Retrospective Determination of the Area at Risk for Reperfused Acute Myocardial Infarction With T2-Weighted Cardiac Magnetic Resonance Imaging

Histopathological and Displacement Encoding With Stimulated Echoes (DENSE) Functional Validations

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Background—The aim of this study was to determine whether edema imaging by T2-weighted cardiac magnetic resonance (CMR) imaging could retrospectively delineate the area at risk in reperfused myocardial infarction. We hypothesized that the size of the area at risk during a transient occlusion would be similar to the T2-weighted hyperintense region observed 2 days later, that the T2-weighted hyperintense myocardium would show partial functional recovery after 2 months, and that the T2 abnormality would resolve over 2 months.

Methods and Results—Seventeen dogs underwent a 90-minute coronary artery occlusion, followed by reperfusion. The area at risk, as measured with microspheres (9 animals), was comparable to the size of the hyperintense zone on T2-weighted images 2 days later (43.4 ± 3.3% versus 43.0 ± 3.4% of the left ventricle; P = NS), and the 2 measures correlated (R = 0.84). The infarcted zone was significantly smaller (23.1 ± 3.7; both P < 0.001). To test whether the hyperintense myocardium would exhibit partial functional recovery over time, 8 animals were imaged on day 2 and 2 months later. Systolic strain was mapped with displacement encoding with stimulated echoes. Edema, as detected by a hyperintense zone on T2-weighted images, resolved, and regional radial systolic strain partially improved from 4.9 ± 0.7 to 13.1 ± 1.5 (P = 0.001) over 2 months.

Conclusions—These findings are consistent with the premise that the T2 abnormality depicts the area at risk, a zone of reversibly and irreversibly injured myocardium associated with reperfused subendocardial infarctions. The persistence of postischemic edema allows T2-weighted CMR to delineate the area at risk 2 days after reperfused myocardial infarction. (Circulation. 2006;113:1865-1870.)

Key Words: contractility ■ edema ■ magnetic resonance imaging ■ myocardial infarction ■ myocardial strain

The area at risk1–3 is defined as hypoperfused myocardium at the time of an ischemic episode. What fraction of the area at risk sustains reversible or irreversible injury depends on a number of factors, including the duration of the occlusion and the presence of collateral vessels. To evaluate therapeutic procedures and drugs aimed at modulating infarct size, it is important not only to measure the size of an infarct but also to know how much myocardium was at risk.4,5 Thus, the percentage of infarcted myocardium within the area at risk provides an index that controls for factors that modulate infarct size other than the intervention or treatment.6–8

Measuring the area at risk is difficult in patients with acute myocardial infarction (MI). Ideally, imaging the area at risk should be simple, yield high-quality images that are easily registered with infarct images, and avoid interfering with acute patient management. Sestamibi single-photon emission computed tomography (SPECT) allows imaging after the patient is stabilized but requires radiotracer administration during ischemia. The need for radioactive tracers in an emergency department setting and the low spatial resolution of the method have limited this approach. Similar approaches may be feasible with manganese-enhanced cardiac magnetic resonance (CMR).9 Prolonged abnormalities in metabolism may highlight the area at risk and allow administration of newer SPECT agents after intervention.10
T2-weighted (T2W) CMR may provide an alternative approach. Many studies have correlated T2W CMR against infarct size, but the results generally show that T2 overestimates infarct size.\textsuperscript{11} Instead, T2W imaging may highlight myocardial edema and therefore could delineate the area at risk.

The aim of the present study was to determine whether edema imaging by T2W CMR could be used as an imaging tool for retrospectively delineating the hypoperfused area at risk in reperfused MI. It was hypothesized that the area at risk by fluorescent microspheres during a transient occlusion would be of similar size to the T2 abnormality observed 2 days later with CMR, and the infarcted zone would be a subset of the area at risk. Also, it was hypothesized that the T2W hyperintense region would resolve and show partial functional recovery after 2 months, which would be consistent with the premise that it encompassed a combination of some stunned myocardium and some infarcted myocardium.

