Diagnostic Efficiency of Creatine Kinase (CK), CKMB, Troponin T and Troponin I in Patients with Suspected Acute Myocardial Infarction

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The goal of this research was to assess the performance of serum creatine kinase (CK), creatine kinase MB (CKMB) [mass and activity], troponin I (TnI) and troponin T (TnT) in the diagnosis of acute myocardial infarction in patients admitted to the Coronary Care Unit at Queen Alia Heart Institute, Amman, Jordan, between March and July 2001. Blood samples collected for cardiac enzyme determination (CK, CKMB activity) were stored at –20°C for later determination of CKMB mass [Abbott Axsym, Ortho Clinical Diagnostics (OCD) ECi and Roche Elecsys], TnI (Abbott Axsym) and TnT (Roche Elecsys). The relative index (RI = CKMB mass/CK), for CKMB mass measurements, was calculated. Clinical notes and/or discharge diagnosis for each patient were reviewed to obtain the diagnosis of acute myocardial infarction. Fifty samples were from acute myocardial infarction (AMI) patients. Area under Receiver Operating Curve values were: CK 0.56, CKMB activity 0.72, percentage of CKMB activity 0.73, CKMB mass (Abbott) 0.76, CKMB mass (Roche) 0.77, CKMB mass (OCD) 0.78, RI (Roche) 0.83, RI (Abbott) 0.87, RI (OCD) 0.86, TnI 0.95, TnT 0.94. Sensitivity: TnI 88%, TnT 93%; specificity TnI 99%, TnT 99%. There was no significant difference in performance between TnI and TnT assays or between any of the CKMB mass measurements. Present results show that TnI and TnT are better cardiac markers than CK and CKMB, mass or activity.

Key words —— acute myocardial infarction, troponin I, troponin T, creatine kinase, creatine kinase MB

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INTRODUCTION

Patients with acute chest pain, in the emergency room, represent a diagnostic challenge.[1)] Diagnosis of acute myocardial infarction (AMI) is usually established based on the clinical symptoms, electrocardiographic (ECG) changes and the activities of conventional cardiac enzymes.[2)] On the other hand, symptoms may be atypical and the ECG is often nondiagnostic.[3,4)] Indeed, conventional cardiac enzymes, creatine kinase (CK) and its isoenzyme MB (CKMB) activities, do not rule out AMI in the early stage.[5,6)]

New markers of myocardial injury have challenged the conventional tests of cardiac cell damage. In several laboratories, the measurement of CKMB mass has replaced CKMB activity to become the "gold" standard for detection of acute myocardial injury.[7,8)] However, the role of CKMB mass measurement is being questioned with the appearance of highly specific troponin T (TnT) and troponin I (TnI) tests.[9,10)] While CKMB is not specific for the myocardium,[11)] cardiac TnT and TnI are considered highly specific for the myocardium and are present only in cardiomyocytes, the contractile apparatus. They are highly sensitive and are specific markers for myocardial injury.[12–14)] Measurement of troponin has the potential to replace not only CKMB but also lactate dehydrogenase-1 (LD-1).[15)] It has been suggested[16)] that elevation in troponin levels should replace elevation of other enzymes in the World Health Organization (WHO) definition of AMI, which is currently two out of three: typical history, unambiguous electrocardiographic changes and unequivocal enzyme changes.[17)] troponin measurement may also be of great importance in risk stratification of patients with unstable angina.[18–20)]

This study was designed to evaluate the performance of markers of cardiac damage in the diagnosis of AMI in patients presented to the emergency room with chest pain.

Three different CKMB mass measurements were assessed as well as total CK, CKMB activity and TnT and TnI. The performance of newer tests was compared with the routine cardiac markers (total CK, CKMB activity).

MATERIALS AND METHODS

Subjects and Samples —— The study group consisted of 100 consecutive patients presented to the

Table 1. Analyzed Factors for Non AMI and AMI Samples on Admission

Analyzed Factor	Non AMI sample ($n = 50$)	AMI sample ($n = 50$)
Time of onset since admission (hr)	18.8 ± 15	19.6 ± 17
%CKMB (%)	7.1 ± 5.1	11 ± 8.4
CKMB activity (U/l)	59 ± 12.4	82 ± 21
CK (U/l)	745 ± 274	876 ± 320
CKMB mass-Roche (μg/l)	10.8 ± 6.8	31.3 ± 7.5
CKMB mass-Abbott (μg/l)	10.4 ± 4.6	33.8 ± 22.3
CKMB mass-OCD (μg/l)	8.3 ± 6.8	28.2 ± 19.6
RI Roche	1.49 ± 1.24	8.04 ± 3.2
RI Abbott	0.97 ± 1.26	4.79 ± 1.87
RI OCD	0.62 ± 0.37	4.41 ± 1.63
Troponin I (μg/l)	0.6 ± 1.3	$> 50^{a)}$
Troponin T (μg/l)	0.034 ± 0.06	2.76 ± 1.42

a) Not quantified; $>$ more than. Data expressed as average ± S.D. and range.

emergency room of Queen Alia Heart Institute, Amman, Jordan, between March and June 2001, with acute chest pain which had lasted for less than 12 hr before admission. There were no prespecified ECG or enzyme criteria required for inclusion at admission. Immediately, following the qualifying episode of ischemic chest discomfort, ECG was performed and venous blood samples were obtained for the initial assays. Subsequent blood samples were drawn at 2, 4 and 6 hr after the initial specimens for the determination of CKMB isoforms and at 2, 4, 6, 12, 24, 36, 48 and 72 hr for all other assays. The control group were those patients (n = 50) who had chest pain and no AMI diagnosis was established.

