DERLEME REVIEW

DOI: 10.5336/cardiosci.2021-84307

Diagnostic Considerations and Analytical Characteristics of Methods for the Determination of Cardiac Troponins: Traditional Review

Kardiyak Troponinlerin Belirlenmesine Yönelik Yöntemlerin Tanısal Önemi ve Analitik Özellikleri: Geleneksel Derleme

Aleksey Michailovich CHAULIN[a]

[a]Department of Cardiology, Samara State Medical University, Samara, RUSSIA

ABSTRACT The laboratory methods for the determination of cardiac troponins (cTnI, cTnT) used nowadays are extremely diverse, which has a significant impact on our understanding of the biology and diagnostic value of cTnI and cTnT as biomarkers. The main classification of methods for the determination of cTnI and cTnT is based on the sensitivity of the immunoassay. Low- and moderately sensitive detection methods are known to be relatively low sensitive, which leads to a relatively late confirmation of cardiomyocyte death. Due to new highly sensitive methods used for the determination of cTnI and cTnT, designated as highly or ultrasensitive immunoassays (hs-TnT and hs-TnT), we received new, revised data about the biology of cardiac troponin molecules. In particular, it became clear that they can be considered products of normal myocardium metabolism since hs-TnT and hs-TnT are detected in almost all healthy patients. It also turned out that hs-TnT and hs-TnT are differ by gender (in men, troponin concentration in the blood is higher than in women), age (in elderly patients, the levels of troponins are higher than in young ones) and circadian cycles (morning concentrations of troponins are higher than in the evening). A huge variety of methods for determining cTnI and cTnT, differing in their diagnostic capabilities, creates the need for tests in order to perform an unbiased assessment of analytical characteristics of each method. This review focuses on the most pressing issues related to the discussion of the biological characteristics of cardiac troponins and the analytical characteristics of troponin immunoassays from a historical and contemporary point of view.

Keywords: Troponin T; troponin I; analytical properties; 99th percentile; acute myocardial infarction

ÖZET cTnI ve cTnT'nin biyolojik belirteçler olarak biyolojisini ve tanı değerini anlamamızda önemli bir etkiye sahip olan günümüzde kullanılan kardiyak troponinlerin (cTnI, cTnT) tayini için kullanılan laboratuvar yöntemleri son derece çeşitlidir. cTn1 ve cTnT'nin tayini için kullanılan yöntemlerin ana sınıflandırması immunoassayin duyarlılığına dayanır. Düşük ve orta derecede duyarlı tespit yöntemlerinin görece düşük duyarlılığa sahip olduğu bilinmektedir, ki bu da kardiyomyosit ölümünün görece geç doğrulanmasına yol açar. cTnI ve cTnT'nin belirlenmesi için kullanılan, yüksek veya aşırı duyarlı immünolojik testler (hs-TnT ve hs-TnT) olarak adlandırılan yeni yüksek duyarlı yöntemler nedeniyle, kardiyak troponin moleküllerinin biyolojisi hakkında yeni, gözden geçirilmiş veriler aldık. Özellikle, hs-TnT ve hs-TnT hemen hemen tüm sağlıklı hastalarda tespit edildiğinden, normal miyokard metabolizmasının ürünleri olarak kabul edilebilecekleri ortaya çıktı. Ayrıca hs-TnT ve hs-TnT'nin cinsiyete (erkeklerde kandaki troponin konsantrasyonu kadınlardan daha yüksek), yaşa (yaşlı hastalarda troponin seviyeleri gençlerden daha yüksek) ve sirkadiyen döngüler (sabah troponin konsantrasyonları akşamdan daha yüksektir) ile farklılık gösterdiği ortaya çıktı. cTnI ve cTnT'yi belirlemek için kullanılan, teşhis yeteneklerinde farklılık gösteren çok çeşitli yöntemler her yöntemin analitik özelliklerinin tarafsız bir değerlendirmesini yapmak için ihtiyaç yaratır. Bu derleme, kardiyak troponinlerin biyolojik özelliklerinin ve troponin immünolojik tahlillerinin analitik özelliklerinin tartışılmasıyla ilgili en acil konulara tarihsel ve çağdaş bir bakış açısıyla odaklanmaktadır.

Anahtar Kelimeler: Troponin T; troponin I; analitik özellikler; 99. yüzdelik dilim; akut miyokard infarktüsü

Correspondence: Aleksey Michailovich CHAULIN
Department of Cardiology, Samara State Medical University, Samara, RUSSIA/RUSYA
E-mail: alekseymichailovich22976@gmail.com

Peer review under responsibility of Turkiye Klinikleri Cardiovascular Sciences.

Received: 05 May 2021 ***Received in revised form:*** 05 Oct 2021 ***Accepted:*** 04 Nov 2021 ***Available online:*** 11 Nov 2021

BASIS OF CARDIAC TROPONIN BIOCHEMISTRY

The troponin complex of striated cardiac muscle tissue consists of three protein molecules [troponin I (cTnI), troponin T (cTnT), troponin C (cTnC)], which together with the protein tropomyosin are essential in the regulation of contraction and relaxation of the heart muscular layer.[1] The amino acid structure of the protein molecules of the troponin complex is important for maintaining the functional role of these proteins. Thus, genetic studies revealed a huge number of different mutations in genes encoding protein molecules of the troponin complex, which caused severe and life-threatening hereditary disorders of the contractile function of cardiac muscle tissue, known as cardiomyopathies.[2,3] Two of the 3 components of the troponin complex of the myocardium (cTnI and cTnT) are different in the amino acid structure from the protein components of the troponin complex of skeletal muscles, which makes them unique and allows using them as biomarkers for detecting ischemic myocardial damage in acute myocardial infarction (AMI). The amino acid structure of cTnC is the same in both cardiac and skeletal muscle tissues, and therefore this protein has no specificity for laboratory diagnosis of AMI. Although it is believed that cTnI and cTnT are localized only in the myocardium, some researchers have reported extramyocardial expression of cTnI and cTnT -namely, in the walls muscle coat of the vena cava and pulmonary veins in humans and other mammals.[2,4-8] Considering these data, cTnI, and cTnT can by no means be called absolutely specific cardiomarkers, and further study of the causes and mechanisms of extramyocardial expression of cardiac troponins should be continued.

