Biochemical markers of acute myocardial infarction

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ABSTRACT

Introduction: A number of biomarkers have been used to aid in the diagnosis of acute myocardial infarction (AMI), in the risk stratification of patients and for predicting events after acute coronary syndrome (ACS). Some of these biomarkers are widely used in clinical practice while others still have no scientific evidence to support their clinical use. Objectives: To analyze the clinical significance of the various biochemical markers for AMI, alone or in combined use, seeking a description according to the source, behavior in the evolution of the disease, including validation, diagnostic limitations, and clinical significance in different contexts. Methods: We performed a literature review using the PubMed database, including original articles and reviews on the use of biomarkers for AMI. The search was limited to articles written in English, published in the last five years. Results: 90 articles were accessed, 57 of which were excluded. Of the 32 selected studies, 14 were randomized and controlled. After judicious analysis, several biomarkers were described: troponin, creatine kinase-MB fraction, myoglobin, H-FABP, BNP and its inactive N-terminal fragment, ANP, copeptin, growth/differentiation factor-15, interleukin-1 receptor antagonis, cardiotrophin, myeloperoxidase, endothelin-1, among others. Conclusions: troponins T and I (at baseline and 6-9h after event) followed by CK-MB mass are the suggested biomarkers for assessing myocardial injury. Hs-cTnI and hs-TnT are very sensitive in the early stages and CK-MB mass has been useful in reinfarction diagnoses given its short half-life. All other biomarkers studied, either for hypotheses inclusion or exclusion, were important for the diagnosis and/or prognosis of AMI. However, further studies are needed to confirm the present data.

Key words: Biomarkers, Pharmacological; Myocardial Infarction.

RESUMO

Introdução: Número crescente de biomarcadores tem sido usado para auxiliar no diagnóstico do infarto agudo do miocárdio (IAM), estratificação de risco dos pacientes e predição de eventos após a síndrome coronariana aguda (SCA). Alguns desses biomarcadores são amplamente usados na prática clínica, outros ainda não apresentam evidências científicas que sustentem seu uso clínico. Objetivos: analisar o significado clínico dos diversos marcadores bioquímicos descritos para o IAM, isolados ou combinados, buscando-se a descrição de acordo com a origem, comportamento na evolução da doença, incluindo validação, limitação diagnóstica e significado clínico em diferentes contextos. Métodos: realizada pesquisa bibliográfica usando a base de dados PubMed, por intermédio de artigos originais e revisões sobre biomarcadores para o IAM. A busca limitou-se aos artigos escritos em inglês, publicados nos últimos cinco anos. Resultados: foram obtidos 90 artigos, dos quais 57 foram excluídos. Dos 32 estudos selecionados, 14 eram randomizados e controlados. Após análise judiciosa, foram descritos vários biomarcadores: troponina, creatinquinase fração MB, mioglobina, H-FABP, BNP e seu fragmento N-terminal inativo, ANP, copeptin, fator-15 de diferenciação e crescimento,

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METHODS

This review was performed based on the following question: Which are, currently, the biochemical markers that can be used for diagnosis and prognosis of AMI and their clinical meaning when isolated or associated with other biomarkers? A bibliographic research was carried out on the PubMed and CAPES databases to answer this question, enabling to search original articles and reviews about AMI biomarkers. Association between the following terms was used in the search: “biological markers”, “myocardial infarction”, “coronary artery disease”, “myocardial ischemia”, “cardiac troponin”, “sensitive troponin”, “ck-mb mass”, among several others using the names of biomarkers from articles and reviews. This search was only on articles in English, published in the last five years and their availability as complete and free texts.

The selection was based on titles, abstracts, and full-text evaluation as a way to reduce the chance of excluding important studies. Studies with abstract and full-text content with an insufficient description about the concentration variability of drug administration, as biomarkers, and those evaluating surgical techniques with biomarkers were excluded. Out of 90 articles initially selected, 65 were excluded; out of the 25 remained articles, 14 were randomized and controlled studies. The results are presented succinctly and in an integrated manner, after a judicious analysis, with emphasis in the main aspects of each study.

