Cardiovascular Topics

Association between apelin-12 and creatine kinase-MB, depending on success of reperfusion in STEMI patients

Xhevdet Krasniqi, Josip Vincelj, Masar Gashi, Blerim Berisha, Dardan Kocinaj

Background: Acute myocardial infarction is characterised by an imbalance in the supply and demand of oxygen in the heart. It requires urgent reperfusion, and poor outcomes are attributed to myocardial ischaemia–reperfusion injury. We aimed to evaluate the association between apelin-12 levels and creatine kinase-MB activity in predicting the effectiveness of reperfusion therapy in ST-segment elevation myocardial infarction (STEMI) patients.

Methods: In this study we included 72 patients with the following criteria: chest pain suggestive of myocardial ischaemia for at least 30 minutes, an electrocardiogram with ST-segment elevation (measured at the J-point) ≥ 2 mm in leads V2–V3 and/or ≥ 1 mm in the other leads, rise of specific biomarkers such as cardiac troponin and the MB fraction of creatine kinase (CK-MB), and those who underwent reperfusion therapy. Blood samples for the measurement of apelin-12 and creatine kinase-MB were collected 12 hours after the reperfusion therapy.

Results: In patients with thrombolysis in myocardial infarction (TIMI) flow grade ≤ 2, the median of the apelin-12 level was 1.80 ng/ml (0.46–9.20), and with TIMI flow 3, it was 5.76 ng/ml (1.14–15.2). Variability was observed in the apelin values (Mann–Whitney test) based on TIMI flow grade ($p < 0.001$), while no variability was observed for creatine kinase-MB ($p < 0.18$). The degree of association between apelin-12 and creatine kinase-MB levels was analysed with Pearson's correlation, enabling us to determine patients with successful reperfusion (determined as TIMI flow 3) ($p < 0.004$), and those with unsuccessful reperfusion (with TIMI flow ≤ 2) ($p = 0.86$).

Conclusion: In STEMI patients undergoing reperfusion therapy, apelin-12 level was associated with creatine kinase-MB activity according to the success of the reperfusion.

Keywords: apelin, myocardial infarction, reperfusion

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Myocardial ischaemia results from an imbalance between the oxygen supply and demand in the heart preceding the development of myocardial infarction.[1]

It is known that in cardiomyocytes, creatine kinase (CK) appears as three isoenzymes: creatine kinase M-type, creatine kinase B-type and mitochondrial sarcomere creatine kinase.[2] On the other hand, adenosine triphosphate (ATP) as a primary carrier of energy in cardiac cells is produced from a variety of substrates (fatty acids and glucose) generally in the mitochondria through oxidative phosphorylation. The creatine kinase M-type (CKM) coupled to ATPase regenerates ATP according to the actual energy requirements.[3] The high-energy phosphoryl bonds of ATP are available at the sites of utilisation and require a phosphagen system that consists of a reversible interaction of creatine and ATP under the control of cytosolic CKM:
Creatine + ATP ↔ phosphocreatine + ADP + H^+.[4,5]

In myocardial infarction, the levels of ATP and phosphocreatine are rapidly depleted due to lack of oxygen, resulting in tissue damage and elevating the levels of the MB fraction of creatine kinase (CK-MB).[6]

During hypoxia, apelin gene expression and secretion are increased through the activation of hypoxia-inducible factor.[7-9] The apelin gene (located on the human X chromosome) encodes a 77-amino acid preproprotein (prepro-apelin) that is cleaved by endopeptidases into a biologically active peptide such as apelin-12. This is followed by positive effects on the cardiovascular system.[7,10-15]

After the binding of apelin with angiotensin receptor like-1 (APJ), phospholipase C is activated, generating inositol trisphosphate and diacylglycerol from phosphatidyl inositol bisphosphate. Furthermore, diacylglycerol activates protein kinase C through which apelin activates its sites on troponin I, thereby regulating ATPase activity in the myocardium influenced by CK (Fig. 1).[16-19]

Acute myocardial infarction due to occlusion of the coronary artery is characterised by an imbalance between the heart's

