Usefulness of Rapid Quantitative Measurement of Myoglobin and Troponin T in Early Diagnosis of Acute Myocardial Infarction

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Background New equipment, the Cardiac Reader™, which can measure blood concentrations of troponin T (T) and myoglobin (M) in only 15 min at the bedside was evaluated for early diagnosis of acute myocardial infarction (AMI).

Methods and Results A total of 34 consecutive patients with AMI who came to hospital within 24 h after onset were studied. Blood samples were collected from the patients at admission and 6, 12, 24, 48 h after onset to qualitatively and quantitatively measure T, M and creatine kinase-MB fraction. There were 20 patients with positive results by qualitative troponin T test and 29 with positive results by quantitative test. Of the patients who visited hospital within 3 h of onset, 17% were positive by the qualitative test and 67% cases had positive results in the quantitative test. The patients were divided into 2 groups according to the flow grade in the infarct-related coronary artery. In the TIMI 0–1 group (n=28), serum myoglobin concentrations were higher than in the TIMI 3–4 group (n=6) at admission and at their peak.

Conclusion The rapid quantitative test of T and M is useful for early diagnosis of AMI and as an indicator of its severity, which can be evaluated from the myoglobin concentration in the hyper-acute phase. (Circ J 2004; 68: 639–644)

Key Words: Acute myocardial infarction; Early diagnosis; Myoglobin; Troponin T

Reperfusion therapy has improved the prognosis of acute myocardial infarction (AMI). Early accurate diagnosis of acute coronary syndrome (ACS) and rapid evaluation of its severity may influence the patient's prognosis. Chest symptoms are the first diagnostic indicator of AMI, but many female patients complain of atypical symptoms¹ and approximately one-third of patients with diabetes mellitus and elderly patients, in particular, develop non-specific symptoms.^{2,3} Although electrocardiography is a simple and highly useful diagnostic method, its sensitivity is approximately 50%.^{4–7} Because 40% of patients with myocardial infarction have a non-specific electrocardiogram (ECG) without ST elevation and 10% of patients have a normal ECG,⁸ general physicians other than cardiologists may have difficulty in diagnosing AMI. Therefore, serum markers may have an important role in the earlier and more accurate diagnosis of AMI. Conventionally, an increase in the creatine kinase-MB fraction (CK-MB), a biochemical marker, has been 'golden standard' for the diagnosis of AMI.⁹ The peripheral total creatine kinase (CK)

concentration begins to increase at 3–8 h after the onset of AMI, peaks at 10–24 h after onset, and the increase continues for 3–4 days. CK-MB is detected peripherally in an earlier stage and disappears more quickly.¹⁰ Many patients show no increase in total CK within 3 h of the onset of AMI. Further, the total CK value is a less specific marker of AMI because it is often increased in patients with skeletal muscle disorders or other traumatic injuries. Cardiac troponin is a highly specific marker for myocardial disorders and myoglobin increases during the early stage of AMI, although it is not so specific for cardiac muscle cells. Cardiac troponin T (cTnT) cannot be detected in healthy subjects without impaired renal function but a trace of CK-MB can be detected. That means cTnT is also a more specific marker of AMI.¹² cTnT increases in parallel with CK-MB and is detectable at 4 h after onset and peaks at approximately 14 h after onset. cTnT shows a biphasic increase and is detectable for almost 10 days.^{13,14} Myoglobin, on the other hand, starts to increase 2–3 h after the onset of AMI, peaks at 6–9 h after onset and then disappears at 18–24 h.^{15,16} Because myoglobin shows such a specific pattern of increase and disappearance, it has been used for the diagnosis of hyper acute AMI and has received much attention as a parameter of reperfusion after thrombolytic therapy.^{17–20} The Cardiac Reader™ (Roche Diagnostics, Germany; Sanwa Kagaku Kenkyusho, Japan) is a device to quantitatively measure troponin T (T) and myoglobin (M) at the bedside in less than 15 min. Compared to Trop T, a quantitative examination method, the Cardiac Reader™ can detect smaller

