European Heart Journal
Acute Cardiovascular Care
EUROPEAN SOCIETY OF CARDIOLOGY®

Article

European Heart Journal: Acute Cardiovascular Care
1–10

DOI: 10.1177/2048872617708973
journals.sagepub.com/home/acc
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What to do when you question cardiac troponin values

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Abstract

High-sensitivity cardiac troponin assays enable cardiac troponin measurement with a high degree of analytical sensitivity and a low level of analytical imprecision at the low measuring range. One of the most important advantages of these new assays is that they allow novel, more rapid approaches for ruling in or ruling out acute myocardial infarctions. The increase in the early diagnostic sensitivity of high-sensitivity cardiac troponin assays comes at the cost of a reduced acute myocardial infarction specificity of the biomarker, because more patients with other causes of acute or chronic myocardial injury without overt myocardial ischaemia are detected than with previous cardiac troponin assays. Increased troponin concentrations that do not fit with the clinical presentation are seen in the daily routine, mainly as a result of a variety of pathologies, and if tested in the same sample, even discrepancies between high-sensitivity cardiac troponin I and troponin T test results may sometimes be found as well. In addition, analytically false-positive test results occasionally may occur since no assay is perfect. In this review, we summarise the biochemical, pathophysiological and analytical background of the work-up for such a clinical setting.

Keywords

Cardiac troponin I, cardiac troponin T, high sensitivity, discrepancy, interference, mismatch

Date received: 7 January 2017; accepted: 19 April 2017

Introduction

Cardiac troponin I (cTnI) and cardiac troponin T (cTnT) are the biomarkers of choice for the diagnosis of myocardial injury, because they are the most sensitive and cardiac-specific laboratory measures of myocardial injury currently available.[1] Recently, high-sensitivity cTn (hs-cTn) assays have been introduced into clinical practice.[2] Their improved analytical performance increases their early diagnostic sensitivity for the detection of acute myocardial infarctions (AMIs); however, this increase comes at the cost of a reduced AMI specificity because more patients with other causes of acute or chronic myocardial injury are detected as well.[2] Thus, increased cTn concentrations due to a variety of non-AMI-related cardiac and primarily non-cardiac pathologies with cardiac involvement (see Box 1), such as heart failure, rhythm disorders, pulmonary embolism, drug toxicity or renal failure, are increasingly seen in the daily routine.[1,2] Usually, although at first glance such increases are unexpected, they are clinically explainable well. With hs-cTn assays, these

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Box 1. Non-acute coronary syndrome-related troponin increase caused by other cardiac diseases or non-cardiac diseases with cardiac involvement.

Cardiac oxygen supply demand imbalance (AMI type II)
For example, prolonged tachyarrhythmias or bradyarrhythmias, coronary vasospasm or coronary microvascular dysfunction, coronary vasculitis (e.g. in Kawasaki or Churg–Strauss syndrome) or coronary embolism, shock, hypertensive urgency or crisis, severe anaemia or respiratory failure, pulmonary embolism
Other causes of myocardial injury
For example, myocarditis, cardiac contusion, cardiac surgery, cardiac ablation therapy, frequent defibrillator shocks, cardiotoxic agents (e.g. anthracyclines or Herceptin), severe neurological diseases (e.g. haemorrhage or ischaemic stroke leading to massive central sympathicus activation)
Non-cardiac diseases with cardiac involvement leading to myocardial injury
For example, sepsis (multifactorial causes of myocardial injury), infiltrative diseases (e.g. amyloidosis or sarcoidosis)

AMI: acute myocardial infarction.

situations may increase.[2] At times, the cTn values may not seem to fit the clinical presentation, and occasionally, if tested in the same blood sample, discrepancies between hs-cTnI and hs-cTnT test results may even be found, which adds to the potential confusion of non-specialised clinical practitioners. Analytical false-positive cTn may explain some of these discrepancies. In this review, we summarise the biochemical, pathophysiological and analytical background for such problems in order to help educate clinicians about how to approach these issues.

