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Circulating myosin light chain I levels after coronary reperfusion: a comparison with myocardial necrosis evaluated from single photon emission computed tomography with pyrophosphate

Hiroshi YOSHIDA,* Mamoru MOCHIZUKI,** Kazuyuki SAKATA,* Mitsuru TAKEZAWA,** Yasunori MATSUMOTO,** Masami YOSHIMURA,** Noriko MORI,* Shoichi YOKOYAMA,* Tsuneo HOSHINO** and Tsuneo KABURAGI*

**Department of Cardiology and **Department of Nuclear Medicine, Shizuoka General Hospital, Shizuoka, Japan*

This study was performed to assess the influence of coronary reperfusion on the serial serum myosin light chain (LC)I levels and to evaluate the relationship between the peak LCI level and the infarct size calculated from single photon emission computed tomography (SPECT) with technetium-99m pyrophosphate (Tc-99m PYP) in 11 patients who underwent coronary reperfusion. Blood was drawn before reperfusion, immediately after reperfusion, and once a day for 14 days, to estimate the time course of serum LCI release. The infarct size estimated by Tc-99m PYP ranged from 7.3 to 62.4 m*l*. The LCI levels obtained before reperfusion were less than 2.5 ng/m*l* but those obtained immediately after reperfusion were much higher. The value ranged from 2.7 to 9.7 ng/m*l* and that expressed as a percentage of peak LCI (% peak LCI) ranged from 19 to 83%. Collateral circulation, reperfusion arrhythmia and the degree of residual stenosis had no influence upon the % peak LCI. The correlation between peak LCI levels and SPECT-determined infarct size was good, with a correlation of 0.76 ($p<0.01$, regression line by least squares method $y=-3.31+1.53x$). Early serum LCI might be influenced by coronary reperfusion but the peak LCI value reflected acute myocardial necrosis in patients who underwent coronary reperfusion.

Key words: myosin light chain I, coronary reperfusion, myocardial necrosis, ^{99m}Tc-PYP/^{201}Tl dual SPECT

INTRODUCTION

ESTIMATING INFARCT SIZE in acute myocardial infarction is very important, because morbidity and mortality are related to the extent of myocardial damage. To limit the infarct size and to improve patients' survival, intracoronary thrombolysis, emergent percutaneous transluminal coronary angioplasty, and intravenous infusion of tissue plasminogen activator have been proposed. Several methods have been developed to evaluate infarct size in man. The 12-lead electrocardiogram (ECG), cumulative or peak creatine phosphokinase-MB (CPK-MB), ejection fraction of the left ventricle, and several radionuclide methods have been used. Myosin light chains are structural protein that are specific to cardiac muscle.[1,2] During myocardial infarction, persisting intracellular acidosis or activation of proteolytic enzymes, or both, finally leads to the release of structurally bound myosin light chains. This results in the continuous appearance of myosin light chains in serum well beyond the first week after the onset of myocardial infarction.[3] The purposes of this study were to assess the influence of coronary recanalization on the serial serum myosin light chain (LC) I levels and to evaluate the relationship between the serum peak LCI level and the infarct size calculated from the single photon emission computed tomography

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For reprints contact: Hiroshi Yoshida M.D., Department of Cardiology, Shizuoka General Hospital, Kitaandou 4–27–1 Shizuoka 420, JAPAN.

Table 1 Characteristics of study patients

Patient No.	Age/Sex	Infarct related artery	Interven-tion	Residual stenosis (%)	Interval to reflow	Reperfusion arrhythmia	Collateral flow	LCI (ng/ml)	
								before reflow	immediately after reflow
1	61/M	Seg. 1	ICT	50	1H	AIVR	poor	<2.5	6.5
2	49/M	Seg. 1	ICT	50	6H	(−)	good	<2.5	5.4
3	77/M	Seg. 1	ICT	90	2H	(−)	good	<2.5	6.3
4	72/M	Seg. 1	PTCA	25	3H	VT	poor	<2.5	9.6
5	67/F	Seg. 2	PTCA	25	3.5H	(−)	good	<2.5	3.9
6	65/M	Seg. 15	ICT	90	4H	(−)	poor	<2.5	5.5
7	71/M	Seg. 6	ICT	90	3H	AIVR	good	<2.5	5.8
8	76/M	Seg. 6	ICT	90	2H	(−)	poor	<2.5	7.0
9	53/M	Seg. 6	ICT	0	5H	(−)	poor	<2.5	2.7
10	38/M	Seg. 7	ICT	0	3.5H	AIVR	poor	<2.5	9.7
11	61/M	Seg. 9	ICT	90	4H	VT	poor	<2.5	4.0

