REPRODUCIBLE COMPUTER-ASSISTED QUANTIFICATION OF MYOCARDIAL PERFUSION WITH CONTRAST-ENHANCED ULTRASOUND

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Abstract—Myocardial perfusion can be quantified by myocardial contrast echocardiography (MCE) and is used for the diagnosis of coronary artery disease (CAD). However, existing MCE quantification software is highly operator dependent and has poor reproducibility and ease of usage. The aim of this study was to develop robust and easy-to-use software that can perform MCE quantification accurately, reproducibly and rapidly. The developed software has the following features: (i) semi-automatic segmentation of the myocardium; (ii) automatic rejection of MCE data with poor image quality; (iii) automatic computation of perfusion parameters such as myocardial blood flow (MBF). MCE sequences of 18 individuals (9 normal, 9 with CAD) undergoing vasodilator stress with dipyridamole were analysed quantitatively using the software. When evaluated against coronary angiography, the software achieved a sensitivity of 71% and a specificity of 91% for hyperemic MBF. With the automatic rejection algorithm, the sensitivity and specificity further improved to 77% and 94%, respectively. For MBF reproducibility, the percentage agreement is 85% (κ = 0.65) for inter-observer variability and 88% (κ = 0.72) for intra-observer variability. The intra-class correlation coefficients are 0.94 (inter-observer) and 0.96 (intra-observer). The time taken to analyse one MCE sequence using the software is about 3 min on a PC. The software has exhibited good diagnostic performance and reproducibility for CAD detection and is rapid and user-friendly. (E-mail: mengxing.tang@imperial.ac.uk) © 2017 The Authors. Published by Elsevier Inc. on behalf of World Federation for Ultrasound in Medicine & Biology. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

Key Words: Myocardial contrast echocardiography, Myocardial perfusion, Computer-assisted quantification, Coronary artery disease, Reproducibility.

INTRODUCTION

Myocardial contrast echocardiography (MCE), which utilises microbubbles, is clinically employed for the assessment of myocardial perfusion and the detection of coronary artery disease (CAD) (Kaul 1997, 2008; Senior et al. 2009a). Current MCE analyses performed in hospitals rely mainly on human visual assessment (Dwivedi et al. 2007; Janardhanan et al. 2003; Jeetley et al. 2004). Such qualitative assessment has poor reproducibility and is highly dependent on the experience level of the clinician (Ma et al. 2009; Yu et al. 2004). This has precluded widespread use of this technique. Hence, there is a need for more effective quantitative assessment of MCE data. Quantitative MCE is performed with the replenishment–destruction method first proposed by Wei et al. (1998). Time–intensity perfusion curves can then be derived and relevant perfusion parameters can be computed to quantify myocardial blood flow.

Accurate quantification is, however, hindered by the noise and high variability inherent in the MCE data (Tang et al. 2011). There is also a lack of robust computer software that allows clinicians to perform myocardial perfusion quantification accurately. In general, automatic and sophisticated image processing and quantitative tools designed specifically for MCE are lacking (Ma et al. 2009; Marwick et al. 1998). This results in the use of existing software to be time consuming, operator dependent and less reproducible. Current quantification studies are conducted by using either custom-designed research software (Peltier et al. 2004; Wei et al. 2001) or commercial software such as QLAB (Philips Ultrasound) (Hayat et al. 2008; Senior et al. 2005), HDI-Lab (Philips Ultrasound in Med. & Biol., Vol. 43, No. 10, pp. 2235–2246, 2017 © 2017 The Authors. Published by Elsevier Inc. on behalf of World Federation for Ultrasound in Medicine & Biology. Printed in the USA. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/) 0301-5629

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These quantification software require the user to manually and arbitrarily select several regions of interest (ROIs) within the myocardium that the operator considers to be suitable for analysis and representative of each vascular territory (Palmieri et al. 2004; Peltier et al. 2004; Wei et al. 2001). This is highly subjective and different users tend to choose ROIs that vary significantly in size, shape and location. This, in turn, affects the fitting of the perfusion curve, and the extracted perfusion parameters can vary considerably with different ROI selections. This makes MCE quantification highly operator dependent, and accurate quantification requires significant user expertise or training (Wei et al. 2001). In addition, the ROI chosen for a particular time frame has to be adjusted manually for all the other frames in the sequence so as to account for motion artefact and avoid left ventricle cavities (Malm et al. 2006; Peltier et al. 2004). This is time consuming and further increases operator dependence. The aforementioned factors could be important contributors to the high variability reported for MCE quantification in several reproducibility studies conducted using the existing software (Ghanem et al. 2007; Palmieri et al. 2004).