Summary of the analytical characteristics of hs-cTn assays

The criteria defining hs-cTn assays are based on convention, incorporating analytical sensitivity and precision.[2,3] They are summarized in Box 2. hs-cTn assays must have high precision in routine use with a total analytical coefficient of variation of <10% at the 99th percentile concentration of the reference population (upper reference limit [URL]). The analytical lower limit of detection (LoD) is in the range of single digits of ng/L or below. In contrast to previous assays, hs-cTn assays permit measurement of cTn concentrations (by convention >LoD) in a significant proportion (by convention >50%) of tested, apparently pathology-free individuals, which permits a more precise calculation of the URL.[3] Whether this criterion is met is obviously also dependent on the composition of the studied reference population. Only a hs-cTnT assay (Roche® Diagnostics) and a hs-cTnI assay (Abbott® Diagnostics) are so far commercially available for routine use in Europe and most parts of the world outside the USA.[4,5] hs-cTn assays show lower URLs for women than men.[5,6] Such a difference is biologically plausible, and the detectability of such a difference may be considered as a surrogate for the high analytical sensitivity of a cTn assay. However, there is still controversy regarding the importance of sex-specific cut-offs for diagnosis and risk stratification in routine use. The increased analytical sensitivity of hs-cTn assays also means that biological and analytical confounds may now be more relevant then with previous cTn assays.

Box 2. Analytical characteristics of high-sensitivity cardiac troponin assays.

1. hs-cTn assays have high precision in routine use at lower concentration ranges with an analytical CV of <10% at the 99th percentile concentration of the reference population (URL)
2. The analytical lower limit of detection is in the range of single digits of ng/L and is markedly lower than the URL
3. hs-cTn assays enable detection of cTn in a significant proportion of the reference population, thereby allowing for a more accurate calculation of the 99th percentile URL with its confidence interval
4. hs-cTn assays must be highly specific for the detection of cTn isoforms

CV: coefficient of variation; hs-cTn: high-sensitivity cardiac troponin; URL: upper reference limit.

Clinical scenarios

Case 1: emergency department presentation of an incidentally discovered elevated hs-cTnI

A 48-year-old male with a bicuspid aortic valve and stable moderate to severe aortic valve regurgitation was referred to the emergency department by a practicing cardiologist because of an elevated cTnI. The patient had no cardiac symptoms and the reason for troponin testing was retrospectively unclear. On admission, a markedly increased hs-cTnI test result (345.9 ng/L, URL <27 ng/L) was confirmed, but hs-cTnT was only 15.8 ng/L (URL <14 ng/L). There was no clinical or laboratory evidence of infection (C-reactive protein 1 mg/L, leukocytes 6.1 g/L) and the remaining parameters including creatinine (1.16 mg/dL, estimated glomerular filtration rate [eGFR] >60 mL/minute/1.73 m^2) were also normal. There was evidence of minor skeletal muscle injury (creatine kinase [CK] 503 U/L [URL <190 U/L] and myoglobin 127 µg/L [URL <72 µg/L]). hs-cTnI and hs-cTnT were retested 5 hours later with similar results (hs-cTnI 368.8 ng/L and hs-cTnT 16.3 ng/L). Computed tomography coronary angiography revealed normal coronary arteries, and stenosis of the aortic isthmus had been ruled out previously. Despite valvular heart disease, the observed marked mismatch between the elevation of hs-cTnI and the disproportionally lower, albeit still modestly elevated, hs-cTnT was striking, and analytical interference with one of the assays may be suspected clinically and has to be ruled out (for a suggested work-up, see below).

