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Baseline serum levels of cardiac biomarkers in patients with stable coronary artery disease

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Abstract

Stable coronary artery disease (CAD) can cause repetitive reversible myocardial ischaemia, and it seems to be possible that reversibly injured myocardium releases small amounts of soluble cytoplasmic proteins. Hence, the aim was to evaluate the effect of stable CAD on baseline serum levels of cardiac biomarkers. We studied 68 consecutive outpatients referred for gated myocardial perfusion imaging. Before a treadmill exercise test, blood samples for measurement of creatine kinase (CK), CK-myocardial band (CK-MB) mass, myoglobin, aspartate aminotransferase (AST) and lactate dehydrogenase (LDH) were collected. Normal perfusion patterns were detected in 29 (43%) patients (group 1) and perfusion defects were detected in 39 (57%) patients (group 2). Baseline serum levels of biomarkers except CK were significantly higher in group 2 ($p = 0.001$). Stable CAD increases baseline levels of CK-MB mass, myoglobin, AST and LDH in the serum and this increase is related to the extent and severity of the perfusion defect and to some extent the ejection fraction of the left ventricle.

Keywords: *Tc-99m MIBI, myoglobin, creatine kinase (CK), CK-MB mass, aspartate aminotransferase, lactate dehydrogenase*

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Introduction

Creatine kinase (CK), CK-myocardial band (CK-MB) isoenzyme, lactate dehydrogenase (LDH) isoenzymes, aspartate aminotransferase (AST), myoglobin, and more recently cardiac troponins T and I, are biomarkers of myocardial injury. They are of fundamental importance especially in the emergency room for evaluation of acute coronary diseases, particularly when electrocardiographic (ECG) findings do not allow a diagnosis (Panteghini et al. 1999, Wu 1999, Collinson 2000, Apple et al. 2001). They are also valuable for qualitatively estimating the size of the infarction, to detect the presence of complications such as re-infarction and to assess the success of acute revascularization (Razek et al. 2000, Cavallini et al. 2005, Turer et al. 2005).

The important question of whether or not the release of intramyocardial proteins is always an indicator of myocardial necrosis is still a matter of debate. Many

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investigators believe that biomarkers of myocardial injury are only released from cells after irreversible injury (Ahmed et al. 1976, Ishikawa et al. 1997, Roberts & Fromm 1998), and some authors think that the physiological impermeability is a metabolically controlled property of the cell membrane and extracellular rise of myocardial proteins may also follow, in smaller amounts, reversibly, disturbances of cell metabolism. Therefore, prevention of enzyme or protein leak is directly or indirectly an energy-consuming process and myocardial plasma membranes become permeable for intracellular proteins if cells come into an energy-deficient state (Mair 1999).

Stable coronary artery disease (CAD) can cause repetitive reversible myocardial ischaemia in the daily life of the patients. The sequence of events occurring during the genesis of regional myocardial ischaemia in patients with CAD comprise a continuum from minor relative differences in flow without metabolic or regional functional consequences, to the full expression of myocardial ischaemia with systolic and diastolic dysfunction, ECG signs and angina. Although, it seems possible that reversibly injured myocardium releases small amounts of soluble cytoplasmic proteins (Michael et al. 1985, Remppis et al. 1995), the effect of stable CAD on the serum levels of cardiac biomarkers related to myocardial injury is not clear.

The aim of this study was to evaluate the effect of stable CAD, detected by gated myocardial perfusion imaging (gMPI), on baseline levels of cardiac biomarkers related to myocardial injury, which were measured in the serum.

