Inter- and intra-study reproducibility of contrast echocardiography for assessment of interventricular septal wall perfusion rate in humans

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Abstract Aims To assess inter- and intra-study reproducibility of myocardial contrast echocardiography (MCE) imaging for quantitative measurement of myocardial perfusion rate in humans in ambulatory setting.

Methods and results In 20 subjects, we performed 2 MCE tests 20–40 min apart on the same day under the same standardized protocol, and evaluated single-reader between-study and between-reading reproducibility of assessment of indicators of myocardial perfusion rate, such as the slope of video-intensity change $k$, and the factors $A$ (peak video-intensity) and $B$ (background video-intensity after bubble destruction) and the product $k \times A$. The region of interest was placed at the mid-posterior interventricular septal wall visualized in apical 4-chamber view. In a sub-analysis, we evaluated indicators of myocardial perfusion rate comparing subjects with normal vs. those with subnormal ejection fraction (EF). Inter-study reproducibility of assessment of $k$ was relatively low (intraclass correlation coefficient $= 0.36$), whereas intra-study reproducibility was fair (intraclass correlation coefficient $= 0.61$). The parameters $k \times A$ and $B$ showed higher reproducibility than the $k$ (inter- and intra-study intraclass correlation coefficients 0.64 and 0.75, 0.74 and 0.91, respectively). For reference, reproducibility data of the depth of the region of interest, of EF and CO were excellent. $k$ and $k \times A$ were lower in subjects with low vs. those with normal EF. Only $k$ and $k \times A$ were lower in subjects with subnormal than in those with normal EF.

KEYWORDS Echocardiography; Reproducibility; Contrast.

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Non-invasive evaluation of myocardial blood flow represents a major advance in medicine. Myocardial contrast echocardiography (MCE) offers new opportunities for assessment of myocardial perfusion rate thanks to new ultrasound tools and software applications.\(^1\)\(^-\)\(^3\) MCE has been used for semi-quantitative evaluation of heterogeneity of myocardial blood flow in different segments of the left ventricular (LV) walls as consequence of known hemodynamically significant coronary artery disease.\(^4\)\(^,\)\(^5\) In addition, there is great interest to employ MCE to study coronary blood flow reserve to be able to target coronary artery dysfunction in a pre-clinical stage. While the feasibility of MCE is undoubted, reproducibility of quantitative assessment of myocardial perfusion rate by MCE has been tested in experimental setting,\(^6\) but not in patients in an ambulatory setting. Knowledge of this methodological aspect is important. In fact, a method having elevated inter-test variability is not useful for clinical decision-making because of high chance of random between-study difference, and consequently, in research field large study sample size may be needed to dilute the statistical error.\(^7\)\(^-\)\(^9\)

Therefore, we sought to evaluate inter- and intra-study reproducibility of MCE for quantitative assessment of parameters of myocardial perfusion rate.

**Methods**

We performed MCE twice in 20 subjects who were candidate to an echocardiographic exam with clinical indications for heart cavity opacification. No exclusion criteria but arrhythmias and poor echocardiographic images were applied. The study protocol consisted of acquisitions within a single echocardiographic exam of 2 separate sets of MCE images of the same LV walls (LV posterior septal wall in apical 4-chamber view) in each patient by the same sonographer. The first MCE image acquisition was performed before, and the second after a standard echocardiographic exam, 20–40 min apart, to minimize physiologic variability. Standard echocardiographic procedures were followed to visualize LV structures.\(^10\) LV volumes were derived from the 4-chamber apical chamber view of the opacified left ventricles, using the Simpson’s method.\(^11\) Stroke volume (SV) was calculated as end-diastolic volume—end-systolic volume; ejection fraction (EF) was calculated as 100 \(\times\) SV/end-diastolic volume; cardiac output (CO) was calculated as SV \(\times\) heart rate. All subjects gave written informed consent to the study. The study is compliant with the Declaration of Helsinki, and has been approved by local ethics committee.

