Enhanced Detection of the Evolution of Tissue Changes After Acute Myocardial Infarction Using Color-encoded Two-dimensional Echocardiography

ALFRED F. PARISI, M.D., MARKKU NIEMINEN, M.D., JOSEPH E. O'BOYLE, B.S.
PAUL F. MOYNIHAN, B.S., SHUKRI F. KHURI, M.D., ROBERT A. KLONER, M.D., PH.D.
EDWARD D. FOLLAND, M.D., AND FREDERICK J. SCHOFEN, M.D., PH.D.

SUMMARY  Acute myocardial infarction was produced in 26 dogs by ligation of the left anterior descending coronary artery. Two-dimensional echocardiograms (2-D echoes) were performed through the chest wall before and serially after coronary ligation. The dogs were then killed in four groups at the following intervals: 24-48 hours, 1-2 weeks, 3 weeks and 6-8 weeks. Each 2-D echo was processed through a video quantizer, which encoded echo amplitudes progressively into eight regions of color. The myocardium was graded with respect to color composition in regions that showed any abnormally contracting segment (ACS). The ACS exhibited a progressive increase in echo intensity that became maximal 6-8 weeks after coronary ligation. Histopathologic and histochemical studies verified that these increases in echo amplitude correlated with the evolution of healing and myocardial scar formation. At 6-8 weeks, the mean collagen content of infarcted myocardium had increased by a factor of 4; concurrently, ACS echo amplitude had increased two- to threefold. These observations suggest that color-encoded 2-D echo promotes facile perception of serial changes in tissue characteristics that result from acute myocardial infarction.

THE CLINICAL EXPERIENCE of M-mode echocardiographers indicates that the hearts of patients with remote myocardial infarction often demonstrate abnormalities of left ventricular wall motion and thickening as well as enhanced intensity of the reflected signal, usually referred to as "increased echo density." 1-3 Rasmussen et al. 4 qualitatively interpreted areas of increased echo density on M-mode echocardiograms and demonstrated a high degree of correlation with the presence or absence of scar at surgery and at autopsy. 5 More recent in vitro and in vivo experiments have shown that increased acoustic reflectivity can occur in ischemic and infarcted myocardium. 6-11

By allowing sampling of the entire myocardial circumference and better spatial localization of the sampled region, real-time two-dimensional echocardiography (2-D echo) has spurred investigation of the regional motion and thickening of the myocardium in ischemia and infarction. 12-15 However, very little has been done to exploit the advantages of 2-D echo in expanding upon the characteristics of acoustic reflectivity of the myocardium observed by M-mode echocardiographers.

Technical limitations of most commercially avail-

able 2-D echo equipment have hindered the investigation of the amplitude patterns of reflected signals. The most important being that video display monitors have a dynamic range of approximately 30 db, while the dynamic range of echo information is 80 db or more. This necessitates significant signal compression in the signal processing and scan conversion. Color processing can increase the dynamic range of echo information because the eye can distinguish considerably more colors than gray shades. While color encoding has been accomplished with M-mode echocardiography, 16 2-D echo allows a spatial integration difficult to achieve with M-mode echocardiography. Rogers et al. 17 showed that useful information about the ultrasonic reflectivity of atherosclerotic coronary artery lesions can be extracted from 2-D echo imaging systems by processing the images so as to "window" upon the range of image intensity of interest, and "expand" the information output from this range.

The purpose of this study was to investigate the characteristics of 2-D echo intensity in experimental acute myocardial infarction with the aid of color image processing techniques. Specifically, we wished to determine whether the evolution of color changes correlated with the evolution of histologic changes and biochemical analysis of collagen content. The potential for this technique was suggested in a previous preliminary report from our laboratory. 17

Methods

Studies were conducted in healthy 20-40-kg mongrel dogs with high-quality 2-D echo images before any intervention. Acute myocardial infarction was produced at open thoracotomy by ligation of the left anterior descending coronary artery. After chest closure, 2-D echo examinations were performed as described previously. 18 All dogs were imaged serially before and periodically over the 8 weeks after coronary artery ligation. The
dogs were killed at systematic intervals (24–48 hours, 1–2 weeks, 3 weeks and 6–8 weeks) after coronary ligation and the results of histopathologic examination related to the 2-D echo image findings immediately before death.