The cardiac muscle tissue contains approximately 4.0-6.0 mg of troponin I and 10.0-11.0 mg of troponin T. Approximately 95% of this amount is part of the troponin complex (structural troponin fraction) and, accordingly, participates in the contractile function of the myocardium. The other 5% of the cTnI and cTnT molecules from the total amount of troponins are localized directly in the cytosol of myocardial cells (cytoplasmic troponin fraction) and do not regulate the contractile activity of the cardiac muscle tissue.[9,10]

Among the huge number of biomarkers proposed for the diagnosis of AMI, only cTnI and cTnT are the most reliable and in-demand in clinical practice. However, they do not fully meet all the criteria of an ideal biomarker for the diagnosis of AMI, since they elevate relatively late after the onset of myocardial ischemia (pain syndrome), have no absolute specificity for detecting cardiomyocyte necrosis of ischemic etiology. The latter circumstance is expressed in the fact that the levels of cTnI and cTnT can be significantly elevated in other (non-ischemic) myocardial injuries that are not associated with AMI, which can lead to false diagnoses.[11,12] Given this circumstance, a more literate and modern formulation for cTnI and cTnT should run as follows: cTnI and cTnT are specific cardiomarkers for identifying any damage to cardiomyocytes (regardless of etiology) but can not be considered specific cardiomarkers for diagnosing any particular type of damage, including ischemic necrosis in AMI. Therefore, medical practitioners should not rely solely on the result of laboratory diagnostic methods (positive cTnI and/or cTnT) during the treatment and diagnostic process when patients with signs of AMI are admitted. It is notable that in the early stages of many pathological processes causing irreversible (AMI, myocarditis, and others) or reversible myocardial damage (physical exertion, stressful conditions, etc.), the dynamics of the elevation of cTnI and cTnT can be practically similar, which hampers differential diagnosis.[11-15] The exact mechanisms of damage to cardiomyocytes in these cases have not yet been finally established by researchers, but the complex nature of several adverse effects in many pathologies is likely. For example, in systemic inflammation (sepsis), it was found that circulating cytokines in the blood cause direct damage to myocardial cells. But in addition to this type of damage, sepsis increases myocardial oxygen demand, which leads to myocardial ischemia with intact coronary arteries, which in this case corresponds to the pathogenesis of AMI Type 2.[16] Considering the mechanisms of increasing cTnI and cTnT in chronic kidney disease (CKD), some factors also play a role. According to some data, an elevation of troponins in CKD occurs due to a decrease in the rate of their elimination from the blood into the urine. In patients

with reduced glomerular filtration rate (GFR), the degree of troponin elevation can be quite dramatic and serum cTnT levels rise more significantly with lower GFR.[17] There are suggestions that in CKD in damage to cardiomyocytes and an increase in serum levels of cTnI and cTnT, a key role is played by the direct damaging effect on myocardial cells of toxic products of metabolic processes, in particular products of nitrogen metabolism.[5,18] And finally, it was hypothesized that in CKD, the expression of cTnI and cTnT in skeletal muscle is activated, providing another interesting mechanism.[5,18]

Reversible damage to myocardial cells under certain physiological conditions (physical activity during prolonged running or severe stressful situations) and pathological conditions (for example, transient ischemic episodes in angina pectoris) are specified by a less sharp dynamics of the elevation of cTnI and cTnT levels. The degree of excess normally does not exceed 5-10 times from the initial values. A small degree of elevation in cTnI and cTnT indicates that only the cytoplasmic troponin fraction provides for this, while the structural troponin fraction of the contractile apparatus of cardiomyocytes in its turn conditions higher concentrations of cTnI and cTnT, which is destroyed in case of irreversible damage to the myocardium.[12,19]

HIGH-SENSITIVE IMMUNOASSAYS: HOW THE UNDERSTANDING OF BIOCHEMISTRY AND THE DIAGNOSTIC VALUE OF TROPONINS HAVE CHANGED

Improved laboratory determination methods and the creation of so-called high-sensitive immunoassays for the detection of cTnI and cTnT (hs-cTnI and hs-cTnT), significantly expanded the diagnostic capabilities, and promising directions for further research.[1,12,13] In particular, it became possible to determine lower concentrations of cTnI and cTnT molecules, which previously remained invisible for moderately sensitive immunoassays. This enabled identifying prognostically unfavorable hs-cTnI and hs-cTnT concentrations in many pathologies. The 99th percentile-serum troponin levels found in 99% of completely healthy people-was proposed as the upper reference value to indicate prognostically unfavorable levels.

High-sensitive methods for determining cTnI and cTnT have changed a number of the ideas about biochemical characteristics, in particular, it was demonstrated that serum troponin levels in healthy patients depend on gender, age, and time of biomaterial sampling during the day.[20-22] Based on this, it was proposed to calculate 99th percentile depending on some of these factors. Thus, the influence of gender specificities on troponin levels turned out to be very significant and was taken into account when calculating the values of 99th percentile in new fast algorithms for the AMI diagnosis.[21,23] It is assumed that the gender specificities of troponin levels (higher levels of hs-cTnI and hs-cTnT in men) are explained by the mass of the left ventricular myocardium, which is larger in men than in women.[23] The age-related effect on hs-cTnI and hs-cTnT levels was noted. In younger healthy patients, serum troponin levels are significantly lower than in elderly patients. According to the researchers, the age-related features of hs-cTnI and hs-cTnT are associated with the presence in elderly patients of some chronic (latent) comorbid abnormalities that can negatively affect myocardial cells and promote the greater release of cTnI and cTnT molecules from them.[24] And, finally, the reports of researchers about the existence of circadian rhythms hs-cTnI and hs-cTnT are very interesting, i.e. the dependence of the concentration of the latter on the time of taking serum samples from patients. Thus, it has been demonstrated that the serum levels of hs-cTnI and hs-cTnT in the same patients are significantly higher in the morning than in the evening.[20] Moreover, this trend is characteristic of both healthy patients and patients with CKD.[20,25] The specific mechanisms of the hs-cTnI and hs-cTnT circadian rhythms formation are not completely clear, but there are assumptions about the connection with the circadian rhythms of other systems, which may have a certain adverse effect on myocardial cells. So, for example, in the morning, the sympathetic and renin-angiotensin-aldosterone systems have maximum activity, which causes load pick-up on the heart muscle tissue, an increase in heart rate and blood pressure. The mechanisms of ac-

tion of these factors on the myocardium are somewhat similar to those that occur during physical exertion and stress, accompanied by an elevation of hs-cTnI and hs-cTnT. It is believed that the increase in the activity of these systems in the morning was formed in the process of evolutionary development to ensure a normal period of wakefulness. Nevertheless, these systems play critical roles in the pathogenesis of cardiovascular disease (CVD), including AMI, and, accordingly, may produce an extra adverse effect on the myocardium of those-patients who have supplementary CVD risk factors (atherosclerosis, dyslipidemia, impaired hemostasis, etc.).[26] It should be noted that the influence of age and time of day on the levels of hs-cTnI and hs-cTnT has conflicting data and has not yet been studied enough, therefore, they are not taken into account in modern fast algorithms for diagnosing AMI.