M=male; F=female; Seg.=the segment of the coronary artery as defined by the AHA committee report; ICT=
PTCA=percutaneous transluminal coronary angioplasty; H=hour; AIVR=accelerated idioventricular rhythm;
CPK-MB=creatine phosphokinase-MB; PYP-uptake=value of uptake of technetium-99m pyrophosphate calculated
computed tomography. LCI=myosin light chain I; % peak LCI=LCI obtained immediately after reperfusion ex-

(SPECT) with technetium-99m pyrophosphate (Tc-99m PYP).

MATERIALS AND METHODS

Study patients

The study population comprised 11 patients with acute myocardial infarction. The diagnosis of acute myocardial infarction was determined on the basis of a history of precordial chest oppressive sensation due to cardiac ischemia lasting more than 30 minutes and an electrocardiographic ST elevation of at least 2 mm in 2 or more adjacent leads. All patients were transferred to the catheter laboratory immediately after arrival and emergency coronary angiography was performed. All the infarction-related arteries had been totally or subtotally occluded. Six patients had good collateral flow from the contralateral coronary artery. Urokinase was infused into the infarct-related arteries at the rate of 24,000 U/min, to a dose of 960,000 U, in every patient. Angiography was repeated every 10 minutes to determine the precise timing of reperfusion. Nine of 11 patients had rapid distal opacification of the coronary artery involved. The remaining 2 patients did not achieve successful reperfusion by intracoronary thrombolysis, so percutaneous transluminal coronary angioplasty was attempted, and good antegrade coronary flow was obtained. Accelerated idioventricular rhythm or ventricular tachycardia was followed by reperfusion in 5 of 11 patients. The interval from the onset of acute myocardial infarction to recanalization was within 6 hours in all patients. The site of infarction was inferior in 6 of 11 patients and anterior in the remaining 5 patients (Table 1). After successful reperfusion, all patients were transferred to the coronary care unit. Hemodynamics were stable and no fatal arrhythmias were noted in any of the patients. A standard 12-lead ECG was recorded every 4 hours.

Measurements of serum CPK-MB

Blood samples for CPK-MB assay were obtained before and immediately after the reperfusion, every 2 hours within 24 hours from the onset of infarction, and every 4 hours during the next 24 hours. Total CPK activity was determined by the modified Rosalki method.[4] CPK-MB was measured by cellulose acetate membrane electrophoresis.

Measurement of serum myosin light chain I

Blood was drawn before and immediately after the reperfusion, and once a day for 14 days, to estimate the influence of reperfusion on serum LCI levels and to obtain the peak LCI level. The LCI value obtained immediately after reperfusion was expressed as the percent of the peak LCI value (% peak LCI). We use a sensitive immunoradiometric assay kit (Myosin LI Assay Kit "Yamasa") for cardiac myosin light chain I by using anti-myosin light chain I monoclonal antibodies. This kit measures circulating LCI in the range of 2.5 to 100 ng/ml and shows little intra- or inter-assay variation.[5]

Radionuclide studies

Within 7 days from the onset of acute myocardial infarction (range 2–7 days), scintigraphy of Tc-99m PYP and thallium-201 (Tl-201) was simultaneously imaged at the nuclear medicine laboratory. Three hours after the injection of 740 MBq Tc-99m PYP, 111 MBq Tl-201 was injected. Five minutes later,

peak	% peak LCI (%)	CPK-MB (IU/l) peak	Infarct size (ml) (PYP-uptake)
24.8	26	507	62.4
21.2	26	331	17.3
13.1	29	45	8.4
32.1	30	648	52.3
11.1	35	126	9.7
6.6	83	132	15.7
30.5	19	574	27.9
10.9	64	220	12.2
5.6	48	135	11.1
14.4	67	502	17.6
11.4	35	388	7.3

intracoronary thrombolysis;
VT=ventricular tachycardia;
on single photon emission
pressed as the percentage of peak LCI.