Immediately postmortem, all hearts were removed. The left ventricle was dissected free of other tissue and then sectioned transversely at 1-cm intervals. The slices were stained with triphenyl tetrazolium chloride (TTC) to enhance delineation of the infarct zone.

Histologic sections of normal and infarcted tissue were stained with hematoxylin-eosin for overall morphology and by Masson’s trichrome method to demonstrate collagen. Histologic grading was done after semiquantitative procedures similar to those described by Mallory et al. and later by Fishbein et al. The following features were specifically sought (Table 1): coagulation necrosis, waviness and thinning, and focal myocyte lysis of myocardial fibers; intercellular edema and hemorrhage; infiltrates of polymorphonuclear leukocytes, lymphocytic plasmacytic, pigmented and nonpigmented mononuclear, and eosinophilic cells; and vascular, fibroblastic and collagenous proliferation. All features were graded as: 0 = absent; +1 = mild; +2 = moderate; +3 = prominent, and +4 = severe. These broad categories indicate the relative magnitude of each histopathologic finding in infarcts at any given time and aid in assessing whether these changes increased or decreased in prominence as the infarcts evolved. A mean score for each feature was calculated for all specimens at each interval. All specimens were examined by a pathologist who was unaware of the results of the 2-D echo examinations.

Biopsies (50–100 mg) were obtained from the cross section of the left ventricular wall in which the infarction was located. Individual biopsies were taken from both normal tissue and the infarction site. Samples were then acid hydrolyzed using 6N HCl at 120°C for 12 hours. After neutralization and decoloration of the hydrolyzate, hydroxyproline was measured by the method described by Newman and Logan. The collagen content was calculated by multiplying the hydroxyproline content by a factor of 7.46 and was expressed as mg/g dry myocardial weight. From these data, the ratio of mean collagen content of infarcted tissue to mean collagen content of normal tissue was calculated.

Echocardiographic studies were performed with a Varian V-3000 phased-array sector scanner. The dogs were imaged from the right side to obtain a minimum of three short-axis sections: at the mitral valve, papillary muscle and apex. Transducer position and orientation were adjusted to produce the most nearly circular sections of the left ventricle in a standardized approach to obtain true orthogonal short-axis sections. Gain and reject settings were adjusted so that the crystal artifact just saturated the video display and discernible gray levels were present throughout the myocardium. The time-gain compensation ramp was set for a uniformly sloping ramp, with no more than a 5-db gain difference between adjacent controls. The identical gain settings used to produce an optimal image in the baseline (control) state were subsequently used in each study. All studies were stored on a Panasonic NV-3160 reel-to-reel video recorder and displayed on a high-resolution Conrac SNA-14/c monitor.

Image postprocessing and analysis were performed on taped studies. Figure 1 demonstrates the analytic system in a block diagram. The recorded images were processed through a Colorado Video 606A real-time video processor and quantizer. A band of input signal amplitudes encompassing the range of signals of interest was selected and divided into eight ranges. The processed video was led into the input of a Conrac 5211C19 RGB color monitor so that each point of the image appeared as one of eight colors in direct proportion to one of the eight amplitude quanta to which it corresponded. Figure 2 demonstrates the type of input/output function performed by the processor. Echo intensities were encoded in equal increments above the minimum values. The highest intensity (red) represents a minimum sixfold increase in echo intensity over the lowest (blue) intensity encoded. Because of gain and attenuation differences between different animal subjects, the input band selected for quantization was taken from just above the weakest signals received from the myocardium as the lower limit and the lowest amplitude signals of the crystal artifact as the upper limit.

Each processed study was evaluated in real-time and
still-frame modes. Because of the potential for introducing artifact in single-frame images, a region of myocardium was considered to produce abnormal high-intensity echoes only when these were identified throughout systole and diastole in an akinetic myocardial segment.