Case 2: a 4-year-old boy with Duchenne muscular dystrophy (deletion 3–30 in the dystrophin gene)

In 2012, heavily increased CK activity (27,757 U/L, CKMB activity 6%) was first noticed when this patient presented with a viral infection. Myoglobin (2144 µg/L) and hs-cTnT (1082 ng/L) were markedly increased as well. The patient had no cardiac symptoms, and the electrocardiogram (ECG), echocardiogram and N-terminal pro-B-type natriuretic peptide (NT-proBNP) were consistently normal during the course of his disease without any evidence of a cardiac phenotype. Cardiac magnetic resonance imaging (MRI) was not done in this small child because it would have required general anaesthesia in order to obtain good imaging quality. Analytical interferences with the hs-cTnT assay were excluded by the addition of blocking antibodies and a normal dilution profile (see below for the rationale). The diagnosis of Duchenne muscular dystrophy was proven by genetic analysis (deletion 3–30 in the dystrophin gene), as well as the mild but typical clinical symptoms. In 2015, hs-cTnT and hs-cTnI could be tested in the same blood sample during a routine follow-up visit. hs-cTnT was 359.8 ng/L, but hs-cTnI was undetectable (<2 ng/L), CK was 36,161 U/L, myoglobin was 2627 µg/L and NT-proBNP (231 ng/L; age- and sex-specific URL <327 ng/L) was still normal. In the persisting absence of a cardiac phenotype, which usually starts to manifest in second decade of life, this cTn discrepancy is consistent with the hypothesis of the maintenance of foetal TnT gene expression or re-expression of foetal TnT genes in Duchenne skeletal muscle myopathy, with a potential for release of cTnT from the diseased skeletal muscle (see below).

Case 3: a 64-year-old female on chronic haemodialysis with increased hs-cTnT referred for coronary angiography

This female was admitted to a local hospital because of angina. A coronary angiography performed 1 year prior had been without significant coronary artery disease (CAD). Her renal failure was due to hereditary polycystic kidney disease and she had been on haemodialysis for 3 years. The admission ECG showed negative T waves in chest leads 5 and 6 and in leads I and aVL. The admission hs-cTnT was 142 ng/L (URL 14 ng/L). Echocardiography revealed normal left ventricular function without regional wall motion abnormalities, left ventricular hypertrophy and no significant valvular disease, but a chronic, haemodynamically non-significant pericardial effusion (retrospectively, there was no change compared with a previous echocardiogram). There was no evidence of infection and the patient was transferred for acute coronary angiography for suspected acute coronary syndrome. There again was no evidence of significant CAD. Pulmonary embolism (because of a history of breast cancer and a D-dimer of 1194 µg/L) and aortic dissection were ruled out by computed tomography. In contrast, hs-cTnI was normal on admission (17.6 ng/L, URL <27 ng/L), but NT-proBNP was strongly elevated (21,004 ng/L). After diagnostic work-up, haemodialysis was performed and cTns were retested thereafter and showed no significant change (hs-cTnT 131 ng/L; hs-cTnI 16.6 ng/L). This case likely represents the reported increased frequency of hs-cTnT versus hs-cTnI elevations observed in patients with end-stage renal disease and demonstrates the importance of serial cTn testing for establishing an AMI diagnosis (see below).

Possible analytical interferences of troponin assays

hs-cTn assays are optimised to reduce analytic confounds by using so-called chimeric mouse–human antibodies and by the addition of heterophilic antibodies blocking antibodies to assay reagents.[4,5] Thus, analytical interferences are a rare cause of false test results, but they are possible. In cases where cTn test results and the clinical presentation are strikingly discordant, analytical false-positive test results have to be considered. The ideal work-up is shown graphically in Figure 1.

There are several possible reasons for erroneous results. First, there are what are called ‘fliers’ or ‘outliers’, which can be excluded by retesting. Outliers are spurious, random, non-repeatable, usually false-positive values. They are not due to analytical imprecision. The reported outlier rate for hs-cTn assays is low (hs-cTnT assay 0.13%; hs-cTnI assay average 0.59%).[7,8] However, misclassifications of patients as false positive or negative due to outliers have not been observed for the hs-cTnI assay,[8] providing confidence that the risk of patient misclassification due to fliers is low. By contrast, analytical confounds usually manifest as stable, unchanging elevations in clinical situations where elevations are not expected. These analytical confounds are described in the following subsections.

