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Review Article

Biochemistry Section

Cardiac Troponins: Analytical Characteristics and Diagnostic Capabilities of Modern (High-sensitive) Determination Methods

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ABSTRACT

Cardiovascular diseases have a leading role in terms of morbidity, mortality, and disability of the population, causing significant socio-economic damage to all countries of the world. This circumstance requires researchers to constantly seek for new biomarkers and improve methods for determining existing biomarkers, and search for new therapeutic targets to improve diagnostic and treatment strategies. Recently, there have been some important changes in laboratory diagnostics of patients with acute coronary syndrome, due to the introduction into the routine practice of new high and ultrasensitive methods for the determination of biomarkers of injury, specific to cardiac muscle tissue, namely cardiac troponins. A key advantage of highly sensitive immunochemical assays is the ability to detect cardiac troponins in the early stages of myocardial infarction. This allows making the optimal decision on the early choice and conduct of reperfusion therapy, which significantly improves the further prognosis of patients. Among the most significant generally recognised disadvantages of highly sensitive determination methods are low specificity and a huge variety of troponin immunoassays. The decrease in specificity is reflected in the fact that cardiac troponins are no longer considered the "gold standard" of diagnosis related to Acute Myocardial Infarction (AMI) (irreversible ischaemic damage to cardiomyocytes). As a result, any damage to the myocardium, even insignificant and reversible under physiological state (physical activity, stress) and several pathological conditions, can lead to an increase in serum levels of cardiac troponins and affect the accuracy of the diagnosis. Each method for the determination of cardiac troponins, among the existing wide variety of troponin immunoassays, possesses different analytical characteristics, and detects different concentrations of troponins in the same patient. This article provides a view of current data on the biology of cardiac troponins, and defines the analytical characteristics of new high-sensitive methods for the determination of cardiac troponins.

Keywords: Acute myocardial infarction, Biomarkers, Coronary vascular diseases, Coefficient of variation, Laboratory diagnostics, Limit of detection

INTRODUCTION

The troponin complex of the human cardiac cross-striped muscle consists of three troponin proteins like Cardiac Troponins I and T (cTnI, cTnT), and cTnC, which normally regulate the processes of optimal myocardial contraction and relaxation [1,2]. The structure (aminoacid profile) of cardiac muscle troponin proteins determines its entire functioning. A large number of minor mutations (by the type of substitution or deletion of one or several nucleotides) were found, which, nevertheless, cause significant dysfunction of the myocardium and are responsible for the development of cardiomyopathies [3,4]. The aminoacid composition of two of the three troponin myocardial proteins (cTnI, cTnT) differs from the structure of troponins in skeletal cross-striped muscle, while the aminoacid composition of the calcium-binding subunit (troponin C) is similar to that in skeletal muscle. Cardiospecific troponins (cTnI, cTnT) are positioned mainly in the myocardium, but at the same time, there is evidence of troponin expression in cross-striped muscle tissue, as well as in the wall of the vena cava and pulmonary veins [1,4-9]. For this reason, they still cannot be considered absolute cardiospecific biomarkers.

The total content of cTnI and cTnT in the human myocardium is 4-6 mg and 10-11 mg per 1 g of wet tissue weight, respectively. Most of the troponins (about 95%) are found in the troponin-tropomyosin complex (contractile apparatus) and regulate the contractile function, while about 5% of troponin proteins are freely located in the cardiac myocytes cytosol and do not participate in the contractile function of the cardiac muscle [10-12].

Today, cardiac troponins (cTnI and cTnT) are still the most demanded biomarkers for the diagnosis of Acute Myocardial Infarction (AMI), having, however, certain disadvantages. These include the relatively late time to establish the fact of the death of cardiomyocytes and insufficient specificity in respect of ischaemic necrosis of cardiomyocytes. Therefore, cTnI and cTnT may be considered specific biomarkers of myocardial cell injury, but cannot be regarded as specific biomarkers of AMI making it impossible to rely only on the results of laboratory studies in establishing this diagnosis. An increase in the level of troponins in the early stages of pathological processes (AMI, myocarditis, etc.,) also does not allow differentiating irreversible injury from reversible, since the concentration of troponins in the early stages is low and may be associated with their cytosolic fraction.