Because of the complexity of the color patterns, myocardial segments were graded using the scheme outlined in table 2. This allowed echo intensity and its distribution to be integrated into a simplified system. Irrespective of the color, all normally moving areas were graded as 0. An abnormally contracting segment (ACS) with a low echo intensity (blue) received a score of 1. Segments with colors representing echo amplitudes of intermediate (brown-yellow) intensity were assigned scores of 2 to 3, depending on the extent of the high-intensity echo abnormality, while the highest echo amplitudes (red) was assigned a score of 4–5 on a similar basis.

The presence of high-intensity echoes was noted for each study, with particular regard to changes in serial studies of dogs after coronary occlusion. After tabulation of results, echocardiographic and pathologic findings were compared to evaluate serial changes in echo amplitude and their relationship to the time course of the evolution of healing myocardial necrosis and the resultant scar formation.

Statistical analyses were performed by standard formulas using a programmable Monroe 325 desktop calculator. The significance of serial observations was determined by analysis of variance.

Results

Twenty-six dogs were studied. Thirteen were killed at 1–2 days, four at 1–2 weeks, five at 3 weeks and

![Figure 1](http://ahajournals.org/)

**Figure 1.** Video chain for color analysis. The output from the Panasonic videotape recorder is processed off-line through a video quantizer, which separates signal intensities into different colors for display on the Conrac monitor.

Four at 6–8 weeks. Infarcted regions showed a progressive increase in high-intensity echo targets, which became more widespread within the myocardium of the ACS over the course of observations. Figure 3 shows color-encoded 2-D echo examinations that typify findings at different stages of this study, with examples of gross and microscopic findings at sacrifice. Areas of uninvolved myocardium occasionally showed high-intensity echoes limited to endocardial surfaces usually just posterior to the crystal artifact. These were never associated with wall motion abnormalities.

Figure 4 shows the frequency distribution of this increasing echo intensity within the ACS of each of the subgroups as a function of time. At 0–2 days, the mean echo intensity score was 2.1. Ten of 13 dogs showed some increase in echo intensity in their akinetic zones. In seven this consisted of a small amount of intermedi-

![Figure 2](http://ahajournals.org/)

**Figure 2.** Function of the videoquantizer. The input voltages above a baseline threshold (A) are separated into colors representing equal echo intensity increments (B) and are so displayed in the video output. Normal myocardium appears as shades of blue; intermediate echo amplitudes are displayed as brown-yellow colors; highest intensity echoes appear in the red region of the display. Input voltages above a predetermined maximum (C) also appear red.

<table>
<thead>
<tr>
<th>Interpretation</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low-intensity (blue) color in ACS</td>
<td>1</td>
</tr>
<tr>
<td>Intermediate-intensity (brown-yellow) color in ACS:</td>
<td></td>
</tr>
<tr>
<td>&lt; 25% of ACS involved</td>
<td>2</td>
</tr>
<tr>
<td>Intermediate-intensity (brown-yellow) color in ACS:</td>
<td></td>
</tr>
<tr>
<td>&gt; 25% of ACS involved</td>
<td>3</td>
</tr>
<tr>
<td>High-intensity (red) color in ACS:</td>
<td></td>
</tr>
<tr>
<td>&lt; 25% of ACS involved</td>
<td>4</td>
</tr>
<tr>
<td>High-intensity (red) color in ACS:</td>
<td></td>
</tr>
<tr>
<td>&gt; 25% of ACS involved</td>
<td>5</td>
</tr>
</tbody>
</table>

Abbreviation: ACS = abnormally contracting segment.
ate color intensity (grade 2), while three dogs showed even higher echo intensity zones. The most prominent microscopic features were necrosis, polymorphonuclear leukocyte infiltration, waviness and thinning of myocardial fibers and intercellular edema (table 3). Biochemical analysis did not show collagen deposition above control values (fig. 5).

The four dogs killed at 1–2 weeks had color intensi-

![serial color-encoded two-dimensional echocardiograms](image)

**Figure 3.** Serial color-encoded two-dimensional echocardiograms from one dog, with a gross, pathologic cross section and microscopic, histologic specimens at the level of infarction. Myocardium to the left of the white arrows was akinetic after coronary ligation. Two hours after left anterior descending coronary artery ligation, color intensity or distribution had not changed since the baseline study; 1 week after ligation, echo intensity had increased (yellow) in the septal and anterior portion of the myocardium; 2 weeks after ligation, there were islands of higher intensity echoes (yellow/red) encompassing a greater portion of the myocardium both transmurally and circumferentially; the study 6 weeks after coronary artery ligation showed further increase in highest intensity (red) echoes in the involved area. The progressive pericardial reaction (white P) described in the text is also evident.

