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Review

Measurement of circulating concentrations of cardiac troponin I and T in healthy subjects: a tool for monitoring myocardial tissue renewal?

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Abstract

The increased analytical sensitivity of the new generation of methods for cardiac troponin I (cTnI) and T (cTnT) has demonstrated that measurable troponin is present in the blood of healthy adult subjects. These data are not in accordance with the prevailing opinion that any reliably detected increase in cardiac troponins should be considered abnormal and potentially caused by cardiac necrosis. The goal of the present review is to discuss the hypothesis that cardiac troponins can be released from cardiomyocytes, even in healthy adult subjects as a result of a process related to ''physiological renewal'' of the human myocardium and possibly enhanced by physical exercise or aging. The latest generation of high-sensitive cTnI and cTnT immunoassays are characterized by detection limits (DLs) as low as a few picograms. This clearly represents a greater increase in discrimination than that obtained by the most sophisticated cardiac imaging techniques that are commercially available at present. However, the critical question is whether high-sensitive troponin assays are clinically useful and in particular, whether some specific laboratory biomarkers (such as cTnI and cTnT) yield better diagnostic (or prognostic) accuracy and cost-effectiveness when compared with echocardiography in patients with cardiovascular disease. Only specific and well-designed clinical trials will answer this important question.
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Keywords: cardiac markers; cardiac troponin I; cardiac troponin T; healthy subjects; physical exercise.

Introduction

Cardiac troponin I (cTnI) and T (cTnT) are considered the most sensitive and specific biochemical markers of myocardial damage at present (1–5). International guidelines (1, 2) and quality specifications (3, 4) recommend that increased concentrations of cardiac troponin should be defined as a measurement value that exceeds the 99th percentile upper reference limit (99th URL); a very low threshold. Moreover, assay imprecision $\leq$10% was also recommended for values corresponding to the 99th URL. However, this condition was not verified in clinical practice when these recommendations were introduced (5–9). Thus, a new generation of more sensitive and standardized cTnI immunoassays has been advocated. These new high-sensitive methods should show a ratio $\leq$1 between the value at which a 10% coefficient of variation (CV) is achieved and the 99th URL value (5–7) (see Table 1).

In the last few years, a new generation of cTnI and cTnT assays was developed by manufacturers in order to improve the analytical performance of cTnI and cTnT methods in accordance with international guidelines and quality specifications (10–23). Some of these methods are characterized by improved low-end analytical sensitivity which should increase the precision at the cut-off threshold (i.e., 99th URL) to about 10% or better (Table 1).

The increased analytical sensitivity of the new generation cTnI and cTnT methods demonstrated that measurable troponin was also present in the blood of healthy adults (10, 12, 13, 17–19, 24) (Table 2). These data are not in agreement with the prevailing opinion that any reliably detected increase in cardiac troponins should be considered abnormal and possibly caused by cardiac necrosis (25). Consequently, the demonstration of measurable circulating troponin concentrations in apparently healthy subjects should lead to abandonment of this clinical paradigm, as another incorrect scientific theory. Unexpected findings should generate new hypotheses or other considerations.

The goal of the present article is to discuss the hypothesis that cardiac troponins can be released from cardiomyocytes even in healthy adult subjects, due to a process related to ''physiological renewal'' (26) of the human myocardium which may be enhanced by physical exercise or aging.

Biochemical and physiological characteristics of cardiac troponins

The troponin complex consists of three integrated proteins called troponin C (TnC), I (TnI) and T (TnT).

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Table 1 DL, analytical sensitivity and 99th URL of some highly sensitive immunoassay methods for cTnI and cTnT.

Method	DL, ng/L	10% CV, ng/L	99th URL, ng/L	Ratio	References
cTnI Assay					
Ultra ADVIA Centaur Siemens	6	57	72	0.8	(10–13)
Singulex Erenna	0.2	0.91	9	0.1	(14)
Ultra Accu TnI Beckman	6	14	40 (all ages)	0.35	(11, 15)
			21 (for age <60 years)	0.67	
cTnT Assay					
Elecsys hs TnT Roche	2	12	14	0.85	(17–19)

DL, detection limit; URL, upper reference limit; cTnI, cardiac troponin I; cTnT, cardiac troponin T; CV, coefficient of variation.

These proteins are included in the thin sarcomeric filaments and are essential for contraction and relaxation of both cardiac and skeletal muscle, but not smooth muscle (27, 28). Although troponins are found in both skeletal muscle and cardiac muscle, the specific troponin isoforms differ between muscle types.

