

Validation Study of a Semi-Automated Program for Quantification of Atherosclerotic Burden

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ABSTRACT

Practical implementation of cardiovascular magnetic resonance (CMR) for the noninvasive screening of atherosclerosis is limited by inter- and intra-observer variability and labor intensity of morphometric analysis by manual planimetry (MANU). We assessed the hypothesis that a semi-automated quantification program (AUTO) for CMR would be faster and more accurate than MANU without loss of reliability. In the analysis of carotid atherosclerosis in asymptomatic hypercholesterolemic patients ($n = 17$), AUTO was superior to MANU in speed and histopathologic correlation without significant differences in inter- and intra-observer variability. Implementation of AUTO may facilitate CMR for the screening of the burden of atherosclerotic disease.

INTRODUCTION

Currently available cardiovascular disease risk assessment methodologies (*i.e.*, risk-factor based algorithms such as the Framingham Heart Study coronary heart disease prediction score (1) fail to detect a substantial portion of asymptomatic patients whose first presentation may be death or disability (2). Although more robust in the high-risk population, (3) Framingham-based risk scoring is only weakly correlated with detection of subclinical atherosclerosis (4) which suggests that the multiple risk factor assessment approach has limitations in predicting disease in the low-to-moderate risk population. While stress testing and traditional coronary angiography are diagnostic for flow-limiting lesions, acute coronary syndrome (ACS) is twice as likely to occur from disruption and subsequent thrombosis of non-severely stenotic plaques compared to the severely stenotic (5). Accurate assessment for

coronary risk in the low-to-moderate risk population remains elusive.

Recent studies have demonstrated that pharmacologic treatment can regress atherosclerotic lesions in the primary prevention population (6–8), and if regression prevents subsequent cardiovascular events, earlier detection of asymptomatic atherosclerotic disease is necessary. The only imaging-based test demonstrated to provide incremental predictive benefit over traditional cardiovascular risk factor assessment is carotid ultrasound (9), and its noninvasive nature and lack of ionizing radiation are further appeals of this modality. However, its implementation as an effective screening tool is limited by lack of method standardization, observer variability, measurement imprecision and the time-consuming nature of analysis (10, 11). Screening of carotid atherosclerosis by cardiovascular magnetic resonance (CMR) shares some of the same benefits and drawbacks as ultrasound, but its superior test variability and ability to distinguish plaque characteristics may make CMR the modality of choice for carotid imaging (12).

For CMR to be a successful screening tool, certain disadvantages of this modality must be overcome for implementation into a broader population. First, the labor intensity of morphometric analysis, commonly done by manual planimetry, must be reduced so that the test can be cost effective. Second, inter- and intra-observer variability must be demonstrated to be low so that serial changes in plaque volume are valid and image interpretation across observers would result in valid comparisons. Third, the images acquired must accurately reflect the underlying pathology of the disease.

In this study, we developed a semi-automated plaque quantification program for CMR image analysis (AUTO) and evaluated its reproducibility and reading time compared to manual

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planimetry (MANU) in the assessment of carotid atherosclerosis in humans. Accuracy of measurement was further assessed by comparing *in vivo* measurement results to a histopathologic reference—rabbits with induced experimental atherosclerosis.

METHODS

Study design

Asymptomatic, untreated, hyperlipidemic (LDL cholesterol ≥ 130 mg/dL, triglycerides ≤ 445 mg/dL) patients ($n = 17$) underwent carotid artery imaging as previously reported (6). For the histopathologic correlation, atherosclerotic lesions in the abdominal aorta of New Zealand White rabbits ($n = 3$) were induced and imaged as previously reported (13–17). All of the investigators were blinded to clinical or personal data of the study subjects (human or rabbit), and image order was randomized before each tracing. Two operators (AH and AL) extensively trained in morphometric analysis of CMR images (8, 18, 19) traced the arterial lumen wall and outer vessel wall for each image. Each image was traced in 4 series by each operator: twice by manual planimetry (MANU) and twice by using the semi-automated border detection program (AUTO) described below. Images were analyzed non-sequentially to minimize order bias. A stopwatch recorded the elapsed time for image analysis. Results from the rabbit images were matched to corresponding histopathologic sections, which were used as reference for actual vessel dimensions.

