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Gülinnaz ALPER[1]
Ömür ERKIZAN[2]
Can HASDEMİR[3]
Azem AKILLI[3]
Taner ONAT[1]

Cardiac Troponin T in Diagnosis of Acute Myocardial Infarction

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Departments of [1]Biochem, Ege Univ. Medical Faculty Cardiology [2]Buca SSK Hospital Dept. of Biochem, Buca-İzmir-Turkey

Abstract: Cardiac Troponin T (TnT) is a regulatory contractile protein not normally found in blood. Its detection in the circulation has been shown to be a sensitive and specific marker for myocardial cell damage. This study was designed to evaluate the diagnostic efficiency of TnT enzyme immunoassay for the early diagnosis of AMI (Acute Myocardial Infarction) and to compare this newly developed test with the conventionally used cardiac enzyme tests in need of an alternative test of greater sensitivity and specificity.

The study population consisted of 13 patients with a mean age of 52±8 years who were hospitalized for AMI. Blood samples for TnT, CPK and CK-MB measurements were drawn at the time of admission (2.9±1.7 hours after the onset of chest pain) and at the 4th, 14-16th, 32-34th and 100-130th hours thereafter. We used an enzyme immunologic assay for the quantitative determination of serum TnT and enzymatic methods for CPK and CK-MB.

TnT appeared in circulation slightly earlier than CPK and CK-MB and increased to a peak value of 46 times the detection limit during the first day. Relative peak values of TnT after onset of pain were 6.4 and 6.8 fold higher than CK-MB and CPK results. The sensitivity of TnT for detecting AMI was 100% from the 4th hour to the 100-130th hour after the onset of symptoms and the diagnostic sensitivity of TnT was superior to that of CPK and CK-MB (100% versus 23% and 15%).

Thus the results of this study indicate that; TnT test improves the efficiency of serodiagnostic tools in detection of AMI and gives a larger diagnostic window that allows serological detection of subacute myocardial infarction.

Key Words: troponin T, acute myocardial infarction

Introduction

Acute myocardial infarction (AMI) is a medical emergency and continues to be the major cause of cardiovascular morbidity and death even nowadays (1,2). The diagnosis of AMI is usually straightforward and based on the peresence of two of three classic findings; 1) a history of characteristic chest pain, 2) evolutionary changes on the ECG and, 3) increased serum concentrations of cardiac enzymes, especially CK isoenzyme MB (2-5).

The measurement of CK-MB is currently the test of choice for the confirmation or exclusion of the diagnosis of AMI (6,7) Though CK-MB determination is an excellent tool for the diagnosis of AMI in the majority of patients, in certain groups (eg. those who do not seek early medical attention, those with concomitant skeletal muscle injury), AMI is often more difficult to assess (5). Yet, variable normal serum levels of CK-MB, its presence in noncardiac muscular tissues, and its small and short-lasting increase in serum during the course of MI limits the diagnostic value of CK-MB determination (8).

Recently, Katus et al. developed an enzyme immunoassay for the cardiac Tn T isoform, which showed a cross-reactivity with Tn T extracted from mixed skeletal muscle of only 1-2% (2,3,8-10) and by using this assay, they provided evidence that measurement of serum Tn T levels offers a diagnostic advantage over CK-MB in the subgroups of patients mentioned above (5,6,8).

Cardiac TnT is a 37-kDa polypeptide subunit of the myofibrillar regulatory troponin complex (9,11). Because the amino acid sequence is unique to cardiac muscle, it is immunologically possible to differentiate skeletal muscle and cardiac protein isoforms (3,9,12). In the cardiomyocyte, TnT is compartmentalized into a minor cytosolic (5%) and a major myofibrillary bound (95%) fraction (9). When the myocytes are damaged,

loss of cell membrane integrity occurs and proteins of the cardiac contractile apparatus diffuse first into the interstitium and subsequently into the intravascular space and lymphatics (5,13). Their pattern of appearance in blood depends on their intracellular location, molecular weight, local blood and lymph flow and the rate of elimination from the blood (5).

