Review

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High sensitivity cardiac troponin assays in the clinical laboratories

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Abstract: Immunoassays measuring cardiac troponins I or T have become firmly established as critical tools for diagnosing acute myocardial infarction. While most contemporary assays provide adequate diagnostic performance, the increased sensitivity and precision of the new, high sensitivity assays that have already been introduced into clinical practice, provide the potential to further shorten intervals between blood draws or the time needed to detect the first significant troponin elevation. In addition to the relatively modest benefits at the diagnostic end, the high sensitivity assays and the investigational ultrasensitive cardiac troponin assays offer improvements for predicting major adverse cardiovascular events, development of heart failure or transition to end-stage kidney disease. These novel high sensitivity assays can measure troponin concentrations in 50%–100% of healthy individuals and therefore allow for the distribution of troponin values within a healthy cohort to be measured, patient’s baseline troponin levels to be monitored, and clinicians to be alerted of deteriorating cardiorenal conditions. We envisage that the high sensitivity assays will become important tools for predicting each patient’s risk of future adverse events and for guiding and monitoring corresponding adjustments of preventative therapeutic interventions.

Keywords: acute myocardial infarction; cardiac troponin I; cardiac troponin T; diagnosis; high sensitivity assay; prognosis; ultrasensitive assay.

Introduction

Assays for cardiac troponins I and T (cTnI and cTnT) have become widely accepted tools for diagnosing acute myocardial infarction (AMI). According to the latest universal definition of AMI, serial biomarker testing, preferably cTn measurement is a critical step for confirming or ruling out AMI. The fundamental clinical utility of cTn measurement in this regard is well established. However, a new generation of high sensitivity (hs) cTn assays has forced a reassessment of cTns, specifically of whether and how to best use increasingly sensitive cTn assays. While the increased sensitivity and precision of these novel assays promises somewhat faster rule-in and rule-out of AMI, this benefit comes with somewhat reduced specificity for acute ischemic cardiac events since the hs assays detect minor cTn elevations and changes in a multitude of other acute and chronic cardiac, as well as non-cardiac conditions. The first three parts of this article therefore review the basic facts about the biology and genetics of cTns and the classification of cTn assays into low, medium and hs, and ultrasensitive categories, and discuss the use of cTn assays for diagnosing AMI and the related questions of diagnostic cut-off and timing of testing.

The association between high cTn concentrations and adverse outcomes has been known for more than two decades. Using the hs assays that allow quantitation of cTns in essentially all individuals, we are now in a position to look beyond the established link between marked cTn elevations and adverse events. The new assays allow us to assess one’s baseline cTn concentration, monitor it over time, or quantitate cTn increases after an exercise tolerance test. All this information can be linked with the likelihood of developing heart failure (HF), another ischemic event, or with a probability of event-free survival. The final part of this review therefore focuses on potential use of cTn assays as short- and long-term prognostic tools.

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Structure and function of cardiac troponins

The heterotrimeric troponin complex, which plays an essential role in the regulation of excitation-contraction coupling in skeletal and cardiac muscle, consists of three troponin molecules with specific functions that are the basis for their designations. Troponin C (TnC, 18 kDa) binds calcium; troponin I (TnI, 24 kDa) inhibits the ATPase activity of the actomyosin complex; and troponin T (TnT, 37 kDa) mediates the attachment of the ternary complex of troponins C, I and T to tropomyosin. cTnI and cTnT are products of specific genes that are only expressed in the adult heart and are distinct from TnI and TnT expressed in skeletal and smooth muscles. This exquisitely specific pattern of expression underlies the use of cTnI and cTnT as biomarkers of cardiac injury. In contrast to cTnI and cTnT, TnC is also expressed in slow-skeletal muscle and has therefore not been considered as a potential cardiac marker [1].

There is a strong amino acid sequence homology between fast and slow skeletal and cardiac TnI in the region which binds to actin and is responsible for inhibition of the actomyosin ATPase, i.e., in the central stable region, while the degree of homology decreases towards the N-terminus [2]. Similarly to TnI, the homology between the three forms of TnT is the strongest in the central tropomyosin binding region and decreases towards the N- and C-terminal regions [3]. These homologies have to be taken into account when selecting antibodies specific for cTnI and cTnT.

The human genome holds three pairs of TnI and TnT genes. These undoubtedly arose by gradual triplication of an ancestral pair of TnI and TnT genes, which most likely originated by a duplication of an ancestral TnI-like gene. These three pairs of genes are located on chromosomes 1, 11, and 19. The genes encoding cTnI and cTnT are not located on the same chromosome; the gene encoding cTnI, TNNI3, is located on chromosome 19 together with the slow skeletal TnT gene, while the gene encoding cTnT, TNNT2, resides on chromosome 1 next to the slow skeletal TnI gene [1, 4]. Given their crucial function for muscle contraction, troponin genes are generally well conserved. Mutations in cTnI and cTnT have been implicated in the pathogenesis of hypertrophic, dilated, and restrictive cardiomyopathies [1, 4, 5].

Expression of TnI and TnT genes differs significantly between embryonic and adult hearts. The slow skeletal TnI is expressed in the hearts of embryos alongside cTnI [6]. At about 9 months after birth this expression pattern transitions to expression of cTnI alone [6, 7]. The cTnT gene is expressed in the heart both in prenatal and postnatal periods, yet its expression is more complex in that it involves four main alternatively spliced transcripts (cTnT1 through cTnT4) that are numbered according to decreasing molecular weight. cTnT1 and cTnT3 predominate in the embryo. cTnT4 along with a minor cTnT3 component become the predominant alternate transcripts postnatally [1]. There is evidence of a tendency towards reverting to fetal expression patterns in the ailing heart, and there are some rare cases of cTn expression outside the heart, which may complicate the overall picture [1, 8]. In addition, expression of cTnI in a uterine leiomyosarcoma has been described [9].