The troponin complex interacts with two key molecules for the contractile process, the thin actin and the thick myosin filaments. Troponin is linked to the tropomyosin protein and positioned among the actin filaments within muscle tissue. The three subunits share different physiological properties. TnT binds the troponin group to tropomyosin, forming a troponin-tropomyosin complex which is responsible for contraction. TnI binds to actin, secures the troponin-tropomyosin complex and leads to muscle relaxation by interrupting the actin-myosin linkage. TnC binds calcium ions producing a structural change in TnI in order to interrupt relaxation and to begin the contraction cycle.

During fetal heart development, skeletal isoforms of TnT and TnI are replaced by cardiac specific isoforms (i.e., cTnI and cTnT). In the 1990s, some cTnI and cTnT immunoassays, based on antibodies specific for these cardiac isoforms, were developed in order to obtain very specific methods for identifying cardiac muscle damage and which were free from interferences due to skeletal muscle isoforms (29). The first generation of cTnT immunoassays encountered false positive results due to cross reactivity with skeletal TnT antibody (29). However, second generation immunoassay methods based on more specific antibodies solved this problem of interference with skeletal muscle isoforms and showed comparable results with cTnI assays (30).

Distribution, degradation and release of troponins from cardiomyocytes

Cardiac troponins (cTnT and cTnT) are predominantly bound (94%–97%) via tropomyosin to actin filaments of sarcomeres, with only a small proportion of cTnT (6%–8%) and cTnI (3%–8%) found in the soluble cytoplasmic pool (31, 32). Intracellular compartmentalization of cardiac troponins has significant impact on their rate of release following myocardial damage.

When a cardiac cell is irreversibly injured, the free cytoplasmic pool is immediately released followed by a slow continual release of the myofibril-bound proteins resulting in the prolonged increases in troponin that are observed (33). In patients with acute myocardial infarction (AMI), cardiac troponins appear in the serum relatively early after the onset of chest pain (from 2 h to 10 h), peak at 12–24 h and may remain abnormal for 4–14 days (i.e., cTnI from 4 days to 7 days and cTnT from 10 days to 14 days) (25).

In a recent study (34), cultures of rat neonatal cardiomyocytes were exposed to mild metabolic inhibition (with 1 mmol/L sodium azide) to induce a necrotic cell death process characterized by a reversible (0–12 h) and irreversible phase (12–30 h). During the first 12 h of metabolic inhibition, cell viability was unchanged with no release of intact cTnI and cTnT or their degradation products. Between 12 h and 30 h of treatment with azide cardiomyocytes showed progressive cell death accompanied by release of intact cTnI (29 kDa), intact cTnT (39 kDa), four cTnI degradation products of 26, 20, 17 and 12 kDa, and three cTnT degradation products of 37, 27 and 14 kDa (34). These findings suggest that metabolic inhibition of cardiomyocytes induces a parallel release of intact

Table 2 Reference values and DLs of some immunoassay methods for cTnI and cTnT.

Method	DL, ng/L	Number of subjects studied	99th URL, ng/L	Range, ng/L	References
cTnI Assay					
Ultra ADVIA Centaur Siemens	6	638	72	0–200	(12)
Ultra ADVIA Centaur Siemens	6	309	39	NR	(24)
Ultra Accu TnI Beckman	NR	442	80	0–300	(11)
cTnI-Architect Abbott	9	479	13	NR	(16)
cTnT Assay					
Elecsys hs TnT Roche	2	533	14	NR	(17–19)
Elecsys hs TnT Roche	1	479	16	NR	(16)

DL, detection limit; cTnI, cardiac troponin I; cTnT, cardiac troponin T; URL, upper reference limit; Range, minimum–maximum value measured; NR, not reported.

cTnI and cTnT and their degradation products beginning only after onset of irreversible cardiomyocyte damage.

However, increased serum concentrations of cardiac troponins have been observed in patients without acute coronary syndromes (ACS) in whom irreversible myocardial cell injury was not a prominent aspect. Examples include patients with cardiomyopathy, heart failure, unstable angina pectoris, cardiac intervention (including cardioversion and ablation), pericarditis, renal insufficiency, stroke, pulmonary embolism, septic shock, and ultra-endurance athletes (7, 8, 33, 35–37). In cases where there is no irreversible necrosis and lethal disruption of sarcolemma, the exact mechanism underlying troponin release remains to be elucidated and likely differs from troponin release due to cell necrosis.