**Echocardiographic myocardial contrast imaging**

Procedures to acquire and analyze MCE imaging were standardized. SonoVue\(^\circ\) (Bracco) was the echocardiographic contrast agent, consisting of sulphur hexafluoride gas encapsulated in a phospholipid monolayer forming stable microbubbles, with the property of resonating at very low acoustic pressure. All exams were performed by the same sonographer using an echocardiographic machine VIVID 7 (GE Vingmed Ultrasound System, Horten, Norway, software ver. 2.2.1) equipped with a matrix array transducer M3S. Each subject underwent acquisitions of 2 digitalized loops of a 4-chamber apical view of the left ventricle 20–40 min apart. All studies were performed in a time period of 4 months, digitally stored and read 4–10 months later. The reader (VP) performed quantitative analyses in a random order in a time window of 1 month to minimize the likelihood that images of a single patient could be analyzed in the same day.

**Image acquisition protocol**

While the subject was resting on the bed, the right antecubital vein was cannulated and a saline solution was infused continuously. Subsequently, with the subject on the left shoulder, an apical 4-chamber view was obtained with the interventricular septal wall in the center of the 2D sector. This approach avoided that contrast in cardiac cavities could shadow the interventricular septum. The contrast agent was injected manually for a total of 1.2 ml in 1 min (0.2 ml pushed every 10 s), with a saline infusion rate of 50 ml/h. Each MCE acquisition was obtained 10 beats after the end of the contrast infusion. The same procedure was followed 20–40 min after the first MCE acquisition. Therefore, all patients received the same amount of contrast agent per study at a similar infusion.

**Conclusions**

The MCE-derived indicator of myocardial perfusion rate \(k \times A\) showed fairly good between-study and between-reading reproducibility.

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rate, and all MCE acquisitions were obtained with the same delay from the end of the contrast infusion. The ultrasound signal related to the contrast agent was detected and visualized using low ultrasound power, real-time mode based pulse inversion power Doppler technique, with color coding of the signal amplitudes coming from the contrast agent, and was displayed as an overlay signal on top the 2D picture (Fig. 1). The ultrasound transmission frequency was set at 1.5 MHz and ultrasounds were received at 3.3 MHz. Two-dimensional images were acquired at a frame rate of 20 s⁻¹ while the mechanical index was ≤0.06. Color gain was as low as possible since its increase does not change the signal-to-noise ratio. Pulse repetition frequency was set at 3.0 kHz, low velocity reject was at 7.61 cm/s and color sample volume was 0.9 mm (factory set-ups). Subsequently, a transient increase of the mechanical index to 1 (so called “flash”) was used to determine bubble destruction and allow detection of the velocity of the bubble replenishment of the interventricular septal wall. Default flash duration was set at 16 frames at mechanical index equal to 1, which represents the theoretical steady state reached while the perfusion images were acquired (usually 15 beats). A digital loop of the MCE images was stored. Subsequently, the standard echocardiographic exam was performed, at the end of which (20–40 min later), a new MCE study was performed following the same procedure as previously described and the same LV walls were visualized as in the first study. A second set of images of the MCE study was digitally stored.

Readings were performed using the off-line digital review system ECHOPAC (GE Vingmed Ultrasound A/S, Horten, Norway, software ver. 2.0). The software allows ECG-triggered measurement of the video-intensity of the myocardium in a restricted region of interest set as an ellipsoid area 8 mm long and 4 mm wide, placed at mid-interventricular septal wall (Fig. 1). Video-intensity was measured in the region of interest gating the onset of the QRS, since coronary blood flow is greater and wall tension is lower at end-diastole than end-systole. Such a procedure also avoids biases from impaired wall thickening. A tracking function was employed to re-position the region of interest to make allowance for any drift. A standard equation was used to describe myocardial perfusion rate: \( Y(\text{dB}) = A(1 - e^{-kt}) + B \). This function represents the change over time of the video-intensity (Y, dB) in the region of interest, and is characterized by 3 terms: A, B and k. According to the equation, for \( t = 0 \) (i.e. right after the flash), Y is equal to B. Therefore, B represents the background video-intensity in the region of interest after bubble destruction. B is a blood-volume dependent parameter, which is representing myocardial capillaries sectional area, but its values are also function of the amount of contrast agent present in the region of interest after the flash. Therefore, B is influenced by the blood concentration of the circulating contrast agent and by the procedure of bubble destruction, which was fixed at 16 frames at mechanical index equal to 1 for all studies. A is a parameter also depending on the blood volume in the region of interest, and in our setting aiming at the detection of the scattered echo-signal from the bubbles, is therefore dependent on the blood concentration of the contrast agent. For \( t = \infty \), Y = A + B, which represents the theoretical steady state reached by the system assuming negligible destruction of bubbles and constant myocardial blood flow. k is the time-constant describing the velocity of bubble replenishment and therefore function of myocardial perfusion rate. The higher the k the faster is the transition from B to B + A. Assuming almost complete destruction of the bubbles by the flash and negligible bubble destruction at plateau during