Typical analytical interferences (e.g. haemolysis, hyperlipidaemia or hyperbilirubinaemia)

These are not a matter of concern in the usual clinically seen concentration ranges.[4,5] However, pronounced haemolysis (>1 g haemoglobin/L), for example, may cause falsely low hs-cTnT values.[4] In addition, biotin, a water-soluble B-complex vitamin, has become a popular over-the-counter dietary supplement (typical content 5–10 mg) for the strengthening of hair and nails or easing peripheral neuropathy, for example. The unintended consequences of its use are spurious test results in immunoassays using biotin–streptavidin technology with the potential for false-negative results in the hs-cTnT sandwich immunoassay.

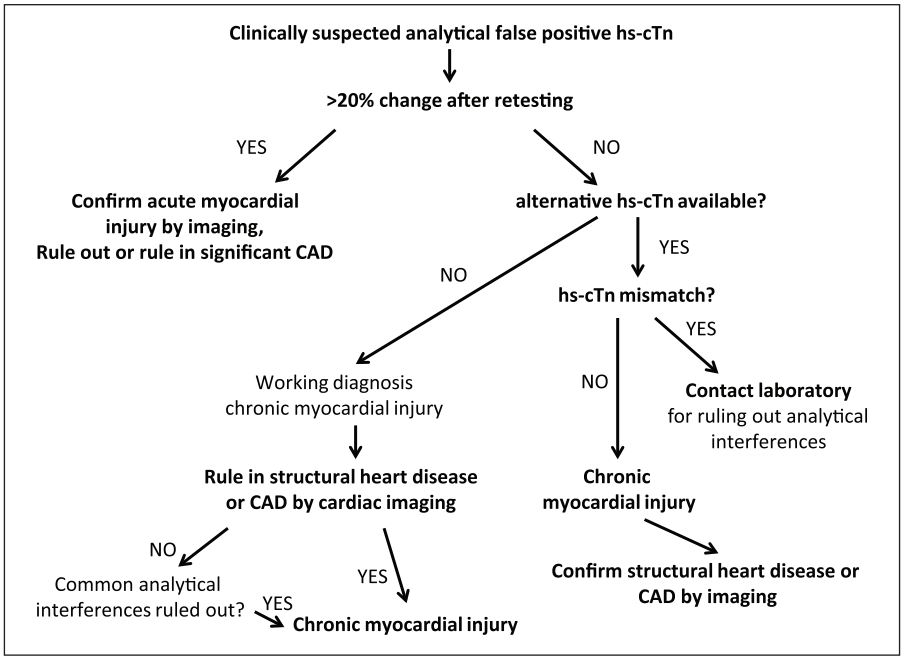

Figure 1. Work-up of a clinically suspected analytical false-positive high-sensitivity cardiac troponin test result. It has to be stressed that very small acute or chronic myocardial injury detected by hs-cTn testing may not always be seen with cardiac imaging, including cardiac magnetic resonance imaging (MRI), because of the limited sensitivities of all of the currently available imaging technologies. Therefore, costly and not easily available imaging technologies, such as cardiac MRI, should be restricted to patients with markedly increased hs-cTn concentrations without substrate in previous imaging.
CAD: coronary artery disease; hs-cTn: high-sensitivity cardiac troponin.

Heterophilic or human anti-mouse antibodies

Occasionally, very high titres of heterophilic antibodies can exist in individual blood samples. These are endogenous human antibodies that bind to immunoglobulins of other species and may interfere with hs-cTn assays (see Figure 2). In addition, specific human anti-mouse antibodies may occur, for example, after treatment with mouse monoclonal antibodies or, presumably, after contact with mice, and may cause false test results in immunoassays, which usually use mouse monoclonal antibodies.

