage that is matched across all four scans. Locations < 4 or > 5 were excluded from statistical analysis. JV = jugular vein.

This coverage is usually sufficient to image the complete carotid atherosclerotic plaque. Average scan time was 35–45 minutes.

Four MRI scans (baseline, 4 weeks, 8 weeks, and 13 weeks) were scheduled for each subject. The four scans from the same subjects were centered on the carotid bifurcation of the index side. The index side was defined to be the side of carotid artery with the higher degree of lumen area reduction compared with the contra-lateral side by MRI.

2.4. *Intra-scan and inter-scan matching*

All images obtained for this study were randomized at a Core Reading Center. Reviewers were blinded to subject, time point, and site information. Previous studies (1, 4, 12) have shown that information from multiple contrast weighted images are needed for accurate identification of plaque tissue components. Therefore, cross-sectional locations from all the five contrast weightings of each carotid artery from a single MRI examination were matched relative to the bifurcation. The bifurcation was assigned to the location 2mm below the location, where the lumen of the common carotid artery separates into the two lumen of the internal and external carotid artery. In order to insure a similar coverage of the plaque for volume-based measurements, only image locations that could be matched across the 4 time points were reviewed. Figure 1 illustrates the inter-scan matching across the four exam time points.

2.5. *Image review*

Four reviewers (A.K., B.C., J.C., and T.S.) with proper training and experience in carotid plaque imaging reviewed the images. Each scan was read by two reviewers who reached consensus decision. All scans of the index side of each subject were reviewed simultaneously. An image-quality (ImQ) rating (5-point scale, 1 = poor, 5 = excellent) for each contrast weighting was assigned to all MR images (4) before the review. Imaging locations with an average image quality < 3 indicating severe blurring due to subject motion or low signal to noise ratio were excluded from the study.

Area measurements of the lumen, outer wall, and tissue components were obtained using a custom-designed image analysis tool QVAS (16). The outer wall area included the lumen and wall areas. The wall area for each location was calculated as the difference between the outer wall and lumen areas. Volumes were calculated by multiplying the sum of all areas per artery by the coverage. The wall/outer wall (W/OW) ratio was calculated as wall volume divided by outer wall volume. LR/NC and Ca volumes were measured as percentages of the vessel wall volume.

The LR/NC and Ca were identified based on previously published MRI criteria. Briefly, the LR/NC as visualized by MRI is iso- to hyperintense (relative to the adjacent sternocleidomastoid muscle) on T1W images and has varied signal intensity on PDW, T2W and TOF images, depending on the amount and age of hemorrhage within the core, if present. Ca is characterized by defined areas with a hypointense signal on all five weightings.

2.6. *Data analysis*

The paired *t*-test was used to compare quantitative measurements of the baseline scan versus scan 4. Measurement variability was assessed by using the quantitative data of the index carotid artery of the subjects assigned to the placebo group, assuming that the biological change that occurred over the duration of the trial (3 months) was small compared to the measurement variability. We used random effects ANOVA to

