Analysis of Creatine Kinase, CK-MB, Myoglobin, and Troponin T Time–Activity Curves for Early Assessment of Coronary Artery Reperfusion After Intravenous Thrombolysis

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Background. Thrombolysis has become the standard therapeutic approach in patients with acute myocardial infarction. To identify patients who may benefit from early invasive procedures, reliable noninvasive assessment of success or failure of thrombolytic therapy is mandatory.

Methods and Results. In a prospective study in 63 consecutive patients undergoing thrombolysis for their first myocardial infarction, serial measurements of creatine kinase (CK), its isoenzyme CK-MB, myoglobin, and troponin T were done to determine their value for noninvasive prediction of coronary artery patency. Blood samples were drawn every 15 minutes during the first 90 minutes, every 30 minutes during the first 4 hours, every 4 hours during the first 24 hours, and every 8 hours during the first 72 hours. The perfusion status of the infarct-related artery was assessed angiographically 90 minutes after initiation of thrombolysis. For each marker, time to its peak concentration and its early initial slope (start of thrombolysis to 90 minutes thereafter) were determined. Areas under receiver operator characteristic (ROC) curves were 0.83, 0.76, 0.82, and 0.80 for maxima of CK, CK-MB, myoglobin, and troponin T, respectively (p < 0.05 by univariate Z test). The corresponding values for early slopes of CK, CK-MB, myoglobin, and troponin T were 0.79, 0.82, 0.89, and 0.80 (p = 0.23 for comparison between myoglobin and CK-MB; p = 0.07 between myoglobin and CK). Sensitivity, specificity, and positive and negative predictive values regarding noninvasive prediction of coronary artery patency after 90 minutes were 80%, 82%, 95%, and 61% for time to CK maximum; 91%, 77%, 91%, and 77% for time to myoglobin maximum; 87%, 71%, 89%, and 67% for early CK slope; and 94%, 88%, 94%, and 82% for myoglobin slope, respectively. When myoglobin slope was assessed together with other clinical reperfusion markers (resolution of chest pain or ST segment elevation, occurrence of reperfusion arrhythmias) by logistic regression analysis, only the myoglobin slope was an independent predictor of coronary artery patency (p < 0.0001).

Conclusions. With regard to noninvasive prediction of coronary artery patency after thrombolytic therapy, measurement of the early initial slopes of the serum markers within only 90 minutes after the initiation of therapy is as accurate as the determination of the time to their peak concentration. Compared with the other markers examined, myoglobin appears to have advantages because of its earlier rise, yielding a better negative predictive value and a higher area under the ROC curve for determination of its early initial slopes. (Circulation 1993;87:1542–1550)

Key Words • myocardial infarction, acute • thrombolysis • reperfusion

The value of coronary artery reperfusion by means of intravenous thrombolysis in patients with evolving myocardial infarction has been rigorously evaluated.1–4 An impressive improvement in survival rates has been demonstrated in patients with successful reopening of the infarct-related artery. In addition, left ventricular function has been shown to be superior in patients treated with thrombolysis compared with placebo-treated patients.5 It is evident from both laboratory and clinical studies that the benefits of thrombolytic therapy are conferred by restoration of patency of the infarct-related artery.5–9 Recently, Abbottsmith and coworkers10 have reported that salvage angioplasty after failed thrombolysis improves outcome in these patients. Thus, invasive means are valuable in selected patients to establish coronary artery patency.11,12 Rescue thrombolysis with readministration of the same or a different thrombolytic agent may also have a role after failed initial thrombolytic therapy.13 On the other hand, acute routine angiography in all patients receiving thrombolytic therapy for acute infarction is unnecessary and may eventually cause harm to some.14–16 Hence, reliable methods other than angiography are needed to detect coronary reperfusion early during the hospital course. In previous studies, the normalization of ST segment elevation in the surface
ECG,17-21 the occurrence of reperfusion arrhythmias,19,22-24 the resolution of infarct-related chest pain,25,26 and various cardiac enzymes have been used for this purpose. For example, a short time to peak creatine kinase (CK)27 or CK-MB concentration28,29 has been proposed as an indicator of reperfusion. More recently, new serum markers such as myoglobin,28,30 CK-MB isoenzymes,31 and the specific cardiac antigen troponin T32 have become available and have been examined in small groups of patients with acute myocardial infarction. Up to now, however, the value of these new reperfusion markers has not been vigorously evaluated, and they have not been compared with each other in a reasonably large cohort of patients undergoing thrombolysis. The aims of the present prospective study, therefore, were 1) to develop simple indexes for success or failure of reperfusion therapy according to the initial CK and CK-MB time-activity curves and 2) to evaluate whether new reperfusion markers such as myoglobin and troponin T yield a higher diagnostic accuracy in prediction of thrombolysis-induced reopening of the infarct-related artery compared with conventional serum markers. Particular attention was paid to the question of whether analysis of early increases in enzyme or protein concentrations (within 120 minutes after start of thrombolysis) is as accurate regarding prediction of coronary artery patency as the determination of the time to their peak concentration, since this would be of marked clinical utility.