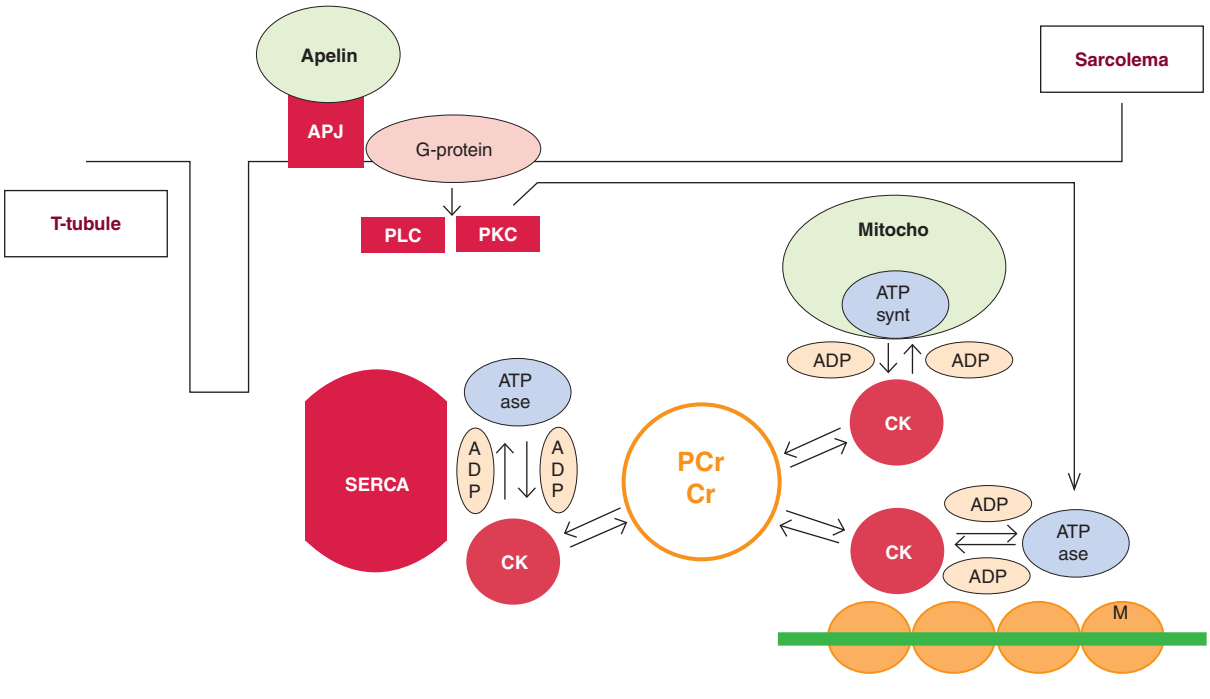

Fig. 1. Relationship between apelin-12 levels and CK-MB activity.

oxygen demand and supply, which requires urgent reperfusion to rescue the ischaemic myocardium from expected death. Poor outcomes are attributed to myocardial ischaemia–reperfusion injury, which is largely mediated by the cytotoxic effects of free radicals generated during ischaemia, complement activation, injury of endothelial cells and inflammation.[20-25]

Apelin activates components of the reperfusion injury salvage kinase pathway, such as phosphatidylinositol-3-OH kinase, Akt/protein kinase B and p44/42 mitogen-activated protein kinase, protecting the myocardium from ischaemia–reperfusion injury.[26-28] The aim of this study was to evaluate the association of apelin-12 and CK-MB levels to predict the effectiveness of reperfusion therapy in ST-elevation myocardial infarction (STEMI) patients,

Methods

This prospective, observational study included 72 patients with STEMI. Inclusion criteria were: chest pain suggestive of myocardial ischaemia for at least 30 minutes, an electrocardiogram (ECG) with ST-segment elevation, measured at the J-point, ≥ 2 mm in leads V2–V3 and/or ≥ 1 mm in the other leads, increased specific biomarkers [cardiac troponin (cTn) and CK-MB], and patients who underwent reperfusion therapy.

All patients received aspirin 250 to 500 mg, clopidogrel 300 to 600 mg and heparin 4 000 to 5 000 IU before primary percutaneous coronary intervention (PCI). The use of glycoprotein IIb/IIIa inhibitors or vasodilators in the catheterisation laboratory was left to the decision of the treating cardiologist.

Based on coronarography, coronary perfusion was determined according to the thrombolysis in myocardial infarction (TIMI) flow grade, with a grade 3 blood flow indicating normal flow and a grade 0 indicating no flow within the vessel. The PCI procedure was performed using standard methods and success was determined by the TIMI grade flow.

Unsuccessful myocardial reperfusion was defined by a 12-lead ECG one hour after successful recanalisation of the infarcted artery as > 30% persistent ST-segment elevation and by coronarography as TIMI grade ≤ 2. Transthoracic echocardiography was performed for assessment of left ventricular ejection fraction.