Troponin

Troponin is a muscular protein that together with tropomyosin regulates the interaction between actin and myosin in the muscular contraction process. There are three polypeptides of troponin linked to tropomyosin (TnT), actin (TnI), and calcium (TnC). The TnT and TnI were described as biomarkers for AMI because of their cardiac specificity, different from TnC that has sequence of amino acids shared with its skeletal isofrom, responsible for the absence of diagnostic value for myocardial lesions.
After a cardiomyocyte lesion, a low percentage of free troponin in the cytoplasm is released into the blood circulation followed by a gradual increase due to the dispersion of troponin linked to complexes. In transmural necrosis, this happens after two to four hours of the occurrence of lesions reaching the systemic peak in about 12 hours and remaining high for up to 4-7 days for TnI, and 10-14 days for TnT. The troponin systemic concentration can vary according to the collateral circulation, intermittent coronary obstruction, lesion size, and cardiomyocyte sensitivity.

Its utility in recurrent myocardial injury detection is limited because its levels remain high for a long period. Daubert et al. showed that the intervals for TnI and TnT sensitivity, according to time, were respectively, lower than 45% and between 25% and 65% at hospitalization; 69% and 82% and 59% and 90% from two to six hours after hospitalization; and 100% and close to 100% from six to 12 hours after hospitalization. The specificity of troponin does not vary significantly over time, being between 83% and 98% for TnI and 86% and 98% for TnT. The positive predictive values of TnI and TnT were 25% and 35% when arriving at the hospital, and 89% and 57% after 12 hours of hospitalization. The negative predictive values of TnI and TnT were 85% and 88% when arriving at the hospital, and 98% and 99% after 12 hours of hospitalization. The dosing of TnI and TnT when arriving at the hospital and between six and nine hours after cardiac surgery are recommended to optimize the validity of the troponin test.

The Brazilian Society of Cardiology recommends that at least two markers should be used in the AMI investigative process: an early marker (myoglobin and CK-MB) and a late and definitive marker (including CK-MB and troponins). Troponins are considered the gold-standard because that they can increase with small (micro) heart attacks, even without CK-MB elevation. It is necessary to consider that myocardial troponin can also be released in several clinical situations such as myocarditis, electrical cardioversion, cardiac trauma, myositis, pulmonary embolism, and kidney failure.

The sensitivity of troponin conventional tests is low for an early AMI diagnosis. Because the American and European consensuses recommend a troponin level up to the equivalent value of 99% of the population, the diagnosis importance and clinical meaning of the troponin test with more sensitivity have been discussed. With the use of an ultra-sensitive test for TnI (hs-TnI), with a cutoff point of 0.04 ng/mL in hospitalization, the clinical sensitivity was 90.7% and specificity was 90.2%, regardless of the duration of thoracic pain, while the ultra-sensitive TnT (hs-TnT) test, under the same conditions, had sensitivity between 84% and 95% and specificity between 80% and 94% for the AMI diagnosis.

The prognosis value of hs-TnT and BNP combination was evaluated due to the chronic cardiac failure based on the median concentration of BNP (97 pg/mL) and hs-TnT (0.0124 ng/mL) and according to clinical risk factors. Mortality increased to 32% in patients with both markers above the median concentration, confirming that the elevation of TnT is proportional to the disease severity favoring the prognosis value of BNP. Another study with women submitted to cardiac surgery during post-menopause demonstrated that TnI levels below 7.6 ng/mL constitute risk factors for the development of adverse events within 30 days, such as operative death, low cardiac output state, and AMI with sensitivity averages of 82%, specificity of 77%, positive predictive value of 40%, and negative predictive value of 96% (CI 95%).

**Creatine kinase (CK)**

Total-CK is the enzyme regulating production and use of high-energy phosphate in contractile tissues. It is composed of subunits B (brain) and M (muscle) that combined form the CK-MM (muscle), CK-BB (brain), and CK-MB (myocardial). The specificity of total-CK is low for cardiac muscle lesions, different from CK-MB, which is found predominantly in the cardiac muscle. The dosing of CK-MB determines enzyme activity, whereas CKMB mass test detects its concentration, regardless of activity, including active and inactive enzymes, which makes the CK-MB mass test more sensitive and reliable than the CK-MB activity tests.