There are two potential explanations for troponin release in the absence of lethal sarcolemma disruption: 1) cellular release of proteolytic troponin degradation products; 2) troponin leakage from reversibly damaged cardiomyocytes as intact protein (Figure 1) (33). Mechanical stretch of cardiomyocytes due to pressure or volume overload may activate some intracellular proteases, such as metalloproteinase (MMP) 2 and 14. These proteases are able to intracellularly degrade cTn (38). Overload induced stretch at the cardiomyocyte level is sensed by integrins. These mechanotransducer molecules link the extracellular matrix to the intracellular cytoskeleton (39). This mechanism may be involved in the stretch-induced release of troponin and its degradation products. However, if cTnI is degraded only at the N- and C-terminal ends, the remaining protein will still have substantial molecular mass (about 12 kDa), and this relatively large molecule may be unable to traverse the myocyte cell membrane. Smaller fragments, being able to traverse the cell membrane, may not be recognized by some two-site immunoassays which require epitope recognition at two portions of the troponin molecule (6).

Hessel et al. (33) recently evaluated whether the stretch-related process, through which cTnI can be released from viable cardiomyocytes, is mediated by integrin stimulation. Using cultures of rat cardiomyocytes, these authors reported that stimulation by integrins was not associated with cTnI degradation, despite the ability of active MMP2 to degrade cTnI in vitro (33). The results of their study suggests that the release mechanism of cTnI from viable cardiomyocytes following integrin stimulation differs from cTnI release from necrotic cardiomyocytes, which is associated with extensive cTnI degradation. These findings also suggest that stretch stimulation of viable cardiomyocytes may lead to release of intact cTnI. The mechanism responsible for the release of intact cTnI from integrin stimulated cardiomyocytes may be the leakage of free intact cTnI from the cytosolic pool. Indeed, several studies have demonstrated mechanically-induced transient disruptions (wounding) of the sarcolemma in vivo (40–43). This mechanism may account for the release of proteins, such as myocyte derived growth factors (i.e., fibroblast growth factors 1 and 2) which are released despite the lack of the classic signal peptide normally associated with exocytotic secretion. These mechanically induced alterations in the permeability of the cardiomyocyte's sarcolemma may similarly be involved in the release of cTnI from the cytosolic pool of cardiomyocytes, in the absence of cell necrosis.

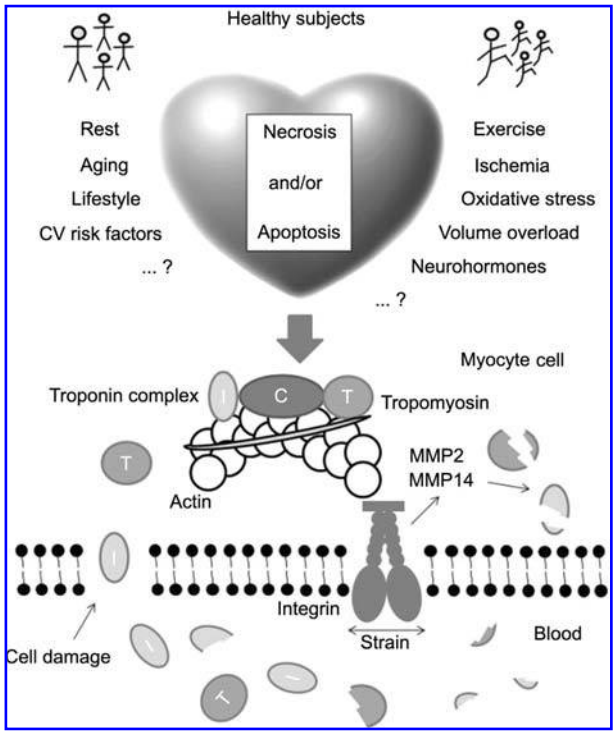

Figure 1 Schematic model illustrating the release mechanisms of cTnI and cTnT from cardiomyocytes following reversible or irreversible damage.
The release of cardiac troponins may be the result of leakage from reversibly damaged myocardial membranes as intact non-degraded protein chain (left side corner of the lower panel) or by release of proteolytic troponin degradation products through the intact myocyte membrane (right side corner of the lower panel). In the latter case, troponins are presumed to be degraded by matrix metalloproteinases (MMP) activated by integrin mediated myocardial stretch. The biggest fragments of asymmetrical troponin degradation are unlikely to directly traverse the intact myocyte membrane and may be released only in cases of membrane leakage.

In conclusion, some recent findings (33, 38–43) may explain why plasma troponin concentrations are increased in the absence of myocardial necrosis in some pathological conditions. However, we cannot exclude other mechanisms, independent of myocardial necrosis and integrin mediated myocardial stretch that might be involved in the degradation and release of troponins from myocardial cells. Further studies are necessary to accurately describe the cellular mechanisms responsible for the release of cTnI and cTnT from damaged and/or viable cardiomyocytes.