imaging was performed with a rotating single-headed digital gamma camera (SHIMADZU SNC-500R) equipped with a low-energy, high resolution, parallel-hole collimator. This gamma camera was interfaced to a dedicated computer (SHIMADZU Scintipac 700). A 15% window was centered over the 140 keV photopeaks characteristic of Tc-99m PYP, and a 20% window was centered over the 75 keV photopeaks characteristic of Tl-201. Data were obtained with the gamma camera from 32 equally spaced stops over a 180 degree arc, from 45 degrees right anterior oblique to 45 degrees left posterior oblique. All projection data were acquired for 30 sec and yield 120,000 to 180,000 counts of Tl-201 and 120,000 to 160,000 of Tc-99m PYP. On the average, 7% of these respective counts were within the myocardial region with thallium and technetium activity. Tomographic data were acquired in a 64×64 matrix with a 1.3×hardware zoom factor. The image displays of SPECT were a vertical long axis, a horizontal long axis, and a short axis. To eliminate bone activity, reconstructions were masked by drawing a region of interest around the heart. The weight of the myocardial infarction was measured by automatically drawing a region of interest around the myocardial Tc-99m PYP uptake for each short axial slice. Thresholds were determined from phantom studies. A 2 ml capsule phantom was enclosed in a cone shaped myocardial phantom which was suspended eccentrically in a 20 cm diameter cylinder. The capsule phantom was mounted with 1.85 MBq of technetium-99m and the remainder of the cone shaped myocardial phantom was mounted with 18.5 MBq of thallium-201. Both 18.5 MBq of technetium-99m and thallium-201 were enclosed in the cylinder simulated back ground, then dual nuclide SPECT was performed with photopeaks and windows identical to those in the *vivo* study. A 70% cutoff method yielded the SPECT-estimated volume that was closest to the actual volume of the uptake. Therefore, we used the 70% cutoff level in the present study. In short axial slices, all pixels within the region of interest that were equal to or greater than 70 percent of the maximal count rate were incorporated into the infarct weight. The total number of pixels in all slices demonstrating increased tracer uptake were then added together and multiplied by the voxel volume (0.05758 ml). The voxel volume was calculated from the pixel size.

Statistical analysis

Comparison of peak LCI levels, peak CPK-MBs and infarct size determined by Tc-99m PYP uptake was performed by regression equations calculated by least squares methods. The correlation coefficients reported are Pearson r values calculated by regression analysis. The difference in the % peak LCI was compared by two sample Wilcoxon test.

RESULTS

In all 11 patients, Tc-99m PYP accurately accumulated in the infarcted area on SPECT images corresponding with the territory of the infarct-related artery and the serial ECG (Fig. 1). The Tc-99m PYP estimates of infarct size ranged from 7.3 to 62.4 ml (Table 1).

The LCI levels obtained before reperfusion were less than 2.5 ng/ml in all 11 patients. But the LCI levels obtained immediately after reperfusion were considerably increased, even within 6 hours from the onset of acute myocardial infarction. The values ranged from 2.7 to 9.7 ng/ml. The % peak LCI ranged from 19 to 83% (Fig. 2). Good collateral circulation, reperfusion arrhythmia and residual stenosis less than 90% were noted in 4, 5 and 6 of 11 patients, respectively. There was no difference in the % peak LCI between cases with and without collateral circulation. Reperfusion arrhythmia and the degree of residual stenosis had no significant influence upon the % peak LCI. (Fig. 3). The peak LCI levels ranged from 5.6 to 30.5 ng/ml and peak CPK-MBs ranged from 45 to 648 IU/l (Table 1).

The correlation between peak LCI levels and SPECT-determined infarct size was good, with a correlation of 0.76 ($p<0.01$, regression line by least squares method $y=-3.31+1.53x$). Peak CPK-MBs correlated less satisfactorily with SPECT-determined infarct size, with a correlation of 0.71 ($p<0.05$, regression line by least squares method $y=1.39+0.06x$) (Fig. 4). The correlation between peak LCI

