

Analysis of serum cardiac biomarkers and treadmill exercise test-electrocardiogram for the diagnosis of coronary heart disease in suspected patients

Zhenhua Zhu, Yan Yan*, Qibing Wang, Juying Qian, and Junbo Ge

Shanghai Institute of Cardiovascular Diseases, Zhongshan Hospital, Fudan University, Shanghai 200032, China

*Correspondence address. Tel: +86-13501829900; E-mail: jackyan000@hotmail.com

The serum proteins creatine kinase isoenzyme MB (CK-MB) and cardiac troponin T are classic biomarkers of cardiac ischemic damage in clinical practice, which can sensitively detect myocardial necrosis, while other two serum proteins, ischemia-modified albumin and N-terminal pro-B-type natriuretic peptide (NT-proBNP), have been recently identified as novel biomarkers of myocardial ischemia. In this study, the four biomarkers were detected in sera from 44 eligible patients with suspected coronary heart disease (CHD) before and after treadmill exercise test (TET), electrocardiogram (ECG) was measured during TET (TET-ECG) and invasive examination of coronary angiography (CAG), which is the ‘gold standard’ of CHD diagnosis, was also performed. For CAG, 25 patients were positive and 19 were negative, whereas for TET-ECG the numbers were 19 and 25, respectively. Among these four biomarkers, the NT-proBNP level in CAG positive group was much higher than those in CAG negative group both before and after TET, with statistical significance before TET ($P = 0.008$). Furthermore, according to receiver operating characteristic (ROC) curve, the serum biomarker NT-proBNP showed diagnostic effect of CHD and its cutoff value was 67 pg/ml, thus 30 of the patients in this study were NT-proBNP positive and 14 were negative. And it was found that NT-proBNP obviously enhanced the sensitivity of examinations whether analyzed alone or in combination with TET-ECG. More importantly, all the patients who were negative in both NT-proBNP and TET-ECG tests turned out to be CAG negative, which means that the combination of these two non-invasive examinations might take the place of invasive examination of CAG for CHD diagnosis. Further studies with more patients are warranted to validate the findings in this paper.

Keywords myocardial ischemia; coronary heart disease; serum biomarker; N-terminal pro-B-type natriuretic peptide (NT-proBNP); coronary angiography; non-invasive examination

Introduction

Myocardial ischemia is the main pathophysiologic characteristic of coronary heart disease (CHD) and its detection is very important for the diagnosis, treatment, and prognosis of CHD. But unfortunately, the identification of myocardial ischemia and early diagnosis of CHD is one of the bottlenecks in medical practice of cardiology. In large heart centers in the USA, only 25% of patients suspected with acute coronary syndrome (ACS), which is the acute attack clinical pattern of CHD, have the same diagnosis when discharged, thus resulting in huge waste of clinical resources [1]. On the other hand, there are still 2% ACS patients who miss being diagnosed annually, which leads to serious outcomes [1,2].

So far, coronary angiography (CAG) is the ‘gold standard’ of CHD diagnosis. However, it is not sensitive for detecting myocardial ischemia. Besides, it is an invasive procedure, which has the risks of serious complications and is not available in all hospitals. Therefore, CAG is only cost-effectively conducted in highly suspected patients.

The non-invasive electrocardiogram (ECG) can reflect myocardial ischemia in terms of ‘ST-T changes’, but it is non-specific and the ischemic changes may not be shown in mild cases or when patients are at rest status. Treadmill exercise test (TET) can give extra load to heart and increase oxygen consumption of myocardium, so as to arouse ischemia in potential cases. Thus ECG measured during TET (TET-ECG) is a more sensitive way of identifying CHD.