Is there physiological renewal of cardiomyocytes?

The pathophysiological mechanisms discussed previously can explain why plasma troponin is increased in some cardiac diseases independent of myocardial necrosis. However, they are not able to explain the recent findings of measurable circulating concentrations of cTnI and cTnT in healthy subjects (10, 12, 13, 17–19, 24). These data require further explanation and pathophysiological considerations.

At present, the prevailing opinion is that any reliably detected increase in cardiac troponins is abnormal and may represent cardiac necrosis (25). This consideration originates from the belief that the heart is a terminally differentiated organ and unable to regenerate working myocytes (44). The concept that myocytes cannot divide originated in the 1920s (45) and was probably due to the difficulty of identifying cardiomyocyte mitotic figures in tissue sections (44, 46). According to this belief, the heart should respond to an increase in workload by hypertrophy of existing myocytes. When myocardial hypertrophy reaches a maximum, cell death and heart failure supervene (44, 47).

However, there is increasing evidence that there is a slow, continuous turnover of cardiomyocytes in the normal heart with cardiomyocyte death and generation of new cardiomyocytes (44, 47). The development of this concept of cardiac remodeling coincided with the development of the concept of apoptosis and the recognition that this phenomenon represents an important mode of cell death in physiological cell turnover and pathological processes (48). Indeed, apoptotic cells have been described in the normal adult heart (44), thus, suggesting that myocyte replication is a significant component of physiological cellular processes. Loss of cardiomyocytes involves apotosis, autopagy and oncosis. These processes can occur simultaneously in a heart undergoing remodeling (47). These observations led not only to the inference that cardiomyocyte death contributes to progression to congestive heart failure, but also that there may be turnover and replacement of cardiomyocytes under normal conditions (49, 50). According to this hypothesis, other studies reported mitotic figures in cardiomyocytes as well as images consistent with cardiomyocyte cytokinesis (50–52). The thesis was then proposed that cardiomyocytes are not terminally differentiated cells, and myocardium is maintained by significant cardiomyocyte turnover and self-renewal (44, 47).

Most recently, the debate regarding the biological basis for cardiomyocyte renewal has undergone a major shift based on the recognition of the importance of stem cells in the biology of all organs of the body, and not only maintenance of the bone marrow (53, 54). It was documented that the myocardium, like other parenchymal organs, contains endogenous cardiac stem cells and cardiomyocyte progenitor cells (55–57). These stem cells are localized in specialized regional environments referred to as niches. These stem cell populations are now considered an important source of cells involved in cardiomyocyte turnover and renewal (58–60). Thus, there is increasing recognition that the heart is a self-renewing organ.

Some concerns remain regarding the occurrence and frequency of cardiomyocyte division, as well as its biological significance and clinical relevance in the mammalian heart. However, a recent experimental study, based on the integration of the isotope ^{14}C into DNA was able to establish the age of cardiomyocytes in humans (26). The results of this study suggested that cardiomyocytes can renew themselves, with a gradual decrease in annual turnover from 1% at the age of 25 years to 0.45% at the age of 75 years, with, on the whole, fewer than 50% of cardiomyocytes exchanged during a normal life span (26).

Circulating concentrations of cTnI and cTnT in healthy subjects

An increasing amount of data supports the hypothesis that low amounts of cardiac troponins can be released from cardiomyocytes, even in apparently healthy subjects, due to a process related to the ''physiological renewal or remodeling'' of human myocardium (10, 12, 13, 17–19, 24, 61, 62). According to this hypothesis, cTnI and cTnT circulating concentrations in healthy subjects should be proportional to the individual's cardiac mass which is continuously renewed. There are some interesting considerations regarding this important issue. First, since heart mass is proportional to body mass, gender-dependent cTnI and cTnT values should be found in healthy subjects because adult males have, on average, greater body mass (and consequently heart mass) than females. Second, from an analytical point of view, better precision of the high-sensitive cTnI and cTnT methods should enable more reliable measurement of very low troponin concentrations. As a consequence, the most sensitive cTnI and cTnT immunoassays should detect a greater fraction of measurable normal values below the 99th URL value.

Taking these considerations into account, Apple (63) recently suggested to divide the new cTnI and cTnT methods into four levels according the percentage of measurable normal values below the 99th percentile: level 1 (contemporary) $<50\%$; level 2 (first generation with high-sensitivity) from 50% to $<75\%$; level 3 (second generation with high-sensitivity) from 75% to $<95\%$; level 4 (third generation with high-sensitivity) $\geq 95\%$. However, it is conceivable that the fraction of measurable values should depend on demographic characteristics of the reference population studied, such as gender, age, and myocardial ventricular mass. Indeed, many studies support the hypothesis that cTnI and cTnT depend strongly on the demographic characteristic of the population enrolled in the study, particularly gender and age (10, 12, 13, 17–19, 24, 61, 62).