Approximately equimolar amounts of cTnI, cTnT and TnC are expressed at the protein level. These three comprise the ternary complex that is incorporated into the contractile apparatus of cardiac myocytes. cTnI and cTnT undergo regular turnover and replacement, with half-lives of 3.2 and 3.5 days, respectively [10, 11]. Replacement of cTn molecules occurs randomly along the thin filaments of differentiated adult rat cardiac myocytes rather than in an ordered fashion. This implies that maintenance of these filaments is performed while maintaining functionality, allowing for consistent cardiac activity [10]. The action of cTns may be modified through phosphorylation, mainly by protein kinase C, or cleavage by proteolytic enzymes [1, 12]. Different patterns of cTn phosphorylation have been reported in ventricular hypertrophy, HF and other conditions [13].

Most cTn molecules are bound to thin filaments as part of the structural pool of a cardiac myocyte. A small portion of cTn proteins, estimated at 2%–8% of the total cellular troponin [14] exists free in the cytoplasm as the cytoplasmic pool. This cytoplasmic pool is likely the source of the initial rise in serum cTn after myocardial injury, with subsequent release of cTn originating from the disintegrating structural pool.

Despite the widespread use of cTnI and cTnT for the diagnosis of myocardial damage, the mechanism of cTn release from cardiac myocytes is not completely understood. One obvious mechanism is that gradual degradation of non-viable cells results in cTn release; however, potential mechanisms leading to release of cTn in the absence of acute injury, if existent, are more problematic. The expression ‘troponin leak’ is frequently used, but it is unclear how the 24 kDa and 37 kDa cTnI and cTnT molecules would be released directly into circulation from viable cardiac myocytes. Perhaps the only mechanism that would allow for limited release of cTn and leave behind
viable cardiac cells is the formation of exosomes containing small amounts of the free, cytoplasmic cTn. Indeed, the formation of blebs in cardiac myocytes exposed to ischemia has been reported [15].

In our opinion, the most likely mechanism leading to the presence of cTn in circulation is myocyte death occurring either physiologically as part of the continuous renewal of cardiomyocytes or as a result of ischemic injury. The process of cardiomyocyte renewal is generally accepted. Recent data suggest that this process is rather slow, leading to a renewal of only approximately 40% of cardiac myocytes during one’s lifetime [16]. Further work is needed to determine if this level of cardiomyocyte turnover is feasible as a source of the low levels of serum cTn measured outside of acute coronary syndromes (ACSs).

The major forms of troponin released into circulation are cTnT and the cTnI-TnC complex. Additional forms found circulating in plasma are the cTnT-cTnI-TnC ternary complex and free cTnI [17, 18]. After release from the cardiac myocyte, cTn is degraded, fragmented, and gradually cleared from the circulation. Assessment of the overall cTn kinetics in the circulation is complicated by the fact that different cTn assays detect different cTn fragments of varying stabilities and half-lives [17–19].

The clearance largely depends on kidney function as has been demonstrated by multiple investigators. Wiessner et al. reported approximately 50% (38.4 h vs. 25.1 h) longer half-life of cTnI in patients with impaired renal function compared to subjects with normal creatinine clearance [20]. The effect of kidney function on cTnT levels is even more pronounced. Lippi and Cervellin compared the effect of decreasing glomerular filtration rate on plasma levels of cTnI and cTnT and found a several-fold higher effect on the cTnT levels when compared to cTnI [21]. This effect is undoubtedly at least partly responsible for suboptimal diagnostic performance of the hsTnT assay in patients with impaired kidney function [22]. Still, once the cut-off values are adjusted, the hsTnT assay retains solid predictive value in the stable dialysis population [23].

Cardiac troponin assays

History of cardiac troponin testing

The first cardiac-specific TnI assay was described in 1987 by Cummins et al. [24] and the first commercial cTnI assay was brought to the market by Dade Behring for use on the Stratus I analyzer. Compared to these first assays, current commercial cTnI assays are 100–1000 times more sensitive. Sensitivity of the investigational assays described below is an additional 10–100 times higher.

The first cTnT assay was developed by Katus and coworkers in 1989 [25]. The initial limit of detection (LOD) was 500 ng/L. As with cTnI, sensitivity of the original assay was about two orders of magnitude lower than that of the current hsTnT assay with LOD of 5 ng/L.

Classification of cardiac troponin assays according to sensitivity

Several ways of classifying cTn assays have been proposed. Some are based on their availability for clinical use [26] and some sort the assays by different generations [27]. Another term that has been used is ‘guideline-compatible’ [26]. However, such classification schemes are not ideal. Due to constantly changing market availabilities and guidelines, assays would be shuffled into different categories. A more systematic approach was proposed by Wu and Christenson who classified the assays based on the percentage of samples from healthy subjects that exceed the assay’s LOD [28]. In other words, a 30% assay would be able to detect cTn in 30% of reference population. Along similar lines, we use a simple designation of low, medium and hs assay, with the term ultrasensitive reserved for investigational assays that exceed requirements for the hs assays.

Low sensitivity (ls) assays

Low sensitivity (ls) assays refer to the oldest, first generation, and already outdated cTn assays (see ‘History of cardiac troponin testing’). These assays detected only marked elevations of cTn and would miss the less pronounced increases in cTn concentrations.

Medium sensitivity (ms) assays

Apple and others proposed to designate the assays that are currently on the market, in particular on the US market, as contemporary assays [26]. However, as previously mentioned, one drawback of this classification scheme is that as the clinical availability of the different cTn assays changes, so would their categorization as contemporary assays. Additionally, what is contemporary for a US reader of this article, is now outdated for readers outside US. We therefore prefer to call the assays currently available in the
US medium sensitivity (ms) assays. These assays reliably detect cTn concentrations exceeding the 99th percentile of a healthy reference population but can only quantitate cTn in a small fraction of healthy subjects. Ms assays have a performance target of measuring cTn with a 10% coefficient of variation at the 99th percentile of their reference population but this goal has been achieved by only a fraction of the ms assays.