In this study, we developed a myocardial perfusion quantification software for MCE with the following main advantages. (i) A software and graphical user interface was developed for easy and fast semi-automatic segmentation and motion tracking of the myocardium. (ii) An algorithm was developed to automatically exclude from the analysis those myocardial segments with poor image quality. (iii) MCE quantification using the software is likely to be reproducible and less operator dependent compared with existing software and qualitative visual assessment. The diagnostic performance of the MCE quantification software in detecting CAD was evaluated on clinical patients and compared with that of qualitative MCE and single-photon emission computed tomography (SPECT), using coronary angiography as the gold standard.

**METHODS**

**Study population and coronary angiography**

From a cohort of 95 patients who had previously participated in a trial in which patients underwent simultaneous MCE and SPECT after dipyridamole infusion, 18 patients were selected (Senior et al. 2013) for this exploratory study. The demographic characteristics of the patients are summarized in Table 1. The patients underwent rest MCE and SPECT on the same day. These patients also underwent coronary angiography within 1 month of the imaging study on clinical grounds. Patients with CAD were defined as those with ≥70% luminal diameter stenosis of any major epicardial artery or major branch by qualitative coronary angiography. Among the recruited individuals, 9 did not exhibit CAD, 4 had single-vessel disease and 5 had multivessel disease. In total, there were 9 left anterior descending (LAD), 1 left circumflex (LCX), 4 right coronary artery (RCA) and 2 diagonal (DIAG) coronary artery cases of stenosis. The results from coronary angiography are used as the standard for the evaluation of diagnostic performance of the MCE quantification software. The study was approved by the institutional review board, and all patients gave informed consent.

**Myocardial contrast echocardiography**

Myocardial contrast echocardiography was performed using a commercial ultrasound machine iE33 (Philips Medical Systems, Best, Netherlands) and SonoVue (Bracco Research, Geneva, Switzerland) as the contrast agent. Triggered images were recorded within 3–4 min in the three apical views (apical four-chamber, apical two-chamber and apical three-chamber) using low-power MCE (power modulation technique) at a mechanical index of 0.1. The focus was set at the mitral valve level. SonoVue was initially started at 60 mL/h using an infusion syringe pump VueJect (BR-INF 100, Bracco Research), which gently rotates and maintains the contrast agent in homogenous opacification of the myocardium. Thereafter, the rate was set between 48 and 60 mL/h to allow homogenous opacification of the myocardium. Once optimised, the machine settings were held constant throughout each study. Flash-impulse imaging at a high mechanical index (1.0) was performed to achieve complete myocardial bubble destruction, after which end-systolic frames were recorded digitally. All the frames occur at the end-systolic phase of the cardiac cycle (end of T-wave on the electrocardiogram). One MCE sequence typically consists of several end-systolic frames (around 10 frames) which spans the same number of cardiac cycles. Once the resting images were acquired, dipyridamole was infused at 0.56 mg/kg over a 4-min period. After a 2-min interval, peak-stress images were subsequently recorded within 3–4 min of the same procedure.
**MCE quantification**

A specialised software was developed in MATLAB R2014b (The MathWorks, Natick, MA, USA) and used for MCE quantification. Figure 1 summarises the steps taken by the software to analyse each MCE sequence. First, ROIs in the myocardium were semi-automatically detected for each frame of the sequence. Next, the time–intensity data for each ROI were generated and fitted to a mono-exponential perfusion curve model (Wei et al. 1998). An automatic algorithm was also built in to remove data of poor quality from subsequent analysis. Finally, perfusion parameters were extracted from the fitted curve model and used for the clinical diagnosis of CAD.

**Semi-automatic segmentation and tracking of the myocardium.** A graphical user interface (Fig. 2) was developed for the semi-automatic segmentation of the myocardium, as well as the subsequent perfusion quantification. First, a reference frame from the MCE sequence was chosen, and the myocardium was manually segmented as the ROI for that frame. We chose the middle frame of the sequence as the reference when the contrast signals have reappeared in the myocardium. By visual inspection, we also ensured that the chosen reference frame had good image quality and no out-of-plane motion. To account for patient and probe motion during scanning, all frames in the sequence (target frames) were aligned to the reference frame using an intensity-based rigid image registration algorithm (Ashburner and Friston 2011). The registration only allowed rigid body transformation, which includes translation and rotation, resulting in 3 degrees of freedom in 2-D space. Because all the images in the triggered MCE sequence were taken at the same phase (end-systole) of the cardiac cycle, we can assume that the myocardium is a rigid body. The rigid transformation between a pair of target and reference images is found by optimising a similarity metric which we chose to be the sum of squared differences. After registration, the transformations found were used to propagate the manual ROI of the reference frame to the other frames to automatically detect the motion-corrected ROIs of these frames. The software then automatically divided each myocardium ROI into six smaller segments, which comply with the 16-segment model. Note that each patient has three apical chamber views which give rise to a total of 18 segments for analysis. The apical-lateral and apical-anterior segments from the 16-segment model appear twice in these 18 segments. For each segment region, the mean video intensity is computed and the background-subtracted time–intensity curve is subsequently generated. The subtraction removes the background myocardial signals immediately after the flash impulse to normalise the image intensity across sequences. The mono-exponential perfusion model is then fitted to the time–intensity data, and relevant perfusion...
parameters are extracted and computed by the software automatically.

