Possible pathophysiological mechanisms of cardiac troponin level elevations in blood serum and urine in arterial hypertension

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Abstract
The review aimed to discuss and detail the main mechanisms of myocardial cell injury and increased concentrations of cardiac specific troponin isoforms (cTnI and cTnT) in blood serum and urine in hypertension. The search and analysis of foreign and domestic literature were carried out using the MedLine, EMBASE, Scopus and eLibrary databases to achieve this goal. According to recent experimental and clinical researches using high and ultra-sensitive methods for determining cTnI and cTnT, cardiomyocytes are extremely sensitive to many damaging factors in a number of physiological and pathological conditions. The serum concentrations of cTnI and cTnT can increase at the earliest stages of cardiovascular diseases (for example, in prehypertension, latent forms of coronary heart disease, arterial hypertension) and are important for predicting subsequent complications in the form of acute and life-threatening cardiovascular diseases (myocardial infarction, stroke, heart failure, and others). Moreover, troponin molecules can be detected not only in blood serum but also in non-invasively obtained biological fluids, including urine and oral fluid, which in the future can be used as new methods for the non-invasive diagnosis of many cardiovascular diseases. Although elevated levels of cTnI and cTnT in blood serum and urine in hypertension have a fairly high diagnostic and prognostic value, the pathophysiological mechanisms of cardiac troponins level elevations in human biological fluids in this pathological condition remain unclear.

Keywords: review, troponin T, troponin I, highly sensitive immunoassays, pathophysiological mechanisms, prehypertension, arterial hypertension.


Background
Cardiac troponins, namely, cTnI, cTnT, and cTnC, are the most important regulatory proteins that are localized as part of the troponin complex and play key roles in the contraction and relaxation of the muscular membrane of the heart. cTnI is an inhibitory subunit that blocks adenosine triphosphate (ATP) hydrolysis and the interaction of actin with myosin in the absence of calcium ions in the diastolic phase. cTnT is a tropomyosin-binding subunit that attaches troponin subunits to actin filaments. cTnC is a calcium-binding subunit that binds calcium ions entering the cytoplasm during the systolic phase [1–3].

The importance of cardiac troponins in the regulation of myocardial function is highlighted by the fact that mutations that cause changes in the amino acid sequence in cTnI, cTnT, and cTnC proteins are accompanied by significant and life-threatening disorders of the contractile function of cardiac muscles and hereditary cardiomyopathies [4–6].

The amino acid composition of cTnC is similar to that of troponin C in skeletal muscle fibers; therefore, this protein is not used as a biomarker of myocardial infarction. Moreover, the amino acid compositions of cTnI and cTnT are unique, which impart them with the necessary specificity, and are very important for use in diagnosing myocardial infarction [2, 7].

In addition to the specific structure, determination methods are important in laboratory diagnostics, as they continuously improve and change our understanding of the biology and diagnostic value of many biomarkers, including cTnI and cTnT [8, 9]. For example, the original methods by Cummins and Katus for determining cTnI and cTnT [10–12] were characterized by low sensitivity and speci-
Possible causes of increased levels of serum cardiac troponins in addition to acute myocardial infarction

Myocardial damage in cardiac pathologies
- Inflammatory heart disease (such as endocarditis, myocarditis, and pericarditis)
- Cardiac arrhythmias (such as atrial fibrillation and supraventricular tachycardia)
- Cardiomyopathy (all types) and heart failure
- Drugs with cardiotoxic properties (such as chemotherapeutic drugs for the treatment of oncological diseases, adrenomimetics, and cocaine)
- Heart surgery (such as coronary bypass grafting and catheter ablation)
- Takotsubo syndrome
- Arterial hypertension
- Central nervous system disorders (stroke and subarachnoid hemorrhage)
- COVID-19

Myocardial injury in noncardiac and systemic physiological and pathological conditions
- Physiological conditions (such as heavy physical activity, stressful situations, old age, male gender, and morning time lapse)
- Systemic inflammation (sepsis)
- Chronic diseases (such as chronic renal failure, chronic obstructive pulmonary disease, diabetes mellitus, and oncological diseases)
- Pulmonary embolism
- Central nervous system disorders (stroke and subarachnoid hemorrhage)
- COVID-19

False-positive causes of elevated troponin levels
- Heterophilic antibodies
- Rheumatoid factor
- Alkaline phosphatase
- Biotin
- Hemolysis
- Fibrin clots in serum
- Cross (nonspecific) reactions of diagnostic antibodies with skeletal troponin isoforms

