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Troponin I (cTnI) fragments immunoreactivity and the development of high-sensitivity diagnostic assays for the associated diagnosis of acute myocardial infarction

Imunorreatividade de fragmentos da troponina I (cTnI) e desenvolvimento de ensaios de alta sensibilidade para o diagnóstico associado do infarto agudo do miocárdio

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ABSTRACT

Among the cardiovascular diseases (CVD), acute myocardial infarction (AMI) is currently considered the most common cause of death and disability worldwide. Several laboratory tests have been developed for the detection of cardiac injury, including troponins that are considered the gold standard marker (surrogate biomarker) of myocardial injury. The high specificity of troponin for cardiomyocyte necrosis is related to a single unique peptide sequence present in troponin at the cardiac muscle. As a result, studies are currently focused on the development of troponin (hs-cTnI) determination tests with high diagnostic sensitivity value. These diagnostic tests aim to detect increasingly lower serum concentrations of cTnI biomarkers, from the detection of peptide fragments that are released after structural biochemical changes. This article discusses the differences between troponin fragments immunoreactivity to the development of cTnI determination tests, such as the high-sensitivity tests, which arise with the proposal of guaranteeing greater efficiency in the AMI associated diagnosis.

Key words: acute coronary syndrome; troponin I; immunodominant epitopes.

INTRODUCTION

Cardiovascular diseases (CVD) are still the most common cause of death worldwide. Out of the 57 million deaths in 2008, 17.3 million (30%) occurred due to CVD[1]. Among these, acute myocardial infarction (AMI) is the main cause of death and disability, and coronary atherosclerosis is one of its main causes. AMI may be a minor life event or a chronic illness; it may not even be detected, but it may be one of the main critical events, leading to sudden death or severe hemodynamic impairment. AMI may be the first manifestation of coronary artery disease, or may happen, repeatedly, in patients with the established disease[2]. Nowadays, serum concentrations of tissue damage markers are assessed, such as cardiac enzymes and isoenzymes, which are essential for diagnosis or exclusion of myocardial lesion. Several laboratory tests have been developed for detection of cardiac injury, including troponins. Troponins are represented as a protein complex composed of three units that regulates the binding between actin and myosin, filaments that interact to produce muscle contraction. While the complex troponin, troponin T (TnT), troponin I (TnI), and troponin C (TnC) is found in both the skeletal and the cardiac muscle, the high specificity of troponin for necrosis of cardiomyocytes is due to a unique peptide sequence present in the troponin originated in the cardiac muscle (cTn), which is not found in the troponin of the skeletal muscle (sTn)[3].

Therefore, this update article approaches aspects related to the presence of structural biochemical changes present in fragments of cardiac troponins I (cTnI), as well as the importance of these changes in these immunoreactive fragments for the development of new highly sensitive diagnostic tests to assure greater efficiency and performance in the AMI associated diagnosis.

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STRUCTURAL CHANGES AND IMMUNOREACTIVITY OF CTN FRAGMENTS

Although troponin (cTn) is specific for lesions of the cardiac muscle, we need to be aware of situations that can alter immunoreactivity, and, consequently, modify its structure. In those cases, both TnT and TnI are released in different molecular forms. For instance, TnT is mainly found as intact TnT:I:C complex, free TnT, and smaller immunoreactive fragments. TnI is mainly released as intact TnI:I:C and I: C complex. The binary form TIc:C seems to be the predominant molecule, besides having forms deriving from proteolysis (degraded) and other biochemical changes, such as phosphorylation, oxidation, and reduction[4]. Thus, a lot of effort has gone into developing monoclonal antibodies to be used in assays of different manufacturers for the troponins that are comparable with each other[5]. We can cite several instruments with their automated assays that are used in the diagnostic support of AMI, such as Architect – Abbott Diagnostics, Centaur – Siemens, Access – Beckman Coulter, Immulite – Siemens Dimension series – Siemens, and Vitros – Ortho Clinical Diagnostics, all of them used for determination of TnI; and Cobas/Modular – Roche Diagnostics, the only one for TnT. There are also non-automated tests; rapid tests, such as i-STAT – Abbott Diagnostics; Triage Cardiac Panel – Alere; Reader – Roche Diagnostics and Radiometer AQT90 for TnI[5].

IMMUNOREACTIVITY OF CTNI FRAGMENTS

As already mentioned, due to heterogeneity of the circulating TnI forms and their sequential changes, the prospects of diagnostic tests for TnI must provide their reactivities or the degree of equimolarity (equimolar ratio) for determination of the several forms of troponin[4, 6, 7]. As a result, the midfragment of TnI (amino acid residue 30-110) has been universally recommended for its stability[4], based on the definition of regions for the increased specificity and identification of epitopes (antigen determinants) or target that develops the new generation of high-sensitivity assays for determination of cTnI[7]. For those assays, the use of N-terminal target region (stable region) of cTnI is recommended in two- or three-site sandwich assays[4], avoiding the terminal regions of the protein that normally degrade due to the effect of active proteinases (for example, calpain-1 and caspase-3) and post-translational modifications. The use of at least two antibodies for this region has demonstrated significant improvements in analytical sensitivity and performance[8].