In a study from our laboratory (12), 692 apparently healthy subjects (311 males and 381 females) with a

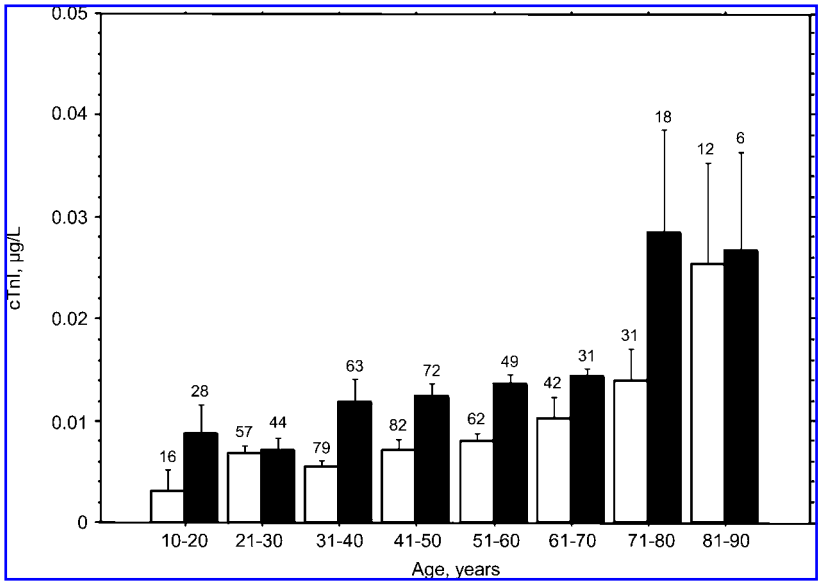

Figure 2 Mean and SEM cTnI concentrations according to gender and age, in decades, as measured by the ADVIA TnI-Ultra method in healthy males (black bars) and females (white bars).
The number of subjects included in each decade is indicated above the bars. Data obtained in the author's laboratory (modified from reference 12 with the permission of the Authors and Publisher of Clinical Chemistry and Laboratory Medicine).

mean (SD) age of 45.3 (17.3) years [range 11–89 years; females 46.5 (17.3) years, males 43.8 (17.1) years], showed significant difference in cTnI values between men and women (men: median 0.012 μg/L, range from undetectable values to 0.196 μg/L; women: median 0.008 μg/L, range from undetectable values to 0.130 μg/L; $p < 0.0001$) using the ADVIA TnI-Ultra method (Siemens Medical Solutions Diagnostics SrL) (Figure 2). Undetectable cTnI was found in 168 individuals (24.3% of total samples). For this study all people were recruited from subjects screened in a preventive medicine program (laboratory staff, blood donors, or voluntary subjects), with no acute or chronic disease as determined by history, clinical examination, ECG, and laboratory tests, nor use of drugs for at least 2 weeks before sample collection.

Gender-dependent cTnI values were also found in another study (61) that used the recently refined Access AccuTnI assay (Beckman-Coulter) to assess the distribution of cTnI results in a community population of elderly individuals [Prospective Study of the Vasculature in Uppsala Seniors (PIVUS) study; $n = 1005$]. Multivariate logistic regression analysis was performed on the entire population in the PIVUS study. Male gender was independently associated with detectable cTnI concentrations > 0.006 μg/L (61).

Gender-dependent difference in the 99th URL for the highly sensitive Roche Diagnostics cTnT assay was also reported by Mingels et al. (16) using a reference population of 479 apparently healthy individuals. The observed 99th percentile was 0.008 μg/L in 215 females and 0.018 μg/L in 264 males ($p < 0.001$). The dependence of cTnT values on age and gender was confirmed by the results of a more recent and larger study (19) that included 545 healthy volunteers between 20 years and 71 years of age (270 females, 49.5%).

Contrary findings were reported by Collinson et al. (24) who found that cTnI values, measured with the ADVIA method, were not dependent on age or gender. In particular, a higher number (53.4%) of the subjects had no measurable cTnI concentration. In this study, the 309 individuals enrolled were randomly selected from a population of ostensibly healthy individuals who were screened to exclude any present or previous history of vascular disease, diabetes mellitus, hypertension, heavy alcohol intake, cardiac medications, or pathologic echocardiogram. In this study, a more rigorous study protocol for screening and selection of the reference population, including echocardiography, resulted in a narrower reference range and a lower 99th URL value compared with findings reported in other studies that used the same highly sensitive cTnI method (12, 13, 24).