Histopathology

Within 24 hours of the final CMR, the rabbits were euthanized by intravenous injection of 150 mg/kg sodium pentobarbital (Sleepaway; Fort Dodge Animal Health; Fort Dodge, Iowa, USA). Prior to euthanasia, the animals received heparin (100 U/kg; American Pharmaceutical Partners; Schaumburg, Illinois, USA) to prevent postmortem thrombosis. The aortas were cannulated at the level of the diaphragm and immediately flushed proximally and distally with 250 mL of 0.1 M phosphate-buffered saline (PBS), pH 7.4. The abdominal aorta was further flushed with 250 mL of cold (4°C) perfusion fixative at 100 mmHg (4% paraformaldehyde in PBS). Using anatomic landmarks observed by CMR, the abdominal aorta was excised, immersed in fresh fixative with preserved *in situ* configuration, and then stored at 4°C for 1 week to fix the tissue. A 6 cm section distal to the left renal artery, which corresponded to the segment analyzed by CMR, was then washed free of formaldehyde using distilled water and immersed in PBS. The aorta segment analyzed by CMR was cut into 3 mm sections corresponding to the imaging analysis segments. Specimens were paraffin-embedded and cut into serial 5 μm thick sections. Within each 3 mm segment, a section was stained with Masson's trichrome elastic stain for quantitative analysis. This method preserved longitudinal configuration without shrinkage during specimen processing, but axial shrinkage is estimated to be approximately 5%.

CMR

In brief, image acquisition was performed on a 1.5-T whole-body MRI system (Signa CV/I, GE Medical Systems; Milwaukee, Wisconsin, USA) with a gradient performance of 40 mT/m and a slew rate of 150 T/m/s. Subjects were scanned in supine position, and 4 element phased-array coils were used. After localization with a fast-gradient-echo sequence, all images were obtained with a double-inversion recovery (*i.e.*, black-blood) fast-spin-echo sequence, and 4 lead ECG signals were used for triggered CMR data acquisition during free breathing. A series of 25 to 30 transverse proton density-weighted (PDW) images centered at the carotid bifurcation (human subjects) were taken with the following parameters: repetition time (TR), 2 R-R intervals; echo time (TE), 12 ms; field of view, 12 cm; slice thickness, 3 mm with no interslice gap; acquisition matrix, 256 $\times$ 256; 512 zero filling; no phase wrap; number of signals averaged, 1; echo-train length, 32; receiver bandwidth, ± 64 Khz. A chemical shift suppression pulse was used to suppress the signal from peri-vascular adipose tissue. The in-plane resolution was 469 $\times$ 469 μm . The rabbit subject image acquisition was similarly obtained of the abdominal aorta from the left renal artery to the iliac bifurcation.

Image analysis

MR images were converted to Tagged Image File Format (TIFF) and exported to a Macintosh computer system (Apple;

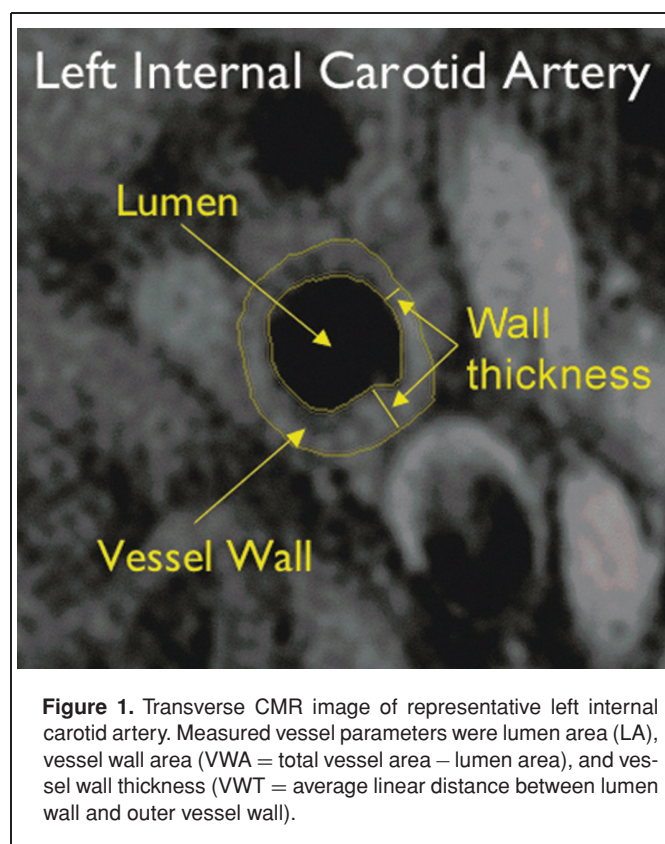


Figure 1. Transverse CMR image of representative left internal carotid artery. Measured vessel parameters were lumen area (LA), vessel wall area (VWA = total vessel area – lumen area), and vessel wall thickness (VWT = average linear distance between lumen wall and outer vessel wall).