The gross, pathologic cross section of the same heart 6 weeks after coronary ligation corresponds to the echocardiogram at 6 weeks. Intersecting arrows indicate the orientation of the two-dimensional echocardiograms and gross specimen. S = septal; P = posterior; L = lateral; A = anterior.

At the bottom right are histologic sections. Collagen is stained blue. The specimen on the left is from a dog killed 2 weeks after ligation. There is early collagenization with numerous inflammatory cells and fibroblasts. There is a delicate, capillary-like vascularity within this area. Note residual, as yet unremodeling, necrotic myocardium delineated by short black arrows. The specimen on the right is a sample from the infarct zone of the gross pathologic specimen (bottom middle). There is a dense, virtually acellular collagen matrix with occasional large, thickwalled blood vessels. Masson's trichrome stain; magnification × 145.
were in early evidence and collagen deposition had begun and increased by a factor of two above control values (fig. 5).

The five dogs killed at 3 weeks had some evidence of the highest intensity (red) echo intensity present in their ACS. The mean echo intensity score was 4.6. Fibroblasts and collagen deposition were even more prominent. Biochemical analysis showed a fivefold increase in collagen above control values.

The four dogs killed at 6–8 weeks achieved an echo intensity score of 5 (fig. 4). Microscopically, collagen was the most prevalent histologic feature (table 3). Histochemical analysis at this time showed the ratio of collagen content of infarcted regions to collagen content of uninvolved myocardium had increased to a value of 5.4 ($p < 0.001$).

**FIGURE 4.** Frequency distribution of color-encoded two-dimensional echocardiography score as a function of time. The horizontal axis indicates the intensity of the color two-dimensional echocardiogram as determined by the scoring system outlined in Table 2. The vertical axis indicates the number of dogs with each score. The distribution of scores increased progressively with time. ACS = abnormally contracting segment.

**TABLE 3.**Histologic Features of Infarction (Mean Scores vs Time)

<table>
<thead>
<tr>
<th>Feature</th>
<th>0–2 Days</th>
<th>1–2 Weeks</th>
<th>3 Weeks</th>
<th>6–8 Weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Necrosis</td>
<td>2.9</td>
<td>4.0</td>
<td>1.4</td>
<td>0.0</td>
</tr>
<tr>
<td>Waviness and thinning</td>
<td>1.5</td>
<td>0.8</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Intercellular edema</td>
<td>1.2</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Hemorrhage</td>
<td>0.5</td>
<td>0.2</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Vascularity</td>
<td>0.0</td>
<td>3.3</td>
<td>3.2</td>
<td>1.9</td>
</tr>
<tr>
<td>Polymorphonuclear leucocytes</td>
<td>2.6</td>
<td>2.4</td>
<td>1.0</td>
<td>0.5</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>0.0</td>
<td>1.0</td>
<td>1.0</td>
<td>0.9</td>
</tr>
<tr>
<td>Pigmented macrophages</td>
<td>0.0</td>
<td>0.5</td>
<td>2.1</td>
<td>1.4</td>
</tr>
<tr>
<td>Nonpigmented mononuclear leucocytes</td>
<td>0.0</td>
<td>3.4</td>
<td>2.5</td>
<td>1.3</td>
</tr>
<tr>
<td>Fibroblasts</td>
<td>0.0</td>
<td>1.0</td>
<td>3.2</td>
<td>1.9</td>
</tr>
<tr>
<td>Collagen</td>
<td>0.0</td>
<td>0.5</td>
<td>2.8</td>
<td>3.8</td>
</tr>
</tbody>
</table>

**FIGURE 5.** Collagen content of tissue as a function of time. Horizontal axis indicates time of sacrifice after coronary artery ligation. Vertical axis at bottom indicates collagen content of samples; at top, axis indicates ratio of collagen content from normal and infarct regions. Values are mean ± SEM. Analysis of variance indicated that the differences between the means of the ratios were statistically significant ($F = 26.03$, $p < 0.001$).
In the course of this study, echo intensity also increased progressively throughout the pericardial regions that were imaged posterior to the heart. At pathologic examination, the pericardium, which had been entered to perform coronary ligation, showed progressive thickening and fibrosis in all dogs killed later than 2 weeks after injury.