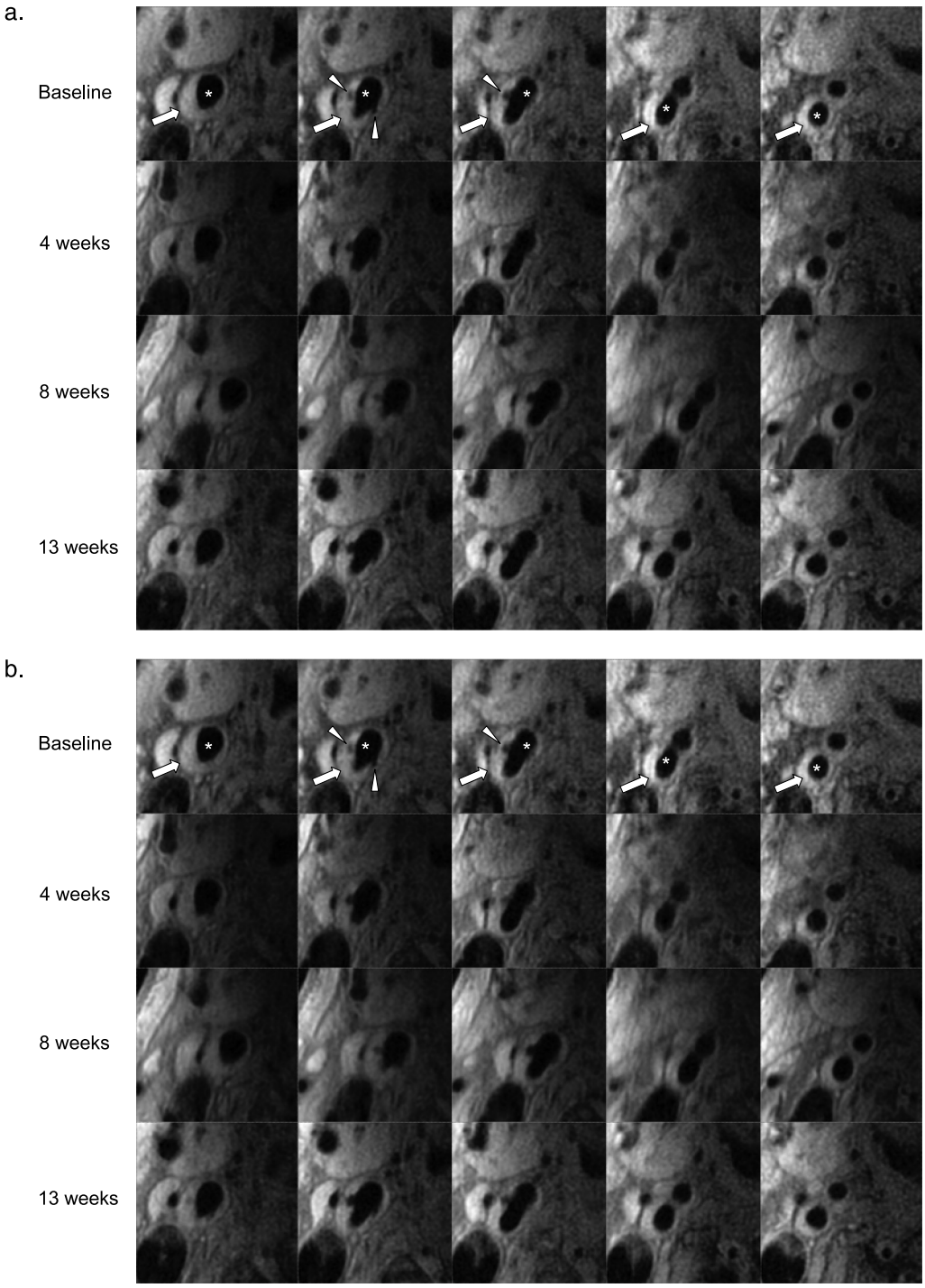

Figure 2. a. T1-weighted images with good image quality (ImQ = 4–5) of a lesion in the right carotid artery (lumen = asterisk) with a large lipid-rich/necrotic core (arrow) with a small piece of calcification (arrowhead). Images are well matched and appear almost identical (1st and 2nd column: common carotid artery, 3rd column: bifurcation, 4th and 5th column: internal carotid artery). b. Contrast-enhanced T1-weighted images of the same patient. The lipid-rich/necrotic core (arrow) does not show any contrast enhancement and the boundaries are clearly delineated by the surrounding enhanced fibrous tissue (calcification = arrowhead; lumen = asterisk).

estimate between- and within-patient variance of each measurement. We calculated the measurement errors for area and volume data of lumen, wall, W/OW ratio and the plaque components. Measurement error was calculated as 100%* $\sqrt{}$ [within-patient variance]/Mean (all measurements). The Intraclass Correlation Coefficient (ICC) was calculated to measure the level of agreement between two measurements repeated within subjects in comparison to the variation in the