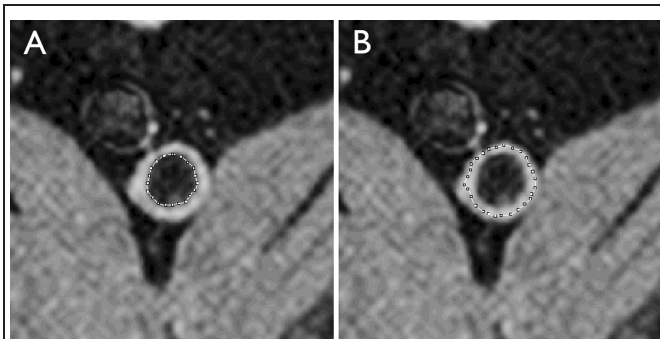


Figure 2. Representative images of lumen wall (A) and outer vessel wall (B) delineations as determined by the semi-automated plaque quantification program (AUTO) in the rabbit aorta. Observer supervision of program results may correct measurement by adjustment of calculated boundary points.

Cupertino, California, USA) for further analysis. MANU was conducted on Image Pro Plus (Media Cybernetics; Carlsbad, California, USA). The custom AUTO tool utilized developer's interface of ImageJ (National Institutes of Health; Bethesda, Maryland, USA) for user interaction and measurements. The algorithm was designed to closely reproduce what a human observer would define as vessel border based on shape and change in signal intensity. In brief, signal intensity histograms were calculated for at least 360 (number dependent on vessel size) radial projections from the lumen center. For each ray, an averaging filter was applied to reduce artifact, and then minimum, maximum and average intensity points were calculated. Maximum intensity points following approximate circular shape were counted as lying within vessel wall. Two-step search for predetermined empiric pattern of signal intensity change identified inner (lumen wall) and outer (vessel wall) boundaries. Boundaries were interpolated and displayed as spline fit curve for interactive adjustment (Fig. 2, step 1—A, step 2—B). Lumen area (LA) was defined as part of image encircled by inner boundary; vessel wall area (VWA) as area between lumen wall and vessel outer wall; vessel wall thickness (VWT) as average distance from lumen wall to vessel outer wall (Fig. 1), and errors in automated border detection were corrected by observer adjustment of the boundary points.

Statistical analysis

Data was exported to MedCalc (MedCalc Software, Mariakerke, Belgium) for statistical analysis. After testing for normal distribution and equality of variances with Levene's *F* test,

Table 1. Mean inter- and intra-observer differences between observations of tracing by automated program (AUTO) or by manual planimetry (MANU) for 17 patients with carotid artery CMR. No statistically significant differences were observed.

	Lumen Area (%)	Vessel Wall Area (%)	Vessel Wall Thickness (%)
MANU			
Inter-observer	6.6 (p = 0.32)	4.6 (p = 0.27)	5.3 (p = 0.09)
Intra-observer	2.8 (p = 0.68)	3.0 (p = 0.47)	2.3 (p = 0.47)
AUTO			
Inter-observer	3.9 (p = 0.50)	4.0 (p = 0.45)	4.2 (p = 0.61)
Intra-observer	0.7 (p = 0.91)	2.1 (p = 0.70)	0.2 (p = 0.96)

paired *t*-test was used to compare inter- and intra-observer measurements in carotid analysis between the two methodologies. Method bias between AUTO and MANU for carotid analysis was assessed by Bland-Altman analysis. Paired *t*-test was also used to compare mean analysis time per image between AUTO and MANU in the carotid measurements, and correlation between each planimetry method for CMR of the rabbit abdominal aorta and the true histopathologic specimen measurements was calculated using Pearson product-moment correlation coefficient. All statistical analyses were performed blindly to AUTO versus MANU, and all probabilities were two-sided with *p* < 0.05 considered statistically significant.