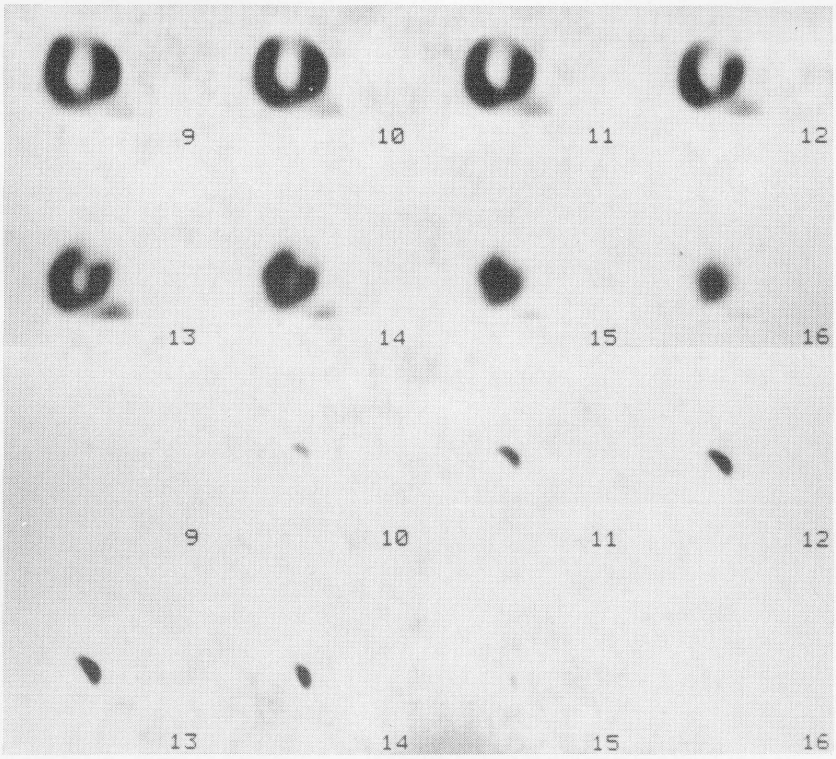

Fig. 1 Short axial reconstructions from simultaneous thallium-201 chrolide (top) and technetium-99m pyrophosphate (bottom) SPECT acquisitions from patient 11. The threshold level of thallium-201 (Tl-201) was 30% and that of technetium-99m pyrophosphate (Tc-99m PYP) was 70%. The uptake of Tl-201 was reduced in the anterior and anterolateral walls, and the uptake of Tc-99m PYP was observed in the same region. The infarct size obtained from Tc-99m PYP was 7.3 m*l*.

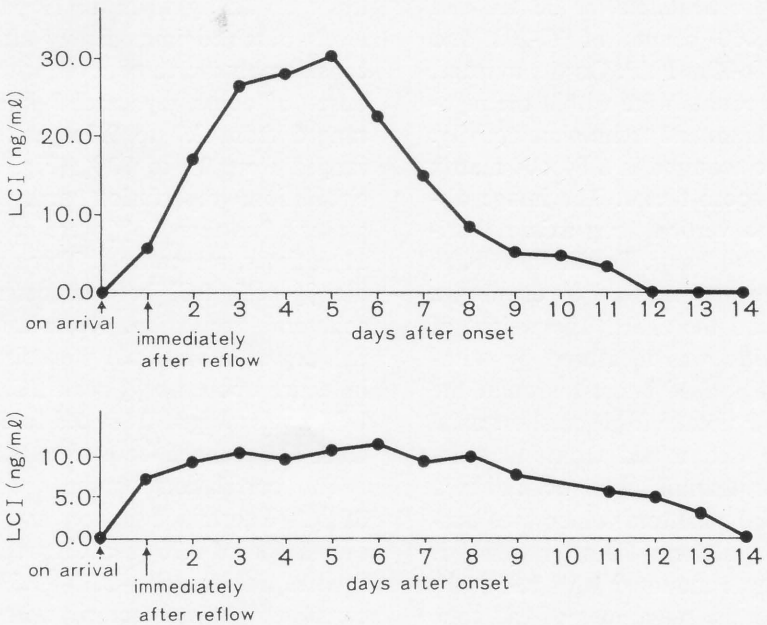

Fig. 2 Time course of serum myocardial light chain I (LCI) following acute myocardial infarction in two patients. Serum LCI increased significantly after reperfusion and a prolonged increase in LCI was noted. Upper panel, Case 7; lower panel, Case 6.

