Ultrasonic tissue characterization: detection of acute myocardial ischemia in dogs

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ABSTRACT Ultrasonic tissue characterization is a new area of investigation in the field of cardiac ultrasound. The amplitude and frequency of the ultrasound signal are normally altered as the signal penetrates through tissue. It is assumed that the amplitude distribution and frequency shift of diseased or edematous tissue are different than those of normal tissue. A statistical approach to the analysis of the unprocessed radiofrequency signal in the amplitude domain was used to study the effect of acute myocardial ischemia on the parameter mean amplitude/standard deviation of the amplitude (MSR). Ten dogs were anesthetized and underwent left lateral thoracotomy. Baseline mean MSR from the interventricular septum was 1.99 ± 0.05, but increased by 30 min after coronary artery occlusion and started to plateau at 1 hr (mean 2.24 ± 0.06). Reproducibility in noninfarcted myocardium (left ventricular inferoposterior wall) was good, with a mean MSR of 2.00 ± 0.05 at baseline and 1.98 ± 0.04 3 to 4 hr later. There was no difference in mean MSR when data were obtained through chest wall and when they were obtained directly from the surface of the heart. We conclude that statistical analysis in the amplitude domain of the unprocessed radiofrequency signal can detect acute myocardial ischemia within 30 min after coronary artery occlusion, provides reproducible measurements, and is unaffected by chest wall filtering.


DETECTION OF early acute myocardial ischemia in man is currently suboptimal. The electrocardiogram may not always reveal acute ischemia because it may demonstrate only nonspecific changes. The technetium pyrophosphate scintigram usually requires a delay of 24 hr before it can be interpreted as positive for myocardial injury. The information obtained from serum creatine kinase–MB levels is also delayed. A history of angina pectoris may be the only evidence of ongoing myocardial infarction in these patients. It would be highly desirable to have a noninvasive tool available for diagnosis and localization of early acute myocardial injury.

Echocardiography has traditionally been used as an imaging device. Recent work by several investigators suggests that interactions between the ultrasound energy and tissue can be analyzed to characterize the histologic state of tissue, both in vitro1,2 and in vivo.3–6 However, several quite different approaches to ultrasonic tissue characterization have been pursued. Miller, Mimb, and Cohen and their colleagues1,2,4 have measured quantitated integrated backscatter from the unprocessed radiofrequency (rf) signal. In vivo, lack of an absolute calibration standard and the presence of the chest wall, with its variable acoustic characteristics, have been problems. Skorton et al.3,5 have analyzed the rf signal after it has been processed and displayed on the traditional image, applying various texture algorithms to extract information about the tissue. The potential disadvantage of image analysis is its dependence on the transducer and machine used to collect and process data and on the position of the region of interest within the scan field.7 We have previously worked with a statistical approach to the study of the unprocessed rf signal8 in which we analyzed the amplitude domain.9,10 We have shown that the measured parameter is independent of the measurement site when tested in a tissue-mimicking phantom. In the phantom there was no statistically significant difference in the parameter mean amplitude/standard deviation of the amplitude (MSR) whether it was sampled at a depth of 1 to 2, 5 to 6, or 9 to 10 cm.9 This

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approach is unaffected by time-gain compensation (mean amplitude) over the range of 16% to 50% of the full scale of the A/D converter.9

Using ultrasonic tissue characterization, Cohen et al.,4 reported an increase in integrated backscatter within 2 hr of coronary artery occlusion but used a constant factor to compensate for chest wall–dependent attenuation of the signal. Although their results indicate that myocardial backscatter could be quantified in the intact dog when chest wall attenuation was compensated for, humans are more likely to have a wider variation in chest wall thickness than the five dogs reported in that study. Skorton et al.,9 using texture algorithm on the processed rf signal, could detect myocardial injury 2 days after coronary artery occlusion in closed-chest dogs. Parisi et al.11 have described serial changes in tissue characteristics of myocardial injury in vivo starting 24 hr after coronary occlusion. They used a color-encoded two-dimensional echocardiographic image technique. Rasmussen et al.,12 using computer enhancement and a mathematically defined integrated backscatter ratio, found changes in backscatter within 12 min of coronary artery occlusion in open-chest dogs. However, a calibration measurement using internal landmarks such as endo-myo-epicardium in an area close to the infarcted region was also required for this study. The need for such internal calibration requires normal reference tissue.

In the present protocol we investigated the possibility of use of our statistical amplitude analysis of the unprocessed rf signal as a noninvasive “biopsy” in the detection of early acute ischemic injury in the dog. Our hypothesis was that the measurements would be unaffected by chest wall characteristics even without external or internal calibration.