Baseline and procedural characteristics of all STEMI patients were documented upon admission and included age, gender, coronary risk factors (diabetes mellitus, dyslipidaemia, hypertension and smoking), family history of cardiovascular disease, previous medications and time from onset to admission. Routine laboratory parameters were measured at admission.

Blood samples for measurement of apelin-12 and CK-MB levels were collected 12 hours after reperfusion therapy. The serum was obtained by allowing the blood to solidify in a serum tube for 30 minutes, centrifuged for 15 minutes at 1 600 rpm, and stored at –80°C to prevent degradation. According to the manufacturer's instructions, apelin-12 was measured with enzyme-linked immunosorbent assay (ELISA), and CK-MB was measured with the immunological inhibition method.

The institutional ethics committee of Dubrava University Hospital, Zagreb and the University Clinical Centre of Kosova, Pristina approved this study. Informed consent was obtained from all individuals participating in the study.

Statistical analysis

The main objective of the study was to investigate the association of apelin-12 level with CK-MB activity according to the success of reperfusion in patients with STEMI. Mean ± standard deviation (SD) and median (range) are used to present

Table 1. Baseline characteristics of patients

Characteristics	*Results*
Age (years)	61.02 ± 11.35
Gender (male)	44 (61.11)
Coronary risk factors	
Hypertension	44 (61.11)
Diabetes mellitus	16 (22.22)
Dyslipidaemia	32 (44.44)
Smoking	32 (44.44)
Family history of cardiovascular disease	20 (27.77)
Killip class > 1	13 (18.05)
Ejection fraction	50.87 ± 10.9
Laboratory values	
Haemolobin (g/dl)	13.68 ± 1.51
Creatinine (µmol/l)	94.0 (67.21–125.34)
Apelin 12 (ng/ml)	4.65 (0.46–15.25)
Creatine kinase-MB (IU/l)	163.5 (15.0–929.0)
Creatine kinase (IU/l)	1414.0 (42.0–7550.0)
Troponin I (ng/ml)	42.89 ± 59.37
Coronary angiographic findings	
Culprit lesion	
RCA	30 (41.66)
LAD	31 (43.05)
LCx	11 (15.27)

Data are expressed as a mean ± standard deviation, median (range) and n (%). RCA, right coronary artery; LAD, left coronary artery; LCx, left circumflex artery.

continuous variables, while categorical variables are expressed as counts and percentages. Comparison of laboratory variables between patients with different TIMI flows (TIMI flow ≤ 2 or 3) was performed using the Mann–Whitney test. The association between variables was assessed with Pearson's correlation. Statistical analyses were performed using SPSS statistics v 21.

Results

Baseline characteristics of the study population are presented in Table 1. The average age was 61 ± 11.35 years and 61.11% of the patients were male. The median value of the apelin-12 level was 4.65 ng/ml (0.46–15.25), while the median value of CK-MB was 163.5 IU/l (15.0–929.0). Other baseline characteristics are presented as mean, median and percentage.

In patients with TIMI flow ≤ 2, the median of the apelin-12 level was 1.80 ng/ml (0.46–9.2), and with TIMI flow 3, it was 5.76 ng/ml (1.14–15.2). Variability was observed in the apelin values (Mann–Whitney test) based on TIMI flow grade ($p < 0.001$), while no variability was observed for CK-MB levels ($p < 0.18$) (Table 2).

Table 2. Comparison of different laboratory variables between patients with TIMI flow ≤ 2 and 3

Variable	*TIMI flow ≤ 2*	*TIMI flow 3*	p-*value*
Apelin-12 (ng/ml)	1.8 (0.46–9.2)	5.76 (1.14–15.2)	< 0.001
Creatine kinase-MB (IU/l)	145.0 (16.0–929.0)	171.5 (15.0–910.0)	0.18
Creatine kinase (IU/l)	1640.0 (57.0–4316.0)	1351.0 (42.0–7550.0)	0.9
Troponin I (ng/ml)	78.27 ± 68.94	25.21 ± 45.17	< 0.001
Haemolobin (g/dl)	13.53 ± 1.95	13.75 ± 1.25	0.32
Creatinine (µmol/l)	91.5 (70.0–124.0)	95.0 (67.21–125.34)	0.51

Data are expressed as a mean ± standard deviation or median (range). TIMI, thrombolysis in myocardial infarction.

