

Are enzymatic tests good indicators of coronary reperfusion?

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Abstract

Objective—To assess the accuracy of four enzymatic tests, including early release rates of creatine kinase and α -hydroxybutyrate dehydrogenase, in assessing coronary reperfusion after thrombolytic therapy.

Design—A prospective clinical trial identifying patients with a successful thrombolytic treatment.

Patients—Eighty nine patients with acute myocardial infarction were studied. Arteriography showed a closed infarct related artery in all of them. Reperfusion due to thrombolysis occurred in 74 patients and there was no reperfusion in 15 patients.

Results—The 74 patients showing coronary reperfusion had a significantly shorter time to peak creatine kinase activity, higher early release rates for creatine kinase and α -hydroxybutyrate dehydrogenase, and a more rapid release of α -hydroxybutyrate dehydrogenase (ratio of cumulative release of α -hydroxybutyrate dehydrogenase during the first 24 hours to that 72 hours after infarction). All these differences were statistically significant ($p < 0.001$). Optimum cut off levels were determined with decision level plots and the accuracy of the four enzymatic tests was calculated. Accuracy was low for all four tests (73%, 70%, 70%, and 82%).

Conclusion—None of the four enzymatic tests accurately predicted the perfusion state of the infarct related coronary artery after thrombolysis. These tests cannot be used reliably in routine clinical practice as non-angiographic markers of coronary reperfusion.

early peaking of plasma creatine kinase and creatine kinase MB activity⁹⁻¹² as well as other cardiac enzymes^{13,14} were found. These non-invasive criteria are neither sensitive nor specific, however, as indicators of recanalisation.^{15,16} Even when they are used in combination, their predictive accuracy is high only when they are concordant. Unfortunately, concordance occurs in only 14% of patients with reperfusion and in 79% of patients without.¹⁵ Recently, several investigators have evaluated the rapid initial increases in plasma creatine kinase and creatine kinase MB activity as non-angiographic markers of reperfusion.¹⁷⁻¹⁹ The onset of these increases closely reflected the time of angiographic documentation of reperfusion. As indicators of coronary artery reperfusion they seemed to be highly sensitive and specific.

Analogous to this rapid initial increase in plasma creatine kinase activity another enzymatic marker of reperfusion could be the rapid initial increase in plasma activity of α -hydroxybutyrate dehydrogenase. To determine the accuracy of enzymatic tests in assessing coronary reperfusion we compared four different enzymatic variables: (1) the time to peak creatine kinase activity; (2) the early release rate of creatine kinase activity; (3) the early release rate of α -hydroxybutyrate dehydrogenase; and (4) the rate of α -hydroxybutyrate dehydrogenase release represented by the ratio of quantities released in 24 hours and 72 hours. The diagnostic effectiveness and efficiency of these tests were evaluated by cumulative distribution analysis graphs²⁰ (as an alternative to receiver operating characteristic curves²¹) and decision level curves.²²

Methods

PATIENTS

Patients studied were part of a prospective trial with 201 consecutive patients treated with intravenous and intracoronary streptokinase for acute myocardial infarction according to a standardised protocol (750 000 IU intravenous streptokinase and 250 000 IU intracoronary streptokinase). The aims of this prospective trial were to identify patients with a successful thrombolytic treatment and to study the effects of elective percutaneous transluminal coronary angioplasty (PTCA) which was used for prophylactic reasons after successful thrombolysis. Acute coronary angiography was performed in all patients. Only patients with an occluded infarct related

Thrombolytic therapy for acute myocardial infarction is used to achieve reperfusion of the infarct related coronary artery. Intracoronary thrombolysis needs emergency cardiac catheterisation and so is of limited value. This has led to widespread use of intravenous administration of thrombolytic drugs. Hence, reliable methods, other than angiographic evidence, are needed to detect coronary reperfusion. In previous studies, relief of chest pain,¹⁻³ resolution of ST segment elevations,^{4,5} and occurrence of specific arrhythmias⁶⁻⁸ were used as markers of reperfusion. Also, after reperfusion of an acutely occluded coronary artery

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artery before the start of intracoronary streptokinase infusion were included in the present analysis. Two groups of patients were compared: (1) patients without reperfusion—namely, those in whom the infarct related artery remained occluded at the end of intracoronary administration of streptokinase (Thrombolysis in Myocardial Infarction Trial (TIMI) grade 0 or 1²³), and (2) patients with reperfusion—that is, with a patent infarct related artery at the end of intracoronary infusion of streptokinase (TIMI grade 2 or 3). Patients without a significant rise in serum enzymes, defined as a cumulative release of α -hydroxybutyrate dehydrogenase in the first 72 hours ≤ 150 U/l, were excluded from the analysis, as they had minimal or no myocardial necrosis after successful thrombolytic therapy. Other exclusion criteria were first blood sample taken \geq six hours after the onset of symptoms and an interval of ≥ 10 hours between the first two blood samples.

