Abstract. – Background and Objectives: Chest pain is one of the most frequent presenting complaints among patients arriving to the Emergency Department. In this population, the prevalence of acute coronary syndrome is about 25% instead that of acute myocardial infarction ranges from 5 to 15%. The diagnostic challenge for the emergency physician is the early rule-in or rule-out of acute myocardial infarction within first few hours after chest pain presentation, particularly in those patients with normal or not significant electrocardiogram.

Evidence and Information Sources: The current review is based on an analysis of most important clinical trials on this topic.

State of the Art: The universal current guidelines established that the term "myocardial infarction" should be used when there is evidence of myocardial necrosis of ischemic origin, as documented by an increase of myocardial necrosis markers. Actually, cardiac troponins are considered the standard biomarkers for acute myocardial infarction diagnosis, because are more superior in sensitivity and specificity to the other available markers.

Perspectives: More recently, high sensitive troponin assays have been developed, permitting the early measurement of very low concentrations.

Conclusion: In this review, we analyzed the diagnostic and prognostic significance of these new high sensitive troponins in Emergency Department chest pain management.

Key Words: Chest pain, Acute coronary syndrome, High sensitive troponin

Introduction

Acute coronary syndrome (ACS) is the most important cause of death and disability in the Western Countries. The prevalence of ACS among patients presenting to Emergency Department (ED) with chest pain ranges from 5% to 25%. The rapid process of diagnosis is crucial for early confirmation of ACS, risk stratification and start of effective therapy. On the other hand, exclusion of non-ACS chest pain patients can facilitate a safely discharge, reducing the costs and the overcrowding in the ED.

According to current international guidelines, the diagnosis of acute myocardial infarction (AMI) is based on the demonstration of an increase of myocardial injury markers. Cardiac troponins are established to be the standard biomarkers for the detection of AMI and for prognostic evaluation of ACS patients1-4.

Cardiac troponins (troponin I [cTnI] and troponin T [cTnT]) are structural proteins unique to the heart because they are components of the contractile apparatus of cardiomyocytes. They are released after cardiomyocytic necrosis, first from the unbound cytoplasmatic pool (early peak) and after from the structural pool (late peak)5.

It is well known that cardiac troponins are superior in sensitivity and specificity to the all other available biomarkers including markers indicative of myocardial ischemia, inflammation, dysfunction and plaque rupture, including myoglobin and the fraction of creatine kinase (CK-MB). In a recent study, Apple et al6 investigated a multimarker strategy for early diagnosis of AMI, testing seven biomarkers (myeloperoxidase, soluble CD40 ligand, placental growth factor, matrix metalloproteinase 9, high-sensitivity C-reactive protein, cTnI, N-terminal pro-brain natriuretic peptide [NT-proBNP]) in 457 patients presenting on ED with symptoms suggestive of ACS. Biomarker sensitivity ranged from 20% to 96%, and specificity ranged from 19% to 89%. cTnI resulted the most clinically accurate biomarker with a sensitivity of 72% (95% CI, 51%-88%) and a specificity of 89% (95% CI, 85%-92%). cTnI alone was also superior to a multiple-biomarker approach.
Despite their high sensitivity and specificity as markers of myocardial injury, the early plasmatic increase of troponin, measured using standard assay, is slower than those of myoglobin and CK-MB. Consequently, it requires prolonged monitoring and at least three serial blood sampling (0-6-12 hours) to rule in or rule out AMI. A delayed start of diagnosis and therapy can increase the risk of cardiac complications. On the other hand, the delay can cause an increase of the patients length of stay and contributes to the over-crowding in the ED.

Moreover, although an increase of cardiac troponin is highly specific for myocardial injury, it does not always indicate the origin of heart damage. In fact, a wide spectrum of pathologies are associated with troponins release including non-ischemic cardiac diseases (cardiac contusion and surgery, percutaneous coronary intervention, tachyarrhythmias, cardioversion, myocarditis, heart failure, cardiac amyloidosis, hypertrophic cardiomyopathy) or noncardiac diseases (pulmonary embolism, chronic renal insufficiency, stroke, subarachnoid hemorrhage, chemotherapy, sepsis).