Fig. 1. Pathological and physiological conditions that increase cardiac troponin levels in addition to acute myocardial infarction, according to [2] as amended and supplemented.

ficity, which were manifested by the relatively late detection of diagnostically significant concentrations in patients with myocardial infarction and a significant number of nonspecific (cross) reactions of anti-cTnI and anti-cTnT antibodies with troponins released from damaged skeletal muscle fibers during rhabdomyolysis and/or physical load [13–15]. With the existence of low-specific research methods, probably erroneous hypotheses regarding the expressions of cTnI and cTnT in human striated skeletal muscles were formed, which were described back in the 1990s by Apple [16] and Messner et al. [17]. Meanwhile, modern research methods have not confirmed the expressions of cardio-specific troponins outside the myocardium [18, 19].

Our understanding of the biochemistry of cTnI and cTnT has changed, as highly sensitive methods of analyses have demonstrated that the levels of cTnI and cTnT depend on several biological factors, including sex, age-related aspects, and circadian characteristics [19–23].

Regarding the sex-specific characteristics of cTnI and cTnT, the serum levels of the latter are significantly higher in men than in women, which is typical for nearly all currently used highly sensitive immunoassays. By analogy with another cardio-specific enzyme (i.e., creatine kinase) and a product of protein metabolism (i.e., creatinine), sex differences in troponin concentrations are ascribed to the higher mass of striated muscles, including cardiac muscles, in men [2, 21].

As regards the age-related aspects of cTnI and cTnT, the levels of troponins are higher in elderly patients than in young patients, which is associated with the presence of chronic latent comorbid pathologies that cause subclinical damage to the cardiomyocytes [2, 24, 25].

The circadian aspects consist of the higher morning levels of troponins over evening levels, which is due to the increased activity of several body systems (sympathoadrenal, hypothalamic–pituitary–thyroid, coagulation, etc.). The increased activity of these systems is an evolutionarily developed adaptive mechanism necessary for the wakefulness of a healthy person [26]; however, these systems also have negative effects on myocardial cells [27].

Several studies using highly sensitive immunoassays have revealed that cTnI and cTnT can be determined not only in the blood serum but also in the urine and oral fluids [28–32], and the levels of cTnI and cTnT differ significantly in the experimental and control groups, which will enable in the future the use of these noninvasively obtained biological fluids in practical medicine. The idea of creating test strips for the determination of cTnI and cTnT in noninvasively obtained biological fluids, particularly in urine, was also proposed [30].

Highly sensitive methods for determining cTnI and cTnT also demonstrated that cardiomyocytes are extremely sensitive to any damage, and the levels of cTnI and cTnT can be increased under many pathological and some physiological conditions (Fig. 1). Moreover, even in healthy patients, cTnI and cTnT molecules are released from the cardiomyocytes, but their levels generally do not exceed the 99th percentile [8, 9]. The mechanisms underlying the release of cTnI and cTnT molecules from the cardiomyocytes and, accordingly, the increase in their levels in the blood serum of healthy people have not been considerably established.
To date, several review articles have emphasized the mechanisms of increased cTnI and cTnT levels in many cardiac (myocarditis, cardiomyopathies, heart failure, and arrhythmias) [33–36] and noncardiac (sepsis, physical load, renal failure, oncological diseases, and use of chemotherapeutic drugs) [37–40] pathologies; however, they do not pay attention to such an important pathological condition as arterial hypertension (AH). According to clinical studies, elevated cTnI and cTnT levels in AH in the blood serum [41–43] and urine [28] have high diagnostic and prognostic values and enable the identification of patients with a higher risk of adverse cardiovascular events and complications. However, the mechanisms of troponin elevation in AH remain unclear and undisputed.

This study aimed to discuss the pathophysiological mechanisms of increased cTnI and cTnT levels in the serum and urine.

Mechanisms of increasing cTnI and cTnT levels in AH

Potential effect of AH on the processes of proteolytic cleavage of troponin proteins and increase in membrane permeability. Although the molecules of cardiac troponins, taking into account their molecular weight (25 kDa for cTnI and 37 kDa for cTnT), are low-molecular-weight proteins, intact troponin molecules cannot pass through the intact membrane of the cardiomyocytes. However, like any protein molecules, cTnT and cTnI are extremely sensitive to the action of proteases, which can be activated under certain pathological conditions.

