"Ultra-sensitive" cardiac troponins: Requirements for effective implementation in clinical practice

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Abstract

The measurement of cardiac troponins, either cardiac troponin I or T, has become the culprit of clinical decision making in patients with suspected acute coronary syndrome (ACS), especially in those with non-ST elevation myocardial infarction (NSTEMI). The leading analytical mainstays of cardiac troponin immunoassays include the limit of blank (LoB), limit of detection (LoD), functional sensitivity, the 99th percentile of a healthy reference population, along with the percentage of "ostensibly healthy" subjects displaying measurable values < 99th percentile. The latest generation of cardiac troponin immunoassays, conventionally defined as "high-sensitive" (HS), is characterized by a LoD over 100-fold lower compared to the first commercialized techniques and a percentage of measurable values consistently > 50% in the general healthy population. The very recent commercialization of methods with further improved analytical sensitivity (i.e., "ultra-sensitive" assays), which allow to measure cardiac troponin values in the vast majority of healthy subjects, is now challenging the diagnostic paradigm based on early rule-out of subjects with cardiac troponin values comprised between the 99th percentile and LoD. New diagnostic strategies, entailing assay-specific cut-offs, must hence be developed and validated in large multicenter studies. The aim of this article is to provide an update on commercially available HS and "ultra"-sensitive techniques for measuring cardiac troponins, along with possible implications of increasingly enhanced analytical sensitivity on diagnostic algorithms for evaluating patients with suspected ACS.

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

Received: March 12, 2018 Accepted: May 16, 2018

Introduction

Despite many efforts made through the adoption of widespread preventive strategies, both morbidity and mortality for acute coronary syndrome (ACS) remain extremely high. Thus, myocardial ischemia has become one of the leading health care challenges worldwide (1). Unlike many other human diseases, the diagnostic approach for patients with suspected ACS has undergone sizable and revolutionary changes since the release of the first diagnostic criteria by the World Health Organization in the early 1970s (2). Irrespective of the presence of typical signs and symptoms of myocardial ischemia and suggestive electrocardiographic (ECG) abnormalities, the measurement of cardiac troponins (cTns), either cardiac troponin I (cTnI) or cardiac troponin T (cTnT), has become the culprit of making a specific clinical decision, particularly for diagnosing non-ST elevation myocardial infarction (NSTEMI) (3,4). Very recent evidence, combining organization and economic endpoints with diagnostic efficiency, also confirms that the measurement of additional biomarkers, such as the creatine kinase isoenzyme MB (CK-MB), impose a considerable financial burden for the health care system, without providing incremental value to patient care (5).
Clinical use of high-sensitivity immunoassays

The universally agreed analytical mainstays of cTn testing are summarized in Tables 1 and 2, and substantially include limit of detection (LoD), limit of blank (LoB), functional sensitivity (also known as “Limit of Quantitation”; LoQ) and the 99th percentile of a healthy reference population (6-8).

The development and commercialization of cTn immunoassays started nearly 40 years ago, and progressed with the release of methods characterized by gradually enhanced analytical performance, which are now gradually and irreversibly replacing the former generation of “contemporary-sensitive” techniques (8). For example, the first-generation cTn immunoassays was characterized by LoD of approximately 500 ng/L and 0% of measurable values (i.e., concentration > LoD) in healthy subjects, while the latest generation of immunoassays is characterized by a LoD over 100-fold lower compared to the original techniques and a percentage of measurable values typically > 50% (9). A substantial revolution has followed the

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**Table 1.** Analytical quality specifications of cardiac troponin immunoassays

| Analytical quality specification | Description |
|----------------------------------|-------------|
| LoB                              | Lowest signal generated in a fluid (i.e., typically the buffer or diluent of the assay) with zero cTn concentration. |
| LoD                              | Value generated in a biological sample with the lowest measurable cTn concentration. |
| LoQ                              | Minimal concentration of cTn that can be measured with ≤ 10% imprecision. |
| 99th percentile                  | Value of cTn corresponding to the 99th percentile of a reference population of ostensibly healthy subjects. |

Percentage (%) of measurable values in healthy subjects: Percentage of cTn values < 99th percentile that can be obtained in a reference population of ostensibly healthy subjects.

LoB - limit of blank. LoD - limit of detection. LoQ - limit of quantitation (i.e. functional sensitivity).

| Percentage (%) of measurable values < 99th percentile in healthy subjects | Assay designation |
|--------------------------------------------------------------------------|-------------------|
| < 50                                                                     | Contemporary-sensitive (CS) - Level 1 |
| 50 - 75                                                                  | First-generation high-sensitive (HS) - Level 2 |
| 75 - 95                                                                  | Second-generation high-sensitive (HS) - Level 3 |
| > 95                                                                     | Third-generation high-sensitive (HS) - Level 4 |
| ~ 99 - 100                                                               | Latest-generation high-sensitive (HS) - Level 5 |

Ratio between 99th percentile and LoD

| Ratio | Assay designation |
|-------|-------------------|
| < 1   | Clinically useable, high-sensitive (HS) |
| ≥ 10  | Extremely high-sensitive (HS) |
| ≥ 20  | Ultra-sensitive (US) |

LoD - limit of detection. 99th percentile - 99th percentile of a reference healthy population. Modified from (8).

