

## Measurement of cardiac troponins revisited

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### ABSTRACT

Elevated cardiac troponins indicate myocardial necrosis and have a high clinical sensitivity and complete myocardial tissue specificity. The recently released document by the Global Task Force on the universal definition of myocardial infarction (MI) has strengthened their role as the main criterion for MI definition. It is therefore pivotal that these clinically relevant biomarkers, on which important diagnostic decisions will rest, are measured with highly reliable methods. This paper will specifically address the characteristics of the currently marketed assays for the measurement of cardiac troponins, discussing the science underlying the use of these biomarkers and how to interpret their values properly.

### INTRODUCTION

Troponin is a component of a protein complex that regulates the contraction of striated muscle. It is found periodically along the thin filament of the myofibrils, in conjunction with the protein tropomyosin. The troponin complex consists of three distinct components: troponin C (the calcium binding element), troponin I (the actinomyosin ATPase inhibitory element), and troponin T (the tropomyosin binding element). The ICT troponin complex serves to regulate the calcium-dependent interaction of myosin and actin and thus plays an integral role in muscle contraction (1). Troponins I and T exist in three distinct molecular forms, which correspond to specific isoforms found in fast-twitch skeletal muscle, slow-twitch skeletal muscle, and heart tissue, respectively. The skeletal isoforms are similar in molecular size, approximately 20 kDa, and exhibit amino acid sequence heterogeneity of approximately 40%. The cardiac isoform also exhibits about 40% sequence heterogeneity with respect to the skeletal isoforms and has an additional 31 residues at the amino (N)-terminus. Thus, it is possible to distinguish immunologically cardiac troponins I (cTnI) and T (cTnT) from skeletal troponins (2). In the myocyte, troponins are found as structural (bound) proteins and as a small free pool that exists in the cytosol, which is about 6-8% for troponin T and 3.5% for troponin I. Immunoassays have been developed, which recognise cardiac forms of both troponins and react with complexed ternary (ICT) and binary (IC) forms, but do not crossreact with skeletal forms.

Troponins can be found in blood in at least two main forms, free and complexed. However, other forms are also found in blood following progressive modification of the molecule including oxidation, reduction and phosphorylation of proteins, and degradation forms. While some assays can differentiate between these forms, the clinical significance of some of these forms is not known at this time. Investigators have examined the

release of cTnT and cTnI into the blood of patients after acute myocardial infarction (AMI). Following myocardial damage, cardiac troponins appear in blood after 2-4 h. They persist long enough (up to 5-14 days) for convenient clinical use. The main form of cTnI antigen found in blood of AMI patients after tissue release is the binary IC complex, with smaller amounts of the ternary ICT complex, binary IT complex and free cTnI (3). cTnI is sensitive to proteolysis; in particular the N- and carboxyl (C)-terminal parts of molecule are less stable compared with the mid-fragment region of cTnI, at amino acid residues 30-110, which is more stable possibly due to protection by troponin C. cTnI can undergo proteolytic degradation not only in necrotic cardiomyocytes, but also in the bloodstream or in collected blood. This progressive modification of cTnI in tissue and in blood leads to a number of different plasma forms of troponin. Labugger et al. (4) showed there were up to 11 degradation products of cTnI present in sera from AMI patients, three of these being of a higher molecular mass.

Recent evidence suggests that cTnT is fragmented into pieces of 8-25 kDa in size that are physiologically released in very small quantities into the blood and are sufficiently small enough to be cleared by the healthy kidney (5).

### PRINCIPLES OF ANALYSIS AND CURRENT TROPONIN ASSAYS

The first RIA for cTnI determination using polyclonal antibodies was described in 1987 (6). Five years later, two independent groups of researchers developed specific monoclonal antibodies for cTnI (7, 8). In 1993, the first commercial immunoassay - a manual ELISA - for cTnI became available from Sanofi Pasteur (9) and the first clinical application of cTnI measurement to diagnose myocardial injury was described (10). In the same year, a prototype automated assay for cTnI on the Baxter Stratus, which utilized two monoclonal antibodies

specific for the cardiac isotype, was presented and the first clinical results were published (11). As time from initiation of a measurement run to first result was only 10 min, this became the first widely-used assay for cTnI in clinical practice.

A double antibody, two-step ELISA for detecting cTnT in serum was obtained in 1989 and the first clinical evaluation related to this assay published two years later (12, 13). Practically, some problems occurred about specificity of this first generation cTnT assay, using an antibody showing a significant crossreactivity with skeletal isoforms of troponin T (14). A later reformulation of the cTnT assay, structured with two cTnT-specific antibodies, avoided, however, this interference effect (15).

A large number of assays for cTnI and cTnT are currently commercially available, using a range of different formats and combinations of mouse monoclonal and/or polyclonal antibodies. Assays for cTnT and cTnI are two- or three-site immunoassays. Only one vendor (Roche Diagnostics) offers the cTnT assay because it is protected by patents. All the assays are of the capture type where an immobilized antibody specifically binds the troponin present in the sample. The captured troponin is then reacted with a second antibody and in some assays a third antibody that is coupled to an indicator molecule. The assays vary from each other by the types of antibody used, by the epitopes to which they bind, and by the type of indicator molecule that is used. Detection is by spectrophotometric, fluorescent, chemiluminescent, and electrochemical methods. A summary of troponin methods is presented in the Appendix.

Differences in clinical utility between cTnT and cTnI results have not been demonstrated. The most important clinical considerations for a troponin assay may be its accessibility (how easily can the assay be run at all hours), its analytical sensitivity and overall imprecision.

## STANDARDIZATION OF TROPONIN MEASUREMENTS

Differences in results exist between cTnI assays due to the nonstandardization of measurements. Variations of between 20- to 40-fold and as much as a 100-fold have been reported among first generation assays (16-18). More recently, however, this variation is reported to be reduced to approximately 2- to 5-fold (19). Lack of comparable cTnI patient values may lead to confusion and problems of interpretation by clinicians who use results from different laboratories.

As cTnT assays are commercially available from only a single manufacturer, the comparability of results for cTnT is not a problem. The 3<sup>rd</sup> and 4<sup>th</sup> generation cTnT methods use recombinant human cTnT as calibration material. Purified recombinant human cTnT has been characterized by SDS-PAGE electrophoresis and its purity checked by western blotting and immunological activity (20). Its cTnT concentration is determined by protein assay and 4<sup>th</sup> generation cTnT values are traceable to the previous 3<sup>rd</sup> generation method.

Assay standardization is a top priority. It is important that clinically relevant biomarkers such as cardiac troponins, on which clinical decisions are based, are measured with standardized methods to achieve comparable results, regardless of the assay system or laboratory where the measurement is performed. The standardization of troponin measurements requires the availability of suitable reference materials for cTnI and cTnT and their value assignment through the use of higher-order reference measurement procedures (21, 22). Another factor that is likely to contribute to quantitative differences between cTnI assays is the variable antibody immunoreactivity to different forms that may be present in blood. Significant heterogeneity has been demonstrated in the reactivities of antibodies of different cTnI assays to the various forms in blood (23). This may result in the over- or under-estimation of the troponin concentrations in individual patient samples. Ideally, assays should recognize both complexed and free cTnI equally and their degraded fragments, oxidized, reduced, phosphorylated and nonphosphorylated forms to allow monitoring of total cTnI present in samples from the same subject over the course of an AMI. Due to proteolytic susceptibility of N- and C-terminal parts of cTnI, most currently available cTnI assays use antibodies that are directed to the mid-fragment epitopes, i.e. amino acids 30-110, which represent the more stable part of the molecule (24).

Apart from differing cTnI antibody specificity between assays, the main reason for poor agreement of values among methods is the lack of a suitable reference material for cTnI. Commutable serum-based material has been shown to improve harmonization of cTnI results (18, 25, 26). In addition to the differences in antibody specificity and calibration, differences in results may be due to the reaction formats used in different methods. For example, assays that use the same three anti-cTnI antibodies and declare traceability to the same purified ICT standard, but are performed on different platforms (i.e. Abbott AxSYM and Architect), still give different cTnI results (19).

