

## Editorial

# Cardiac troponins: what we knew, what we know – where are we now?

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According to the recent report from the American Heart Association (AHA) Statistics Committee and Stroke Statistics Subcommittee, coronary heart disease (CHD) caused about one of every five deaths in the US in 2005. It is also estimated that 785,000 Americans will suffer from a new coronary attack, and 470,000 will have a recurrent attack in 2009. More worrisome, the AHA also highlights that ~37% of the people who experience a coronary attack in a given year will die from it. From 1995 to 2005, the annual death rate from CHD declined 34.3%, but the actual number of deaths only declined by 19.4% (1). These concerning estimates deserve major focus, since acute myocardial infarction (AMI) remains the leading healthcare problem worldwide. This calls for additional efforts for understanding the pathophysiology, identifying risk factors and reliable diagnostic and prognostic markers.

Remarkable advances in the understanding of the pathophysiology of myocardial injury over the past 20 years have enabled the identification of structural proteins and intracellular macromolecules to be used as effective biomarkers for both diagnosing and risk stratification of acute coronary syndrome. Proteins of the sarcomeric pool, particularly those of the troponin complex, were recognized as the most potentially effective markers for a variety of biological and technical reasons. As underscored by Hugo A. Katus, the discovery of cardiac troponin T (cTnT) was a typical example of serendipity. While analyzing the specificity of polyclonal goat antihuman cardiac myosin-light-chains antisera, Katus detected incidentally “a cardiospecific antibody fraction directed against cTnT contaminations of the myosin light-chains antigen” (2). This observation led to the purification of cTnT, the generation of monoclonal antibodies, and the development of a pilot enzyme immunoassay for circulating cTnT (3). It is remarkable that the same report by the group of Katus on myosin-light-chains (4) prompted the work on the “other cardiac troponin”, that is cardiac troponin I (cTnI), by Jack H. Ladenson and coworkers. As stressed by Ladenson, “... the results attributed to myosin light chain by Katus were probably attributable to cTnT”, and “... as there was no cardiac specificity with the myosin light chain assay developed by Edman Daud in my laboratory... we started to work with cTnI and to develop specific

antibodies” for the measurement of that biomarker (5). The discovery of cardiac troponins and the development of assays for their measurement in serum and/or plasma specimens has to be regarded as a major breakthrough in the biochemical approach to the diagnosis of the acute coronary syndrome. Foremost to its success was the proof of absolute cardiospecificity for both cTnT and cTnI, along with improved risk prediction in chest pain patients in many prospective multicenter trials. However, after the initial publications on cTnT and cTnI, more than 11 years of continuous work was required before the measurement of troponins as cardiac markers could finally be established in the clinical community. The milestone in this process was a paper published in 2000 by the working group of the European Society of Cardiology/American College of Cardiology/AHA (6). This paper introduced the redefinition of AMI, replacing the previous WHO definition established back in 1979 (7). This redefinition endorsed the diagnostic use of cardiac troponins instead of the so-called “cardiac enzymes”, which also included creatine kinase isoenzyme MB (CK-MB) mass. According to the recent Universal Definition of Myocardial Infarction (8) and the guidelines of the National Academy of Clinical Biochemistry (NACB) (9), cardiac troponins have been identified as the preferred biomarkers, whereas CK-MB mass is the second marker of choice, when troponin measurements are unavailable.

The current prevailing opinion, based on a huge amount of evidence collected to date, is that any reliably detected increase in cardiac troponins is abnormal, and may underlie cardiac necrosis (9). In turn, this led to the development and release of international guidelines and quality specifications that recommend the definition of “increased concentration of cardiac troponin” as a measurement value exceeding the 99th percentile upper reference limit (99th URL) (6). A desirable imprecision of <10% as estimated by the coefficient of variation (CV) is also recommended for values corresponding to the 99th URL. However, new generations of cardiac troponin assays have been developed in the last few years in order to improve the analytical performance of contemporary assays, with particularly increased low-end analytical sensitivity. Data gathered using these new generation cTnI and cTnT methods demonstrated that measurable troponin values might also be present in the blood of healthy subjects. While there are major concerns regarding the criteria used in some studies for recruiting and selecting healthy subjects, as well as other analytical problems, it seems to be time for revising the current paradigms on cardiac troponin release into blood. In this issue of *Clinical Chemistry and Lab-*

oratory Medicine, two interesting papers provide stimulating new insights and hypotheses on the mechanisms underlining the release of cardiac troponins and the associated analytical problems.

In the first paper, Giannoni et al. (10) discuss the intriguing working hypothesis that cardiac troponins can be released from cardiomyocytes of healthy adult subjects due to a process related to the "physiological renewal" of the human myocardium. This process, in turn, might be enhanced by physical exercise and/or aging. According to recent findings several pathological conditions are characterized by increases in plasma troponin concentrations in the absence of myocardial necrosis. However, the cellular mechanisms responsible for the release of these proteins in damaged and/or viable cardiomyocytes need to be better elucidated.

In the second paper, Mauro Panteghini provides a critical appraisal of factors influencing the definition of the 99th percentile limit of cardiac troponins which represents a key issue in the clinical interpretation and utilization of troponin results (11). Finally, Lippi et al. (12) review the potential clinical and analytical implications of antibody specificity in immunoassays, immunoreactivity of plasma isoforms released into the blood after myocardial injury, along with the interference from a variety of antibodies; all factors that might contribute to decreased diagnostic efficiency of troponin. An additional key point is the presence of polymorphisms in the genes encoding for both cTnI and cTnT. Although most of these genetic variants encode for dysfunctional protein, and are hence associated with inherited cardiomyopathies, such as hypertrophic (HCM), dilatative (DCM) and restrictive (RCM) cardiomyopathy, the cTnT Arg129Lys polymorphism and those observed in the stable domain of cTnI do not virtually alter the functional properties of the molecules in the myocyte, and are thereby predictably asymptomatic. Although the prevalence of these polymorphisms in the general healthy population is mostly unknown, it is predicted that they might influence (most likely decrease) the binding of monoclonal antibodies in vitro, thereby affecting the diagnostic performance of commercial immunoassays for detecting myocardial injury.

It seems mandatory that advancements in cardiac troponin assay methods, namely the development of highly sensitive assays, cannot create a "conundrum for clinicians and laboratory scientists" (13) and thus question the clinical usefulness of troponin measurements. To overcome the barrier for accurate interpretation of cardiac troponins in clinical practice, two proposals should be advanced. First, the validation of cardiac troponins must be based on evidence-based quality specifications. The recent proposal of an assay-dependent scorecard based on designations of the total imprecision (CV%) of each assay at the 99th percentile (12) seems to be an important tool for educating clinicians and laboratory professionals on the strengths and weaknesses of the cardiac troponin assays used in clinical practice. Then, further studies are needed for better elucidating the pathophysiology of release of cardiac troponins and to understand the

clinical significance of measurable concentrations in apparently healthy subjects, as well as in patients with chronic myocardial disease when troponins are measured using the new generation of high-sensitivity assays. Merging the analytical and pathophysiological developments, the clinical value of cardiac troponins will be maintained and even improved.

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