Methods

Patient Population

Sixty-three consecutive patients with acute myocardial infarction treated with thrombolysis were studied prospectively between May 1990 and October 1991. Patients with typical chest pain of ≥30 minutes' duration, unresponsive to nitrates, and with ECG ST segment elevation of ≥0.1 mV in at least two limb leads or ≥0.2 mV in at least two precordial (V) leads were eligible for the study. Patients with cardiogenic shock were excluded. Thrombolytic therapy had to be initiated within 6 hours after onset of symptoms. The thrombolytic agent used was prourokinase in 32 patients combined with recombinant tissue plasminogen activator (rt-PA) in 20 patients and anisoylated plasminogen streptokinase activator complex (APSAC) in 31 patients. After thrombolysis, all patients received a bolus of 7,500 to 10,000 units of heparin followed by a continuous infusion of 1.250 units/hr for the next 72-96 hours (partial thromboplastin time adjusted to two times the upper normal). All patients gave their informed consent to the study. The usual exclusion criteria for thrombolysis were applied.

Coronary Angiography

Coronary angiography was performed within 90 minutes after onset of thrombolytic therapy and repeated 24 hours later. The angiographic findings were assessed according to the Thrombolysis in Myocardial Infarction (TIMI) criteria for reperfusion.4 For this purpose, the first injection of contrast was taken as the patency assessment. TIMI grades 2 and 3 were defined as coronary artery patency. For statistical analysis, early patency within the first 90 minutes was considered successful coronary artery opening.

Serum Markers

Blood samples (10 mL) were taken before initiation of thrombolysis, every 15 minutes for the first 90 minutes after start of therapy, every 30 minutes for the first 4 hours, every 4 hours up to 24 hours, and every 8 hours up to 72 hours. The samples were stored at room temperature to allow clotting and frozen at −20°C after centrifugation until analysis. Samples were analyzed by investigators who were unaware of the angiographic results. Plasma total CK activity was assayed according to the method of Rosalki.33 The mass of the CK-MB isoenzyme was determined by means of a rapid fluorometric enzyme immunoassay based on a two-site sandwich immunoassay methodology (Baxter Stratus, Miami, Fla.).34 Units are given in nanograms per milliliter. Myoglobin was determined nephelometrically by a quantitative latex agglutination test developed from an earlier-described semiquantitative test (Behring Werke, Marburg, Germany).35 Assay times are 15-30 minutes. The test was validated against the commonly used radioimmunoassay determination of myoglobin30 (Behring Werke; data on file). Troponin T was measured with a recently developed enzyme immunoassay (Böhringer Mannheim, Germany) that has been described in detail elsewhere.32,36,37 The upper limits of the normal range were 70 units/L for creatine kinase, 5.6 ng/mL for CK-MB (mass), 85 ng/mL for myoglobin, and 0.2 ng/mL for troponin T. For each of these serum markers, time to peak concentration after start of therapy was calculated. In addition, the slope of each serum marker within the first 90 minutes was calculated and expressed in units per liter per hour or nanograms per milliliter per hour by dividing the difference of the corresponding values at initiation of therapy and 90 minutes thereafter by 1.5. In Figure 1, raw data from a patient with successful reperfusion and from another without reperfusion are shown to illustrate the technique of slope determinations.

