

Do we need additional laboratory markers of ACS?

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ABSTRACT

Biochemical markers are playing an important role in the diagnosis of acute coronary syndrome (ACS). Due to their high sensitivity and absolute specificity for myocardial damage, cardiac troponins represent the most widely used biomarkers for the diagnosis of ACS. Recently, a number of novel biomarkers have been evaluated as alternative markers that would bring added value to the measurement of troponins. This manuscript gives an overview of the most commonly investigated markers in this field.

Key words: acute coronary syndrome, high sensitive troponin, copeptin

INTRODUCTION

Suspected acute coronary syndrome (ACS) is one of the most common tentative diagnoses in emergency departments (ED). It implies a series of clinical symptoms caused by ischemia of the heart muscle. The most common cause of ACS is plaque rupture with acute thrombosis leading to partial or complete occlusion of the blood vessel (coronary arteries) at the site of thrombus formation and subsequent myocardial necrosis. In an emergency admission, it is very important to distinguish acute myocardial infarction - AMI (STEMI, NSTEMI) from non-AMI (UA, ischemic episodes) in order to accelerate therapeutic treatment. Additionally, holding patients presenting with chest pain to the ED creates a significant burden and increases costs to the healthcare system. Given the relatively low sensitivity of ECG in the detection of AMI (35-50%), measuring markers of myocardial damage in plasma is a standard procedure for patients with suspected ACS. Depending on the timing of their appearance in the circulation and

the analytical sensitivity of the measurement method, diagnostic accuracy and significance of a particular cardiac marker varies.

ERA OF CARDIAC TROPONIN

By the early 90s of the last century, different biochemical markers (mostly enzymes as AST, LDH, CK, CK-MB) were used as markers of myocardial damage. Accordingly, more than 30 years ago one of three defined criteria for the diagnosis of ischemic heart disease reported by the International Society and Federation of Cardiology and World Health Organization was "temporal changes in serum enzymes".

(1) Currently, the measurement of cardiac troponins is an established gold standard for the diagnosis of acute myocardial infarction and troponins are recommended to be used as biochemical markers in the universal definition of acute myocardial infarction. (2) Due to high diagnostic sensitivity and specificity, the measurement of troponin as a marker of myocardial necrosis is required in all patients with suspected ACS according to the "Guidelines for the Diagnosis and Treatment of Acute Coronary Syndromes in Patients Presenting Without Persistent S-T-segment" - Croatian Society of Cardiology and the European Society of Cardiology. (3)

Troponin is a protein complex located on the thin actin filament of the striated muscle contractile apparatus, consisting of three subunits: I, T and C. It seems to be released in circulation very early in the phase of ischemia and therefore its plasma concentration increases already at minimal myocardial injury. The diagnostic sensitivity of troponin T and I are equal, and both markers have absolute specificity for myocardium due to the unique amino-acid sequence in the cardiac-specific protein isoforms identified only for troponin

T and I. (4, 5) This exclusivity makes TnT and TnI so special among cardiac biomarkers that they are termed cardiac TnT (cTnT) and cardiac TnI (cTnI).

CONVENTIONAL VS HIGH SENSITIVE TROPONIN ASSAY

At first, "conventional" assays to determine cTnT and cTnI were used. They did not have the acceptable diagnostic sensitivity and specificity until six to nine hours after the onset of ischemia. Since cTn measured with a conventional assay begins to rise in plasma at about the same time as CK-MB, initially CK-MBmass was considered as a substitute for cTn when a cTn assay was not available. (6) However, it was shown that cTn and CK-MB are not equivalent because cTn has a much greater increase in relation to reference value, and elevated values after AMI persist much longer (seven to 10 days in relation to two to three days when CK-MB falls to normal levels). (4) A limitation of these conventional cTn assays is time-dependent sensitivity and its inability to detect low levels of Tn, which imposes a necessity to prolong serial sampling for six to 12 hours. In order to increase the accuracy for detection of AMI, a highly sensitive cTnT and cTnI assay (hs or ultra cTn) was developed, which allows for a measurement of concentrations that are 10 times lower than the previous ones. This assay allows lower cut-off, greater accuracy and earlier detection of AMI. (7, 8) By using hs cTn it is possible to detect troponin concentration three hours after the onset of ACS symptoms and to monitor a rise and/or fall of concentrations in a short-time period after presentation (after three or even one hour). (3, 9) Highly sensitive cTn assays have an important analytical characteristic: adequate assay precision <10% CV at or below 99th percentile value so that they meet improved

analytical requirements recommended in Guidelines for biomarkers of ACS and heart failure. (2, 6) However, despite the ability for earlier diagnosis of AMI, there is still a “troponin-blind” interval after the onset of chest pain due to delayed troponin release. (8) Therefore, there is a need for a biomarker that is released immediately due to acute myocardial ischemia. On the other hand, use of the highly sensitive cTn assay enables detection of myocardial injury due to a broad spectrum of pathologies which increases the number of positive tests on admission as compared to less sensitive assays. It complicates triage and management of patients with chest pain because only a minority are ultimately diagnosed with ACS, and many fewer are diagnosed with AMI. (10, 11)