Methods

Animal Preparation
All experiments were approved by the Animal Care and Use Committee of the National Heart, Lung and Blood Institute of the National Institutes of Health. Seventeen dogs (Marshall Farms, North Rose, NY) were anesthetized with subcutaneous administration of acepromazine (0.2 mg/kg), intravenous administration of thiopental sodium (15 mg/kg), and inhaled isoflurane (0.5% to 2.0%). Surgical preparation included a left jugular 8F Hickman catheter, a left atrial appendage infusion catheter (Brovic 6.6F), a femoral arterial line, and a balloon occluder around the left anterior descending coronary artery usually distal to the first diagonal branch. A 90-minute total left anterior descending coronary artery occlusion was performed to induce MI, followed by reperfusion as previously described.\textsuperscript{15} The animals were euthanized immediately on completion of CMR (9 on day 2, 8 on month 2) with a lethal injection of potassium chloride after heparin administration (10 000 U).

Relation Between Measures of Area at Risk
To compare the size of the T2W abnormality with the area at risk and the infarcted zone, 9 animals were studied. To obtain an adequate range of area at risk measurements for correlation statistics, 3 short-axis slices per animal were used (basal, mid, apical). Approximately 5\times10\textsuperscript{5} fluorescent microspheres (Interactive Medical Technologies, Irvine, Calif) were injected into the left atrial catheter at the time of the occlusion to determine the area at risk. Two days later, this group of 9 animals underwent 1.5-T CMR edema imaging (T2W imaging). After euthanasia, infarct size was defined by triphenyltetrazolium chloride (TTC) staining.

Resolution of T2 Abnormality and Functional Recovery Within the Area at Risk
To demonstrate that the T2W abnormality included both stunned and infarcted myocardium, the functional recovery of systolic strain was imaged in another group of 8 animals 2 days after occlusion and was repeated 2 months later. For these 8 animals, infarct size was quantified 2 days after occlusion by gadolinium-diethylenetriamine pentaacetic acid (Gd-DTPA) delayed-enhancement CMR images rather than relying on 2-month chronically remodeled TTC histopathological samples. One midventricular slice per animal was used for the functional recovery analysis.

Imaging Parameters
First, edema imaging was performed with T2W double-inversion blood-suppressed fast-spin-echo magnetic resonance imaging (MRI)\textsuperscript{14} with parameters as follows: 1.1\times1.1\times4-mm\textsuperscript{3} voxel, ±62-kHz bandwidth, 62.2-ms echo time (TE), 4- to 6-heartbeat repetition time (TR), 12-echo train length, and 4 averages. Second, left ventricular (LV) systolic strain was imaged using displacement encoding with stimulated echoes (DENSE)\textsuperscript{14,15} acquired at a spatial resolution of 1.0\times1.0\times7.0 mm\textsuperscript{3}, ±32-kHz bandwidth, 6.5-ms TE, 2-heartbeat TR, 12-echo train length, 1.2-mm/π encoding strength, and 4 averages. The DENSE gradient encoding strength was adjusted so that the antiecho was shifted out of the sampled k-space window. Last, delayed-enhancement viability imaging was performed ~20 minutes after injection of intravenous Gd-DTPA with the following parameters: 1.1\times1.1\times8-mm\textsuperscript{3} voxel, ±32-kHz bandwidth, 3.6-ms TE, 2- to 3-heartbeat TR, and phase-sensitive image reconstruction.\textsuperscript{16} The inversion time was adjusted to null the normal myocardium.\textsuperscript{17} A 20-heartbeat maximum per breathhold was allowed, and all acquisitions were done during suspended respiration.