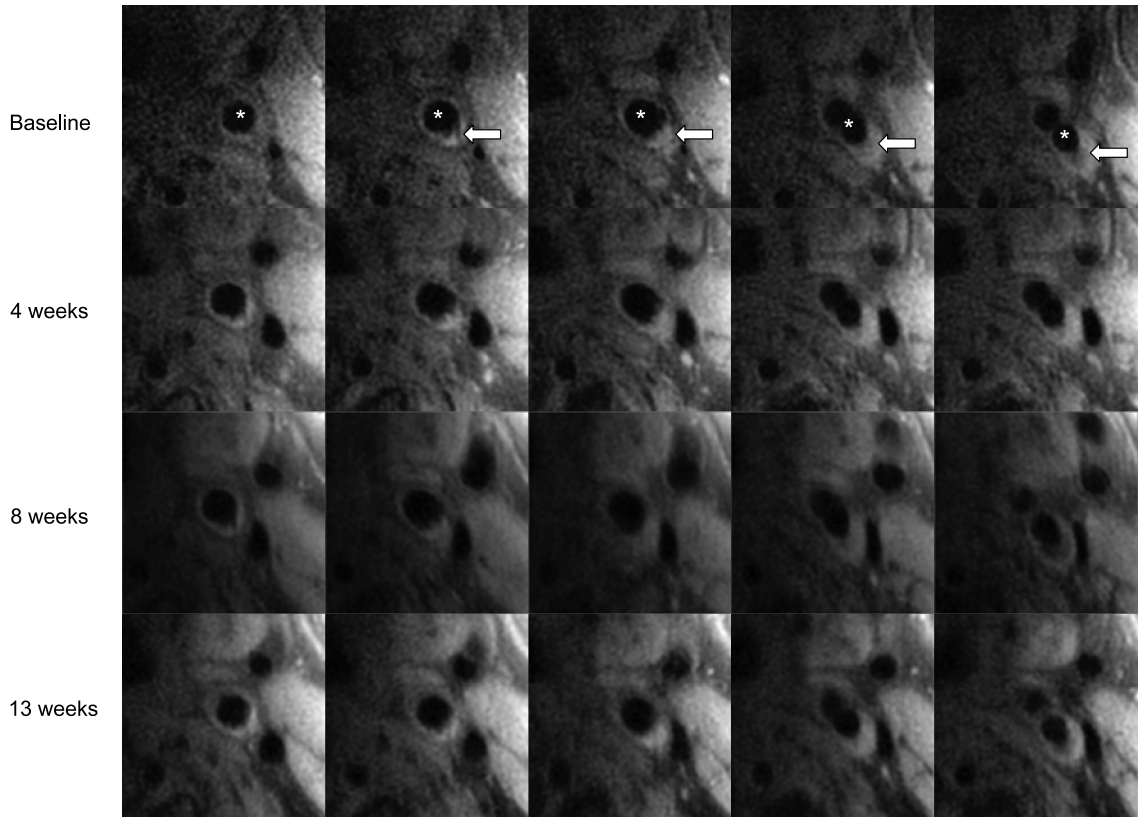

Figure 3. T1-weighted images with moderate image quality (ImQ = 3) of a lesion in the left carotid artery (lumen = asterisk). The different time points are well matched and the lesion with a medium sized lipid/necrotic core (arrow) appears almost identical. The arrow points to a medium sized lipid/necrotic core. 1st–3rd column: common carotid artery, 4th column: bifurcation, 5th column: internal carotid artery.

measurement across subjects. An ICC close to 1.0 indicates that measurement error is small relative to the range of values encountered. For the LR/NC or Ca plaque components, only those subjects that exhibited a non-zero area at all four time points were used in the analysis. We used normal quantile-quantile plots of the patient and within patient effects to detect atypical measurements. Power analysis based on a two sample unpaired *t*-test (two-sided) was performed with 80% power and $p < .05$ to denote statistical significance. The measurement error (as a percent) for each endpoint was used as the standard deviation for sample size determination. Spearman's Correlation Coefficient (r) was used to correlate the measurement error of wall measurements with the mean ImQ across the four scans. Analyses were carried out in SPSS for Windows (version 10, Systat Software Inc., Richmond, CA, USA), R (version 2.0.0, The R Foundation for Statistical Computing, www.r-project.org) or in Stata (version 8, StataCorp LP, College Station, TX, USA).