Improvements in the analytical sensitivity of troponin immunoassays: some clinical considerations

Further pathophysiological considerations can be derived from the results of high-sensitive troponin immunoassays. The incidence of heart failure significantly increases after the age of 55 years, and is the most common cause of death in the elderly (64). Several histological changes in myocardial tissue can be found in most individuals with aging. These changes are characterized by myocyte loss with subsequent hypertrophy of the remaining cells and calcification of several cardiac structures (65, 66). In addition, the age-related loss of arterial compliance contributes to isolated systolic hypertension and left ventricular hypertrophy (65, 66). Despite these changes, the majority of apparently healthy older adults show

preservation of cardiac output in the setting of reduced diastolic filling by means of the Frank-Starling principle (66). In accordance with these findings (65, 66), we can hypothesize that increased concentrations of cTnI measured with high-sensitive immunoassay methods in apparently healthy older adults is the result of increased remodeling of myocardial tissue. This hypothesis is in agreement with results reported by Eggers et al. (67) who studied the prevalence of cTnI increases in an elderly population that included 1005 individuals aged 70 years. This study observed that increased cTnI, measured by a high-sensitive immunoassay method, are relatively common in elderly subjects and are associated with cardiovascular risk and/or impaired cardiac performance (67).

From a clinical point of view, it may not be clear to most patients and physicians whether the new high-sensitive cTnI and cTnT methods will lead to more clarity or confusion. It is likely that the number of patients presenting with values that exceed the limit of detection and the recommended threshold corresponding to optimal precision (%CV $\leq$10) will increase further, thus raising a dilemma regarding the appropriate triage of these patients (61, 68, 69). If increased analytical sensitivity and precision of troponin methods allows earlier identification of AMI (8, 15, 21), it may result in an increase in ''false positive'' results in patients with cardiovascular disease, especially those with advanced age, heart failure, severe co-morbidities, such as chronic renal insufficiency or those using potentially cardio-toxic drugs (8, 61, 68, 69).

Finally, it is well known that some interfering substances, such as fibrin (70), heterophilic antibodies (71) and troponin autoantibodies (72) should be considered as potential causes for false-positive results in troponin immunoassays, as recently reviewed (6, 9). The potential clinical relevance of specific cardiac troponin autoantibodies will be discussed in detail in the next section.

The clinical impact of measurable cTnI and cTnT concentrations in apparently healthy subjects

International guidelines (1, 2) recommend that evidence of an increase or decrease in cardiac TnI or TnT, with one or more values above the 99th URL, be present for diagnosis of AMI. According to this definition, a reliable estimation of the 99th URL assumes a central role in the clinical diagnosis of AMI. Indeed, some recent studies that included more than 300 individuals and used high-sensitive methods for the measurement of cTnI or cTnT, demonstrated that selection of the reference population significantly affects the calculation of the 99th URL (12, 16, 24, 61, 62) (Table 2). As discussed in the previous section, some large studies (12, 16, 19, 24, 61, 62) reported that circulating concentrations of cardiac troponins in ''apparently'' healthy individuals are age and gender dependent when measured with high-sensitive methods.

The results of these studies (12, 16, 24, 61, 62) suggest some relevant clinical considerations. First, the clinical protocol used to exclude the presence of asymptomatic cardiac disease, especially in older subjects, is likely to affect the statistical analysis with respect to the distribution of troponin values measured by highly sensitive cTnI methods. Second, since the calculation of the 99th URL can depend significantly on the reference population studied, demographic and clinical characteristics of the reference population should be clearly stated by manufacturers, as well as by the authors of clinical studies. Finally, the sample size is an important factor to take into account for estimation of the 99th URL. Guidelines recommend a minimum of 120 reference individuals per group for statistical determination of reference limits (73). However, a sample size of at least 300 individuals is required to establish the 95% probability that at least 99% of the population will fall below the highest observed analyte value (74). Also, the uncertainty in defining the actual 99th URL is high because the cut-off concentration is approximately equal to the individual having the third highest cTnI or cTnT concentration. Thus, the inclusion of three apparently healthy individuals with slightly increased troponin concentrations in the reference group can have profound consequences on the calculation of the 99th URL.