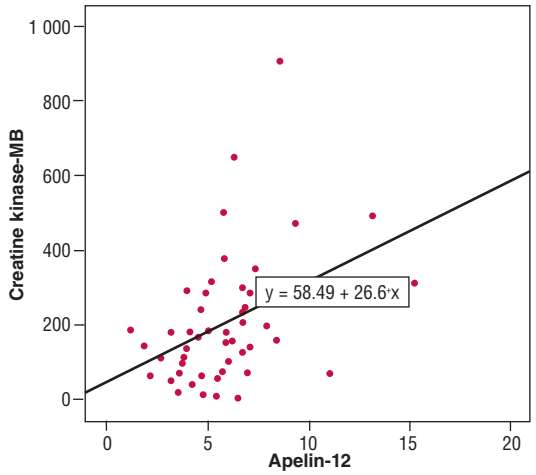

Fig. 2. Correlation between apelin-12 levels and CK-MB activity in patients with TIMI flow 3.

The degree of association between apelin-12 and CK-MB was analysed with Pearson's correlation, predicting patients with successful reperfusion (TIMI flow 3) ($p < 0.004$), and those with unsuccessful reperfusion (TIMI flow ≤ 2) ($p = 0.86$). Fig. 2 shows the correlation between apelin-12 and CK-MB levels in patients with TIMI flow grade 3, while Fig. 3 shows this correlation in patients with TIMI flow grade ≤ 2.

Discussion

Our study was conducted to investigate the association of apelin-12 and CK-MB levels with success of reperfusion (TIMI flow 3 or ≤ 2) in patients with STEMI. During acute myocardial infarction, apelin-12 showed a protective effect in unsuccessful reperfusion, and a high level of apelin-12 resulted in better TIMI flow.

Among the subgroup of patients with successful reperfusion

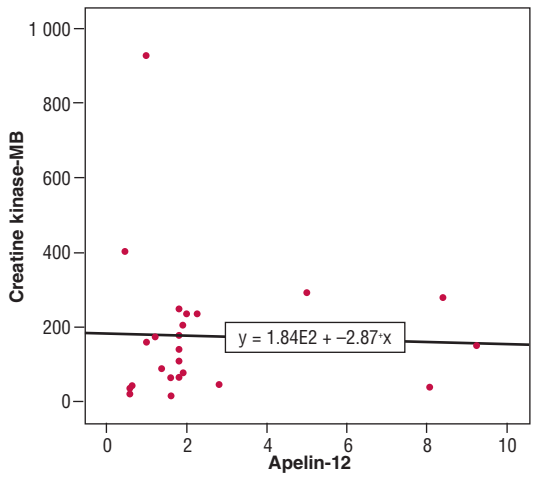

Fig. 3. Correlation between apelin-12 levels and CK-MB activity in patients with TIMI flow ≤ 2.

(TIMI flow 3), a positive correlation was observed between increased levels of apelin-12 and increased activity of CK-MB. Cardiomyocytes are highly oxidative cells where the mitochondria are located at the site of high ATP demand, and together with myofilaments and sarcoplasmic reticulum, they create intracellular energetic units around the sarcomeres.[29]

In the cardiomyocytes, ischaemia and hypoxia represent acute crises of energy provision, followed by increased activity of CK, which catalyses the phosphoryl exchange between ADP and CK. According to a previous study, CK structurally associates with sarcoplasmic reticulum membranes and is capable of linking energy production and utilisation, using phosphocreatine to rephosphorylate all of the ADP produced by the ATPases.[30] One of the enzymes that is activated after the binding of apelin to its receptor is protein kinase C, through which apelin activates its sites on troponin I, thereby regulating Ca^{2+} sensitivity and ATPase activity in the myocardium.[18,19] Therefore, in patients with successful reperfusion (TIMI flow 3), the expression of hypoxia-inducible factor 1-alpha (HIF-1α) increased the level of apelin, which regulated ATPase activity and was associated with increased levels of CK-MB.

In our study, the subgroup with unsuccessful reperfusion exhibited a decreased level of apelin-12 that did not correlate with the level of CK-MB. Reperfusion therapy may induce pathological events in patients with acute myocardial infarction, leading to myocardial tissue injury. Increased generation of highly reactive oxygen species in the heart within minutes of reperfusion has been followed by decreased stability of HIF-1α.[31,32] In patients with unsuccessful reperfusion (TIMI flow ≤ 2), decreased stability of HIF-1α is followed by decreasing levels of apelin-12 and its association with CK-MB. Presumably, the apelin/APJ axis may serve as a potential target for the prevention of myocardial reperfusion injury in patients with STEMI.

One limitation is that this observational study had a relatively limited number of patients.

Conclusion

In STEMI patients undergoing reperfusion therapy, apelin-12 levels were associated with CK-MB activity according to the success of reperfusion. This indicates the role of apelin-12 in the CK system. In the future, apelin-12 could be used as a cardioprotective agent.

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