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*Biochem Med (Zagreb) 2018;28(3):030501*  
https://doi.org/10.11613/BM.2018.030501
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The introduction of these so-called “high-sensitivity” (HS) immunoassays, driven by the recent evidence that patients with values of both cTnI and cTnT comprised between the LoD and the 99th percentile (i.e., > LoD and < 99th percentile) or between the functional sensitivity of the immunoassay and the 99th percentile (i.e., > LoQ and < 99th percentile) have a higher risk of unfavorable clinical outcomes (both total and cardiovascular) compared to those with lower values (i.e., displaying cTn values < LoD or < LoQ) (10-15). Notably, such an enhanced risk of adverse events apparently lasts for a longer period after evaluation in the emergency rooms, since patients with values comprised between the 99th percentile and LoD (or the functional sensitivity) also have an increased rate of 30-day major adverse cardiovascular events (MACE).

According to this paradigm, new diagnostic algorithms no longer use the 99th percentile as the reference diagnostic threshold, but implement lower cTn cut-offs (i.e., conventionally identified with LoD or with the functional sensitivity) and entail shorter-time serial testing (i.e., between 1 and 2 hours after baseline assessment, rather than 3 and 6 hours afterwards) (16-21). The efficiency of this strategy for rapid rule-out of ACS and for identification of patients with enhanced risk of 30-day MACE has already been proven in a consistent number of studies, and it is therefore predictable that this strategy may soon become the standard of care (10-15,22).

Table 3. Analytical performance of the four fully-automated high-sensitive cardiac troponin immunoassays commercially available

| Manufacturer       | Troponin | Platform      | LoB (ng/L) | LoD (ng/L) | CV10% (ng/L) | 99th percentile (ng/L) | Ratio 99th percentile/LoD | Measurable values > LoD (%) |
|--------------------|----------|---------------|------------|------------|--------------|------------------------|----------------------------|-----------------------------|
| Beckman Coulter    | cTnI     | Access        | 0.1        | 0.3        | 1.3          | 16                     | 53                         | 97                          |
| Siemens            | cTnI     | ADVIA Centaur | 0.5        | 2.2        | 2.7          | 48                     | 22                         | 80 - 95                     |
| Abbott             | cTnI     | ARCHITECT    | 0.7 - 1.3  | 1.1 - 1.9  | 5.6          | 19                     | 10 - 17                    | 57 - 75                     |
| Roche              | cTnT     | ELECSYS       | 3.0        | 5.0        | 12           | 14                     | 3                          | 32 - 42                     |

cTnI - cardiac troponin I. cTnT - cardiac troponin T. LoB - limit of blank. LoD - limit of detection. CV10% - value with ≤ 10% analytical imprecision.
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Diagnostic algorithms with “ultra-sensitive” techniques

Given that a significant proportion of subjects with cTn values < 99th percentile are at enhanced risk of adverse cardiovascular events, the use of LoD or functional sensitivity as diagnostic thresholds for early rule-out will be no longer feasible due to the obvious increased rate of false positive results using the so-called “US” techniques (Figure 1). The decrease of the positive predictive value will likely be magnified as long as the analytical performance of the current immunoassays is improved further, or when newer and even more analytically sensitive techniques are commercialized. For example, a recent study aimed to investigate the analytical performance of the new Sgx cTnl Assay (Singulex Inc., Alameda, USA) reported that the LoB, LoD and functional sensitivity of this assay are 0.02, 0.08 and 0.53 ng/L, whilst measurable cTnl concentrations could be observed in as many as 99.5% of healthy subjects (29). Paradoxically, these considerations pave the way to turn back the clock to nearly 40 years ago, when the strategy used for identifying the most efficient diagnostic thresholds of cTns was based on receiver operating characteristics (ROC) curve analysis (Figure 2). Indeed, the new cTn cut-offs for “US” technique will need to combine the best diagnostic performance at patient presentation with the risk of 30-day MACE, yielding a cTn value in the lower end of concentrations comprised between the 99th percentile and the LoD. Although the timing of serial sampling after patient presentation should also be defined with large (and possibly multicenter) clinical studies, it is predictable that 0h/1h or 0h/2h time points may be efficient, reliable and safe using “US” techniques, since the reference delta cTn variation selected for optimal ruling-out or ruling-in ACS will now be characterized by excellent performance in terms of analytical imprecision (i.e., much lower than 10%). Therefore, the less specificity of “US” techniques, especially when using low diagnostic cut-offs, will be probably overcome by the advantage of enabling a safer and earlier rule out of ACS. More specifically, recent studies showed that the use of a low cTn cut-off, equal or close to the LoD of the immunoassay, may allow adopting...
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0h/1h rule-in and rule-out algorithms, maintaining virtually the same diagnostic efficiency of the conventional 0h/3h algorithms, but also generating a favourable impact, both organizational and economic, for patient management in short stay units such as the emergency room (13,30,31). Notably, 0h/2h serial sampling can be seen as a promising and reliable alternative to the shorter 0h/1h strategy, especially suited for those facilities where the 0h/1h algorithm cannot be straightforwardly implemented due to practical reasons (i.e., the emergency room is far from the core laboratory and/or efficient means of samples transportation such as pneumatic transport system are unavailable) (16).

Conclusions

In conclusion, the gradual introduction in clinical practice of the so-called “US” cTn immunoassays will need to be anticipated by large and robust clinical studies aimed to identify the most suitable cut-offs and the most appropriate timing for serial sampling to get the most from these new techniques. For the time being, thoughtful translation of current diagnostic algorithms to these “US” and other incoming cTn immunoassays seems unadvisable.

Potential conflict of interest

None declared.
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