MEASUREMENTS OF SERUM ENZYME ACTIVITIES

Samples of 5 ml of venous blood were obtained at admission, then every six hours until creatine kinase activity reached its highest value and thereafter daily for the next three days. The blood samples were allowed to clot and the activities (U/l) of creatine kinase and α -hydroxybutyrate dehydrogenase in the serum were measured with an autoanalyser (DuPont ACA) in the Department of Clinical Chemistry at our hospital.

CALCULATION OF CUMULATIVE

α -HYDROXYBUTYRATE DEHYDROGENASE

Van der Laarse *et al* used a two compartment model to account for the kinetics of myocardial proteins between the intravascular and extravascular spaces.²⁴ We used this model to calculate the amount of α -hydroxybutyrate dehydrogenase that entered a litre of plasma up to a certain time. The total α -hydroxybutyrate dehydrogenase activity released by the infarcted myocardium in the first 24 hours (Q_{24}) and the first 72 hours (Q_{72}) were calculated and expressed in U/l; Q_{72} is considered to represent the ultimate infarct size.²⁴

CALCULATION OF EARLY RELEASE RATE OF CREATINE KINASE AND α -HYDROXYBUTYRATE DEHYDROGENASE

For both enzymes the difference in total activity of the first two samples was divided by the time between these samples. This gave the early release rate of enzyme expressed in U/l/h.

CALCULATION OF α -HYDROXYBUTYRATE DEHYDROGENASE RELEASE RATE

Another method to measure the rate at which α -hydroxybutyrate dehydrogenase which is released from the myocardium into the circulation is to calculate the ratio of the quantities of α -hydroxybutyrate dehydrogenase released in the first 24 hours and the first 72 hours (Q_{24}/Q_{72}). This method was also used in the randomised streptokinase trial conducted by The Netherlands Interuniversity Cardiology Institute.¹³

ANALYSIS

For comparative analysis we used cumulative distribution analysis graphs,²⁰ directly plotting sensitivity and specificity against all possible cut off points. To select the optimum decision threshold a decision level curve was used,²² with which the difference between the true positive rate (sensitivity) and the false positive rate (100-specificity) was plotted against all possible cut off levels.

The Mann-Whitney rank sum test was used to assess differences between means of data in reperfused and non-reperfused patients. Two tailed p values are reported. A p value of < 0.05 was regarded as statistically significant.

Results

From September 1987 to July 1989, 201 consecutive patients were treated with intravenous and intracoronary streptokinase for acute myocardial infarction according to a standardised protocol. The initial coronary angiogram showed that the infarct related coronary artery was patent in 89 patients (44.3%). These patients were excluded from the analysis. Twenty three of the 112 remaining patients were excluded because of (a) minimal or no myocardial necrosis—that is, a $Q_{72} \leq 150$ U/l (one patient), (b) time of first blood sample \geq six hours after onset of infarction (four patients), (c) time interval between the first two blood samples ≥ 10 hours (seven patients), and (d) incomplete data due to early death in hospital or transfer to other departments (11 patients).

Thus 89 patients formed our study population; 74 showed reperfusion of the infarct related artery after intracoronary streptokinase and 15 showed no reperfusion.

Table 1 presents the results of the four enzymatic tests for these patients. Reperfused patients showed a shorter time to peak creatine kinase activity, more rapid early release rates of creatine kinase and α -hydroxybutyrate dehydrogenase and also a higher Q_{24}/Q_{72} ratio than non-reperfused patients. The differences between groups were highly significant for all enzymatic tests. The enzymatic infarct size as measured by cumulative release of α -hydroxybutyrate dehydrogenase during the first 72 hours (Q_{72}) was not different between groups.

Figure 1 presents the cumulative distribution analysis graphs for the four enzymatic tests. It seemed that none of the four tests was sufficiently sensitive or specific to assess reperfusion. Using decision level plots, we determined the optimum decision threshold value for all four enzymatic tests and measured sensitivity, specificity, accuracy, and the predictive values of a positive test and a negative test. Accuracy is defined as true positives plus true negatives divided by the number of patients tested. Table 2 summarises the results. Despite optimal cut off values the accuracy of the tests was low with 82% as the highest value for Q_{24}/Q_{72} . The predictive value of a negative test was very low for all tests. The ratio Q_{24}/Q_{72} had the best predictive value of a negative test (50%). This is still low and not

Table 1 Results of enzymatic tests for reperfusion in patients with reperfused and non-reperfused infarct related coronary arteries as documented angiographically

Variable	Reperfusion (mean (SD))	No reperfusion (mean (SD))	p Value*
No of patients	74	15	
Time to peak CK (h)	13.1 (8.3)	24.0 (8.5)	<0.0001
Early release rate of CK (U/1/h)	89.3 (84.8)	29.2 (28.9)	0.0008
Early release rate of HBDH (U/1/h)	54.9 (49.3)	17.6 (17.4)	0.0005
Release rate of HBDH (Q_{24}/Q_{72})	0.65 (0.16)	0.50 (0.09)	0.0002
Enzymatic infarct size (Q_{72} (U/1))	1336 (841)	1213 (587)	NS

*Mann-Whitney rank sum test (two sided).