Methods

Tissue characterization: statistical analysis of rf. A volume of healthy myocardium can be modeled as a large number of independent scatterers. Ultrasound scattered from such a volume will show a particular distribution of amplitudes, called a Rayleigh distribution. Disease processes such as collagen formation, calcium deposits, and edema may cause the scatterers to become correlated (i.e., dependent). A situation in which some fraction of the scatterers are dependent and some are independent leads to a different, non-Rayleigh, amplitude distribution. The ratio MSR is a computationally simple way to distinguish between Rayleigh and non-Rayleigh distributions. The MSR for a Rayleigh distribution is 1.91 and is expected to increase above this value for tissue that contains some fraction of correlated scatterers.13

Data acquisition. Two-dimensional echocardiograms were obtained with a commercially available sector scanner (Hewlett-Packard 77020A) with a focused phased-array 3.5 MHz transducer. The region of suspected infarct or presumed normal tissue was imaged in the parasternal short-axis view and the M mode cursor (one scan line) was directed through the area of interest. The gain settings (transmit power and time-gain compensation controls) were adjusted to obtain an optimal image. The unprocessed rf signal from the cursor-selected scan line was sampled at 20 MHz and digitized with a Biomation 8100 transient recorder (8 bits) under microprocessor control (Cromemco, Z-2 Mountain View, CA). One repetition consisted of 40 M mode scan lines sampled sequentially over 40 msec. The first five to 10 ultrasonic scan lines were used to calculate a MSR for that repetition. An average MSR was then calculated from 10 such repetitions. This averaged MSR represented a single measurement at a given point in time. All data were acquired in mid-diastole to minimize any potential cyclic variation in MSR such as that reported for integrated backscatter.14 Each scan line contained 1000 data points corresponding to 4 cm. The data were stored on a nine-track magnetic tape and later transferred to a Hewlett-Packard HP1000 F-series minicomputer for processing. For the last two animal experiments in this series we used a different acquisition system. This system consisted of a Hewlett-Packard A-series computer with a Hewlett-Packard model 5180 waveform recorder. The signal was digitized at 20 MHz and stored on the computer’s disk.

Data analysis. The unprocessed rf signal was displayed as amplitude vs tissue depth (figure 1). The operator selected the depth corresponding to the anatomic region of interest. The computer derived a histogram (figure 1) representing the number of occurrences of rectified peak amplitudes of the digitized signal on the y axis and the amplitudes on the x axis. In each window of interest, the amplitude of the peaks of the rectified signal were calculated. The ratio of the mean to the standard deviation of these amplitudes was described by the MSR value. Considerable care was taken in the setting of the data window within the rf tracing. The window width was kept constant at approximately half a centimeter and adjusted as needed in the middle of the myocardial wall to avoid high-amplitude endocardial or epicardial reflections. In a tissue-mimicking phantom and in human blood we have previously shown that gate size does not significantly alter MSR if kept at 0.5 to 1 cm.9 To assess problems with improper gate settings, the window was automatically shifted 15 points (1.2 mm) to each side of the original position to ensure that specular interfaces were not included in the window. If the MSR changed more than 3% as the window shifted, the primary window was moved, if possible, to find an area that did not show this change. If such a window could not be found, then the rf was rejected.

Animal preparation. Ten mongrel dogs were sedated with morphine sulphate (2 mg/kg, im) and anesthetized with intravenous chloralose (85 mg/kg) and urethane (625 mg/kg). Ventilation was provided by a Harvard pump, which delivered room air through auffed endotracheal tube. Depth and rate of respiration were adjusted to maintain arterial pH between 7.35 and 7.45, PO2 between 80 and 110 mm Hg, and PCO2 between 35 and 45 mm Hg. A large-bore catheter was inserted into the left femoral artery of each dog to monitor the systemic arterial pressure with a Statham P23Db transducer. A second catheter was placed in the right femoral vein for administration of additional anesthetic agent, which was given to maintain deep anesthesia throughout the experiment. The right jugular vein was catheterized for infusion of antiarrhythmic drug. The chest of each dog was opened by means of a left lateral thoracotomy and the heart was exposed and supported in a pericardial sling. A bipolar pacemaker electrode was attached to the right ventricle. Ventricular pacing rate was maintained at 150 beats/min with one channel of a WPI 800 series stimulator.