Cardiac Tn T is one of these regulatory contractile proteins not normally found in blood (8, 14,15). In a group of recent studies its detection in the circulation has been shown to be a sensitive and specific marker for myocardial cell damage (12,14,16).

The aim of this study is to evaluate the diagnostic efficiency of Tn T enzyme immunoassay for the early and subacute diagnosis of AMI and to compare this newly developed test with the conventionally used cardiac enzyme tests in need of an alternative test of greater sensitivity and specificity.

Materials and Methods

The study population consisted of 13 patients (12 men and a woman) with a mean age of 52±8 years who were hospitalized in Coronary Intensive Care Unit of Cardiology Department of Ege University Medical Faculty, İzmir, Turkey for AMI. Only the patients suffering from chest pain that met electrocardiographic and enzymatic criteria of the World Health Organization were included in the study. Patients who have stable or unstable angina pectoris or other cardiovascular disorders were not included in the study. All the patients were given bed rest and an intensive medical regimen that included nitrates, beta-blockers, calcium-channel blockers and intravenous thrombolytic therapy. Standart 12-lead electrocardiography (ECG) was performed routinely at the time of admission and thereafter. The procedures followed in the study were in accord with the ethical standards of the committee on human experimentation of the hospital of Ege University Medical Faculty.

Blood samples for Tn T, CPK and CK-MB measurements were drawn at the time of admission (2.9±1.7 hours after the onset of chest pain) and at the 4th, 14-16th , 32-34th and 100-130th hours thereafter. Blood samples were stored at room temperature for 30 minutes to allow clotting. After centrifugation at 5000 g for 10 minutes, the serum samples were stored frozen at -30°C as aliquots for a maximum of two months until the analysis were done.

For the quantitative determination of serum Tn T, an enzyme immunoassay (ELISA Troponin T; Boehringer Mannheim) was used. Based on a technique using spreptavidin, this single step sandwich assay allows serial determination of blood samples to be made within two hours (3,8,9,12,17).

The test requires streptavidin-coated tubes as the solid phase and two monoclonal antihuman cardiac TnT antibodies (12,17). During 60-minutes incubation period, the antigen (in this case, TnT in patient's serum) is bound by one biotinylated and one peroxidaselabeled antibodies. This complex adheres to the test-tube wall because of the high-affinity streptavidin-biotin interaction. After two washing steps, the substrate chromogen solution (ABTS) is added. Substrate conversion is quantified by the occurrence of a change in color at 420 nm. Measurements were made against substrate-chromogen solution as blank with a LKB spectrophotometer using semimicro cuvette of 1.0 cm light path. For TnT, the reference interval (0.0-0.2 ng/ml) was established from samples of ten healthy blood donors from laboratory staff and values greater than 0.20 ng/ml were considered positive for AMI.

Catalytic activities of CPK and CK-MB were measured at 37°C with a Hitachi 705 autoanalyser using commercial reagent supplied by Boehringer Mannheim (CK-NAC activated and CK-MB NAC activated, respectively). The upper reference limits used for these assays were 190 U/L for CPK and 24 U/L for CK-MB at 37°C.

Data Analysis:

The history of the patients, 12-lead ECG were evaluated by cardiologists, who were unaware of the serological test results. The test results are expressed as "relative increases" which is defined as the ratios of the patients serum activities (mean±SEM) to the upper limit of normal in the case of CPK and CK-MB or to the analytical sensitivity of the TnT test (0.2 ng/ml). Also, the results are expressed in terms of "sensitivity" which is defined as the number of true positive test results in all patients with AMI and "specificity" as the number of true negative test results in all healthy blood donors without AMI.

Results

The mean±SEM values of cardiac marker proteins (TnT, CPK and CK-MB) after hospital admission of the patients with AMI were presented in Table 1.