CK creatine kinase; HBDH, α -hydroxybutyrate dehydrogenase.

relevant for clinical practice. The predictive value of a positive test was high for all tests but this was due to a high pretest probability for a positive test result.

Discussion

The results, using the methods in this study, show that none of the four enzymatic tests is accurate enough to be of clinical use in identifying coronary reperfusion. These observations contrast with the findings of Garabedian *et al*, Lewis *et al*, and Ong *et al*.¹⁷⁻¹⁹ Neither the early increase in creatine kinase activity nor the early increase of α -hydroxybutyrate dehydrogenase was accurate (70% for both; table 2) in assessing the reperfusion state of the infarct related artery. In comparison with the other investigations there are differences in methods and the definition of reperfusion. In the other studies¹⁷⁻¹⁹ blood samples for enzyme measurements were taken at shorter intervals (15-120 minutes) than ours. Ong *et al* stated, for their reperfusion model, that the most important discriminating factor between reperfusion and non-reperfusion is the time to onset of increase in creatine kinase MB activity.¹⁹ In the case of reperfusion, the initial increase occurred at the time of reperfusion;

there was an early rapid rate of increase, which then progressively declined. So, with a longer interval between taking the first two blood samples, as in our study, the initial rapid increase rate could be missed. Garabedian *et al* and Lewis *et al* also determined the rate of increase in creatine kinase MB activity in the very early phase of reperfusion and found that several empirical estimates of the rate of increase could differentiate between patients with reperfusion and without.^{17,18}

The definition of reperfusion is not the same for all studies mentioned earlier. Garabedian *et al* defined reperfusion as complete distal opacification of the previously occluded coronary artery (TIMI grade 2 or 3).¹⁷ The same definition was used in our study. This definition includes patients with slow and rapid filling of the distal part of the coronary artery, and hence with slow and rapid appearance patterns of cardiac enzymes. Lewis and coworkers, however, studied only reperfused patients with a complete and rapid filling of the infarct related artery (TIMI grade 3), and they found that the rates of absolute increase in creatine kinase and creatine kinase MB activity completely separated the patients with from the patients without reperfusion.¹⁸ These findings indicate that a rapid initial increase in cardiac enzyme activity in plasma depends upon the re-establishment of perfusion with normal coronary blood flow rather than simply patency of the infarct related artery. Although in our study reperfused patients showed a shorter time to peak creatine kinase activity and more rapid early release rates of creatine kinase and α -hydroxybutyrate dehydrogenase than non-reperfused patients, and the differences reached significance, we could not determine an index of coronary artery reperfusion with plasma enzyme tests that clearly distinguished between reperfused and non-reperfused patients. Even the optimum cut off points for the different

Cumulative distribution analysis graphs for the four enzyme tests.