Materials and methods

Patients

We studied 68 consecutive outpatients (all men, age range 32–80 years, mean ± SD 55.1 ± 12.4) referred for gMPI because of risk factors for CAD, chest pain, abnormal exercise ECG findings or known stable CAD, including patients with prior myocardial infarctions, prior revascularization, such as percutaneous transluminal coronary angioplasty or coronary artery bypass grafting, angiographically proven coronary atherosclerosis, or reliable non-invasive evidence of myocardial ischaemia. They had not had a recent heart attack or cardiovascular event during the previous 3 months and they did not have complicated cardiovascular disease. As serum levels of these biochemical markers are related to muscle mass, women were excluded from the study (Sorichter et al. 2001, Wiviott et al. 2004). Patients who had known renal disease, liver disease and muscle injury or disease were also excluded from the study. All patients gave informed consent, according to the approved clinical protocol of the local ethical committee.

Biochemistry

Before the treadmill exercise test, blood samples for the measurement of CK, CK-MB mass, myoglobin, AST and LDH were collected by venipuncture from the antecubital vein. The blood samples were centrifuged to obtain serum before being transported to the laboratory for enzymatic analysis by conventional methods. AST, LDH and CK were measured on an Olympus AU 2700 autoanalyzer using a commercially available kit (Olympus Diagnostica GmbH, Hamburg, Germany). CK-MB mass and myoglobin assay were carried out on an ADVIA Centaur analyzer (Bayer Corporation,

Tarrytown, NY, USA) using chemiluminometric immunoassay technology where constant amounts of two antibodies were used.

Gated myocardial perfusion imaging

gMPI was performed with a 2-day stress–rest protocol. β-blockers and calcium channel antagonists were discontinued 48 h before testing, and nitrate compounds were discontinued at least 6 h before testing. A symptom-limited treadmill exercise test was performed by all patients, using the standard Bruce's protocol. Exercise endpoints included achievement of $\geq$85% of maximal predicted heart rate and predetermined symptoms and signs limiting the patient such as angina, fatigue and vertigo. All the patients were injected with 740–925 MBq (20–25 mCi) methoxyisobutylisonitrile labelled with technetium or Tc-99m (Tc-99m MIBI) (Medi-Radiopharma, Budapest, Hungary) at peak stress. Gated SPECT imaging was initiated 30–60 min later, using a 15% window centred over the 140-keV photopeak. Acquisitions were performed using a 2-detector 90° camera (Optima; GE, Milwaukee, WI, USA) with 64 projections over 180° (right anterior oblique 45° to left posterior oblique 45°). The stress SPECT acquisition was gated to the R wave and the cardiac cycle was divided into eight frames. The following day, 740–925 MBq (20–25 mCi) Tc-99m MIBI was injected intravenously at rest, and SPECT imaging was initiated 60 min later using the same protocol with stress.

Image analysis

Scintigraphic images were analysed by three experienced observers in a blinded manner and results were reported as normal or defective. In addition, semiquantitative scintigraphic parameters such as stress total severity score (STSS) showing the extent and severity of a perfusion defect and cardiac mass were derived by using commercially available software (Emory ECToolbox). The quantitative regional left ventricular functional parameters such as left ventricular end-systolic volume (ESV) and left ventricular ejection fraction (LVEF) were assessed by Cedars–Sinai's Quantitative automated gated SPECT (QGS) software. These parameters have been extensively validated (Germano et al. 1995, Kang et al. 1997, Yoshioka et al. 1999, Taillefer et al. 2003). According to the results of the gMPI, the study population was divided into two subgroups. Patients in group 1 had normal perfusion patterns and patients in group 2 had perfusion defects on their scans.

Statistical analysis

The distribution of the data was tested using the Kolmogorov–Smirnov test. Since the distribution of the data was not normal, non-parametric statistical methods were preferred for statistical evaluation of the data. The differences between the groups were tested using the Mann–Whitney U test. The relationships between variables were detected by the Spearman's correlation test. All statistical calculations were performed with SPSS 10.0 for Windows statistical software package (Chicago, IL, USA). All values in the text are given as median values (min–max). A p value <0.05 was considered statistically significant in all analyses.

Table I. Patient characteristics.