The increase in circulating cTnI and cTnT concentrations following physical exercise

Regular exercise is part of a healthy lifestyle and aids in the prevention of cardiovascular disease (75). Although habitual physical activity reduces coronary heart disease events, vigorous activity can also acutely and transiently increase the risk of sudden cardiac death and AMI in susceptible individuals (75). Many studies have reported increases in cTnT or cTnI concentrations following strenuous exercise, such as marathons or other endurance races, even in well-trained athletes (76–89). The clinical implication of troponin increases after strenuous exercise has not been clarified. Participants in endurance sport events can have abnormal laboratory parameters, including electrolytes and biomarkers for cardiac function. Usually, these abnormal values return to normal within 24–48 h after a strenuous event, suggesting these effects are transient (89).

Michielsen et al. (89) reviewed the available literature on cTnT release following prolonged strenuous exercise. Although the exact mechanism of release remains to be clarified, it has been hypothesized that these increases most likely reflect reversible myocardial stunning and/or true, irreversible, ischemic cell injury and even cell death. Transient increases could represent loss of cTnT from the cytosolic pool due to membrane damage induced by oxidative stress, hypoxia, or transient ischemia. Indeed, exercise has been shown to induce overload of free oxygen radicals leading to increased membrane leakage (90). However, as discussed above, the majority of intra-

cellular cTnT is bound to the myofibrils in a complex with cTnI and TnC. Mechanical stretch of cardiomyocytes, as occurs during pressure or volume overload, can activate some intracellular proteases, such as MMP which are able to degrade cardiac troponin intracellularly (36) (Figure 1). Therefore, atrial stretch due to an increase in venous return after physical exercise may be involved in the stretch-induced release of troponin by cardiomyocytes.

Middleton et al. (80) recently examined the kinetics of cTnT release in nine well-trained men during and after completion of a marathon to investigate the time course of exercise-related increases in cTnT. Between 60 min and 120 min following start of the marathon, cTnT increased in all participants. However, at completion of the race, or within 1 h of completion, cTnT had returned to baseline values in all subjects (80). However, all but one subject showed further release of cTnT within the 24 h recovery period, with five subjects having an increased cTnT 24 h after exercise. These authors suggested that: 1) the release of cTnT within the first 60 min of exercise indicates that exercise-induced cTnT release is not necessarily limited to prolonged endurance exercise; 2) the consistent increase and pattern of cTnT observed in all participants during and after exercise likely reflects a physiologic, as opposed to pathologic, mechanism (80).

In conclusion, an increasing amount of data indicates that the post-exercise troponin release may reflect part of the process of remodeling. However, the clinical significance of chronic exposure to endurance exercise is unknown. The development of myocardial fibrosis has been suggested as a long-term outcome to chronic exposure to repetitive bouts of endurance exercise, and has been linked to an exercise-induced inflammatory process observed in an animal model (84). This hypothesis is supported by a limited number of postmortem studies in athletes and an increased prevalence of complex arrhythmia in veteran athletes (84). Further studies are necessary to clarify the clinical significance of increased circulating cTnI and cTnT following strenuous physical exercise in athletes.

Cardiac troponin concentrations and autoantibodies

Recently, human IgG reacting with cTnI and cTnT was suggested as a potential cause of false-negative results in some troponin immunoassays (91, 92), and as a possible contributor to idiopathic cardiomyopathy (92–95) and poor outcome following myocardial infarction (96). The incidence of autoantibodies to cTnI and cTnT is relatively high in apparently healthy blood donors (12.7% for cTnI and 9.9% for cTnT, respectively) (72, 91, 97), as well as in patients with cardiac, infectious, and autoimmune diseases (92–96, 98).

The mechanisms for the appearance of troponin autoantibodies in healthy subjects or cardiac patients are not known, as recently reviewed (93, 94). It is conceivable that a protein, sequestrated in sarcomeric filaments and not present in plasma, may be inaccessible to the immune system. When released into the extra-cellular space from damaged cardiomyocytes, the protein may then be able to activate an immune response leading to the production of autoantibodies. However, the recent demonstration of measurable (even if very low) plasma cTnI and cTnT concentrations seems to be incompatible with this hypothesis. Furthermore, recent studies found specific autoantibodies against not only sarcomeric proteins, but many other cardiac proteins as well. In particular, autoantibodies to contractile proteins, structural proteins, proteins of energy metabolism/transfer, ion channels, and sarcolemmal receptors have been identified in patients with idiophatic dilated cardiomyopathy (IDCM) (93, 94). The prevalence of autoantibodies in patients with IDCM is between 65% and 70%, ranging from 20% to 95% depending on the screening techniques and the antibody in question (93).