Histopathology and Image Processing
The hearts were excised and immersed in isotonic agar solution. Once the agar solidified, hearts were sliced in 4-mm-thick sections with a commercial meat slicer. TTC staining was performed to demarcate the infarcted region.\textsuperscript{18} Briefly, the myocardial slices were immersed in a 1% TTC solution in saline at 37°C for ~4 minutes and then rinsed with physiological saline. Subsequently, 2 consecutive 4-mm slices were matched to the 8-mm CMR slice of interest by registering the total number of short-axis ex vivo slices obtained to the total number of short-axis CMR images obtained from the apex to the mitral valve plane. These two 4-mm slices were each sectioned into 16 transmural circumferential sectors for fluorescent microsphere counting. Every 2 tissue samples corresponding to the same transmural circumferential sector of the two 4-mm slices were paired together for this purpose. The anterior right ventricular insertion point was used to align these samples to the CMR images. For comparison with the microsphere sectors, T2W images were similarly sectioned by software into circumferential sectors and analyzed in that manner. Infarct size was determined automatically with in-house software by counting the number of pixels within the TTC-negative stained area. To allow for better cross-registration along the circumferential direction, both microsphere and CMR 16 sector data were linearly interpolated by a factor of 2 (32 circumferential sectors total).

The CMR infarcted region was quantified by a computer algorithm after manual image tracing of epicardial and endocardial borders.\textsuperscript{19,20} T2W and contrast-enhanced areas were measured on the basis of 50% of the peak myocardial signal intensities (half-height threshold). Color maps of intramural myocardial strain were reconstructed by custom software.\textsuperscript{14,21} Hypokinetic myocardium was defined as areas with <10% radial thickening. Abnormal blood flow was defined as <50% blood flow relative to a remote zone, which encompassed the sectors of highest blood flow within the slice.

Statistical Analysis
All areas are described as percent of the LV area. Results are mean±SEM. The sizes of the T2 abnormality, area at risk, and infarct zone were compared by use of paired t tests with Bonferroni correction for multiple comparisons. Linear correlation and Bland-Altman analysis were used to compare the size of the T2 abnormality with the area at risk. Because 2 to 3 myocardial slices were analyzed for each of the 9 animals used for area at risk comparisons (a total of 24 slices), a generalized linear model for repeated measures was performed with SPSS (version 11.0.1, SPSS Inc, Chicago, Ill). Repeated-measures ANOVA was used to test for differences in strain by zone over time. Value of P>0.05 were considered not significant. Signal-to-noise ratio was defined as the mean signal intensity within a region of interest divided by the mean value of the noise. Contrast-to-noise ratio within 2 regions of interest was defined as the difference of their signal-to-noise ratios.

The authors had full access to the data and take full responsibility for its integrity. All authors have read and agree to the manuscript as written.
Results

Relation Between Measures of Area at Risk

Figure 1 shows that the T2 abnormality was comparable in size to the area at risk but also that the T2 abnormality was significantly larger than the infarcted region. The average size of the area at risk (43.4±3.3% of LV area), as measured by fluorescent microspheres at the time of the occlusion, was not significantly different than the size of the hyperintense area seen in T2W CMR images 2 days later (43.0±3.4% of LV area; P=NS; n=24). The area at risk and the T2 abnormality were significantly larger than the infarcted territory (23.1±3.7% of LV area; both P<0.001; n=24), as measured by TTC-negative staining on day 2. Thus, the 90-minute occlusion resulted in a partially infarcted area at risk.

Good correlation (R=0.84) was observed between area at risk measurements by microspheres and the size of the corresponding T2W abnormalities (Figure 2, top). The Bland-Altman plot (Figure 2, bottom) showed no bias. Intrasubject correlations were not significant in the generalized linear model for repeated measures.