POTENTIAL „NEW“ CARDIAC BIOMARKERS IN ACS - MULTIMARKER STRATEGY

In recent years, a search has been underway for new biomarkers that would give added value to troponins and thus contribute to risk stratification of patients with possible ACS. Furthermore, some proteins that reflect different pathophysiological mechanisms in myocardial ischemia have been evaluated as potential new cardiac biomarkers: early biomarkers of cardiomyocyte injury (H-FABP, GPBB), markers of neurohormonal activation (copeptin, NT-proBNP), markers of vascular inflammation (hsCRP, MPO, MMP9), haemostatic activation and thrombosis (D-dimer, fibrinogen, homocysteine). It is clear that simultaneous elevation of these biomarkers in a patient's plasma indicates the onset of several harmful mechanisms placing that individual patient in a high-risk status. In general, new markers are always compared to troponin as the gold standard with the highest diagnostic accuracy.

The results of numerous studies indicate benefits of a multimarker strategy over the use of a single marker, particularly for predicting short-term and long-term risks in patients with non-ST elevation ACS or in predicting adverse cardiac events (death, IM, congestive heart failure). (12-14) At present, however, there is no recommended multimarker panel for this use in practice. (13)

The identification of combinations of biomarkers to facilitate early exclusion of ACS at the time of presentation has been

of growing interest. Considering the high sensitivity of the hsTn assay, researchers usually investigate a combination of a conventional cTn assay and early biomarkers as markers of ischemia, myoglobin or CK-MB. For example, many ED utilise triple marker testing with CK-MB, myoglobin and cTnI to exclude AMI at presentation with increased diagnostic sensitivity and negative predictive value (NPV – rule-out) instead of a single marker use. (15, 16) Although combination of a conventional cTn assay with “early” markers increases diagnostic sensitivity for ACS or AMI – especially in the first 12 hours after chest pain onset – still none of investigated multimarker rule-out strategies have been recommended for clinical practice. (17, 18) While a highly sensitive cTn assay has improved diagnostic accuracy and is capable of detecting myocardial injury earlier than conventional troponin assay, there is still a need for a biomarker that is released immediately due to acute myocardial ischemia (before troponins?) to achieve a more accurate early diagnosis excluding ACS.

Some biomarkers that can predict ischemic myocardial injury have been evaluated in combination with hsTn assays. Among them, two markers have perhaps the greatest complementary potential: heart-type fatty acid protein (HFABP) and copeptin. The significance of these two biomarkers was stated in the NICE guidelines, but further evaluation is needed before they enter into clinical practice. (19)

COPEPTIN

Copeptin is a C-terminal part of the precursor pre-vasopressin (pre-proAVP). It consists of 39 aminoacids and has glycopeptide structure. When the vasopressin system is activated, copeptin will be released into circulation from the posterior pituitary gland in equimolar amounts with vasopressin and can be used as a surrogate biomarker of vasopressin secretion. Even mild to moderate stress situations contribute to copeptin release. If the body is exposed to endogenous stress such as AMI, this activates the vasopressin system and copeptin is excreted into circulation independently of necrosis of cardiac cells. (20) Also, an inadequate filling of the left ventricle caused by AMI stimulates cardiac baroreceptors or causes direct damage to baroreceptors which subsequently leads to

vasopressin and copeptin secretion from the posterior pituitary gland. (21) After the onset of chest pain, copeptin is elevated within three to four hours, reaching a peak value in the first day. (22)

Copeptin is referred to as a BRAHMS copeptin assay (Brahms UK Ltd – Thermo-Fisher Scientific). It is an automated immunofluorescent assay intended to be used together with cTn testing for ruling out myocardial infarction in patients presenting with chest pain.

According to results, a combined assessment of conventional troponin and copeptin improves diagnostic performance in triage of a large number of patients with suspected ACS, specifically in the first hours after cardiac pain onset, and thus might accelerate therapeutic decision-making. (23) Early after the onset of chest pain, the combined measuring of conventional cTn and copeptin provides greater sensitivity (85.1% vs 75.9%) and the highest NPV (92.4% vs 89.9%) when compared to the combined measuring of conventional cTn and myoglobin as well as to a single marker measurement. (23) The rapid ruling out of AMI at presentation using conventional cTn and copeptin can exclude about 65% of ACS suspected patients, with a sensitivity of 98.8% and NPV of 99.7%. (24)

Based on initial clinical experience, the combined determination of hs cTn and copeptin enables a 100% early exclusion of AMI in one-third of patients with NPV in general hospital ED. (25) Also, one prospective study demonstrated that this dual marker strategy slightly increased detection of ACS at admission – compared with use of hs cTn alone – to a level similar to that achieved by repetition of hs cTn measurement. (26)

Recommendations of NICE medical technology guidance MTG4 (published in 2011, in March 2016 NICE commenced the review of this guidance) state the following:

- “BRAHMS copeptin assay is a promising new development for the early ruling out of myocardial infarction in patients presenting with chest pain. However, there was uncertainty about the proportion of patients presenting with chest pain who would benefit from its use, and about the amount of time and resources that would be saved in practice.”