From a pathophysiological point of view, at least two conditions appear to be necessary for the appearance of autoantibodies to cardiac proteins: a mechanism producing chronic cardiac damage (generally based on a sustained inflammatory process) and some abnormalities of cell-mediated and/or humoral immunity (93, 94). B-cell recognition of antigen is not the only element necessary for B-cell activation. Most antigens are T-dependent, meaning that T-cell help is required for maximal antibody production. Therefore, activation of the immune system network is necessary to stimulate the development of a B-cell to a mature plasma cell. According to this hypothesis, autoimmune abnormalities, viral infection and genetic abnormalities appear to be major predisposing factors for IDCM (94). Furthermore, recent findings have suggested that at least some of these antibodies may be directly related to the pathophysiology of IDCM. In particular, cTnI autoantibodies increase the L-type calcium current, which is known to be related to myocardial damage (92–100).

From an analytical point of view, troponin autoantibodies can interfere in cTnI and cTnT immunoassays (72, 91, 97). Both false positive and false negative values may occur depending on whether the autoantibody-analyte complex is distributed in the free or bound analyte fraction (9). The effect of the presence of autoantibodies on troponin assays is complex and requires experiments to isolate the interfering factor and test assay recovery after its addition to high troponin samples (9). In some immunoassays, the major interfering effect may occur at low troponin concentrations, affecting accurate determination of the 99th URL.

High-sensitive cTnI and cTnT methods: a powerful tool for monitoring renewal and remodeling of myocardial tissue?

Any improvement to the analytical precision and clinical sensitivity of a laboratory test is always good; this concept should be also applied to cTnI and cTnT

immunoassays. More sensitive assays with better precision should permit more reliable estimation of very low troponin concentrations. It is conceivable that improvements in assay sensitivity is required in order to measure near or even below ng/L concentrations. As a result, a new generation of cTnI and cTnT assays have been developed by manufacturers in order to improve the analytical performance and standardization of cardiac troponin assays in accordance with international guidelines and quality specifications. Some of these methods are characterized by improved low-end analytical sensitivity and precision, which should increase the precision at the cut-off threshold (99th percentile of the reference population) to about 10% or better (Table 1).

The last generation of cTnI and cTnT immunoassays was characterized by a detection limit (DL) of a few picograms of protein (Table 1). A simple calculation may better explain the impact that the increase in analytical sensitivity of the new generation immunoassays for cTnI and cTnT may have on clinical practice. For example, considering that a cardiomyocyte has a cTnI content of about 70 mg/g myocardial tissue (101), a cTnI amount of about 10 pg should be contained in a 1-mg mass of myocardial tissue. According to these findings, it is conceivable that necrosis (damage) of 10–50 mg of myocardial tissues should be detected by high-sensitive troponin methods, with DL $<$10 pg, owing to a significant increase in circulating concentrations following release of the protein by injured cardiomyocytes (102).

This represents an increase in tissue discrimination and spatial resolution significantly better than that obtained by sophisticated cardiac imaging techniques (103). In addition, the cost of a cTnI or cTnT assay is about 10–100 times less than that of cardiac imaging (echocardiography, SPECT, CT-scan, NMR, or PET) (103). Finally, cardiac imaging results are operator dependent, and very difficult to standardize and compare in an external, inter-laboratory quality scheme. Despite these drawbacks and limitations, cardiac imaging, especially echocardiography, is considered the gold standard for the diagnosis and risk stratification of patients with cardiovascular disease and heart failure, according to international guidelines (104–108).

A new generation of highly sensitive cTnI and cTnT methods may have some beneficial clinical effects. First, some troponin concentrations measured in the reference population with the first generation high-sensitive troponin methods (from 25% to 55%), are still below the analytical sensitivity of the new generation immunoassay methods (12, 13, 24). This suggests that the imprecision of these methods at low troponin concentrations should be improved (63). Thus, a new generation of more sensitive cTnI and cTnT methods is necessary to better define the true normal concentrations and the 99th URL (63). Second, a new generation of troponin methods will enable better risk stratification of patients who, with the current assays, do not have increased protein concentrations, and may allow risk stratification of patients with chronic stable angina and heart failure. It is important to note that increased values of cTnI and cTnT are an index of cardiac tissue damage, even in cases of extra-cardiac diseases including chronic inflammatory disease, end stage renal disease, or treatment with powerful cardio-toxic drugs. This should allow for an appropriate diagnosis and, when necessary, specific treatment.

From a clinical point of view, the critical question is whether highly sensitive troponin assays are clinically useful in patients with cardiovascular disease and, in particular, whether some specific laboratory biomarkers such as cTnI and cTnT should share better diagnostic or prognostic accuracy and cost-effectiveness when compared with echocardiography (103). Only well designed clinical trials can answer these important questions.

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