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Comparison of the Diagnostic Utility of CK, CK-MB (Activity and Mass), Troponin T and Troponin I in Patients with Suspected Acute Myocardial Infarction

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ABSTRACT

Objective: The aim of the study was to investigate the clinical performance of serum creatine kinase (CK), CKMB (mass and activity), Troponin T (TnT) and Troponin I (TnI) in the diagnosis of acute myocardial infarction (AMI) in patients admitted to the Coronary Care Unit at Tan Tock Seng Hospital between June and July 1998.

Methods: Routine blood samples sent to the laboratory 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), Troponin I (Abbott Axsym) and Troponin T (Roche Elecsys). For CKMB mass measurements, the relative index (RI = CKMB mass/CK) was calculated. The diagnosis of acute myocardial infarction was obtained from inspection of clinical notes and/or discharge diagnosis for each patient.

Results: Forty-four of fifty-nine specimens were from 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) 0.86, RI (OCD) 0.87, TnT 0.94, TnI 0.95. Sensitivity: TnI 88%, TnT 93%; specificity TnI 100%, TnT 92%. There was no significant difference in performance between Troponin T and Troponin I assays or between any of the CKMB mass measurements.

Conclusion: Troponin T and I are superior to CKMB (mass or activity) and CK in the identification of patients with AMI. Combining multiple sampling of the percentage of CKMB with single confirmatory troponin testing may provide a cost-effective testing protocol for suspected AMI patients.

Keywords: myocardial infarction, cardiac troponin, creatine kinase MB

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INTRODUCTION

In recent years, newer markers of myocardial damage have challenged the place of traditional tests of cardiac injury. In many laboratories, the measurement of creatine kinase – MB (CKMB) mass has replaced CKMB activity to become the "gold" standard for biochemical detection of acute myocardial injury[1]. In turn, the role of CKMB mass measurement is being questioned with the emergence of cardiospecific troponin assays[2,3]. While CKMB is not specific to the myocardium[4], cardiac Troponin T and I show greater cardiospecificity and are found only as components of the contractile apparatus in cardiomyocytes. They are highly sensitive and are specific markers of myocardial necrosis[5]. Troponin measurement has the potential to replace not only early markers of myocardial damage such as CKMB but also late markers such as LD1[6]. It has been suggested[7] that Troponin elevation should replace enzyme elevation in the World Health Organisation (WHO) definition of acute myocardial infarction (AMI), which is presently 2 out of 3 from: typical history, unequivocal electrocardiographic changes and unequivocal enzyme changes[8]. Troponin measurement may also be of value in risk stratification of patients with unstable angina[9-11]. However increasing diagnostic performance comes with increased cost and clinicians and laboratories must choose between a variety of markers from different vendors when deciding on a cost-effective menu of cardiac markers.

This study was designed to assess the performance of different markers of cardiac damage in the diagnosis of acute myocardial infarction in patients admitted to the Coronary Care Unit at Tan Tock Seng Hospital. Three different CKMB mass measurements were evaluated as well as total CK, CKMB activity and Troponin T and I. The performance of newer assays was compared with the present routine panel of cardiac markers (total CK, CKMB activity).

METHODS

Subjects and Samples

Serum aliquots were taken from routine cardiac enzyme (total CK, CKMB activity) samples collected from patients in the Coronary Care Unit at Tan Tock Seng Hospital between June – July 1998. Wherever possible, the earliest sample, including those from the Emergency Department, were retrieved. 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 batch analysis of other cardiac markers (CKMB mass, Troponin T, Troponin I). Patient case notes and/or discharge summaries were inspected to establish the time of onset of symptoms (where available), time of presentation to Tan Tock Seng Hospital and the diagnosis of AMI by WHO criteria[8].

Laboratory analysis

The routine cardiac panel consisted of total CK and CKMB activity with calculated percentage of CKMB (CKMB activity as percentage of total CK activity). Total CK and CKMB activity were measured on the Hitachi 917 clinical chemistry analyser (Roche Diagnostics, Singapore) using reagents supplied by the instrument 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 diagnosis of AMI is 6%.