- “...good quality clinical studies are needed to support the potential of the BRAHMS copeptin assay to offer advan-

tages to patients and the NHS. The Committee wished to give strong encouragement to further research, with relevant outcome measures, on the use of the BRAHMS copeptin assay for the early ruling out of myocardial infarction.”

HEART-TYPE FATTY ACID BINDING PROTEIN (H-FABP)

H-FABP is a low molecular weight protein (15kDa), abundantly present in the cytoplasm of cardiomyocytes. It can also be expressed (to a lesser extent) in other tissues with an active fatty acid metabolism (skeletal muscle, distal tubular cells of the kidney, specific parts of the brain). However, H-FABP is highly specific to myocardium, 15-20 times more specific than myoglobin. It is smaller than myoglobin, troponin T, I and CK-MB. Due to the low molecular weight and cytoplasmic location of H-FABP, it is released extremely quickly from ischemic myocardium and it can be determined in plasma only 30 minutes after an ischemic episode (usually one to four hours). It achieves peak concentrations after six hours and rapid return to baseline after 24 hours, which offers significant potential to detect reinfarction. (27) Because of these characteristics, H-FABP is intensively investigated and effort has been put into trying to determine its diagnostic significance in ACS, especially for ruling out AMI in combination with troponin. (27) Unlike conventional cTn and CK-MB, H-FABP has greater diagnostic sensitivity and the highest NPV within the first four hours of the symptoms onset, but cTn is more sensitive for patients whose chest pain lasts longer than 12 hours. (17) Simul-

taneous determination of diverse markers (CK-MB, myoglobin, H-FABP, cTnI) can increase sensitivity and NPV related to individual marker testing, and serves as a good strategy for accurate exclusion of non-AMI patients at presentation in ED. (17) The hs cTn assay is much improved over the conventional cTn assay and offers similar diagnostic performance as H-FABP in patients presenting with chest pain in ED. But the present results show that many more false-positive hs cTn tests than false-positive H-FABP tests occur in patients with non-ACS. (28) After examining the combinations of a few potential biomarkers in patients with early onset ACS, the multicenter study concluded that the diagnostic performance of hs cTnT and H-FABP is similar, and that combined use of H-FABP and myeloperoxidase (MPO) is optimal for improving the early diagnosis of ACS. (28) Another large study showed that high sensitive troponin negative patients with H-FABP concentrations above cut-off had a significantly increased risk of adverse outcome after the minimum follow-up period of 12 months. (29) In a large cohort of patients presenting across the spectrum of ACS, prognostic utility of H-FABP was assessed. Results show that elevation of H-FABP is associated with an increased risk of death and major adverse cardiac events (MACE) when measured during the first days after hospitalization for ACS. (30) In an observational study, a new accelerated diagnostic protocol has been derived for patients with a negative value of hs cTnT, H-FABP and with no ECG ischemia. To reduce unnecessary admissions and health care costs, these “triple negative” patients have been immediately discharged from ED and followed up for 30 days without

statistically significant increase in MACE. (31) A similar study that involved a larger number of patients showed that the combination of H-FABP, hs cTnT and ECG had a sensitivity of 99.1% and NPV of 99.7%. Using a single blood test at presentation, this combination could exclude AMI in 48.8% of patients and identify 99.1% AMI, while missing 0.95% AMI. (32)

CONCLUSION

Cardiac troponins are the most sensitive and the most heart-specific markers of myocardial damage. As assays for cTn have become more and more sensitive and capable to reliably detect the parameter from smaller and smaller quantities in blood, it is often hard to find interpretation for obtained results. For this purpose, revised guidelines have been prepared according to new research findings.

New algorithms incorporating new promising markers with highly sensitive troponin assays hold promise for more rapid diagnosis or the ruling out of AMI. Studies have shown that elevated H-FABP is an independent predictor of high risk of adverse outcome in troponin-negative patients, even in conjunction with a highly sensitive troponin assay. Combining high sensitive troponins and H-FABP in a rule-out biomarkers-based model provides the opportunity to safely discharge non-AMI patients early after presentation, without their evaluation pending in ED and thus saving the healthcare system much aggravation and cost.

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