Clinical Reperfusion Markers

Resolution of maximal ST segment elevation was judged from two surface ECGs taken before and 120 minutes after start of thrombolysis. In addition, Holter monitoring was started before injection of the thrombolytic agent for assessment of reperfusion arrhythmias within the first 90 minutes. Accelerated idioventricular rhythm (AIVR) was defined by a heart rate of ≤100 beats per minute, a long coupling interval, a regular configuration and rate, and termination by sinus escape. Sinus bradycardia was defined by a heart rate of <55 beats per minute. Patients were also interviewed for the severity of chest pain on a subjective scale ranging from 0 to 10 before and 90 minutes after thrombolysis.

Statistical Methods

Values are given as mean±SD. The calculation of sensitivity, specificity, positive predictive value, and negative predictive value was performed according to the formulas described in our previous report.19 A χ² test was used to test the univariate correlation of noninvasive markers to patency of the infarct artery as well as differences of numerical parameters between
groups. Linear regression analysis was performed for related parameters, and coefficients of correlations were calculated and tested for significance. The independent correlation of serum and clinical markers to coronary artery patency was determined by means of logistic regression analysis with coronary artery patency as the outcome variable. Markers were entered as categorical variables (positive or negative) by use of their respective cutoff values (see below). For statistical purposes, the following definitions of positive reperfusion markers were applied. According to two previous studies at our institution, a maximum of CK activity within the first 12 hours after start of thrombolysis was prospectively defined as a positive marker of coronary artery patency. For the CK slope, a cutoff of 40 units·L⁻¹·hr⁻¹ was prospectively derived from the report of Lewis et al. As reported by Hogg et al. and a previous study from our institution, a positive ST marker was defined as a reduction of ST segment elevation of ≥50%. With respect to pain score, a decrease of at least two points was considered suggestive of reperfusion. AIVR and sinus bradycardia <55 beats per minute within the first 90 minutes (only in patients with inferior infarctions) were prospectively defined as reperfusion arrhythmias. The cutoffs for the time to peak CK-MB, myoglobin, and troponin T were retrospectively defined at 10, 3, and 15 hours after start of thrombolysis, respectively, since no previously described cutoff values were available from the literature. Accordingly, the cutoffs for the absolute rise of each marker over the first 90 minutes of therapy were chosen as 10, 150, and 0.2 ng·mL⁻¹·hr⁻¹ for CK-MB, myoglobin, and troponin T, respectively. To allow comparison with the CK data, these numbers were chosen to represent similarly good but not optimal cutoffs. Since potential bias concerning the choice of a single cutoff cannot be excluded (even if prospectively defined), sensitivity and specificity data are represented over the entire cutoff range in the applicable figure (Figure 2). Furthermore, receiver operator characteristic (ROC) curve analysis as an objective means for determination of the utility of the various markers for noninvasive prediction of coronary artery patency was applied. The major advantage of this technique is provided by its independence of definitions of cutoff values. This statistical method was initially developed in the field of signal detection theory and provides a powerful tool to assess the predictive quality of a given test. With this technique, sensitivity (true positives) is plotted against 1-specificity (true negatives) for the possible

range of cutoff points of a specific marker. The area under the ROC curve represents a single number for the predictive quality of the test, with a maximum of 1.0 representing an ideal test (100% accuracy) compared with an area of 0.5 indicating that the test is no better than chance. Thus, ROC curve analysis yields a single value for each marker evaluated. To compare the different areas under the ROC curves of several markers (i.e., to compare the predictive quality of tests), a univariate Z-test has been described in detail by Hanley and McNeil. Significance was considered at a value of p<0.05.
**Results**