Cardiomyocytes can be damaged in many physiological states (physical exertion, psychoemotional stress) and pathological conditions (myocarditis, sepsis, renal failure, chemotherapy treatment of cancer, etc.,), that are not associated with AMI, which, on the one hand, provides additional diagnostic opportunities, and on the other hand, it can complicate the differential diagnosis of AMI from these conditions [13-18]. The mechanisms of damage to cardiomyocytes and the subsequent increase in the level of troponins in these conditions have not been completely established, moreover the dynamics (kinetics) of concentration, is as a rule, nonspecific and may be similar to the kinetics in AMI. The mechanisms of damage are likely multiple and complex. So, for example, in sepsis, both a direct damaging effect of inflammatory cytokines and an increase in myocardial oxygen demand were noted, which in some way is similar to type 2 myocardial infarction development mechanisms. In renal failure, the mechanism responsible for the growth of serum troponins constitute, according to some data, a decrease in the glomerular filtration rate [19,20], and according to others, it has a direct damaging effect of accumulated toxic metabolic products, and

activation of the expression of cardiac troponins in skeletal muscles [6,21]. Under physiological conditions, like prolonged exertion, psychoemotional stress, or transient episodes of ischaemia, damage to cardiomyocytes is usually reversible, and the level of troponins in serum increases due to the release of those troponins that are freely located in the cardiomyocyte. At the same time, under very heavy and prolonged loads, for example, when running for a marathon distance, the level of troponins increases by ten folds, which may indicate irreversible damage (destruction and release of troponins from the contractile apparatus) and death of cardiomyocytes.

The development and implementation of highly sensitive methods for the determination of troponins (hs-cTnI and hs-cTnT) have significantly expanded the diagnostic capabilities and prospects for the use of these biomarkers [13,14,17,18]. As a rule, an increase in the concentration of High-Sensitive Cardiac Troponins I and T (hs-cTnI and hs-cTnT) > 99th-percentile, that is the troponin concentration, which is detected in 99% of truly healthy individuals, is considered an unfavorable prognostic sign, and an increased result is allowed only in 1% of truly healthy examined people.

High-sensitive methods enabled to establish the dependence of the concentration of troponins on biological characteristics: gender, age and circadian rhythms [22,23]. Thus, the level of cardiospecific troponins in men is higher than in women, which is recommended to be applied to establish the values of the 99th-percentile used in modern algorithms for the AMI diagnosis [24]. The higher level of cardiac troponins in men lies in the fact that the mass of the left ventricle in them is bigger than in women [25,26]. The age-related features of the troponin level are that, in elderly patients the concentrations are higher than in young ones [26,27]. It is assumed that the reason for this is co-morbidity, which can negatively affect cardiomyocytes [28,29]. Besides, the level of troponin T is slightly higher in the morning than in the evening, both in healthy patients and in patients with renal insufficiency [25,30,31]. The exact mechanisms of the formation of circadian features of the concentration of cardiac troponins have not been established [23]. However, it can be assumed that this is due to the circadian features of other human systems, which to a various extent, affect the cardiovascular system. In particular, there is relation to the activation of the sympathoadrenal and renin-angiotensin-aldosterone systems, an increase in the heart rate and blood pressure, as well as to the activation of the haemostasis system. These features have developed evolutionarily and are necessary for a healthy person during wakefulness, however, they can have an extra adverse effect in the presence of other risk factors (for example, atherosclerosis) and chronic cardiovascular diseases (angina pectoris, transient ischaemic attacks) [32].

Age and circadian features of the level of cardiac troponins have not been sufficiently studied, and the data available on this issue are contradictory, which is why they have not yet been widely used in routine practice. For example, a study conducted by van der Linden N et al., reported a pronounced effect of hs-cTnT circadian rhythms on the early diagnosis of myocardial infarction [31]. Whereas, according to another study by Klinkenberg LJJ et al., hs-cTnT circadian rhythms do not have a significant effect on early diagnostic algorithms for AMI [22]. When analysing the design of these two studies, which have conflicting results in terms of the clinical significance of hs-cTnT circadian rhythms, some differences in the clinical characteristics of patients can be noted. In particular, the degree of diurnal variations in hs-cTnT levels in the van der Linden N et al., study appears to have been influenced by a concomitant disease, this being a chronic renal failure [31].