The blood samples were collected in evacuated gel tubes for serum preparation and allowed to clot at room temperature before centrifugation. Serum aliquots were stored at –20°C for later analysis of other cardiac markers (CKMB mass, TnT and TnI). Patients files and discharge notes were inspected to establish the time of onset of symptoms, time of presentation to Queen Alia Heart Institute and the diagnosis of AMI according to the WHO criteria.[17)]

The protocol of this study was approved by the Ethics Committee of Jordan University of Science and Technology, School of Medicine. A written consent was obtained from all participated patients.

Laboratory Analysis —— Total CK and CKMB activity were measured on the Hitachi 911 clinical chemistry analyzer (Roche Diagnostics, Germany) using kits supplied by the manufacturer. CKMB activity was measured by an immunoinhibition method. The upper reference interval for total CK was 210 U/l for men and 164 U/l for women. The manufacturer's recommended percentage of CKMB cutoff for the diagnosis of AMI is 6%.

CKMB mass was measured immunochemically on the Axsym (Abbott Diagnostics, Germany), using kits supplied by the manufacturer. Relative Index (RI) values for each CKMB mass measurement were calculated (RI = CKMB mass in μg/l divided by total CK in U/l). TnT was measured on the Roche Elecsys 1010 and TnI was measured on the Abbot Axsym. The manufacturer's recommended cutoffs for the diagnosis of AMI were: 5 μg/l for CKMB mass Roche Elecsys, 0.1 μg/l for TnT and 2 μg/l for TnI.

Statistical Methods —— Analyze-It Add-on software (Analyze-It Software Ltd., U.K.) for Microsoft Excel was used to analyze Receiver Operating Characteristic (ROC). Results of ROC analysis were expressed as areas under the individual ROC curves and the 95% confidence intervals. The software was also used to calculate specificity, sensitivity, positive and negative predictive values and accuracy at various cutoffs.[21–23)]

RESULTS

Table 1 show average values ± standard deviation (S.D.) and ranges for each analyzed factor at the time of admission. Figure 1 demonstrates the overall performance of the different tests in the diagnosis of AMI that were assessed by comparison of the areas under the ROC curves (AUC). There was no difference in AUC between TnI and TnT or among any of the CKMB mass measurements. Sensitivity, specificity, positive and negative predictive values and accuracy at specified cutoffs are shown

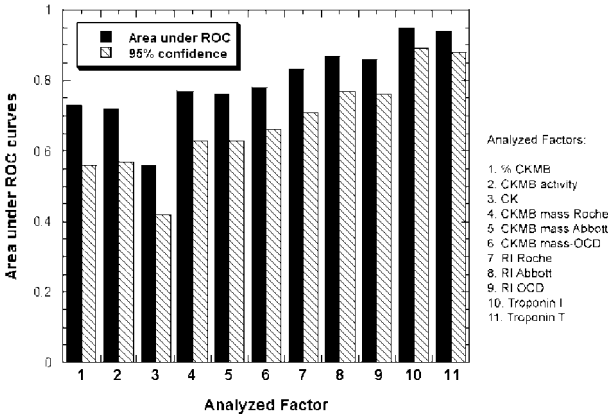

Fig. 1. Area under ROC Curves for Acute Myocardoal Infarction Diagnosis

in Table 2.

According to the manufacturers AMI diagnostic cutoffs, there were 6 false negative results for TnI and TnT, reflected in the negative predictive values of 0.74–0.80. These were the initial samples taken shortly after admission (average 2.5 hr, range 0.5–3.5) and in all other cases, subsequent samples were positive for both TnI and TnT. The median time since onset of symptoms (n = 5) was 5.5 hr, compared to 40 hr for true positive samples (n = 44). There were no false positive TnI and TnT results.