Resolution of T2 Abnormality and Functional Recovery Within the Area at Risk

Figure 3 (top row) shows delayed-enhancement, end-diastolic cine, T2W, and DENSE radial thickening strain images acquired from 1 of the 8 animals followed up over 2 months. The MI seen in the acute delayed-enhancement image is a subendocardial infarction. On average, the T2W abnormality on day 2 was brighter than the remote zone (signal-to-noise ratio, 10.9±1.1 versus 8.0±1.1; P=0.001; n=8). Two months later, the 2 regions were isointense (9.3±1.5 versus 9.1±1.4; P=NS; n=8). The contrast-to-noise ratio on day 2 was significantly different than 2 months later (2.9±0.8 versus 0.3±0.4; P=0.01).

Figure 4 summarizes regional recovery of function over a 2-month period within the hyperintense area in acute T2W images for all 8 animals in the 2-month follow-up group. Two days after acute MI, the DENSE systolic radial strain was severely reduced in the myocardium within the abnormal T2 zone compared with remote myocardium (4.9±0.7% versus 29.5±2.1% strain; P<0.001). Significant recovery of regional contractile function was observed in the abnormal T2 zone after 2 months (13.1±1.5% strain; P=0.001 versus day 2). However, the recovery of function was only partial, and this zone still exhibited significantly lower radial strain than remote myocardium (24.2±2.0% strain; P=0.001). Initial hyperkinesis in the remote zone decreased at the month 2 follow-up (P=0.017).

Similar results were seen for circumferential strain on day 2 (T2 zone, 5.9±0.7% strain; remote, 22.0±1.4% strain; P<0.001). Circumferential strain in the abnormal T2 zone recovered to 12.6±1.2% strain (P=0.001 versus day 2) but only partially relative to the remote zone (17.6±2.2% strain; P=0.006). Initial circumferential strain hyperkinesis in the remote zone decreased at the month 2 follow-up (P=0.015).

Discussion

The hyperintense region observed in T2W CMR images 2 days after reperfused infarction demarcated the area at risk at the time of the ischemic episode. This edematous region, visible in all 17 animals, was consistently larger than the infarcted region. The nearly transmural T2 abnormality re-

Figure 1. The area at risk was not significantly different than the edematous region seen by T2W CMR (P=NS). Both the area at risk and the T2 abnormality were significantly larger than the infarcted territory, as identified with TTC (both P<0.001). The area at risk was mapped by fluorescent microspheres during the 90-minute occlusion. T2W CMR for edema sizing and TTC-negative staining for infarct sizing were performed 2 days after occlusion. T2W CMR for edema sizing and TTC-

Figure 2. The area at risk measured by microspheres injected during ischemia correlated with the T2W abnormalities observed 2 days later (R=0.84; top). There was no systematic error by the Bland-Altman plot analysis (bias, 0.4%; SD, 9.0% LV area; bottom).
Pathological studies performed by Reimer and Jennings suggest that edema and inflammation are present transmurally, not limited just to the infarcted myocardium, with a reported 25% increase in total water content. In vivo imaging studies, Garcia-Dorado et al showed that T2 abnormalities closely correlated with increased total water content and accurately depicted the area at risk in a porcine ischemia reperfusion model. In infarcted perfused hearts, Boxt et al found similar correlations between T2 and myocardial total water content.

Ischemic myocardium shifts from aerobic metabolism to anaerobic glycolysis and ceases to contract. Lactate starts increasing 4-fold in the damaged tissue within minutes after the onset of ischemia. As the high-energy phosphates are depleted and the adenine nucleotide pool is catabolized, a range of osmotically active particles accumulates. Therefore, an osmolar load builds within the cell, resulting in water influx. T2W MRI potentially reflects this increase in water content with increased signal intensity. This effect lasts for days after the initial ischemic episode.

In acute MI, edema in the peri-infarct zone results from balanced water content increases in both the extracellular and intracellular compartment volumes of viable myocardium. Within acutely infarcted myocardium, there is a conversion of intracellular space into extracellular space as a result of cell lysis. Because of the increased total water content and increased water mobility, both the peri-infarct and the infarcted zones appear hyperintense on T2W images.

