Assessment of Location and Size of Myocardial Infarction With Contrast-Enhanced Echocardiography. II. Application of Digital Imaging Techniques

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Contrast echocardiography can be used to identify non-perfused regions of myocardium and localize and quantify infarcted myocardium. Analysis is usually undertaken by visual inspection of an analog two-dimensional echocardiographic image. The purpose of our study was to apply digital imaging techniques to contrast-enhanced echocardiograms for the determination of myocardial infarct size. Myocardial contrast was produced by an injection into the aortic root of a mixture of hydrogen peroxide and blood. Sixteen open chest dogs were studied 4 hours after coronary artery occlusion. Echocardiograms were evaluated by two independent observers. The results were compared with infarct location and size determined with nitro-blue tetrazolium staining of the corresponding slice of the left ventricle. Both the routine analog echocardiographic image and the digital subtraction image were analyzed. For the latter, three pre-contrast and three postcontrast echocardiographic end-diastolic fields were digitized in a 256 × 256 × 6 bit matrix and then averaged. Average pre- and postcontrast images were mathematically subtracted to form the digital subtraction image.

There was excellent correlation between the percent of infarct determined with digital subtraction contrast echocardiography and results of nitro-blue tetrazolium staining (r = 0.97, SEE = 0.04, p < 0.001). Using linear regression, the relation between infarct size by the two studies was best described by the equation DSI = 0.92 NBT + 0.03, where DSI = digital subtraction image and NBT = infarct size by nitro-blue tetrazolium. Inter- and intraobserver variability were also excellent (r = 0.93 and 0.96, respectively).

It is concluded that the digital imaging technique can be applied to contrast-enhanced echocardiography and is an accurate and reproducible method for determining infarct percent in a single slice of the left ventricle.

Contrast echocardiography, using either intracoronary or aortic root injections of a variety of newly developed contrast agents, has been demonstrated to be an accurate means of localizing myocardial perfusion defects in canine models of coronary occlusion (1–6). Analysis of these images has involved visual inspection of the echocardiographic image, coupled with planimetry to quantify the regions of perfused and nonperfused myocardium. The newer technique of digital subtraction imaging has proven to be a valuable adjunct for radiographic examinations (7). Maurer and Tei and their coworkers (8,9) recently applied digital imaging techniques.
to contrast echocardiography for the study of myocardial perfusion. The purpose of this study was to apply digital imaging techniques to two-dimensional echocardiograms and assess the accuracy of digital subtraction imaging for the determination of myocardial infarct size from contrast-enhanced echocardiograms. In addition, we compared digital subtraction imaging to our previously reported analog image analysis with respect to determination of infarct size and inter- and intraobserver variability (3).

**Methods**

**Animal preparation.** Sixteen mongrel dogs (18 to 26 kg) were sedated and maintained with sodium pentobarbital anesthesia. They were ventilated using a Harvard veterinary respirator and a standard endotrachial tube. The heart was exposed through a right thoracotomy and the pericardium was entered. A snare was placed on the left anterior descending (nine dogs) or circumflex (seven dogs) coronary artery for future occlusion. The arterial pressure and electrocardiogram were monitored continuously. A 7 French straight aortic catheter with one end hole and four side holes was advanced from the right femoral artery into the aortic root. Its position was confirmed by palpation and echocardiographic visualization.

**Contrast echocardiography.** Ultrasound contrast was obtained by mixing 3 ml of 0.3% hydrogen peroxide with 6 ml of the dog's blood in a 10 ml syringe and then immediately injecting this into the aortic root as a bolus (3, 5, 10, 11). After recording a baseline contrast echocardiogram, the snare was tightened to totally occlude the coronary artery. Dogs were pretreated with 2 mg/kg lidocaine and maintained with a 2 mg/min lidocaine infusion. After 4 hours of coronary occlusion, the echocardiographic transducer position was fixed using a stand attached to the procedure table, and final pre- and postcontrast two-dimensional echocardiograms were recorded. All echocardiograms were recorded on videotape (VHS format) using commercially available two-dimensional scanners (Advanced Technology Laboratory) and a 5 MHz transducer. The transducer was placed directly on the free wall of the right ventricle; an acoustic standoff was not used.

**Infarct size determination.** After recording the final echocardiogram, needles were passed along the plane of the echocardiographic examination to mark the slice of the left ventricle for analysis. Saturated potassium chloride was injected into the right ventricle to induce ventricular fibrillation and fix the heart in diastole. The heart was then removed, flushed with cold water and sliced in a breadloaf fashion. The slices were then incubated for 5 minutes in nitro-blue tetrazolium to mark the region of myocardial infarction (12). This slice was then photographed. The photographic image was projected, and the epicardial and endocardial contours traced. From the epicardial and endocardial areas, the total myocardial area was calculated. The area of the infarct, as delineated by nitro-blue tetrazolium, was also traced and its area calculated. The fraction of infarcted myocardium in the single slice was then calculated as: area of infarcted myocardium divided by total myocardial area. In this study, no attempt was made to reconstruct the three-dimensional geometry of the infarct or the total mass of the left ventricle infarcted.