**Perfusion curve modelling and parameter extraction.** The mono-exponential model, developed by Wei et al. (1998), was used to fit the time–intensity data. The model equation is given by

\[ I(t) = A(1 - e^{-\beta t}) \]

where \( A \) is the plateau contrast intensity, which represents the myocardial blood volume, and \( \beta \) is the rate of increase of contrast intensity, which represents the myocardial blood velocity. The product of \( A \) and \( \beta \) gives the myocardial blood flow (MBF). After the model is fitted, relevant perfusion parameters can then be extracted from the fitted perfusion curve. Table 2 lists the perfusion parameters studied. Figure 3 illustrates the mono-exponential model fitted to the time–intensity data of an example myocardial segment, as well as the perfusion parameters extracted from the fitted curve. In addition, we computed the ratio of each parameter at stress over that at rest, which is representative of the coronary flow reserve (Wei et al. 1998).

**Automatic rejection of myocardial segments of poor quality.** Some of the time–intensity data (especially the basal segments) fit poorly to the perfusion curve model. This can be due to poor image quality, noise, uncorrected attenuation and/or non-linear propagation artefacts (Tang et al. 2010; Tang and Eckersley 2006). An algorithm was developed that can automatically identify these segments with poor model fitting and subsequently discard them from the analysis. The coefficient of determination \( R^2 \), the values of which range from 0 to 1, is used as a measure of the goodness of fit of the perfusion model to the time–intensity data. An empirical threshold is set for \( R^2 \). Any segment whose perfusion curve is fitted with \( R^2 \) smaller than the threshold is considered a poor fit and that segment is not used for further analysis. As the \( R^2 \) threshold increases, more segments are considered of poor quality and hence removed from the analysis. However, this also results in better diagnostic performance because only the better-quality data are analysed. We validated with a range of \( R^2 \) threshold values using the area under the receiver operating characteristic.

![Graphical user interface of the myocardial contrast echocardiography perfusion quantification software.](image)

**Table 2.** Perfusion parameters computed for the mono-exponential perfusion model

<table>
<thead>
<tr>
<th>Perfusion parameter</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>( A )</td>
<td>Myocardial blood volume</td>
</tr>
<tr>
<td>( \beta )</td>
<td>Myocardial blood velocity</td>
</tr>
<tr>
<td>MBF</td>
<td>Myocardial blood flow, ( A \times \beta )</td>
</tr>
<tr>
<td>( t_{90} )</td>
<td>Time required to reach 90% of the plateau value, ( A )</td>
</tr>
</tbody>
</table>
(ROC) curve as an indicator of diagnostic performance. It was found that a threshold value of 0.6 gives a good compromise between the diagnostic performance and the amount of data discarded.

**Qualitative MCE and SPECT**

Qualitative MCE and qualitative SPECT were carried out as described in Senior et al. (2013). The 16-segment model was used for all the assessments. Qualitative MCE is done using the following scoring system based on contrast intensity after microbubble destruction: 1 = homogenous opacification, 2 = heterogenous opacification, 3 = minimal or absent contrast opacification. CAD was considered to be present when two contiguous segments exhibited perfusion defects.

Qualitative assessment with SPECT was carried out using the following scoring system: 0 = normal tracer uptake, 1 = mildly reduced tracer uptake, 2 = moderately reduced tracer uptake, 3 = severely reduced tracer uptake and 4 = absent tracer uptake. A rest defect was defined as a score ≥2 in at least one segment. A fixed defect was any resting defect that remained unchanged during stress with an accompanying wall thickening abnormality on gated SPECT. A reversible defect was defined as a reduction in tracer uptake by at least one grade except when the resting score was 0, when the change in score should be ≥2. CAD was considered present when a resting defect and/or a reversible defect were detected in two or more contiguous segments.

For MCE, SPECT and coronary angiography data, on-site analysis performed at the time of the trial was used.