These interfering antibodies can be unmasked by the addition of further blocking antibodies (so-called heterophilic blocking antibodies) or by dilution studies. The former eliminate the antibodies, leading to a change in test results. For the latter, when an interferent is present, samples will not dilute linearly until the substance causing the interference is eliminated. Thus, samples fail to dilute and then, when the interferent is gone, the values become remarkably reduced.

Troponin autoantibodies

Circulating anti-cTnI and anti-cTnT autoantibodies have been described.[9,10] Autoantibodies can occur in association with autoimmune disorders. Interestingly, there is an association between the presence of autoantibodies against cTnI and cardiomyopathy.[11] Historically, autoantibodies to cTnI have been reported to cause negative interference in some cTnI immunoassays by blocking the binding of the assay antibodies to the mid-fragment of cTnI.[9] At very high titres, they may mask the release of small amounts of cTnI. Although antibodies to cTnT have been described, disruptions in assay results have not been reported so far.

Macrotroponins

Macrotroponin I causing elevated hs-cTnI has been reported as well.[12] Macroanalytes consist of an analyte bound to analyte-specific autoantibodies, resulting in high-molecular-weight complexes that are cleared more slowly from the circulation than the free analyte, resulting in persistently elevated concentrations. It appears that the interference of macrotroponin is more common with the hs-cTnI assay than with the hs-cTnT assay.[4,12]

In conclusion, in case of a clinically suspected analytically false-positive or -negative hs-cTn test result, an additional blood sample should be obtained in order to exclude random error, and if necessary, procedures such as re-centrifugation (e.g. to remove fibrin clots or microparticles in specimens), dilution studies and/or incubation with heterophilic blocking reagents or polyethylene glycol precipitation to remove high-molecular-mass interferents should be performed. If available, an alternative hs-cTn can be tested

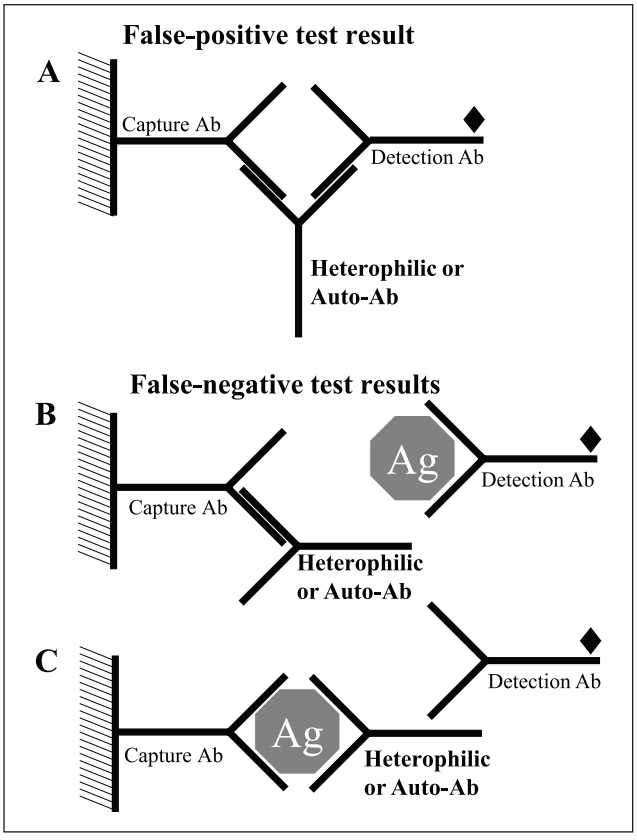

Figure 2. Possible analytical interferences by antibodies in cardiac troponin immunoassays. (A) False-positive test results may occur due to antibodies bridging the capture and detection antibodies in the absence of the antigen to be detected (i.e. cardiac troponin) in the sample leading to a positive signal. False-negative test results may occur by (B) binding of antibodies to the capture antibody, which blocks the binding of the troponin detection antibody complex, or (C) binding of antibodies to the antigen (i.e. troponin) capture antibody complex, which blocks the binding of the detection antibody to troponin, causing a negative test signal in the presence of cardiac troponin in the sample.
Ab: antibody; Ag: antigen.

in the same sample as well. If dilution testing suggests an interference, identification of the exact nature of the interfering substance may require more sophisticated procedures, but knowing the value is spurious is all that is needed for clinical care.