**Echocardiographic analysis system.** Echocardiograms were evaluated by two independent observers. One observer analyzed each echocardiogram on two separate occasions. Both the routine analog image from the videotape and the digital subtraction image (see later) were evaluated. The analysis system consisted of a commercially available microprocessor-based off-line echocardiographic viewing system. The digital imaging system included an IBM microprocessor (personal computer model #5150) and a high speed analog to digital converter that is capable of digitizing echocardiographic fields in real time (Micro Sonics, Inc.). The analog image is digitized at 3.9 MHz; thus, 16.7 ms is required to digitize an echocardiographic field in a 256 x 256 matrix. Because we need a 256 x 256 format, which does not conform precisely to the dimensions of the recorded analog image, the lateral, superior and inferior image margins were reduced. This resulted in the loss of screen width and identification markings, but not of the actual echocardiographic image. Echocardiographic fields were selected by playing the videotape in slow motion and then freezing the desired image on the screen. The digital image was then stored for future use in a 256 x 256 x 6 bit matrix in the 512 K byte system memory. The memory capacity of the system allowed for storage of eight echocardiographic fields. Using the microprocessor, mathematical manipulations such as addition, averaging and subtraction could be performed.

*The actual analysis sequence was as follows. The videotape was advanced to a position immediately before (within 10 cardiac cycles) injection of the ultrasound contrast agent. Three pre- and three postcontrast end-diastolic fields were stored. End-diastolic was defined as the image associated with the upstroke of the electrocardiographic R wave. To minimize the effects of overall cardiac motion, the actual fields were selected in the following manner. The precontrast fields were selected as those immediately preceding appearance of any contrast medium in either the left ventricular cavity or myocardium. To allow sufficient time for perfusion of the normal segments of myocardium, five cardiac cycles were then counted and the subsequent three end-diastolic fields stored. The endocardial and epicardial contour from the initial precontrast field was retained on the video screen and slow motion forward and reverse modes were used to ensure equivalency of ventricular geometry and screen position before storage of the image. If equivalency was not present, the echocardiographic field was*
rejected and the subsequent end-diastolic image evaluated. The three precontrast fields were then mathematically averaged to form a single precontrast image. The three postcontrast fields were averaged to form the postcontrast image. The precontrast "mask" was then digitally subtracted from the postcontrast image to form the digital subtraction image. An example of this process is presented in Figures 1 and 2.

Echocardiographic infarct fraction determination. Analysis of the analog image was performed before analysis of the digital subtraction image, and has been previously reported (3). Analysis of the analog image was completed on an earlier generation off-line system that was identical to the current system, with the exception of the analog to digital converter and IBM microprocessor incorporated in the latter. The analog image was played from the videotape onto the video monitor through a video disk. The video disk allowed for presentation of a stable, flicker-free image from which accurate measurements could be made. All areas were determined using the electronic planimeter of the off-line echocardiographic analysis system. Contours were outlined using a "joystick" control to trace the image area on the video screen. The areas bounded by the epicardium and endocardium were calculated and the total myocardial area was calculated as the difference between these two area measurements. The boundary of the nonperfused region was defined as the line providing the best visual separation of the myocardium which did not increase in image intensity and that which did. Real time analysis of the contrast appearance was often necessary for this assessment because of the wide range of echo intensities in the precontrast image. The area of nonperfused myocardium from a contrast-enhanced frame was then calculated. The fraction of infarcted myocardium was calculated as for the nitro-blue tetrazolium study.

The digital subtraction images were analyzed for infarct size using the same overlay and electronic planimetry system as used for the analog image analysis. The myocardial area was calculated from the epicardial and endocardial contours of the averaged analog image. The digital subtraction image was then presented and the area of nonperfused myocardium determined. For the digital subtraction image, the boundary between perfused and nonperfused myocardium was established as the most distinct separation between the myocardium with a contrast effect and the myocardium without contrast. In two instances in which the infarct was large, the region of perfused myocardium was measured and subtracted from the total myocardial area to calculate the area of the nonperfused region. The fraction of infarcted myocardium was calculated as for the analog image analysis and the nitro-blue tetrazolium data.

Statistical analysis. Data are presented as the mean ± 1 standard deviation. Standard correlation coefficients and the standard error of the estimate were calculated. The relation between different data sets was described with linear regression. One-way analysis of variance with repeated measures was used to test for statistical significance between results of the two types of observations for determination.