**Statistical analysis**

The perfusion parameters obtained from MCE quantification were validated against coronary angiography as the gold standard. The perfusion parameters for each stenosis group were expressed as the mean ± standard deviation (SD), and their distribution was studied using box-and-whisker diagrams. The two stenosis severity groups were compared using the Mann–Whitney U-test as the spread of the perfusion parameter values is skewed and not normally distributed. A $p$ value < 0.05 was considered to indicate statistical significance. ROC curve analysis was used to determine the optimal cutoff values of the perfusion parameters for the detection of significant stenosis. The diagnostic performance of the parameters was evaluated with the area under the ROC curve. The sensitivity, specificity and concordance at the cutoff value were computed and compared against those of qualitative MCE visual assessments and SPECT. All analyses were done on a vascular territory basis. The vessel was considered to have significant stenosis if two or more myocardial segments that supplied blood to that vessel had significant stenosis.

**Reproducibility study**

The inter-observer and intra-observer variability of MCE quantification using our software was studied. For inter-observer variability, the MCE data of 7 randomly selected patients (126 myocardial segments) were reanalysed using our software by three observers separately. For intra-observer variability, one observer (Y.L.) used the software to re-analyse the MCE data of the same 7 patients more than 3 mo after his first analysis. The variability of the continuous perfusion parameter value was assessed by computing the intra-class correlation coefficient (ICC). ROC curve analysis was used to obtain binary classification (>70% or <70% stenosis) of each myocardial segment.
segment. The variability of the binary CAD stenosis group classification was assessed by computing the percentage agreement and $\kappa$ statistics. For inter-observer variability, the mean percentage agreement and mean $\kappa$ statistics between all pairs of observers were calculated.

**RESULTS**

**Diagnostic performance of quantitative MCE**

Two hundred eighty-two of 288 (98%) myocardial segments were analysed. Among these, 191 (68%) segments were subtended by <70% luminal diameter stenosis, and 91 (32%) segments, by >70% luminal diameter stenosis. Table 3 lists the value of each perfusion parameter during stress/hyperemia as well as their stress/rest ratio for each stenosis group. Figure 4 illustrates the distribution of each perfusion parameter for the two stenosis groups in box-and-whisker diagrams. The diagrams depict skewed and non-normal distributions for all the perfusion parameters. This was further confirmed by a Shapiro–Wilks test, which is used to test for normal distribution. As such, the Mann–Whitney $U$-test was used for comparison of perfusion parameter values between the two stenosis groups because it does not assume a normal distribution.

Receiver operating characteristic curves were generated to obtain the optimal cutoff value for each perfusion parameter. Table 4 compares, on a vascular territory basis, the diagnostic performance of the perfusion parameters at their optimal cutoff values with that of qualitative MCE and SPECT.

**Automatic rejection of myocardial segments of poor quality**

Before application of the rejection algorithm, a total of 282 segments were analysed. The algorithm discarded 77 (27%) segments, which it considered to fit poorly the mono-exponential model. This left 205 (73%) segments for analysis. Figure 5 illustrates a distribution of the 77 discarded segments based on the 16-segment model. Of the 77 discarded segments, 48 (62%) were basal segments, 18 (24%) were mid-segments and 11 (14%) were apical segments. In terms of vascular territories, 13 of a total of 60 vessels (22%) were discarded by the rejection algorithm.

To examine the effect of this automatic rejection algorithm, we used the perfusion parameter, MBF, as an example. Its diagnostic performance improved after the removal of poor-quality segments, as assessed by the increase in sensitivity, specificity and concordance (Table 5). The area under the ROC curve increased from 0.76 to 0.80 after removal of poor-quality segments (Fig. 6). Table 6 also lists a smaller coefficient of variation for the parameter MBF for the two stenosis groups after the rejection algorithm was applied. Similar diagnostic improvements were seen for other perfusion parameters.

**Reproducibility study**

Table 7 outlines the inter-observer and intra-observer variability in the measurements of the perfusion parameters on a segment basis. In general, parameter $A$ had the lowest variability followed by MBF, $t_{90}$ and $\beta$.