RESULTS

Analysis time

The time to quantify carotid vessel dimensions (LA, VWA, and VWT) with AUTO was significantly shorter than with MANU. Analysis time per image was 17.4 ± 3.1 for AUTO versus 87.3 ± 19.4 for MANU, (seconds $\pm$ SD; *p* < 0.001; Fig. 3); therefore, image analysis was 80% faster with AUTO.

Inter- and intra-observer variability

No statistically significant differences in inter- or intra-observer variability in carotid artery measurements were found with either AUTO or MANU for any of the vessel parameters measured (Table 1). We observed consistently lower variability with AUTO than MANU for both inter- and intra-observer mean differences. This per image analysis is comparable, if not superior, to prior measurement errors seen with carotid plaque evaluation by CMR (6). Bland-Altman plots do not suggest any

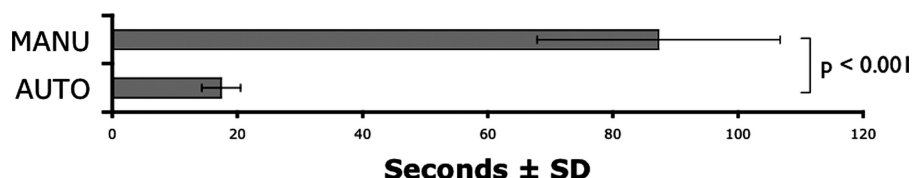


Figure 3. Evaluation time per image. Use of the automated program (AUTO) was significantly faster than manual planimetry (MANU).