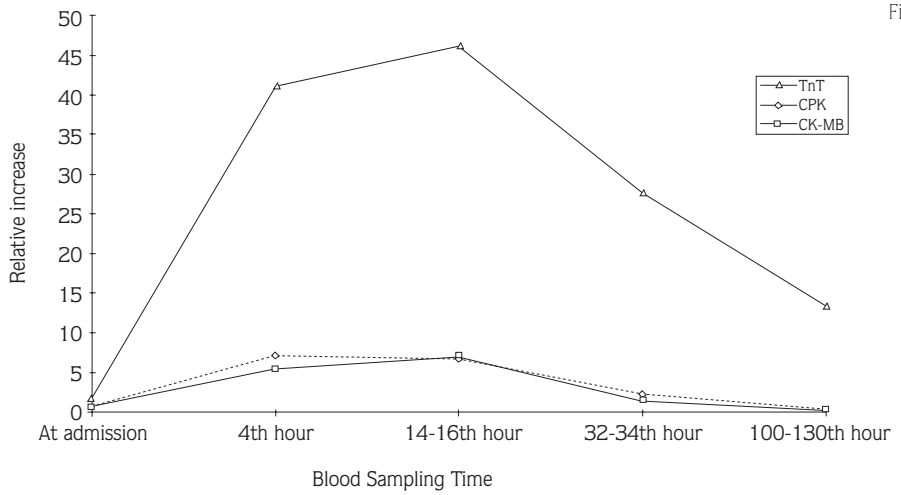

Figure 1. The relative increase of cardiac marker proteins by time in AMI.

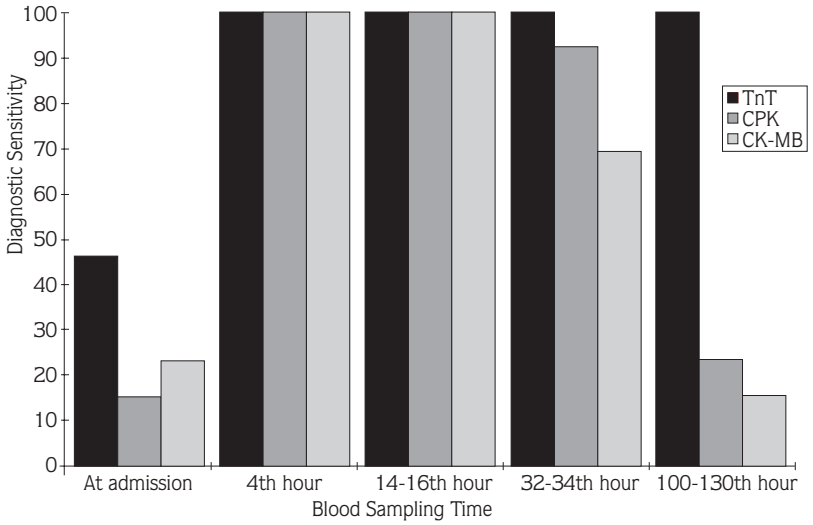

Figure 2. The diagnostic sensitivity of cardiac marker proteins by time in AMI.

Table 1. The mean±SEM values of TnT, CPK and CPK-MB after hospital admission of the patients with AMI.

	TnT	CPK	CK-MB
	ng/ml	U/L	U/L
At admission	0.33±0.14	138.9±36.5	16.2±2.4
4 th hour	8.26±2.53	1366.4±300.1	135.5±26.8
14-16 th hour	9.22±1.57	1290.6±184.8	172.0±56.0
32-34 th hour	5.54±1.19	483.7±98.7	40.2±8.0
100-130 th hour	2.69±0.55	110.7±23.7	12.8±4.2

The relative increase of the mean±SEM values and the diagnostic sensitivity of the cardiac marker proteins by time in AMI were presented in Table 2 and were shown in Figure 1 and Figure 2, respectively.