Currently, there is no cTnI reference method that is able to value-assign a matrixed (serum-based) commutable reference material. Analytical methods commonly used in reference measurement procedures, e.g., mass spectrometry, lack the analytical sensitivity to measure the low cTnI concentration levels that occur in blood. As an alternative, the development of immunochemical reference procedures based on the availability of monoclonal antibodies with well-defined epitope specificity has been proposed (21, 22, 27). This approach requires first there is a clear definition of the measurand, which for cTnI is defined as a unique, invariant part of the molecule that is common to all forms present in blood and is unaffected by complexing with other molecules (28). Analytical requirements of the immunologic reference procedure should include: 1) comparable analytical specificity to commercial assays; 2) acceptable measurement uncertainty; 3) calibration against the purified troponin reference material SRM

2921 of the National Institute of Standards and Technology (NIST); and 4) technical validation by a network of laboratories (22). The specificity of the antibody for the defined epitope on the cTnI molecule and the ability of the assay to be unaffected by interferences will be most important in deciding on a reference procedure. Depending on the fulfilment of these requirements, the next step in standardization would be preparation of a commutable secondary reference material for cTnI and its value-assignment by the selected reference method (21). This material would be available for the calibration of manufacturers' internal procedures through a value transfer process (Figure 1).

**LIMIT OF BLANK, LIMIT OF DETECTION, AND LIMIT OF QUANTITATION OF TROPONIN ASSAYS**

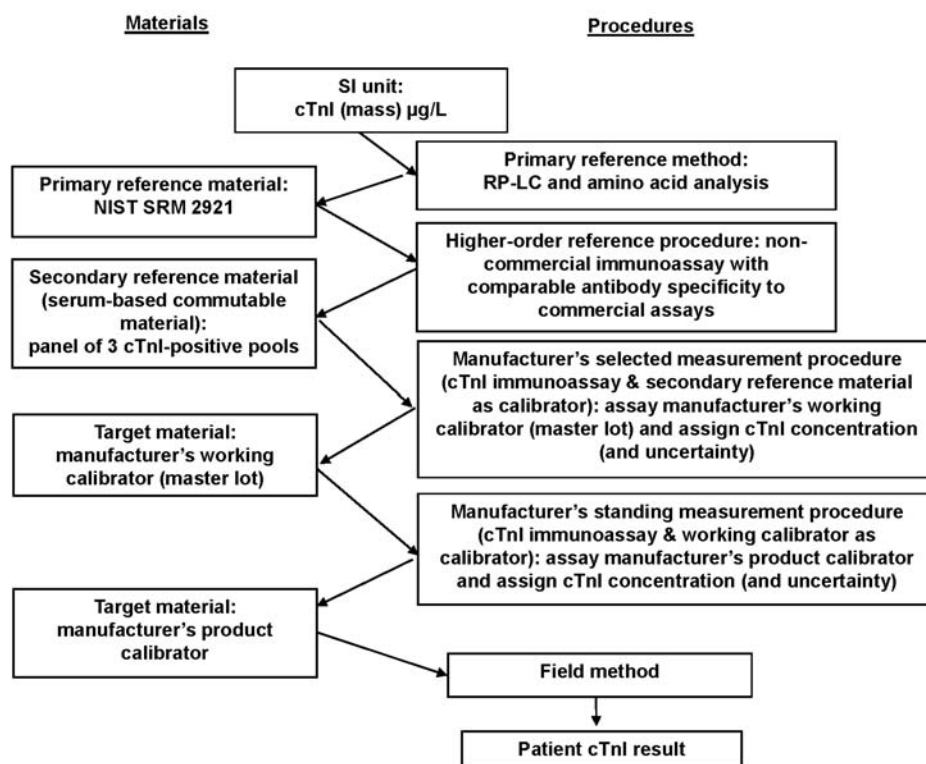
The assay detection limit (LoD) is defined as the lowest amount of cardiac troponin in a biological sample that can reliably be detected by a given analytical procedure (29). In defining LoD, it is important to distinguish it from limit of blank (LoB), which is defined as the highest measurement result that is likely to be observed (with a stated probability) for a sample that contains no troponin (29). Thus, the major difference in the estimation of LoD and/or LoB is the sample type

used. Zero calibrators, assay diluents, or serum troponin-free (recommended) are only useful for determining LoB. By contrast, adequate samples for LoD estimation should have troponin concentrations in the range from LoB to 4 x LoB (29). Calculations are  $LoB = mean_{blank} + 1.645 SD_{blank}$  (n=60) and  $LoD = mean_{blank} + 1.645 SD_{blank} + 1.645 SD_{sample}$  (n=60), respectively (29). As in the majority of cases (see Appendix), LoD is practically estimated as the troponin concentration corresponding to a signal 2 SD above the mean (n=20) for a sample in which troponin is absent, e.g. zero calibrator, it is not surprising that many commonly reported LoD values are lower than they would be if more rigorous procedures are used.

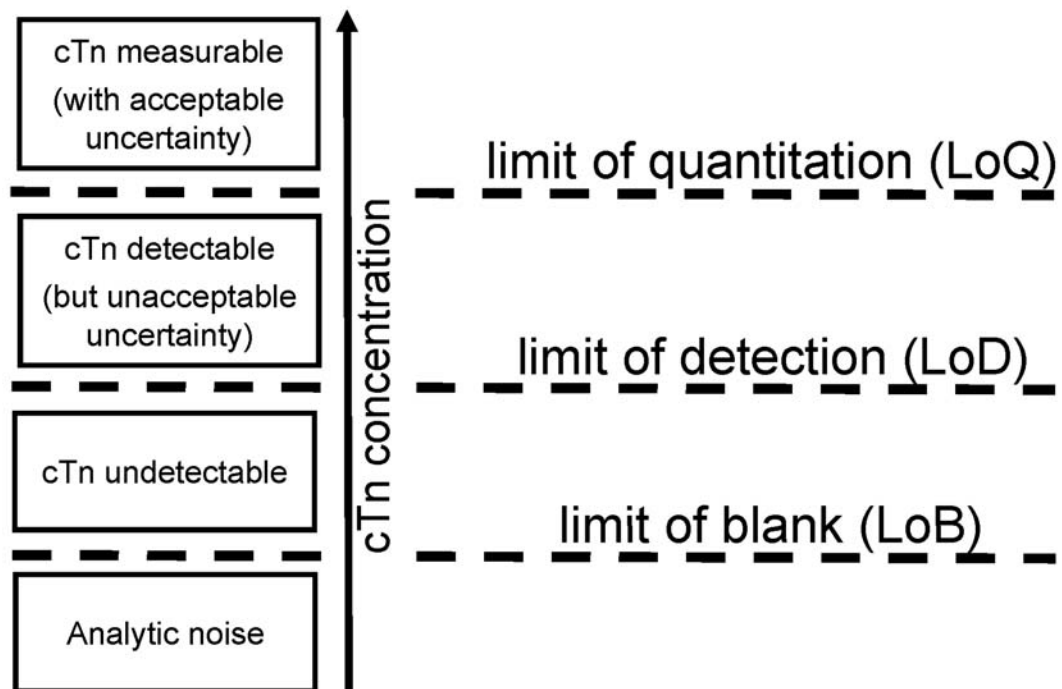
For the clinical application of cardiac troponin, the most important assay characteristic is, however, the limit of quantitation (LoQ), defined as lowest amount of troponin that can be quantitatively determined with stated acceptable uncertainty (30). As the experimental evidence has shown that an uncertainty  $\leq \pm 10\%$  at troponin decision limit permits physicians to keep diagnostic misclassification of evaluated patients lower than 1% (31), a maximal total error of  $\pm 10\%$  at the AMI decision limit is recommended.

Figure 2 displays the different types of limits defining the troponin assay performance.