Biochemistry and pathophysiology of cTnI and cTnT

The science undergirding these issues has been reviewed in detail recently.[1,13–15] In short, the troponin complex plays a central role in the calcium regulation of actin thin filament function and is essential for the contraction of striated muscles. Only troponin I (TnI; the actomyosin ATPase inhibitory subunit) and troponin T (TnT; the tropomyosin binding unit), but not troponin C (TnC; the calcium binding subunit) are encoded as cardiac-specific isoforms (cTnI and cTnT). However, many aspects of troponin functioning are still incompletely understood. The molecular mass of human cTnT is 39,700 Da and of human cTnI is 24,000 Da.[13]

The underlying mechanisms for cTn release from normal hearts are still uncertain. Since analytical interferences could be ruled out,[4,5] a constant limited turnover of cTn and/or cardiomyocytes is indicated by detectable hs-cTn concentrations in people with normal hearts. cTnT undergoes rapid turnover with a half-life of approximately 3.5 days within cardiomyocytes.[16] When not incorporated into myofilaments, cTnT is rapidly degraded (e.g. by caspase or μ-calpain) in cardiomyocytes in order to avoid toxic effects.[13]

During foetal development, cTnT is expressed in cardiac, embryonic and neonatal skeletal muscles.[14,15,17–23] cTnT is downregulated in skeletal muscle during development, and after birth, cTnT gradually disappears from skeletal muscle, such that healthy adult human skeletal muscle does not contain cTnT. By contrast, cTnI has not been reported to be expressed at the protein level in foetal and adult human skeletal muscle.[13,24,25] When human skeletal muscle is chronically damaged, such as in patients with chronic skeletal muscle myopathies, it recapitulates embryonic myogenesis with re-expression of foetal proteins, potentially including cTnT isoforms. Such a process is likely to be dependent on disease severity. These re-expressed forms can then be released from the injured skeletal muscles into the circulation chronically or, if the muscle is damaged, acutely, with a rise and fall in serial testing. Previous studies in human skeletal muscle biopsies have shown evidence of re-expression of cTnT in skeletal muscle at the protein level by immunohistochemistry and western blotting.[17–21,25,26] Given the inherent problems of the specificity of these technologies, these observations cannot be regarded as proof.

Recently, however, cTnT expression in skeletal muscle biopsy specimens of patients with Pompe disease was demonstrated with the more sophisticated analytical technology of proteomics (i.e. detection of cTnT fragments using nanoflow liquid chromatography mass spectrometry, and by simultaneous detection of cTnT mRNA).[27] By contrast, cTnT was not detected in the skeletal muscle of healthy controls.[27] Wens et al.[27] detected cTnT isoform 6, which is the cTnT isoform that is expressed in healthy hearts, in the skeletal muscle of some patients with Pompe disease, which identifies the skeletal muscle as a potential source of circulating cTnT in these patients in case of the lack of cardiac involvement.

However, austere protein chemists might request amino acid sequence analysis of the positive bands detected using the antibodies of the hs-cTnT assay in western blotting, and finding cTnT by amino acid sequencing is a definitive proof of its expression in human skeletal muscle samples and its interference with the commercial hs-cTnT assay. This has

yet to be demonstrated, and thus, despite accumulating clinical evidence in several chronic neuromuscular diseases,[17–31] this issue is still not definitively solved.

