Creatine kinase isoform electrophoresis for the early confirmation of myocardial infarction detected by timed sequential CK slope analysis

Paula Chattington, D. Clarke and W.D. Neithercut

Department of Medicine for the Elderly and 1 Department of Chemical Pathology, Wirral Hospital NHS Trust, UK

Summary: Creatine kinase (CK)-MM and -MB isoforms were evaluated for the early diagnosis of myocardial infarction in patients aged over 65 years admitted to a district general hospital with acute chest pain.

Samples were collected for standard cardiac enzymes, timed CK slope analysis, and CKMM and CKMB isoform analysis from 48 patients admitted with acute chest pain. CKMM and CKMB isoform analyses were conducted using a Helena Rep electrophoresis system under standard conditions supplied by the company. In addition to the results of the biochemical tests the discharge diagnosis of the patients were also recorded.

CKMM isoform analysis resulted in three false-negative classifications of patients and one false-positive. The predictive value of this test was 100% for a positive result and 94% for a negative result. CKMB isoform analysis was less accurate and there were six false-negative results and five false-positive results. The predictive value of a positive result was 75% and 85% for a negative result. CK isoform analysis became unreliable when mean total CK levels in serum were 210 IU/l (± 171).

CK isoform analysis may be of use in the investigation of patients whose samples have a total CK concentration greater than the reference range but was no better than timed CK slope analysis for the detection of myocardial infarction in patients aged more than 65 years.

Introduction

Standard three-day cardiac enzymes provide a retrospective diagnosis of myocardial infarction which does not contribute to the immediate management of patients. Timed sequential creatine kinase (CK) slope analysis may be used for early confirmation of myocardial infarction in both younger patients admitted to coronary care units and in individuals aged over 65 years presenting to general medical units with acute chest pain.1-4 Earlier confirmation of myocardial infarction using this test has been shown to be of benefit in the subsequent management of patients.1

Although timed sequential CK slope analysis is of value in the early confirmation of myocardial infarction, reservations concerning its use for the exclusion of myocardial infarction exist.5,6 Even in those studies in which the test was seen to perform well there were both false-positive and false-negative classifications of patients. It has been suggested that this test needs to be used in conjunction with other tests for confirmation of the diagnosis of myocardial infarction.7

Measurement of the isoforms of creatine kinase, CKMM and CKMB, have also been proposed for the early diagnosis of myocardial infarction.8,9 An increase in the concentrations of these CK isoforms occurs within a few hours after myocardial infarction. As these tests are costly, samples may need to be batch analysed. Dedicated equipment may also be required. These tests may therefore be more suitable for the rapid confirmation of the results of timed slope CK analysis than as the primary test for confirmation of myocardial infarction. We have therefore investigated the value of CKMM and CKMB isoform analysis in conjunction with timed slope CK analysis for the early diagnosis of myocardial infarction in patients admitted to a district general hospital aged over 65 years with a presenting complaint of acute chest pain.

Patients

A total of 49 patients (18 women) aged more than 65 years were included in the study. All had a
presenting complaint of acute chest pain. On admission to hospital blood was collected into a plain tube for CK analysis and some was also collected into a tube containing 5 mmol/L EDTA for CK isof orm analysis. The specimen for CK isof orm analysis was sent immediately on ice to the laboratory. Eight to 12 hours after admission, a second blood sample was collected into a plain tube for CK analysis and calculation of the logarithm of the rate of increase in CK activity. A slope value of greater than 0.015 was considered to indicate myocardial infarction. The validity of this cut-off had previously been confirmed in a study of elderly individuals.

Throughout their stay in hospital, patients' treatment and further investigation continued under the direction of the consultant who was responsible for their care. The results of timed CK analysis and CK isof orm analysis were not made available to the consultant responsible for the patient's care. The results of serial cardiac enzymes which included serum CK and lactate dehydrogenase (LDH) activity which were collected on the 3 days after admission were recorded. A rise in total CK activity outwith the reference range, with a peak 24–48 h after onset of chest pain or a later peak in LDH activity was used to confirm a diagnosis of myocardial infarction.

Three serial electrocardiograms (ECGs) were recorded. Standard ECG changes were used for the diagnosis of myocardial infarction. The discharge diagnosis was also recorded.

The study was approved by the Wirral District Ethics Committee and all patients gave informed consent.