Optimal discrimination between small myocardial



**Figure 1**  
Suggested approach for the standardization of cardiac troponin I (cTnI) measurements through traceability implementation to the reference measurement system. From ref. 22.  
RP-LC, reversed-phase liquid chromatography; NIST, National Institute of Standards and Technology; SRM, Standard Reference Material.



**Figure 2**  
Limits at low cardiac troponin (cTn) concentrations.

injuries vs. analytical noise requires assays that have low LoD and lower uncertainty at low troponin concentrations. Efforts to improve precision of troponin assays are warranted, therefore: irrespectively of how the testing is performed (in the central laboratory or at the bedside), an optimal precision performance is needed at the low end of the assay measurement range. Whereas the literature has been enriched with studies appropriately addressing the total imprecision of troponin assays, as to what the lowest concentration will be to attain a 10% CV (17, 19), the manufacturers' package inserts often report imprecision data primarily based on within-run or between-day evaluation of samples with troponin concentrations much higher than the corresponding AMI cutoff. Furthermore, troponin concentrations at 10% CV determined in clinical laboratories is frequently higher than quoted by the manufacturer, e.g., Siemens TnI-Ultra™ concentrations at 10% CV determined in two laboratories were 0.05 and 0.07  $\mu\text{g/L}$  compared with 0.03  $\mu\text{g/L}$  according to the manufacturer's package insert (19, 32).

### TROPONIN PERFORMANCE GOALS

Total imprecision values differ significantly among the currently available assays. The current recommendation for maximal allowable imprecision in troponin assays has been set at 10% CV at the proposed decision limit for AMI, i.e. the 99<sup>th</sup> percentile upper reference limit (URL) (33-36). However, assay imprecision at cTnI and cTnT concentrations close to this limit is approximately 20% CV for current assays (19). Survey data for two samples from the 2007 EQAS of the

Reference Centre for Quality Control of the Azienda Ospedaliero-Universitaria Careggi (Florence, IT) show inter-laboratory intra-assay CV of 7.1-20.6% for sample 02-2007 (concentrations ranged from 0.19 to 0.29  $\mu\text{g/L}$  for cTnI assays and 0.11-0.12  $\mu\text{g/L}$  for cTnT assays), and 7.6-27.2% for sample 07-2007 (concentrations from 0.16 to 0.24  $\mu\text{g/L}$  for cTnI assays and 0.12-0.13  $\mu\text{g/L}$  for cTnT assays).

Quality procedures should be implemented within the laboratory for reliable and reproducible performance of troponin assays, particularly at low concentrations, to avoid reporting of falsely positive results. In addition to regular monitoring of manufacturers' quality controls, daily measurement of a control material with a troponin concentration close to the cutoff level (in-house or manufactured if available) can detect assay drift or deterioration of assay performance (37). Long-term monitoring of troponin imprecision will take into account new troponin reagent lots, changes in reagent formulation, and any sub-optimal analyzer performance compared with a short-term evaluation.

### SPECIMEN

#### Sample type

Recommendations by the IFCC Committee on Standardization of Markers of Cardiac Damage (C-SMCD) and National Academy of Clinical Biochemistry (NACB) should be followed when collecting, transporting, and processing patient samples (28, 35, 38). If serum samples are used, blood should be allowed to clot. Care should be taken when preparing specimens

for testing from patients who have received anticoagulant therapy. These specimens may require additional time to clot. If plasma samples are used, blood is usually collected in a tube containing heparin anticoagulant. EDTA can split  $\text{Ca}^{2+}$ -dependent troponin complexes and decrease troponin concentration in assays that measure preferentially these molecular forms (39). Either serum or heparinized plasma may be used as sample type for most commercially available assays, or whole blood for some point-of-care methods. However, several studies report significant differences in troponins measured in serum and plasma (40). Binding of heparin to troponins may reduce their immunoreactivity as a result of interaction between the negatively charged glycosaminoglycan and basic amino acid residues on the troponin molecule. This effect is also dependent on the heparin concentration in sample collection tubes (41, 42). In some assays, the effect of heparin may be induced by changes within the sample matrix itself (43).

### **Sample stability**

Depending on the assay antibody configuration, sample stability is method-dependent creating a need for specific data for each commercially available assay (39). Diagnostic manufacturers should specify sample storage and stability conditions in their package inserts.

Results may be confounded if there is poor pre-analytical handling of troponin specimens, e.g., insufficient sample centrifugation and/or late separation of red cells from serum or plasma, presence of fibrin due to incomplete serum separation, etc.

### **INTERFERENCES**

Studies indicate that hemolysis can interfere with some troponin immunoassay procedures and both false positive and false negative results have been reported (44-46).

Other sources of interference that may affect the assay detection process and cause false-negative troponin values include: 1) ascorbic acid in immunoassays using alkaline phosphatase (ALP) in the detection reaction; 2) biotin in assays using biotinylated antibody; 3) streptokinase in the presence of streptavidin; and 4) high titres of antibodies to ruthenium or streptavidin in electrochemiluminometric cTnT assays. Generally, interference is method-dependent and may vary for each commercially available assay.

### **Heterophile antibodies**

One of the possible sources of interference for the sandwich-type immunometric troponin assays is endogenous antibodies directed against the proteins of nonhuman species, i.e. heterophile antibodies (HA). HA consist of natural antibodies and autoantibodies that are polyreactive against heterogeneous, poorly defined antigens of different chemical composition and generally

show low affinity, weak binding. Natural or autoimmune rheumatoid factors (RF) account for most heterophile interference in immunoassays. False positives in troponin assays arise by binding of RF to the Fc constant domain of antigen-antibody complexes if the detection antibody is labelled anti-human IgG (47-50). Antibody Fab fragments may prevent interference mediated by the Fc-part of intact antibodies (51).

Human anti-animal antibodies (HAAA) are high affinity, specific polyclonal antibodies produced against a specific animal immunogen whole immunoglobulin of IgG or IgM class. They show strong binding with antigen of a single chemical composition and are produced in a high titre such that they compete with the test antigen by cross-reacting with reagent antibodies of the same species to produce a false signal. HAAA are most commonly human anti-mouse antibodies (HAMA), but also include antibodies to rabbits, goats, sheep, etc. As with any assay employing mouse antibodies the possibility exists of interference by HAMA (52-54). One stimulus that is increasingly responsible for HAMA production is mouse monoclonal antibodies, which are used in diagnostic image analysis and immune directed therapy. A large percentage (~40%) of patients treated with radiolabeled mouse monoclonal antibodies developed HAMA within a few weeks of treatment (54).

While some first generation troponin assays were prone to interference from HA, next generation assays may be less susceptible to false-positive values (55). Using one of these assays, the current incidence of HA interference is reported to be 1 in 5000 (0.02%) (56). Apart from false-positive results, HA can cause falsely low results if they bind to the variable regions of the capture antibody, mimicking the antigen to be measured, and preventing troponin from binding. The most commonly used technique for minimizing the impact of HAMA on commercial immunoassays is the addition of nonimmune mouse immunoglobulin (IgG). The IgG should neutralize the more frequently encountered HAMA. Steps the laboratory can take to evaluate the possible presence of HA include the use of a larger dilution of the sample with reagent containing the nonimmune mouse IgG or performing the analysis on a differently configured assay, preferably one employing different species of reagent antibodies.

### **Autoantibodies**

Autoantibodies also have the potential to cause interference in troponin immunometric assay methods. False-positive or false-negative values may arise depending on whether the autoantibody-analyte complex partitions into the free or the bound analyte fraction. Bohner et al. (57) reported on a false-negative cTnI due to the presence of a circulating autoantibody (probably IgG), which showed high affinity for cTnI and prevented its recognition by the two-site immunoassays used. More recently, Eriksson and co-workers have suggested the incidence of circulating cTnI autoantibodies causing falsely negative troponin values as high as 3.5% (58, 59).

The practical importance of the phenomenon remains, however, unclear (60).

