Mini Review

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Cardiac troponins – a paradigm for diagnostic biomarker identification and development

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Abstract: The introduction of cardiac troponins into clinical diagnostics has not only improved diagnostic pathways for myocardial infarction but also profoundly influenced the definition of myocardial infarction. The term troponin appeared in the literature almost 60 years ago, i.e. shortly after this journal was founded. The development of cardiac troponins from proteins involved in muscle contraction, which were in the focus of few specialized research groups from physiology and biochemistry, to one of the most frequently measured protein biomarkers in medicine is a paradigmatic success story which is also reflected in almost 300 publications on the topic in this journal. From the viewpoint of biomarker development the critical success factors were medical need, timely generation of medical evidence, and the rapid development of robust and precise laboratory assays.

Keywords: acute coronary syndrome; cardiac troponin; myocardial infarction.

Introduction

Cardiac troponins (cTn) are an outstanding example of successful translational biomarker research in medicine, which provides important clues for future biomarker development. On the occasion of CCLM's anniversary it appears appropriate to review the development of cardiac troponins over these six decades. Early research on troponins was dominated by physiologists and biochemists interested in muscle structure and function. The term "troponin" appears 1966 for the first time in an article by Setsuro Ebashi and Ayako Kodama. They show that the tropomyosin-like protein which they call troponin is critical for the function of tropomyosin [1]. In this article the authors speculated already that troponin was more than one protein. In the years to follow, three troponins were identified by traditional protein chemistry [2, 3]: one bound to tropomyosin, one to calcium, and one inhibited actomyosin ATPase. In the mid-1970-ies these were named troponin T (TnT), troponin C (TnC), and troponin I (TnI), respectively. It was soon discovered that three different variants of TnT and TnI were detectable in striated muscle, one in cardiac muscle and two in slow and fast skeletal muscle fibres [4, 5]. Protein sequences of troponins were obtained by Edman degradation [6]. The discovery of different isoforms enabled the generation of specific antibodies against the different variants which were used in the late 1970-ies to identify the cellular localization of the different troponin components [7–9]. With the advent of recombinant DNA technology things became even more complicated as alternative splicing of troponin pre-mRNAs was observed [10]. In fact, substantial heterogeneity was also confirmed by protein analysis [11]. By analysis of cDNA and genomic DNA it was discovered that the three isoforms of TnI and TnT were encoded by three independent genes, respectively, which confirmed the differences of the primary structure of the skeletal and cardiac isoforms already known from protein chemistry [for review see 12].

The obvious specificity of cardiac TnI (cTnI) and TnT (cTnT) and the proven option to generate isoform specific antibodies made them candidates in the search for more specific and sensitive biomarkers of myocardial infarction (MI). Biomarkers were part of the diagnostic approach to patients with suspected MI since the 1950-ies when John LaDue and coworkers showed that serum glutamic oxaloacetic transaminase (aspartate aminotransferase) was elevated in patients with MI [13]. However, the biomarkers available by 1980, i.e. creatine kinase and creatine kinase MB, myoglobin, aspartate aminotransferase, and lactic dehydrogenase had limited cardiac specificity. This led to an active search for more specific and ideally more sensitive markers. Accordingly, specific immunoassays for cardiac troponins I and T were developed and cTnI and cTnT were proposed as markers for MI by Cummins and

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coworkers in 1987 [14] and by Katus and coworkers in 1989 [15], respectively. Since then, cTn have not only become the laboratory markers of choice for suspected MI. They have also profoundly changed the definition of MI itself. In fact, cTn are part of the universal definition of MI put forward by the leading cardiology societies. This development was only possible by the combination of rapid assay development and continuous assay improvement on the one hand and clinical studies which assessed the predictive value of cTn for short and long term clinical endpoints on the other hand. The importance of this linkage is impressively demonstrated by the fact that the determination of cTn came into clinical routine essentially after seminal endpoint studies were published in the 1990-ies. It should be noted that this journal published its first article on cTn in the diagnostics of suspected myocardial infarction by Bakker et al. in 1993 [16]. This was followed by almost 300 further publications until today, including original research, reviews, and recommendations from scientific societies or working groups.

In spite of this tremendous success story several open questions remain regarding the use and interpretation of cTn in clinical routine and the pathophysiologic implications. In this short review the current status of cTn in clinical diagnostics will be summarized followed by a discussion of some of the unresolved issues.