High sensitivity (hs) assays

As manufacturers have worked towards increasing the sensitivity of their assays, the definition of hs in the area of cTn testing has been a moving target. The IFCC task force suggested in 2012 that in order to label a cTn assay as highly sensitive, cTn should be measurable in more than 50% of healthy subjects, and preferably in more than 95% [29]. In our opinion, an ideal hs assay should quantitate cTn in all healthy subjects, i.e., be a 100% assay in the Wu and Christenson classification described above [28]. This level of performance is achievable; in fact, it has been achieved by the latest investigational assays.

Clinical, fully automated assays with increased sensitivity are typically developed using multiple standard steps known to improve immunoassay performance. These interventions may include increases in sample volume and antibody concentrations, along with changes in antibody selection and extra blocking reagents suppressing background noise. Two hs assays are currently available in most countries – the fifth generation cTnT assay by Roche Diagnostics [30] (hsTnT) and the hs cTnI assay by Abbott Diagnostics (hsTnI) [31]. As of the time of writing this article, these assays are available in a number of countries but they have not been cleared by the FDA for clinical use in the US.

Ultrasensitive assays

While it is not clear that performance exceeding the standard proposed above, i.e., ability to quantitate cTn in all individuals, would bring additional value in the clinical setting, several investigational cTn assays have achieved further increase in sensitivity. We suggest designating such assays, which are capable of quantitating cTn at levels well below the lowest cTn concentrations seen in healthy subjects, as ultrasensitive. This additional sensitivity may be beneficial for selected novel applications of the cTn assays, such as measuring changes in cTn levels after exercise stress testing or using them for early detection of a rise in baseline cTn in patients receiving cardiotoxic chemotherapy or monitoring for drug-induced cardiac toxicity in experimental animal models [32].

Three novel platforms have been developed, which are utilizing different approaches to ultrasensitive analyte detection. The Singulex cTnl assay is a modified immunoassay with capture antibodies conjugated to paramagnetic particles. Multiple washes are used throughout the procedure to eliminate background noise. The fluorescently labeled detection antibodies captured on the surface of the cTnl-adsorbed beads are then eluted from the beads and quantitated using an instrument employing single molecule flow cytometry [33]. The Nanosphere cTnl assay is a doubly amplified sandwich immunoassay, which leverages the company’s Verigene nucleic acid detection protocol [34]. The Quanterix cTnl assay is another modified sandwich immunoassay. Troponin is captured by antibodies conjugated onto magnetic beads, followed by binding of an enzyme-labeled detection antibody. The beads are then deposited into femtoliter-scale wells slightly larger than the particles and enzyme substrate is added. The CCD-camera based reader counts the number of fluorescent wells on the grid, which reflects the cTnl concentration in the specimen. Quanterix introduced the term digital ELISA to describe this type of assay [35, 36]. Analytical characteristics of most ms, hs and ultrasensitive cTn assays are summarized in Table 1.

Harmonization of cardiac troponin assays

Necessary steps to standardize cTnl testing and the progress so far have been described in depth by Wu and Christenson [28]. Standardization of cTnl testing is indeed a formidable effort. In addition to various combinations of capture and detecting antibodies used in different cTnl assays (Figure 1A), each assay comes with its own incubation conditions, heterophile blocking reagents, and detection technology. It therefore comes as no surprise that well over a decade long effort to standardize cTnl assays has yielded only limited results, that there is no gold standard reference method available, and that it therefore is still difficult, if not impossible to compare patient results obtained using different cTnl assays. Multiple reasons why this effort is bound to fail were discussed by Apple who concluded that standardization of various cTnl assays is unlikely to happen anytime soon [38]. Given the multitude of reasons why each assay detects different forms of cTnl with different efficiency, we concur with his conclusions. While cTnl standardization is currently not achievable, there is an ongoing effort to harmonize available cTnl
Table 1  Analytical characteristics of cTn assays.