The CK-MB activity increases within 4-6 hours after a heart attack with a peak around 18 hours and returns to the normal level after 48 hours. It has a diagnostic sensitivity of 93% to 100% after 12 hours of first symptoms, however, it is little sensitive for diagnosis in the first six hours of evolution. It is recommended then, that its use should be serial at every 3-4 hours and evaluation for at least nine hours to confirm or dispel the AMI diagnosis in suspected patients.

Among other parameters, at 25 °C, the activity of CK-MB in normal conditions is less than 10 U/L and must correspond to less than 6% of total CK. The CK-MB has a reference value below 5 ng/mL. The CK-MB
Biochemical markers of acute myocardial infarction

FABP (38.5%) was greater than CK-MB (34.6%), myoglobin (34.6%) and troponin T (23.1%). In the fourth hour, the specificity of H-FABP (88.5%) was similar to CK-MB (88.5%) and troponin T (88.5%) surpassing myoglobin (73.1%). However, there are some limitations in the measurement of H-FABP determined by: surgeries, kidney disease, and FABPs elevation in the skeletal muscle, which has similar structure to the cardiac muscle and may overestimate the actual values of H-FABP. The diagnosis can be established by combining the values of H-FABP with other biomarkers.

The association between H-FABP and troponin values helps in AMI risk stratification. H-FABP levels above 5.8 mcg/L are considerably associated to increasing risk of death in proportion to increased troponin concentration. The sole dosing of H-FABP does not have high validity for an AMI early diagnosis if the positive predictive value equal to 65.8% and negative predictive value equal to 82.0% are observed. H-FABP association with other biomarkers for AMI early diagnosis could be considered.

Brain natriuretic peptide (BNP) and its inactive N-terminal fragment (NT-proBNP)

Levels of brain natriuretic peptide (BNP) and its inactive N-terminal fragment (NT-proBNP) are used for the diagnosis and prognosis of heart failure, risk stratification after acute coronary syndrome, and prediction of adverse events associated with stable angina in apparently healthy people. They are considered the best mechanical stress markers.

BNP dosing associated with cardiac imaging methods allows evaluation of left systolic ventricular dysfunction, from the cardiac remodeling. Since this process involves synthesis and degradation of collagen, which is a component of the extracellular matrix, BNP was correlated with collagen metabolism markers, suggesting that it modulates the formation of rich collagen scars after AMI. In chronic heart failure, BNP and NT-proBNP are markers with strong prognostic implication. However, they may suffer the influence of age and renal function in addition to naturally varying over time. The sensitivity, specificity, and positive and negative predictive values for the prediction of deaths in patients with heart failure were 50%, 79.2%, 28.6%, and 90.5% for BNP and 53.1%, 79.9%, 30.4%, and 91.1% for NT-proBNP.
Atrial natriuretic peptide (ANP) and its MR-proANP fragment

ANP is primarily synthesized in the atrium and its secretion occurs in response to atrial distension and myocytes stretching. The natriuretic peptides play an important role in the regulation of blood pressure and volume, once they induce diuresis, natriuresis, and vasodilation in congestive heart failure. They act as antifibrotic and anti-hypertrophic agents preventing cardiac remodeling after AMI. ANPs play an important role in the diagnosis and prognosis of heart failure and late left ventricular remodeling after AMI. MR-proANP is a more stable peptide than ANP, it if is at least equal to NT-proBNP as a predictor of death and heart failure, and considered important predictor of adverse events after AMI.

ANP administered in human beings with myocardial ischemia and undergoing coronary reperfusion has been associated with the size reduction of the necrosis area and to little improvement in cardiac function for a period of 30 days. However, it is not able to determine a significant difference regarding mortality. ANP can also interact with immune cells and cause pro-inflammatory effects, inducing reactions associated to its cytotoxic effects in neutrophils.

Copeptin

Copeptin is a stress hormone, considered a new biomarker for the prognosis in acute diseases. The response to stress is characterized by activation of the hypothalamic-pituitary-adrenal axis, involving the corticotropin releasing hormone (CRH), being the main responsible for the secretion of the adrenocorticotropic hormone (ACTH), a cortisol production stimulant. However, these hormones are unstable and quickly purified from the plasma. Because the precursor of vasopressin gives rise to copeptin, which is a more stable compound and easier to determine, it was considered as the biomarker of choice in the clinical practice.