Another non-invasive method is blood test. The classic biomarkers of myocardial ischemic damage are creatine kinase isoenzyme MB (CK-MB) and cardiac troponin T (cTnT), which are sensitive for myocardial necrosis and have been used as diagnostic tools of acute myocardial infarction in clinical practice [3]. Biologically, creatine kinase (CK) is an important enzyme that catalyzes the conversion of creatine. CK-MB is one of the three isoenzymes of CK, and it occurs mainly in the heart. As for TnT, it is

one of the three subunits of troponin complex, which regulates the calcium-mediated contractile process of striated muscle. Because of different amino acid sequence, cTnT can be identified from TnT of skeletal muscle and is specific for myocardium. Recently, ischemia-modified albumin (IMA) and N-terminal pro B-type natriuretic peptide (NT-proBNP) have been studied as novel biomarkers, which are promising in identifying myocardial ischemia. It was reported that the N-terminus of human serum albumin could change its structure and thus reduce the *in vivo* transitional metal-binding capacity by the oxidative stress effect of myocardial ischemia, forming the so-called 'ischemia-modified albumin (IMA)', which could be determined by albumin cobalt-binding test method [4–6]. In February 2003, the Food and Drug Administration (FDA) of USA approved its use in clinical practice. Brain natriuretic peptide (BNP) is a neuroendocrine hormone secreted by cardiac ventricular cells. It has the effects of promoting natriuresis, diuresis, vasodilation and suppression of the sympathetic nervous system and renin–angiotensin–aldosterone system. Its synthesis and secretion are enhanced when the ventricular load and ventricular wall tension increase. NT-proBNP is a co-product during the generation of BNP, a neuroendocrine hormone secreted by cardiac ventricular cells [7]. Because of its longer biologic half-life and higher concentration in sera, NT-proBNP is easier to be detected in peripheral circulation than BNP [7]. Both BNP and NT-proBNP have been approved by the FDA since November 2000 as diagnostic biomarkers of congestive heart failure. Afterwards, it was realized that myocardial ischemia is another important stimulating factor for the secretion of BNP and NT-proBNP, and the levels of BNP and NT-proBNP correlate well with the degree of ischemia [8–10]. However, there is little information about the serum levels of those biomarkers in suspected CHD patients, even though they are the most common group of people doctors encounter in the clinic and need to make diagnosis of.

In this study, we subject the suspected CHD patients to CAG and TET-ECG, and also detected the levels of cTnT, CK-MB, IMA and NT-proBNP in their sera. For all these examinations, CAG is the 'gold standard' to make a definite CHD diagnosis, while others were analyzed for their diagnostic effect of CHD.

Materials and Methods

Patients and general procedures

Forty-four patients with complaints of 'chest pain, chest pressure or discomfort' and suspected of CHD were enrolled in Zhongshan Hospital (Shanghai, China) from November 2007 to March 2008. All the patients of contraindications of treadmill exercise test (TET), peripheral

artery disease, myopathy and renal function insufficiency were excluded. For each patient, 10 ml venous blood samples were drawn before and 2 h after TET. After centrifugation, the sera were collected for determination of the biomarkers, i.e. IMA, NT-proBNP, cTnT, and CK-MB. And all patients underwent CAG at the end to identify CHD diagnosis.

Materials

HITACHI-7600 automatic biochemistry analyzer (HITACHI, Japan) and Elecsys 2010 automatic electrochemistry luminescent immunoanalyzer (Roche Diagnostics GmbH, Switzerland) were used in biomarker assays, while TET was conducted by means of X12-Scribe system (Mortara Instrument, USA). The reagents used in biomarker analyses were as follows: N-terminal pro B-type natriuretic peptide (NT-proBNP), Troponin T STAT (CARDIAC T), Creatine Kinase-MB liquid (Roche Diagnostics GmbH, Switzerland) and IMA kit (Yikang Technological Development Ltd, Changsha, China).

Assays of serum biomarkers

All the biomarkers were measured automatically by instruments using ready-made reagents mentioned above according to instructions. NT-proBNP and cTnT were determined by Elecsys 2010 automatic electrochemistry luminescent immunoanalyzer, whereas IMA and CK-MB were determined by HITACHI-7600 automatic biochemistry analyzer. All these assays were under Roche Qcs international quality control.

Treadmill exercise test-electrocardiogram (TET-ECG)

Maximal or sub-maximal exercise of Bruce scheme was adopted, that is, the patients had to reach 100% or 85% of their maximal heart rate (220–age). Synchronous 12-lead electrocardiogram was recorded during the TET-ECG.