### TROPONIN REFERENCE INTERVALS

The European Society of Cardiology (ESC), American College of Cardiology Foundation (ACCF), American Heart Association (AHA), and World Heart Federation (WHF) recommend a single decision cut-point for cardiac troponins corresponding to the 99<sup>th</sup> percentile URL for the diagnosis of patients presenting with suspected AMI (36). In most studies on reference values, cTnI and cTnT are, however, largely undetectable in blood. Missov and DeMarco have suggested that the plasma concentration for cardiac troponins in healthy subjects is ~0.001-0.002 µg/L (1-2 ng/L) and results from a continuous microscopic loss of cardiomyocytes during normal life (61). Further improvement in troponin assay sensitivity is required to measure down to these suggested nanogram concentrations where troponin reference values may well be Gaussian-distributed. Using a highly sensitive assay Wu et al. (62) have suggested that the cTnI reference values are at least 10-fold lower than values detected by current assays.

Definition of the 99<sup>th</sup> percentile URL may also vary depending on the reference population used. In different studies, the sources of the reference individuals were apparently healthy subjects with no history or evidence of cardiac disease, blood donors, or individuals from the local community without evidence of diabetes mellitus, hypertension, known cardiac disease, or being treated for known hyperlipidemia and who tested negative for asymptomatic coronary artery disease by stress echocardiography (19, 43, 56, 63-69).

Detectability of troponin in apparently healthy individuals may indicate those at increased cardiovascular risk due to an underlying co-morbidity. In a general population study, a proportion of Swedish elderly men with no cardiac symptoms at the time of cTnI elevation detected went on to develop cardiac disease or had increased mortality at follow-up several years later (70). In a separate study of 3,557 American residents the estimated population prevalence of cTnT elevation was 0.7% (71). Follow-up of subjects with elevated cTnT indicated that small troponin elevation in this community population was associated with underlying cardiovascular disease or a high-risk phenotype for cardiovascular disease.

### POSTANALYTICAL PHASE: INTERPRETATION OF TROPONIN RESULTS

#### Diagnosis of AMI

A joint ESC/ACC/AHA/WHF Global Task Force has recently reaffirmed the definition of AMI to be used where there is evidence of myocardial necrosis due to prolonged myocardial ischemia (36). Criteria for diagnosis of AMI due to a primary coronary event (Type 1) or secondary due to an imbalance between oxygen supply and

demand (Type 2) are the detection of rise and/or gradual fall of cardiac troponin (the preferred marker) with at least one value above the 99<sup>th</sup> percentile URL *and* clinical evidence of ischemia, with at least one of the following:

- ischemic symptoms;
- electrocardiographic changes of new ischemia;
- development of pathologic Q waves on the electrocardiogram, or
- imaging evidence of new loss of functioning myocardium or new regional wall motion abnormality (36).

Whereas ST-segment elevation AMI (STEMI) requiring revascularization therapy does not require troponin assessment to begin therapy, serial biomarker testing is recommended for patients without diagnostic STEMI but who have clinical suspicion of AMI. When chest pain is atypical or electrocardiographic changes are nondiagnostic or absent, troponins provide central diagnostic information.

Various studies have shown that the degree of troponin elevation also has significant prognostic value with mortality rates increasing as troponin concentrations increase both in clinical trials of patients with acute coronary syndrome (ACS) and in the Emergency Department setting (72-74).

### Troponin elevations in the absence of acute coronary syndrome

Myocardial necrosis as signified by cardiac troponin elevation may not necessarily be due to prolonged myocardial ischemia related to coronary atherothrombotic disease as in AMI. Other non-ischemic pathophysiological conditions can cause myocardial necrosis and therefore elevations in cardiac troponin concentrations, e.g., hypoxia (lack of oxygen), chemical injury, physical (electrical, temperature, radiation) injury, immunologic injury, or infectious agents (Table 1) (75-77). Causes such as these should be considered when interpreting troponin results. In general, in Emergency Department populations, where there is a low pre-test probability (prevalence) of thrombotic coronary artery disease, elevation of troponin may be less predictive of ACS. Troponin elevation in the absence of ACS still has prognostic value and can predict adverse short- and long-term survival in different clinical conditions associated with myocyte necrosis.

### APPENDIX

#### SYNOPSIS OF CARDIAC TROPONIN METHODS

##### Abbott AxSYM ADV cTnI (2<sup>nd</sup> generation assay) (19, 78)

*Method:* microparticle enzyme immunoassay (MEIA) with fluorometric detection of enzyme-hydrolyzed fluorescent product.

*Principle:* cTnI in serum or plasma binds to microparticles coated with two different monoclonal anti-cTnI antibodies; each antibody is directed against a different antigenic site on the cTnI molecule, namely

**Table 1**

*Diseases causing elevation of cardiac troponins in blood in addition to acute myocardial infarction*

- Acute neurological disease (including ischemic stroke, subarachnoid hemorrhage)
- Acute limb ischemia
- Acute liver failure
- Acute rheumatic fever
- Cardiac trauma (including contusion, ablation, pacing, firing, cardioversion, catheterization, cardiac surgery)
- Carbon monoxide poisoning
- Cardiotoxicity from cancer therapy
- Congestive heart failure
- Critically ill patients, especially with sepsis
- Diabetic ketoacidosis
- End-stage renal failure
- Extreme exertion
- Heart transplantation
- Hypertension, including gestational
- Hypotension, often with arrhythmias
- Hypothyroidism
- Infiltrative diseases [including amyloidosis, scleroderma, glycogen storage disease type II (Pompe's disease), hemoglobinopathy with transfusion hemosiderosis, idiopathic hypereosinophilic syndrome]
- Mushroom poisoning
- Myocarditis/Pericarditis
- Postoperative noncardiac surgery
- Pulmonary embolism
- Thiamine deficiency (beriberi)

amino acids 24-40 and 87-91. After reaction, unbound substances are washed away and a third monoclonal anti-cTnI antibody (to amino acids 41-49) labeled with biotin conjugate added; after reaction, unbound conjugate is washed away and a second conjugate, ALP-anti-biotin antibody, is added. The fluorogenic substrate 4-methylumbelliferyl phosphate (MUP) reacts with the ALP antigen-antibody complex and the intensity of fluorescence generated by the product, 4-methylumbelliferone, measured at 450 nm is directly proportional to cTnI in the sample.

*Specimen:* serum or plasma (heparin).

*LoD:* <0.01 µg/L.

*Dynamic reportable range:* 0.02-22.8 µg/L.

*Imprecision (10% CV):* 0.16-0.56 µg/L.

#### **Abbott Architect cTnI (2<sup>nd</sup> generation assay) (19, 56, 79)**

*Method:* two-step, three-site immunometric assay with chemiluminescence detection.

*Principle:* cTnI in serum or plasma binds to paramagnetic microparticles coated with two distinct monoclonal anti-cTnI antibodies specific for different epitopes on the cTnI molecule, namely amino acids 24-40 and 87-91. After incubation and washing away of

unbound substances, a third monoclonal anti-cTnI antibody (directed to amino acids 41-49) labeled with acridinium conjugate is added; after reaction, unbound conjugate is washed away and hydrogen peroxide and sodium hydroxide are added to trigger a chemiluminescent reaction; the measured luminescence [as Relative Light Units (RLU)] is directly proportional to cTnI in the sample.

*Specimen:* serum or plasma (heparin).

*LoD:* <0.01 µg/L.

*Dynamic reportable range:* 0.01-50 µg/L.

*Imprecision (10% CV):* 0.032-0.055 µg/L.

#### **Abbott i-STAT cTnI (19, 41, 80)**

*Method:* two-site ELISA with electrochemical detection.

*Principle:* cTnI in whole blood or plasma contacts an electrochemical sensor in the single-use cartridge allowing the monoclonal (capture) and polyclonal (ALP-conjugated) antibody pair (both directed within the central 30 to 100 amino acids of cTnI) to dissolve into the sample. cTnI becomes labeled with ALP and is captured onto the sensor surface during the 7 min incubation step; wash fluid containing enzyme substrate is then applied to remove unbound substances and, at the same time, ALP bound to the antigen-antibody complex reacts

releasing an electrochemically detectable product; the generated amperometric signal is directly proportional to cTnI in the sample.