To overcome these limitations, technology improvements have allowed a new generation of sensitive assays of cardiac troponins allowing to early detect very small cTn concentration.

**High-Sensitive Troponin Definition**

The new high-sensitive (hs) troponin assays are able to measure troponin concentrations that are lower by a factor of ten than those measurable with conventional assays. They have shown an analytic sensibility (measurement limit) equal or even lower to 6 pg/ml. Since cTnI and cTnT are present in human myocardial muscle at the concentration of 70 µg and 100-200 µg every tissue gram respectively, it is likely that high sensitive methods could measure the quantity of protein corresponding to 1 mg of myocardial tissue, below the sensitivity of imaging methods. Since many assays entered in the market place to be used in clinical practice and many will enter in the next future, the problem is how to compare the different assays. According to Wu et al, a rationale approach could be to use a score card using a normal reference healthy young population to establish 99th percentile values with an acceptance designation for clinical practice depending on the total imprecision (CV%) of each assay (≤10% guidelines acceptable; >10 ≤20% clinically usable; ≥20% not acceptable).

The designation of the assay depends on the percentage of measurable normal values below the 99th percentile in a healthy population (<50%: level 1 or contemporary; 50 to <75%: level 2 or first generation; 75 to <95%: level 3 or second generation; ≥95% level 4 or third generation). The term “high sensitivity” should be reserved only to level 2, 3 and 4.

**Troponins as Cardiac Biomarkers: Usefulness and Limitation in Chest Pain Patients**

**Acute Coronary Syndrome Diagnosis**

In the last years, several studies were performed to examine the diagnostic performance of these new sensitive cardiac troponin assays for the early diagnosis of AMI in ED patients.

In particular, in a prospective, multicenter study, Keller et al evaluated new hs-TnI assays in 1818 patients presenting with new-onset chest pain at three German ED centers. All patients underwent an initial clinical assessment that included a clinical history taking, a physical examination, 12 lead ECG, pulse oximetry, standard blood measurements, and chest radiography. cTnT, CK-MB, myoglobin and hs-TnI were measured at presentation and 6 to 9 hours after presentation or as long as clinically indicated. The hs-TnI assay was previously tested in 5000 population-based subjects and a value of 0.04 ng per milliliter was defined as the 99th percentile for that population. The concentration of 0.04 ng per milliliter (a 10% coefficient of variation at 0.03 ng per milliliter) was used as the upper reference limit. The diagnosis of AMI was established if one value of more than 0.04 ng per milliliter (a 10% coefficient of variation at 0.03 ng per milliliter) was used as the upper reference limit. The diagnosis of AMI was established if one value of more than 0.04 ng per milliliter was documented, combined with a rise or fall in the value of 30% or more within 6 hours after admission. To determine the final diagnosis for each patient, two independent cardiologists who were unaware of the results of the troponin I assay reviewed all data.

The Authors reported that the diagnostic accuracy (area under the receiver-operating-characteristic curve [AUC]) for samples obtained on admission was highest with the hs-TnI assay (0.96), as compared with the cTnT assay (AUC, 0.85) and other myocardial biomarkers. The clinical sensitivity for the hs-TnI assay was 90.7% and the specificity was 90.2%, as compared with 72.7% and 94.1% for cTnT assay, respectively.
The Authors\textsuperscript{11} also evaluated the association between an elevated value on admission and the diagnosis of AMI, according to the time of chest-pain onset. In patients presenting within 3 hours after chest-pain onset, 81.1% of AMI patients had a single troponin I level of more than 0.04 ng per milliliter on admission, with a negative predictive value of 84.1% and a positive predictive value of 86.7%. A total of 88% of AMI was detected on admission in patients presenting within 6 hours after the onset of chest pain, and 95% of AMI were detected in those presenting between 6 and 12 hours after the onset of chest pain. With serial measurements (on admission and 3 or 6 hours after admission), the rate of detection of AMI was 100%.