Clinical Data

Sixty-three consecutive patients were included in the protocol. There were 54 men and nine women with a mean age of 59±11 years (range, 33–81 years). Twenty-eight patients suffered from anterior and 35 from inferior myocardial infarction. Angiography revealed the left anterior descending coronary artery in 26, the right coronary artery in 32, and the circumflex artery in five patients as the corresponding infarct-related artery. The time interval from onset of chest pain to start of thrombolysis averaged 190±112 minutes. In 46 patients (73%), angiography revealed TIMI grade 2 or 3 reperfusion of the infarct-related vessel at 90 minutes. In the remaining 17 patients, the infarct-related artery was found to be occluded (TIMI grade 0 or 1). Late reperfusion of the infarct vessel between 90 minutes and 24 hours occurred in 10 patients, whereas two patients had reocclusion within this time frame. Among the 17 patients with an occluded vessel on the first coronary angiogram, late reperfusion (within 24 hours) did not significantly influence the time elapsed to peak concentrations of the various serum markers. Thus, these patients are referred to as one group throughout the paper. Four patients had to be defibrillated within the first hours because of the occurrence of ventricular fibrillation, and one other patient had femoral bleeding consequent to angiography. Since the overall predictive value of the markers examined was not significantly influenced by the data of these five patients, they were included in the analysis.

Analysis of CK

The mean peak CK value was 1,041±686 units/L for patients with a patent versus 942±758 units/L for patients with an occluded infarct artery (p=NS). Time to peak concentration averaged 9.0±6.2 hours in patients with versus 17.1±6.4 hours for patients without coronary artery patency (p<0.001). Sensitivity and specificity for prediction of vessel patency at the chosen cutoff were 80% and 82%, respectively, and they were comparable for a range of cutoffs between 10 and 15 hours (Figure 2, Table 1). This resulted in a high area under the ROC curve of 0.83 and a univariate $\chi^2$ of 18.5 (Figure 3). For the initial CK slope between 0 and 90 minutes after start of therapy, sensitivity and specificity were almost comparable at 87% and 71%, respectively (Figure 1 and Table 1). Accordingly, ROC curve area

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<th>Positive predictive value (%)</th>
<th>Negative predictive value (%)</th>
<th>$\chi^2$</th>
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ROC, receiver operator characteristics; CK, creatine kinase.

![Figure 3](http://circ.ahajournals.org/doi/figure/10.1161/01.CIR.87.5.1546)

**Figure 3.** Receiver operator characteristic (ROC) curves for noninvasive prediction of coronary artery patency by means of peak and slope measurements of creatine kinase (CK), CK-MB, myoglobin, and troponin T (n=63).
and $\chi^2$ were slightly lower, at 0.79 and 17.4 (Table 1). The optimal range of cutoffs was between 40 and 80 units \( \cdot \text{L}^{-1} \cdot \text{hr}^{-1} \) (Figure 2). An inverse correlation was found between CK slope and maximum ($r= -0.40$, $p<0.001$).

**Analysis of CK-MB Mass**

Peak CK-MB averaged 216±151 ng/mL for patients with a patent versus 167±130 units/L for patients with an occluded infarct artery ($p=NS$). Time to peak was similar to that of CK and averaged 7.5±1.1 hours in patients with versus 12.0±5.3 hours for patients without coronary artery patency ($p=0.004$). Sensitivity and specificity for prediction of vessel patency at the chosen cutoff of 10 hours were 78% and 65% for CK-MB maximum, respectively. The best range of cutoffs was 8.5–12 hours. For the slope of CK-MB, the range of acceptable cutoffs was 15–20 ng \( \cdot \text{mL}^{-1} \cdot \text{hr}^{-1} \) (Figure 2 and Table 1). ROC curve analysis disclosed values of 0.76 for CK-MB maximum and 0.79 for CK-MB slope.

**Analysis of Myoglobin**

Myoglobin peaked after 2.1±2.8 hours in patients with successful (peak value, 2.361±1,824 ng/mL) compared with 4.9±4.2 hours ($p<0.02$) in patients with failed reperfusion (peak value, 1.037±1137 ng/mL, $p=0.001$). Sensitivity was 91% together with a specificity of 77% at the predefined cutoff of 3 hours. For the myoglobin slope, a sensitivity of 94% and specificity of 88% at the preselected cutoff of 150 ng \( \cdot \text{mL}^{-1} \cdot \text{hr}^{-1} \) were obtained (Figure 2). ROC curve area was comparable to the other markers for myoglobin maximum at 0.82; however, the univariate $\chi^2$ value was highest of all maxima with 25.6. Myoglobin slope revealed the highest ROC curve area and univariate $\chi^2$ value of all studied markers (ROC, 0.89; $\chi^2=36.7$) (Figure 3, Table 1). There was an inverse correlation between myoglobin slope and maximum ($r=-0.35$, $p<0.01$).