Figure 1. Creation of a digital subtraction image. A, Single echocardiographic field before injection of contrast medium. A3 on the right. The image produced by averaging three such end-diastolic fields. B, The postcontrast image showing an area of myocardium that is not contrast-enhanced (arrowheads). B3, The image produced by averaging three postcontrast end-diastolic fields. There has been substantial smoothing of the image and the nonperfused area of myocardium is again apparent.
**Results**

Infarct size. Complete data including image analysis, digital subtraction image and nitro-blue tetrazolium studies were available from the 12 dogs that survived the 4 hour coronary occlusion. In one dog, there was no myocardial infarction present at the termination of the experiment either by contrast echocardiography or by nitro-blue tetrazolium; data from this dog were excluded from analysis. Therefore, final data consisted of studies in 11 dogs.

Myocardial infarction fraction ranged from 0.07 to 0.55 (mean 0.23 ± 0.14). The infarct was partially subendocardial in six studies, involved a papillary muscle in six studies and was purely transmural in only two studies. The right ventricular free wall was involved in two studies; the right ventricular component of the infarction was excluded from both echocardiographic and nitro-blue tetrazolium analyses.

Contrast echocardiograms. These were of good quality in 10 studies. Qualitatively, the contrast level was judged to be poor by both observers in one study. Subjectively, both observers thought their level of confidence for separation of normally perfused from nonperfused myocardium was improved markedly by digital subtraction imaging.

At baseline study, all areas increased in image intensity. Contrast studies recorded after 4 hours of coronary occlusion revealed definite areas of nonperfused myocardium in the 11 dogs with documented infarction (Fig. 1 to 4). The location of the infarct was predicted accurately in all instances. The comparison of nitro-blue tetrazolium and digital subtraction imaging for determination of infarct size is presented in Figure 5. The comparisons of multiple observations for determination if inter- and intraobserver correlations are presented in Table 1. As previously reported, the correlation between analog image analysis and nitro-blue tetrazolium was good ($r = 0.92$, SEE = 0.05). The relation of nitro-blue tetrazolium (NBT) infarct size to predicted size was described by the equation: analog image = 0.73 NBT + 0.06 (3). Although the correlation was good, the line described by this equation is statistically different from the line of identity. Furthermore, analog image analysis underestimated infarct size at higher ranges and overestimated it at lower ranges (3). The reasons for this remain unclear; however, this phenomenon was not noted in the digital subtraction image data.

**Discussion**

Relation to prior studies. Prior studies, by ourselves and other investigators, have demonstrated the utility of contrast-enhanced echocardiography for assessing the location of myocardial perfusion deficits (1–6), determination of myocardial infarct size (3) and detection of partial coronary occlusions (8,9,13). The new information from this study suggests that digital imaging technology can be applied to two-dimensional echocardiograms and may offer greater accuracy than analysis of the analog image, both with respect to determination of infarct size and inter- and intraobserver variability.
DIGITAL SUBTRACTION CONTRAST ECHOCARDIOGRAPHY

Figure 3. Digital subtraction image (DSI) of contrast echocardiogram and corresponding anatomic specimen. The digital subtraction contrast echocardiogram recorded after 4 hours of coronary artery occlusion with superimposed epicardial and endocardial contours is in the upper panel. Note the region of noncontrast enhancement (arrowheads). This region corresponds in location and size to the infarct in the lower panel which shows the pathologic specimen. Note also the second region of apparent decreased contrast (white arrow). This apparent region of low contrast is an artifact arising from the presence of abnormally bright intramyocardial echoes in the myocardium at baseline. The lower panel shows the nitro-blue tetrazolium (NBT) stained slice of the left ventricle corresponding in location to the echocardiogram. Note the excellent correspondence between location and size of the infarct by these two techniques.

As with all prior studies, visual inspection of the echocardiogram accurately localized the region of nonperfused myocardium. The correlation between nitro-blue tetrazolium (NBT) and digital subtraction image (DSI) was higher than between nitro-blue tetrazolium and analog image analysis. Additionally, the line described by the regression equation (DSI = 0.92 NBT + 0.03) is not statistically different from the line of identity. Although the difference between the ability of digital subtraction image and analog image analysis to predict infarct size was not statistically significant, we believe the improved slope and intercept of the equation derived from digital subtraction image data may offer some improvement in the ability to predict actual infarct size when compared with analysis of the analog image. There was also an improvement in inter- and intraobserver correlations with the digital subtraction image as compared with analog image analysis. These differences, although encouraging, did not reach statistical significance.

Advantages of digital subtraction. Our current analysis relies on a subjective differentiation between contrast-enhanced and nonenhanced myocardium. Similar criteria were used for both analog and digital analysis (see Methods). The broad range of baseline echo intensities from the myocardium often make the subjective determination of contrast appearance difficult. This is especially true in regions where high intensity echoes are already present within the myocardium before contrast injection.