![Figure 1](http://ehjcimaging.oxfordjournals.org/) Example of analysis of myocardial perfusion rate. The graph shows the change in video-intensity (Y, dB) over time (X). The graph is obtained as best fit through the discrete data points of measured video-intensity (squared dots in the graph) on ECG trigger (onset of QRS on the X axis).
acquisition, \( B + A \) is function of \( A \). Consequently, while \( k \) is function of the myocardial perfusion rate independent of myocardial blood volume, \( k \times A \) (i.e. \( k \) times \( A \)) is an estimate of myocardial perfusion rate accounting for myocardial blood volume. We analyzed 15 beats from the flash to provide enough data points for estimation of the 3 parameters \( A \), \( B \), and \( k \) by best fitting procedure.

**Statistical analysis**

Paired \( t \)-test was used to evaluate intra-reader between-study as well as intra-reader intra-study reproducibility of assessment of parameters of the wash-in curve, \( A \), \( B \), and \( k \), \( k \times A \), depth of region of interest, EF and CO. Mean and standard deviations as well as absolute difference were estimated. Intraclass correlation coefficients and their 95% confidence interval were used as indicators of reproducibility,\(^{13}\) as in previous study.\(^{10,14}\)

In addition, Bland–Altman method\(^{8}\) was used to verify systematic error across the range of the values of each parameter, plotting mean of the paired sets of measurements vs. their absolute difference. Unpaired \( t \)-test was used to assess differences between subjects with low (\(< 55\%\) vs. those with normal EF (\(\geq 55\%\)). In all cases, a 2-tailed \( p < 0.05 \) indicated the statistical significance.

**Results**

The study sample comprised 20 subjects (3 women) with mean age of \( 67 \pm 15 \) (range \( 49–87 \)) and mean blood pressure \( 141/82 \pm 9/11 \) mmHg. Mean LV internal diameter was \( 5.8 \) cm (range \( 4.9–6.8 \) cm), and mean EF was \( 51\% \) (range \( 34–72\% \)). Of the study sample, 16 subjects had history of arterial hypertension, 8 had history of diabetes, and 12 referred previous myocardial infarction. Wall motion abnormalities were found in 12 subjects, 3 of whom had global wall motion dysfunction. Moderate or severe mitral regurgitation was found in 1 subject whereas none had aortic regurgitation \( \geq 2+ \).

**Reproducibility of assessment of parameters of myocardial perfusion velocity**

Table 1 shows inter- and intra-study reproducibility analyses. The \( k \) was slightly but not significantly higher in the second than the first study