Keller et al\textsuperscript{11} concluded that hs-TnI assay significantly improves early diagnosis of AMI and in particular the measurement obtained on admission still provides good levels of accuracy and discrimination for the diagnosis of AMI. However, better sensitivity may thus lead to a higher rate of false positive results and other causes of increased troponin concentration should be excluded.

Similar results were reported by Reichlin et al\textsuperscript{7}, who conducted a multicenter study to examine the diagnostic accuracy of sensitive cardiac troponin assays in 786 patients, who presented to the ED with symptoms suggestive of AMI and in whom the onset of symptoms had occurred within 12 hours before presentation. All patients underwent an initial clinical assessment that included a clinical history taking, a physical examination, 12 lead ECG, pulse oximetry, standard blood measurements, and chest radiography. cTnT, cTnI, CK-MB, and myoglobin were measured at presentation and 6 to 9 hours after presentation or as long as clinically indicated. Cardiac troponin levels were determined with the use of four sensitive assays (Abbott, Architect Troponin I, Roche High-Sensitive Troponin T, Roche Troponin I, and Siemens Troponin I Ultra) and a standard assay (Roche Troponin T). To determine the final diagnosis for each patient, two independent cardiologists reviewed all available data. The diagnostic accuracy for AMI, as quantified by the AUC, was significantly higher with the four sensitive cardiac troponin assays (ranging from 0.94 to 0.96) than with the standard assay (area under the curve: AUC = 0.90) and was also significantly higher than that with the other traditional biomarkers. The diagnostic accuracy was similar among the four sensitive assays. The diagnostic performance of the four sensitive cardiac troponin assays was similar both in the case of non-ST-segment-elevation AMI and ST-segment elevation AMI. The superiority of the sensitive cardiac troponin assays was most pronounced among patients with recent onset of chest pain (within 3 hours): the AUC for the sensitive assays (from 0.92 to 0.93) was significantly higher than AUC standard assay (0.76). Finally, sensitivity, specificity, negative predictive value and positive predictive value for new troponin assays ranged from 75% to 95%, 80% to 97%, 95% to 99% and 50% to 83% respectively, compared with 72%, 97%, 94% and 85% of standard troponin assay.

The Authors\textsuperscript{7} concluded that the accuracy of the sensitive cardiac troponin assays was higher than that of the standard assay, particularly among patients with a recent onset of chest pain. This property may make it possible to reliably rule out the diagnosis of AMI in many patients on the basis of the initial measurement.

As study limitations, no information was done about diagnostic accuracy of sensitive cardiac troponin assays among patients with severe kidney failure. Moreover, some patients with positive results of sensitive cardiac troponin assays had not a final diagnosis of AMI suggesting that they might have small AMI below the limit of detection of the conventional assays.

To examine the cardiac anatomic causes of elevation of high sensitive troponin, Januzzi et al\textsuperscript{12} recently tested the diagnostic performance of hs-TnT and correlated hs-TnT both with clinical syndrome and with cardiac abnormalities as demonstrated by 64-slice computed tomography (CT) angiography. A cohort of 377 low- to intermediate-risk patients presenting to ED with chest pain and clinical suspicion for AMI were enrolled. A final diagnosis of ACS was made retrospectively by 2 physicians on the basis of the history and available data.