The possibility of detecting hs-cTnI and hs-cTnT in biological fluids other than blood deserves special attention for researchers and medical practitioners. Such biological fluids (for example, urine and oral fluid), obtained non-invasively and painlessly, have great prospects for practical medicine. In addition to being atraumatic, painless, and easy to obtain, this biomaterial reduces the risk of personnel becoming infected with dangerous blood-borne infections, such as HIV and viral hepatitis B and C. In addition, when obtaining biomaterials there is no need to involve trained medical personnel and there are great opportunities for quick obtaining and preliminary diagnosis of diseases directly by the patient at home. It is important to note that high-sensitive immunoassays should be used to determine cTnI and cTnT molecules in non-invasive biological fluids since moderately sensitive methods do not detect such relatively low troponin concentrations. According to a recent study, troponin T molecules are detected in the urine of all the examined (both in the experimental and control groups). Moreover, in the experimental group (patients with arterial hypertension) hs-TnT levels were significantly higher than in the control group (patients with normal blood pressure).[27] At present, the diagnostic value of a non-invasive biomaterial is being studied for the diagnosis of many diseases, including endocrine, oncology, and CVDs, in particular, AMI.[28-31]

ON IMMUNOCHEMICAL ANALYSES FOR THE DETERMINATION OF CARDIAC TROPONINS: METHODOLOGY PRINCIPLES AND DEVELOPMENT HISTORY

For the identification of cTnI and cTnT molecules, many different immunochemical methods have been developed, including radioimmunoassays, enzyme immunoassays, immunofluorescence, and chemiluminescence assays. The measurement principle of these methods is based on several main stages: immunological, chemical and detection. The immunological stage is the interaction of diagnostic antibodies with the corresponding antigen (for anti-cTnI and anti-cTnT antigens are cTnI and cTnT molecules, respectively). The chemical step of the determination methods usually consists of an enzymatic reaction (the reaction between the enzyme used as a label and the substrate with the formation of a colored reaction product). The stage of detection depends on the label used, which differs from one immunoassay to another. So, for example, in the case of using radioisotopes as a label, the intensity of radioactive radiation will be estimated. When using fluorophores, the signal is recorded using a fluorometer, and when using an enzyme, the color intensity is estimated using a photocolorimeter or spectrophotometer. The more labeled diagnostic antibodies bind to the desired antigen, the stronger the signal obtained at the detection stage will be, which is calculated from the calibration curve. The results obtained are expressed as an exact quantifiable concentration (ng/L, μg/L, and others) or assessed visually using diagnostic test strips (qualitative methods). The latter is very convenient and useful for home use, at the patient's bedside, and in ambulances.

The need for specific immunochemical analyzes for the determination of cTnI and cTnT to diagnose AMI goes back a long way. The first methods (first-generation immunoassays) were developed more than 35 years ago and their analytical performance has since then improved. In 1987, Cummins et al. announced the development of the first cTnI method. This method had an extremely high minimum detectable concentration (about 10 μg/L or 10,000 ng/L). However, the degree of such an elevation in troponin level was observed only in micro focal AMI

and at a later date after admittance. Therefore, such an immunoassay was unsuitable for practical medicine and was significantly inferior to the diagnostic value of creatine kinase-MB, which at that time was generally recognized as the gold standard for diagnosing AMI.[32] Over the next few years, a research group led by Katus et al. reported on the development of an enzyme immunoassay for the determination of cTnT. The minimum detectable concentration for this method was 100 ng/L, and the laboratory test time took 90 minutes. According to a study using this immunoassay, cTnT levels were closely correlated with serum creatine kinase-MB enzyme activity. Although this immunoassay was superior in diagnostic value to other AMI biomarkers (aspartate aminotransferase, lactate dehydrogenase, and creatine kinase-MB), it had a very significant drawback, namely, the presence of a large number of interferences (nonspecific or false-positive) between diagnostic antibodies with skeletal troponins, which led to frequent false positives in damage and/or disease of skeletal muscle. Second-generation troponin assays have increased specificity and decreased false positives. Further improvement of determination methods of cardiac troponins resulted in the immunoassays of the third and fourth generations, almost completely spare of nonspecific reactions, and reduced minimum detectable concentration. This led to an earlier diagnosis of AMI-within 6-8 hours from the moment of admission of patients with complaints of chest pain.[33,34] Thus, cTnI and cTnT have become the new gold standard in the diagnosis of AMI, which was finally documented in 2000 by leading experts from the European and American Cardiac Communities.[34,35] However, the time required for laboratory confirmation of ischemic necrosis of cardiomyocytes remained relatively long and was not suitable for the early diagnosis of AMI; therefore, further work on the search for new biomarkers and improving the sensitivity of troponin determination methods was continued. So, in 2007-2010, the first data appeared on high-sensitive methods for the determination of troponins (hs-cTnI and hs-cTnT), also called fifth-generation immunoassays. The minimum detectable concentration of these immunochemical methods was only 1-10 ng/L, which was tens and hundreds of times less than some earlier moderately sensitive methods and thousands of times more than the first-ever prototypes created more than 35 years ago. The time spent on laboratory research using high-sensitive immunoassays was only 20-30 minutes.[36-39]

The contemporary market offers a large number of diagnostic immunoassays for troponin cTnI and cTnT determination. All of them differ in their analytical characteristics, and laboratory results obtained using the same serum by different methods do not coincide. The problem of standardizing different methods for determining cTnI and cTnT is significant.[40,41] For example, if a patient needs to be transferred to another hospital, which uses a different method for determining troponin, then the results can not be compared to reveal the AMI-peculiar elevation kinetics, and it is necessary to conduct repeated studies, which is costly and time-consuming.