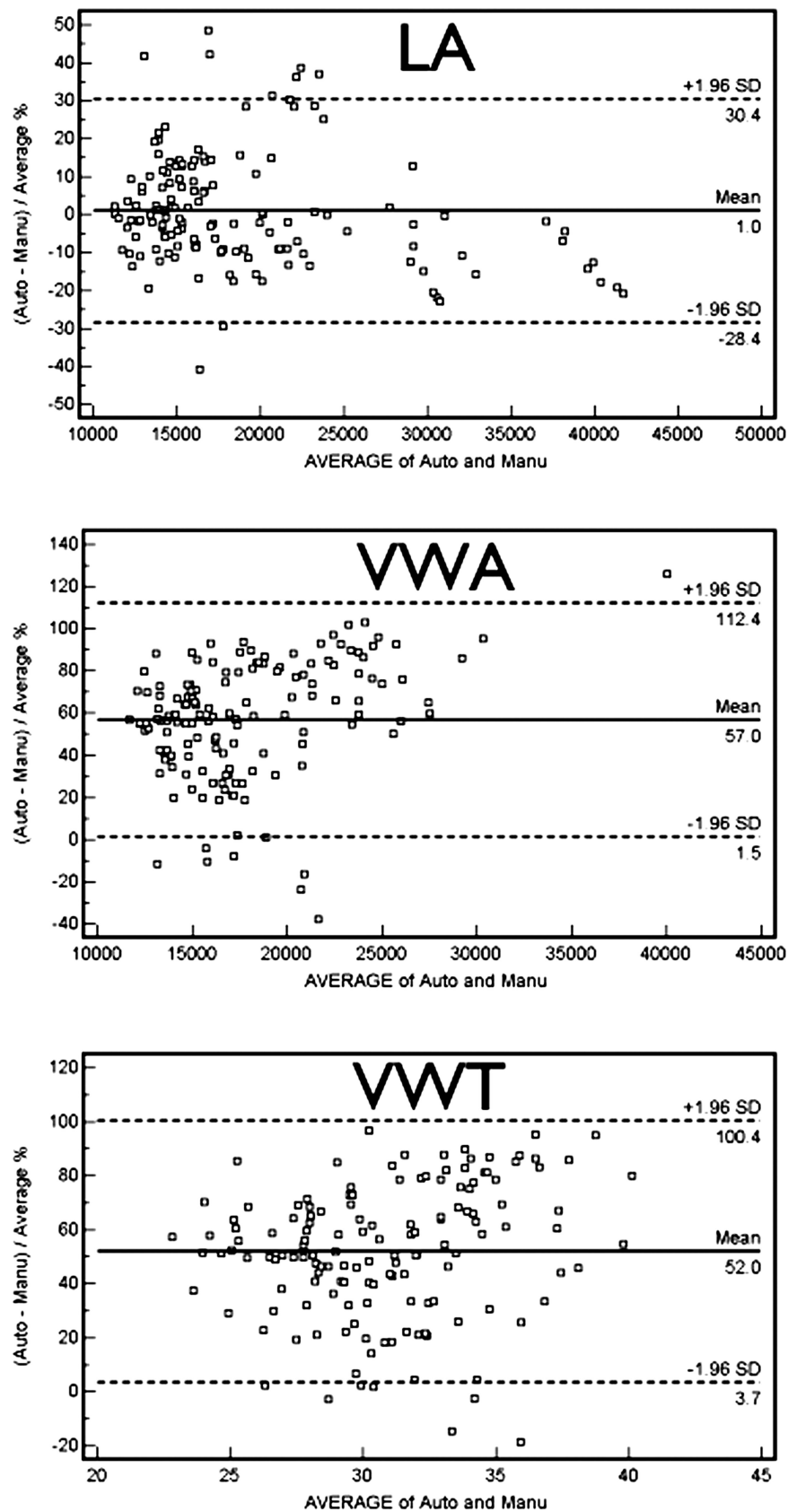
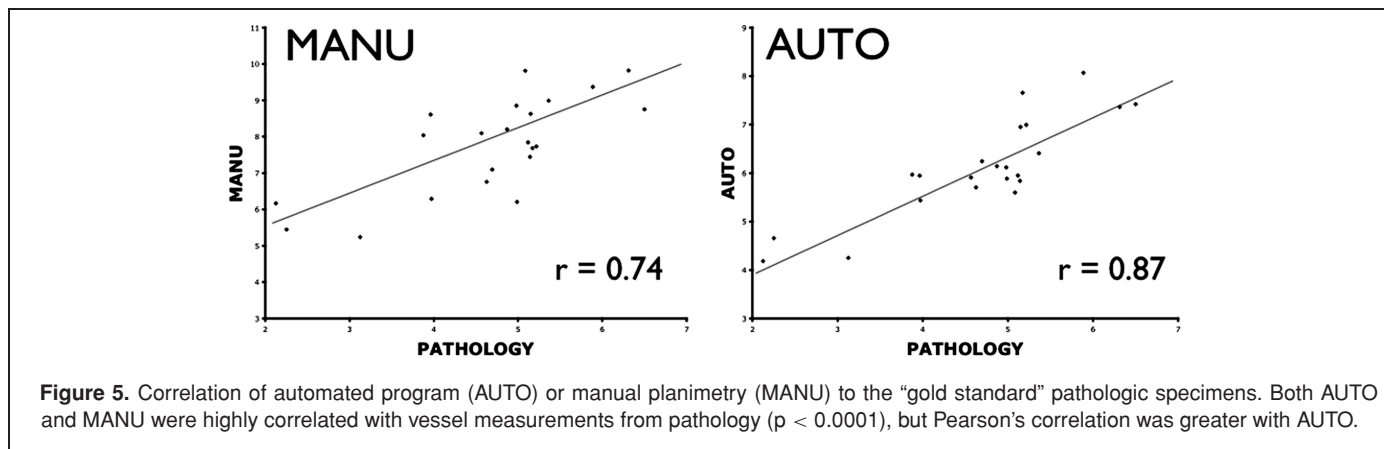


Figure 4. Bland-Altman plots depicting measurement agreement between AUTO and MANU for lumen area (LA), vessel wall area (VWA), and vessel wall thickness (VWT). No systematic biases were observed.



systematic bias between AUTO and MANU for any of the vessel dimensions measured (Fig. 4). The error detected was small with minimal differences beyond 2 standard deviations, and the differences were not correlated with the magnitude of the measured parameter.

Histopathologic correlation

Both AUTO- and MANU-evaluated animal images were highly correlated with the measurements from pathology (Fig. 5). Close agreement was seen with both AUTO and MANU to the “gold standard” pathology, but correlation was greater with AUTO ($r = 0.87$, $p < 0.0001$, 95% confidence interval [CI] 0.69–0.94) compared to MANU ($r = 0.74$, $p < 0.0001$, 95% CI 0.46–0.88).

