Myocardial Contrast Echocardiography With a New Calibration Method Can Estimate Myocardial Viability in Patients With Myocardial Infarction

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OBJECTIVES We have developed a novel calibration technique applicable for myocardial contrast echocardiography (MCE). We assessed the value of this technique in the recognition of myocardial infarction (MI) and its spatial extent, and we also performed a validation study in normal subjects.

BACKGROUND The heterogeneity of contrast intensity (CI) among myocardial segments limits the clinical use of MCE.

METHODS We performed MCE with a slow-bolus injection of Levovist and recorded end-systolic harmonic power Doppler images at intervals of four heart beats in 15 normal volunteers and 30 patients with MI. We divided the left ventricular (LV) wall into 12 segments and placed the region of interest in the subendocardial region in each segment and in the adjacent LV cavity. We measured calibrated CI (dB) by subtracting the cavity CI from myocardial CI.

RESULTS The mean intersegmental difference in myocardial CI was 15.8 dB at baseline, whereas it was reduced to 6.3 dB after calibration (p < 0.01). Calibrated CI was higher in the kinetic segments than in the akinetic segments (−14.5 ± 2.3 dB [range −18.7 to −9.9 dB] vs. −22.5 ± 2.6 dB [−27.8 to −17.7 dB], p < 0.001), and −18.0 dB was the optimal cutoff point to discriminate these from each other. Color-coded mapping of calibrated CI may identify the spatial extent of persistently akinetic myocardium as areas of calibrated CI of ≤−18.0 dB.

CONCLUSIONS This new calibration method reduces the intersegmental difference in CI in normal subjects. Calibrated CI provides an estimate of persistently akinetic myocardium in patients with MI, and its color-coded mapping is comprehensive and identifies the spatial extent of MI. (J Am Coll Cardiol 2004;43:1799–806) © 2004 by the American College of Cardiology Foundation
The inclusion criteria were: 1) confirmed acute MI; and 2) percutaneous coronary intervention (PCI) successfully performed in the acute stage. Patients with severe valvular disease and/or atrial fibrillation were not included. Fifteen normal volunteers were also examined to obtain normal values of CI (all male; mean age 33 ± 6 years). The study protocol was approved by the ethics committee of our hospital. All volunteers and patients gave written, informed consent to participate in this study.

**Study protocol.** The MCE examination was performed two weeks after PCI. The SONOS 5500 (Philips Medical Systems, Andover, Massachusetts) with an S3 probe was used. We depicted the apical four- and two-chamber views with harmonic power Doppler (HPD) imaging (6,16–20), and ultrasound was transmitted at 1.8 MHz and received at 3.6 MHz. Focus was placed at the mitral level, and the mechanical index and dynamic range were set to maximum values—1.6 and 40 dB, respectively. Overall, gain was adjusted to minimize artifacts on the baseline study.

A solution of Levovist (concentration of 300 mg/ml, Schering Japan, Osaka, Japan) (16,19–21) was administered as a bolus (3 ml) at a rate of 0.5 ml/s with a volumetric pump (PULSAR, Medrad, Indianapolis, Indiana). The MCE images were recorded for at least 180 s after the contrast injection. End-systolic images were obtained every four heart beats and stored on a magneto-optical disk.

Imaging was performed with two triggers (multiframe triggering mode). The first pulse was used for myocardial opacification. The second pulse was used only for bubble destruction to assess the quality of images, and we reduced motion artifact as much as possible by adjusting the triggering time. This technique might have an influence on MCE imaging, but minimizing artifact is essential for the quantitative analysis. Two-dimensional echocardiography was repeated at a mean of five months (range three to seven months) after PCI.