<table>
<thead>
<tr>
<th>Company/platform(s)/assay</th>
<th>LOD, ng/L</th>
<th>99th percentile, ng/L</th>
<th>%CV at 99th percentile</th>
<th>Epitopes recognized by antibodies used in the assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abbott Architect TnI</td>
<td>9</td>
<td>28</td>
<td>14.0</td>
<td>C: 87–91; D: 24–40</td>
</tr>
<tr>
<td>Abbott Architect hs-cTnI</td>
<td>1.1–1.9</td>
<td>19.3</td>
<td>4.0–6.0</td>
<td>C: 24–40; D: 41–49</td>
</tr>
<tr>
<td>Abbott AxSYM ADV TnI</td>
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<td>40</td>
<td>14.0</td>
<td>C: 87–91; D: 24–40</td>
</tr>
<tr>
<td>Abbott i-STAT TnI</td>
<td>20</td>
<td>80</td>
<td>16.5</td>
<td>C: 41–49; 88–91; D: 28–39, 62–78</td>
</tr>
<tr>
<td>Alere Triage Cardi 3 TnI</td>
<td>2</td>
<td>22</td>
<td>17.0</td>
<td>C: 27–39; D: 83–93, 190–196</td>
</tr>
<tr>
<td>Alere Triage SOB TnI</td>
<td>50</td>
<td>NA</td>
<td>NA</td>
<td>C: NA; D: 27–40</td>
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<tr>
<td>Beckman Coulter Access Accu-TnI</td>
<td>10</td>
<td>40</td>
<td>14.0</td>
<td>C: 41–49; D: 24–40</td>
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<tr>
<td>Beckman Coulter Access hs-cTnI</td>
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<td>8.6</td>
<td>10.0</td>
<td>C: 41–49; D: 24–40</td>
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<td>bioMerieux Vidas Ultra TnI</td>
<td>10</td>
<td>10</td>
<td>27.7</td>
<td>C: 41–49; 22–29; D: 87–91, 7B9</td>
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<tr>
<td>Mitsubishi PATHFAST TnI</td>
<td>8</td>
<td>29</td>
<td>5.0</td>
<td>C: 41–49; D: 71–116, 163–209</td>
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<tr>
<td>Nanosphere VeriSens hs-cTnI</td>
<td>0.2</td>
<td>2.8</td>
<td>9.5</td>
<td>C: 136–147; D: 49–52, 70–73, 88, 169</td>
</tr>
<tr>
<td>Ortho VITROS Troponin I ES</td>
<td>12</td>
<td>34</td>
<td>10.0</td>
<td>C: 24–40, 41–49; D: 87–91</td>
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<tr>
<td>Quanterix SiMoA TnI</td>
<td>0.01</td>
<td>NA</td>
<td>NA</td>
<td>C: 24–40; D: 86–90</td>
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<tr>
<td>Radiometer AQ90 FLEX TnI</td>
<td>9</td>
<td>23</td>
<td>17.7</td>
<td>C: 41–49, 190–196; D: 137–149</td>
</tr>
<tr>
<td>Radiometer AQ90 FLEX TnT</td>
<td>8</td>
<td>17</td>
<td>15.2</td>
<td>C: 125–131; D: 136–147</td>
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<tr>
<td>Response Biomedical RAMP TnI</td>
<td>30</td>
<td>NA</td>
<td>18.5 at 50</td>
<td>C: 85–92; D: 26–38</td>
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<tr>
<td>Roche Cardiac Reader cTnT</td>
<td>30</td>
<td>NA</td>
<td>NA</td>
<td>C: 125–131; D: 136–147</td>
</tr>
<tr>
<td>Roche Cobas h 232 TnT</td>
<td>50</td>
<td>NA</td>
<td>NA</td>
<td>C: 125–131; D: 136–147</td>
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<td>Roche Elecsys TnT 4th generation</td>
<td>10</td>
<td>NA</td>
<td>NA</td>
<td>C: 125–131; D: 136–147</td>
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<td>Roche Elecsys hsTnT</td>
<td>5</td>
<td>14</td>
<td>8.0</td>
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<td>Roche Elecsys TnI</td>
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<td>160</td>
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<td>Siemens ADVIA Centaur Tnl-Ultra</td>
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<td>8.8</td>
<td>C: 41–49, 87–91; D: 27–40</td>
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<td>Siemens Dimension VISTA CTNI</td>
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<td>45</td>
<td>10.0</td>
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<td>56</td>
<td>10.0</td>
<td>C: 27–32; D: 41–56</td>
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<tr>
<td>Siemens Dimension® RxL CTNI</td>
<td>40</td>
<td>70</td>
<td>20</td>
<td>C: 27–32; D: 41–56</td>
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<tr>
<td>Siemens IMMULITE® 1000 TnI</td>
<td>100</td>
<td>190</td>
<td>11</td>
<td>C: 87–91; D: 27–40</td>
</tr>
<tr>
<td>Siemens IMMULITE® 1000 Turbo TnI</td>
<td>150</td>
<td>300</td>
<td>14</td>
<td>C: 87–91; D: 27–40</td>
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<td>Siemens IMMULITE® 2000 XPI TnI</td>
<td>200</td>
<td>290</td>
<td>10.3</td>
<td>C: 87–91; D: 27–40</td>
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<tr>
<td>Siemens IMMULITE® 2500 STAT TnI</td>
<td>100</td>
<td>200</td>
<td>NA</td>
<td>C: 87–91; D: 27–40</td>
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<tr>
<td>Siemens IMMULITE®1000 Turbo TnI</td>
<td>150</td>
<td>NA</td>
<td>NA</td>
<td>C: 87–91; D: 27–40</td>
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<tr>
<td>Siemens Stratus® CS cTnI</td>
<td>30</td>
<td>70</td>
<td>10.0</td>
<td>C: 27–32; D: 41–56</td>
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<td>Singulex Erenna hs-cTnI</td>
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<td>10.1</td>
<td>9.0</td>
<td>C: 41–49; D: 27–41</td>
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<tr>
<td>Tosoh ST AIA-PACK TnI</td>
<td>60</td>
<td>60</td>
<td>8.5</td>
<td>C: 41–49; D: 87–91</td>
</tr>
</tbody>
</table>

Adapted with permission from the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) [37] December 2012 Version and from additional references [31]. LOD, limit of detection; 99th percentile, 99th percentile concentration; %CV at 99th percentile, percent CV (total imprecision) at the 99th percentile; C, D, amino acids recognized by the capture (C) and detection (D) antibodies on the cTnI or cTnT molecule. NAD, the 99th percentile concentration of the value distribution of a reference population is indeterminate, i.e., the 99th percentile is not adequately determined since more than 99% of control subjects have cTn concentrations < LOD. NA, data are not available.

assays by defining the extent of standards and by making the use of these assays and interpretation of test results as consistent as possible.

In contrast to cTnI, standardization of the cTnT assay is not an issue. Due to patent protection, the cTnT assay is only available from Roche Diagnostics and does not have to be standardized against cTnT tests from other manufacturers. Currently the major inconvenience of cTnT testing is the coexistence of a less sensitive fourth generation cTnT assay in the US and the hsTnT assay in most other countries. These two assays use the same monoclonal antibodies (Figure 1B) and only differ in ancillary reagents and reaction conditions. They should therefore be expected to produce practically identical results. Nevertheless, correlation between the two generations of the cTnT assays at the low end of the reportable range is non-linear, giving higher numerical results for the hsTnT assay than for the fourth generation cTnT [30]. An important consequence of
the concurrent availability of two cTnT assays is the existence of different diagnostic cut points and different diagnostic performance of the two assays.

Since cTnI and cTnT are part of the ternary complex with one cTnI, cTnT and TnC molecule each, they should be released from damaged cardiomyocytes in equimolar amounts. Nevertheless, cTnI and cTnT levels correlate poorly. This may be due to several mechanisms – different stability of cTnI and cTnT in the circulation [17], impact of kidney function on cTn clearance [20], and potentially different ratios of free cTnI and cTnT pools in the cytoplasm of the damaged myocytes, which is all further complicated by different performance characteristics of individual cTnI assays.