Experimental studies have revealed that the stretching of cardiac muscle tissue, oxidative stress, and ischemia of cardiomyocytes lead to the activation of matrix metalloproteinase-2, which cleaves the cTnI molecule into small peptide fragments that can pass through the cardiomyocyte membrane into the extracellular space and then enter the bloodstream [44–47]. Calpain-1 is another intracellular enzyme that can cause the degradation of the cTnI molecule, and its activity intensifies with an increase in myocardial load under experimental conditions [47]. Blocking calpain-1 with a specific inhibitor reduced the degradation of troponin molecules [48]. Under AH conditions, the load on the myocardium increases significantly, and this mechanism of fragmenting troponins into smaller ones and the release of the latter from cardiomyocytes can be considered very feasible.

In addition to the proteolytic cleavage of troponins, activated proteases can cause proteolysis and cleavage of cardiomyocyte membrane protein components, facilitating the release of cytoplasmic proteins. In cardiomyocytes, approximately 5% of the total mass of troponin proteins is located outside the troponin complex directly in the cytoplasm (cytoplasmic or nonstructural fraction).

The troponin proteins constituting this fraction are believed to be the first to be released during pathological and physiological conditions. Moreover, given the relatively small volume of this fraction, troponin levels in reversible myocardial damage, for example, during severe physical exertion or stressful situations, do not exceed the 99th percentile by more than 3–5 times [49, 50]. The degree of increase in the serum content of cardiac troponins in AH is also small.

According to Afonso, approximately a third of patients with AH have cTnI levels of 4.06 ± 14.6 ng/mL, which is several times higher than the norm for the troponin test system used (<2 ng/mL) [41]. Other clinical studies obtained quite similar data on the prevalence and degree of increase in the counts of cardiac troponins in AH [42, 43].

Given the small degree of increase in the concentration of cardiac troponins in AH, according to clinical studies [41–43], the key contribution to the increase in serum levels of cardiac troponins is possibly made by the cytoplasmic fraction of troponins, which molecules are split into small fragments and enter the bloodstream. In myocardial infarction, the levels of cardiac troponins increase by several tens and hundreds of times relative to the norm, which indicates pronounced and irreversible damage to cardiomyocytes, and generally, the prevalence of increased results is 100% [21, 51].

Along with intracellular proteolytic cleavage of troponin molecules, membrane permeability of cardiomyocytes also play an important role and can change under certain physiological and pathological conditions. The membrane permeability of cells in AH was widely studied by Russian researchers (Yu.V. Postnov, S.N. Orlov, etc.), who proposed the membrane theory of primary AH [52]. According to this theory, the root cause of AH is genetically determined structural and functional anomalies of ion channels that transport monovalent cations. The impaired ion transport results in the accumulation of excess calcium levels inside the cells, which leads to a decrease in the synthesis of ATP in the mitochondria. The latter circumstance disrupts the work of several other enzymes and ion channels, particularly sodium–potassium–ATP-ase, which will cause a decrease in the transmembrane potential of the cell and an increase in the transport of calcium ions into the cell through voltage-dependent channels. Thus, a vicious pathogenetic circle is closed, which underlies the subsequent progression of AH [52–54].

According to Hessel et al., myocardial volume overload leads to cardiac muscle stretching and
activation of transmembrane glycoprotein receptors called integrins. These proteins function as mechanotransducers, increasing the membrane permeability and activating the enzymes matrix metalloproteinase-2 and calpain-1, which additionally enhance the proteolytic degradation of troponins [55]. Thus, the cleavage of troponins into small fragments and an increase in the permeability of the cell membrane of cardiomyocytes create the necessary conditions for the release of the cytoplasmic pool of troponin molecules and an increase in the concentration of the latter in AH.

**Apoptosis of cardiomyocytes** is initiated by several mechanisms that may be associated with AH development and progression. According to Cheng et al., stretching of the heart muscle walls increases oxidative stress and increases the expression of the Fas protein, which is one of the key inducers of apoptosis [56]. The action of the adrenergic system is another mechanism that causes an increase in the apoptosis of cardiomyocytes, and an activity increase is very characteristic of AH. Experimental studies have revealed that β-adrenergic receptor agonists (norepinephrine and isoproterenol) trigger intracellular apoptotic signals via cAMP-dependent and NF2-dependent mechanisms on cardiomyocytes [57–59].