**Analysis of MCE data.** The MCE images were analyzed off-line using a newly developed image analyzing system (VoluMap–445, YD, Ikoma, Japan). This system retrieves MCE images from a magneto-optical disk. The LV myocardium was divided into six segments by using both apical two- and four-chamber views, according to the segmentation proposed by the American Society of Echocardiography. We placed the variable size of the region of interest (ROI) (3 mm width) in the subendocardial layer of each segment and in each adjacent LV cavity to measure CI (Fig. 1). The distance between these was about 3 mm. The CI of any pixel in ROI was confirmed within the dynamic range, and we measured the mean CI of each ROI. We analyzed MCE images obtained 60 and 100 s after injection in normal subjects to determine an appropriate time for the correction. At each myocardial segment, the calibrated CI was determined as the difference (dB) between the mean myocardial CI and cavity CI. Because the measurements are made in decibels, we subtracted cavity value from myocardial value to derive a relative blood volume fraction. Calculation was not performed if any of the following was present: 1) CI of LV cavity beyond the dynamic range; 2) absolutely no contrast enhancement—called “dropout”; and 3) apparent motion artifacts.

This system constructs a color-coded map of calibrated CI with semi-automatic ROI. Apical two- or four-chamber

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**Abbreviations and Acronyms**

- **CI** = contrast intensity
- **HPD** = harmonic power Doppler
- **LV** = left ventricular
- **MBV** = myocardial blood volume
- **MCE** = myocardial contrast echocardiography
- **MI** = myocardial infarction
- **PCI** = percutaneous coronary intervention
- **ROI** = region of interest

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**Figure 1.** Measurements of contrast intensity (CI) in the left ventricular (LV) myocardium and adjacent LV cavity on the myocardial contrast echocardiography (MCE) image. The LV myocardium was divided into six segments in each apical four- and two-chamber view (left). As shown in an HPD image of the apical four-chamber view (right), for example, the ROIs (12 blue ovoid shapes in the image) were placed in each segment and each adjacent LV cavity. See text for details. Myocardial segments: AA = apical anterior; AL = apical inferior; AL = apical lateral; AS = apical septum; BA = basal anterior; BI = basal inferior; BL = basal lateral; BS = basal septum; MA = mid-anterior; MI = mid-inferior; ML = mid-lateral; MS = mid-septum.
views were used. We traced endocardial and epicardial borders and set the long-axis of the LV cavity. The software divides myocardial segments into layers of 5 mm along the long axis. It establishes a sample width of 3 mm along the endocardial border in both the subendocardial layer and LV cavity. After determining that the CI of all pixels within the sample width is within the dynamic range that is automatically shown, the CI was measured in each sample width of 3 mm on both sides of the endocardial border in every layer. Finally, calibrated CI in the subendocardial layer was calculated and superimposed on the myocardial segment of each layer, using a color-coded system. Values of calibrated CI in the subendocardial layer in the corresponding segments are indicated by different colors every 2 dB.

Analysis of wall motion. Wall motion in the apical four- and two-chamber views was analyzed by two experienced observers blinded to all clinical information. The segments ascribed to each of the three vascular bed territories are mentioned by the American Society of Echocardiography. A myocardial segment was defined as “persistently akinetic” if it showed akinesia or dyskinesia at a mean of five months. Other segments, the majority of which were normal, were defined as kinetic.

Statistical analysis. Data are expressed as the mean value ± SD. The Student paired t test was used to compare two continuous variables. Univariate differences of variables between groups were determined by one-way analysis of variance (Scheffé F test) for continuous variables. The chi-square test was used to compare categorical variables. For differences, a value of p < 0.05 (two-sided) was considered statistically significant. Interobserver and intraobserver variabilities were assessed for measuring calibrated CI in 30 randomly selected segments. Interobserver variability was calculated as the standard deviation of the differences between the measurements made by two independent observers who were unaware of the other patient data and expressed as a percentage of the average value. Intraobserver variability was calculated as the standard deviation of the differences between the first and second determinations (3-week interval) for a single observer and expressed as a percentage of the average value.

RESULTS

Reproducibility of data. Interobserver variability for measuring calibrated CI was 7.3%. Intraobserver variability for measuring calibrated CI was 8.5%.

Study in normal volunteers. Among 180 myocardial segments in 15 normal volunteers, quantitative CI analysis was possible in 170 segments (94%) at 60 s after injection. The number of analyzable segments was reduced to 151 (84%) at 100 s after injection. Contrast enhancement disappeared in some normal contractile areas by 100 s after injection, and this was a main cause of failed CI analysis. It was sometimes hard to analyze CI of the basal segments. Myocardial CI was lower at 100 s than at 60 s after injection (p < 0.01), but calibrated CI was not different between these (Fig. 2). Therefore, we used MCE images at 60 s after injection for further analysis.