Cardiac troponin assays as a diagnostic tool

Universal definition of myocardial infarction

The first five World Health Organization (WHO) definitions in the 1960s and 1970s and subsequent WHO/MONICA (Multinational MONItoring of trends and determinants in Cardiovascular disease) definition in 1980 made ECG results and epidemiological data the basis for the diagnosis of AMI. As the number of cases with atypical symptoms and normal ECG kept increasing, it was becoming clear that the new definitions should take into account the increasing importance of biomarkers. The universal redefinition of myocardial infarction (MI) was formulated by a joint committee of the European Society of Cardiology and the American College of Cardiology and published in 2000 [39], followed by the second definition in 2007 [40], and the current third definition in 2012 [41–44]. According to these definitions, the classic, so-called type 1 MI is diagnosed based on the detection of a rise and/or fall of cardiac biomarker values (preferably cTn) with at least one value above the 99th percentile upper reference limit (URL) and with at least one of the following: 1) symptoms of ischemia; 2) new or presumed new significant ST-segment-T wave (ST-T) changes or new left bundle branch block; 3) development of pathological Q waves in the electrocardiogram; 4) imaging evidence of new loss of viable myocardium or new regional wall motion abnormality; and 5) identification of an intracoronary thrombus by angiography or autopsy.

Thus, in the three latest definitions, biomarkers, and namely cTn, have come to the center stage of the diagnostic podium, yet the use of cTn assays, their required
What constitutes the rise and/or fall in cTn concentrations?

The magnitude of the rise and fall in cTn values that is diagnostic of an AMI has been another subject of involved discussion and multiple publications. Some authors claim...
that absolute differences in cTn concentrations should be used, while others prefer relative changes, and yet others use a combination of absolute changes at lower concentrations and relative changes at higher troponin levels [50–52]. The hs cTn assays now allow monitoring patient’s cTn concentrations over time. An objective tool for the assessment of the significance of differences in serial results from an individual is provided by the reference change value (RCV) [53, 54]. RCV can be calculated from the imprecision of an assay and the short- and intermediate-term intra-individual variation in an analyte, in this case cTn. In order to use RCV for an early rule-in or rule-out of an AMI, one would have to know the baseline cTn value, which is typically only established upon presentation of a patient to ED. Another important, measurable parameter is the inter-individual variation, which reflects biological variations among healthy individuals from a defined population. A number of investigators measured intra- and inter-individual variations using various cTn assays [15, 55–60]. The results ranged from 4% to 30% for intra-individual variations and from 30% to 200% for inter-individual variations as summarized by Nordenskjöld and coworkers [60].

While not explicitly mentioned in the definition, the notion of rise and fall of cTn concentrations is fundamentally associated with the timing of testing. The constantly increasing sensitivity and associated higher precision of novel cTn assays allows for gradual reduction in time intervals between onset of symptoms and detection of cTn positivity [61]. Hs assays may allow reduction of the recommended 3-h intervals to 2 h [62]. However, after an attempt to further shorten the initial interval to 1½ h, investigators concluded that testing at 3 h is superior to testing at 90 min [63].

In a thorough and detailed evaluation of the diagnostic performance of cTn concentrations measured by the ms and hs cTnI assays and of their serial changes in the diagnosis of AMI, Keller and coworkers concluded that among patients with suspected AMI, hsTnI determination 3 h after admission may facilitate early rule-out of AMI. They also reported that the relative change in hsTnI levels from admission to 3 h after admission may facilitate an early diagnosis of AMI. Interestingly, the conclusions were similar when either an hs or an ms cTnI assay was used [64].

This is seemingly contradicted by Reichlin et al., who claimed that a simple algorithm incorporating hsTnT baseline values and absolute changes within the first hour allowed a safe rule-out and an accurate rule-in of AMI in 77% of randomly selected patients with acute chest pain [65]. This conclusion may have been aided by the high pre-test probability of MI in the patient cohort typical for the centers publishing these observations and may not apply to hospitals with significantly lower pre-test probabilities or EDs where cTn testing is sometimes broadly, albeit incorrectly, employed as a screening test.

Since essentially any significant acute increase in cTn concentrations should be apparent after 3 h from the initial blood draw, when one uses the hs assays, the question whether or not it is necessary to test at both 3 and 6 h was addressed by Biener et al. who concluded that non-ST-elevation MI may be ruled-in or -out at either 3 or 6 h with similar predictive values [66]. The authors also
concluded that absolute changes in cTn concentrations performed better than relative changes at both time points [66].

A very radical approach was undertaken in the recently presented Biomarkers in Cardiology-8 (BIC-8) study. This multicenter clinical trial assessed whether or not the addition of hs copeptin, a marker of cardiac stress, to hs cTn in patients with suspect AMI can increase the rate of early, safe hospital discharge. Investigators of this study claimed that a high proportion of patients could be discharged early, thus saving unnecessary treatments and ED time [67]. We regard this approach with caution as it does not include the essential concept from the universal definition of MI that a rise and/or fall in biomarker concentration should be observed. Additional studies are needed before this approach can be considered validated.

Based on the studies described above and additional analyses of the optimum timing of testing, it appears reasonable, for the time being, to follow the recommendation of the European Society of Cardiology for ruling in and ruling out AMI [68] and to measure cTn concentrations at 0, 3, and 6 h, with the 6-h time point offering rather reassurance than added diagnostic value.

**Additional considerations in diagnosing AMI**

The 99th percentile cut-off recommended for the diagnosis of AMI is useful only when applied to patients with a high pretest probability of AMI. Ordering of cTn testing in patients with a very low pretest probability of AMI, often as a precaution to prevent malpractice litigation, adversely affects the positive predictive value of cTn assays for diagnosing AMI. The markedly different pre-test probability of AMI among various centers, ranging from carefully triaged patients in specialized chest pain centers to unselected patients presenting to EDs leads to the discrepancies between remarkable performance of cTn assay reported by some authors [69, 70] and less impressive performance encountered by others [71]. This issue is currently further exacerbated by the simultaneous use of ms and hs assays.

