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Serum TGF-β1 in patients with acute myocardial infarction

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As acute myocardial infarction (AMI) is a major cause of death and disability, rapid and accurate diagnosis is critical for effective evidence-based management and treatment,[1,2] but there still an unmet clinical need in that diagnosis is often unclear. The use of cardiac biomarkers has become the frontline diagnostic tools for AMI, and has greatly enabled the clinicians in the rapid diagnosis and prompt treatment planning, thereby reducing the mortality rate to a great extent. The World Health Organization defined the term myocardial infarction (MI) by the presence of two of the three following characteristics [3,4]: acute chest pain, development of Q waves in electrocardiogram (ECG) and increase of enzymes in the blood. Later guidelines included cardiac troponins in the diagnosis of AMI.[5] However, a disadvantage of troponin T (TnT) is the late peak in serum levels that makes it difficult in identifying a recurrent MI.[6] Creatine kinase MB (CK-MB) is also used to diagnose AMI. It rises in the serum at 4-9 h after the onset of chest pain, peaks ~24 h and returns to baseline values at 48-72 h. One advantage of CK-MB over the troponins is the early peak in serum levels that helps in the detection of re-infarction. Thus, the serum level of troponin along with the level of the CK-MB is used to assess the diagnosis of AMI.[7] Despite this, CK-MB may not be significantly increased in non-ST-segment elevation MI (NSTEMI),[8] which often make the NSTEMI patients misdiagnosed because they frequently lack typical symptoms and elevated ST-segment in their ECG, in contrast to ST-segment elevation myocardial infarction (STEMI). Therefore, the search for novel sensitive biomarkers for early AMI diagnosis is very important.

Transforming growth factor-β1(TGF-β1) is a multifunctional cytokine linked to vascular remodelling processes, myocardial hypertrophy, renal fibrosis, ventricular remodelling and the early response to MI. [9–11] Under stress of ischemia and hypoxia, TGF-β1 is activated and released into the blood, and is markedly induced and rapidly activated in the infarcted myocardium.[12,13] Bioactive TGF-β1 is released in the cardiac extracellular fluids 3–5 h following reperfused infarction.[14] The purpose of the present study was to test the hypothesis of raised levels of serum TGF-β1 in AMI patients, to compare levels between STEMI and NSTEMI, and to determine whether serum TGF-β1 could serve as sensitive biomarker for the early diagnosis of AMI compared to TnT.

This study was approved by the Research Ethics Committee of The affiliated hospital of Qingdao University, Qingdao, China. Patients > 18 years of age presenting to the emergency department with symptoms suggestive of AMI with an onset or peak within the last 24 h were recruited after providing written informed consent. No extramural funding was used to support this work. This was a prospective, observational study. One hundred and three patients with AIM [65 with STEMI and 38 with NSTEMI] were enrolled. The clinical characteristics of all patients are given in Table 1. All the AMI patients were diagnosed according to the international criteria.[3,4] Patients with previous MI or percutaneous coronary intervention, any chronic peritoneal or hemodialysis, acute or chronic infection, significant hepatic dysfunction, kidney failure (glomerular filtration rate (GFR)<15 mL/min/1.73 m^2 or on dialysis) or known or treated malignancies were excluded. One hundred and three healthy adult volunteers (normal ECG and no history of cardiovascular diseases, same exclusion criteria) were enrolled as controls. All controls, who had the same exclusion criteria and no history of any cardiovascular disease, came from the medical examination centre of the affiliated hospital of Qingdao University (n = 37) and People's Hospital of Linyi (n = 66). Full blood count and routine biochemistry indices were determined invenous blood. Creatine kinase MB (CK-MB) and cardiac specific troponinT (TnT) were measured in serum immediately after arrival at the hospital as markers of myocardial damage.

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Table 1. Baseline characteristics of the patients.