Table 2.
It shows the relative increase of the mean±SEM values and the diagnostic sensitivity of TnT, CPK and CK-MB in patients with AMI.

Blood Sampling	Relative Increase			Diagnostic Sensitivity		
	TnT	CPK	CK-MB	TnT	CPK	CK-MB
At admission	x1.7	x0.7	x0.7	46%	15%	23%
4th hour	x41.3	x7.2	x5.6	100%	100%	100%
14-16 th hour	x46.2	x6.8	x7.2	100%	100%	100%
32-34 th hour	x27.7	x2.5	x1.7	100%	92%	69%
100-130 th hour	x13.5	x0.6	x0.6	100%	23%	15%

At the time of admission six of the 13 patients with AMI had elevated TnT values (46%) while only two of them had elevated CPK (15%) and three of them had elevated CK-MB activities (23%) (Table 2,

Figure 2). The patients with and those without elevated TnT values did not differ with respect to clinical charcteristics and treatment.

At the 4th and 14-16th hours after admission, all of the patients had elevated TnT values, besides increased CPK and CK-MB activities (Table 2, Figure 2). However, relative peak value of TnT at the 14-16 th hours was 6.4 and 6.8 fold higher than CK-MB and CPK values, respectively (Table 2, Figure 1).

At the 32-34th hours after admission TnT was detectable in all of the patients (100%) while CPK activities showed an increase in 12 patients (92%) and CK-MB was elevated in 9 patient (69%) (Table 2, Figure 2). At the 100-130th hours all of the patients still had increased TnT values (100%) while only 3 of them had increased CPK values (23%) and 2 of them had elevated CK-MB activities (15%) (Table 2, Figure 2).

The specificity of TnT test was evaluated with the measurement of serum TnT concentrations in controls, comprised of 10 healthy blood donors from laboratory staff who do not have cardiac discomfort, and was found 100%.

Discussion

The diagnosis of AMI has traditionally been based on the triad of a characteristic clinical history, electrocardiographic abnormalities and increased serum concentrations of cardiac enzymes (3,4). For the early diagnosis of AMI, the diagnostic value of chest pain is limited. The same is true for specific changes in the electrocardiographic recordings at admission (8). Therefore, measurement of biochemical markers has become a cornerstone in the diagnosis of AMI (11). However, in pratice, physicians are sometimes left with patients in whom a definitive diagnosis of MI can not be made by measurement of conventional plasma enzymes or isoenzymes (8,18). There are problems with the diagnosis of small myocardial infarctions, which from the therapeutic and prognostic points of view are at least comparable in importance with the transmural infarctions. The ECG offers little help in such cases and the sensitivity of the laboratory parameters CPK, CK-MB, lactate dehydrogenase (LD) and LD1 isoenzyme is not sufficient because of the variations in normal serum levels and of the short-lasting and small increases in serum activity after such events. The available diagnostic methods are also unsatisfactory in patients with multiorgan diseases or with additional skeletal muscle lesions, because in such cases a definite differentiation of skeletal muscle and heart muscle necrosis is impossible because of the organ distribution of CPK and LD or its isoforms. And also, current diagnostic procedures are unable to give definite evidence of infarction in patients who arrive at the hospital in a subacute phase (5).

In recent years, considerable efforts have been made to improve the specificity and sensitivity of methods for diagnosing AMI. Myoglobin is an early and sensitive marker of cardiac cell damage but lacks specificity. The use of LD and its LD1 isoenzyme improves specificity in diagnosing AMI; however, these isoenzymes are not restricted to cardiac muscle tissue and increases in their serum concentrations have been observed in noncardiac conditions (3). The sensitivity of the CK determination is limited because the increase in serum CK is relatively small, lasts only a short time after the onset of pain and may be obscured by differences in normal serum levels. Clinicians would therefore benefit from the introduction of a new cardiac-specific marker of myocardial muscle cell damage. A recently introduced alternative, proposed as a very sensitive and specific marker for myocardial damage, is constituted by the immunological determination of cardiospecific troponin T, a 37 kDa polypeptide subunit of the myofibrillar regulatory troponin complex (11).