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Test 1</th>
<th>Test 2</th>
<th>Test 1–Test 2 difference</th>
<th>Inter-test reproducibility(^a)</th>
<th>Intra-test reproducibility(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>( k ), ( \text{dB/s} )</td>
<td>2.7 \pm 0.5</td>
<td>2.9 \pm 0.5</td>
<td>(-0.2 \pm 0.5)</td>
<td>0.70 (0.56–0.82)</td>
<td>0.63 (0.45–0.72)</td>
</tr>
<tr>
<td>( A ), ( \text{dB} )</td>
<td>4.4 \pm 2.1</td>
<td>4.4 \pm 2.1</td>
<td>(0.0 \pm 0.3)</td>
<td>0.70 (0.56–0.82)</td>
<td>0.63 (0.45–0.72)</td>
</tr>
<tr>
<td>( B ), ( \text{dB} )</td>
<td>1.65 \pm 0.20</td>
<td>2.3 \pm 0.20</td>
<td>(-0.65 \pm 0.20)</td>
<td>0.70 (0.56–0.82)</td>
<td>0.63 (0.45–0.72)</td>
</tr>
<tr>
<td>Depth of ROI, cm</td>
<td>7.2 \pm 0.6</td>
<td>7.2 \pm 0.6</td>
<td>(0.0 \pm 0.3)</td>
<td>0.70 (0.56–0.82)</td>
<td>0.63 (0.45–0.72)</td>
</tr>
<tr>
<td>EF, %</td>
<td>53 \pm 10</td>
<td>53 \pm 10</td>
<td>(0.0 \pm 0.3)</td>
<td>0.70 (0.56–0.82)</td>
<td>0.63 (0.45–0.72)</td>
</tr>
<tr>
<td>CO, l/min</td>
<td>4.1 \pm 1.1</td>
<td>4.1 \pm 1.1</td>
<td>(0.0 \pm 0.3)</td>
<td>0.70 (0.56–0.82)</td>
<td>0.63 (0.45–0.72)</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>70 \pm 9</td>
<td>70 \pm 9</td>
<td>(0.0 \pm 0.3)</td>
<td>0.70 (0.56–0.82)</td>
<td>0.63 (0.45–0.72)</td>
</tr>
</tbody>
</table>

\(^a\): Intraclass correlation coefficients and their (2.5th, 97.5th) confidence limits.
(p = NS). The inter-study variability of \( k \) was large and intraclass correlation coefficient low. On average, the intra-test intra-reader between-reader intraclass correlation coefficient was higher than the inter-study. As it may be seen in Fig. 2 panel A, a few cases showed significantly higher \( k \) in second than first study, whereas in the panel D it may be appreciated no systematic error of between-reading differences of assessment of \( k \).

On the contrary, both inter- and intra-study reproducibility of assessment of \( B \) was very good. As shown in Fig. 2 panels B and E, no appreciable systematic error was seen in both inter- and intra-study of assessments of \( B \).

The inter- and intra-study reproducibility of \( A \) was both fairly good, and was higher than those obtained for \( k \) but slightly worse than those obtained for \( B \).

The inter-study reproducibility of the product \( k \times A \) was relatively good, and intra-study between-reading reproducibility approached a good level (Table 1). Overall, the reproducibility of assessment of \( k \times A \) was greater than \( k \). Fig. 2, panels C and F, shows no systematic errors for the inter- and intra-study assessments of \( k \times A \).

Since the position of the region of interest on the septal wall may be an issue for reproducibility, we also tested the variability of the placement of the region of interest expressed as depth from the position of the probe on the skin. Findings were that minimal variability was seen with regard to the placement of the region of interest on the septal wall.

Inter-study reproducibility of EF and that of CO were both very good, and the intra-study reproducibility coefficients were excellent (Table 1). There was no significant between-study difference in heart rate.

**Myocardial perfusion rate between subjects with normal vs. those with subnormal ejection fraction**

To verify whether our assessment of parameters of myocardial perfusion rate had pathophysiologic correlates with their physiologic determinants, we divided the study population into those with normal EF (\( \geq 55\% \), \( n = 12 \)) vs. those with subnormal LV systolic function (\( n = 8 \)). Values of \( k \), \( k \times A \), \( A \), \( B \), and EF were those from the 1st MCE test, and the 2 readings were averaged. Compared to those with normal EF, the group with subnormal EF showed lower \( k \) and \( k \times A \), whereas \( B \) and \( A \) did not differ between the 2 groups (Table 2).