Table 2. Analysis of Various Factors for Specific Cutoffs

Test	Cutoff	Cutoff source	Sensitivity	Specificity	Positive Predictive Value	Negative Predictive Value	Accuracy
% CKMB	6	Recommended cutoff (Manufacturer)	0.95	0.42	0.82	0.77	0.81
	7	ROC	0.96	0.55	0.85	0.83	0.85
CKMB mass Roche	3.1	97.5 centile from normal Population (Manufacturer)	0.97	0.18	0.80	0.69	0.76
	5	Recommended cutoff (Manufacturer)	0.93	0.25	0.72	0.52	0.75
	7	ROC	0.92	0.41	0.81	0.60	0.80
CKMB mass Abbott	3.8	95 centile from normal Population (Manufacturer)	0.95	0.23	0.77	0.62	0.73
	9.3	95 centile from hospitalized non-AMI Population (Manufacturer)	0.83	0.42	0.83	0.47	0.75
	3	ROC	0.97	0.20	0.77	0.76	0.80
CKMB mass OCD	3.38	97.5 centile from normal Population (Manufacturer)	0.93	0.34	0.81	0.65	0.77
	4.55	97.5 centile from hospitalized non-cardiac Population (Manufacturer)	0.94	0.41	0.80	0.68	0.82
	5.31	97.5 centile from hospitalized cardiac non-AMI population (Manufacturer)	0.91	0.42	0.83	0.62	0.79
	3	ROC	0.94	0.41	0.81	0.66	0.79
RI — Roche	0.7	ROC	0.94	0.52	0.87	0.68	0.81
RI — Abbott	0.9	ROC	0.92	0.69	0.89	0.70	0.83
RI — OCD	0.7	ROC	0.94	0.68	0.90	0.76	0.85
TnI	2	Recommended cutoff (Manufacturer)	0.88	0.99	0.99	0.74	0.92
	2	ROC	0.90	0.99	0.99	0.74	0.92
TnT	0.1	Recommended cutoff (Manufacturer)	0.93	0.94	0.98	0.80	0.93
	0.1	ROC	0.94	0.95	0.98	0.80	0.93

Value derived from Receiver Operating Curve (ROC). Ortho Clinical Diagnostics (OCD). Relative index (RI). Troponin I (TnI). Troponin T (TnT).

DISCUSSIONS

This research on cardiac markers showed better diagnostic accuracy for TnI and TnT when compared to the conventional markers such as total CK and CKMB activity. Total CK alone was a poor diagnostic marker of AMI but it is an important factor of calculated values such as percentage of CKMB and RI. The performance of %CKMB is relatively good when compared to the CKMB mass. The disadvantages of the CKMB activity include interference due to hemolysis, macro-CK and CKBB.[24–26] Nonetheless it remains popular due to low cost and ability to be run on general clinical chemistry analyzers, unlike the more costly CKMB mass methods that generally require dedicated immunoassay platforms. At both the recommended and ROC-derived cutoffs, it was highly sensitive but displayed poor specificity. There was no difference in the performance of the three CKMB mass methods despite absolute differences in their measured levels necessitating assay-specific cutoffs. The choice of the cutoff is complicated by the lack of recommendation from manufacturers. Regardless of the cutoff chosen, all assays gave relatively poor specificity and performed less well than the traditional percentage of CKMB parameter.

The performance of RI values derived from the CKMB mass results were much better. Serum CKMB can originate from both skeletal and cardiac muscles.[27–29] In skeletal muscle, CKMB can comprise up to 2% of total CK while in cardiac muscle, CKMB makes up 20–46% of total CK.[21] It is possible to differentiate myocardial from skeletal muscle damage by expressing the CKMB result as a percentage of total CK. Since CKMB is expressed in mass units (μg/l) and total CK in activity units (U/l), the term "relative index" rather than percentage is used to describe the ratio of CKMB to total CK. RI may not be suitable for all cases,[26] especially those with low total CK results where the absolute CKMB mass result is more meaningful.

Among investigated cardiac markers, TnI and TnT were the best. No difference in their performance was seen, in spite of differences in their absolute values and release kinetics. The two assays have previously been shown to have a prognostic value for prediction of AMI and cardiac death in unstable angina patients.[27]

Depending on a single test to exclude AMI is risky; that was shown by the early false negative results for both TnI and TnT. Therefore, the need for multiple timed samples is recommended.[21] troponin appears in the blood 4–8 hr following symptom onset and remains abnormal for 4–12 days.[30,31] Interpretation of results from early sampling can be deceptive and physicians should be aware of that a single test at the time of arrival may be insufficient for the diagnosis of MI.[32,33]

Evaluated markers varied in their diagnostic performance. Investigated markers displayed high sensitivity but only TnI and TnT revealed equally high specificity. The use of multiple sampling of a sensitive assay such as the percentage of CKMB along with a more sensitive and specific assay such as TnI or TnT could provide a cost-effective alternative to multiple troponin sampling as was recommended by others.[16] Myoglobin instead of the percentage CKMB could be used as an alternative approach as it appears in the blood earlier than other cardiac markers but is not cardiac specific marker and expensive.[33]

Cardiac TnI and TnT were the best cardiac markers for the diagnosis of AMI among all other investigated markers such as CKMB (mass and activity) and total CK. There was no difference between either TnI or TnT. CKMB mass showed better performance when combined with total CK and expressed as RI values. Total CK alone was a poor marker for AMI diagnosis. Combining multiple sampling of the percentage of CKMB with single confirmatory troponin testing may provide a reliable and cost-effective testing protocol for suspected AMI patients.

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