The left anterior descending coronary artery (LAD) was dissected free and a snare was placed around the artery at the level of the mid-LAD (beyond the first large diagonal branch). Before
FIGURE 1. Top. One sample of unprocessed rf signal displaying amplitude (RF AMP) on the y axis and distance on the x-axis. The rf signal was obtained from a closed-chest measurement with the cursor cutting slightly obliquely through the septum. A definable window (dashed vertical lines) was set in the septum, within the myocardium, for further analysis with use of a histogram of the data. Endo = endocardium; IVS = interventricular septum; PW = posterior wall. Bottom. Histogram constructed with data from 10 samples such as those in the top panel and displaying the number of occurrences of a certain amplitude plotted on the y axis and amplitudes on the x axis. The mean amplitude, standard deviation of amplitudes, and MSR are automatically derived by the computer. Gates that were too close to specular interfaces were detected by comparing the MSR values for the selected window with the ones obtained when the window was shifted by a predetermined amount to the left or the right. In this study, the gate was shifted 15 points (1.2 mm) and the corresponding MSR values are displayed to the right in this panel.

occlusion of the artery, 16.7 μg/kg/min diltiazem was infused for 30 min as antifibrillatory drug therapy (total dose of diltiazem, 0.5 mg/kg). At the completion of this infusion the artery was tied off to create an infarct in the LAD distribution.

Open-chest measurements. The dog was placed supine on its right side in a specially constructed wooden cradle. Open-chest measurements were obtained by placing the transducer directly on the interventricular sulcus angling steeply toward the left ventricular apex. The transducer was hand held and a very light pressure was applied on the heart to ensure contact between the transducer and the beating heart. No gel or stand-off device was used. An image from the parasternal short-axis view was obtained at the level of the papillary muscles.

Radiofrequency data were collected in all 10 dogs from the midinterventricular septum and left ventricular inferoposterior wall before occlusion of the coronary artery. After occlusion, data were sampled from the septum at 30 and 60 min after occlusion and then hourly for 4 hr. Data from the left ventricular inferoposterior wall were again obtained at 4 hr. Although there was no absolute way to determine that data were being sampled from the same area before and after occlusion, the level of the papillary muscles was imaged similarly at all times and a conscientious effort was made to sample rf from the same area throughout the study.

Closed-chest measurements. The closed-chest measurements were obtained with the animal in the same position as described for the open-chest measurements. Data were collected with the transducer placed under the wooden cradle in a specially built opening to allow images to be obtained from underneath the dog. The ultrasound beam then passed through the right chest wall, right ventricle, and finally the left ventricle. A short-axis parasternal view was obtained at the level of the papillary muscles. Radiofrequency data from the midinterventricular septum were obtained through the right chest wall at the same time intervals as for the open-chest measurements.

Histology. A biopsy sample was obtained from the infarcted area at the time each dog was killed. To ensure that samples were from the area of ultrasonic sampling, a long needle was passed through the interventricular sulcus and into the midseptum along the direction of the ultrasound beam. Tissue was analyzed by light and electron microscopy to allow examination of the ultrastructure of the tissue after occlusion that reflected the change observed in the ultrasonic parameter.

Statistical analysis. The MSR obtained for each point in time for each animal was the result of averaging 10 MSRs. The unpaired Student t test was used to compare posterior wall measurements at different times. Two-factor analysis of variance was used to compare open- and closed-chest measurements and MSRs at baseline and after coronary occlusion. Multiple simultaneous comparisons were done to determine if baseline MSR was different from MSR after coronary occlusion.

Results

MSR as a function of time after occlusion of the LAD in 10 dogs is illustrated in figure 2. The data used in the following analyses are listed in table 1. All MSRs are mean ± SD. A two-factor analysis of variance was done with one factor being the measurement of the midinterventricular septum in a closed or open position and the other time after occlusion.

Table 2 represents the results of the analysis of variance. The F statistic for open vs closed chest was 0.41, which was not statistically significant (p > .5). The comparison of MSR at baseline and at five intervals after occlusion yielded a F statistic of 23.0, p < .0001.

A set of multiple comparisons of the differences between the baseline MSR and each MSR after occlusion is shown in table 3. The 99% confidence interval did not overlap zero, so the baseline MSR was significantly different from each postocclusion ratio.