(Received December 16, 2003; revised manuscript received April 15, 2004; accepted May 11, 2004)

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Qualitative Trop T	Cardiac T	Cardiac M
Negative (<0.1 ng/ml)	<0.05 ng/ml $0.05\sim0.1$ ng/ml $0.1\sim2.0$ ng/ml >2.0 ng/ml	<30 ng/ml $30\sim700$ ng/ml >700 ng/ml
Positive		

Fig 1. Expression of data. Cardiac T test can distinguish a small amount of troponin T from 'negative' Trop T.

Table 1 Comparison of the Trop T; Cardiac Reader™ (CR) and CK-MB

Time after onset (h)	Trop T (qualitative)	Cardiac T		Cardiac M		CR (T+M)		CK-MB	
		(-)	(+)	(-)	(+)	(-)	(+)	(-)	(+)
0-3 h									
(-)	10	6	4	7	3	4	6	6	4
(+)	2	0	2	0	2	0	2	0	2
>3 h									
(-)	4	2	2	2	2	1	3	0	4
(+)	18	0	18	1	17	0	18	0	18

Less than 3 h from onset, CR is the most sensitive test.

Cardiac T (+), not negative*; cardiac M (+), >70 ng/ml; CR (+), positive for T or M; CK-MB (+), >4 IU/L.

amounts of T, as shown in Fig 1. The present study was conducted to determine whether the Cardiac Reader™ (CR) is useful for the early diagnosis of AMI and accurate evaluation of its severity.

Methods

Patients

We enrolled 34 consecutive patients (24 males, 10 females, aged 43-89 years (mean age: 67 ± 11 years)), who first developed AMI between December 2000 and February 2002 and who underwent emergency coronary angiography within 24 h of the onset of chest pain.

Patients with renal failure, hypothyroidism, myopathy and traumatic injury, and those requiring defibrillation were excluded from the study. Informed consent was obtained from each patient before participation in this study.

Study Protocol

Emergency coronary angiography was conducted at the time of admission to determine the infarct-related coronary artery (IRA). Percutaneous coronary intervention (PCI) was subsequently conducted in patients without spontaneous recanalization. Coronary artery flow in the IRA before PCI was classified according to the TIMI (Thrombolysis In Myocardial Infarction study) flow grade scale using the conventional method. Two experienced angiographers independently and blindly graded each film. If there was a discrepancy in the grading, a third angiographer made the final decision.

Venous blood samples were taken into test tubes with heparin at the time of admission and at 6, 12, 24, 48, 72, and 120 h after onset. Cardiac T and M were measured using the new immunochromatography system (CR), which requires only 150 μ l of whole blood. In the reader, there is a test strip containing 2 monoclonal antibodies specific for M and T (metal-labeled antibody and biotin antibody) and these form a sandwich complex that moves along the test strip by capillary action. The complex binds to streptavidin

fixed at the detection site and its accumulation forms a red line. The intensity of the red line increases in proportion to the M or T concentration. The CR measures them using 555 nm LED colored light with a CCD photo sensor to convert their concentrations. CK-MB was determined by the automated immunity obstruction method.

Evaluation of Angiography

The patients were divided into the following 2 groups according to the TIMI flow grade of the IRA before PCI: TIMI 0-1 and TIMI 2-3.

The M concentrations at the time of the hospital visit were compared between the following 2 groups of patients: Group P consisted of patients whose lesion was in the proximal portion of #1, #5, #6 or #11 and Group D consisted of patients whose lesion was in the distal portion.

Statistical Analysis

The data of this study was analyzed by Stat View 5.0 (Abacus Inc, Berkley, CA, USA). Continuous variables were reported as mean $\pm$ 1SD. The Mann-Whitney U test was used to evaluate the significance of differences in the T, M and CK-MB concentrations between the groups. In all comparisons, $p<0.05$ was considered to be significant.