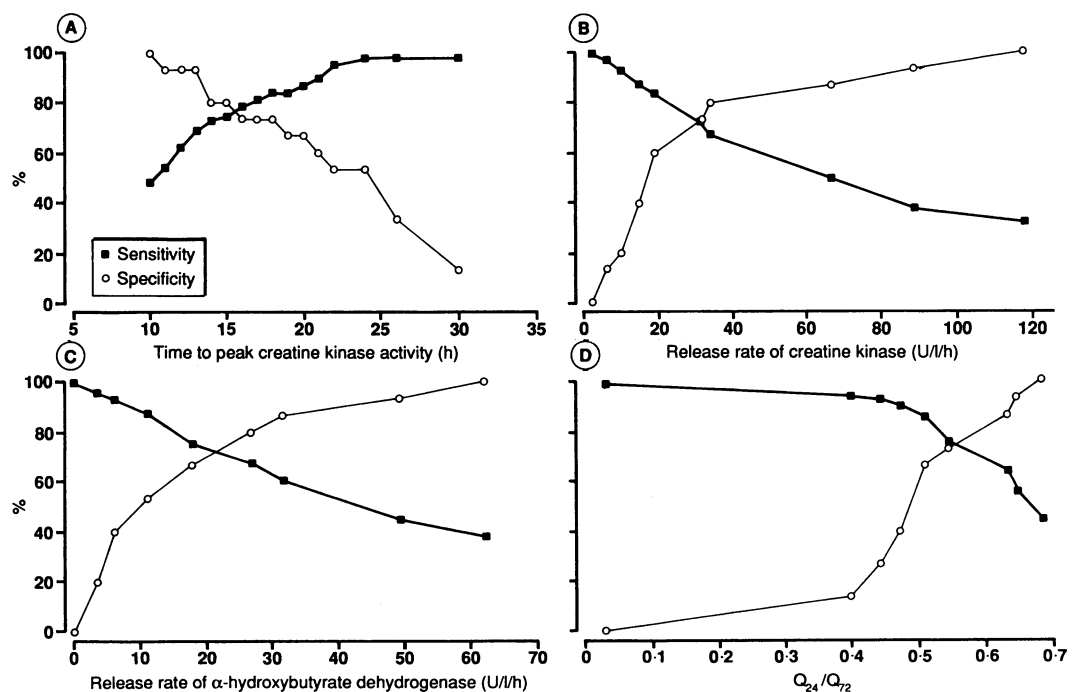


Table 2 Sensitivity, specificity, accuracy, and predictive values at optimal decision threshold levels of four enzymatic tests for reperfusion applied to patients with acute myocardial infarction having reperfusion (74) and no reperfusion (15), after early thrombolytic therapy.

Enzymatic test	Decision threshold	Sensitivity	Specificity	Accuracy	PV+	PV-
Time to peak CK (h)	≤ 13	69	93	73	98	38
Early release rate CK (U/1/h)	≥ 34.1	68	80	70	94	33
Early release rate HBDH (U/1/h)	≥ 27.0	68	80	70	94	33
Release rate HBDH (Q ₂₄ /Q ₇₂)	≥ 0.51	86	67	82	93	50

All values are in percentages.

PV+, predictive value of a positive test result; PV-, predictive value of a negative test result; CK, creatine kinase; HBDH, α -hydroxybutyrate dehydrogenase.

tests determined with the use of decision level curves were not useful to separate reperfused from non-reperfused patients. These findings indicate that the rapid release of cardiac enzymes after reperfusion does not depend on reperfusion alone but also depends on several other factors. The recovery of coronary blood flow after recanalisation affects the rate of appearance of cardiac enzymes, which is in accordance with the bi-exponential model of Ong *et al.*¹⁹ Another factor is the ultimate size of infarction. When the amount of necrosis is small and the total release of enzyme activity (Q₇₂) is low, the early release rate will also be low and may approach the values seen in patients without reperfusion. Also, the interval between infarction and reperfusion determines the early release rate of cardiac enzymes. In late reperfusion, irreversible microvascular changes occur and nutritive flow is not completely re-established even with a patent infarct related coronary artery.^{25,26} Therefore, even with successful thrombolysis in late reperfusion, the pattern of appearance of cardiac enzymes may resemble that of permanent occlusion. These factors can easily explain the overlap in early release rate of creatine kinase or α -hydroxybutyrate dehydrogenase from reperfused and non-reperfused patients, making these enzymatic tests of little use in routine clinical practice.

Early reocclusion of the infarct related artery after initial successful thrombolysis may also be responsible for the discrepancy between enzymatic indices of reperfusion taken over a long period (24 hours) and angiographic assessment of reperfusion obtained during the 30 minute period of intracoronary infusion of streptokinase. We found no symptomatic reocclusion during the first 24 hours after recanalisation, however. Early asymptomatic reocclusion cannot be excluded because early repeated coronary arteriography was not performed, but it is unlikely to have occurred in many cases.

With regard to intravenous thrombolysis it is of great importance to detect accurately and in an early phase patients with persistent occlusion of the infarct related artery despite thrombolytic treatment, because these patients might benefit from further interventional procedures such as "rescue" PTCA to restore coronary blood flow.^{27,28} The predictive value of a negative test result is very low for all enzymatic tests (table 2), especially for the early release rates of creatine kinase and α -hydroxy-

butyrate dehydrogenase, the two tests that will give a result within the first hours of infarction, when rescue PTCA may be considered. In the presence of a low pretest probability of non-reperfusion, enzymatic tests will not discriminate accurately between patients with and without reperfusion of the infarct related coronary artery. It seems likely that other more refined or sophisticated assays, such as those for creatine kinase isoforms,^{29,30} have the same limitations in predicting coronary reperfusion because their rate of appearance is affected by the same factors as affect the enzymes we measured.

Possibly the results would have been improved if we had taken blood samples more frequently; however, a high sampling rate makes the test cumbersome in routine clinical practice. It seems that currently only coronary arteriography allows accurate assessment of the perfusion state of the infarct related artery, particularly in the very early stage of myocardial infarction when further interventional procedures may still be beneficial to the patient.

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