The criteria for 'positive' were: ECG showed ST segments of adjacent leads descended horizontally or downslopingly for at least 0.1 mV, and last for more than 2 min, with or without concomitant typical angina symptoms. The criteria for 'negative' were: objective load achieved without ST-T changes.

Coronary angiography

All patients underwent coronary angiography. Seldinger technique was adopted to puncture right femoral, and Judkins method was used to make multipart coronary angiography. 'Positive' was defined as existing at least 70% stenosis of one or more coronary branches whose diameter is >2 mm, whereas 50% stenosis for the left main branch.

Table 1 Determination of biomarkers in CAG positive (+) and negative (–) patients

CAG	IMA (U/ml)		NT-proBNP (pg/ml)		CK-MB (U/L)	
	Pre-TET	Post-TET	Pre-TET*	Post-TET	Pre-TET	Post-TET
+	51.08 ± 5.69	52.20 ± 5.66	187.97 ± 166.29	197.00 ± 178.82	11.72 ± 3.36	12.96 ± 4.86
–	51.05 ± 5.88	51.58 ± 5.26	107.27 ± 105.15	117.91 ± 93.34	11.95 ± 6.92	13.21 ± 6.43

* $P < 0.05$ in the test of NT-proBNP difference between CAG positive and negative patients.

Data analysis

Data were presented as mean ± SD. For biomarkers other than NT-proBNP, mean comparisons before and after TET were tested by paired-samples *t*-test and comparisons between two groups by independent samples *t*-test. For NT-proBNP, non-parametric tests such as Wilcoxon signed ranks test and Mann–Whitney test were used instead because of its large variances in people (tens to hundreds pg/ml). Enumeration data were reported as absolute value (constituent ratio). Receiver operating characteristic (ROC) curve was used for general evaluation of biomarkers. Calculation and illustration were made by software SPSS 15.0. Statistical significance was established at $P < 0.05$.

Results

Determinations and tests of four serum biomarkers

Each studied patient was given eight assays for four biomarkers as IMA, NT-proBNP, CK-MB, and cTnT both before and 2 h after TET. As shown in **Table 1**, among those biomarkers, only NT-proBNP values in CAG positive group were much higher than those in negative group both before and after TET, and the difference between positive and negative groups before TET had statistic significance ($P = 0.008$). In other words, NT-proBNP values were statistically different between CHD patients and non-CHD persons. In all patients, sera cTnT levels were less than its lower detection limit (0.01 ng/ml), so cTnT levels were not listed in the table.

ROC curve of NT-proBNP for CHD diagnosis

ROC curve is a most often used method to determine the best cutoff value for a diagnostic test. When an examination established as ‘gold standard’ and several different values defined as cutoff values of a diagnostic test, a series of sensitivity and specificity values can be calculated accordingly. Then the curve can be made with the true positive rate (sensitivity) as *y*-axis and the false positive rate ($1 - \text{specificity}$) as *x*-axis, so that it can show the relationship between sensitivity and specificity. For a desirable diagnostic test, the ROC curve is shaped with the convexity towards the upper-left quadrant of the reference frame. The point most close to the upper-left angle is chosen as the best cutoff value that it leads to the minimal

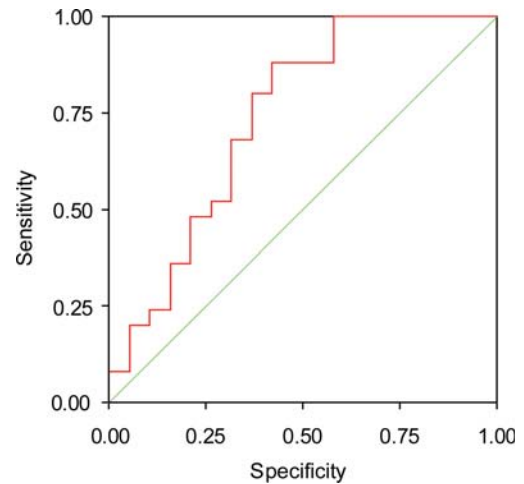


Figure 1 ROC curve of NT-proBNP for CHD diagnosis The AUC was 0.74 (0.58, 0.89), $P = 0.008$. The best cutoff value was 67 pg/ml, which made a largest sum of sensitivity and specificity (0.88, 0.58, and 1.46, respectively). Green, reference curve; red, experimental curve.

sum of false positive rate and false negative rate, in other words, the best combination of sensitivity and specificity. On the other hand, through calculation of the area under the curve (AUC), statistic test can be done to identify the ability of the test as a diagnostic tool.