The International Federation of Clinical Chemistry (IFCC) provides an independent peer review and systematization of data on methods for cardiac troponins determination. According to the IFCC, the most reliable and sound high-sensitive immunoassays are produced by the following companies: Roche-Diagnostics, Abbot, Beckman-Coulter, Ortho, Siemens, Singulex, bioMerieux, LSI-Medience.[42,43] Roche-Diagnostics manufactures kits for hs-TnT determination only, while other above mentioned manufacturers produce diagnostic kits for hs-TnI determination, therefore, the problem of standardization mainly concerns hs-TnI. The results of hs-TnI in the same patient, obtained with high-sensitive methods from different manufacturers, can differ several times. One of the main reasons for the inconsistent results is that different manufacturers use different multiple antibodies in their kits that target separate epitopes of the cTnI and cTnT molecules. With AMI, a large number of fragments of troponin molecules circulate in the blood serum of patients, which have different stability, half-life and elimination.[44] When using antibodies against unstable troponin molecules epitopes, the laboratory result may be underestimated, while when using antibodies against more stable epitopes, the result may be overestimated. The processes of decay and elimination of troponins are poorly understood, but it is likely that they occur continuously and

may depend on several factors, including the medications prescribed. In addition, some antigenic epitopes of the cTnI and cTnT molecules are targets of autoantibodies and heterophilic antibodies, which cause false positive and false negative laboratory test results. The study of the processes of decay and elimination of troponin molecules, as well as the effect of autoantibodies and heterophilic antibodies, is the subject of further research aimed at improving the quality of troponin immunoassays.

Many healthcare institutions are gradually transitioning to the use of high-sensitive troponin immunoassays in routine clinical practice. Anand et al. studied the prevalence of the use of high-sensitive immunoassays and the recommendations of the "The fourth universal definition of myocardial infarction (2018)". The researchers analyzed 1,902 medical organizations from 23 countries located on 5 different continents. According to the study, about 41% of medical organizations are using high-sensitive troponin immunoassays in everyday clinical practice for the early diagnosis of AMI. Attention is drawn to the heterogeneity of the prevalence of high-sensitive methods in different parts of the world, i.e., in North America, only 7% of medical institutions use high-sensitive immunoassays and up to 60% in Europe. Institutions using high-sensitive immunoassays adhere to accelerated algorithms for diagnosing AMI (0-3 hours), and use the 99th percentile recommended by immunoassay manufacturers as the upper reference limit. A small number of organizations use the 99th percentile values according to the gender specificities of AMI patients.[43]

HIGH-SENSITIVE TROPONIN IMMUNOASSAYS: BASIC ANALYTICAL CHARACTERISTICS AND CLASSIFICATION

The huge variety of high-sensitive troponin immunoassays calls for their classification and comparative assessment of their analytical characteristics to find the best method for practical medicine. The key analytical characteristics by which the quality of immunoassays should be assessed according to IFCC data are:[22,43,45]

- Limit of blank (LoB) - the maximum concentration of hs-cTnI and hs-cTnT detected in a biological sample that does not contain molecules of cardiac troponins,

- Limit of detection (LoD) - the minimum detectable concentration of hs-cTnI and hs-cTnT in a biological sample containing cardiac troponin molecules,

- Limit of quantitation (functional sensitivity, LoQ), is the lowest analyte concentration that can be quantitatively detected with a stated accuracy and precision.[45]

- 99th percentile general (without regard to gender) - serum levels of hs-cTnI and hs-cTnT, detected in 99% of absolutely healthy people, regardless of gender.

- 99th percentile, regarding gender - serum levels of hs-cTnI and hs-cTnT, detected in 99% of completely healthy people, considering gender specificities.

- The percentage of measurable values in healthy individuals - the number of healthy people (in%), in which troponin molecules in the blood serum are determined,

- Coefficient of variation (CV) - scatter of results (in%) in the same sample during the serial determination of hs-cTnI and hs-cTnT levels,

- Ratio of 99th percentile to LoD (99th percentile/LoD).

Many troponin immunoassays, which are labeled as high-sensitive by manufacturers, may not appear as such. As criteria for assessing and determining the high-sensitive immunoassay, IFCC experts suggested using 2 parameters: 1) CV should not be ≤10%, 2) the concentration of troponins in healthy people should be higher than LoD in at least 50% of the subjects. To avoid confusion with excessive zeros and decimal points in laboratory results, it is recommended that all results obtained from high-sensitive immunoassays be expressed in ng/L.[22]

It is important to realize that the diagnostic value of the results of laboratory troponin studies in human biological fluids directly depends on the analytical

TABLE 1: The main parameters of troponin immunoassays.[22,23,27,57]

Parameter	Abbreviation	Definition, additional comment
Limit of Blank	LoB	This is the lowest signal generated in a liquid (an empty sample) with zero troponin concentration. The lower this indicator is, the less the probability of error and the immunoassay is better.
Limit of Detection	LoD	This is the lowest detectable concentration of troponin molecules in the biological fluid. The lower this indicator is, the higher the sensitivity of the immunoassay.
Limit of Quantitation	LoQ	This is the lowest concentration of troponin molecules in the biological fluid, which can be detected with an error of 10% or less. The lower this indicator, the better the immunoassay.
99th percentile general (without regard to gender)	-	This is the average concentration of troponin molecules in the biological fluid, detected in 99% of a healthy population without taking into account gender and age.
99th percentile, regarding gender	-	This is the average concentration of troponin molecules in the biological fluid, detected in 99% of a healthy population, taking into account gender. At the same time, depending on the immunoassay used, the 99th percentile in men is 1.5-2.5 times greater than in women.
Threshold value (cut-off level)	-	This is the lowest concentration of troponin for the diagnosis of AMI. This parameter was widely used only in moderately sensitive troponin immunoassays, whereas 99th percentile is now used in highly sensitive immunoassays.
Coefficient of variation	CV	Distribution of random measurement values in the same sample. The lower this indicator is, the more accurate the immunoassay is.
The percentage of measurable values in healthy individuals	-	This indicator corresponds to the number of healthy people (in%) who will have a detectable (above LoD) concentration of troponin molecules in biological fluids. The higher this indicator is, the more sensitive the immunoassay is.
Ratio of 99th percentile to LoD	99th percentile/LoD	This is a calculated indicator obtained by dividing the value of the 99th percentile and the LoD. The higher this indicator is, the more sensitive the immunoassay is.