Our software achieved relatively high reproducibility compared with some existing software. For instance, our software obtained good reproducibility for the perfusion parameters with ICC values of 0.99 ($A$), 0.93 ($\beta$) and 0.96 (MBF) for intra-observer variability and 0.98 ($A$), 0.91 ($\beta$) and 0.94 (MBF) for inter-observer variability. In contrast, Palmieri et al. (2004) used ECHOPAC software (GE Vingmed Ultrasound A/S, version 2.0) and reported ICC values of 0.91 ($A$), 0.61 ($\beta$) and 0.74 (MBF) for intra-observer variability. Another study by Yang et al. (2012) used QLAB software (Philips Ultrasound, version 6.0) and reported ICC values of 0.950 ($A$), 0.820 ($\beta$) and 0.873 (MBF) for intra-observer variability and 0.950 ($A$), 0.869 ($\beta$) and 0.851 (MBF) for inter-observer variability. Ghanem et al. (2007) also reported high variability for MCE quantification, especially for parameter $\beta$, when using the software tool HDI-Lab (Philips Ultrasound).

In addition, our MCE quantification software also achieved good reproducibility for CAD stenosis group classification. Intra- and inter-observer percentage agreement of MBF using our software was 88% ($k = 0.72$) and 85% ($k = 0.65$) respectively. This is superior to the reproducibility of MCE visual assessment reported in several studies. For instance, Senior et al. (2009b) and Arnold et al. (2010) reported inter-observer agreement of 73% and 81%, respectively. Tsutsui et al. (2005) and Porter et al. (2001) reported inter-observer agreement of 87%

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Table 3. Stress or hyperemic values and the stress/rest ratios of each perfusion parameter for the two stenosis groups

<table>
<thead>
<tr>
<th>Parameter</th>
<th>&lt;70% luminal diameter stenosis (n = 191)</th>
<th>&gt;70% luminal diameter stenosis (n = 91)</th>
<th>$p$ value (Mann–Whitney U-test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stress or hyperemic value</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$A$</td>
<td>$0.71 \pm 0.16^*$</td>
<td>$0.67 \pm 0.14$</td>
<td>0.03</td>
</tr>
<tr>
<td>$\beta$</td>
<td>$3.0 \pm 1.8$</td>
<td>$1.7 \pm 1.1$</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>MBF</td>
<td>$2.1 \pm 1.3$</td>
<td>$1.2 \pm 0.7$</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>$t_{90}$</td>
<td>$1.0 \pm 0.5$</td>
<td>$1.9 \pm 1.7$</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>Stress/rest ratio-reserve</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$A$</td>
<td>$1.2 \pm 0.3$</td>
<td>$1.1 \pm 0.3$</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>$\beta$</td>
<td>$2.1 \pm 1.6$</td>
<td>$1.6 \pm 1.0$</td>
<td>0.006</td>
</tr>
<tr>
<td>MBF</td>
<td>$2.5 \pm 2.0$</td>
<td>$1.7 \pm 1.3$</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>$t_{90}$</td>
<td>$0.83 \pm 0.85$</td>
<td>$1.1 \pm 1.3$</td>
<td>0.006</td>
</tr>
</tbody>
</table>

MBF = myocardial blood flow.

* Mean ± standard deviation.
(κ = 0.41) and 84% (κ = 0.63), respectively. Senior et al. (2004) reported intra- and inter-observer agreement of 80% (κ = 0.72) and 85% (κ = 0.62).

**Software analysis time**

The total time taken to analyse one MCE sequence using our software is about 3 min using a standard PC with Intel Core i7-4770 processor. The software took about 20 s to perform the automatic motion tracking of myocardium and the computation of perfusion parameters. The remaining time was spent by the user on manual segmentation and manual adjustment of ROIs. MCE quantification using this software is fast because of the software’s semi-automatic segmentation features. The main limiting factor was the time taken for manual adjustment of ROIs.

The software developed in this study is available upon request, subject to a general licence, by emailing ultrasound-imaging-group@imperial.ac.uk. The data underlying this article are from a previous industry sponsored study and are not available to protect their commercial confidentiality.

**DISCUSSION**

This study illustrates the use of computer-assisted tools and quantification methods that were specifically

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Table 4. Diagnostic performance of quantitative MCE, qualitative MCE and SPECT on a vascular territory basis