With the result of CAG as ‘gold standard’ for CHD, according to the ROC curve (**Fig. 1**), and along with calculations and test results of the AUC, there was statistic significance of NT-proBNP as a diagnostic biomarker for CHD [AUC = 0.74 (0.58, 0.89), $P = 0.008$]. Also, we got the best cutoff value of 67 pg/ml according to the curve.

Examinations of CAG and TET-ECG

All patients underwent TET-ECG and CAG. As for CAG, ‘positive’ was defined as existing at least 70% stenosis of one or more coronary branches whose diameter is over 2 mm; whereas 50% stenosis for the left main branch. Thus, 25 (56.8%) were positive and 19 (43.2%) were negative in the cohort as shown in **Table 2**. For TET-ECG the criteria were as follows: ‘positive’ referred to those whose ECG showed ST segments of adjacent leads descended horizontally or downslopingly for at least 0.1 mV, and last for more than 2 min, with or without concomitant typical angina symptoms, whereas ‘negative’ meant objective load

Table 2 Comparison of different examinations for the 44 patients with suspected CHD

Examination	CAG		TET-ECG		NT-proBNP	
	Number	%	Number	%	Number	%
+	25	56.8	19	43.2	30	68.2
-	19	43.2	25	56.8	14	31.8

achieved without ST-T changes. So among the 44 studied patients, 19 (43.2%) were positive and 25 (56.8%) were negative as shown in **Table 2**.

Different examinational strategies for CHD diagnosis

Taking CAG results as diagnostic gold standard of CHD, we compared other four different examinational strategies for CHD diagnosis, which were TET-ECG alone, NT-proBNP alone, and the combination of these two for either serial (TET-ECG × NT-proBNP) or parallel (TET-ECG + NT-proBNP) tests. For NT-proBNP, cutoff value of 67 pg/ml according to the ROC curve was used to differentiate positive and negative patients. As for the combinational analysis, parallel tests positive was defined as either NT-proBNP or ECG was positive, whereas in serial tests, only both examinations positive could be presumed as positive.

As shown in **Tables 3 and 4**, compared with the gold standard diagnosis of CAG, the sensitivity values of those four examinational strategies were 52%, 88%, 40% and

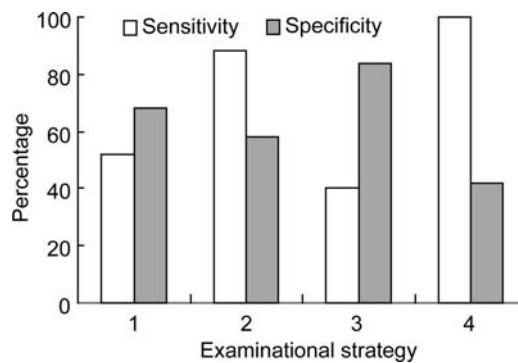


Figure 2 Comparison of different examination strategies for CHD diagnosis 1, TET-ECG; 2, NT-proBNP; 3, TET-ECG × NT-proBNP; 4, TET-ECG + NT-proBNP. TET-ECG × NT-proBNP represents serial tests strategy of which positive is defined as both are positive, whereas ‘TET-ECG + NT-proBNP’ stands for the parallel tests strategy of which positive is defined as either of two is positive.

100%, respectively (see blue columns in **Fig. 2**); and the specificity values of them were 68%, 58%, 84% and 42%, respectively (see red columns in **Fig. 2**). These data indicated that NT-proBNP obviously enhanced the sensitivity of examinations whether analyzed alone or in combination with TET-ECG as parallel tests. Besides, all the patients who were negative in both NT-proBNP and TET-ECG turned out to be CAG negative (which was shown as ‘0’ of negative TET-ECG × NT-proBNP patient in CAG positive subgroup in **Table 4**), which meant that these two alternative non-invasive examinations combined could potentially take the place of invasive CAG for ruling out CHD diagnosis.