Release and clearance of cTns

As discussed above, there appears to be normal turnover of the troponin complex and/or apoptosis with caspase degradation in healthy hearts, leading to detectable cTn concentrations in most individuals.[4–6,13,16] The troponin complex plays a significant role in the sensitivity of cardiac muscle to declines in intracellular pH (e.g. N-terminal proteolytic truncation of TnT may be an early adaptive response to myocyte injury when myofilament-associated calpain is activated by intracellular calcium overload).[13] Small, loosely bound, rapidly releasable cTnI and cTnT pools exist within cardiomyocytes.[13,16,32] However, recent data indicate that the fraction of cTnT that can dissociate from myofibrils *in vivo* is substantially larger than the 5–10% that has been previously reported.[33] Consequently, cTns manifest rapid release after myocardial injury that is comparable to cytosolic proteins, such as myoglobin, or CKMB mass.[34] The sustained increase in cTn after AMI is probably a combination of slow washout and local tissue degradation. Unrestricted blood flow results in faster extraction and clearance of cTns after myocardial injury.[33] cTns are more sensitive to minor myocardial injury than CKMB because their release ratio (the amount of protein found in the circulation over that depleted from the heart) is higher and because the CKMB content of normal hearts is lower. Both cTns generally detect myocardial necrosis equally well. After AMI, cTnT increases tend to continue for longer than cTnI increases.[35] cTns appear to be predominantly degraded and cleared by organs with a high metabolic rate (e.g. the reticuloendothelial system), with the kidneys being significantly involved.[36,37] cTns are cleared from the circulation with a half-life of 1–2 hours, similar to myoglobin or CKMB.[33]

Troponins present substantial challenges to measurement by immunoassay technology. They occur as various complexes or fragments and post-translational modifications (e.g. phosphorylation).[1,13,38,39] Intact cTns rapidly disappear from the circulation during the early hours after AMI, but immunoreactive fragments persist for longer.[38,39] The clinical relevance of complex formation, post-translational cTn modifications and cTn degradation after myocardial injury is that they may lead to changes in the availability of specific epitopes and thus varying recoveries of cTn variants in different cTn assays.[1–3] Most assays detect intact cTn (free or complex bound) and a varying mixture of cTn degradation products.

hs-cTns in chronic renal failure

Renal dysfunction is a condition in which elevated hs-cTn concentrations are commonly detected, and a previous generation of the cTnT assay, which is no longer available in most parts of the world, has been approved by the US Food and Drug Administration for risk stratification in end-stage renal disease patients. Deteriorating renal function appears to be more apt to increase hs-cTnT than hs-cTnI.[36,37] Increased hs-cTnT values (>14 ng/L) are common in chronic renal failure patients starting with an eGFR of <60 mL/minute/1.73 m^2.[36] In dialysis patients, hs-cTnT levels are more frequently elevated than hs-cTnI levels.[40] The underlying causes of this are still not fully understood, and are probably multifactorial. They include ongoing myocardial injury, but impairment in renal clearance is likely to be involved as well. Intact free cTnT is too large to be cleared by the kidneys, but smaller cTn fragments would be small enough for renal clearance, and a renal tubular component may contribute to excretion as well. Thereby, renal dysfunction also leads to cTn fragment accumulation. It has been argued that, in contrast to some cTnI assays, cTnT continues to be detected even as small fragments, because the epitopes detected (aa 125–131 and aa 136–147) are centrally located and close together.[4] However, a similar situation exists for the hs-cTnI assay,[5] which uses antibodies that detect epitopes in the stable, centrally located part of cTnI (aa 24–40 and aa 41–49), which are also close together. A significantly greater reduction after haemodialysis of approximately 50% for hs-cTnT has been reported as compared to hs-cTnI (median 30%).[40] These differences may be explained by differences of adherence of cTnI and cTnT and their immunoreactive complexes or fragments to the dialyser membrane, and may also be dependent on the specific membrane used for haemodialysis. At first glance, published data on cTnT expression in the skeletal muscles of patients with end-stage renal failure appear to be conflicting.[22,41] Haller et al. reported the absence of cTnT expression in the abdominal wall or back skeletal muscle biopsy specimens of five patients with end-stage renal failure.[41] However, truncal skeletal muscles are not typically involved in uremic skeletal myopathy, which usually affects proximal–extremity muscles, such as the deltoid, biceps and vastus lateralis. By contrast, Ricchiuti and Apple[22] reported cTnT mRNA expression and cTnT protein expression by western blotting analysis without cTnI expression in approximately 50% of the skeletal muscle specimens of haemodialysis patients in whom abdominal wall muscles, back muscles and arm muscles were tested. Unfortunately, it was not reported which muscle specimens tested positive. For future studies, it will be important to focus on muscles that are usually affected by uremic skeletal muscle myopathy. On the other hand, there was not a close relationship noted between the presence of uremic skeletal muscle myopathy and elevated cTnT values in 50 chronic haemodialysis patients.[42] Given the high prevalence of concomitant cardiac diseases, the high cardiovascular risk and the lack of clear results, it is still only possible to speculate about the origin of cTnT in many patients with severe renal failure.