**Analysis of Troponin T**

Troponin T peaked after 11.6±9.3 hours in patients with successful reperfusion (peak value, 23.6±18.8 ng/mL) compared with 20.3±14.4 hours ($p=0.03$) in patients without reperfusion (peak value, 101±6.3 ng/mL; $p<0.001$). Sensitivity and specificity were 74% and 71%, respectively, at the preselected cutoff of 15 hours, with a narrow range of favorable cutoffs between 14 and 16 hours (Figure 2). This range was also narrow for the analysis of troponin T slopes, for which sensitivity was 80% at a specificity of only 65% for the predefined cutoff of 0.2 ng \( \cdot \text{mL}^{-1} \cdot \text{hr}^{-1} \). ROC curve areas were 0.80 for both maximum and slope analysis of troponin T (Figure 3, Table 1). Univariate $\chi^2$ values were low, with 8.6 for troponin maximum and 9.7 for troponin slope.

**Comparison of Serodiagnostic Markers**

The ROC curves of the four serum markers were plotted to compare the value of each serum marker regarding noninvasive prediction of reperfusion. Analysis of time to peak concentration revealed similar values for all four markers evaluated. Comparison of early slopes demonstrated the highest ROC area for analysis of myoglobin (Figure 3, Table 1). The difference in ROC areas between myoglobin and CK was close to significance ($p=0.07$), whereas the difference in
minutes and 91% at 60 minutes after start of therapy (Figure 5).

Comparison With Other Clinical Reperfusion Markers

Table 2 demonstrates that resolution of ST segment elevation and the occurrence of reperfusion arrhythmias were also predictors of coronary artery patency. The calculated $\chi^2$ values indicate that both markers were comparable to those serum markers with the lowest predictive values for coronary artery patency (Tables 1 and 2). ROC curve analysis for ST segment resolution resulted in an area under the ROC curve of 0.74 (versus 0.79 for CK slope, $p=NS$; versus 0.89 for myoglobin slope, $p<0.02$). Because of the lack of variable cutoffs with respect to reperfusion arrhythmias, ROC curve analysis cannot be performed for this marker. The chest pain score did not yield statistically significant prediction of the reperfusion status (Table 2). When myoglobin slope was assessed together with other clinical markers by means of logistic regression analysis, only the myoglobin slope was an independent predictor of coronary artery patency ($p<0.0001$). The probability values for the other markers examined were 0.10 for the occurrence of reperfusion arrhythmias, 0.13 for ST segment resolution, and 0.28 for resolution of chest pain.

Discussion

The present study indicates that noninvasive prediction of coronary artery patency after thrombolytic therapy in patients with acute myocardial infarction is possible by assessment of time–activity curves of serum markers. Of particular clinical importance is the observation that analysis of the initial slopes of activity curves over the first 90 minutes after initiation of thrombolysis yields the same and for myoglobin even better predictive accuracy than the determination of the time to peak concentration of each marker. Decision making as to further invasive procedures can be improved by use of analysis of these early slopes in a substantial proportion of patients. This allows early mechanical interventions such as salvage angioplasty in selected individuals in whom the infarct-related artery remains occluded after thrombolysis. It has been demonstrated that these patients benefit from invasive interventions, whereas routine coronary angiography is unnecessary and may even be harmful to some individuals.