The combination of troponin T (level < 0.01 ng/mL) with copeptin (level < 14 pg/mL) allows high accuracy in the exclusion of AMI, with 99.7% negative predictive value, and able to eliminate the need for monitoring and serial blood testing for their confirmation in most patients. The combination of copeptin with BNP improves the prediction of its outcome. As vasopressin is released in response to changes in plasma osmolarity, and in order to increase the post-charged, the values of plasmatic copeptin released in stoichiometric proportion to vasopressin are increased in patients with chronic heart failure and related to disease severity, being considered better predictors of mortality than BNP. Sensitivity, specificity, and positive and negative predictive values of copeptin for the prediction of death in patients with heart failure were equal to 67.7%, 82.5%, 39.6%, and 93.8%, respectively.

Differentiation factor-15 and growth (GDF-15)

GDF-15 is a member of the TGF-ß superfamily, initially identified as a macrophage inhibitory cytokine. GDF-15 is expressed in the myocardium due to stress and seems to have cardio protective effect since its anti-apoptotic and anti-hypertrophic action in the heart have been demonstrated. The highest concentration of this biomarker is positively associated with age, females, history of hypertension, and diabetes mellitus and it has been used in the stratification of risk of death in cardiovascular diseases in addition to providing prognostic information when used together with other biomarkers. The best level of GDF-15 considered for the prediction of mortality in one year was 1808 ng/L, with sensitivity of 68.5%, specificity of 68.8%, and risk of death equal to 14.1% when the upper limit of the normal range is equal to 1200 ng/L. GDF-15 levels are strongly related to the magnitude of cardiac ischemia or AMI evolution. In AMI without ST segment elevation, NT-proBNP, GDF-15, and age are considered independent predictors of death. In patients after AMI, GDF-15 allows additional information for levels of NT-proBNP higher than 763 pmol/L, making these biomarkers strong identifiers of death and heart failure, favoring the recognition of patients with high risk of death.

Interleukin 1 receptor protein (ST2)

ST2 is a member of Interleukin-1 receptors that exist in transmembrane (ST2L) and soluble forms (sST2). Interleukin-33 acts as a functional ST2L ligand resulting in cardio protective effect to mechanical stress. The plasmatic concentration of sST2 in its up-
Biochemical markers of acute myocardial infarction

Phospholipase A2 associated to lipoprotein (Lp-PLA2)

The phospholipase-A2 secreted by monocytes, macrophages, and T lymphocytes can circulate in the plasma linked to the LDL particle. In the ACS, higher levels of Lp-PLA2 are observed when compared with healthy individuals. Lp-PLA2 is significantly associated with lipid levels, but only weakly or not related to other risk markers in ACS. Risk of cardiovascular or mortality events does not correlate with the Lp-PLA2 plasmatic levels. Hatoum et al. examined the association between Lp-PLA2 activity and coronary artery disease incident in diabetics mellitus type 2. The higher levels of Lp-PLA2 activity were associated with increased risk of coronary disease. This indicates that the biological role of Lp-PLA2 on risk evaluation in patients with ACS has not yet been elucidated. New studies are needed to clarify its prognosis meaning in ACS.

Biomarkers for collagen processing

Amino-terminal pro-peptide of pro-collagen type I (PINP) and type III (PIIINP) are considered markers for collagen synthesis; and the tissue inhibitor of matrix-I metalloproteinases (TIMP-I) inhibits proteinase involved in the breakdown of collagen; and the telopeptide type I collagen (ICTP) is considered a type I collagen degradation marker. The behavior of these markers was evaluated in 476 patients after AMI. PINP increased significantly from the beginning until the first month of AMI, modestly decreasing later; PIIINP showed similar profile but it remained in the per tercile (sST2 > 700 ng/L) is considered a strong independent predictor of all causes of mortality in patients with acute heart failure, in one year period.

The overall assessment of ST2 and NT-proBNP in AMI patients with ST segment elevation showed that their basal levels are significantly higher in those who suffered cardiovascular death or with ICF. In AMI patients, high level of ST2 is a strong predictor of cardiovascular death or heart failure in 30 days and adverse cardiovascular outcomes, regardless of traditional risk factors; it provides prognosis and complementary information to the NT-proBNP levels. ST2 is elevated in acute bronchial asthma and autoimmune diseases, which requires judicious assessment for its use in the clinical practice.