Table 3 Analysis of NT-proBNP and TET-ECG for CHD diagnosis in suspected patients subject to TET-ECG × NT-proBNP serial test

CAG	Positive patients			Negative patients ^a	Total
	TET-ECG	NT-proBNP	TET-ECG × NT-proBNP		
+	13 (52)	22 (88)	10 (40)	15 (60)	25 (100)
-	6 (32)	8 (42)	3 (16)	16 (84)	19 (100)

Values are represented by number (%). ^aPatients other than those who got positive of both NT-proBNP and TET-ECG were actually the serial tests (TET-ECG × NT-proBNP) negative group of patients, of whom either NT-proBNP or TET-ECG was negative.

Table 4 Analysis of NT-proBNP and TET-ECG for CHD diagnosis in suspected patients subject to TET-ECG + NT-proBNP parallel test

CAG	Negative patients			Positive patients ^a	Total
	TET-ECG	NT-proBNP	TET-ECG + NT-proBNP		
+	12 (48)	3 (12)	0 (0)	25 (100)	25 (100)
-	13 (68)	11 (58)	8 (42)	11 (58)	19 (100)

Values are represented by number (%). ^aPatients other than those who got negative of both NT-proBNP and TET-ECG were actually the parallel tests (TET-ECG + NT-proBNP) positive group of patients, of whom either NT-proBNP or TET-ECG was positive.

Discussion

This study was about the analyses of cardiac ischemic biomarkers in the sera of suspected CHD patients. The cardiac ischemic biomarkers we refer to here include not only cTnT and CK-MB, which have been generally acknowledged and used in the clinical practice of myocardial infarction diagnosis, but cannot be detected in serum until myocardial cells necrotize; but also new biomarkers such as IMA and NT-proBNP, which can potentially identify reversible myocardial ischemia. For each of them, serum assays were performed both before and 2 h after TET. TET here had two roles: first, it was *per se* an examination which could assist CHD diagnosis; second, for patients who did have lesions in coronary arteries, it might arouse myocardial ischemia to let us study the changes of those four biomarkers under that circumstance.

However, when the serum levels of biomarkers were compared in pairs before and after TET, we did not find any significant changes. Despite of further CAG classification, there were still no significant differences, even in the positive groups. We presumed that the load treadmill exercise provided might not be heavy enough to provoke myocardial ischemia, or the ischemic state was too short to create biomarker level changes (patients were asked to stop exercising immediately if ST segment depressed for 2 min). Another alternative explanation was that maybe some ischemic biomarkers did rise after TET, but the single time point of blood drawing (2 h post-TET) was too early to capture that.

When the biomarkers were compared between CAG positive and CAG negative subgroups, one statistically significant difference was found: NT-proBNP serum levels before TET in CAG positive group were higher than those in CAG negative group. In other words, NT-proBNP sera levels were different between CHD patients and non-CHD persons, which made it a potential biomarker to help diagnose CHD. So we used ROC curve to testify this hypothesis, which showed that NT-proBNP did have diagnostic effect of CHD, and the best cutoff value of the assay for identifying positive or negative was 67 pg/ml.

Thus besides CAG, we have another four examinational strategies for CHD diagnosis, which are TET-ECG, NT-proBNP, and combination of both as serial or parallel tests. As a result, we found that the NT-proBNP examination has higher sensitivity of CHD diagnosis (88%) than that of the TET-ECG examination (52%). When those two were used in combination as parallel tests, the sensitivity reached 100% (positive patients in CAG positive subgroup was 100% as shown in **Table 4**), that is, no CHD patient was missed when those two examinations were tested jointly. This has important clinical meanings. Although