The main biological fluid for determining troponin levels is blood. However, high-sensitive methods allowed the determination of troponins in other biological fluids, that can be obtained noninvasively, which is another important and promising advantage. Obtaining this biomaterial from patients is atraumatic and painless, reduces the risk of developing blood-borne infections (HIV, viral hepatitis, etc.,), and does not require trained medical personnel. Biomaterial sampling can also be carried out by the patient himself at home. For example, the concentrations of troponins in urine are quite small and are not captured by moderately sensitive test systems, whereas the high-sensitive test method detected hs-TnT in morning urine in all subjects. Moreover, in the urine of patients with hypertension, the levels of hs-TnT were significantly higher than in patients with normal blood pressure [33]. Oral fluid is another promising noninvasive biomaterial for the diagnosis of many endocrine, oncological, and cardiovascular diseases, including AMI [34,35]. For instance, a recent single-site pilot study has shown that the concentration of hs-cTnI in the oral fluid in AMI patients is significantly higher than in the control group of patients, and the levels of hs-cTnI in serum and oral fluid are moderately correlated [36]. Further studies on larger samples of patients are planned to establish reference values and standardise the pre-analytical stage to increase the clinical and diagnostic value of hs-cTnI levels in the oral fluid.

Methods for the Determination of Cardiac Troponins: A Brief History of the Troponin Immunoassays Development

The determination of troponins in the blood is carried out according to different immunochemical methods (radioimmunoassay, enzyme multiplied immunoassay, immunofluorescence, and chemiluminescence immunoassay), the basic principle of which includes several sequential stages: immunological, chemical, detection. At the first (immunological) stage, specific interaction of commercial kit diagnostic antibodies with an antigen, which in this case is troponin, takes place. At the second and third stages, either an additional immunological reaction of antibodies and the formation of a sandwich-type complex occurs, or a chemical (enzymatic) reaction and registration of the received signal happens. Signal detection methods also differ depending on the antibody label used: in the case of an enzyme immunoassay, the colour intensity is assessed using a photometer/spectrophotometer; in the case of radioimmunoassay, where radioisotopes (radionuclides) are used as a label, it is evaluated with a radiometer (radio spectrometer), and in the case of using fluorophores, the signal is recorded on a fluorometer. The level (strength) of the developed signal is directly proportional to the concentration of troponins in the biological sample. The result is more often expressed in quantitative values (ng/mL, ng/L, μg/L), or a visual assessment of the number of strips formed is carried out, which is specific for qualitative methods (diagnostic test strips) applied at the patient's bedside.

The development of immune assay methods for the determination of cardiac troponins started almost 35-year-ago and has been characterised by a gradual improvement in analytical parameters and, as a consequence, diagnostic capabilities. The very first method for the determination of cardiac troponin I, which was based on radioimmunoassay, was developed in 1987. It had a detection threshold of 10 μg/L (10,000 ng/L), the run time being quite long (1-2 days). Due to such a low sensitivity and long duration of the study, the time for detecting diagnostically significant concentrations of troponin in the blood was late and this method could only detect extensive myocardial infarctions, therefore troponin I was significantly inferior to the enzyme Creatine Kinase-Myocardial Band (CK-MB) widely used at that time and considered the «gold standard» for AMI diagnosis [37]. A few years later, Katus HA et al., presented the first fully automated enzyme multiplied immunoassay (EIA, ELISA) for the determination of troponin T with a detection limit of 100 ng/l and a run time of 90 minutes. Peak troponin T levels correlated with those of CK-MB. This immunoassay, also called the «first-generation assay» method, was superior to the standard biomarkers used at the time to diagnose AMI [38]. However, this method had a significant drawback, consisting of a cross-reaction of diagnostic antibodies with troponin isoforms peculiar of skeletal muscles, and a high percentage of positive results in diseases of skeletal muscles (myopathies), or heavy