Results

Patients

There were 18 patients in group P and 16 in group D; 28 patients were classified into the TIMI 0-1 group and 6 into the TIMI 2-3 group.

Comparison According to Trop T

Results of the conventional qualitative Trop T test, cardiac T/M and CK-MB are shown in Table 1. Of the 14 patients with negative results by quantitative Trop T test, it is noteworthy that 6 had a positive Cardiac T. In addition, 10 of those 14 patients would have been diagnosed as AMI according to the combined results of M and CK-MB.

Table 2 Comparison of the Cardiac Reader™ and CK-MB

	Cardiac Reader™	
	(+)	(-)
CK-MB		
(+)	26	1
(-)	2	4

Cardiac Reader™ combines the results for T and M.

Table 3 Rate of Correct Diagnosis of the 3 Methods

	Trop T	CR	CK-MB	CR + CK-MB
<3 h	16.7%	66.7%	50.0%	66.7%
>3 h	81.8%	81.8%	100%	100%
Total	58.8%	76.5%	82.4%	88.2%

Cardiac Reader™ (CR) surpasses CK-MB in diagnosis performance even in the early phase.

Fig 2. Correlation between myoglobin and CK-MB of the maximum concentrations of each. The cases in which the max myoglobin >700 ng/ml were excluded.

Fig 3. Comparison of myoglobin (M) and CK-MB concentrations between patients with a distal (D) or proximal (P) culprit lesion. Data are expressed as mean + 1SD.

Cardiac Reader™ and CK-MB

The comparison between CK-MB and CR in terms of performance is shown in Tables 2 and 3. Twenty-six patients were positive by CR and CK-MB, and 4 had negative results with both techniques. Two patients were positive by CR, but negative by CK-MB, and one was positive by CK-MB but negative by CR. Of the patients examined within 3 h of onset, 66.7% were positive by CR, and 50.0% were positive by CK-MB, whereas only 16.7% had a positive Trop T test on admission. In all patients, the rate of correct diagnosis was 88.2% when we used both the CR and CK-MB. It was higher than when we used CK-MB alone (82.4%). The Qualitative Trop T test had the lowest diagnostic rate in any cases.

Relationship Between Maximum Concentrations of M and CK-MB

As shown in Fig 2, the maximum concentration of M was closely related to the maximum concentration of CK-MB ($R=0.81$, $p<0.0001$). This was in fact obtained by comparison of the M concentration at 12 h after onset and the CK-MB concentration at 24 h after onset because of the limitation of the study protocol.

Classification According to Lesion

No difference in the concentrations of M and CK-MB was found at any time point between groups P and D (Fig 3).

Fig 4. Comparison of myoglobin and CK-MB concentrations between patients with TIMI 0-1 flow and TIMI 2-3 flow. Data are expressed as mean + 1SD.

Fig 5. Relationship between clinical severity and the concentration of myoglobin. Myoglobin has a high concentration at an earlier stage in serious cases (filled circles) than in mild cases (open circles). One patient died because of cardiac rupture on the seventh day of the disease. Three patients who required intra aortic balloon pumping (IABP) had concentrations of myoglobin >700 ng/dl at 5-6 h after onset.

Classification According to TIMI Flow Grade

Fig 4 shows the relationships between each value of M and CK-MB, and the TIMI flow grade. Patients with lower TIMI flow grades showed higher M concentrations and higher maximum CK concentrations. In contrast to the CK-MB concentrations, the M concentrations at the time of the hospital visit, showed significant difference between the 2 groups.

M Concentration of Serious Cases at the Time of the Hospital Visit

Fig 5 shows the relationship between clinical severity and myoglobin concentration at the time of the hospital visit. Patients with serious symptoms had a tendency to reach higher myoglobin concentrations earlier than those with mild ones. One patient died from cardiac rupture on the seventh day of the disease. Patients requiring intra-aortic balloon pumping (IABP) and artificial respirator showed more than 700 ng/dl of myoglobin at 5-6 h after the onset. The result suggests that the development of serious symp-

toms can be predicted by the myoglobin concentration at the time of the hospital visit.