AMI: Acute myocardial infarction.

characteristics of troponin immunoassays (Table 1). The guidelines developed by IFCC should be used to calculate analytical performance. For example, for the correct calculation of 99th percentile, considering gender, it is necessary to measure the concentration of hs-cTnI and hs-cTnT in at least 300 women and the same number of men. Moreover, these parameters may differ in individual populations, therefore, in the ideal case, each laboratory should calculate its values of 99th percentile, considering a specific population. But given the cost and complexity of such work, the 99th percentile levels, which are provided by the manufacturer, can be used.[22,45] In addition to this, there are several important and problematic questions when calculating the 99th percentile: how to select healthy patients for the study, and what statistical calculation method should be used. The selection of healthy patients is associated with several controversial points since different researchers and manufacturers adhere to separate criteria for designating "healthy people" and select different age patients.[22,46] So, for example, to determine healthy patients, different methods can be used, ranging from a simple survey (questionnaire) up to a complex laboratory and functional examination methods (for example, echocardiography, studies of natriuretic peptide, creatinine, and other indicators). Full screening is the best option for selecting a healthy population, but it is expensive and, accordingly, inaccessible. It has been shown that when selecting a healthy population according to more stringent criteria, the 99th percentile values are significantly lower than those provided by manufacturers using their standard criteria.[47] When choosing an age group, the question arises about the most optimal age for including patients for calculating the levels of the 99th percentile, whether to use

young (30 years old) patients or older patients (40-90 years old), in whom AMI develops more often in real clinical practice.[22,47] Finally, another problem in the calculation of the 99th percentile is the choice of the method of static processing of the results, which is not currently standardized, and different manufacturers and researchers use multiple methods, which makes the values of the 99th percentile vary. Among the calculation methods, 2 are most often used, these being the nonparametric (Harrell-Davis method) and the robust (stable) statistics method, which result in different values of the 99th percentile when analyzing the same results obtained.[48,49] Thus, using the 99th percentile as an example, it is shown that the problem of calculating analytical characteristics for high-sensitive immunoassays has not been finally solved.

It should be noted that some of the most advanced AMI diagnostic algorithms (0-1 hour and 0-2 hours) no longer use the 99th percentile values suggested by manufacturers, but are guided by significantly lower cut-off levels when deciding on the tactics of patient management. This is since many patients with hs-cTnI and hs-cTnT levels below the 99th percentile (with no confirmed AMI) have a higher risk of developing adverse cardiovascular events than patients with less LoD (or LoQ) values within 30 days from emergency admittance for pain syndrome. The success of these ultra-rapid management strategies for AMI patients has been demonstrated in a series of studies aimed at identifying patients at high risk of adverse cardiovascular events within 30 days.[50-54]

One of the most important analytical characteristics of immunoassays is LoD. This parameter determines the time the diagnosis is established/confirmed. For example, the second-third generation immunoassay methods had an LoD in the range of 100-500 ng/L, and this concentration in serum was formed relatively late (12-24 hours later) from the moment of pain syndrome, and in some micro focal infarctions it could even remain undetected by these methods, and, accordingly, the optimal treatment of patients with AMI began with a delay. No person in a healthy population had troponin molecules in the blood serum. However, at the present stage, the LoD of high-sensitive immunoassays is only several ng/L, and in some ultrasensitive immunoassays, it may even be less than 1 ng/L. This is several hundred times more sensitive and allows identifying myocardial damage almost at the level of single cells, and the percentage of measurable values of hs-cTnI and hs-cTnT in a healthy population ranges from 50 to 100%.[21,46] Garcia-Osuna et al. and colleagues studied the analytical characteristics of a new test method capable of detecting troponin I at the level of individual molecules. The study showed that this test method has about 10 times greater accuracy compared to the currently used hs-TnI determination method. The LoD value of this test method is 0.08-0.12 ng/L, and the proportion of healthy people with measurable troponin concentrations reached 99.5%. In addition, healthy people were very strictly selected (based on a complete medical examination as well as an assessment of the levels of natriuretic peptides). The median hs-cTnI value was significantly higher in men compared to women and in the elderly compared to young people. This indicates the need to reflect age specificities when assessing hs-cTnI levels. This hypersensitive immunoassay is significantly superior to other existing high-sensitive test methods.[55] Such high sensitivity could be achieved using 4 types of antibodies, including 2 antibodies against epitopes located in the middle of the troponin molecule and 2 antibodies against epitopes located at both ends of the molecule. This approach provides a broader coverage of troponin I molecules and fragments compared to high-sensitive troponin immunoassays based on the use of only 2 types of antibodies. The main limitation of this study is that in its current format the assay takes about 40 min to process a sample. Thus, until the analytical time was not decreased, the Singulex cTnI assay cannot be applied for the rapid rule-out and rule-in AMI protocols in the daily practice.[55]

The most important quality-determining parameter of troponin immunoassays is CV-the spread of values (in%) in a serial study of the concentration of troponins in the same sample. In high-sensitive and high-precision immunoassays, the CV should not exceed 10% (CV≤10%).[56] However, such immunoassays are expensive and therefore may not be available to some institutions. For practical use, troponin immunoassays with CV not exceeding 20% are allowed. In immunoassays with a CV over 20%, false positive

TABLE 2: Troponin immunoassays: accuracy and sensitivity.[50,51]

Coefficient of variation	
CV≤10%	This immunoassay has a high accuracy (the most optimal)
10%≤CV≤20%	This immunoassay has an average accuracy, but is acceptable for diagnosis
CV≥20%	This immunoassay has low accuracy and is unacceptable for diagnosis
The percentage of measurable values in healthy individuals	
<50	Moderate-sensitive troponin immunoassay (1st generation immunoassay)
50-75	Highly sensitive troponin immunoassay (2nd generation immunoassay)
75-95	Highly sensitive troponin immunoassay (3rd generation immunoassay)
>95	Highly sensitive troponin immunoassay (4th generation immunoassay)
99-100	Highly sensitive troponin immunoassay (5th generation immunoassay)
Ratio of 99th percentile to LoD	
<1	This immunoassay is clinically acceptable (highly sensitive 2nd generation)
≥10	This immunoassay has an extremely high sensitivity (highly sensitive of the 3rd and 4th generations)
≥20	This immunoassay is designated as ultra-sensitive (highly sensitive of the 5th generation)

CV: Coefficient of variation; LoD: Limit of detection.

and false negative laboratory test results are highly possible, and therefore such immunoassays are not recommended for use (Table 2). Due to a significant improvement in the analytical characteristics of high-sensitive immunoassays, an additional (functional) classification of troponin immunoassays and an additional analytical criterion-the ratio of the 99th percentile to LoD (99th percentile/LoD) were introduced. The higher the value of this ratio, the more sensitive the test, and the more healthy people will have of the molecules cTnI and cTnT in the blood serum. Based on this value, some researchers distinguish hypersensitive and ultrasensitive immunoassays (Table 2).