<table>
<thead>
<tr>
<th>Perfusion parameters</th>
<th>Optimal cutoff value</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Concordance</th>
<th>Area under ROC curve</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quantitative MCE (stress value)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>0.58</td>
<td>57% (8/14)</td>
<td>80% (37/46)</td>
<td>75% (45/60)</td>
<td>0.69</td>
</tr>
<tr>
<td>β</td>
<td>1.2</td>
<td>64% (9/14)</td>
<td>98% (45/46)</td>
<td>90% (54/60)</td>
<td>0.84</td>
</tr>
<tr>
<td>MBF</td>
<td>0.90</td>
<td>71% (10/14)</td>
<td>91% (42/46)</td>
<td>87% (52/60)</td>
<td>0.82</td>
</tr>
<tr>
<td>t90</td>
<td>2.0</td>
<td>64% (9/14)</td>
<td>98% (45/46)</td>
<td>90% (54/60)</td>
<td>0.84</td>
</tr>
<tr>
<td>Quantitative MCE (stress/rest ratio-reserve)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>1.0</td>
<td>71% (10/14)</td>
<td>85% (39/46)</td>
<td>82% (49/60)</td>
<td>0.73</td>
</tr>
<tr>
<td>β</td>
<td>1.1</td>
<td>79% (11/14)</td>
<td>78% (36/46)</td>
<td>78% (47/60)</td>
<td>0.76</td>
</tr>
<tr>
<td>MBF</td>
<td>1.4</td>
<td>79% (11/14)</td>
<td>72% (33/46)</td>
<td>73% (44/60)</td>
<td>0.79</td>
</tr>
<tr>
<td>t90</td>
<td>0.93</td>
<td>79% (11/14)</td>
<td>78% (36/46)</td>
<td>78% (47/60)</td>
<td>0.76</td>
</tr>
<tr>
<td>Qualitative MCE</td>
<td>—</td>
<td>79% (11/14)</td>
<td>85% (34/40)</td>
<td>83% (45/54)</td>
<td>—</td>
</tr>
<tr>
<td>SPECT</td>
<td>—</td>
<td>71% (10/14)</td>
<td>90% (36/40)</td>
<td>85% (46/54)</td>
<td>—</td>
</tr>
</tbody>
</table>

MCE = myocardial contrast echocardiography; SPECT = single-photon-emission computed tomography; MBF = myocardial blood flow; ROC = receiver operating characteristic.
developed to improve the accuracy and reproducibility of MCE quantification in detecting CAD stenosis. This software uses a graphical user interface to allow fast semi-automatic segmentation and tracking of the myocardium. It can also automatically detect myocardial segments with poor image quality so that they are removed from subsequent analysis. The main results are summarised as follows: (i) The MCE quantification software had excellent inter (three observers)- and intra (one observer)-reader reproducibility. (ii) The software achieved diagnostic performance comparable to that of qualitative MCE and SPECT. (iii) An MCE sequence could be analysed rapidly. (iv) The algorithm automatically rejected segments of poor image quality, which improved the diagnostic performance of MCE quantification in CAD detection.

Semi-automatic segmentation and tracking of myocardium

For our MCE quantification software, the user selects the entire myocardium in a single frame as a "template" ROI (Fig. 2). This avoids the arbitrary selection of a ROI and constrains the ROI size, shape and location so that it does not vary significantly across different users. This helps to ensure consistency and agreement in the

Table 5. Diagnostic performance of parameter MBF before and after the removal of poor-quality segments on a vascular territory basis

<table>
<thead>
<tr>
<th>Poor-quality segments removed</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Concordance</th>
</tr>
</thead>
<tbody>
<tr>
<td>No</td>
<td>71% (10/14)</td>
<td>91% (42/46)</td>
<td>87% (52/60)</td>
</tr>
<tr>
<td>Yes</td>
<td>77% (10/13)</td>
<td>94% (32/34)</td>
<td>89% (42/47)</td>
</tr>
</tbody>
</table>

ROIs selected and, therefore, improves the reproducibility of MCE quantification. Furthermore, our software also automatically divides the myocardium ROI into six smaller myocardial segments. Thus, the user does not need to manually place six ROIs within the myocardium separately and there is no inconsistency in determining the location of each segment ROI. Finally, our software does not require the user to manually select the ROI for each time frame of the video sequence. Based on the manual myocardium segmentation on one frame, the software uses a rigid registration algorithm to track the

Fig. 5. Distribution of the myocardial segments (16-segment model) discarded by the rejection algorithm at $R^2 = 0.6$.

Fig. 6. Receiver operating characteristic curves for the parameter myocardial blood flow (MBF) before and after the removal of poor-quality segments.
myocardium ROIs in all the other frames automatically. Furthermore, we have observed that our algorithm is robust even in the presence of perfusion defects. This is because the algorithm takes into account the global image statistics when performing image registration. Any regional changes in intensity pattern caused by perfusion defects do not affect the final registration results significantly. All the above semi-automatic segmentation and tracking methods have helped to reduce the variability and operator dependence of MCE quantification, reduce the analysis time and increase the ease of use of our software.

One limitation of our rigid registration algorithm is that it works on the assumption that the myocardial shape remains unchanged throughout the MCE sequence. The algorithm is unable to account for any non-rigid motion of the myocardium. This is currently not a problem for the analysis of our triggered MCE sequences in which the myocardial shape remains approximately unchanged. However, the problem would become prominent if we apply the algorithm to real-time MCE sequences which capture significant myocardial motion and deformations throughout the entire cardiac cycle.