Discussion

Early Diagnosis of Acute Myocardial Infarction

Because some patients within 3 h of onset showed no increase in CK, the sensitivity and specificity of CK to diagnose AMI are questionable.^{21,22} To determine the best treatment for patients with chest pain, cTnT has been proven as a useful marker. In the present study, the method using all of the test for CK-MB, cTnT and M improves the diagnostic accuracy in the hyper-acute phase compared with using CK-MB or cTnT alone, and quantitative determination using the CR may have been more useful for early diagnosis compared with the method using CK-MB alone. Although the diagnosis of AMI is not only made by blood chemical tests, they are useful as a means of assisting the diagnosis of patients with non-typical symptoms or ECG. A simplified determination of cTnT and M at the bedside

using the CR is considered to be useful for the diagnosis of patients with chest pain in small clinics or on remote islands.

However, it should be noted that the positive rate of the CR in patients in the hyper-acute phase within 2 h of onset was low.

Early Diagnosis of the Severity of Myocardial Infarction

Some studies have indicated that biochemical markers such as CK-MB, cTnT and cTnI are useful for classifying the risk of patients with ACS. The present study investigated whether a short-term prognosis could be estimated from the M concentration. The results suggested that if a patient had a high concentration at approximately 6 h after onset, the symptoms may progress to Killip's class 3–4, including pulmonary edema. The M concentration at 5–6 h after onset may be useful for predict short-term prognosis such as the need for the IABP or an artificial respirator. The CR may be useful for selecting patients who need emergency and intensive treatment.

Relationship Between Maximum Concentrations of Serum Myoglobin and Myocardial Damage

The maximum concentration of CK-MB is related to the grade of myocardial damage^{23–26} and the present study showed a close relationship between the maximum concentrations of M and CK-MB. The study data suggested that the maximum concentration of M, which was obtained at 6–12 h after onset, could be related to the grade of myocardial damage. Therefore, it may be used for an earlier evaluation of damage than by CK. According to the evaluation of the measurement accuracy for cTnT and M by the CR in the test facilities, the correlation coefficients in comparison with the control were 0.962 and 0.974. However, the M concentration tended to be lower than the concentration obtained using EVATEST (Nissui Seiyaku, Japan).²⁷ Although it may be a problem that the measured values depend on the reagents, the good reproducibility and linearity of correlation of this method are together sufficiently practical for the evaluation of severity. The fast diagnostic method in the hyper-acute phase of AMI using heart-type fatty acid-binding protein, which has been recently developed, has a higher incidence of false positive results²⁸ Therefore, that is used only qualitatively and is not regarded as a 'golden standard'.

The interpretation of symptoms, ECG and echocardiogram is not reliable for the diagnosis of small infarctions, including non-Q wave infarctions, and the CR may be a convenient tool for cardiologists as well as general physicians because it can be used to quickly eliminate the suspicion of AMI.

Study Limitations

The present study had a relatively small number of patients. The CR has an upper limit of measurement for T and M. The data beyond the upper limit were excluded from the statistical analysis.

Serum myoglobin concentrations vary greatly over a short period of time. In the present study, the M concentration at 12 h after onset was used as the maximum concentration. In order to obtain the true maximum, blood samples would need to be collected more frequently, but it was difficult to collect blood samples at 1-h intervals at most clinical sites. Therefore, we investigated the relationship between the M concentration at 12 h after onset and the

CK-MB concentration at 24 h after onset.

Conclusions

The M concentration obtained by the CR may be a useful marker of early AMI and for evaluation of the reperfusion status of the IRA. The maximum concentration of M may be a useful marker of the severity of AMI, even in the hyper-acute phase.

Acknowledgments

The support and cooperation from Professor Fumio Nomura and Keiko Saito in the Department of Clinical Laboratory, Chiba University Hospital, and from the clinical technologists in the 2 community hospitals, are greatly appreciated.

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