Methods

The clotted blood samples were centrifuged at 3,000 r.p.m. when received by the laboratory and a portion of the serum was analysed for CK and LDH at the time of receipt in order to simulate the provision of a routine service. EDTA samples collected for CK isof orm analysis were stored at 4°C overnight for subsequent analysis the following day. It was not possible to store these samples longer as the stability of the isof orms at 4°C was limited to 24 hours.

Total CK concentrations were measured at 37°C using a Synchron CX7 analyser (Beckman Instruments, High Wycombe, UK) using commercial reagents (Randox, Dublin, Eire). The between-batch imprecision of this method was 4.1% at a level of 147 IU/L and 2.3% at a level of 467 IU/L. The laboratory reference range for CK was 195 IU/L for males and 170 IU/L for females.

LDH was measured using the same instrument and commercial reagents from the same supplier. The between-batch imprecision of this method was 1.6% at a level of 354 IU/L and 1.0% at a level of 923 IU/L. The laboratory reference range for LDH was 230–460 IU/L.

CK isof orms were analysed using a Helena Rep Electrophoresis System (Helena Laboratories, Gateshead, UK). Standard conditions supplied by the company were used. Electrophoresis time was 15 minutes at 1,400 V. Samples were prepared by centrifugation and a portion of the EDTA plasma added to each plate. The plates were read by two operators. The electrophoretic scans were recorded for subsequent analysis. A ratio of CKMM1 to CKMM3 isof orm activity of greater than 1.0 was considered to indicate myocardial infarction and a ratio of CKMB2 to CKMB3 isof orm activity of greater than 1.5 was considered to indicate myocardial infarction.

Results

The mean age (± s.d.) of the 15 patients who had suffered a myocardial infarction, was 74 years (± 7) compared with 77 years (± 6.5) for the 33 patients who had another cause of chest pain (P = NS) (Table 1).

Complete collection of samples for CK slope analysis occurred for only 33 patients. This test apparently produced three false-negative results for individuals who had a myocardial infarction and one false-positive result for an individual who had a final diagnosis of angina pectoris. Review of the casesheets indicated that one false-negative was due to a late myocardial infarction occurring after both timed samples had been collected and the false-positive result was due to diagnosis of angina pectoris when the patient may have suffered a myocardial infarction. CK slope analysis therefore had a sensitivity of 83% and specificity of 91% with a predictive value for a positive result of 90% and of 87% for a negative result.

CK isof orm analysis was conducted on samples from all the patients studied.

CKMM isof orm analysis also failed to classify patients correctly according to final diagnosis. There were three false-negative results. One was due to the individual who developed a myocardial infarction following collection of samples. There was also one false-positive result due to the possible misdiagnosis of a patient with angina pectoris. This gave a sensitivity of 92% and specificity of 97%, giving a predictive value of 92% for a positive result and of 91% for a negative result.

CKMB isof orm analysis predicted the final diagnosis less accurately than CKMM isof orm analysis or timed slope CK analysis. This was due to low total CK levels in many of the admission samples, making it impossible to detect bands
accurately in some patients' samples. As a result there were six false-negative and five false-positive results giving a sensitivity of 60%, specificity of 85% and a predictive value of 64% for a positive result and of 82% for a negative result. The mean total CK concentration in these samples was 210 IU/l (± 171).

Individually, none of the tests proved completely satisfactory for the early diagnosis of myocardial infarction. To determine whether CKMM isoforms could be used to confirm borderline slope CK results, the interpretation of CKMM isoforms analysis was restricted to patients who had raised total CK activity in either of the timed samples but a normal log slope timed CK analysis.

The data from 33 patients with both CKMM and CK slope analyses were reanalysed considering the CKMM isoform result only when the CK slope analysis was negative and the total CK concentration in either sample greater than the reference range. Two individuals who had a discharge diagnosis of myocardial infarction were misclassified by this protocol (Figure 1), while one individual who had a discharge diagnosis of angina pectoris appeared to have actually suffered a myocardial infarction. The predictive value of this protocol for the analysis of samples from individuals with myocardial infarction was 90% for a positive result and 95% for a negative result.

Discussion

This study has demonstrated that it is difficult to produce complete certainty of confirmation of diagnosis of myocardial infarction or its exclusion, even using sensitive tests currently available for the early diagnosis of myocardial infarction. Timed sequential CK slope analysis has been shown to be a valuable test for the early diagnosis of myocardial infarction. While this test is better than the standard cardiac enzymes at rapidly predicting the final diagnosis of myocardial infarction, it still produces false-positive and false-negative results. Timed sequential CK slope analysis proved less sensitive in this study than in previous studies. This may have been due to the small number of patients investigated.