A sample of blood for hs-TnT and standard cTnT was taken and cardiac CT imaging and interpretation were performed with a 64-slice scanner. Interpretation of the CT angiogram included assessment for presence and extent of coronary artery disease. In particular, the Authors\textsuperscript{12} assessed the number of segments affected by atherosclerotic plaque, the characteristics of the plaque (calcified or noncalcified), the presence of significant coronary stenosis, the cardiac structure and function, including measures of chamber volume in end systole and end diastole, left ventricular ejection fraction, left ventricular mass, and regional left ventricular dysfunction.
As results, the Authors reported that median concentrations of hs-TnT were significantly higher among patients with ACS than among those without. Moreover, they were highest in patients with AMI, intermediate in those with unstable angina, and lowest in those without ACS. The area under the curve for the diagnosis of ACS was of 0.79 for hs-TnT compared with 0.74 for cTnT with a significant discrimination improvement (hs-TnT detected 27% more cases of ACS). Using the 99th percentile cut point for a healthy population, hs-TnT had a better sensitivity and negative predictive value for ACS than cTnT (62% vs 35%, 96% vs 93%, respectively), but a worse specificity and positive predictive value (89% vs 99%, 38% vs 72%, respectively). Interestingly, Januzzi et al. also reported the predictors of hs-TnT increase in patients with chest pain and they found that, among all subjects, independent predictors of elevated hs-TnT levels included age, presence/extent of coronary artery disease (CAD), cardiac structure, cardiac function, and NT-proBNP. On the other hand, subjects with elevated hs-TnT but without ACS were more likely to be older and have more complex medical histories (including prior CAD) and more cardiac abnormalities, with more prevalent and extensive CAD, larger cardiac chambers and greater left ventricular mass.

The Authors concluded that high sensitivity troponins are highly accurate tests for myocardial injury and anatomical abnormalities rather than a test for AMI. For these reasons, they should be considered a signal for structural heart disease irrespective of an ACS, and only in a correct evaluation of the clinical context a positive result should be interpreted as consistent with ACS.

Although these results are certainly impressive and emphasize the role of new sensitive troponin assays in ACS diagnosis, some aspects still remain unclear, as suggested by two recent editorials. First of all, according to hs-cTnT definition, only few currently used cTn methods could be denominated as high-sensitive; secondly, although new troponin measurement obtained on admission of patients still provides good levels of accuracy, it is not clear how long it takes to rule out AMI; thirdly, new troponin assays could change the concept of unstable angina, as suggested by a recent pilot study using a nanoparticle assay for cTnl, showing that myocardial injury is detectable in a high percentage of patients currently classified as having unstable angina and than suggesting that ischemia with rest pain without injury is rare. Finally, better sensitivity and reduced specificity may lead to a higher rate of false positive results and a wide range of non-ischemic diseases should be excluded.

Biologic variation of troponin levels due to different release and clearance kinetics should be also considered, as a suggested by Wu et al., that found a 46% increase or a 32% decrease in hourly hs-TnT measurements for 4 hours in 17 healthy individuals. Appropriate use of information about biologic variation of troponin concentrations is consequently needed in ED patients for AMI diagnosis. This point was recently under lighted by White, that suggested to use different Δ changes to diagnose IMA on the basis of baseline troponin level. In particular, for patients with baseline fourth-generation troponin T level >53 ng/ml, AMI can be diagnosed if a change >20% is observed in 3 hours later troponin measurement; for patients with baseline level <53 ng/ml, a change >50% is needed for AMI diagnosis.

**Risk Stratification and Prognosis**

Actually there are few studies assessing whether high sensitive troponin assays can be useful for risk stratification and prognosis of patients affected by acute or chronic cardiac diseases.

In 2009, Omland et al. tested the levels of hs-TnT in 3679 patients with stable coronary artery disease without heart failure or left ventricular systolic dysfunction and the correlation with the risk of future cardiovascular events. The lower limit of detection of the high sensitive assay was 0.001 µg per liter. The value at the 99th percentile in a sample of 1338 apparently healthy blood donors was 0.0133 µg per liter. The Authors reported that concentrations of troponin T were at or above the limit of detection in 97.7% of the patients and equal to or greater than the 99th percentile value for apparently healthy subjects in 11.1% of the patients. CTT levels were positively associated with several conventional risk factors. During follow-up, there were 125 cardiovascular deaths in the study cohort. Interestingly, both in the univariate and multivariate model, the cumulative incidence of cardiovascular deaths was significantly associated with the cTnT level (hazard ratio 2.78 and 2.39, respectively), suggesting that the new troponin T assay provided powerful prognostic information in patients who would have undetectable levels with the conventional assay. Moreover, during follow-up there were 104 first hospitalizations for fatal
or nonfatal heart failure. In unadjusted analyses, the incidence of heart failure increased with increasing levels of troponin T. After adjustment, the association remained highly significant (hazard ratio, 2.20). No significant associations were found between fatal or nonfatal AMI and increasing troponin T levels. This study demonstrated that very low circulating levels of cardiac troponin T are detectable in the great majority of patients with stable coronary artery, are associated with multiple conventional risk factors and have a graded relationship with the incidence of cardiovascular death and heart failure, not AMI, in these patients.