Delayed-enhancement CMR for acute viability assessment relies on Gd-DTPA, which does not traverse cell membranes but remains within the extracellular space. Importantly, delayed-enhancement imaging shows the relative sizes of the extracellular and intracellular volumes, i.e., the volume of distribution. For edematous viable myocardium, this ratio has not been substantially altered; therefore, the peri-infarct zone does not exhibit significant contrast enhancement. On the other hand, the acute infarct demonstrates delayed contrast enhancement as a result of the disproportionately increased apparent extracellular space.

DENSE14 allowed mapping intramural systolic strain at a resolution comparable to that of the infarct and the T2W images. The fundamental resolution of the DENSE experiment is equivalent to ≈1000 ultrasonic crystals implanted in a single short-axis slice of myocardium, all spaced a uniform 1 mm apart. The strain maps derived from the DENSE CMR show that the functional deficit in the acute setting is more...
comparable in size to the T2 enhanced zone than the infarcted region. Functional recovery after 2 months, measured by DENSE, confirms the presence of reversible injury within the partially infarcted area at risk.

Study Limitations
The present study does not address the issue of potentially increased water content in stunned myocardium in the absence of infarction and does not test how well these methods will work in nonreperfused infarctions. For comparing the area at risk by microspheres to the T2 abnormality, the average myocardial mass interrogated with microspheres was 650 mg, whereas the T2 voxels corresponded to 4.8 mg. Thus, the reference standard is much lower resolution than the T2W images being validated. The differences in resolution may contribute to the scatter between these measures. Using smaller tissue samples for microsphere analysis might not provide accurate flow measures. Also, the remote zone was defined by the highest myocardial flow within each slice. Selecting a single remote zone within the basal slice to analyze all 3 slices yielded similar results (not presented here), but different methods of threshold selection could potentially influence the magnitude of the results. Finally, for the correlation and agreement analyses, 1 apical and 2 basal slices of 27 could not be analyzed because of arrhythmias, which resulted in poor image quality.

Conclusions
Microsphere area at risk measurements correlate with the corresponding T2 abnormalities. The area at risk and the T2 abnormality were similar in size, and both were significantly larger than the infarcted zone. This suggested that the T2 hyperintense region encompassed both viable and infarcted myocardium associated with the area at risk. In support of this hypothesis, partial functional recovery was observed with high-resolution DENSE during the time when the T2 abnormality resolved. Therefore, edema imaging with T2W CMR delineates the area at risk 2 days after reperfused MI.

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Disclosures
None.

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**CLINICAL PERSPECTIVE**

The study of reperfusion therapies for acute myocardial infarction (MI) has been aided by an understanding of the importance of the initial area at risk, the degree of salvage, and the final infarct size. Infarct size can be determined by radionuclide single-photon emission computed tomography (SPECT) imaging, cardiac magnetic resonance (CMR), and computed tomography (CT). Measurement of area at risk by SPECT 99mTc sestamibi imaging was validated, but these images can be challenging to obtain in the setting of a clinical trial. This study uses microspheres to validate that high-quality T2-weighted (T2W) CMR imaging can determine the area at risk up to 2 days after an experimental acute infarction, taking advantage of the concept that the bright abnormality on T2W images likely represents myocardial edema. These T2W images of area at risk can be performed as part of a more comprehensive CMR examination of MI that also includes infarct size (by delayed-enhancement gadolinium imaging), regional and global function, and myocardial perfusion. Moreover, we also demonstrate that within the partially infarcted area at risk, strain maps of regional contractile function using a method known as displacement encoding with stimulated echoes (DENSE) demonstrate that noninfarcted tissue within the area at risk involve a reversible contractile abnormality in these reperfused infarcts. These CMR methods have excellent potential for translation to clinical practice. Thus, it should be possible to assess the area at risk, infarct size, and myocardial salvage in 1 examination scheduled after patient stabilization (approximately 2 days after MI). This should be a powerful way of assessing the efficacy of infarct reduction and reperfusion therapies.
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