physical exertion (during marathon running). Low sensitivity was also considered to be another disadvantage. Second-generation methods were characterised by higher specificity and sensitivity, which was reflected in a decrease in cross-reactivity with skeletal troponins and the possibility of earlier AMI diagnosis (on average, 6-12 hours after its development). Measurement of troponin levels in AMI patients surpassed the use of all other available biomarkers (CK-MB, lactate dehydrogenase, aspartate aminotransferase) in frequency, and in 2000 a joint document of the European and American Societies of Cardiology recommended the use of troponin T for the diagnosis of AMI [39]. Subsequent improvement of methods of determination and development of analyses of "third" and "fourth-generation" eliminated cross-reactivity, improved analytical characteristics, including the LoD (minimum detectable concentration). The analysis time was approximately halved, and the advantage in the diagnosis of AMI finally passed from CK-MB to troponins [40]. The disadvantage of such moderately sensitive methods was still considered to be a low sensitivity, and the prolonged average time required for accurate laboratory diagnosis of AMI (detection of diagnostically significant levels of cardiac troponins in the blood) comprising 6-12 hours from the moment of the development of the AMI clinical picture. In 2007-2009, the first reports appeared on high-sensitive methods of analysis ("fifth-generation"), the detection threshold of which was 1-10 ng/l, which is about tens and hundreds of times higher than that of moderately sensitive methods and thousands of times higher than those of the prototypes of 30-years-ago, and the analysis time is 20-30 minutes [41-43].

Troponin I immunoassays have evolved similarly since the prototype developed by Cummins (1987). In 1992, monoclonal antibodies were used to develop an enzyme multiplied immunoassay: the LoD was 1900 ng/l, and the study execution time was 3.5 hours. Unlike the then existing methods for determining troponin T, this analysis was highly specific for detecting myocardial damage. At the same time, false-positive results were practically not observed even in conditions of skeletal muscle diseases (myopathies), chronic renal failure, and marathon running [44]. Over the past 25 years, researchers and manufacturers have developed several assay methods with different combinations of antibodies on multiple platforms. There are currently more than 30 troponin I test kits in the market. The methods available range from older, less sensitive models to modern, high- and ultra-sensitive ones. Given the heterogeneity of cTnI analysis methods, the quantitative results obtained for the same patient using different methods and analysers (devices) do not match. Standardisation of cTnI determination methods based on different platforms remains a difficult task and is one of the major problems [45]. So, if it is necessary to transport a patient to another hospital that uses a different test system, the results of troponin determination cannot be compared, which is accompanied by additional economic and time costs.

According to the International Federation of Clinical Chemistry (IFCC), the most popular and commercially available test kits for performing highly sensitive analyses are produced by the following companies: Abbot, Beckman Coulter, bioMerieux, LSI Medience, Roche, Ortho, Siemens, Singulex, etc., [17,23,46] Among them, only Roche manufactures kits for hs-TnT determination, all others produce kits for hs-TnI. A huge drawback of these tests is the lack of standardisation, which is expressed in diverse results in the same patient when determined by different commercial kits, in which case the results can differ by 5-10 times or more. This is primarily related to the fact that different kits use different antibodies directed to different epitopes (antigenic determinants) of troponin molecules: in AMI, troponin fragments with different stability circulate in the blood to a greater extent. Thus, the use of antibodies directed against unstable troponin epitopes will result in underestimation of the results compared to antibodies against stable fragments of the molecule. At the same time, some epitopes of troponin molecules are targets of autoimmune antibodies and heterophilic antibodies, while the latter can contribute to the production of both false-positive and false-negative results. In this direction, further research continues to clarify the mechanisms of influence on the analysis result (interference), which will contribute to improving the quality and standardisation of analyses.

By now, many healthcare institutions have switched to the use of high-sensitive troponin test systems. Anand A et al., recently conducted a global study, in a form of a specially designed telephone questionnaire, to evaluate the implementation of the key recommendations of the Universal Definition of Myocardial Infarction (2018) towards the use of high-sensitive troponins. The authors interviewed physicians in 1902 medical centers in 23 countries on five continents. Cardiac troponin was used as the main AMI diagnostic marker in 96% of centers; the use of CK-MB continues in some countries of Latin America (Argentina, Mexico). Only 41% of centers employed high-sensitive assays, with a wide range from 7% in North America to 60% in Europe. In institutions where high-sensitive methods of analysis were applied, a serial measurement strategy with a predominance of accelerated diagnostic pathways (0-3 hours) was more often used. In this case, more attention was paid to the diagnostic threshold of the 99th-percentile, however, only 18% of centers turned to the 99th-percentile threshold values associated with gender characteristics [47].