Discussion

The observations herein indicate that the intensity of echoes from acutely infarcted myocardium increases soon after the insult and becomes greater and more widespread within the area of tissue damage as a function of time. In 10 of 13 dogs, myocardial echo intensity increased to some degree within 48 hours after coronary ligation. By 1 week or later, all dogs showed these changes. This progressive increase in echo amplitude paralleled the histopathologic evolution of myocardial infarction. Echo amplitude was greatest when hydroxyproline synthesis had increased and collagen deposition was at its fullest. These observations confirm and extend the observations of Rasmussen et al., who noted high-intensity echoes as a feature of myocardial scarring. They are also consistent with the increase of ultrasonic backscatter from the collagen-rich zones of experimentally induced infarction obtained in excised canine and rabbit hearts. In our series of experimental animals, in which calibrated measures of tissue echo intensity could be directly compared to baseline observations, intermediate gradations of echo intensity were discerned during the inflammatory and neovascular stages that preceded collagen deposition. By the time dense collagen tissue had formed, the prevalence of red (highest intensity), echoes within akinetic segments indicated a two- to threefold increase in echo intensity. These observations also concur with reports of the ultrasonic characteristics of remote infarction both in intact animals and in man.

The system we used is considerably simpler than computer-based analysis systems being evaluated that are designed to assess ultrasonic backscatter from myocardial tissues soon after injury. While our observations are consistent with backscatter experiments in showing increased signal after myocardial infarction, the color-encoded system is not designed to be as sensitive or to convey the extent of information contained in changes in ultrasonic frequency distribution found in backscatter analysis. Unlike backscatter experiments that perform detailed analysis of a small segment of myocardium within a tomographic section, this color-encoded system increases perceptibility so as to allow immediate characterization of an entire tissue cross section. The system demonstrated a systematic progression of echo intensity in the evolution of acute myocardial infarction. Similar information might be obtained by using gray-scale intensity as a guide to tissue changes. If results are compared, i.e., fully developed scar as the result of replacement of necrotic tissue by dense collagen, a specific advantage is not necessarily gained by color processing. However, the evolution of changes before the "healed scar" stage of infarction is considerably more difficult to appreciate solely with a gray-scale system. We could not grade comparably the black-white images in this series using the same visual inspection technique used in evaluating our color images.

The color-encoded approach has limitations, some of which have become apparent in the course of these experiments. Because of the angle dependence of reflected signal amplitude, transducer-target spatial relationships must be consistently and systematically sought. Excessive increases in depth-compensated gain can also artifactually produce high-intensity echoes in the corresponding portion of the image field. Specular reflections from epicardial and endocardial targets in the near field, close to the crystal artifact, can also cause some confusion. Reflected signals are also subject to phase cancellation effects which, in theory, can limit their value. These problems suggest that the use of the system in a setting that allows serial observations is less likely to present difficulties than when judgments are made on isolated single studies. With serial observations, each subject can provide a baseline (control) image. Using identical gain settings, changes in echo intensity from a controlled baseline observation may be of greater significance than interpretations based on any individual study. Thus, the state of nonfunctioning myocardium might be considerably different in two patients with acute myocardial infarction, one of whom develops progressively diffuse high-intensity echoes within the akinetic segment while the other does not.

These experiments suggest that the quality of myocardial echoes must be considered concurrently with thickness, thickening and endocardial motion to characterize fully the tissue under examination, particularly in the process of acute myocardial infarction. Echo signals from myocardium are part of a more complex and potentially helpful diagnostic process, and contain information that has not been systematically exploited. Increased echo intensity is but one aspect of this information, which under appropriate conditions can be rapidly accessed using color-encoded video processing.

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References