Pretest probability of AMI must also be considered alongside likelihood of other conditions known to elevate cTn levels. These include a multitude of acute cardiac and non-cardiac conditions that may all present with both an elevation of cTn over the 99th percentile as well as dynamic changes in cTn concentration. No algorithm alone can distinguish with certainty AMI from other acute cardiac conditions such as myocarditis, arrhythmias, acutely decompensated HF and non-cardiac conditions such as pulmonary embolism or even strenuous exercise [72] in perfectly healthy individuals. A combination of clinical judgment, additional biomarkers, and diagnostic procedures may have to be employed to arrive at the correct diagnosis. Nevertheless, optimization of ‘troponin algorithms’ described in the previous section is an essential step in the standardization of this process.

Numerous chronic conditions are also associated with cTn elevations over the 99th percentile; however these are not associated with appreciable rise or fall in cTn levels. Conditions known to be associated with cTn elevations are listed in Table 2.

Figure 3 shows the typical ranges of cTn concentrations for some of the conditions associated with cTn increase. While the lowest and highest values are characteristic for healthy subjects and for patients with large AMI, cTn concentrations for many conditions are in the gray zone of greatest diagnostic uncertainty. In such a case, while cTn concentration and the kinetics of cTn rise and fall provides valuable information, this information must be interpreted in clinical context with the aid of additional diagnostic tools.

The recommended 3-h testing intervals may be relatively short for some patients and unnecessarily long for others whose cTn levels rise very quickly. Adherence to such schedules could be obviated by continuous monitoring of cTn levels using one of the cTn sensors in development [73–76]. Even if cTn is not monitored on a continuous basis, it is nearly impossible to adhere to an exact schedule of 3-h blood draws. We propose to determine the clinical significance of cTn changes by calculating the rate of cTn change, a ‘troponin velocity’, i.e., change in ng/L/h, in lieu of absolute changes.

Diagnosis of AMI would be aided by the knowledge of individual reference ranges, or rather individual baseline, together with each person’s the intra-individual analyte variability, or at least average intra-individual variability in a given population. Barring intra- and inter-day variability, baseline cTn levels are relatively stable and change only slowly with time [28, 59, 77, 78]. Hs assays allow establishing each person’s baseline cTn levels. Whenever available, these baseline cTn concentrations should be taken into account and may, at least at times, accelerate the rule-in/rule-out process. While the concept of an individual baseline is appealing, the evidence for its use is limited and additional studies of its utility are needed.

In addition to their potential diagnostic benefit, knowledge of patient’s baseline cTn levels and their monitoring over time would help to predict future adverse events and decide about optimum therapy for a given patient. Such use of cTn assays for risk prediction is described in the following section.
Cardiac troponin assays

The ability to identify patients at increased long-term risk of cardiovascular (CV) death, HF or stroke is a crucial step in prevention, which may include more aggressive pharmacotherapy, lifestyle changes, and other interventions. The short-term, in particular 30-day, prognosis is of particular importance to ED clinicians contemplating patient discharge versus admission with additional observation and diagnostic procedures. In addition, the 30-day readmission rate in patients with HF and other conditions is a closely watched parameter in today’s health care. Consequently any biomarker that may allow such a determination would be a valuable additional diagnostic and prognostic tool. cTn assays, especially the hs and ultrasensitive versions, can play an important role in that respect. The following sections describe some of the numerous studies addressing the short- and long-term predictive value of cTn assays. These studies are arranged by increasing sensitivity of the assays used. It should be emphasized that when mentioning patients with cTn> LOD of a particular assay, percentages of these subjects not only reflect the sensitivity of the assay used but also the patient population studied, with increasing detection rates in older patients, men, and patients with more serious morbidities.
Early reports on prognostic value of cTn assays

Prognostic applications of cTn testing are by no means limited to the hs assays. Reports on association between elevations in cTn and future adverse events date back more than two decades. As early as 1992, Hamm and coworkers [79] reported that measurable cTnT in the serum of patients with unstable angina (UA) was a short-term prognostic indicator for death or MI. However, due to the is of the assay with LOD of 200 ng/L, more than 10 times the 99th percentile of today’s cTnT assays, it appears that the positive results were rather diagnostic for AMI than for UA, which itself is a rapidly disappearing disease entity [80].

A similar study that followed a larger group of patients with unstable coronary artery disease (CAD) for 5 months concluded that the maximum cTnT value obtained during the first 24 h provides independent and important prognostic information for death or MI. In this study, the low risk group of patients with TnT <60 ng/L was somewhat closer to today’s 99th percentile [81].

The relationship between mortality at 42 days and the level of cTnI in the specimen obtained on enrollment of 1404 symptomatic patients with UA or non-Q-wave AMI was studied by Antman et al. [82]. Just as in the previous studies that used cTnT, the authors concluded that cTnI provides useful short-term prognostic information and permits early identification of patients at an increased risk of death. Patients in this study were stratified according to a cut point of 400 ng/L, again a very high value compared to the 99th percentiles of current hs and ultrasensitive cTnI assays that range from <10 to approximately 30 ng/L.

In sum, early prognostic applications of the ls cTn assays clearly demonstrated that marked elevations at baseline are associated with worse short-term outcomes. From today’s point of view this comes as no surprise as such elevations are diagnostic of significant cardiac damage which, in turn, is known to be associated with a decreased probability of event-free survival.

Prognostic applications of medium sensitivity cTn assays

Over the following decade, the sensitivity of cTn assays increased by about an order of magnitude. Improved assays allowed detection of all cTn concentrations exceeding the 99th URL cut-off but they still only probed the high end of the reference range. Consequently, they allowed stratification of patients according to the 99th percentile, i.e., as normal versus abnormal, an improvement over the earlier stratification at high cTn concentrations selected typically to offer the best discriminatory value. In addition, an increasing number of studies started to address the longer term predictive value of cTn by following patients for extended periods of time.

Evaluating the short-term prognosis, James et al. used an ms cTnT assay to study 30-day outcomes in patients with ACS from the GUSTO IV trial. They found that cTnT>100 ng/L was associated with greater 30-day mortality and that cTnT levels below the assay’s LOD of 10 ng/L had the highest predictive value for event-free survival [83]. In an additional study of subjects from the GUSTO IV trial, the fourth generation cTnT assay not only assisted in risk stratification of patients with non-ST-segment elevation ACS, but also helped to identify patients who benefited from early coronary revascularization.