Cardiotrophine (CT-1)

CT-1 is a member of the superfamily of Interleukin-6 (IL-6), produced by cardiomyocytes and cardiac fibroblasts in biomechanical stress situations and under exposure to humoral factors such as angiotensin II. Once secreted, activation of different signaling pathways occurs, leading to growth and cardiomyocytes dysfunction.

The CT-1 myocardial expression is greater in elder people, hypertensive cardiovascular disease, left ventricular hypertension (LVH), and heart failure (HF). In 31% of hypertensive patients without LVH, the CT-1 concentrations are above the upper normal limit, which suggests that CT-1 increases early during the development of hypertension. The detection of LVH by echocardiography shows 70% sensitivity and specificity of 75% for CT-1, being also a potent biomarker for the evaluation of development, progression, and regression of LVH in hypertensive patients.

The high plasmatic levels of CT-1, BNP, and IL-6 are independent predictors of mortality, while those of CT-1 increase with the severity of congestive heart failure (CHF). CT-1 seems to play an important role in the vascular inflammation process and pathogenesis of atherosclerosis stimulating genes and expression of ICAM-I and MCP-1 proteins in human aortic endothelial cells, which induces adhesion and migration of monocytes.

Endothelin-1 (ET-1)

ET-1 is a peptide mainly produced in the endothelium, playing a fundamental role in vascular homeostasis that presents two receptors, the endothelin A (ETA) and endothelin B (ETB). ETA can be found in smooth muscle tissue from blood vessels and its activation promotes vasoconstriction. ETB can be found in endothelial cells and its activation mediates the release of nitric oxide, natriuresis, and diuresis. ET-1 is an important mediator of many cardiovascular complications; its high plasmatic concentrations are associated to the worst prognosis after AMI, and is considered a predictive factor of death or IC. ET-1 is a very unstable compound and difficult to be measured. Thus, the ET-1 C-terminal residue gives more reliable results and with clinical meanings of similar value.

Phospholipase A2 associated to lipoprotein (Lp-PLA2)
range of basal levels until the ninth month after AMI; TIMP-1 remained with elevated levels throughout the study and ICTP was above the reference value at the beginning of the research, gradually reducing in one month, and subsequently stabilizing with values within the reference interval. One can infer that there is probably elevation of extracellular matrix degradation in the early stage, followed by an increase in its synthesis after AMI, contributing to the process of cardiac remodeling.

In the clinical evaluation, PIIINP seems to be a more of an accurate marker of cardiovascular events in chronic conditions than of events in the acute stage; biomarkers of collagen and BNP correlated positively with high levels of ultra-sensitive CRP, and the association between high levels of ICTP and BNP may indicate greater extension of the heart attack area.

CONCLUSION

All biomarkers mentioned above, whether on the inclusion or exclusion of hypotheses, have their differential importance in the clinical contexts of AMI. Currently, the Brazilian Society of Cardiology suggests the use of troponins T and I and CK-MB mass as myocardial lesion markers for the diagnosis of AMI. Similarly, the American and European Consensuses of Cardiology suggest the use of troponin (T or I) as a biomarker for myocardial lesion. If troponin tests are not available, the best alternative is CK-MB mass; total CK is not recommended. In the absence of such tests, any other available tests are chosen, being fundamental the recognition of its diagnostic limitation and inability to assist the real needs of each patient.

Troponin (I or T) is the preferred marker for myocardial lesion, having almost absolute specificity and high sensitivity. HsTnT increases the prognostic value of BNP in the prediction of mortality in patients with heart failure, a condition that can also be predicted by the ANP, ST2, ET-1, and copeptin levels. The combination of TnT with copeptin or normal myoglobin concentrations (in the first hours) show high accuracy in the exclusion of AMI. Early diagnosis of AMI is possible through the high sensitivity test of troponins or H-FABP and myoglobin when associated with other parameters. Further studies in different conditions are needed to extend and confirm the prognostic values of AMI biomarkers in order to establish the criteria for more accurate treatments.

REFERENCES

Biochemical markers of acute myocardial infarction