3. Results

3.1. *MRI scans, image quality and mean coverage*

Of the 20 recruited asymptomatic subjects, one subject was excluded from analysis due to image quality < 3 on all four scans. The measurement variability was assessed by analyzing quantitative data from the index carotid artery of the 19 placebo group subjects with image quality ≥ 3. Of those, seventeen completed the 4 scans study within 13 weeks, and two terminated the study after 2 and 3 MRI scans, respectively. One subject developed appendicitis, and the other subject developed a pleural effusion. Both adverse events were unrelated to the study. In total, 73 arteries on the index side were reviewed (scan 1 and 2: 19 arteries, scan 3: 18 arteries, scan 4: 17 arteries). All individual cross-sectional locations of the 73 arteries had an ImQ ≥ 3, yielding 660 matched locations for comparison. Mean coverage was 1.8 cm, ranging from 1.4–2.2 cm, with a mean image quality of 4.1. Figures 2 and 3 show sample images with moderate and good ImQ matched across the four time points. Of note, there is excellent registration of the cross-sectional locations across the four scans with almost identical configuration of the lumen, wall and the lesion appearance.

3.2. *Descriptive characteristics*

The mean age of the 19 subjects in the placebo group (10 male, 9 female) was 68 years, ranging from 52–78 years. The risk factor profile and the mean values for the lipid panel and plaque characteristics at baseline are described in Table 2. The

Table 2. Baseline characteristics

	Mean ± SD or %	Range (if applicable)
I. Demographics and risk factors		
Age (years)	68 ± 7	52–78
Weight (kg)	78 ± 13	61–113
Height (cm)	167 ± 7	155–180
Male Sex	53% (10/19)	
Lipid-lowering drugs	42% (8/19)	
CAD	53% (10/19)	
Hypertension	53% (10/19)	
Hypercholesterolemia	68% (13/19)	
II. Lipid panel		
Total cholesterol (mg/dl)	178 ± 35	111–238
HDL (mg/dl)	53 ± 18	33–104
LDL (mg/dl)	109 ± 31*	66–177
VLDL (mg/dl)	15 ± 8*	4–29
III. Plaque characteristics scan 1		
Mean lumen volume (mm^3)	557 ± 214	295–1050
Mean wall volume (mm^3)	746 ± 256	363–1455
Mean LR/NC volume (mm^3)	98 ± 161	0–630
Mean Ca volume (mm^3)	18 ± 34	0–185
Mean %LR/NC volume (%)	10 ± 12	0–46
Mean %Ca volume (%)	2 ± 3	0–17
Mean W/OW ratio	0.6 ± 0.1	0.4–0.8

CAD = Coronary artery disease diagnosed by cardiac catheter or status post myocardial infarction; LR/NC = Lipid-rich/necrotic core; Ca = Calcification; W/OW = Wall divided by outer wall.
*One value missing.

between-patient standard deviations were 252 mm^3 for wall volume, 0.1 for W/OW ratio, 4.3% for %Ca volume, and 12.7% for %LR/NC volume. Of note, 16 of 19 subjects exhibited features of advanced atherosclerotic disease, such as LR/NC (13/19) and/or Ca (11/19).