	Group 1 ($n = 29$)	Group 2 ($n = 39$)
Age (years) (mean)	49	62
Hypertension	16	8
Diabetes mellitus	3	5
Smoking	14	14
Angina	10	17
Q-MI	–	13
PTCA ± stent	1	12
Bypass surgery	3	10

MI, myocardial infarct; PCTA, percutaneous transluminal coronary angioplasty.

Results

The clinical characteristics of the 68 patients in this study are shown in Table I. There were 34 patients (50%) who had cardiac catheterization performed; 27 of 34 patients (79%) were found to have CAD, as defined by a >70% stenosis of one or more vessels. Of these, 13 (48%) patients had a percutaneous intervention (angioplasty or stent placement). There were also 13 patients (48%) who had undergone coronary artery bypass surgery. According to the results of the gMPI, the study population was divided into two subgroups. Patients in group 1 had normal perfusion patterns and patients in group 2 had perfusion defects on their scans. Normal perfusion patterns were detected in 29 (43%) patients (group 1) and perfusion defects were detected in 39 (57%) patients (group 2). Twenty-five (64%) patients in group 2 had only reversible perfusion defects indicating ischaemia and 14 (36%) patients had fixed perfusion defects indicating myocardial infarct on their scans.

Descriptive statistics of the biochemical and scintigraphic parameters are shown in Table II. According to the results of the scintigraphic parameters, extent and severity of the perfusion defects were significantly higher in group 2, and functional indices of the left ventricle in group 2 were significantly lower than those of group 1.

Table II. Descriptive statistics of biochemical and scintigraphic parameters. Results are median values (min–max).

	Group 1 ($n = 29$)	Group 2 ($n = 39$)	p-Value*
Biochemical parameters			
Myoglobin (ng ml^{-1})	32.0 (17.8–44.0)	40.9 (13.93–150.95)	0.001
CK-MB mass (ng ml^{-1})	0.5 (0.00–1.7)	1.2 (0.3–4.9)	0.001
CK (U l^{-1})	60.0 (22.0–195.0)	79.0 (25.0–267.0)	0.103
AST (U l^{-1})	18.0 (11.0–33.0)	21.0 (8.0–265.0)	0.010
LDH (U l^{-1})	121.0 (98.0–175.0)	141.0 (68.0–790.0)	0.036
Scintigraphic parameters			
Mass (g)	135.0 (98.0–209.0)	152.0 (94.0–290.0)	0.040
STSS	81.0 (0–707.0)	566.0 (59.0–1431.0)	0.001
EF (%)	62.0 (24.0–75.0)	54.0 (21.0–75.0)	0.011
ESV (ml)	30.5 (12.0–102.0)	44.0 (13.00–224.0)	0.009

CK, creatine kinase; CK-MB, CK-myocardial band; AST, aspartate aminotransferase; LDH, lactate dehydrogenase; STSS, stress total severity score; EF, ejection fraction;ESV, end-systolic volume. *Mann–Whitney U test.

Table III. Results of the correlation analysis between biochemical and scintigraphic parameters.

	Mass (g)	STSS	EF (%)	ESV (ml)
Myoglobin (ng ml^{-1})	0.163	0.327**	−0.253*	0.196
CK-MB mass (ng ml^{-1})	0.195	0.477**	−0.318**	0.287*
CK (U l^{-1})	0.188	0.143	−0.237	0.242*
AST (U l^{-1})	0.086	0.223	−0.051	0.056
LDH (U l^{-1})	−0.130	0.169	0.083	−0.126

CK, creatine kinase; CK-MB, CK-myocardial band; AST, aspartate aminotransferase; LDH, lactate dehydrogenase. Spearman's correlation test: *$p \leq 0.0$; **$p \leq 0.01$.

According to the results of the biochemical measurements, serum levels of biochemical markers, except for CK, were significantly higher in group 2, and the statistical differences in myoglobin and CK-MB mass levels in group 2 were more prominent ($p = 0.001$).