Daniels et al. used the fourth generation TnT assay in specimens collected between 1997 and 1999 from 957 older adults participating in the Rancho Bernardo study. Study subjects were stratified into two cohorts depending on the absence or presence of detectable (≥10 ng/L) TnT and followed for mortality through 2006. Those with detectable baseline TnT were at increased risk of death compared to subjects with undetectable TnT, and this increased risk persisted for years [84]. Importantly, this conclusion was not only valid for the complete patient cohort, which included patients with known CAD, but also for the adults with no signs and symptoms of CAD at enrollment.

Using the TnI-Ultra assay, Leistner et al. concluded that minor increases in cTnI were associated with increased mortality and major adverse CV events independently of traditional risk factors in a large primary prevention cohort [85].

Blankenberg and coworkers evaluated 30 novel biomarkers from different pathophysiological pathways in 7915 subjects from the FINRISK97 population cohort with 538 incident CV events at 10 years. The researchers developed a biomarker score and validated it in 2551 men from the Belfast Prospective Epidemiological Study of Myocardial Infarction (PRIME) cohort (260 events). They demonstrated that cTnI was one of the few independent risk markers contributing information beyond the traditional model of CV risk assessment with hazard ratio per SD of 1.18 in the FINRISK97 male subjects [86].

Assessment of cTn levels can also be used in the prognosis of other medical conditions. cTn is frequently, and often very significantly, elevated in septic patients. A large retrospective study assessed whether or not elevations in the fourth generation cTnT levels measured at
admission are independently associated with in-hospital, short-term (30 days), and long-term (3 years) mortality in intensive care unit (ICU) patients admitted over the period of 6 years with sepsis, severe sepsis, and septic shock. Of the 926 patients studied, 645 (69.7%) patients had detectable cTnT levels. After adjustment for severity of disease and baseline characteristics, cTnT levels remained associated with in-hospital and short-term mortality [87]. There was no statistically significant association with long-term mortality.

Elevations in cTn have also been repeatedly reported in patients with chronic kidney disease (CKD) [20, 88, 89]. Lamb et al. compared the predictive value of ms cTnI and cTnT assays in 227 patients with CKD. Interestingly, all-cause mortality during a median 24-month follow-up was associated with abnormal, i.e., detectable cTnT, but not with abnormal cTnI in this study [90]. However, more sensitive cTnI assays displayed similar data to the cTnT results [91].

Fourth generation TnT assay was used to study 1000 patients with anemia, type 2 diabetes and CKD from the TREAT trial [92]. The TnT levels were elevated, i.e., detectable, in 45% of subjects and the TnT elevations were associated not only with the composite of all-cause death and end-stage renal disease (ESRD) but also with ESRD alone during a 4-year follow-up. It was concluded that measurement of TnT may improve the identification of patients with CKD who are likely to require renal replacement therapy, supporting thus a link between cardiac injury and the development of ESRD [93, 94]. It is quite likely that serial monitoring of cTnT, or cTnI, would help to identify patients with gradually worsening prognosis. However, the fact that only 45% had measurable baseline TnT levels precludes additional effective risk stratification. This and many similar results illustrate the limitation of the ms assays.

Prognostic applications of high sensitivity cTn assays

Our ability to measure cTn concentrations in at least 50% of all subjects using hs cTn assays makes these assays an essential tool for short- and long-term prediction of CV and other adverse events. Hs assays can establish each patient’s cTn baseline and monitor it over time. This may prove very important as the cTn reference ranges are quite wide, and most patients’ cTn concentrations are clustered at the low end of the reference range. Consequently, a patient’s troponin concentration may acutely increase by a few hundred percent and still remain below the diagnostic cut-off.

Most hs cTn studies published to date have used the hsTnT assay and have been performed in Europe as the hsTnT assay was the first hs assay approved for clinical use and has been widely available outside the US. Studies coming from the US use this assay on an investigational basis. Additional prognostic studies have been performed using Abbott hsTnI that received the CE mark in 2013 but has not been approved for clinical use in the US either.

Do the hs assays offer a better predictive value compared to the ms assays? In essentially all comparisons, the outcome is clear; the more sensitive the better albeit often not dramatically better. Haaf et al. compared hsTnI with two newer cTnI assays and the fourth generation TnT and concluded that the hsTnT assay was more accurate than the two TnI assays in the prediction of long-term mortality [95].

In the footsteps of their earlier study with third generation TnT assay, Lindahl et al. compared performance of the hsTnT assay in serum samples collected 48 h after randomization in 1452 ACS patients from the GUSTO-IV trial and concluded that the hsTnT assay identified more patients at an increased risk for subsequent cardiac events than the fourth generation cTnT assay [96].

Patients from the EARLY-ACS (Early Glycoprotein IIb/IIIa Inhibition in NSTE-ACS) and SEPIA-ACSI-TIMI 42 (Omapatrilat for the Treatment of Patients with NSTE-ACS) trials were combined for an evaluation of predictive value of cTn concentrations for major CV adverse events within 30 days using the hsTnI assay and the fourth generation cTnT assay. All patients had detectable hsTnI at baseline compared to 94.5% with detectable fourth generation cTnT. After adjustment for elements of the TIMI risk score, patients with hsTnI ≥99th percentile had a 3.7-fold higher adjusted risk of CV death or MI at 30 days relative to patients with hsTnI <99th percentile. Importantly, when stratified by categories of hsTnI, even very low concentrations demonstrated a graded association with CV death or MI. Use of sex-specific cut points did not improve prognostic performance [47].

de Lemos et al. studied 3546 individuals aged 30–65 years at enrollment in the Dallas Heart Study, a multiethnic, population-based cohort study who were followed for a median of 6.4 years. They found that cTnT concentrations, measured using the hsTnT assay, were associated with structural heart disease and subsequent risk for all-cause mortality [97].