Assay development 1990–2020

It should be noted that the initial assays for cTn had a limited analytical sensitivity with a reported measuring range from 10–1,000 µg/L for cTnI [14] and 0.5–25 µg/L for cTnT [15]. The assay for cTnT was rapidly improved to extend the measuring range to 0.1–15 µg/L [17]. Also an early non-radioactive immunoassay for cTnI showed improved analytical sensitivity (1.9 µg/L) compared to the initial radioimmunoassay [18]. Over the following years assays were continuously improved and transferred to platforms enabling high throughput. Currently available high-sensitivity (hs) assays for cTnI and cTnT which became available after 2010 are approx. 100 to 1,000 times more sensitive than the initial cTn assays. The limits of detection are well below 5 ng/L depending on the specific assay. According to the generally accepted definition two requirements must be met by high-sensitive assays for cTn: (1) coefficient of variation at the 99th percentile is below 10%, and (2) cTn is detectable in more than 50% of individuals of a healthy reference population [19]. Due to the rapid development in this area, the IFCC Committee on Clinical Application of Cardiac Bio-Markers has published a summary of the properties of the available cTn assays [20] which is continuously updated on their website (https://www.ifcc.org/ifcc-education-division/emd-committees/committee-on-clinical-applications-of-cardiac-bio-markers-c-cb/). From a technical point of view the lowered limit of detection and the improved analytical precision of the assays at low concentrations is a major breakthrough. However, these improvements have relevant implications for the interpretation of results as will be discussed below.

Diagnostic use of cTn

As of today, cTn are the preferred biomarkers in patients with suspected acute coronary syndrome. They are indispensable for rule-out and rule-in of acute myocardial infarction when the electrocardiogram is inconclusive which is the case in the majority of patients presenting with symptoms compatible with acute coronary syndrome [21, 22]. Particularly, rapid rule-out of MI in these patients has become feasible with the hs-cTn assays. Early clinical studies performed soon after the development of hs-cTn assays showed that reliable rule out of MI could be obtained with repeat determination of cTn after 3 h rather than 6 h which were recommended at that time [23, 24]. In the most recent guidelines even more rapid rule-out procedures have been included for MI in patients with suspected acute coronary syndrome. These protocols are based on the cTn concentration at presentation alone or – if required – on retesting after one or 2 h [21]. They have been shown to be reliable in large patient cohorts with negative predictive values close to 100%. Compared to this great improvement, biomarker based rule-in for NSTEMI is still more difficult with significantly lower diagnostic reliability, especially when cTn concentrations are only moderately increased [21, 22]. This is caused by many confounding factors affecting the plasma concentration of cTn, e.g. age, sex, or kidney function. In addition, analytical interferences causing false positive results have been observed [25, 26]. The much lower 99th percentiles of the hs-cTn assays provide formally “abnormal” results which are outside the measuring range of the older assays. In other words, the older assays had cutoff values far above the current hs-cTn assays. Accordingly, cTn concentrations above the cutoff of the old assays had higher positive predictive values.

Attempts to move from fixed cutoffs based on the distribution of cTn in healthy reference populations to positive and negative predictive values of single or repeated determinations of cTn have gained more interest for obvious reasons [27]. For rule-in the delta between the first and

second cTn concentration has become a critical variable [28]. The relatively low intraindividual variation of cTn has been a prerequisite for the feasibility of this approach [29–31]. A limitation of such rule-in algorithms based on cTn and its short-time changes is the variable pretest probability of patients presenting to chest-pain-units or emergency rooms. Furthermore, calculation of predictive values is more time consuming than comparison of a cTn result with a fixed cutoff. In theory, limitations of predictive values could be overcome by the use of positive and negative likelihood ratios which permit calculation of post-test probabilities from pre-test probabilities. Obviously, this requires clinical assessment of pre-test probability by appropriate risk scores, e.g. GRACE, HEART, TIMI without inclusion of cTn. Unfortunately, only few clinicians are used to apply likelihood ratios to clinical decision making.

Cardiac troponins as part of the definition of myocardial infarction

In 2000 a joint committee of the European Society of Cardiology and the American College of Cardiology published a novel definition of MI [32]. The committee proposed that "any amount of myocardial necrosis caused by ischemia should be labeled as an infarct". cTn as cardiac-specific biomarkers became critical for the definition of MI, because biomarkers were and still are the most sensitive method to detect myocardial necrosis, even though an increase in their blood concentration cannot prove ischemia as the underlying cause. Accordingly, the committee recommended that a concentration of cTn beyond the 99th percentile of the assay within the first 24 h after the index clinical event should be interpreted as an indicator of myocardial necrosis. In combination with clinical, electrocardiographic, or imaging signs of ischemia the diagnosis of MI should be made. This biomarker based definition of MI was based on the observation made in clinical studies that any pathological increase in cTn in patients with acute coronary syndrome would adversely affect short- and long-term outcome. Patients considered to have unstable angina in the past could now be stratified prognostically by cTn. This even extended to the response to treatment as it could be shown that patients with unstable angina and elevated cTnT benefitted from treatment with the platelet inhibitor abciximab while patients with normal cTnT did not [33–37].

The initial version of the new definition of MI was specified and extended in 2007, 2012 and 2018 [38–40]. The current fourth universal definition of MI from 2018 introduced the concept of myocardial injury which is solely based on elevation of cTn, while myocardial infarction still requires additional evidence of myocardial ischemia. It should be noted here that the initial definition of MI implied that cTn are markers for myocardial necrosis. The term myocardial injury takes into account that ample evidence accumulated that cTn can be released into the bloodstream without myocyte necrosis (see below). This has become obvious with the novel high-sensitive assays for cTn (hs-cTn) which detect much lower concentrations than the initially available assays. Unfortunately, the distinction between myocardial injury and myocardial infarction appears somewhat arbitrary. Clinical evidence of an ischemic cause turns acute myocardial injury into myocardial infarction, i.e. myocardial cell death. While infarction implies irreversible cell death, myocardial injury may be considered reversible. This is also obvious from the potential causes of cTn release into the circulation which include increased cellular membrane permeability and even normal cell turnover.