Reproducibility of MSR in noninfarcted tissue of
TABLE 1
Mean (±SD) MSRs

<table>
<thead>
<tr>
<th>Time after occlusion (min)</th>
<th>0</th>
<th>30</th>
<th>60</th>
<th>120</th>
<th>180</th>
<th>240</th>
</tr>
</thead>
<tbody>
<tr>
<td>Septum open</td>
<td>1.99±0.05</td>
<td>2.21±0.06</td>
<td>2.26±0.11</td>
<td>2.24±0.07</td>
<td>2.32±0.17</td>
<td>2.29±0.09</td>
</tr>
<tr>
<td>Septum closed</td>
<td>2.01±0.07</td>
<td>2.22±0.07</td>
<td>2.22±0.05</td>
<td>2.30±0.05</td>
<td>2.34±0.06</td>
<td>2.28±0.06</td>
</tr>
<tr>
<td>Posterior wall</td>
<td>2.00±0.05</td>
<td>2.22</td>
<td>2.22</td>
<td>2.22</td>
<td>2.22</td>
<td>1.98±0.04</td>
</tr>
</tbody>
</table>

TABLE 2
Two-factor analysis of variance with unequal replication

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Sum of squares</th>
<th>d.f.</th>
<th>Variance</th>
<th>F ratio</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Open vs closed chest</td>
<td>0.0031</td>
<td>1</td>
<td>0.0031</td>
<td>0.41</td>
<td>&gt;.5</td>
</tr>
<tr>
<td>Time after occlusion</td>
<td>0.8683</td>
<td>5</td>
<td>0.1737</td>
<td>23.0</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Residual</td>
<td>0.3393</td>
<td>45</td>
<td>0.0075</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

d.f. = degrees of freedom.

FIGURE 3A. Electron microscopic slide of swelling of mitochondria with cristal stacking (arrows, to the right) compared with normal tissue (left).
open-chest dogs (left ventricular inferoposterior wall), tested in six animals, was 2.00 ± 0.05 at baseline and 3 to 4 hr later it showed no significant change by unpaired Student's t test (1.98 ± 0.04; p = .49).

Electron microscopy revealed swelling of the sarco-tubular system, mitochondrial swelling, and "cristal stacking" (figure 3A), as well as swelling of the capillary endothelium (figure 3B), at 3 to 4 hr after coronary occlusion. At this time light microscopy showed only minimal changes associated with ischemic contraction (figure 4).

**Discussion**

Ultrasonic tissue characterization is a new and potentially exciting field of cardiac ultrasound. Several different approaches to ultrasonic tissue characterization have been tested and it is not yet clear which, if any, is superior. We have worked with the statistical approach to ultrasonic tissue characterization and found that we can detect ischemia as early as 30 min after coronary artery occlusion in this study. This is in agreement with what some others have found using very different techniques. Although the current data are expressed as mean ± SD, and our method required a fair amount of averaging of data, the measurement 30 min after coronary artery occlusion was statistically significantly different from baseline. There may be several reasons for the relatively large spread in indi-

![FIGURE 3B](attachment:image_url) Swelling of capillary endothelium (arrows) 3 to 4 hr after coronary artery occlusion (right) compared with normal tissue (left).
individual measurements of MSR. First, the myocardium sampled may not have been homogeneously ischemic from epicardium to endocardium and this may be a patchy process. Second, there is most likely a degree of variation inherent in the method itself. Third, our method is extremely sensitive to any inclusion of high-amplitude signals in analysis of the window of interest, since such a specular reflector immediately will markedly increase the standard deviation of amplitudes and thereby decrease the MSR. Nevertheless, in contrast to that of Cohen et al., our method of analysis was independent of acoustic filtering by the chest wall and did not require any external or internal calibration such as that reported by Cohen and by Rasmussen et al. This is a very important feature since the method therefore becomes potentially applicable in humans. Our amplitude analysis showed excellent reproducibility over time in nonischemic myocardium when tested in the left ventricular inferoposterior myocardium.

The explanation for the altered rf signal in early ischemia, as reflected in the MSR, is not entirely clear. Our MSR parameter did not increase as a matter of increased echogenicity alone (higher mean amplitude) since we also registered a decrease in the standard deviation of the amplitude. The electron microscopic analysis suggests there are changes in tissue structures at the subcellular level and this may alter scattering properties. Although we have not proven that the changes seen on the electron microscopic slides can account for the observed changes in MSR, it is important to know there are changes in the tissue at this early point in time after coronary occlusion. Myocardial cells or bundles of cells can be looked upon as individual scatterers, and as found by others using erythrocytes, when the density of scatterers changes, the backscatter may change. Despite the uncertainty about why the MSR measurement changes with acute myocardial ischemia, we believe the results reported here, obtained with this statistical approach to the study of unprocessed rf data analysis and ultrasonic tissue characterization, are encouraging and warrant further work to better understand and utilize the technique.

References


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