Currently available and approved by the IFCC for practical use, high-sensitive test systems are summarized in Table 3.[57]

CONCLUSION

Laboratory methods of cardiac troponins detection remain an important diagnostic tool that is constantly being improved and changes the understanding of the biochemistry of cardiac troponin molecules and opens up new possibilities for use in laboratory diagnostics. Cardiac troponins are a specific indicator of all myocardial damages, regardless of etiology, and in the future can be used not only in cardiology but also in other fields of medicine. For the most optimal and effective use of cardiac troponins in diagnostics, it is important to realize the close dependence of the research result on the methods of determination and their main analytical characteristics, such as the 99th percentile general and 99th percentile (by gender), Limit of blank, minimum detectable concentration, CV, and others. It is also necessary to continue work on studying the mechanisms and influence of age and time of day on the levels of high-sensitive troponins and assessing new promising possibilities for studying cardiac troponin molecules in non-invasive biological fluids.

Source of Finance

During this study, no financial or spiritual support was received neither from any pharmaceutical company that has a direct connection with the research subject, nor from a company that provides or produces medical instruments and materials which may negatively affect the evaluation process of this study.

Conflict of Interest

No conflicts of interest between the authors and / or family members of the scientific and medical committee members or members of the potential conflicts of interest, counseling, expertise, working conditions, share holding and similar situations in any firm.

Authorship Contributions

This study is entirely author's own work and no other author contribution.

TABLE 3: The main highly sensitive methods for determining cardiac troponins: companies and parameters (according to The International Federation of Clinical Chemistry data, 2020).[57]

Company/diagnostic test system	LoB (ng/L)	LoD (ng/L)	CV, %	99th percentile general (without regard to gender) and 99th percentile, regarding gender, ng/L	The percentage of measurable values in healthy individuals, %	Statistical method for calculating 99th percentile
Abbott/Alinity i systems (hs-cTnI)	1.0	1.6	4.0	Total-26.2; For men-34.2; For women-15.6	Total-85.0; For men-92.0; For women-78.0	Robust
Abbott/ARCHITECT i systems (hs-cTnI)	0.7-1.3	1.1	4.0	Total-26.2; For men-34.2; For women-15.6	Total-85.0; For men-92.0; For women-78.0	Robust
Beckman Coulter/Access 2 (hs-cTnI)	0.0-1.7	1.0-2.3	3.7	Total-17.5; For men-19.8; For women-11.6	Total->50.0; Not specified by the manufacturer for men and women	Non-parametric
Beckman Coulter/Dxl (hs-cTnI)	0.0-1.7	1.5-2.3	5.2	Total-17.9 For men-19.8; For women-14.9	Total->50.0; Not specified by the manufacturer for men and women	Non-parametric
BioMérieux/VIDAS (hs-cTnI)	1.9	3.2	7.0	Total-19.0; For men-25.0; For women-11.0	Not specified by the manufacturer	Not specified by the manufacturer
LSI Medience (formerly Mitsubishi)/PATHFAST (hs-cTnI)	-	1.0	<6	Total-15.48; For men-16.91; For women-11.46	Total-76.0; Not specified by the manufacturer for men and women	Non-parametric
LSI Medience (former Mitsubishi)/PATHFAST-II (hs-cTnI)	1.23	2.33	6.1	Total-27.9; For men-29.7; For women-20.3	Total-66.3 For men-78.8; For women-52.8	Non-parametric
Ortho/VITROS (hs-cTnI)	0.14-0.51	0.39-0.86	<10.0	Total-11.0; For men-12.0; For women-9.0	Total->50.0; Not specified by the manufacturer for men and women	Non-parametric
Roche/cobas e801 (hs-cTnT)	2.5	3.0	<10.0	Total-14.0; For men-16.0; For women-9.0	Total-57.4; Not specified by the manufacturer for men and women	Not specified by the manufacturer
Siemens ADVIA Centaur XP/XPT (hs-cTnI)	0.5	1.6	<4.9	Total-46.5; For men-58.0; For women-39.6	Total-72.0; For men-86.0; For women-57.0	Non-parametric
Singulex Clarity (hs-cTnI)	0.02	0.08	2.39	Total-8.67; For men-9.23; For women-8.76	Total-99.0; For men-100; For women-99.0	Non-parametric

LoB: Limit of blank; LoD: Limit of detection; CV: Coefficient of variation.