Characteristic	Control	AMI	*p*-value
Age (years)	57 ± 12	58 ± 12	0.562
Sex (male/female)	83/20	78/25	0.538
Smoker (No/Yes)	62/41	72/31	0.148
Hypertension (No./total No.)	44/59	64/39	0.182
Hyperlipemia (No./total No.)	23/80	26/77	0.743
Diabetes (Y/N)	14/89	15/88	0.503
Body mass index (kg/m^2)	26.2 (24–28)	26.7 (24–29)	0.193
SBP (mmHg)	124 ± 20	125 ± 21	0.462
DBP (mmHg)	73 ± 13	74 ± 14	0.527
Heart rate (beats/ min)	68 ± 12	70 ± 12	0.68
TC (mmol/L)	3.84 ± 1.25	3.88 ± 1.32	0.74
WBC ($\times 10^3$/ul)	6.36± 1.23	11.15± 2.17	<0.001
Hb (g/l)	128± 18	141± 15	0.184
TG (mmol/L)	1.39 ± 0.86	1.50 ± 0.92	0.165
HDL (mmol/L)	1.32 ± 0.22	1.07 ± 0.25	0.068
LDL (mmol/L)	2.62 ± 1.04	2.53 ± 1.02	0.483
BUN (mmol/L)	6.14 ± 2.36	6.48 ± 2.14	0.142
CK-MB (U/L)	18.27 ± 7.43	56.1 ± 42.3	<0.01
Cardiac troponin T (ng/l)	0.12±.08	1.24± 1.13	<0.001
TGF-β1 (ng/l)	0.172 ±.024	1.26 ± 1.05	<0.001

Serum TGF-β_1 levels were measured using an ELISA kit (TGF-β_1 Emax Immunoassay System kit Promega, Madison, WI, USA), according to the manufacturer's instructions. Serum TGF-β_1 concentrations were measured in four independent experiments. The intra-assay precision, expressed as coefficients of variation, was 4.2–6.3%; the inter-assay precision was 5.8–8.4% and the sensitivity was < 9.2 ng/l. All assays were performed in duplicate. Statistical treatment was performed using SPSS 17.0 software. Continuous variables were compared with the use of the Mann–Whitney test and t-tests, as appropriate, and categorical variables with the use of the Pearson chi-square test. Receiver operating characteristic (ROC) curves were constructed to assess the sensitivity and specificity of TGF-β1 measurements obtained to compare its ability to diagnose AMI. All hypothesis testing was two-tailed, and P values of less than 0.05 were considered to indicate statistical significance without adjustments for multiple testing.

Table 1 shows routine laboratory indices with raised white blood cell count and cardiac markers in AMI patients compared to controls. Table 2 shows AMI patients classified by STEMI or NSTEMI. TGF-β1, but not TnT, was higher in those with STEMI. Figure 1 shows analysis according to the timing of the blood sample from outset of symptoms. TGF-β1 levels peaked at 3–6 h, TnT at 12–24 h. To further evaluate the predictive power of serum TGF-β1 for AMI, ROC curve and areas under ROC curve (AUC) analyses were performed (Figure 2). The AUC of TGF-β1 in AMI patients was 0.76 [95% confidence interval (CI): 0.68-0.82, $p < 0.001$]. ROC curve analysis of TGF-β1 exhibited strong differentiation power between AMI patients and healthy controls during the early phase of AMI. In addition, the AUC of TGF-β1 in STEMI patients was 0.81 [95% CI: 0.79-0.91, $p < 0.001$] and 0.68 [95% CI: 0.63-0.76, $p < 0.001$] in NSTEMI patients.