Because our images are highly dynamic, another issue is the effect of contrast reappearance in the myocardium on registration performance. Our algorithm generally works well for most MCE sequences. Contrast changes caused by reperfusion can occasionally affect the registration performance, especially for images at the beginning of the MCE sequence, where low-contrast signals in the myocardium result in very different myocardial appearance compared with images from the later part of the sequence. Furthermore, the existing algorithm still requires manual segmentation of a reference frame.

Table 6. Coefficient of variation of parameter MBF for the two stenosis groups before and after the removal of poor-quality segments

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Before</th>
<th>After</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1.7</td>
<td>1.2</td>
</tr>
<tr>
<td>β</td>
<td>1.5</td>
<td>0.56</td>
</tr>
</tbody>
</table>

Table 7. Inter-observer and intra-observer variability of the stress perfusion parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Intra-class correlation coefficient</th>
<th>Agreement</th>
<th>κ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inter-observer variability</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>0.98</td>
<td>95%</td>
<td>0.86</td>
</tr>
<tr>
<td>β</td>
<td>0.91</td>
<td>85%</td>
<td>0.69</td>
</tr>
<tr>
<td>MBF</td>
<td>0.94</td>
<td>85%</td>
<td>0.65</td>
</tr>
<tr>
<td>tso</td>
<td>0.94</td>
<td>85%</td>
<td>0.69</td>
</tr>
<tr>
<td>Intra-observer variability</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>0.99</td>
<td>98%</td>
<td>0.94</td>
</tr>
<tr>
<td>β</td>
<td>0.93</td>
<td>83%</td>
<td>0.67</td>
</tr>
<tr>
<td>MBF</td>
<td>0.96</td>
<td>88%</td>
<td>0.72</td>
</tr>
<tr>
<td>tso</td>
<td>0.95</td>
<td>84%</td>
<td>0.69</td>
</tr>
</tbody>
</table>

Automatic rejection of myocardial segments of poor quality

Myocardial contrast echocardiography data are subjected to high levels of noise and variability. Image quality can be corrupted by non-linear artefacts, attenuation or out-of-plane motion (Tang et al. 2011). Poor-quality data do not provide useful diagnostic information, but instead reduce the accuracy of the diagnosis. Hence, discarding poor-quality data from our analysis is an important step that will help to improve diagnostic accuracy. The advantage of MCE visual assessment is that experienced cardiologists can easily pick out poor-quality images and not consider them in making their judgment. But computerised MCE quantification should also include the step of automatically detecting and discarding data of poor quality. However, this step is missing in existing MCE quantification software, and the user has to make the extra effort to manually reject myocardial segments of poor image quality (Ghanem et al. 2007; Malm et al. 2006).

The rejection algorithm we implemented is an automated mechanism that can remove such poor-quality myocardial segments from the analysis. Looking at the distribution of the rejected segments in Figure 5, we can see that a larger proportion of basal segments were rejected compared with the mid- and apical segments. This is in agreement with the manual rejection of poor-quality segments in Malm et al. (2006) and Ghanem et al. (2007). We also observed that more anterior segments are rejected compared with inferior segments. This is because the basal segments and anterior segments are more likely to suffer from poor image quality as they are more susceptible to attenuation and artefacts such as rib shadowing (Porter and Xie 2010; Senior et al. 2009a). Including these segments in the analysis often results in the false-positive detection of perfusion defects. Therefore, the overall diagnostic performance and accuracy of MCE quantification improved after the algorithm discarded these poor-quality data. Poor-quality basal
segments are an inherent limitation of the existing MCE imaging. However, there is usually enough adequate information from other segments to allow perfusion assessment by coronary artery territory (Senior et al. 2009a). But we are still likely to obtain inconclusive diagnoses for some vessels which depend more heavily on the analysis of the basal segments. Future MCE imaging techniques using, for example, high-frame-rate imaging and signal processing could significantly improve the overall image quality and potentially reduce the number of rejections.