REFERENCES

1. Park KC, Gaze DC, Collinson PO, Marber MS. Cardiac troponins: from myocardial infarction to chronic disease. Cardiovasc Res. 2017; 113(14):1708-18. [Crossref] [PubMed] [PMC]
2. Duplyakov DV, Chaulin AM. Mutations of heart troponines, associated with cardiomyopathies [Kardiologiya: novosti, mneniya, obuchenie]. Cardiology: News, Opinions, Training. 2019; 7(3):8-17. Russian. [Crossref]
3. Pasquale F, Syrris P, Kaski JP, Mogensen J, McKenna WJ, Elliott P. Long-term outcomes in hypertrophic cardiomyopathy caused by mutations in the cardiac troponin T gene. Circ Cardiovasc Genet. 2012;5(1):10-7. [Crossref] [PubMed]
4. Messner B, Baum H, Fischer P, Quasthoff S, Neumeier D. Expression of messenger RNA of the cardiac isoforms of troponin T and I in myopathic skeletal muscle. Am J Clin Pathol. 2000;114(4):544-9. Erratum in: Am J Clin Pathol 2000;114(6):986. [Crossref] [PubMed]
5. Ricchiuti V, Apple FS. RNA expression of cardiac troponin T isoforms in diseased human skeletal muscle. Clin Chem. 1999;45(12): 2129-35. Erratum in: Clin Chem 2000;46(3): 437. [Crossref] [PubMed]
6. Wens SC, Schaaf GJ, Michels M, Kruijshaar ME, van Gestel TJ, In 't Groen S, et al. Elevated plasma cardiac troponin T levels caused by skeletal muscle damage in pompe disease. Circ Cardiovasc Genet. 2016;9(1):6-13. [Crossref] [PubMed]
7. Schmid J, Liesinger L, Birner-Gruenberger R, Stojakovic T, Scharnagl H, Dieplinger B, et al. Elevated cardiac troponin T in patients with skeletal myopathies. J Am Coll Cardiol. 2018;71(14):1540-9. [Crossref] [PubMed]
8. Rusakov DY, Vologdina NN, Tulayeva ON. Развитие исчерченной сердечной мышечной ткани в стенках полых и легочных вен [The development of striated cardiac muscle tissue in the walls of the caval and pulmonary veins]. Journal of Anatomy and Histopathology. 2015;4(3):105. Russian. [Link]
9. Dhoot GK, Gell PG, Perry SV. The localization of the different forms of troponin I in skeletal and cardiac muscle cells. Exp Cell Res. 1978;117(2):357-70. [Crossref] [PubMed]
10. Filatov VL, Katrukha AG, Bulargina TV, Gusev NB. Troponin: structure, properties, and mechanism of functioning. Biochemistry (Mosc). 1999;64(9):969-85. [PubMed]
11. Chaulin AM, Karslyan LS, Duplyakov DV. Некоронарогенные причины повышения тропонинов в клинической практике [Noncoronarogenic causes of increased cardiac troponins in clinical practice]. 2019;10(4):81-93. Russian. [Crossref]
12. Chaulin AM, Duplyakov DV. MicroRNAs in atrial fibrillation: pathophysiological aspects and potential biomarkers. International Journal of Biomedicine. 2020;10(3):198-205. [Crossref]
13. Chaulin AM, Abashina OE, Duplyakov DV. Pathophysiological mechanisms of cardiotoxicity in chemotherapeutic agents. Russian Open Medical Journal. 2020;9(3):1-9. [Crossref]
14. Chaulin AM, Duplyakov DV. Arrhythmogenic effects of doxorubicin. Complex Issues of Cardiovascular Diseases. 2020;9(3):69-80. [Crossref]
15. Chaulin AM. Elevation mechanisms and diagnostic consideration of cardiac troponins under conditions not associated with myocardial infarction. Part 1. Life (Basel). 2021;11(9): 914. [Crossref] [PubMed] [PMC]
16. Wilhelm J, Hettwer S, Schuermann M, Bagger S, Gerhardt F, Mundt S, et al. Elevated troponin in septic patients in the emergency department: frequency, causes, and prognostic implications. Clin Res Cardiol. 2014;103(7): 561-7. [Crossref] [PubMed]
17. Dubin RF, Li Y, He J, Jaar BG, Kallem R, Lash JP, et al; CRIC Study Investigators. Predictors of high sensitivity cardiac troponin T in chronic kidney disease patients: a cross-sectional study in the chronic renal insufficiency cohort (CRIC). BMC Nephrol. 2013;14:229. [Crossref] [PubMed] [PMC]
18. Zümrütdal A, Bakinen O, Uçan H, Atalay HV, Bodur H. Relationship between uremic myopathy and false-positive cardiac troponin T test. Nephron. 2000;86(4):522-3. [Crossref] [PubMed]
19. Chaulin AM. Diagnostic value of highly sensitive cardiac troponins and mechanisms of their increase in serum and urine in arterial hypertension. La Rivista Italiana della Medicina di Laboratorio. 2021;17(2):99-107. [Link]
20. Klinkenberg LJ, Wildi K, van der Linden N, Kouw IW, Niens M, Twerenbold R, et al. Diurnal rhythm of cardiac troponin: consequences for the diagnosis of acute myocardial infarction. Clin Chem. 2016;62(12):1602-11. [Crossref] [PubMed]
21. Chaulin AM, Duplyakova PD, Duplyakov DV. Circadian rhythms of cardiac troponins: mechanisms and clinical significance. Russian Journal of Cardiology. 2020;25(S3):4061. [Crossref]
22. Apple FS, Jaffe AS, Collinson P, Mockel M, Ordonez-Llanos J, Lindahl B, et al; International Federation of Clinical Chemistry (IFCC) Task Force on Clinical Applications of Cardiac Bio-Markers. IFCC educational materials on selected analytical and clinical applications of high sensitivity cardiac troponin assays. Clin Biochem. 2015;48(4-5):201-3. [Crossref] [PubMed]
23. Shah AS, Griffiths M, Lee KK, McAllister DA, Hunter AL, Ferry AV, et al. High sensitivity cardiac troponin and the under-diagnosis of myocardial infarction in women: prospective cohort study. BMJ. 2015;350:g7873. Erratum in: BMJ. 2015;350:h626. Erratum in: BMJ. 2016;354:i4840. [PubMed] [PMC]
24. Trupp RJ, Albert G, Ziegler A. Sex-specific 99th percentiles derived from the AACC Universal Sample Bank for the Roche Gen 5 cTnT assay: Comorbidities and statistical methods influence derivation of reference limits. Clin Biochem. 2018;52:173. [Crossref] [PubMed]
25. van der Linden N, Cornelis T, Klinkenberg LJ, Kimenai DM, Hilderink JM, Litjens EJ, et al. Strong diurnal rhythm of troponin T, but not troponin I, in a patient with renal dysfunction. Int J Cardiol. 2016;221:287-8. [Crossref] [PubMed]
26. Chaulin AM, Duplyakov DV. On the potential effect of circadian rhythms of cardiac troponins on the diagnosis of acute myocardial infarction. Signa Vitae. 2021;17(3)79-84. [Link]
27. Pervan P, Svaguša T, Prkačin I, Savuk A, Bakos M, Perkov S. Urine high sensitive Troponin I measuring in patients with hypertension. Signa Vitae. 2017;13(Suppl 3):62-4. [Crossref]
28. Chaulin AM, Karslyan LS, Bazyuk EV, Nurbaltaeva DA, Duplyakov DV. [Clinical and diagnostic value of cardiac markers in human biological fluids]. Kardiologiia. 2019;59(11):66-75. Russian. [Crossref] [PubMed]
29. Mirzaii-Dizgah I, Riahi E. Salivary high-sensitivity cardiac troponin T levels in patients with acute myocardial infarction. Oral Dis. 2013;19(2):180-4. [Crossref] [PubMed]
30. Chaulin AM, Duplyakova PD, Bikbaeva GR, Tukhbatova AA, Grigorieva EV, Duplyakov DV. Concentration of high-sensitivity cardiac troponin I in the oral fluid in patients with acute myocardial infarction: a pilot study. Russian Journal of Cardiology. 2020;25(12):3814. [Crossref]
31. Chaulin A. Clinical and diagnostic value of highly sensitive cardiac troponins in arterial hypertension. Vasc Health Risk Manag. 2021;17:431-43. [Crossref] [PubMed] [PMC]
32. Cummins B, Auckland ML, Cummins P. Cardiac-specific troponin-I radioimmunoassay in the diagnosis of acute myocardial infarction. Am Heart J. 1987;113(6):1333-44. [Crossref] [PubMed]