Figure 3 shows values of myocardial and calibrated CI of each myocardial segment in the apical four- and two-chamber views. Even in normal volunteers, myocardial CI was different, depending on the depth of myocardial segments. It was 19.9 ± 2.1 dB in the apical segments, 12.4 ± 2.1 dB in the middle segments, and 6.0 ± 2.2 dB in the basal segments. Intersegmental differences in myocardial CI were 15.8 ± 2.1 dB in the four-chamber view and 15.5 ± 2.3 dB in the two-chamber view. Calibrated CI was −12.0 ± 1.7 dB in the apical segments, −14.7 ± 1.8 dB in the middle segments, and −16.8 ± 1.9 dB in the basal segments. After calibration, the intersegmental differences were reduced (p < 0.01) to 6.3 ± 1.5 dB in the four-
chamber view and 5.8 ± 1.2 dB in the two-chamber view. The calibrated CI value for all normal segments was −14.5 ± 2.3 dB.

Characteristics of patients with MI. Of the 30 patients (mean age 58 ± 11 years), 25 were male and 5 were female. Twenty-one patients had anterior MI and nine had inferior MI. The mean time from symptom onset to coronary reperfusion was 7.6 ± 5.2 h. Of all 360 myocardial segments, 69 (19%) were akinetic on the two-dimensional echocardiogram at two weeks after PCI and 61 (17%) were akinetic five months after PCI. Infarct-related arteries were patent in all patients, as shown by coronary angiography performed five months later.

Myocardial CI in infarcted segments. Quantitative CI analysis was possible in 218 (75%) of 291 kinetic segments and 59 (86%) of 69 akinetic segments. Most of the dropout segments were in the basal segments. Myocardial CI was significantly lower in the akinetic segments (7.4 ± 2.6 dB [range 3.5 to 12.0 dB]) than in the kinetic segments (12.8 ± 5.8 dB [range 4.2 to 20.9 dB], p < 0.01), but there was a significant overlap between these segments (Fig. 4). Calibrated CI was also significantly lower in the akinetic segments (−22.5 ± 2.6 dB [range −27.8 to −17.7 dB]) than in the kinetic segments (−14.5 ± 2.3 dB [−18.7 to −9.9 dB], p < 0.001), and the overlap was small. Receiver-operating characteristic curve analysis documented that −18.0 dB is an appropriate cutoff point to discriminate akinetic segments from kinetic segments (97% sensitivity, 97% specificity).

Color-coded display to determine spatial extent of MI. We attempted to make a color-coded map of calibrated CI in each patient by using the VoluMap-445. Based on the cutoff value, we colored the segments with calibrated CI (in the subendocardial layer) of ≤−18.0 dB in cool colors, and the segments with calibrated CI >−18.0 dB in warm colors. Figure 5 shows a representative image of a patient with normal contraction, showing all myocardial segments colored in warm colors. Figure 6 shows a representative...
image of a patient with anterior MI. Wall motion of the mid-septum and apical region was akinetic, and VoluMap analysis documented cool colors in the corresponding zone. Based on the color-coded analysis, we compared the relationship between the calibrated CI and remote-stage asynchrony (Table 1). There was a significant relationship between two variables (kappa value 0.86). The majority of kinetic segments (94%) showed calibrated CI $\geq -18.0$ dB. All persistently akinetic segments showed calibrated CI of $\leq -18.0$ dB. Thus, we could clearly predict the irreversibly damaged myocardial areas and their spatial extent with VoluMap.