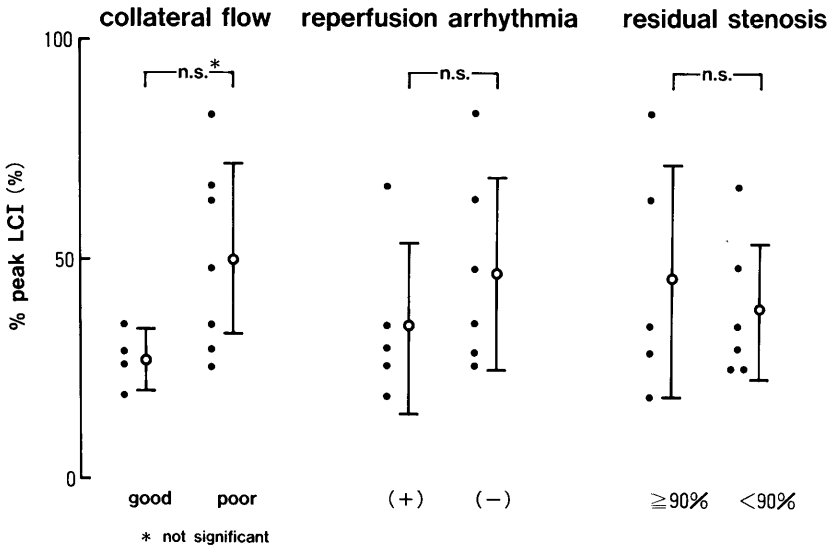

Fig. 3 Comparison of the % peak LCI in the cases with and without collateral circulation, with and without reperfusion arrhythmia, and in the degree of residual stenosis. The % peak LCI was not affected by the presence of good collateral circulation, the presence of reperfusion arrhythmia or the degree of residual stenosis.

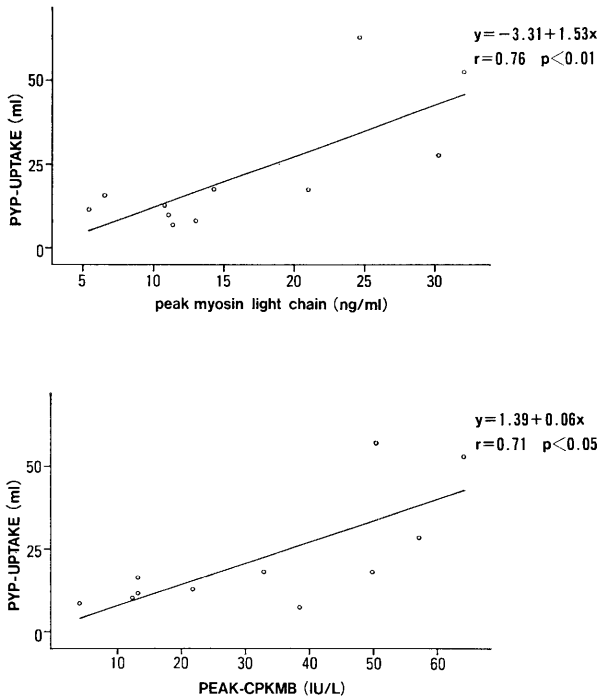

Fig. 4 Correlation of infarct size calculated from technetium-99m pyrophosphate uptake on SPECT with peak myosin light chain (upper) and peak CPK-MB (bottom). The correlation between the peak myosin light chain level and SPECT-determined infarct size was good, with a correlation of 0.76 ($p<0.01$, regression line by least squares method $y=-3.31+1.53x$). Peak CPK-MB correlated less satisfactorily with SPECT-determined infarct size, with a correlation of 0.71 ($p<0.05$, regression line by least squares method $y=1.39+0.06x$).

levels and peak CPK-MBs was also good, with a correlation of 0.83 ($p<0.01$).

DISCUSSION

Our data demonstrated that early serum LCI levels underwent reperfusion influence, but that the peak LCI levels obtained 3 to 7 days after the onset of acute myocardial infarction reflected infarct size in patients with successful reperfusion.