**Discussion**

Reliability of assessment of myocardial perfusion rate by MCE needs to be taken into account when MCE is considered for clinical research and decision-making. In our study, under standardized procedures for acquisition and analysis of MCE images to minimize technical sources of variability, we assessed inter-study reproducibility of myocardial perfusion velocity parameters and assessed in parallel the intra-study between-reading reproducibility to verify the extent to which variability due to reading procedures contributes to inter-study variability. Furthermore, we tested reproducibility of assessment of the position of the region of interest at interventricular septum, since difference in depth of the region of interest may determine different degree of shadowing and affect reproducibility data. For reference within the study sample, we also tested reproducibility of assessment of CO and EF, parameters which are physiologically correlated with myocardial perfusion rate.

Under our study protocol, the time-constant \( k \), characterizing the rate of the change of the video-intensity from \( B \) (minimum) to \( A + B \) (maximum), showed relatively high inter-study variability. However, we also found a good reproducibility of the assessment of the product \( k \times A \), a previously recognized most accurate indicator of myocardial perfusion rate. Intra-study reproducibility of \( k \) was higher than the inter-study, suggesting that the reading procedure is likely to be a relatively small component of the sources of inter-study variability of \( k \). Technical sources of variability, other than the reading procedures, include the contrast agent injection procedure and MCE images acquisition procedures. However, we carefully standardized the echocardiographic protocol and performed studies under controlled and similar conditions. In fact, inter- and intra-study reproducibility of assessment of \( B \) was from "good" to "very good". Because \( B \) is the background video-intensity in the region of interest right after the flash, and since the flash was fixed at 16 frames at a mechanical index of 1 for all subjects, \( B \) is mostly expression of the amount of contrast agent present in the myocardium in the region of interest, which was therefore substantially similar between-test. The elevated reproducibility of \( B \) is reassuring on the procedures of MCE images acquisition that we adopted and argues against major role of contrast agent injection procedures as major source of inter-study variability. Moreover, the inter-study reproducibility performance of
Figure 2  Bland–Altman tests. The 3 upper panels A, B and C show plots of between-study mean values of \( k \), \( B \) and \( k \times A \), respectively, vs. between-study differences (value in study 1—value in study 2). The lower 3 panels D, E and F show plots of between-reading mean values of \( k \), \( B \) and \( k \times A \), respectively, vs. between-reading differences (value in reading 1—value in reading 2).
the assessment of $A$ was greater than that of the assessment of $k$. $A$ is another parameter that can be influenced by the blood concentration of the contrast agent because it is blood-volume dependent. Since we were receiving scattered echo-signal at 3.3 MHz, and therefore most likely to be generated by the contrast agent, $A$ can be function of the blood concentration of the contrast agent. Therefore, our results on reproducibility of $A$ and $B$ argue against a major role of contrast agent injection procedures as source of inter-study variability. In addition, bolus injection of echocardiographic contrast agents has been used successfully in clinical studies testing accuracy of MCE to evaluate coronary heart disease in comparison to nuclear medicine. The reasons for relatively low reproducibility of $k$ are to be understood. In a previous study in experimental setting, reproducibility of measurement of $A$ and $k$ has been found optimal in vitro and in vivo, with values of $k$ ranging between 0.18 and 0.25. However, in vivo data were obtained in open-chest dogs anesthetized and ventilated with the ultrasound transducer placed on a standoff consisting of latex bag filled with saline solution and positioned on the epicardium of the LV anterior wall. Contrariwise, we tested our hypothesis in ambulatory patients.

Another aspect of our study protocol was that we choose to trigger end-diastolic frames rather than end-systolic frames. Coronary blood flow is greater and wall stress is lower in diastole than in systole, and onset of QRS allows objective triggering. Therefore, as demonstrated in experimental setting, video-intensity signal/noise should be highest in end-diastole. Nevertheless, if wall thickening is preserved, septal wall is thicker and better seen in systole, which may facilitate the reading procedure. However, impairment or asynchrony of wall thickening is associated with reduced regional myocardial perfusion rate even in presence of normal coronary blood flow. In an exploratory analysis in a subgroup of 7 patients (data not shown), we re-analyzed myocardial perfusion rate using end-systolic triggering, and found only a marginal trend toward improvement of reproducibility of $k$ and $A$ while the 95% confidence intervals of their intraclass correlation coefficients remained wide; with regard to $B$, findings were not substantially different from those shown in Table 1.