These results were confirmed by a recent investigation testing the prognostic role of hs-TnI in patients with systolic heart failure. A cohort of 258 symptomatic patients with left ventricular ejection fraction <45% were enrolled. Blood samples were collected for measuring serum levels of TnT and hs-TnI (lower limit of detection was 0.006 ng/ml and the lowest concentration at which the CV was <10% was 0.03 ng/ml) and plasma NT-proBNP. The mean follow-up period was of 2.6 years. The Authors found that TnT and hs-TnI were elevated in 12% and 43% of patients, respectively. During the follow-up, 20 cardiac deaths occurred. High plasma hs-TnI and NT-pro-BNP but not TnT resulted to be useful for predicting mortality in these cohort of patients (hazard ratio for mortality of 5.74 in patients with high plasma NT-proBNP and hs-TnI compared to those with low NT-proBNP or hs-TnI).

Finally, we report the results of two trials testing the prognostic role of high-sensitive troponin assays in ACS population.

In the first study, Kavsak et al retrospectively tested a cohort of 383 subjects, originally recruited in 1996, presenting to the ED with symptoms suggestive of ACS whose plasma obtained at presentation was used in 2007 for hs-TnI assay research. On the basis of hs-TnI measurement, the population was divided into 4 groups: <5.00, 5.00-9.99, 10.00-40.00, and >40.00 ng/L. The aim was to evaluate if hs-TnI concentrations were predictive of death/AMI within 10 years after presentation. The Authors found that hs-TnI assay was useful for risk stratification of ACS patients both in the short and long term. In fact, there were significant differences between groups for the probability of death/AMI up to 10 years after presentation on the basis hs-TnI value and, at 3 and 6 months, patients with hs-TnI >10.00 ng/L were at higher risk for death/AMI (hazard ratio 3.7) compared with lower hs-TnI patients.

These results were confirmed by a prospective study by Apple et al, that measured hs-TnI on admission and 6-24 h after admission in 371 acute chest consecutive patients. The end point was the occurrence of cardiac events (AMI, percutaneous coronary intervention or coronary artery bypass graft) or death during 60 days follow-up period. The patients were divided into 4 groups on the basis of hs-TnI levels: <0.006 µg/L, 0.006-0.04 µg/L, 0.04-0.10 µg/L, and >0.10 µg/L. The Authors reported that event rate was significantly lower in the first group than in the others (2.8% vs 11.1%, 24.1%, 55.1%, respectively; p <0.0001). After adjustment for age, diabetes, history of hypertension, previous AMI and estimated glomerular filtration rate, the relative risk of an event compared with the first group was 3.9, 8.9 and 25 for the other groups, respectively. The Authors concluded that hs-TnI is an independent predictors of adverse events in ACS patients, at least in the short-term period.

Large prospective studies are necessary to extend our understanding about the prognostic power of hs-Tn assays for long-term risk in ACS patients.

**Conclusion**

The new higher sensitivity assays are crucial for the more rapid diagnosis of AMI. However, as they are more sensitive and any cardiac damage cause their release into the blood, non ischemic troponin increase can be more frequently found. A rising and/or falling pattern of troponin levels is than essential to distinguish between increased levels caused by chronic, non ischemic diseases from an acute myocardial ischemic damage. However, biological variation of troponin levels should be also considered to interpret serial tests results. Then, the only way to correctly diagnose AMI and to differentiate it from the other cardiac diseases is to integrate hs-Tn results into a correct evaluation of the ED patients clinical context.

Additional basic and clinical studies are needed to clarify the cellular mechanisms for the release of troponins, the diagnostic and prognostic significance in ED chest pain management and the impact on patients outcomes.
References