High-sensitive Methods of Troponins Determination: Analytical Characteristics, Criteria, Classification

The main analytical characteristics of the quality of troponin immunoassays are Limit of the Blank (LoB) representing the maximum concentration of an analyte that can be detected in a sample that does not contain it; LoD, that is minimum detectable concentration), limit of quantification (functional sensitivity, LoQ), 99th-percentile, gender characteristics of the 99th-percentile, percentage of measurable values in healthy individuals, coefficient of variation (% CV) ratio of 99th-percentile/LoD [24,48,49].

Many researchers have a question: which analysis should be considered high-sensitive? The IFCC Task Force of the Committee on the Clinical Application of Cardiac Biomarkers (TF-CB IFCC) has proposed to designate a highly sensitive method that meets two criteria [17]. First, the CV% when establishing the 99th-percentile values should not exceed 10%. Secondly, in more than 50% of healthy people, the concentration of troponins should be higher than the LoD of the analytical method.

However, many of the methods designated as high-sensitive do not meet these criteria. For high sensitive assays, all journals, manufacturers, laboratories, and institutions should use the ng/L unit to avoid confusion and decimal points followed by unnecessary zeros used in moderately sensitive and some modern high-sensitive assays [24].

The clinical and diagnostic value of the results of determining hs-TnT and hs-TnI is directly related to the analytical characteristics of the troponin diagnostic agents used [Table/Fig-1]. To define the analytical parameters of the test system, the recommendations of the IFCC experts should be followed. So, for example, in establishing the values of the 99th-percentile accordingly to gender, the level of troponin in at least 300 women and 300 men should be determined. In the future, these values can be adjusted when new data are received, and in theory, each laboratory should establish its own 99th-percentile, which in this case will correspond not only to the test system and the analyser used but also to the peculiarities of this population. However, given the complexity and cost of such studies, it is permissible to focus on the parameters provided by manufacturers [24,49]. Establishing optimal values for the 99th-percentile is a very important point which involves several key questions: how should the reference groups be selected? Which statistical calculation method should be applied? The definition of a healthy person is a matter of debate [17,23,24]. How should patients be selected by age-young (<30-year-old) or those who

correspond to classic AMI patients (40-90-year-old)? What criteria should be used to designate "healthy" patients, whether using a simple survey (questionnaire) or through a complete medical examination, including both physical and instrumental laboratory studies (electrocardiography, echocardiography, determination of the concentration of natriuretic peptides, creatinine level)? The latter option is perfect, but yet expensive. The selection of the control group according to strict criteria shifts the 99th-percentile to lower values [50]. Also, when calculating the 99th-percentile, there is a need for a unified statistical approach. The proposed calculations, namely, the distribution-free (Harrell-Davis method) and robust (resistant) statistics method provide different values of the 99th-percentile. Discussions are continuing on this matter [51]. Thus, the above conditions have a strong influence on the establishment of the level of the 99th-percentile justifying its significant variation between methods of analysis provided by different manufacturers.

Indicator	Description, note
LoB (Limit of Blank)-Limit of Blank (false response)	Lowest signal generated in liquid with zero troponin concentration (blank sample)-less is better
LoD (Limit of Detection)-Limit of Detection (minimum detectable concentration)	The value obtained in the biological fluid with the lowest troponin concentration-less is better
LoQ (Limit of Quantitation)-Limit of Quantitation (functional sensitivity)	Minimum concentration that can be determined with an accuracy of ≤10%-less is better
General value (gender-blind) of 99th-percentile	The concentration of troponin, detected in 99% of truly healthy individuals and only in 1% of truly healthy subjects, a false-positive result is allowed most often for some unknown reason
Gender values of 99th-percentile	Troponin concentrations detected in 99% of healthy individuals, taking into account gender. In men, the 99th-percentile of the upper limit of the norm is about 1.5-2 times higher than in women, depending on the analysis used
Threshhold level (cut-off level)	Minimum troponin concentration for AMI diagnosis. This indicator is used only in moderately sensitive test systems, while in accelerated algorithms using high- and ultrasensitive test systems, the 99th-percentile level is used as a reference
Coefficient of variation (CV%)	Random scatter of measurements in the same sample. The smaller it is, the more accurate the analysis is.
Percentage of values <99th-percentile in healthy subjects	The number of healthy people (in%), in which the level of troponin in the blood is determined in the range from LoD to the 99th-percentile.
99th-percentile/LoD ratio	The higher the value, the higher is the test sensitivity