In the attempt of understanding other possible sources of variability of the MCE parameters of myocardial perfusion rate, the inter- and intra-study variability of EF and CO was measured in parallel with the parameters of myocardial perfusion rate. Not surprisingly, our results showed excellent reproducibility of EF and CO. In addition, to test whether in our study sample, MCE-derived parameters of myocardial perfusion rate had expected pathophysiologic correlates, we performed a sub-analysis dividing our study sample into those with normal vs. those with subnormal EF. Indeed, low EF was associated with lower myocardial perfusion velocity as physiologically expected and previously reported in literature. The random sequence of readings excluded any potential bias.

### Study limitations

This study comprises a relatively small series of patients. In addition, because we focused only on the interventricular septal wall visualized in apical 4-chamber view, we did not consider alternative views and walls, our setting may be considered as best scenario. By design, the present study did not assess inter-study reproducibility of myocardial perfusion reserve in consecutive MCE evaluations, which requires a controlled and constant injection rate of the contrast agent, but in 2 studies in the same patient 20–40 min apart. Therefore, our findings might not be extrapolated to consecutive MCE acquisitions using contrast infusion by a pump. Moreover, our infusion protocol may not be applicable to stress-echocardiography. However, our goal was to assess reproducibility of MCE for estimation of myocardial perfusion rate under controlled and extremely simple conditions that can be easily implemented in clinical settings such as evaluation of flow-wall motion matching in case of evaluation of chest pain or before and after revascularization procedure. The fact that reproducibility performance indices of the parameters $B$ and $A$ were in the range of good-to-very good, is reassuring with

#### Table 2  Myocardial perfusion rate in subjects with normal vs. abnormal ejection fraction

<table>
<thead>
<tr>
<th></th>
<th>Low EF ($n = 8$)</th>
<th>$p$</th>
<th>Normal EF ($n = 12$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k$</td>
<td>$0.58 \pm 0.39$</td>
<td>$&lt; 0.05$</td>
<td>$0.96 \pm 0.26$</td>
</tr>
<tr>
<td>$k \times A$, dB/s</td>
<td>$1.6 \pm 1.5$</td>
<td>$&lt; 0.05$</td>
<td>$4.6 \pm 2.8$</td>
</tr>
<tr>
<td>$A$, dB</td>
<td>$4.7 \pm 3.6$</td>
<td>NS</td>
<td>$5.0 \pm 4.2$</td>
</tr>
<tr>
<td>$B$, dB</td>
<td>$2.4 \pm 2.8$</td>
<td>NS</td>
<td>$1.3 \pm 1.2$</td>
</tr>
</tbody>
</table>

Values in table are mean ± standard deviation. EF, ejection fraction (low EF = $< 55\%$). $A$, $B$ and $k$ are part of the equation describing myocardial replenishment by microbubbles after their destruction by transient acoustic energy increase (flash): $k$ is the time-constant, indicator of myocardial perfusion rate blood-volume independent. $k \times A$ is a blood-volume dependent parameter of myocardial perfusion rate.
regard to the impact of variability of contrast agent injection procedure on our findings.

Conclusions

Using a simple and clinically feasible standardized protocol for MCE imaging, we found a relatively good reproducibility of the parameter of myocardial perfusion rate $k \times A$. MCE was able to detect differences in myocardial perfusion rate between subjects with low vs. those with normal EF as pathophysiologically expected. However, our data suggest that MCE may be reliable for assessment of myocardial perfusion rate in cohorts, whereas in single subjects, the MCE may be affected by excessive variability that represents impairment for the capability of the method to detect relatively small inter-study changes of the myocardial perfusion rate intra-subject.

References