CKMB mass was measured immunochemically on three different instruments using reagent supplied by the respective manufacturers: Axsym (Abbott Diagnostics, Singapore), Vitros ECi (Ortho Clinical Diagnostics (OCD), Singapore) and Elecsys 1010 (Roche Diagnostics, Singapore). Relative Index (RI) values for each CKMB mass measurement were calculated (RI = CKMB mass in ug/L divided by total CK in U/L). Troponin I was measured on the Abbot Axsym while Troponin T was measured on the Roche Elecsys 1010. The manufacturer's recommended cutoffs for the diagnosis of AMI were: 2 ug/L (Troponin I), 0.1 ug/L (Troponin T) and 5 ug/L (CKMB mass-Roche Elecsys). Manufacturer-supplied reference intervals for other assays are shown in Table III. No diagnostic cutoffs for AMI were provided by the manufacturers for the other assays.

Statistical methods

Receiver Operating Characteristic (ROC) analysis was performed using Analyse-It Add-on software (Analyse-It Software Ltd, UK) for Microsoft Excel. Results of ROC analysis were expressed as areas under the individual ROC curves and the 95% confidence intervals. The same software was used to calculate clinical sensitivity, specificity, positive and negative predictive values and accuracy at different cutoffs[12-13].

RESULTS

During the study period, 59 samples were collected from 37 patients. Forty-four samples were from AMI patients. The median values and ranges for each parameter are shown in Table I. The time of onset of symptoms was available for only 1 of 15 non-AMI samples and 28 of 44 AMI samples, which limited further analysis of this variable. The overall performance of the different tests in the diagnosis of AMI was assessed by comparison of the areas under the ROC curves (AUC) shown in Table II. Examples of ROC curves are shown in Fig 1. There was no difference in AUC between Troponin T and I or among any of the CKMB mass measurements. Sensitivity, specificity, positive and negative predictive values and accuracy at specified cutoffs are shown in Table III.

Using the AMI diagnostic cutoffs suggested by the manufacturers, there were 5 false negative results for Troponin T and I reflected in the negative predictive values of 0.72 – 0.79. All of these samples represented the initial samples taken soon after admission (median 2.8 hours, range 0.4 – 3.0) and in all cases, subsequent samples were positive for both Troponin T and I. The median time since onset of symptoms (n = 4) was 5.5 hours, compared to 40 hours for true positive samples (n = 21). There was 1 false positive Troponin T result in a patient with end stage renal failure (Troponin T 0.2 ng/L) and no false positive Troponin I results.

DISCUSSION

This study of cardiac markers in patients admitted to CCU showed greater diagnostic accuracy for the newer markers cardiac Troponin T and I compared to traditional markers such as total CK and CKMB activity. Based on the ROC areas under the curve, there was a clustering of markers into four groups: total CK, CKMB (activity and mass), RI and Troponin T/I.

Total CK alone was a poor discriminator of AMI but is a necessary component of calculated values such as percentage of CKMB and RI. Percentage of CKMB performed well compared to the CKMB mass methods. Disadvantages of the CKMB activity method used include interference from haemolysis, macro-CK and CK-BB[14]. However it remains popular in Europe and has the advantages of low cost and ability to be run on general clinical chemistry analysers, 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. The improved performance at a cutoff $\geq$ 7% suggests that the present 6% value should be reviewed.

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 Abbott and OCD and the provision of 95%

Table I – Median values and range for non-AMI and AMI samples

Parameter	Non-AMI	AMI
Sample size	15	44
Time since admission (h)	16.5 (0.85 – 257)	19.9 (0.43 – 92)
CK (U/L)	752 (202 – 2330)	862 (57 – 6355)
CKMB activity (U/L)	61 (17 – 165)	77 (23 – 464)
% CKMB (%)	6.9 (3.1 – 15.1)	10 (3.1 – 21.2)
CKMB mass – OCD (ug/L)	7.7 (0.8 – 27.1)	26.9 (1.05 – > 400*)
CKMB mass – Abbott (ug/L)	10.4 (1.3 – 36.2)	33.8 (1.6 – 403.2)
CKMB mass – Roche (ug/L)	10.8 (1.95 – 36.63)	31.3 (2.83 – > 500*)
RI – OCD	0.62 (0.14 – 0.18)	4.41 (0.44 – 11.9)
RI – Abbott	0.97 (0.22 – 3.87)	4.79 (0.59 – 15.2)
RI – Roche	1.49 (0.34 – 3.86)	6.04 (0.52 – 17)
Troponin T (ug/L)	0.034 (0.01 – 0.232)	2.76 (0.01 – > 25*)
Troponin I (ug/L)	0.6 (0 – 2)	> 50* (0.4 – 15840)