33. Katus HA, Looser S, Hallermayer K, Remppis A, Scheffold T, Borgya A, et al. Development and in vitro characterization of a new immunoassay of cardiac troponin T. Clin Chem. 1992;38(3):386-93. [Crossref] [PubMed]
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(3):959-69. Erratum in: J Am Coll Cardiol 2001;37(3) :973. [Crossref] [PubMed]
35. Hermsen D, Apple F, Garcia-Beltràn L, Jaffe A, Karon B, Lewandrowski E, et al. Results from a multicenter evaluation of the 4th generation Elecsys Troponin T assay. Clin Lab. 2007;53(1-2):1-9. [PubMed]
36. Reichlin T, Hochholzer W, Stelzig C, Laule K, Freidank H, Morgenthaler NG, et al. Incremental value of copeptin for rapid rule out of acute myocardial infarction. J Am Coll Cardiol. 2009;54(1):60-8. [Crossref] [PubMed]
37. Mingels A, Jacobs L, Michielsen E, Swaanenburg J, Wodzig W, van Dieijen-Visser M. Reference population and marathon runner sera assessed by highly sensitive cardiac troponin T and commercial cardiac troponin T and I assays. Clin Chem. 2009;55(1):101-8. [Crossref] [PubMed]
38. Chaulin AM, Duplyakov DV. Факторы окружающей среды и сердечно-сосудистые заболевания [Environmental factors and cardiovascular diseases]. Hygiene and Sanitation. 2021;100(3):223-8. Russian. [Crossref]
39. Keller T, Zeller T, Ojeda F, Tzikas S, Lillpopp L, Sinning C, et al. Serial changes in highly sensitive troponin I assay and early diagnosis of myocardial infarction. JAMA. 2011;306(24): 2684-93. [Crossref] [PubMed]
40. Adams JE 3rd, Bodor GS, Dávila-Román VG, Delmez JA, Apple FS, Ladenson JH, et al. Cardiac troponin I. A marker with high specificity for cardiac injury. Circulation. 1993;88(1): 101-6. [Crossref] [PubMed]
41. Apple FS. Counterpoint: Standardization of cardiac troponin I assays will not occur in my lifetime. Clin Chem. 2012;58(1):169-71. [Crossref] [PubMed]
42. International Federation of Clinical Chemistry and Laboratory Medicine. Task Force on Clinical Applications of Cardiac Bio-Markers. Analytical Characteristics of Commercial Cardiac Troponin I and T Assays Declared by the Manufacturer. (date acess 05.05.2021) Available from: [Link]
43. Anand A, Shah ASV, Beshiri A, Jaffe AS, Mills NL. Global adoption of high-sensitivity cardiac troponins and the universal definition of myocardial infarction. Clin Chem. 2019;65(3):484-9. [Crossref] [PubMed]
44. Chaulin AM. Phosphorylation and fragmentation of the cardiac troponin T: mechanisms, role in pathophysiology and laboratory diagnosis. International Journal of Biomedicine. 2021;11(3):250-9. [Crossref]
45. Armbruster DA, Pry T. Limit of blank, limit of detection and limit of quantitation. Clin Biochem Rev. 2008;29 Suppl 1(Suppl 1):S49-52. [PubMed] [PMC]
46. Apple FS. A new season for cardiac troponin assays: it's time to keep a scorecard. Clin Chem. 2009;55(7):1303-6. [Crossref] [PubMed]
47. Collinson PO, Heung YM, Gaze D, Boa F, Senior R, Christenson R, et al. Influence of population selection on the 99th percentile reference value for cardiac troponin assays. Clin Chem. 2012;58(1):219-25. [Crossref] [PubMed]
48. Eggers KM, Apple FS, Lind L, Lindahl B. The applied statistical approach highly influences the 99th percentile of cardiac troponin I. Clin Biochem. 2016;49(15):1109-12. [Crossref] [PubMed]
49. Chaulin AM, Abashina OE, Duplyakov DV. Высокочувствительные сердечные тропонины (hs-Tn): методы определения и основные аналитические характеристики [High-sensitivity cardiac troponins: detection and central analytical characteristics]. Cardiovascular Therapy and Prevention. 2021;20(2):2590. Russian. [Crossref]
50. Cervellin G, Mattiuzzi C, Bovo C, Lippi G. Diagnostic algorithms for acute coronary syndrome-is one better than another? Ann Transl Med. 2016;4(10):193. [Crossref] [PubMed] [PMC]
51. Sörensen NA, Neumann JT, Ojeda F, Schwemer T, Renné T, Schnabel RB, et al. Challenging the 99th percentile: A lower troponin cutoff leads to low mortality of chest pain patients. Int J Cardiol. 2017;232:289-93. [Crossref] [PubMed]
52. Lippi G, Bonfanti L, Dipalo M, Aloe R, Cervellin G. Clinical, organizational and economic analysis of high-sensitivity cardiac troponin testing in the emergency department. Ann Res Hosp. 2017;1(6):1-7. [Crossref]
53. Ferencik M, Mayrhofer T, Lu MT, Woodard PK, Truong QA, Peacock WF, et al. High-sensitivity cardiac troponin i as a gatekeeper for coronary computed tomography angiography and stress testing in patients with acute chest pain. Clin Chem. 2017;63(11):1724-33. [Crossref] [PubMed] [PMC]
54. Jaeger C, Wildi K, Twerenbold R, Reichlin T, Rubini Gimenez M, Neuhaus JD, et al. One-hour rule-in and rule-out of acute myocardial infarction using high-sensitivity cardiac troponin I. Am Heart J. 2016;171(1):92-102.e1-5. [Crossref] [PubMed]
55. Garcia-Osuna A, Gaze D, Grau-Agramunt M, Morris T, Telha C, Bartolome A, et al. Ultrasensitive quantification of cardiac troponin I by a Single Molecule Counting method: analytical validation and biological features. Clin Chim Acta. 2018;486:224-231. [Crossref] [PubMed]
56. Chaulin AM, Duplyakov DV. Высокочувствительные сердечные тропонины: циркадные ритмы [High-sensitivity cardiac troponins: circadian rhythms]. Cardiovascular Therapy and Prevention. 2021;20(1):2639. Russian. [Crossref]
57. High-Sensitivity* Cardiac Troponin I and T Assay Analytical Characteristics Designated by Manufacturer IFCC Committee on Clinical Applications of Cardiac Bio-Markers (C-CB) v122019. Access date (05.05.2021) Available from: [Link]