3.3. *Change baseline versus scan 4*

The underlying assumption of this work is that no changes occurred in the lesions over the three months. To test the validity of this assumption, we compared the baseline and scan 4 results. During the 3 months of the study, there was a slight trend towards progression of atherosclerotic disease, with an increase of wall volume and W/OW ratio by 2.4% ($p = .3$) and 1.7% ($p = .1$), respectively. Lumen volume decreased by 2.4% ($p = .043$). There was a statistically non-significant increase in %LR/NC volume (+ 0.1%; $p = .9$) and %Ca volume (+ 5.7%; $p = .5$). This analysis suggests that some of the variability was due to progression and therefore the reproducibility estimates are conservative.

3.4. *Intraclass correlation coefficient*

The ICC showed excellent agreement for all measurements, indicating that the variation in the measurements across subjects was much higher than the variation of repeated measurements within subjects. The ICC for volume-based measurements ranged from 0.95–0.99 and the ICC for area-based measurements ranged from 0.90–0.97 (Table 3). Figure 4 shows the correlation between baseline and the three other time points for wall volume, W/OW ratio, %LR/NC volume and %Ca volume.

3.5. *Measurement error*

Table 3 illustrates the measurement errors for all variables obtained. One individual was excluded from all analyses of calcium. The mean for this person was 10.5 SDs (standard deviations) away from the overall mean for calcium volume. Measurement errors based on multiple locations (volumes) were in general lower than the ones based on single locations (minimum or maximum areas). Measurement errors for minimum or maximum areas ranged from 4.1–25.2% and volume-based measurement errors ranged from 3.2–30.8%. Specifically, the measurement error was 4.3% for lumen volume, 3.2% for W/OW ratio, 5.8% for wall volume, 10.6% for %LR/NC and 18.6% for %Ca. When the measurement error was calculated only for a subset of subjects which had more severe atherosclerotic disease with a higher %LR/NC, the measurement error for %LR/NC dropped (Table 4).

Table 3. Measurement error and intraclass correlation coefficient

Measurement	Mean scan 1–4 ± SD^b	SD^w of scan 1–4	CV (%)	ICC (95% CI)
Volumes/ratios				
Wall volume (mm^3)	746 ± 252	43	5.8	0.97 (0.95–0.99)
Lumen volume (mm^3)	543 ± 212	23	4.3	0.99 (0.98–1.00)
Outer wall volume (mm^3)	1289 ± 349	43	3.3	0.98 (0.97–1.00)
Mean W/OW ratio	0.58 ± 0.1	0.02	3.2	0.97 (0.94–0.99)
LR/NC volume (mm^3)†	140 ± 180	16	11.1	0.99 (0.98–1.00)
Ca volume (mm^3)†	30.2 ± 40.0	9.3	30.8	0.95 (0.90–1.00)
% LR/NC (%)†	14.4 ± 12.7	1.6	10.6	0.98 (0.97–1.00)
% Ca (%)†	3.8 ± 4.3	0.7	18.6	0.97 (0.95–1.00)
Minima or maxima (areas/ratios)				
Min. lumen area (mm^2)	16.9 ± 8.2	1.7	9.8	0.96 (0.93–0.99)
Max. wall area (mm^2)	59.5 ± 19.5	4.2	7.1	0.96 (0.92–0.99)
Max. outer wall area (mm^2)	101 ± 25	4	4.1	0.97 (0.95–0.99)
Max. W/OW ratio	0.69 ± 0.13	0.03	4.1	0.95 (0.91–0.99)
Max. LR/NC area (mm^2)†	15.9 ± 14.6	2.5	15.2	0.97 (0.94–1.00)
Max. Ca area (mm^2)†	5.5 ± 6.5	1.3	24.1	0.96 (0.92–1.00)
Max. %LR/NC (%)†	26.7 ± 13.5	4	14.0	0.92 (0.85–0.99)
Max. %Ca (%)†	10.9 ± 8.3	2.7	25.2	0.90 (0.80–0.99)

SD^b = Between-patient standard deviation; SD^w = Within-patient standard deviation; CV = Coefficient of variation (measurement error); ICC = Intraclass correlation coefficient; W/OW = Wall divided by outer wall; LR/NC = Lipid-rich/necrotic core; Ca = calcification.
†Only patients that exhibited the feature in at least one of the images were included; n = 13 for LR-NC, n = 11 for calcification.