AMI is the acute necrosis of myocardial tissue due to persistent and severe ischemia, and one of the most frequently occurring cardiovascular diseases and one of the leading causes of morbidity and mortality in both developed and developing countries.[16] AMI is separated into two categories based on the changes seen in electrocardiography: STEMI and NSTEMI. In STEMI, the infarct-related artery is usually totally occluded by fibrin-rich clots, and immediate reperfusion therapy is the initial approach. In contrast, the initial conservative strategy or the initial invasive strategy can be taken in patients with NSTEMI whose infarct-related artery is partially occluded by platelet-rich clots. Prompt diagnosis is critical to controlling the development of AMI and initiating appropriate therapy to reduce the mortality rate and improve prognosis. Traditionally, CK, lactate dehydrogenase and aspartate aminotransferase were used to evaluate for myocardial damage among patients presenting with chest pain. While sensitive for detecting cellular death, these biomarkers lacked specificity for myocardial injury.[17] Later, CK-MB and serum myoglobin levels were utilised to improve specificity for cardiac damage and reduce the time to diagnosis.[7] However, CK-MB is not significantly increased in the early stage of AMI, furthermore, CK-MB may be normal in NSTEMI patients,[8] which limits the application of CK-MB. Cardiac Troponin later replaced CK-MB as the biomarker of choice for diagnosing AMI.[18] Troponin is a protein released from myocytes when irreversible myocardial damage occurs. It is highly specific to cardiac tissue and accurately diagnoses MI. However, following reperfusion therapy, the actual troponin level can be misleading due to the washout phenomenon. Troponin levels peak at 12 h, and stay elevated for 10 days or more.[18] While the use of troponin for diagnosing AMI and risk stratification to aid decision-making has revolutionised the management of patients presenting with chest pain, the 12-h wait for the levels to peak remains a weakness of this biomarker, although high sensitive troponin assays have been introduced to rectify this weakness. A positive Troponin 1 is associated with increased risk of an adverse outcome at 30 days.[19]

Table 2. Baseline characteristics of patients with STEMI and NSTEMI.

Characteristic	STEMI ($n = 65$)	NSTEMI ($n = 38$)	p-value
Age (years)	57 ± 13	57 ± 12	0.632
Sex (male/female)	52/13	31/7	0.472
Smoker (No/Yes)	12/51	9/19	0.127
Hypertension (No./total No.)	54/11	30/8	0.34
Hyperlipemia (No./total No.)	17/48	9/29	0.67
Diabetes (Y/N)	11/54	4/34	0.63
Body mass index (kg/m^2)	26.8 (24–29)	27.0 (24–30)	0.485
SBP (mmHg)	125 ± 22	125 ± 19	0.57
DBP (mmHg)	75 ± 12	74 ± 11	0.74
Heart rate (beats/ min)	71 ± 12	71 ± 11	0.72
WBC ($\times 10^3$/ul)	11.4± 1.16	11.4± 1.15	0.49
Hb (g/l)	143± 16	140± 1.6	0.56
TC (mmol/L)	3.84 ± 1.12	3.89 ± 1.13	0.74
TG (mmol/L)	1.52 ± 1.21	1.48 ± 1.14	0.243
HDL (mmol/L)	1.04 ± 0.32	1.07 ± 0.43	0.080
LDL (mmol/L)	2.58 ± 1.04	2.48 ± 0.98	0.147
BUN (mmol/L)	6.49 ± 3.15	6.56 ± 2.84	0.436
Cardiac troponin T (ng/l)	1.45 ± 0.63	1.29 ±0.43	0.076
TGF-β1 (ng/l)	1.37 ± 0.12	1.13 ±0.12	0.019