**DISCUSSION**

**Advantages of our calibration method.** Even though the content of bubbles should be the same among all myocardial segments in normal subjects, myocardial CI varied significantly, with the highest value in the apical segments and the lowest value in the basal segments. The maximal intersegmental difference in myocardial CI before calibration reached as much as 15.8 dB (about 40 times) in normal subjects, but it was significantly reduced to 6.3 dB (about 4 times) after calibration. Additionally, this calibration method provides an estimate of MBV. Because the measurements of CI are made in decibels, subtraction of the blood value from the myocardial value is equivalent to the acoustic intensity to derive a relative blood volume fraction of myocardium. By doing this process automatically, we drew the color-coded map of calibrated CI, which may represent a map of microvascular damage. The advantages of this calibration method using HPD-triggered imaging are as follows: 1) this parameter is relatively independent of bubble concentration, so long as CI is within a dynamic range; 2) HPD is a feasible technique, because its signal originates exclusively from the bubbles, unless there is an artifact (6,16–20); and 3) although human data are limited (22), the physiologic range of MBV is documented well in animal studies (23,24). Theoretically, infarcted myocardium can be depicted as segments showing calibrated CI lower than the normal range.

**MBV and viability.** Because calibrated CI correlates well with MBV, it may be used for the assessment of myocardial viability. Traditionally, myocardial viability has been measured through a direct or indirect measurement of myocyte function, cell membrane or mitochondrial function, metabolic activity, and contractile reserve. Another way is the measurement of capillary density (25). If no or at most a few capillaries are present within the myocardium, it is very unlikely that sufficient oxygen is delivered to the myocytes within the region (26,27). Experimental studies documented that the size of contrast defect showing no-reflow phenomenon correlates well with the size of myocardial necrosis. Because MCE may be used to detect this process in situ, it is not surprising that calibrated CI reflects myocardial viability in patients with MI. Calibrated CI was significantly higher in the kinetic segments, most of which represent the normal myocardium, than in the persistently akinetic segments.

The range of MBV in the normal myocardium in humans remains unknown. If the concentration of bubbles in the blood is constant in the systemic circulation, the difference in CI between the myocardium and LV cavity should be proportional to the difference in blood fraction. We measured CI in decibels; thus, the subtraction of the cavity CI

**Figure 4.** Comparisons of myocardial contrast intensity (CI) and calibrated CI among myocardial segments in normal subjects, as well as kinetic and akinetic segments in patients with myocardial infarction (MI). Data points indicate all values of myocardial CI (left) and calibrated CI (right). Rectangles and vertical lines indicate the mean value ± SD. Myocardial CI at the akinetic segments was significantly lower than that at the normal and kinetic segments, although it was almost the same at normal and kinetic segments. However, there was a large overlap of data ranges between kinetic and akinetic segments. On the other hand, calibrated CI at the akinetic segments was also significantly lower than that at the other segments. Here, it should be noted that the overlap was obviously small compared with that in plain myocardial CI. It is expected that they will be easily distinguished after the calibration.
from the myocardial CI is equivalent to taking the ratio of these. The calibrated CI is expressed as “negative dB,” but it implies a ratio $<1.0$. Because the LV cavity consists of 100% blood, we estimated MBV (ml/100 g myocardium) of corresponding myocardial segment using the following formula:

$$\text{MBV/100} = 10^{(\text{calibrated CI}/10)}$$

The calibrated CI of normal myocardium ($-14.5 \pm 2.2$ dB) implies an MBV of 4.0 ± 2.2 ml/100 g myocardium in the subendocardial layer. In dogs or pigs, the entire coronary system, which includes epicardial conduit arteries, arterioles, capillaries, venules, and veins, contains about 12 ml of blood/100 g myocardium, and it is one-third in the capillaries (24). The blood present in the LV myocardial vessels, if it is defined as MBV, may reach about 4.5 ml/100 g myocardium, and this is comparable to our human data in this study. The calibrated CI in the subendocardial layer of persistently akinetic segment ($-22.5 \pm 2.6$ dB) was significantly lower, and the estimated MBV was 0.6 ± 0.3 ml/100 g myocardium. This is about 15% of MBV in the normal myocardial tissue. However, further studies are required to determine whether our method truly correlates with MBV measured with the other methods.