Subjectively, both observers thought that the digital subtraction technique conferred greater confidence in separating perfused from nonperfused myocardium than did the analog image. Figure 4 is an example of one such case, where delineation of the perfusion deficit is difficult to identify in the analog image, but is readily apparent in the digital subtraction image. An additional subjective advantage of digital subtraction imaging for determination of infarct size arises from the subtraction of the bright epicardial echoes from the image. In a routine, noncontrast-enhanced image, there is usually little difficulty in separating the actual myocardium from the epicardium or surrounding extracardiac structures. However, after contrast enhancement, the myocardial image intensity may equal that of the epicardium, and accurate separation of these structures may not be possible. An example of this phenomenon occurs in Figures 1 and 2 where the bright epicardial echoes anteriorly may be confused with contrast-enhanced myocardium. Note in the digital subtraction image that these echoes are removed and that easy identification of the actual myocardium is possible.

Limitations. There are several limitations inherent in this technique, and, on occasion, the digital subtraction imaging process can produce artifacts. If bright specular echoes are present within the myocardium before contrast injection, they will then be subtracted from the contrast-enhanced image. This can create a false region of noncontrast enhancement that could be confused with a true nonperfused region of myocardium. This pitfall can be avoided by recognition of this phenomenon and evaluation of the precontrast image for areas of abnormal image intensity within the myocardium at baseline. A similar phenomenon
Figure 4. A, A precontrast and B, postcontrast end-diastolic frame showing a vaguely defined region of nonperfused myocardium (arrowheads). C, The digital subtraction contrast echocardiogram demonstrating the superiority of this technique for delineating this region of nonperfused myocardium in this case. The endocardial and epicardial borders are outlined in C to assist in determination of infarct borders.

occurs with the bright endocardial echoes. The endocardium provides a relatively brighter image than the actual myocardium. These brighter endocardial echoes are subtracted from the image when the digital subtraction image is created. Because of this, there may be some loss of exact endocardial boundaries in the digital subtraction image. The digital imaging system described in this study allows for rapid presentation of any of the archived echocardiographic fields for immediate comparison with one another. Using this technique, little difficulty was encountered in determining the precise endocardial border of the nonperfused region. It is our belief that these limitations are minor. With only moderate experience, very little confusion should ensue in evaluating digital subtraction contrast echocardiograms. We believe the advantages of the technique outweigh these limitations.

Relation to myocardial perfusion. In this study we evaluated only the ability of contrast-enhanced echocardiograms subject to digital subtraction to predict infarct size after 4 hours of total coronary occlusion. The distribution of myocardial ultrasound contrast is a function not of infarction, as such, but of myocardial blood flow. This has been demonstrated in studies in which only brief coronary occlusion was applied and, thus, no infarct created (2,4). In addition, preliminary experience in evaluating partial coronary artery occlusion suggests that there will be abnormalities of appearance of ultrasound contrast in the myocardium distal to a significant partial coronary occlusion (8,9,13). We predict that abnormalities of ultrasound contrast in the myocardium identical to those seen in total permanent occlusion will, therefore, be seen with severe spasm or partial coronary artery occlusion leading to flow reduction at rest. In these later instances, distribution of the noncon-
Contrast-enhanced myocardium will not predict infarct size, but rather should accurately reflect the location and amount of myocardium in jeopardy from the coronary artery stenosis.

**Future directions.** Thus far, this promising new technique of contrast-enhanced echocardiography to visualize myocardial perfusion has been applied only in the animal laboratory. Although intracoronary injections of ultrasound contrast contrast have been performed in a small number of patients in the past (14,15), we believe it is best to further evaluate this technique with respect to both cardiac toxicity and to its effect on the other vital organs before utilizing it in human subjects. Our series of experiments used hydrogen peroxide and blood as a contrast agent. Many other ultrasound contrast agents have been used, including gelatin encapsulated microbubbles (2), saccharide microbubbles (16), and solutions of agitated renografin and saline (4,8,9). We do not believe our work should be construed as an endorsement of any one contrast agent as opposed to others, but rather should accurately reflect the location and amount of myocardial perfusion and function with a single examination. Contrast two-dimensional echocardiography should prove valuable as an adjunct to other studies, including analysis of wall motion (17) or localization of infarcted or ischemic myocardium for other purposes.

**Conclusion.** We have applied new digital imaging technology to two-dimensional echocardiograms and produced digital subtraction images from contrast-enhanced echocardiograms to determine myocardial infarct size. In addition to subjective advantages of digital subtraction imaging for separation of perfused from nonperfused myocardium, this technique may confer an increase in accuracy and reproducibility over analysis of the analog image.

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### References