In a group of recent studies, it has been shown that the diagnostic sensitivity of TnT is considerably better than that of myoglobin, CPK and CK-MB activity (11). The biggest advantage of TnT is its cardiospecificity. Thus, measurements are especially helpful in the assessment of patients with myocardial ischemia and skeletal muscle injury-eg, after cardiac surgery or in multiorgan damage (19).

In an attempt to find a more suitable marker for myocardial cell damage, we assessed the diagnostic properties of TnT and compared it with those of the currently used cardiac enzymes, such as CPK and CK-MB. In most of the cases with AMI, TnT appeared in the circulation within 3 hours after the onset of chest pain-slightly earlier than the rise in CPK and CK-MB activities, thus elevated results at admission were encountered more frequently for TnT (46%) than for CPK (15%) and CK-MB (23%) measurements. ($p<0.01$) (Table 2, Figure 2).

It is seen that, our results are in concordance with the ones of Katus HA et al (8) who had observed increased serum TnT values in 50% of 388 patients

admitted with chest pain and suspected AMI as early as 3 hours after the onset of chest pain and with those of Mair J et al (3) who had reported a 50% TnT sensitivity at the 4th hour after the onset of AMI symptoms.

In a recent report investigating the value of TnT in early diagnosis of AMI, Bakker AJ et al (11) pointed out that within 4 hours after onset of chest pain, the currently used methods for measuring enzyme of CPK and CK-MB attained a low diagnostic sensitivity (20% and 16%, respectively) while TnT showed a considerably higher diagnostic sensitivity (51%) than that of CPK and CK-MB activities.

Also it is interesting to note that during the first day, TnT increased to a peak value of 46.2 times the detection limit, while CPK and CK-MB activities generally did not increase more than 7.2 fold (Table 2, Figure 1). Concordant with our findings, Katus HA et al (8) reported that TnT increased to a first peak value of 40 times the detection limit during the first day and a peak value of 30 times the detection limit on about the fourth day, while serum CPK and LD activities only increased to nine and three times the upper limit of normal, respectively.

The half life of TnT is 120 minutes, so the sustained increase in serum concentration probably reflects continuing release of this protein from disintegrating myofilaments(19). This apparent superriority of TnT to CPK and CK-MB, probably is due to a longer diagnostic window rather than to more release of the protein per gram of damaged tissue (6).

The sensitivity of TnT for detecting AMI was 100% from the 4th hour of admission to the 100-130 th hours and it was still 100% revealing a peak values of 13.5 times the detection limit on about the fifth day by contrast to CPK (23%) and CK-MB (15%) activities which were mostly normalized (Table 2, Figure 1 and 2). Thus, the persistance of the elevation of TnT levels at the 5th day also facilitates retrospective identification of AMI.

These results are in accordance with other investigators' findings (2,3,5,9,11,19,20) so TnT test can be proposed as an ideal late marker for diagnosing subacute infarction in patients who only come to the hospital in the late phase of the infarction, with uncharacteristic symptoms and already normalized CPK and CK-MB activities.

Based on our finding discussed above, it is concluded that in addition to the high diagnostic sensitivity and specificity, the early and persistent rise in serum TnT concentrations provides a wide diagnostic window offering additional reliability in the diagnosis of myocardial infarction both in hyperacute and subacute phases.

Thus, the data of this study indicate that the newly developed TnT test improves the efficiency of serodiagnostic tools for the detection of myocardial cell necrosis as compared with conventionally used cardiac enzymes.

It appears that, TnT will be an attractive marker both for early and late diagnosis of AMI, if more rapid and cheaper assay methods should be developed in the near future.

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