*Specimen:* whole blood or plasma (heparin).

*LoD:* <0.02 µg/L.

*Dynamic reportable range:* 0.02-50 µg/L.

*Imprecision (10% CV):* 0.10-0.14 µg/L.

### Beckman Coulter Access cTnI (2<sup>nd</sup> generation assay) (19, 63, 81-83)

*Method:* two-site immunoenzymometric assay with chemiluminescence detection.

*Principle:* cTnI in serum or plasma reacts with ALP-conjugated monoclonal anti-cTnI antibody and paramagnetic particles coated with a second monoclonal anti-cTnI antibody. The two monoclonal cTnI antibodies are directed to amino acids 24-40 (capture antibody labeled with ALP) and 41-49 (detection antibody attached to paramagnetic particles). After incubation, unbound sample and excess antibodies are washed away; cTnI sandwiched between the two antibodies is bound to the solid phase in a magnetic field; the chemiluminescent substrate Lumi-Phos 530 is added and the intensity of luminescence generated, measured with a luminometer in RLU, is directly proportional to cTnI in the sample.

*Specimen:* serum or plasma heparin (no EDTA).

*LoD:* <0.01 µg/L.

*Dynamic reportable range:* 0.01-100 µg/L.

*Imprecision (10% CV):* 0.06-0.10 µg/L.

### BioMerieux Vidas TnI-Ultra (2<sup>nd</sup> generation assay)

*Method:* one-step immunoassay sandwich method with fluorescent detection.

*Principle:* The Solid Phase Receptacle (SPR) serves as the solid phase as well as the pipetting device for the assay. The sample is transferred into the wells containing anti-cTnI antibodies labeled with ALP. The sample/conjugate mixture is cycled in and out of the SPR several times. This operation enables cTnI to bind with the antibodies fixed to the interior wall of the SPR and the conjugate to form a sandwich. Unbound components are eliminated during washing steps. Two detection steps are then performed successively. During each step, the substrate (MUP) is cycled in and out of the SPR. The conjugate enzyme catalyzes the hydrolysis of this substrate into a product (4-methylumbelliferone) the fluorescence of which is measured at 450 nm. The intensity of the fluorescence is proportional to the concentration of cTnI present in the sample.

*Specimen:* serum or plasma heparin (independent validation of heparin plasma samples not available).

*LoD:* <0.01 µg/L.

*Dynamic reportable range:* 0.01-30 µg/L.

*Imprecision (10% CV):* 0.08-0.10 µg/L.

### DiaSorin Liaison cTnI (43, 67)

*Method:* two-site immunometric assay with chemiluminescence detection.

*Principle:* cTnI in serum reacts with affinity-purified polyclonal goat anti-cTnI antibody (directed to amino acids 27-39) labeled with isoluminol and paramagnetic particles coated with a murine monoclonal anti-cTnI antibody (directed to amino acids 80-110); the intensity of luminescence generated is directly proportional to cTnI in the sample.

*Specimen:* serum or plasma EDTA (no heparin).

*LoD:* <0.023 µg/L.

*Dynamic reportable range:* 0.01-100 µg/L.

*Imprecision (10% CV):* 0.054-0.065 µg/L.

### Innotrac Aio cTnI (2<sup>nd</sup> generation assay) (84-86)

*Method:* "all-in-one" dry chemistry technology with time-resolved fluorometric detection.

*Principle:* all the specific reagents (the tracer and capture antibodies) are provided in a dry stable form within an assay cup. Addition of the sample together with a generic assay solution starts the immunoreaction. The antibody configuration of the assay adds a monoclonal antibody with an epitope in the N-terminal region of cTnI (amino acid residues 20-35) and one with an epitope in the C-terminal region (amino acid residues 185-200) to two mid-fragment cTnI antibodies (epitopes in the region of amino acid residues 35-55 and 80-95). Incubation is carried out under continuous shaking. A wash step is followed by a drying procedure after which the time-resolved fluorescence of the europium-tracer (proportional to cTnI in the sample) is read from the surface.

*Specimen:* whole blood, plasma (heparin) or serum.

*LoD:* <0.01 µg/L.

*Dynamic reportable range:* 0.01-100 µg/L.

*Imprecision (10% CV):* 0.04 µg/L.

### BioSite Triage cTnI (2<sup>nd</sup> generation assay)

*Method:* fluorometric enzyme immunoassay with fluorescence detection.

*Principle:* cTnI in whole blood or plasma is added to reader sample port via a transfer pipette; red blood cells are separated from plasma via a filter contained in the device; a fixed plasma volume reacts with fluorescent-conjugated anti-cTnI antibodies and the reaction mixture flows down the device until fluorescent antigen-antibody complex is captured on a discrete zone and fluorescence is detected; the generated fluorescence is directly proportional to cTnI in the sample.

*Specimen:* whole blood or plasma (EDTA).

*LoD:* <0.05 µg/L.

*Dynamic reportable range:* 0.05-30 µg/L.

*Imprecision (10% CV):* not determined.

### Mitsubishi Kagaku Iatron Pathfast cTnI

*Method:* one-step, three-site immunoenzymometric assay with chemiluminescence detection.

*Principle:* cTnI in whole blood or plasma reacts with magnetic particles coated with monoclonal anti-cTnI antibody (cTnI residues 41-49) and two ALP-conjugated monoclonal anti-cTnI antibodies, recognizing the cTnI

mid (residues 71-116) and C-terminal portion (residues 163-210). After removing unbound enzyme-labeled antibody a chemiluminescent substrate is added; the intensity of luminescence generated is directly proportional to cTnI in the sample.

*Specimen:* whole blood or plasma (heparin).

*LoD:* <0.02 µg/L.

*Dynamic reportable range:* 0.02-50 µg/L.

*Imprecision (10% CV):* not determined.

### **Ortho-Clinical Diagnostics Vitros ECi cTnI (2<sup>nd</sup> generation assay) (19)**

*Method:* three-site immunometric assay with chemiluminescence detection.

*Principle:* cTnI in serum or plasma reacts with horseradish peroxidase (HRP)-conjugated monoclonal anti-cTnI antibody (directed to amino acids 87-91) and biotinylated monoclonal anti-cTnI antibodies (directed to amino acids 24-40 and 41-49); cTnI sandwiched between the three antibodies is captured by streptavidin coated onto wells and unbound substances are washed away; signal reagent containing a luminol derivative and peracid is added together with a transfer reagent that leads to oxidation of luminol by HRP in the bound conjugate; the intensity of chemiluminescence generated is directly proportional to cTnI in the sample.

*Specimen:* serum, plasma heparin or EDTA (independent validation of heparin/EDTA plasma samples not available).

*LoD:* <0.01 µg/L.

*Dynamic reportable range:* 0.01-80 µg/L.

*Imprecision (10% CV):* 0.034 µg/L.

### **Roche Elecsys cTnT (4<sup>th</sup> generation assay) (37, 87)**

*Method:* one-step, two-site ELISA with electrochemiluminescence detection.

*Principle:* serum or plasma is mixed with biotinylated mouse monoclonal anti-cTnT antibody and monoclonal anti-cTnT antibody labeled with a ruthenium complex. After addition of streptavidin-coated microparticles, antibody-antigen-labeled antibody sandwich binds to the solid phase via interaction with biotin and streptavidin. The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of an electrode; application of a voltage emits chemiluminescence, which is measured by a photomultiplier and is proportional to cTnT in the sample.

*Specimen:* serum or plasma (heparin or EDTA).

*LoD:* <0.01 µg/L.

*Dynamic reportable range:* 0.01-25 µg/L.

*Imprecision (10% CV):* 0.03 µg/L.