3.6. *Measurement error of wall volume: ImQ and study sites*

The measurement error for wall volume did not differ substantially across sites and ranged from 5.2–6.3%. When the measurement error was calculated dependent on the ImQ, the measurement error was 7.5% in the 4 subjects with a mean ImQ across the 4 scans < 4, 5.4% for the 10 subjects with mean ImQ 4–4.5 and 3.2% for the 5 subjects with mean ImQ > 4.5. The measurement error of wall volume showed a strong negative correlation with the ImQ ($r = -0.7$; $p = .002$).

3.7. *Power analysis*

Power analysis based on these measurement errors shows that a study with 43 participants in each group could detect a 5%

Table 4. Sample size calculation

			Treatment effect				
			2%	5%	10%	20%	50%
Endpoint	N	CV (%)	Subjects per group*				
Wall volume	19	5.8	264	43	12	4	2
Lumen volume	19	4.3	147	24	7	3	2
Mean W/OW ratio	19	3.2	81	14	4	2	2
> 0% LR/NC	13	11.0	943	152	39	10	3
> 3% LR/NC	11	9.2	660	107	28	8	3
> 6% LR/NC	10	9.0	635	103	27	8	3
> 12% LR/NC	6	6.9	370	61	16	5	3
> 0% Ca	11	18.6	2704	433	109	28	5
Min. lumen area	19	9.8	755	122	31	9	3
Max. wall area	19	7.1	400	65	17	5	2
Max. W/OW ratio	19	4.1	133	22	6	3	2
Max. %LR/NC	13	15.0	1778	285	72	19	4
Max. %Ca	11	25.2	4992	800	201	51	9

CV = Coefficient of variation (measurement error); N = Number of subjects used for analysis; W/OW = Wall divided by outer wall; LR/NC = Lipid-rich/necrotic core; Ca = Calcification.
*Fewer than 10 subjects per treatment group is not recommended.