CAG is a reliable method to make a definite diagnosis of CHD, it is an invasive procedure which has risks of serious complications and costs patients a lot of pain. Since both TET-ECG and NT-proBNP assays are simple, cheap, most importantly, non-invasive, high sensitivities and accessible, they can be used as screen examinations in suspected CHD patients. Although the specificities of tests dropped down (68%, 58% and 42%, respectively, for TET-ECG, NT-proBNP, and combination of these two as parallel tests), it is still acceptable considering the fact that there exist efficient methods such as coronary CT and CAG to further diagnose CHD. Taking the serious consequence of missed diagnosis of CHD into consideration, high sensitivity is more important than low specificity. However, when TET-ECG and NT-proBNP are combined as serial tests, the disadvantage of low specificity can be compensated, for the specificity of that strategy is as high as 84%.

In this study we also found that all the persons with negative TET-ECG and NT-proBNP tests were also CAG negative. Based on the identical results, for this group of people, the non-invasive serial examination of TET-ECG and NT-proBNP could take the place of CAG for ruling out CHD diagnosis, sparing them lots of unnecessary pains and risks.

To summarize, in the suspected CHD patients in our study, the sera NT-proBNP levels of CHD patients were higher than those of non-CHD persons. By means of ROC curve, it was found to have diagnostic effect of CHD and could obviously enhance the sensitivity of examinations whether analyzed alone or in combination with TET-ECG as parallel tests. And because of the identical results, the latter strategy could potentially take the place of invasive examinations of CAG for ruling out CHD diagnosis. However, considering the fact that the number of patients in our study is relatively small, and so far no other similar studies were reported, the role of cardiac ischemic biomarkers such as NT-proBNP and their combination with TET-ECG in the diagnosis of CHD still need to be explored. Further researches and evidence are warranted to validate the findings in this paper.

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References

- 1 Kost GJ and Tran NK. Point-of-care testing and cardiac biomarkers: the standard of care and vision for chest pain centers. *Cardiol Clin* 2005, 23: 467–490.
- 2 Pope JH, Aufderheide TP, Ruthazer R and Woolard RH. Missed diagnoses of acute cardiac ischemia in the emergency department. *N Engl J Med* 2000, 342: 1163–1170.
- 3 The Task Force for the diagnosis and treatment of non-ST-segment elevation acute coronary syndromes of the European Society of Cardiology. Guidelines for the diagnosis and treatment of non-ST-segment elevation acute coronary syndromes. *Eur Heart J* 2007, 28: 1598–1660.
- 4 Bar-Or D, Lau E, Rao N, Bampos N, Winkler JV and Curtis CG. Reduction in cobalt binding capacity of human albumin with myocardial ischemia. *Ann Emerg Med* 1999, 34: S56.
- 5 Bar-Or D, Curtis G, Rao N, Bampos N and Lau E. Characterization of the Co^{2+} and Ni^{2+} binding amino-acid residues of the N-terminus of human albumin. An insight into the mechanism of a new assay for myocardial ischemia. *Eur J Biochem* 2001, 268: 42–47.
- 6 Bar-Or D, Lau E and Winkler JV. A novel assay for cobalt-albumin binding and its potential as a marker for myocardial ischemia: a preliminary report. *Emerg Med* 2000, 19: 311–315.
- 7 Omland T, Aakvaag A and Vik-Mo H. Plasma cardiac natriuretic peptide determination as a screening test for the detection of patients with mild left ventricular impairment. *Heart* 1996, 76: 232–237.
- 8 Eui-Young C, Hyuck MK and Yong-Won Y. Assessment of extent of myocardial ischemia in patients with non-ST elevation acute coronary syndrome using serum B-type natriuretic peptide level. *Yonsei Med J* 2004, 45: 255–262.
- 9 Sadanandan S, Cannon CP and Chekuri K. Association of elevated B-type natriuretic peptide levels with angiographic findings among patients with unstable angina and non- ST segment elevation myocardial infarction. *J Am Coll Cardiol* 2004, 44: 564–568.
- 10 Bassan R, Potsch A and Maisel A. B-type natriuretic peptide: a novel early blood marker of myocardial infarction in patients with chest pain and no ST-segment elevation. *Eur Heart J* 2005, 26: 234–240.