With the hs-cTn assays the long debated question whether cTn is released into the circulation only by necrosis has gained new momentum. This is easily illustrated by the fact that with the initial assays no increase in cTnI could be observed in marathon runners which was taken as evidence of the high cardio-specificity compared to creatine kinase [41, 42]. Interestingly, the first report on possible increases of cTn after strenuous exercise used an assay for cTnT which had on LoD of approx. 0.1 µg/L [43]. This was much lower than the cTnI assays available at that time, even though much less sensitive than current assays. Today one of the best documented and most intriguing cases of acute myocardial injury (according to the current definition) is observed in endurance sports. A relevant proportion of persons participating in such activities as marathon running or long distance biking will experience increases of cTn which exceed the 99th percentile of the respective assay and fulfil the definition of myocardial injury. Practically all affected athletes are free of any symptoms besides perhaps exhaustion. Accordingly, the clinical relevance of such elevations of cTn, can only be assessed by long-term follow-up. Available data on the underlying pathophysiologic mechanisms and the frequency of the phenomenon have been summarized recently in an excellent review [44]. While the evidence suggests that cTn can be released from cardiomyocytes without permanent damage to the heart, data are at present inconclusive. There is some indication that cTn elevations might be associated with an increased risk of future cardiovascular events in older persons, long-term data on younger persons are lacking. This is in contrast to chronic elevations of cTn in the range below the 99th

percentile. Several studies showed that cTn above median of the general population is associated with increased risk of future cardiovascular events [45–47]. A very recent study on this topic adds another piece to the puzzle. Arnadottir et al. determined cTn with three different assays after brief (30–90 s) occlusion of the left anterior descending coronary artery by a balloon catheter in patients without coronary artery disease. With 90 s occlusion all patients were described as having chest discomfort – a typical symptom of ischemia. There were significant increases in cTn within the following hours depending on the duration of vessel occlusion [48]. Of note, depending on the cTn assay 13–63% of patients with 90 s occlusion would have qualified as myocardial injury and in the strict sense myocardial infarction, because they developed symptoms of ischemia. Even though the study was small for obvious reasons, two results are remarkable: (1) short reversible ischemia is sufficient to release enough cTn into the circulation to be detected by the current hs-cTn assays, and (2) different cTn assays appear to have different sensitivity towards the cTn molecules released under these conditions. Under the assumption that short periods of ischemia do not cause myocyte necrosis, this study provides further evidence that cTn release does not require cell necrosis.

Standardization of assays for cTn

This work leads directly over to another open issue, i.e. standardization or at least harmonization of cTn assays. The apparent differences between cTnI and cTnT assays in sensitivity to cTn released by short episodes of cardiac ischemia might be explained by differences of release kinetics between cTnI and cTnT. In the case of the two cTnI assays it is apparent that the assays contrary to their designation do not measure exactly the same thing. It was hypothesized that the use a second capture antibody close to the carboxy-terminus of cTnI in one of the assays affects reactivity with proteolytic fragments of cTnI. This would suggest that perhaps different fragments of cTnI are released during reversible hypoxic stress and irreversible myocyte necrosis [49]. Since proteolysis of cTnI has been described early on, most pairs of antibodies used in cTnI assays target the central region of the protein which is considered to be relatively stable [50]. Thus, if proteolysis explains the difference between the two cTnI assays, one must postulate that it affects primarily the cTnI molecules released during acute ischemic stress rather than the chronically released background cTnI. Further research is needed to clarify, if and to what extent reversible injury to cardiomyocytes leads to the release of different molecular forms of cTnI which might be of diagnostic value. Thus, differences in the assay behaviour are appreciated, and attempts at harmonization of cTnI assays are underway, but have not yet achieved much change [51]. cTnT has been less affected by the issue of standardization simply by the fact that currently there is only one commercially available hs-cTnT assay. While this does not necessarily mean that proteolysis or other modifications are nor relevant for cTnT assays, there are only limited data.

Conclusions

The tremendous success of cTn as biomarker in clinical diagnostics of acute coronary syndromes observed over the last three decades has obvious reasons: First, there was a clear medical need for improved biomarkers of myocardial infarction. Second, cTn were included in large clinical studies which confirmed their diagnostic and even prognostic value. Third, robust and precise assays for high throughput were developed early on making cTn available for routine diagnostics. Of the three factors, medical need is probably the single most important determinant of the fate of novel biomarkers. Nevertheless, even after a quarter century of experience with cTn there are still open issues. Interestingly, some of those arose with the availability of highly sensitive assays and require further research. As in previous years new developments regarding development and application of novel and established biomarkers such as cTn will be a major focus of this journal.

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