* not quantified

Table II – Area under ROC curves (AUC) for AMI diagnosis

Analyte	AUC	95% Confidence limits
CK	0.56	0.42 – 0.71
CKMB activity	0.72	0.57 – 0.86
% CKMB	0.73	0.56 – 0.90
CKMB mass – OCD	0.78	0.66 – 0.91
CKMB mass – Abbott	0.76	0.63 – 0.89
CKMB mass – Roche	0.77	0.63 – 0.91
RI – Abbott	0.86	0.76 – 0.96
RI – OCD	0.87	0.77 – 0.97
RI – Roche	0.83	0.71 – 0.94
Troponin T	0.94	0.88 – 1.00
Troponin I	0.95	0.89 – 1.00

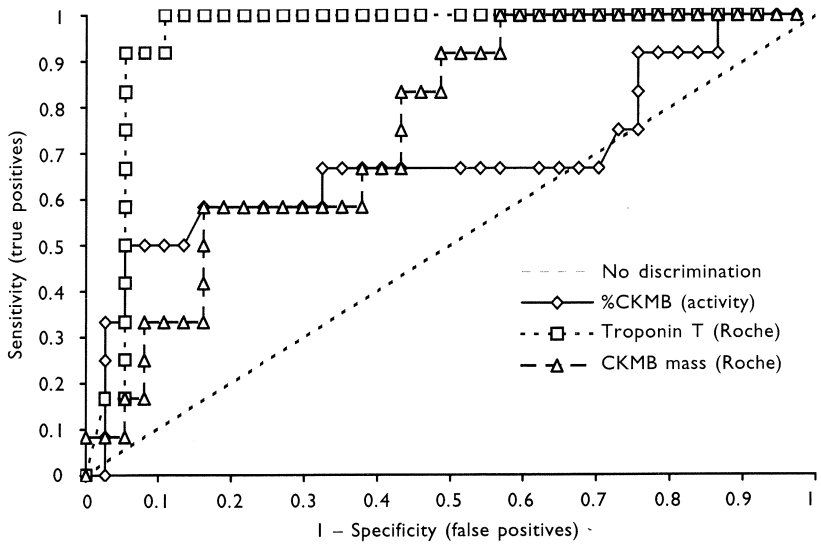

Fig 1 – Receiver Operating Characteristic (ROC) Curve for %CKMB (activity), CKMB mass (Roche) and Troponin T.

reference intervals of Abbott and 97.5% reference intervals by OCD. Regardless of the cutoff chosen, all assays gave relatively poor specificity and performed less well than the traditional percentage of CKMB parameter.

Much better was the performance of the RI values derived from the CKMB mass results. Serum CKMB can originate from both skeletal and cardiac muscles[15]. In skeletal muscle, CKMB can comprise up to 2% of total CK while in cardiac muscle, CKMB makes up 20% – 46% of total CK[4]. By expressing the CKMB result as a percentage of total CK, it is possible to differentiate myocardial from skeletal muscle damage. Since CKMB is reported in mass units (ug/L) and total CK in activity units (U/L), the term "relative index" rather than percentage is used to refer the ratio of CKMB to total CK. Use of RI may not be appropriate in all cases[16], especially with low total CK results where the absolute CKMB mass result is more meaningful. Nevertheless, its indiscriminant use in this study demonstrated an improvement in diagnostic specificity over the CKMB mass results alone. These findings should encourage laboratories offering CKMB mass measurement to also present their results as RI values.