Based on such background, we have developed software that builds a map of calibrated CI on the myocardial MCE images. In this map, kinetic and persistently akinetic myocardium is clearly discriminated in a single MCE image. We used the calibrated CI $\leq -18.0$ dB as a cutoff point for discrimination. For a better understanding, we colored the segments with calibrated CI $\leq -18.0$ dB as blue and black, and those with calibrated CI $> -18.0$ dB as white and red. The segments with calibrated CI $\leq -18.0$ dB were judged as persistently akinetic in a follow-up study, with high sensitivity and specificity. In the color-coded display, the color of each myocardial segment represents the calibrated CI of the subendocardial layer, not the mean value of whole layer. We focused on the calibrated CI in the subendocardial layer, because its myocardial damage is the most severe and the correction may be incomplete in the epicardial layer. Thus, we can assess the spatial extent of irreversibly dam-

**Figure 5.** Color-coded map of calibrated contrast intensity (CI) in a patient with normal contraction. (Left) An HPD image in the apical four-chamber view was analyzed. (Right) In this case, all segments showed warm colors, implying calibrated CI $> -18.0$ dB. Note that the box colors indicate calibrated CI in the subendocardial layer, not the averaged value of the segment. See text for details.

**Figure 6.** Color-coded map of calibrated contrast intensity (CI) in a patient with anterior myocardial infarction (MI). (Left) An HPD image in the apical four-chamber view shows a reduced contrast enhancement zone extending from the distal septum to cardiac apex. (Right) After the analysis with VoluMap, the mid-septum to apical region shows cool colors, indicating calibrated CI $\leq -18.0$ dB. See text for details.
aged myocardium with ease and high reliability using this technique.

**Study limitations.** Several technical limitations need to be addressed. First, there is a dynamic range (40 dB) before which system saturation occurs. If the CI in the LV cavity is beyond the upper limit of the dynamic range, the calibrated CI is underestimated. A similar case occurs when myocardial CI is lower than the lower limit of the dynamic range. We routinely checked that all CI values are within the dynamic range before the quantitative analysis. The pulsing interval, every four heartbeats, may be so short that capillaries cannot be filled with bubbles, and a longer pulsing interval is required to measure MBV. However, it is practically hard to keep the same position and angulation of the transducer with a long pulsing interval.

The HPD signal is affected by several artifacts, including motion artifact, blooming, and shadowing. Motion artifact and blooming could affect myocardial CI. We used the multiframe triggering method to check and minimize motion artifact by adjusting trigger timing.

Our calibration method could substantially reduce an intersegmental difference in CI, but the correction was not perfect. Calibrated CI was still higher in the apical segments than in the basal segments. A possible difference in the attenuation of ultrasound beams between passing through the myocardium and LV cavity, time point of measurement, blood stream in the LV cavity, and focus position might explain the incomplete correction.

By bolus injection, the timing of peak concentration in the LV cavity is earlier than that in the myocardium, and thus continuous infusion might be preferable. However, we could not obtain an adequate HPD signal with continuous infusion in our echocardiograph apparatus. Continuous infusion of contrast agent is preferable for accurate quantitative analysis. Although many advantages of continuous infusion exist, an obvious advantage of bolus injection is that it can be performed in a rapid manner to provide an immediate qualitative assessment with a peak contrast effect (28).

In estimating MBV from the calibrated CI, we hypothesized that the bubble concentration in blood is the same between the LV cavity and coronary microcirculation. The hematocrit of the capillaries is reported to be lower than that in the systemic circulation (29,30). Because bubble behavior is similar to that of red blood cells in the microcirculation, there may be an underestimation of this technique.

Although several problems still remain uncertain, at least in this study, we documented that MCE with the noble correction technique can provide quantitative and accurate evaluation of myocardial infarction in an ordinary clinical setting. With the advancement of a high-resolution imaging modality and improvement of the analyzing process, we could obtain full transmural information on a pixel-by-pixel basis rather than simply on subendocardial information.

**Table 1. Relationship Between Calibrated Contrast Intensity and Remote-Stage Asynergy**

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<tr>
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<th>Kinetic Segments (n = 226)</th>
<th>Persistently Akinetic Segments (n = 51)</th>
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<tbody>
<tr>
<td>Calibrated CI of more than −18.0 dB (n = 213)</td>
<td>213</td>
<td>0</td>
</tr>
<tr>
<td>Calibrated CI of −18.0 dB or less (n = 64)</td>
<td>13</td>
<td>51</td>
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</tbody>
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Kappa value = 0.86. CI = contrast intensity.

**REFERENCES**


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