Giannitsis et al. investigated whether there is an additional contribution of hsTnT to the risk of mortality prediction by N-terminal B-type natriuretic peptide (NT-proBNP) in 1469 patients with stable CAD enrolled in the Ludwigshafen Risk and Cardiovascular Health Study.
(LURIC). They found a significant association between initially abnormal hsTnT concentrations and decreased likelihood of survival. The simultaneous determination of NT-proBNP and hsTnT was superior for risk stratification compared to determining either marker alone. Addition of hsTnT was particularly beneficial for the prediction of 1-year mortality [98].

In patients with stable CAD, any detectable hsTnT level is significantly associated with all-cause mortality, CV death, and MI after adjustment for traditional risk factors and NT-proBNP [99]. Lindahl et al. compared four cTn assays in serum samples from 1335 patients with ACS. All patients were followed for 30 days for death and AMI, and for 1 year for mortality. They concluded that the hs assays had comparable prognostic properties and outperformed the less sensitive cTnI assay [100].

Hs cTn assays can also predict the risk of developing HF and forecast its clinical course. deFilippi et al. evaluated 4221 community-dwelling adults aged 65 years or older without prior HF who had cTnT measured at baseline using the hsTnT assay. The testing was repeated at 2–3 years. They found that baseline cTnT levels and changes in cTnT levels were associated with incident HF and CV death [101].

In another study of HF patients, Gravning and co-authors investigated prognostic value of hsTnT in a subcohort of 1245 patients with ischemic systolic HF from the CORONA trial. They concluded that hsTnT was a risk factor for the primary end point (CV death, non-fatal MI, and non-fatal stroke), as well as all-cause mortality, CV mortality, and the composite of CV mortality and hospitalization from worsening of HF. They concluded that elevated hsTnT levels provide strong and independent prognostic information in older patients with chronic ischemic HF [102].

Using the hsTnT assay, Eggers et al. investigated the associations of cTnT with CV disease and outcome in 940 men aged 71 years participating in the Uppsala Longitudinal Study of Adult Men. cTn was measurable in 872 subjects (92.8%) and independently predicted both fatal and non-fatal CV events including stroke. In this study, the relationship to outcome over 10 years of follow-up was mainly seen in men with prevalent CV disease suggesting that the prognostic value of cTnT in subjects free from CV disease is limited [103].

While the primary use of hs cTn assays is to diagnose ACS and predict adverse events in patients with CV conditions, they can serve as an important short-term outcome predictor in non-cardiac settings. Using the hsTnT assay, the prognostic significance of cTnT elevations in 451 critically ill patients measured within 12 h of admission to a non-cardiac medical ICU was studied. A total of 98% patients had detectable levels of hsTnT and 33% had levels above the diagnostic cut-off of a traditional fourth generation cTnT assay. Patients with higher hsTnT levels had markedly higher rates of in-hospital mortality and longer stays in the hospital and ICU [104]. In a similar outcomes study, hsTnT measured shortly after admission in 197 (62.9%) patients, with significantly higher rates in patients with severe sepsis. Both septic shock and rise of cTn predicted poor outcome [105].

### Ultrasensitive cTnI assays as potential prognostic tools

Once the hs assays reach or exceed 95% detection rate for cTn, further increase in sensitivity may not add significantly to their predictive value. At the same time, the exquisite sensitivity of these assays enables additional applications of cTn testing. Using the single-molecule cTnI assay, Sabatine et al. demonstrated that an increase in cTn concentration exceeding 1.4 ng/L in a specimen collected 4 h after exercise stress testing is associated with transient myocardial ischemia and with higher incidence of adverse outcomes during an 8-year follow-up [106].

Using the same assay, elevated values of cTnI were associated with adverse long-term CV outcomes in patients stable through 30 days after ACS. Moreover, there was a gradient of risk even within the normal range of cTnI. Very importantly, patients with elevated cTnI benefited the most from intensive statin therapy [107].

### Additional considerations in predicting adverse events

Measurement of cTn concentration both at baseline and over time in conditions known to be associated with elevated cTn levels (see Table 2) may prove beneficial and may guide medical therapy and other therapeutic interventions. No major diagnostic company nowadays spares resources when it comes to development of novel, hs cTn assays. This effort comes hand in hand with establishing optimum reference ranges, or diagnostic cut-offs, for each assay. While the primary goal of these efforts is to develop the best assay for diagnostic purposes, prognostic information obtained using these assays is becoming increasingly important.

Due to the fact that the reference ranges are wide due to gender and age differences, interpersonal variability, and other factors, the importance of establishing patient’s...
cTn baseline cannot be overemphasized. This, in fact, may be one of the true advantages of the hs cTn assays that at times offer only incremental benefit when it comes to their diagnostic and prognostic use [108]. While impractical in the general population, a number of settings may warrant repeat cTn testing. As the rise in cTn has been shown to predict rejection of a transplanted heart, serial cTn testing might be warranted in transplant recipients [109]. Similarly, patients who are about to undergo potentially cardiotoxic therapy may benefit from serial cTn testing and a switch to a different therapy when a predetermined allowable increase in cTn is exceeded [32]. Monitoring of cTn concentration could be useful adjunct to serial monitoring of natriuretic peptides in patients with HF, which is gradually becoming accepted [110]. Minor elevations in cTn measured by hs or ultrasensitive assays may be one of the first preclinical signs in hypertrophic cardiomyopathy and may help to detect family members at risk without the need of large-scale genetic screening [111].

**Conclusions**

cTnI and cTnT assays are indispensable tools for the diagnosis of AMI and other acute events associated with cardiomyocyte injury. No other biomarker is likely to replace cTnI and cTnT for the diagnostic purposes in the near future. Clinical implementation of the hs cTn assays will be associated with modest gains on the diagnostic side, with the exception of patients whose baseline cTn concentrations were established prior to the acute event. At the same time, the hs and ultrasensitive cTn assays will be frequently used as valuable prognostic tools, either alone or in combination with other CV biomarkers.

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**References**


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