The size and location of infarcted tissue has an important influence on patient mortality and morbidity. Several methods have been developed to estimate acute infarct size. The ECG is useful for the diagnosis of acute myocardial infarction, but it is not quantitative. Serial and peak CPK-MB is directly related to infarct size,[6] but early contact with the patient and serial blood samples are required for this assay. Furthermore, the CPK-MB release rate was accelerated and the recovery rate of CPK-MB into the serum was increased in the presence of complete reperfusion in dogs.[7] The estimation of infarct size by serial CPK-MB should therefore be done with caution in a patient who has undergone coronary reperfusion. Ejection fraction is an indirect measure of infarct size and a useful parameter in determining post infarction prognosis.[8] However factors other than the extent of myocardial necrosis, including the function of the nonischemic myocardium, the size of the ventricle, preload and afterload of the left ventricle, heart rate, and drugs affect the ejection fraction. In addition, previous myocardial scarring also influences the degree of depression of

the ejection fraction. So the ejection fraction has a good correlation with the clinical outcome in patients who have suffered from myocardial infarction, but it is not suitable for estimating directly the infarct size. Myocardial scintigraphy with Tl-201 has emerged as a noninvasive technique that aids in the detection, localization, and quantification of myocardial necrosis.[9] Tomographic imaging techniques overcome the geometric limitations of the planar scintigraphic methods. However, myocardial uptake of Tl-201 is reduced in the acute and previous necrotic areas, so the acute infarcted area cannot be measured by the extent of the perfusion defect of Tl-201. Furthermore, the larger the infarct size, the more difficult it is to trace the defect by manual techniques or computerized planimetry.

Tc-99m PYP accumulates in acute necrotic myocytes and the myocardial uptake of pyrophosphate reflects myocardial necrosis. SPECT with Tc-99m PYP has been used to determine the size of myocardial necrosis.[10,11] A dual tracer SPECT technique with Tc-99m PYP and Tl-201 enabled us to record thallium-201 and technetium-99m pyrophosphate images simultaneously and to compare both images in the same slices, so the localization and quantification of myocardial necrosis is much easier. Several studies have described an overestimation of infarct size calculated by Tc-99m PYP in preparations of temporary coronary occlusions followed by reperfusion, but Jansen reported that SPECT imaging with adequate threshold makes possible accurate localization and determination of infarct size when Tc-99m PYP was injected 90 min or more after reflow.[12] So SPECT with Tc-99m PYP is attractive for the evaluation of acute infarct size.

Myosin light chains are bound to insoluble myosin heavy chains, whereas only a minor fraction exists as a cytosolic precursor pool of myosin synthesis. In myocardial infarction, myosin light chains are gradually released from myocytes. Nagai et al. and Katus et al. reported that the serum concentration changes in myosin light chains showed a biphasic pattern in acute myocardial infarction. An initial rise found at 6 to 18 h after the onset of pain reflected a release from the cytosolic light chain, and a continuous delayed increase detected up to the 10th day after the onset of pain corresponded to an ongoing breakdown of the insoluble structural cardiac myosin light chain.[13,14] Isobe et al. reported that the influence of drastic changes in coronary flow soon after the onset of acute myocardial infarction would be expected to be minimal on the release of myosin light chains.[5,15] However our study demonstrated that before reperfusion the serum concentration of LCI was within normal limits and immediately after reperfusion the serum concentration of LCI significantly increased, even within 6 hours from the onset of acute myocardial infarction. The % peak LCI ranged from 19% to 83%, which was not considerably influenced by collateral circulation, reperfusion arrhythmia or the degree of residual stenosis. But a significant increase in LCI obtained after reperfusion might reflect not only a release from the pool of soluble cytoplasmic myosin light chains but also a release of structurally bound myosin light chains from myofilament caused by proteolysis. Early restoration of coronary blood flow facilitates the washing out of both CPK-MB and myosin light chains from the infarct myocardium. Washing out of myocardial myosin light chains release by effective reperfusion may affect the early phase of the time course of myosin light chains. The amount of myosin light chains released reflects myocardial necrosis estimated by left ventriculography in patients with successful reperfusion or those treated with conventional therapy.[5] We also found a good correlation between the peak LCI level and the infarct size estimated by Tc-99m PYP accumulation in patients with successful coronary reperfusion.

In the present study, we demonstrated that the early serum LCI might be influenced by coronary reperfusion but the peak LCI level reflected acute myocardial necrosis in patients who underwent coronary reperfusion. An early and prolonged increase in LCI might be valuable for detecting the presence of good reperfusion and evaluating myocardial necrosis.

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