In previous studies from our institution as well as from others, a short time to peak CK or CK-MB concentration has been proposed as an indicator of successful thrombolysis-induced coronary artery reperfusion. Only two studies so far have aimed to examine the usefulness of the initial slopes of time–activity curves of the two serum markers CK and CK-MB concerning assessment of coronary artery patency. These investigators found that a rapid increase in CK and CK-MB concentrations closely reflected angiographic documentation of reperfusion. However, ROC characteristics were not reported. Other newly developed serum markers such as CK-MB isoforms or the specific cardiac antigen troponin T have also been examined with respect to their utility as indicators of coronary reperfusion. Pulko and Perryman analyzed peaks of MB$_2$/MB$_1$ subform ratios in 33 patients undergoing thrombolysis and found this ratio helpful for assessment of coronary artery patency within 2 hours after start of therapy. However, their study appears to be limited by the small number of patients undergoing thrombolytic therapy and by the fact that coronary angiography was performed as late as 5 days after thrombolysis. Katus et al studied the value of the new cardiac antigen troponin T in 57 patients and showed that troponin T peaks significantly earlier if the infarct-related artery is successfully reperfused. However, cutoff values and initial slopes were not specifically assessed in this study.

To the best of our knowledge, the present study is the first to compare the serum markers CK, CK-MB, myoglobin, and troponin T systematically regarding their value for noninvasive prediction of reperfusion. Of all markers examined, myoglobin appears to exhibit several advantages that make this protein particularly suitable for early noninvasive prediction of patency of the infarct-related vessel. First, comparison of the ROC curve characteristics revealed that analysis of the initial myoglobin concentration increase allows the best overall prediction of success or failure of thrombolytic therapy of all studied markers for analysis of both time to peak and early slopes. Although the differences between the ROC areas were small, the difference between myoglobin and CK approached significance ($p=0.07$), indicating potential clinical importance. Furthermore, the highest univariate $\chi^2$ values were also obtained for both myoglobin slope and time to peak concentration. This also resulted in high sensitivity and positive predictive value for prediction of coronary artery patency. Additionally, the negative predictive value and specificity, which reflect identification of patients with occluded vessels, were quite high at 82% and 88%, respectively, numbers that are not reached by the other markers examined. Second, as already mentioned, quantitative nephelometric analysis of myoglobin, which has been validated against the radioimmunoassay used by Ellis et al, provides laboratory results after a short turnover time of only 15–30 minutes. Furthermore, an even more
rapid assay using turbidimetry has recently been introduced into clinical practice. Third, myoglobin concentration exhibited the earliest rise of all serum markers examined after initiation of therapy. Although the purpose of the present investigation was not to examine the reliability of serum markers with respect to diagnosis of myocardial infarction but rather the efficacy of thrombolysis, this protein may offer an additional diagnostic advantage. The usefulness of myoglobin for noninvasive prediction of infarct-artery patency has recently been confirmed by other investigators. However, Clemmensen and coworkers reported no comparison with other serodiagnostic markers in their study. In the present investigation, CK maximum and CK-MB maximum yielded relatively good diagnostic information regarding coronary artery patency as well. However, compared with the time elapsed to reach peak myoglobin levels, it took four to five times as long to reach maximal concentrations of these enzymes. This time frame would therefore significantly reduce the chances to salvage jeopardized myocardium by means of invasive procedures. It has been shown previously that combined analysis of noninvasive markers (i.e., peak CK, resolution of ST segment elevation, occurrence of reperfusion arrhythmias) can improve assessment of success or failure of thrombolytic therapy. In the present study, other clinical reperfusion markers did not add significantly to the predictive power of myoglobin slope analysis, indicating the particular strength of this marker.

According to the results of the present study, troponin T offers no advantages for noninvasive prediction of coronary reperfusion in patients undergoing thrombolysis (Table 1). However, troponin T analysis was helpful in the four patients suffering from ventricular fibrillation. In these patients, CK values were distorted by the enzyme release from skeletal muscles caused by the defibrillation shock, whereas troponin T levels were not affected because of the high specificity of this marker for myocardial tissue.

Clinical Implications

The present study demonstrates that analysis of the early initial rise of several serum markers is as accurate as determination of the time elapsed to reach their peak concentration with respect to noninvasive prediction of success or failure of thrombolysis in patients with acute infarction. This allows early noninvasive prediction of coronary artery patency in the majority of patients within the first 2 hours after initiation of thrombolytic therapy. These time limits allow for measures such as rescue angioplasty that aim to salvage myocardium. Early slope analysis of myoglobin appears to be superior to the other serum markers examined and, in addition, exhibits the most rapid concentration increase of all markers evaluated.

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