### **Siemens (formerly Bayer) ADVIA Centaur cTnI-Ultra (2<sup>nd</sup> generation assay) (19, 32, 88, 89)**

*Method:* three-site immunometric assay with chemiluminescence detection.

*Principle:* cTnI in serum or plasma is captured by polyclonal goat anti-cTnI antibody (directed to amino

acids 27-40) labeled with acridinium ester and two biotinylated monoclonal anti-cTnI antibodies directed to amino acids 41-49 and 87-91. Biotin in the immune complex then binds to magnetic latex particles conjugated to streptavidin; after washing to remove unbound substances, acid and base reagents initiate the chemiluminescence reaction. The photons released are counted by the instrument photomultiplier and the RLUs detected by the system are proportional to cTnI present in the sample.

*Specimen:* serum, plasma heparin or EDTA (independent validation of heparin/EDTA plasma samples not available).

*Lower limit of detection:* <0.006 µg/L.

*Dynamic reportable range:* 0.01-50 µg/L.

*Imprecision (10% CV):* 0.03-0.07 µg/L.

### **Siemens (formerly Dade Behring) Dimension cTnI (2<sup>nd</sup> generation assay) (19, 82, 90, 91)**

*Method:* one-step, two-site immunoenzymometric assay with spectrophotometric detection.

*Principle:* cTnI in serum incubated with chromium dioxide particles coated with a monoclonal anti-cTnI antibody and ALP-labeled monoclonal anti-cTnI antibody, specific for the stable central region of the molecule, to form a particle/cTnI/conjugate sandwich. Unbound conjugate is removed by magnetic separation and washing; the ALP in the antibody conjugate dephosphorylates flavin adenine dinucleotide phosphate (FADP) to produce FAD. FAD then binds to apo D-amino acid oxidase to form active holo D-amino acid oxidase, thereby producing hydrogen peroxide which, in the presence of HRP, reacts with 3,5-dichloro-2-hydroxybenzenesulfonic acid and 4-aminoantipyrine to form a colored product that absorbs at 510 nm. The absorbance change is proportional to cTnI in the sample.

*Specimen:* serum (no plasma heparin or EDTA).

*LoD:* <0.02 µg/L.

*Dynamic reportable range:* 0.04-40 µg/L.

*Imprecision (10% CV):* 0.14-0.26 µg/L.

### **Siemens (formerly DPC) Immulite 2500 cTnI (2<sup>nd</sup> generation assay)**

*Method:* two-site immunometric assay with chemiluminescence detection.

*Principle:* cTnI in serum or plasma reacts with beads coated with monoclonal anti-cTnI antibody (against epitopes 24-40) and ALP-conjugated anti-cTnI antibody (against epitopes 80-110); the intensity of luminescence generated is directly proportional to cTnI in the sample.

*Specimen:* serum or plasma (heparin).

*LoD:* <0.20 µg/L.

*Dynamic reportable range:* 0.01-100 µg/L.

*Imprecision (11% CV):* 0.30 µg/L.

### **Siemens (formerly Dade Behring) Stratus CS cTnI (68, 92-95)**

*Method:* two-site immunoenzymometric assay with

fluorescence detection.

**Principle:** cTnI in serum or plasma is added onto glass fiber paper in a test pack containing a dendrimer-linked monoclonal anti-cTnI antibody; after incubation an ALP-labeled monoclonal anti-cTnI antibody is added; both antibodies are specific for the stable central region of the cTnI molecule. Unbound conjugate is removed by magnetic separation and washing; a substrate wash is applied and the ALP reacts with MUP; the generated fluorescence is proportional to cTnI in the sample.

**Specimen:** whole blood, plasma (heparin) or serum (no EDTA plasma).

**LoD:** <0.018 µg/L.

**Dynamic reportable range:** 0.01-50 µg/L.

**Imprecision (10% CV):** 0.06-0.10 µg/L.

### Tosoh AIA-Pack cTnI (2<sup>nd</sup> generation assay) (69, 91)

**Method:** two-site immunoenzymometric assay with fluorescence detection.

**Principle:** cTnI in serum reacts with ALP-conjugated monoclonal anti-cTnI antibody, directed to amino acids 87-91 of the cTnI molecule, and magnetic beads coated with another monoclonal anti-cTnI antibody, directed to amino acids 41-49. After incubation unbound sample and excess antibodies are washed away; cTnI sandwiched between the two antibodies is bound to the solid phase in a magnetic field; a fluorogenic substrate, MUP, reacts with the ALP antigen-antibody complex and the intensity of fluorescence generated by the product, 4-methylumbelliferone, is directly proportional to cTnI in the sample.

**Specimen:** serum (no plasma heparin or EDTA)

**LoD:** <0.02 µg/L.

**Dynamic reportable range:** 0.02-120 µg/L.

**Imprecision (10% CV):** 0.09-0.13 µg/L.

### REFERENCES

- Zot AS, Potter JD. Structural aspects of troponin-tropomyosin regulation of skeletal muscle contraction. *Annu Rev Biophys Chem* 1987;16:535-59.
- Katus HA, Scheffold T, Remppis A, Zehlein J. Proteins of the troponin complex. *Lab Med* 1992;23:311-7.
- Katrakha AG, Bereznikova AV, Esakova TV, et al. Troponin I is released in bloodstream of patients with acute myocardial infarction not in free form but as complex. *Clin Chem* 1997;43:1379-85.
- Labugger R, Organ L, Collier C, Atar D, Van Eyk JE. Extensive troponin I and T modification detected in serum from patients with acute myocardial infarction. *Circulation* 2000;102:1221-6.
- Diris JH, Hackeng CM, Kooman JP, et al. Impaired renal clearance explains elevated troponin T fragments in hemodialysis patients. *Circulation* 2004;109:23-5.
- Cummins B, Auckland ML, Cummins P. Cardiac-specific troponin-I radioimmunoassay in the diagnosis of acute myocardial infarction. *Am Heart J* 1987;113:1333-44.
- Larue C, Defacque-Lacquement H, Calzolari C, Le Nguyen D, Pau B. New monoclonal antibodies as probes for human cardiac troponin I: epitopic analysis with synthetic peptides. *Mol Immunol* 1992;29:271-8.
- Bodor GS, Porter S, Landt Y, Ladenson JH. Development of monoclonal antibodies for an assay of cardiac troponin-I and preliminary results in suspected cases of myocardial infarction. *Clin Chem* 1992;38:2203-14.
- Larue C, Calzolari C, Bertinchant JP, et al. Cardiac-specific immunoenzymometric assay of troponin I in the early phase of acute myocardial infarction. *Clin Chem* 1993;39:972-9.
- Mair J, Wagner I, Puschendorf B, et al. Cardiac troponin I to diagnose myocardial injury. *Lancet* 1993;341:838-9.
- Adams JE, Bodor GS, Dávila-Román VG, et al. Cardiac troponin I: A marker with high specificity for cardiac injury. *Circulation* 1993;88:101-6.
- Katus HA, Remppis A, Looser S, et al. Enzyme linked immunoassay of cardiac troponin T for the detection of acute myocardial infarction in patients. *J Mol Cell Cardiol* 1989;21:1349-53.
- Katus HA, Remppis A, Neumann FJ, et al. Diagnostic efficiency of troponin T measurements in acute myocardial infarction. *Circulation* 1991;83:902-12.
- Collinson PO. Cardiac troponin T in renal disease. *Clin Chem* 1995;41:1671-3.
- Muller-Bardoff M, Hallermayer K, Schröder AA, et al. Improved troponin T ELISA specific for cardiac troponin T isoforms: assay development and analytical and clinical validation. *Clin Chem* 1997;43:458-66.
- Apple FS, Maturen AJ, Mullins RE, et al. Multicenter clinical and analytical evaluation of the AxSYM troponin-I immunoassay to assist in the diagnosis of myocardial infarction. *Clin Chem* 1999;45:206-12.
- Panteghini M, Pagani F, Yeo KT, et al. Evaluation of imprecision for cardiac troponin assays at low range concentrations. *Clin Chem* 2004;50:327-32.
- Christenson RH, Duh SH, Apple FS, et al. Towards standardization of cardiac troponin I measurements. Part II: Assessing commutability of candidate reference materials and harmonization of cardiac troponin I assays. *Clin Chem* 2006;52:1685-92.
- Tate JR, Ferguson W, Bais R, et al. The determination of the 99<sup>th</sup> centile level for troponin assays in an Australian reference population. *Ann Clin Biochem* 2008;45:275-88.
- Hallermayer K, Klenner D, Vogel R. Use of recombinant human cardiac troponin T for standardization of third generation troponin T methods. *Scand J Clin Lab Invest* 1999;59(suppl 230):128-31.
- Panteghini M. Standardization of cardiac troponin I measurements: the way forward? *Clin Chem* 2005;51:1594-7.
- Panteghini M, Bunk DM, Christenson RH, et al. Standardization of troponin I measurements: an update. *Clin Chem Lab Med* 2008;46: in press.
- Wu AHB, Feng YJ, Moore R, et al. Characterization of cardiac troponin subunit release into serum after acute myocardial infarction and comparison of assays for troponin T and I. *Clin Chem* 1998;44:1198-208.
- Katrakha AG, Bereznikova AV, Filatov VL, et al. Degradation of cardiac troponin I: implication for reliable immunodetection. *Clin Chem* 1998;44:2433-40.
- Tate JR, Heathcote D, Rayfield J, Hickman PE. The lack of standardization of cardiac troponin I assay systems. *Clin Chim Acta* 1999;284:141-9.
- Newman DJ, Olabiran Y, Bedzyk WD, et al. Impact of antibody specificity and calibration material on the measure of agreement between methods for cardiac troponin I. *Clin Chem* 1999;45:822-8.
- Stenman UH. Immunoassay standardization: Is it possible, who is responsible, who is capable? *Clin Chem* 2001;47:815-20.
- Panteghini M, Gerhardt W, Apple FS, et al. Quality specifications for cardiac troponin assays. *International*