Troponin I and T were the best performing markers studied. Despite differences in the absolute values and release kinetics of the two markers, no difference in their performance was seen. The similar performance of the two assays has previously been shown prognostically for prediction of AMI and cardiac death in unstable angina patients[17]. The single false positive Troponin T result illustrates the known presence of Troponin T in many cases of end stage renal failure at a greater frequency than Troponin I[18]. It has suggested that increased levels of Troponin T in dialysis patients without evidence of cardiac ischemia represent extracardiac expression of cardiac Troponin T in uraemic skeletal muscle[19-21]. Others have argued that Troponin T in the serum of end-stage renal failure patients originates from the heart[22] and that increased Troponin T predicts myocardial infarction or death in uraemic patients[23]. The significance of these elevations is still controversial but clinicians should be aware that increased levels of both Troponin T and I may be seen in uraemia.

The early false negative samples for both troponin assays demonstrates the danger in relying on a single test result to rule out AMI and the need for multiple timed samples[7]. Troponin appears in the blood 4 – 8 hours following symptom onset and remains abnormal for 4 – 12 days[24]. Over-interpretation of results from early sampling can be misleading and clinicians should remember that a single test at the time of arrival may be inadequate for clinical decision making[5,25].

Each of the markers evaluated varies in its diagnostic performance and cost. All markers demonstrated high sensitivity but only the troponin assays exhibited similarly high specificity. The combination of multiple sampling of a cheap,

Table III – Performance measures for specified cutoffs

Test	Cutoff	Source of cutoff	Sensitivity	Specificity	PV +	PV -	Accuracy
% CKMB	6	Manufacturer, recommended cutoff	0.95	0.40	0.81	0.75	0.80
	7	ROC	0.95	0.53	0.84	0.80	0.84
CKMB mass – Abbott	3.8	Manufacturer, 95 centile from normal population	0.95	0.21	0.78	0.60	0.71
	9.3	Manufacturer, 95 centile from hospitalised non-AMI population	0.83	0.43	0.81	0.46	0.73
	3	ROC	0.98	0.21	0.79	0.75	0.79
CKMB mass – Roche	3.1	Manufacturer, 97.5 centile from normal population	0.98	0.15	0.78	0.67	0.77
	5	Manufacturer, recommended cutoff	0.93	0.23	0.70	0.50	0.76
	7	ROC	0.93	0.39	0.82	0.63	0.79
CKMB mass – OCD	3.38	Manufacturer, 97.5 centile from normal population	0.93	0.33	0.80	0.63	0.78
	4.55	Manufacturer, 97.5 centile from hospitalised non-cardiac population	0.93	0.40	0.82	0.67	0.80
	5.31	Manufacturer, 97.5 centile from hospitalised cardiac non-AMI population	0.91	0.40	0.82	0.60	0.78
	3	ROC	0.93	0.40	0.82	0.67	0.80
RI – Abbott	0.9	ROC	0.91	0.67	0.88	0.71	0.84
RI – Roche	0.7	ROC	0.93	0.50	0.86	0.67	0.83
RI – OCD	0.7	ROC	0.93	0.67	0.89	0.77	0.86
Tn T	0.1	Manufacturer, recommended cutoff	0.93	0.92	0.97	0.79	0.92
	0.1	ROC	0.93	0.92	0.97	0.79	0.92
Tn I	2	Manufacturer, recommended cutoff	0.88	1.00	1.00	0.72	0.91
	2	ROC	0.88	1.00	1.00	0.72	0.91

ROC = value derived from Receiver Operating Curve. PV + = Positive Predictive Value. PV - = Negative Predictive Value.

sensitive assay such as the percentage of CKMB coupled with confirmatory sampling of a more expensive yet sensitive and specific assay such as troponin could provide a cost-effective alternative to multiple troponin sampling as recommended by some[7]. Another approach would be the use of myoglobin in place of the percentage of CKMB in such a protocol. Myoglobin appears in the blood earlier than other cardiac markers but is not cardiac specific[26]. The greater cost of myoglobin measurement makes this less financially attractive than the percentage of CKMB measurement.

CONCLUSION

Cardiac Troponin T and I demonstrated superior diagnostic performance in the diagnosis of AMI over CKMB (mass and activity) and total CK measurement. There was no difference seen between either Troponin T or I or among any of the CKMB mass assays examined. CKMB mass measurements gave greater performance when combined with total CK and presented as RI values. Total CK alone was a poor indicator of AMI. Combining multiple sampling of the percentage of CKMB with single confirmatory troponin testing may provide a cost-effective testing protocol for suspected AMI patients.

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