Apoptosis of cardiomyocytes can lead to a very significant increase in the level of cardiac troponins, which was demonstrated in a recent experimental study by Weil et al. [60]. In this experiment, the researchers initiated apoptosis in the porcine myocardium by short-term pressure overload of the left ventricle. Moreover, after 30 min, the levels of troponins exceeded the upper limit of the norm, and after 1 and 24 h, the concentrations of troponin T reached relatively high values (856 ± 956 ng/L and 1.462 ± 1.691 ng/L, respectively). Furthermore, histological signs of necrosis of the cardiomyocytes were not detected by the researchers [60], which indicate that the mechanism of apoptosis of myocardial cells was responsible for the increase in the serum levels of troponins.

Another mechanism of apoptosis of cardiomyocytes in AH is associated with the membrane theory of hypertension by Postnov and Orlov [61, 62]. Thus, genetically determined impairment in the transport of ions across the plasma membrane of cardiomyocytes can lead to an increase in the intracellular calcium level, which is accompanied by a decrease in ATP formation, activation of apoptosis, and release of cardiac troponin molecules from the cardiomyocytes.

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**Aspects of elimination of cardiac troponins through the glomerular filter: Influencing factors and possibilities of noninvasive diagnostics**

In addition to the mechanisms of troponin release from the myocardium, the mechanisms of troponin elimination from the bloodstream are also significant, which should be taken into account by researchers and practitioners. Thus, in patients without signs of cardiovascular disease, but with signs of chronic renal failure, elevated levels of troponins are often noted [63, 64]. Moreover, the level of troponins is higher in patients with a lower glomerular filtration rate (GFR) than in patients with a higher GFR, which indicates a direct relationship between the degree of troponin level increase and the functional state of the kidneys [64].

However, direct evidence of the elimination of troponins through glomerular filtration, particularly studies confirming the presence of troponins in the urine, was not available for a long time. In some studies, urinary troponin levels were detected only in isolated cases; therefore, this mechanism of troponin elimination was considered doubtful [65]. Troponin molecules were considered relatively large molecules and, according to the authors, could not pass through the glomerular filter [66].

However, several recent studies have reported the presence of troponin molecules in the urine of patients with cardiovascular diseases. Thus, according to Pervan et al., troponin I was detected in the morning urine of patients in the experimental (patients with AH) and control (normotensive) groups. Special attention should be paid to the fact that the average concentrations of troponin I were higher in patients with AH than in people with normal blood pressure [28]. Since AH enhances GFR, this mechanism probably determines the results obtained. Urinary troponin levels are relatively low, which explains why moderately sensitive assays failed to detect these concentrations. In the study by Pervan et al., a highly sensitive immunoassay was used to determine troponin I in urine [28]. In another study, Chen et al., using a highly sensitive detection method, found troponin I in the urine of patients with diabetes mellitus, and troponin I levels were of prognostic value [29].

A possible explanation for the way troponin molecules penetrate the glomerular filter is the processes of proteolytic cleavage under the influence of several intra- and extracellular proteases. Most probably, troponin molecules, by splitting into small fragments, leak into other noninvasively obtained biological fluids, particularly into the urine and oral fluids [28–32]. However, the processes of proteolytic cleavage of troponins inside

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1 cAMP, cyclic adenosine monophosphate.
cells and blood serum have been insufficiently examined. Although researchers have reported dozens of fragments of various molecular weights and sizes, all the enzymes that are responsible for the cleavage of troponins and the formation of such a significant number of fragments are unknown [66–68].

Furthermore, Katrukha et al. [66] reported that the thrombin enzyme catalyzes the specific cleavage of troponin T into two fragments. Under AH, this enzyme is activated [69], and processes of proteolytic cleavage of troponins into small fragments increase and GFR increases, which contributes to the elimination of formed small fragments through the glomerular filter into the urine.

The identification of all enzymes and factors that influence the proteolytic cleavage of troponin molecules is of great importance for understanding this process and improving laboratory diagnostics, including the use of urine as a biomaterial for noninvasive diagnostics.

The above-described mechanisms for increasing cTnT and cTnI levels in human biological fluids in AH are summarized in Fig. 2.

**Conclusion**

The mechanisms of activation of the proteolytic cleavage of troponin molecules inside the cardiomyocyte, an increase in the permeability of myocardial cell membranes, an increase in apoptotic processes, and the effect of AH on the filtration of troponin molecule fragments through the glomerular filter into the urine underlie the increase in the level of cardiac troponins in AH. In the future, this can be used as a noninvasive method for detecting damage to cardiomyocytes in AH and assessing the prognosis of patients.

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