- Federation of Clinical Chemistry and Laboratory Medicine (IFCC). *Clin Chem Lab Med* 2001;39:174-8.
29. Clinical and Laboratory Standards Institute. Protocols for determination of limits of detection and limits of quantitation; Approved guideline. CLSI document EP17-A. 2004.
  30. WHO. Glossary of terms for biological substances used for texts of the requirements. Expert Committee on Biological Standardization. WHO unpublished document BS/95.1793. Geneva: World Health Organization, 1995.
  31. Sheehan P, Blennerhassett J, Vasikaran SD. Decision limit for troponin I and assay performance. *Ann Clin Biochem* 2002;39:213-6.
  32. Prontera C, Fortunato A, Storti S, et al. Evaluation of analytical performance of the Siemens ADVIA Tni-Ultra immunoassay. *Clin Chem* 2007;53:1722-3.
  33. Panteghini M, Apple FS, Christensen RH, et al. Use of biochemical markers in acute coronary syndromes. IFCC Scientific Division, Committee on Standardization of Markers of Cardiac Damage. *Clin Chem Lab Med* 1999;37:687-93.
  34. Alpert JS, Thygesen K, Antman E, Bassand JP. Myocardial infarction redefined - a consensus document of the Joint European Society of Cardiology/American College of Cardiology Committee for the Redefinition of Myocardial Infarction. *J Am Coll Cardiol* 2000;36:959-69.
  35. National Academy of Clinical Biochemistry and IFCC Committee for Standardization of Markers of Cardiac Damage Laboratory Medicine Practice Guidelines: Analytical issues for biochemical markers of acute coronary syndromes. *Clin Chem* 2007;53:547-51.
  36. Thygesen K, Alpert JS, White HD on behalf of the Joint ESC/ACCF/AHA/WHF Task Force for the Redefinition of Myocardial Infarction. Universal definition of myocardial infarction. *Eur Heart J* 2007;28:2525-38.
  37. Dolci A, Dominici R, Luraschi P, Panteghini M. 10% CV concentration for the fourth generation Roche cardiac troponin T assay derived from Internal Quality Control data. *Clin Chim Lab Med* 2006;44:1495-6.
  38. Panteghini M. The importance of analytical quality specifications for biomarker assays currently used in acute cardiac care. *Acute Cardiac Care* 2006;8:133-8.
  39. Panteghini M. Performance of today's cardiac troponin assays and tomorrow's. *Clin Chem* 2002;48:809-10.
  40. Gerhardt W, Nordin G, Herbert AK, et al. Troponin T and I assays show decreased concentrations in heparin plasma compared with serum: lower recoveries in early than in late phases of myocardial injury. *Clin Chem* 2000;46:817-21.
  41. Apple FS, Murakami MM, Christenson RH, et al. Analytical performance of the i-STAT cardiac troponin I assay. *Clin Chim Acta* 2004;345:123-7.
  42. Speth M, Seibold K, Katz N. Interaction between heparin and cardiac troponin T and troponin I from patients after coronary bypass surgery. *Clin Biochem* 2002;35:355-62.
  43. Pagani F, Stefani F, Chapelle JP, et al. Multicenter evaluation of analytical performance of the Liaison troponin I assay. *Clin Biochem* 2004;37:750-7.
  44. Wenk RE. Mechanism of interference by hemolysis in immunoassays and requirements for sample quality. *Clin Chem* 1998;44:2554-9.
  45. Hawkins RC. Hemolysis interference in the Ortho-Clinical Diagnostics Vitros ECI cTnI assay. *Clin Chem* 2003;49:1226-7.
  46. Lyon ME, Ball CL, Krause RD, Slotsve GA, Lyon AW. Effect of hemolysis on cardiac troponin T determination by the Elecsys 2010 immunoanalyzer. *Clin Biochem* 2004;37:698-701.
  47. Krahn J, Parry DM, Leroux M, Dalton J. High percentage of false positive cardiac troponin I results in patients with rheumatoid factor. *Clin Biochem* 1999;32:477-80.
  48. Dasgupta A, Banerjee SK, Datta P. False-positive troponin I in the MEIA due to the presence of rheumatoid factors in serum. *Am J Clin Pathol* 1999;12:753-6.
  49. Banerjee S, Linder MW, Singer I. False-positive troponin I in a patient with acute cholecystitis and positive rheumatoid factor assay. *Cardiology* 2001;95:170-1.
  50. Yeo KT, Storm CA, Li Y, et al. Performance of the enhanced Abbott AxSYM cardiac troponin I reagent in patients with heterophilic antibodies. *Clin Chim Acta* 2000;292:13-23.
  51. Ylikotila J, Hellström JL, Eriksson S, et al. Utilization of recombinant Fab fragments in a cTnI immunoassay conducted in spot wells. *Clin Biochem* 2006;39:843-50.
  52. White GH, Tideman PA. Heterophilic antibody interference with CARDIAC T quantitative rapid assay. *Clin Chem* 2002;48:201-3.
  53. Covinsky M, Laterza O, Pfeifer JD, Farkas-Szallasi T, Scott MG. An IgM  $\gamma$  antibody to *Escherichia coli* produces false-positive results in multiple immunometric assays. *Clin Chem* 2000;46:1157-61.
  54. García-Mancebo ML, Agulló-Ortuño MT, Gimeno JR, et al. Heterophile antibodies produce spuriously elevated concentrations of cardiac troponin I in patients with *Legionella pneumophila*. *Clin Biochem* 2005;38:584-7.
  55. Makaryus AN, Makaryus MN, Hassid B. Falsely elevated cardiac troponin I levels. *Clin Cardiol* 2007;30:92-4.
  56. Lam Q, Black M, Youdell O, Spilsbury H, Schneider HG. Performance evaluation and subsequent clinical experience with the Abbott automated Architect STAT troponin-I assay. *Clin Chem* 2006;52:298-300.
  57. Bohner J, von Pape KW, Hannes W, Stegmann T. False-negative immunoassay results for cardiac troponin I probably due to circulating troponin I autoantibodies. *Clin Chem* 1996;42:2046.
  58. Eriksson S, Junikka M, Laitinen P, et al. Negative interference in cardiac troponin I immunoassays from a frequently occurring serum and plasma component. *Clin Chem* 2003;49:1095-104.
  59. Eriksson S, Halenius H, Pulkki K, Hellman J, Pettersson K. Negative interference in cardiac troponin I immunoassays by circulating troponin autoantibodies. *Clin Chem* 2005;51:839-47.
  60. Panteghini M. Selection of antibodies and epitopes for cardiac troponin immunoassays: should we revise our evidence-based beliefs? *Clin Chem* 2005;51:803-4.
  61. Missov ED, De Marco T. Clinical insights on the use of highly sensitive cardiac troponin assays. *Clin Chim Acta* 1999;284:175-85.
  62. Wu AHB, Fukushima N, Puskas R, Todd J, Goix P. Development and preliminary clinical validation of a high sensitivity assay for cardiac troponin using a capillary flow (single molecule) fluorescence detector. *Clin Chem* 2006;52:2157-9.
  63. Uettwiller-Geiger D, Wu AH, Apple FS, et al. Multicenter evaluation of an automated assay for troponin I. *Clin Chem* 2002;48:869-76.
  64. Apple FS, Quist HE, Doyle PJ, Otto AP, Murakami MM. Plasma 99th percentile reference limits for cardiac troponin and creatine kinase MB mass for use with European Society of Cardiology/American College of Cardiology consensus recommendations. *Clin Chem* 2003;49:1331-6.
  65. Apple FS, Murakami MM. Serum 99th percentile reference cutoffs for seven cardiac troponin assays. *Clin Chem* 2004;50:1477-9.