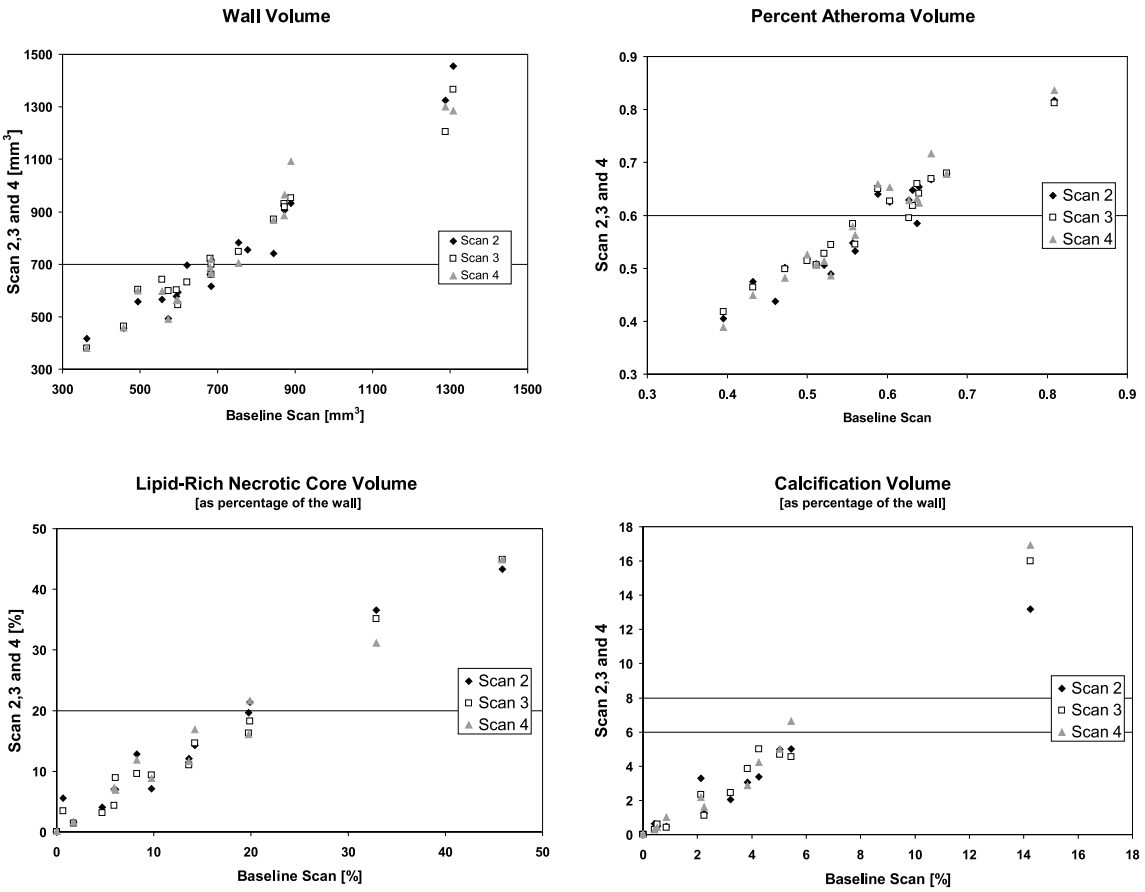

Figure 4. The figure illustrates the correlation between baseline and the three follow-up scans for the quantitative measurements of wall volume, percent atheroma volume, lipid-rich/necrotic core volume and calcification.

change in W/OW ratio, 5% change in wall volume, and 10% change in %LR/NC volume (power = 80%, $p < .05$). A study with only 14 participants in each group could detect a 5% change in W/OW ratio, 10% change in wall volume, and 20% change in %LR/NC volume. The calculated measurement errors include any true biological changes that have occurred in 3 months. Table 4 illustrates that the number of subjects needed to detect changes in plaque composition decreases if a study is planned for subjects with more advanced atherosclerotic disease. For example, all subjects have > 3% LR/NC volume in their carotid artery plaques at baseline, a minimum of 28 subjects per group would need to be recruited in order to detect a 10% change in %LR/NC volume. If all subjects have > 12% LR/NC volume at baseline, then the minimum number per group needed for recruitment to detect a 10% change would be 16.

4. Discussion

The results of this multi-center trial demonstrate that high-resolution MRI has a low variability for quantitative measurements of lumen volume, wall volume, W/OW ratio, LR/NC volume, %LR/NC volume, and %Ca volume. Furthermore, the measurement error of specific cross-sectional location based area measurements, such as minimum lumen area, maximum wall area, and maximum percent atheroma area was less than 10%. With our standardized protocol, we achieved sufficient image quality and coverage to perform quantitative measurements in 19 of 20 subjects and good registration was obtained across the four different time points, using the bifurcation as a landmark (Figs. 1–3). In the planning of future MRI trials the measurement errors for lumen, wall, W/OW ratio, LR/NC and Ca obtained in the current study can be used to calculate the number of subjects needed to detect a variety of levels of treatment effect.

This study showed excellent correlation for baseline measurements compared to the other time points for all measured variables (Table 3). This indicates that the variation between subjects of any of the measurements was much greater than the variation within the repeated measurements and expresses almost perfect agreement on repeated measurements (perfect agreement = 1.00). The intraclass correlation coefficients stated here for all volume-based measurements are comparable to those obtained by IVUS in repeated measurements in coronary arteries, which ranged from 0.97–0.99 (17).