Reproducibility

The reproducibility assessment indicated that the MCE quantification software developed is marginally dependent on operators and the usage of the software. Both inter-observer variability and intra-observer variability are reasonably low compared with existing MCE quantification software and MCE visual assessment, which are known to be subjective and operator dependent (Ghanem et al. 2007; Ma et al. 2009; Palmieri et al. 2004; Yu et al. 2004). The good reproducibility of our software can be attributed to its computerised automation with a limited and minimal amount of human input. These automations include the semi-automatic and non-arbitrary segmentation of the myocardium ROI and the automatic tracking of the myocardium, which are lacking in existing MCE quantification software. In addition, we observed that parameter A has higher reproducibility than the other parameters which agrees with the findings in Ghanem et al. (2007) and Palmieri et al. (2004). This is so because all other parameters have a temporal dependency. As a triggered MCE sequence captures only the end-systolic frames, the changes and information between these frames are lost. This contributes to greater variability in all temporally derived parameters during curve fitting. Hence, obtaining better-quality data with higher frame rate could be the key to more reproducible MCE quantification. A recent development in ultrafast ultrasound imaging (Tanter and Fink 2014) and its application to contrast-enhanced cardiac imaging (Toulemonde et al. 2016) have made this possible with significant improvement in image quality and temporal resolution. However, there would also be greater challenges in the segmentation and quantification of such data because of the movement of the beating heart and flowing blood. The high computational cost resulting from the significant increase in the amount of data is another problem that needs to be addressed. Future work would be directed at the analysis of such high-frame-rate data which could open up new possibilities for MCE quantification.

Diagnostic performance of MCE quantification

All of the perfusion parameters during stress/hyperemia differed significantly (p < 0.05) between the two stenosis groups. Patients with significant stenosis had significantly lower values of A, β and MBF, which correspond to lower myocardial perfusion. On the contrary, values of t90 were higher because it takes longer for the contrast to refill the myocardium in patients with more severe stenosis. The same trend was observed for the stress/rest ratio of each perfusion parameter where patients with significant stenosis had lower ratio values of A, β and MBF, but higher ratio values of t90. However, the standard deviations of the ratio values were generally greater as more variability is introduced when analysing both the rest and stress MCE data. This variability arises from such factors as different microbubble concentrations during rest and stress, difficulty in maintaining identical imaging planes, inhomogeneous ultrasound fields and attenuation-induced and other artefacts (Wei et al. 2001). If such variability can be minimised, the stress/rest ratio can be a useful diagnostic indicator of coronary blood flow reserve, which is used to assess the presence and severity of CAD (Wei et al. 2001).

In terms of sensitivity, perfusion parameters derived from MCE quantification (ranging from 57% to 71%) generally have lower values than those of qualitative MCE (79%) and SPECT (71%). In terms of specificity, perfusion parameters derived from MCE quantification (ranging from 80% to 98%) generally have higher values than those of qualitative MCE (85%) and SPECT (90%). The accuracy/concordance of MCE quantification (ranging from 75% to 90%) is comparable to that of qualitative MCE (83%) and SPECT (85%). Among the perfusion parameters, MBF has the highest sensitivity, which is comparable to that of SPECT, but inferior to that of qualitative MCE. β and t90 have the highest specificity, which is superior to that of both qualitative MCE and SPECT. β and t90 have the highest concordance, followed by MBF, which are all superior to qualitative MCE and SPECT. Apart from that for A, the areas under the ROC curves for the perfusion parameters range from 0.82 to 0.84.

Parameter A is less accurate than β in distinguishing between the two stenosis groups, because A is solely intensity based, and during image acquisition, A can be affected by various factors such as microbubble concentration and scanner settings. On the other hand, β is a temporally derived parameter that is less susceptible to these factors. As such, biased parameter A estimates would lead to biased MBF values, which offers a possible explanation for the slightly poorer diagnostic performance of MBF compared with β evident in Table 4.

In general, except for that of parameter A, the stress/rest ratios of the parameters did not achieve better diagnostic performance compared with the stress values of the parameters alone. The sensitivity and specificity of the stress/rest ratio of A are higher than those of the parameter A itself. However, for the other parameters,
the ratio has higher sensitivity but lower specificity than the parameters themselves. This is because parameter A is solely an intensity-dependent parameter. Taking the ratio acts as a kind of normalisation, which accounts for some variability in the scanner settings. On the contrary, other temporally dependent parameters are subject to high variability because of the low frame rate at which the data are acquired. Hence, analysing both the rest and stress sequences and taking their ratio will add much more variability, which may not be offset by the effects of normalisation.

In future work, we will test our software on a larger clinical data set to further evaluate its diagnostic performance in CAD detection. Specifically, we are interested in determining the capability of the software in distinguishing between patient groups with stenosis of differing severity. We anticipate that the improved features of our software, such as semi-automatic ROI selection, rejection of poor-quality data and lower operator dependence, can help to improve the capability of diagnosing mild stenosis for early CAD detection.

In conclusion, the MCE quantification software developed can achieve clinically useful diagnostic performance comparable to that of qualitative MCE and SPECT. It is reproducible, rapid and user-friendly.

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REFERENCES