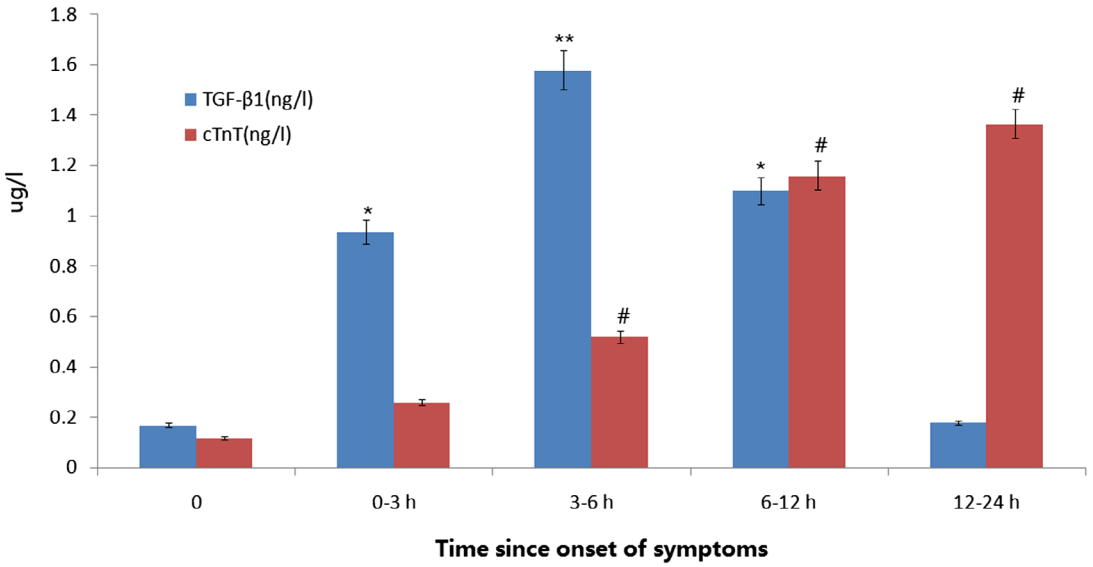

Figure 1. TGF-β1 and troponin T levels at presentation in relation to the time since the onset of symptoms among patients found to have an acute myocardial infarction vs. control,$^*p < 0.01$;$^{**}p = 0.001$;$^{\#}p = 0.001$.

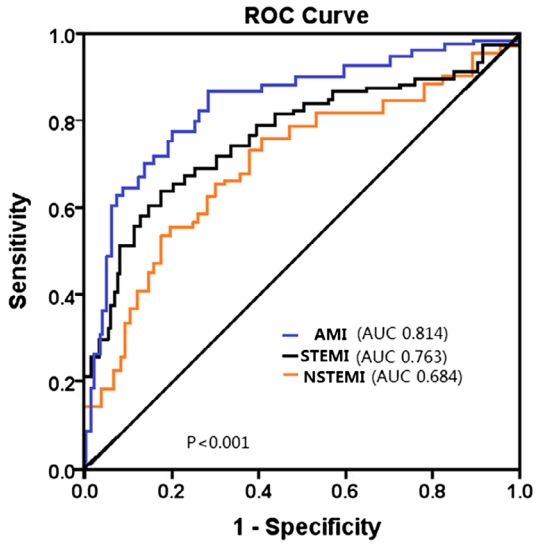

Figure 2. Evaluation of serum TGF-β1 for the diagnosis of STEMI and NSTEMI by ROC curve analysis.

Previous studies have demonstrated that TGF-β is markedly up-regulated in experimental models of MI. Bioactive TGF-β1 is released in the cardiac extracellular fluids 3–5 h following reperfused infarction,[15] we suggested that it could be used as a marker for diagnosis of AMI.

Our analyses showed that the TGF-β1might be a suitable diagnostic marker of AMI, with a distinct difference in levels between the STEMI and NSTEMI groups and the controls. The results of ROC analyses indicated that TGF-β is specific and sensitive for the early diagnosis of STEMI and NSTEMI. In addition, TGF-β1 is more sensitive in STEMI than NSTEMI, suggesting that serum TGF-β1 may potentially have great prognostic value in patients with STEMI. In addition, we found that TGF-β1 levels was significantly increased at 0–3 h, and peak at 3–6 h, and returned to normal at 24 h. However, TnT levels increased at 6hs, further increased at 12 h, and were still elevated

after 12–24 h. We therefore suggested that TGF-β1 could be an early marker for the diagnosis for AMI. However, whether or not TGF-β1 is superior to high sensitive troponin assays needs further investigation.

Although TGF-β1 is activated in AMI, the function and mechanisms of TGF-β is poorly understood. Experimental studies suggest that TGF-β signalling may be crucial for repression of inflammatory gene synthesis in healing infarcts mediating resolution of the inflammatory infiltrate.[18] In addition, TGF-β may play an important role in modulating fibroblast phenotype and gene expression, promoting extracellular matrix deposition in the infarct by up-regulating collagen and fibronectin synthesis and by decreasing matrix degradation through induction of protease inhibitors.[20] Therefore, understanding the signalling mechanisms responsible for TGF-β-mediated effects is important in order to design novel therapeutic strategies targeting the TGF-β signalling cascade in the infarcted and remodelling heart.

This study represents an advance in biomedical science because it shows that the serum levels of TGF-β1 could serve as a novel and useful biomarker in AMI in general and STEMI in specific.

Disclosure statement

No potential conflict of interest was reported by the authors.

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