[Table/Fig-1]: Contemporary analytical characteristics of the quality of cardiac troponin immunoassay methods.

At the same time, some new rapid diagnostic algorithms (one- and two-hour) do not target the 99th-percentile level as a reference diagnostic threshold but use lower cut-off values to make decisions about hospitalisation/invasive interventions or sending the patient home. This is because many patients with hs-Tn concentrations ranging from the LoD (or LoQ) to the 99th-percentile have a higher risk of adverse outcomes compared to those with minimal or absent detectable values (i.e., <LoD or LoQ). The success of these strategies has been demonstrated in several studies devoted to quick excluding acute coronary syndrome and identification of patients with increased risk of 30-day adverse cardiovascular events [52-57].

Limit of detection is very important in the early diagnosis of AMI. For example, the first-second generation immunoassay method had an LoD of 100-500 ng/L due to which AMI was diagnosed too late (after 12-24 hours). In some cases, small-focal infarctions were missed and troponin was not detected in any healthy patient (0% of the measured values in the reference population). At the present stage of development of high-sensitive analyses, LoD can be as little as a few ng/L and even <1 ng/L, which is hundreds of times more sensitive, and allows detecting myocardial damage at the level of single cells; the percentage of measured values of troponins ranges from 50 to 100% [24,49]. Garcia-Osuna A et al., recently studied the analytical characteristics of a new method that detects troponin I at the level of single molecules. The study proved that this method is approximately 10 times more sensitive than the currently used hs-TnI method. The LoD of this method was 0.08-0.12 ng/L, and the proportion of healthy people with measurable troponin concentrations reached 99.5%. At the same time, healthy subjects were very rigidly selected (based on history, normal levels of natriuretic peptides, and creatinine). The median hs-cTnI was significantly higher in men compared to women and in the elderly compared to young people, which indicates the need to reflect age-related characteristics in hs-cTnI levels. This hypersensitive immunoassay is significantly superior to other existing high-sensitive methods [58]. Such sensitivity was achieved through the use of four types of antibodies: two of them are directed to epitopes located in the center of troponin, and other two to epitopes located at both ends of the molecule, which provides a greater uptake of the troponin I molecule and its fragments compared to test systems based on the use of two to three types of antibodies.

An important parameter that determines the accuracy of an immunoassay is CV%. The method is considered highly accurate and meets the requirements of the IFCC if, when serially determining the level of troponin in the same sample, the average spread of the results obtained does not exceed 10% (CV ≤10%). However, due to the low commercial availability of high-sensitive tests, troponin tests with 10%≤CV≤20% are still widely used in many laboratories. The use of these test systems can lead to false-positive and false-negative results. Tests with CV >20% are unacceptable and should be removed from the use in clinical practice [Table/Fig-2]. A significant improvement in the analytical parameters of high-sensitive tests allowed the introduction of an additional, so-called "functional" classification of methods based on the ratio of the 99th-percentile and LoD. The greater the 99th-percentile/LoD ratio, the higher the likelihood of identifying subjects with measurable values.

Coefficient of variation (analysis inaccuracy in %)	
CV ≤10	Highly-accurate (acceptable for diagnosis)
10≤CV≤20	Low-accurate, but clinically acceptable
CV ≥20	Unacceptable for use
Percentage (%) of measurable values of <99th percentile in healthy people	
<50	Moderately sensitive- level 1
50-75	High-sensitive 1st generation- level 2
75-95	High-sensitive 2nd generation- level 3
>95	High-sensitive 3rd generation- level 4
99-100	High-sensitive 4th generation- level 5
99th-percentile/LoD ratio	
<1	Clinically acceptable (High-sensitive)
≥10	Extremely highly sensitive
≥20	Ultra sensitive

[Table/Fig-2]: Accuracy and sensitivity of cardiac troponin immunoassay methods.