Confounding diagnoses that may be present in an elderly population may also increase the likelihood of inappropriate classification of individuals

Table I  Discharge diagnosis of patients studied

<table>
<thead>
<tr>
<th>Discharge diagnosis</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myocardial infarction</td>
<td>15</td>
</tr>
<tr>
<td>Angina pectoris</td>
<td>20</td>
</tr>
<tr>
<td>Left ventricular failure</td>
<td>5</td>
</tr>
<tr>
<td>Dysrhythmia</td>
<td>2</td>
</tr>
<tr>
<td>Chronic obstructive airways disease</td>
<td>2</td>
</tr>
<tr>
<td>Anaemia</td>
<td>1</td>
</tr>
<tr>
<td>Other</td>
<td>3</td>
</tr>
</tbody>
</table>

Figure 1  Confirmation of myocardial infarction using timed CK slope analysis and CKMM isoform analysis for samples with CK activity greater than 196 IU/l and a normal CK slope. X = patients with myocardial infarction; X = patients with other causes of chest pain.
using timed sequential CK slope analysis. Other tests are therefore needed for confirmation of myocardial infarction. CK isoform analysis proved less effective in this study than expected. Both CKMB and CKMM isoforms electrophoresis produced false-positive and false-negative results. A small number (3) of these results may have been the result of clinical misclassification of individuals. Apparent clinical misclassification may have occurred either because of a late myocardial infarction, or as a result of basing a final diagnosis on minor ECG or cardiac enzyme changes.

Analytical misclassification occurred when samples had a low total CK concentration. The electrophoretic method of analysing CK isoforms did not have sufficient sensitivity to determine the presence of increased CKMM and especially CKMB isoforms reliably, when the mean total CK level was 210 IU/l or less. The accurate timing of the collection of samples for CK isoform analysis is also important.

The total CK concentrations in some early samples may have been low because patients presented rapidly following the onset of chest pain. As CKMB isoform and CKMM isoforms may be positive within 3–6 hours of the onset of myocardial infarction, it is unlikely that the samples were collected too soon after the onset of chest pain as the patients in this study presented in the Accident and Emergency Department up to 6 hours after the onset of chest pain.

Following recognition of the difficulty produced by low total CK concentrations, the data were re-examined applying the rule that, if the CK slope was normal but the total CK concentration was greater than the reference range in either of the two timed CK samples, then the CKMM isoform data would be used for confirmation. When this protocol was followed all patients were correctly classified with regard to the final diagnosis except for three. One individual had biochemical evidence of myocardial infarction but had a clinical diagnosis of angina pectoris. One individual had had a late infarct without either of the tests confirming this, and one individual had a diagnosis of myocardial infarction without ECG changes or a significant rise in standard cardiac enzymes.

Combining the two tests would have resulted in a need to conduct isoform analysis on 10% of the samples. These results suggest that it may be possible to combine the use of CKMM isoform analysis with slope CK analysis to confirm the diagnosis of myocardial infarction.

While earlier confirmation or exclusion of acute myocardial infarction has been shown to be of value in the subsequent management of these patients, none of the biochemical tests were able to detect acute myocardial infarction early enough to influence the decision to use thrombolytic therapy. It is unlikely that a suitable biochemical test for use in deciding whether to administer thrombolytic therapy will be developed as current evidence indicates that thrombolytic treatment needs to be administered as soon after the onset of the myocardial infarction as possible and that any delay, while waiting for results, may be to the disadvantage of the patient.

This study has demonstrated that CK isoform analysis might be of use in excluding myocardial infarction in patients with borderline timed CK slope analysis. Isoform analysis could be restricted to samples with a total CK concentration that is greater than the reference range.

References

Creatine kinase isoform electrophoresis for the early confirmation of myocardial infarction detected by timed sequential CK slope analysis.

P. Chattington, D. Clarke and W. D. Neithercut

Postgrad Med J 1994 70: 805-808
doi: 10.1136/pgmj.70.829.805

Updated information and services can be found at:
http://pmj.bmj.com/content/70/829/805

These include:

Email alerting service
Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

Notes

To request permissions go to:
http://group.bmj.com/group/rights-licensing/permissions

To order reprints go to:
http://journals.bmj.com/cgi/reprintform

To subscribe to BMJ go to:
http://group.bmj.com/subscribe/