DISCUSSION

The results from this study imply that a semi-automated quantification program for the CMR determination of atherosclerotic burden in the carotid artery is superior in speed and pathologic correlation without significant difference in observer dependency. AUTO was faster in quantifying vessel dimensions than manual planimetry without increasing observer variability and demonstrated greater correlation with the pathologic specimens of known dimension. Implementation of semi-automated quantification tools may allow atherosclerosis screening by CMR feasible.

The overall accuracy of AUTO in our analysis was impressive. Prior analysis by our group that used manual morphometric analysis had a per image error of 4.1% (6) whereas AUTO had an error of 2.1%. Therefore, with the use of AUTO, changes of as little as 4% may be detected by CMR with the assistance of this semi-automated planimetry method—a reduction of error by approximately 50%. In addition, AUTO and MANU were both highly correlated with a pathologic reference of the *in vivo* atherosclerotic abdominal aorta in an experimental rabbit model. We chose this *in vivo* imaging model to assess the accuracy of these planimetry methods since phantom analysis is not subject to confounding image artifacts and plaque heterogeneity of the *in vivo* animal, and the rabbit aorta is similar in size to relevant

human vessels; thus, this pathologic correlation allows a more “realistic” reference for image interpretation. While other investigators have evaluated similar semi-automated algorithms in healthy human volunteers and evaluated accuracy against a synthetic glass phantom (20) our study is the first to our knowledge to confirm results against a histopathologic standard. Although both were statistically significant, AUTO had higher correlation than MANU.

This increased accuracy and high reproducibility would reduce the necessary sample size to conduct a study to detect differences between groups or for serial assessment of different interventions for the prevention of atherosclerosis. Prior sample size calculations assuming a power of 0.8 and α of 0.05 predicted 108 patients would be needed to detect a 3% true change in vessel wall area and 29 patients to detect a 6 percent true change (12). With the increased accuracy of AUTO, only 59 patients would be needed for a 3% change, and 15 patients would be needed to detect a 6% change (21). This reduction in necessary sample size increases the feasibility of any clinical trial using CMR as an imaging endpoint by reducing the necessary sample size.

The 80% reduction in analysis time makes planimetry endpoints a more viable. Without AUTO, plaque burden quantification of one carotid artery would take 36 to 44 minutes; with AUTO, planimetry is reduced to 7 to 9 minutes. Furthermore, the semi-automated boundary point determination increases the feasibility that this evaluation may be done by a supervised technician by not only decreasing the labor intensity but also removing observer subjectivity. For quantification of atherosclerotic burden, speed and ease of use are imperative for clinical acceptance of this measure.

Since border detection is dependent upon image quality, AUTO requires more observer correction when signal-to-noise ratio is low or luminal flow artifacts are present, but manual planimetry is also slowed down and less accurate with poor image quality. Optimizing protocols with more advanced imaging systems should further improve performance. However, errors in border detection with lipid- or calcium-rich plaques or adjacent high signal-intensity structures are inherent with the AUTO algorithm. These limitations may render fully automated

planimetry impractical and underscore the importance of a semi-automated process: one that is over-read by a technician to correct these errors.

Although a considerable improvement over MANU, AUTO could be even faster if such an algorithm were integrated into workstation function. In our study, images had to be converted to a file format interpretable by ImageJ and exported to another computer. These steps could be saved if image processing were bundled into other workstation features. AUTO could be even faster with advances in computer processing power which would further minimize computational time of the algorithm.

Ultimately, adoption of CMR for risk assessment of atherosclerotic disease requires clinical trials that correlate atherosclerotic burden by this method with cardiovascular events. To date, no definitive trial has been conducted. Furthermore, the carotid arteries have been chosen because these vessels are the most accessible to image; however, coronary imaging, although not yet broadly available by CMR, would be preferable. Nevertheless, the AUTO algorithm is easily adaptable to any vessel dependent upon image quality. Also, this study was conducted with only PDW images for this analysis; however, since AUTO is based on border determination simply by changes in signal intensity, this algorithm would perform similarly in the evaluation of T1W and T2W sequences.

Conclusion

Semi-automated atherosclerosis quantification by CMR is faster and more accurate than manual planimetry methods. By increasing the ease-of-use, decreasing the labor intensity of image analysis and reducing observer subjectivity, semi-automated image processing may increase feasibility of risk assessment by CMR. Integration of this algorithm as a workstation tool would further speed analysis.

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