Some existing modern high-sensitive test systems available for clinical use, as well as their analytical parameters, are summarised in [Table/Fig-3] (according to IFCC, December 2019) [59].

CONCLUSION(S)

High-sensitive methods for the determination of troponins have significantly changed the understanding of the biology of cardiac troponins and made it possible to improve the diagnosis of AMI. However, there exist problems associated with the analysis that can affect the efficiency of the determination of cardiac troponins in routine clinical practice. In order to ensure reliable and optimal

Company/platform/method	LoB (ng/L)	LoD (ng/L)	CV (%)	99th-percentile (general and by gender), ng/L	Percentage of measurable values in the range from LoD to 99th-percentile (general and by gender, %)	Statistical method used to calculate the 99th-percentile
Abbott/Alinity i systems/Alinity i STAT High Sensitive Troponin-I; commercial OUS	1.0	1.6	4	General-26.2 F-15.6 M-34.2	General-85 F-78 M-92	Robust-statistics
Abbott/ARCHITECT i systems/ARCHITECT STAT High Sensitive Troponin-I; commercial	0.7-1.3	1.1	4	General-26.2 F-15.6 M-34.2	General-85 F-78 M-92	Robust-statistics
Beckman Coulter/Access 2, DxI/Access hsTnI; commercial- OUS	0.0-1.7	1.0-2.3	3.7	General-17.5 F-11.6 M-19.8	> 50	Distribution-free method
Beckman Coulter/Access 2,/Access hsTnI; commercial- U.S.: Serum	0.0-0.8	1.0-2.0	6	General-18.2 F-11.8 M-19.7	> 50	Distribution-free method
LSI Medience (formerly Mitsubishi) PATHFAST hs-cTnI; commercial	-	1	<6	General-15.48 F-16.91 M-11.46	General-76	Distribution-free method
LSI Medience (former Mitsubishi) PATHFAST hs-cTnI /PATHFAST cTnI-II	1.23	2.33	6.1	General-27.9 F-20.3 M-29.7	General-66.3 F-52.8 M-78.8	Distribution-free method
Ortho/VITROS/hsTroponin I; commercial	0.14-0.51	0.39-0.86	<10	General-11.0 F-9.0 M-12.0	> 50	Distribution-free method
Roche/cobas e801/cTnT-hs 18-min and STAT; commercial	2.5	3	<10	General-14.0 F-9.0 M-16.0	General-57.4	Distribution-free method
Siemens ADVIA Centaur XP/ XPT High Sensitivity TnI (TNIH), US and OUS; commercial	0.50	1.6	<4.9	General-46.5 F-39.6 M-58.0	General-72.0 F-57.0 M-86.0	Distribution-free method
Singulex clarity cTnI; commercial	0.02	0.08	2.39	General-8.67 F-8.76 M-9.23	General-99 F-99 M-100	Distribution-free method

[Table/Fig-3]: High-sensitive methods for the determination of cardiac troponins T and I according to the data of the committee on the clinical use of cardiac biomarkers IFCC (dated December 2019, provided by the manufacturers) according to with rev [59].
F: Female; M: Male

use of high-sensitive methods for the determination of troponins in clinical practice, it is important to take into account their main analytical characteristics: 99th-percentile, gender characteristics of the 99th-percentile, the LoD (minimum detectable concentration), coefficient of variation, 99th-percentile/LoD, and limit of Blanc. Since modern (high-sensitive) methods are gradually introduced into the routine practice of many healthcare institutions, there is an urgent need for independent assessments of the analytical characteristics as well as clinical and diagnostic value of new immunoassays. It is also essential to study the impact on analysis result by certain factors, such as circadian rhythms and age, and to clarify the new diagnostic possibilities of studying cardiac troponins in biological fluids obtained by a noninvasive way, in particular, in the oral fluid.

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