66. Apple FS, Murakami MAM. Serum and plasma cardiac troponin I 99th percentile reference values for 3 2nd-generation assays. *Clin Chem* 2007;53:1558-60.
67. Venge P, Johnston N, Lagerqvist B, Wallentin L, Lindahl B, and the FRISC-II Study Group. Clinical and analytical performance of the Liaison cardiac troponin I assay in unstable coronary artery disease, and the impact of age on the definition of reference limits. A FRISC-II Substudy. *Clin Chem* 2003;49:880-6.
68. Christenson RH, Cervelli DR, Bauer RS, Gordon M. Stratus CS cardiac troponin I method: performance characteristics including imprecision at low concentrations. *Clin Biochem* 2004;37:679-83.
69. Pagani F, Stefani F, Micca G, et al. Multicenter evaluation of the TOSOH AIA-pack second-generation cardiac troponin I assay. *Clin Chem* 2004;50:1707-9.
70. Zethelius B, Johnston N, Venge P. Troponin I as a predictor of coronary heart disease and mortality in 70-year-old men. A community-based cohort study. *Circulation* 2006; 113:1071-8.
71. Wallace TW, Abdullah SM, Drazner MH, et al. Prevalence and determinants of troponin T elevation in the general population. *Circulation* 2006;113:1958-65.
72. Lindahl B, Diderholm E, Lagerqvist B, Venge P, Wallentin L, and the FRISC II Investigators. Mechanisms behind the prognostic value of troponin T in unstable coronary artery disease: A FRISC II substudy. *J Am Coll Cardiol* 2001;38:979-86.
73. James SK, Armstrong P, Barnathan E, et al. GUSTO-IV-ACS Investigators. Troponin and C-reactive protein have different relations to subsequent mortality and myocardial infarction after acute coronary syndrome: A GUSTO-IV substudy. *J Am Coll Cardiol* 2003;41:916-24.
74. Kontos MC, Shah R, Fritz LM, et al. Implication of different cardiac troponin I levels for clinical outcomes and prognosis of acute chest pain patients. *J Am Coll Cardiol* 2004;43:958-65.
75. Panteghini M. Acute coronary syndrome. Biochemical strategies in the troponin era. *Chest* 2002;122:1428-35.
76. Panteghini M. The new definition of myocardial infarction and the impact of troponin determination on clinical practice. *Int J Cardiol* 2006;106:298-306.
77. Panteghini M. The basics of cardiac marker interpretation. *Biochim Clin* 2007;31:9-12.
78. Storti S, Prontera C, Parri MS, et al. Evaluation of the analytical performance of the advanced method for cardiac troponin I for the AxSYM platform: comparison with the old method and the Access system. *Clin Chem Lab Med* 2006;44:1022-9.
79. Hubl W, Demant T, Gladrow E. Evaluation of the Architect STAT troponin-I assay. *Clin Lab* 2005;51:251-5.
80. Di Serio F, Lovero R, Leone M, et al. Integration between the tele-cardiology unit and the central laboratory: methodological and clinical evaluation of point-of-care testing cardiac marker in the ambulance. *Clin Chim Lab Med* 2006;44:768-73.
81. Venge P, Lindahl B, Wallentin L. New generation cardiac troponin I assay for the Access immunoassay system. *Clin Chem* 2001;47:959-61.
82. Koerbin G, Tate JR, Potter JM, et al. The comparative analytical performance of four troponin I assays at low concentration. *Ann Clin Biochem* 2005;42:19-23.
83. Er TK, Tsai LY, Jong YJ, et al. Effect of seropositive rheumatoid factor on cardiac troponin I measurement using the Access immunoassay analyzer. *Clin Chem Lab Med* 2006;44:1293-4.
84. Pagani F, Stefani F, Panteghini M. Innotracc Aio! second-generation cardiac troponin I assay: imprecision profile and other key characteristics for clinical use. *Clin Chem* 2004;50:1271-2.
85. Weber M, Rau M, Madlener K, et al. Diagnostic utility of new immunoassays for cardiac markers cTnI, myoglobin and CK-MB mass. *Clin Biochem* 2005;38:1027-30.
86. Altinier S, Zaninotto M, Mion MM, et al. Innotracc Aio!: a point-of-care or a routine analyzer? Analytical performance and plasma/whole blood comparison. *Clin Chem Lab Med* 2006;44:1278-82.
87. Hermsen D, Apple F, Garcia-Beltrán L, et al. Results from a multicenter evaluation of the 4<sup>th</sup> generation Elecsys troponin T assay. *Clin Lab* 2007;53:1-9.
88. Casals G, Filella X, Bedini JL. Evaluation of a new ultrasensitive assay for cardiac troponin I. *Clin Biochem* 2007;40:1406-13.
89. van de Kerkhof D, Peters B, Scharnhorst V. Performance of the Advia Centaur second-generation troponin assay TnI-Ultra compared with the first-generation cTnI assay. *Ann Clin Biochem* 2008;45:316-7.
90. Kim WJ, Laterza OF, Hock KG, et al. Performance of a revised cardiac troponin method that minimizes interferences from heterophilic antibodies. *Clin Chem* 2002;48:1028-34.
91. Haug C, Bachem MG, Woehrle H, et al. Evaluation of two modified cardiac troponin I enzyme immunoassays. *Clin Chem Lab Med* 2002;40:837-9.
92. Heeschen C, Goldmann BU, Langenbrink L et al. Evaluation of a rapid whole blood ELISA for quantification of troponin I in patients with acute chest pain. *Clin Chem* 1999;45:1789-96.
93. Altinier S, Mion M, Cappelletti A, et al. Rapid measurement of cardiac markers on Stratus CS. *Clin Chem* 2000;47:991-3.
94. Beneteau-Burnat B, Baudin B, Vaubourdolle M. Evaluation of Stratus CS stat fluorimetric analyzer for measurement of cardiac markers troponin I (cTnI), creatine kinase MB (CK-MB), and myoglobin. *J Clin Lab Anal* 2001;15:314-8.
95. Di Serio F, Amodio G, Varraso L, et al. Integration between point-of-care cardiac markers in an emergency/cardiology department and the central laboratory: methodological and preliminary clinical evaluation. *Clin Chem Lab Med* 2005;43:202-9.