DIAGNOSTIC EFFICIENCY: CTNI DETECTION LIMITS

The thorough investigation of new target epitopes is decisive for overall performance of the diagnostic tests currently most used in the detection of cTnI. With the identification of those epitopes, new tests gained specificity, so, in a short time assays are expected to follow the guidance of the European Society of Cardiology (ESC) and the American College of Cardiology (ACC): detection limit for TnI assays must be very low among healthy individuals, that is, low number of false-positive results, considering the 99th percentile for the definition of AMI[9]. The diagnostic industry is focused on reduction of analytical imprecision and improvement of sensitivity to meet the recommendations necessary for manufacturing of diagnostic kits with highest coefficient of variation (CV) of 10% at the 99th percentile[10]. Alternatively, the use of a lower serum concentration of measurable troponin has been suggested when CV is lower than 10% (CV < 10%)[11]. However, most analytical methods for diagnostic support used in emergency hospitals do not satisfy the rule established by the guidelines, what characterizes lack of uniformity among the used assays[12]. Still as an aggravating factor, although less precise assays of cardiac troponin do not generate relevant false-positive AMI diagnoses, even when CV is 20% at the 99th percentile, they are considered acceptable[7, 13]. According to Bates *et al*. (2010)[14], after AMI, the circulating fragments of cTnI are present in the serum in the complexes TIc:C and IC, and the fragments cTnT present themselves combined as TIc:C, or exclusively in their free form, free cTnT. These serum fragments obtained the following averages of recovery (confidence interval) for patients' cTn (n = 35), that is, 86% (41%-110%) for cTnT, 49% (23%-134%) for cTnI (Siemens Centaur), and 48% (23%-102%) for cTnI (Beckman Access). Waxman *et al*. (2006)[15] demonstrated that at a heterogeneous population of patients with acute disease, the presence of troponin I, even at levels below diagnostic cutoff, is associated to adverse prognosis, with increased risk for mortality. Mortality risk is directly proportional to the order of magnitude, beginning in the lowest detectable concentrations. Therefore, harmonization of diagnostic tests is imperative for detection of cTnI concerning the type of circulating serum fragment.

HIGH-SENSITIVITY TROPONIN: HS-CTN

The definition of epitopes is important for analytical specificity of cTnI assays, and the careful investigation about them has led to the development of a new generation of high-sensitivity TIc:C

assays[7, 8]. The high-sensitivity diagnostic tests used to detect troponin are different from those conventionally used for its higher sensitivity associated with the analytical method[16]. The key differentiating element between hs-cTn assays and the first assays developed for cTn is increased sensitivity, what is only apparent in values close to the 99th percentile. Information originated from concentrations of hs-cTn close to the 99th percentile has challenged clinical interpretation, because findings in this concentration range confirm increased sensitivity. Studies demonstrate higher precision of early diagnosis of AMI with the use of assays for hs-cTn, when compared with old assays for cTn used at admission to an emergency unit[13].

Concerning the clinical utility of cTnI, low cutoff points below the 99th percentile resulted in increased TIc:C detection rate, what can be attributable to chronicle instead of acute processes[7, 17]. The only assay for the high-sensitivity troponin commercially available (except in the USA, for it has no approval by the Food and Drug Administration [FDA]), is that provided by Roche, the hs-TnT assay. Concentrations for high-sensitivity assays are expressed in nanograms per liter or picograms per milliliter, instead of the commonly used unit micrograms per liter[16].

FINAL COMMENTS

Nowadays, the biggest challenge at a hospital emergency room is deciding, after first acute care, between hospitalization and discharge, given the admission of patients with chest pain. With that aim, well-defined clinical procedures and protocols are necessary, including tests and differential diagnostic examinations, since the clinical picture can be atypical, because electrocardiographic changes can be subtle or inexistent. Troponins, gold standard cardiac biomarkers, are currently the cornerstone of AMI associated diagnosis due to their specificity for the cardiac muscle. Studies have been conducted to increase sensitivity and standardize analytical assays, such as high-sensitivity assays for cardiac troponins, which were developed to detect increasing lower serum concentrations of these markers. The need for this development was due to lack of harmonization among assays already conducted for TnI determination, because of the high values of CV observed (20%-40%). Aspects such as molecular degradation, instability of regions used as antigenic determinants, effect of active proteinases, and post-translational modifications are some of the facts determining analytical failure when compared to current high-sensitivity assays used for detection of troponin I (hs-CTnI). Thus, analysis tools [for example, receiver operating characteristic (ROC) curve, sensitivity and specificity analysis, efficiency and predictive value theory] and some clinical algorithms should be used during follow-up in patients with suspected AMI, principally when there is the possibility of obtaining false-negative results due to the several forms of circulating fragments and the lack of harmonization of diagnostic tests used for TnI determination.

RESUMO

Entre as doenças cardiovasculares (DCV), o infarto agudo do miocárdio (IAM) atualmente é considerado a causa mais comum de morte e incapacidade em todo o mundo. Vários testes laboratoriais vêm sendo desenvolvidos para a detecção de lesões cardíacas, entre eles, as troponinas, consideradas marcador (biomarcador sugestivo) padrão-ouro de lesão miocárdica. A alta especificidade da troponina para a necrose dos cardiomiócitos está relacionada com a sequência peptídica única presente na troponina do músculo cardíaco. Em função disso, estudos estão voltados para o desenvolvimento de conjuntos diagnósticos de alta sensibilidade para a determinação das troponinas I (hs-cTnI). Esses conjuntos diagnósticos surgem com o objetivo de detectar concentrações séricas cada vez menores desses biomarcadores a partir da detecção de fragmentos peptídicos que são liberados após modificações bioquímicas estruturais. O presente artigo discorre sobre as diferenças de imunorreatividade dos fragmentos de troponina no desenvolvimento de nossos testes para a determinação da cTnI, a exemplo dos testes de alta sensibilidade, que surgem com a proposta de garantir maior eficiência no diagnóstico associado do IAM.

Unitermos: *infarto do miocárdio; troponina I; mapeamento de epítopos.*

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