The measurement error or modified coefficient of variation ranged from 3.2–5.8 for lumen volume, wall volume and W/OW ratio and was comparable to the coefficient of variation previously reported in an individual institution (4–6% for wall volume measurements) (14). It was also similar to previously reported measurement errors by IVUS in coronaries for plaque volume measurements (CV = 5.6%–6.4%), lumen volume measurements (CV = 8.5%–8.6%) and vessel volume measurements (CV = 6.2%–6.7%) (17) and carotid IMT measurements by ultrasound (CV = 2.4%) (18, 19) −10.6% (19, 20).

The major advantage of MRI compared to other imaging modalities, besides its non-invasiveness and the absence of radiation, is its unique information regarding plaque composition. In vivo plaque tissue characterization is limited with ultrasound (21) and the role of computed tomography, which has achieved promising results for imaging coronary artery calcification (22) has yet to be established for in vivo imaging of other plaque compositional features. Therefore, coefficients of variations for plaque components measured by other imaging modalities have not been previously reported. The measurement error in this study was 11.1% for %LR/NC volume and 18.6% for %Ca volume and dropped when calculated for patients with more severe atherosclerotic disease (Table 4). The measurement error was also lower when volumes instead of areas were measured, suggesting that the use of multiple locations per patient in progression/regression studies can facilitate the detection of real change.

One important outcome of the present study is to provide the basis for power calculation in designing future MRI-based trials. Table 4 can be used as a reference for designing such studies by reading off the number of subjects needed to detect an expected treatment effect. One important consideration that emerges is the importance of subject selection in these trials since those with more advanced disease have comparatively lower coefficients of variation than individuals with very early stages of atherosclerosis. This is especially true for the compositional measurements of LR/NC and Ca. Therefore, a study that includes subjects with any amount of %LR/NC at baseline will require 39 subjects per treatment group, which is 2.4 times more than the 16 subjects that would be required per group in a study that includes subjects with > 12% LR/NC at baseline.

4.1. *Limitations*

The reviewers were blinded to the time point information and clinical information, but all four scans were reviewed simultaneously. The simultaneous review of all scans is necessary to assure identical scan coverage and accurate matching across the four scans, but might have reduced the variability of quantitative measurements compared to a completely blinded review. However, this study design with simultaneous review of 2 or more exams is the standard of practice in clinical trials using imaging methods (23, 24), and it is important to note that this study was conducted as part of a clinical trial in which the reviewers expected true biological change.

We based the analysis of reproducibility measurements on the combined variability of each parameter across all time points, assuming no change has occurred over the three month interval of the study. Because some true biological change has occurred, the stated measurement errors represent an upper bound of the measurement noise. We believe, however, that measurement error is the dominant cause of error and that our estimates are therefore only slightly elevated.

Compared to previously published reports (12), the subjects in this study had less advanced carotid atherosclerotic disease indicated by smaller % Ca volume and % LR/NC volume. Measurement errors would likely decrease when larger volumes are measured. Therefore, the measurement errors reported in this study may be overestimated for a study involving subjects with more advanced carotid atherosclerosis.

Only images with image quality ≥ 3 were considered for the review, resulting in exclusion of 1 of 20 subjects (5%). The number of exclusions secondary to poor image quality should decline with improvements in hardware (e.g. higher field MRI and coil design) and in pulse sequence design. 3T MRI holds great promise for future MRI trials, and we expect significant improvement in the stated coefficients of variations by 1) decreasing the slice thickness/increasing the spatial resolution 2) increasing the scan coverage and 3) better image quality due to shorter scan times and higher signal strength.

5. Conclusion

The results of this multi-center MRI trial in a group of subjects with asymptomatic, early carotid atherosclerotic disease show that quantitative measurements of wall, W/OW ratio, %LR/NC and %Ca have a low variability across scans, and thus that carotid MRI is a viable tool for evaluating clinical trial outcome. MRI provides the opportunity to noninvasively study atheroma volume and plaque composition in longitudinal clinical trials. Power analysis based on these measurement errors shows that a study with 43 participants in each group could detect a 5% change in W/OW ratio, 5% change in wall volume, and 10% change in %LR/NC volume (power = 80%, $p < 0.05$).

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