CK-MB mass and myoglobin levels showed statistically positive significant correlation with STSS and negative significant correlation with the LVEF (Table III). Although the patients with perfusion defects on their scans seemed to have heavier hearts, we could not find any significant correlation between cardiac mass and biochemical parameters. There was also no significant correlation between ESV and biochemical parameters.

Discussion

The early release of cardiac markers is influenced by a variety of factors, the most important influence being their intracellular compartmentation. In contrast to the release of cytosolic proteins, the release of structurally bound proteins requires both a leaky plasma membrane and a dissociation or degradation of the subcellular structure, which is a slower process (Mair 1999). For this reason, we have chosen biomarkers located in the cytosolic compartment of the myocyte. It is obvious that AST, CK, LDH and myoglobin are not specific enough for myocardial injury, but other potential diseases that may cause an increase in serum levels of these biomarkers were eliminated if possible. Hence, the myocardium was the possible origin for the release of biomarkers in this study.

The outstanding finding of the study is that the presence of stable CAD causes an increase in the baseline serum levels of biomarkers related to myocardial injury, except for CK. Although the underlying mechanism is unclear, a theorem concerning a relationship between energy metabolism and the physiological impermeability of the cell membrane may be helpful in understanding the process (Mair 1999). According to this theorem, there is a relationship between energy metabolism and enzyme release in cardiomyocytes (Piper et al. 1984). In other words, prevention of leakiness is, directly or indirectly, an energy-consuming process and myocardial plasma membranes can become permeable for intracellular proteins if cells come into an energy-deficient state. It has already been showed that, at moderate ischaemic stress, myocardial tissue can release small amounts of macromolecules from the cytosolic compartment by mechanical mechanisms other than persistent membrane perforation (Piper et al. 1984, Heyndrickx et al. 1985, Wienen & Kammermeier 1988). If we suppose that stable CAD can cause repetitive ischaemic attacks in the daily life of

patients, it will not be a surprise that it may cause continuing cell membrane dysfunction and biomarker release from myocytes. Interestingly, these biomarkers can also be detected in the sera of patients without detectable CAD. Actually, it has been reported that, under normal non-pathological conditions there appears to be a basal level of enzyme leakage from the myocardial cell to both the blood and lymph, and this basal leakage would not appear to be associated with irreversible damage (Spieckermann et al. 1979). This physiological enzyme loss may be mediated by physicochemical factors (Sakai & Spieckermann 1975).

Another interesting point is that CK-MB mass levels in the study population were under the manufacturer's upper limit of normal but even these normal levels could provide important clues about the presence of CAD. CK-MB is an energy-producing protein in the cytosolic compartment and it has been shown to be upregulated following cellular damage (Voss et al. 1995). It has also been reported that abnormal CK-MB enzyme activity due to the presence of cardiac injury must exceed the residual baseline concentration, which for CK-MB averages 1.50 $\mu g\, l^{-1}$ (Wu & Ford 1999) and our results are consistent with this report.

It has been shown that, gMPI is highly sensitive and specific for the detection and localization of CAD (Maddahi et al. 1989, Van Train et al. 1993, Berman et al. 1995, Benoit et al. 1996, Sharir et al. 2000). It gives peak stress perfusion and the addition of gating to routine myocardial perfusion SPECT provides accurate and reproducible information on left ventricular function and volume at the time of acquisition. In this study, serum CK-MB and myoglobin concentrations showed positive mild correlation with STSS and negative mild correlation with the LVEF. These mild correlations may imply that baseline serum concentrations of both biomarkers can have diagnostic value in patients with stable CAD.

As a limitation of the study, we could not study other cardiac biomarkers including cardiac troponin T and cardiac troponin I. Actually troponin T and cardiac troponin I are more sensitive and specific biomarkers for myocardial injury and, as part of future studies we believe that both can give more encouraging results.

Conclusion

We have shown that stable CAD increases baseline levels of CK-MB mass, myoglobin, AST and LDH in the sera of patients and this increase is related to the extent and severity of a perfusion defect and to some extent the ejection fraction of the left ventricle.

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