Despite more frequently seen increases and irrespective of the mechanisms of increase, hs-cTnT and hs-cTnI maintain their prognostic value in patients with chronic renal failure.[40,43–46] Interestingly, in direct comparison, hs-cTnI was more strongly associated with left ventricular mass and left ventricular dysfunction, and hs-cTnT was more strongly associated with CAD and residual renal function in a population of stable dialysis patients.[43] The presence of both elevated hs-cTnT and hs-cTnI concentrations in patients with chronic kidney diseases is associated with an increased presence of underlying structural heart disease and increased cardiovascular risk. Studies vary with regards to which is found to be a better predictor of mortality in severe renal failure.[44–46] A prospective randomised clinical trial assessing the value of cTn testing in a clinical decision-making context is still needed.

In the setting of suspected AMI and renal failure for hs-cTnT, higher medical decision limits have been suggested (>twofold usual URL, 30 ng/L if eGFR <60 mL/minute/1.73 m^2; 144 ng/L if eGFR <30 mL/minute/1.73 m^2).[47,48] Nonetheless, for the definitive diagnosis of acute myocardial injury, it is still mandatory to detect a significant change in serial testing.[1,2,49]

Possible explanations for reported discrepancies between hs-cTnT and hs-cTnI in clinical studies

There are reported discrepancies in the early diagnostic sensitivities and specificities of the commercially available hs-cTn assays for AMI, as well as in their utilities for risk stratification, at least when using their URLs as medical decision limits.[40,43–46,50–53] A comparative study of hs-cTnI and hs-cTnT for AMI diagnosis suggests that when one uses hs-cTnT as a gold standard biomarker for AMI diagnosis, which obviously leads to a significant bias, hs-cTnI appears to be markedly less sensitive.[50] Since cTnT and cTnI are different analytes, one might also argue that cTnT is simply more sensitive, despite the differences in the number of normal subjects in whom values can be measured,[4–6] which, after all, is an arbitrary surrogate for sensitivity. Indeed, it was postulated that one would need to halve the 99th percentile URL value of hs-cTnI in order to match the sensitivity of hs-cTnT.[50] However, it is also unlikely that other extensive validation studies of the hs-cTnI assay are inaccurate[5,51,52] and that these proposed cut-off values are egregiously too high. In addition, these comparisons of early AMI sensitivities were based on 99th percentile URL decision limits of both hs-cTn assays, which were not obtained from the same reference population and may also influence study results and conclusions, as mentioned above. However, although it is fair to note that when values are directly compared[50] and hs-cTnT is used as the gold standard biomarker it appears that hs-cTnT is more sensitive, the opposite would be true if hs-cTnI was used as the gold standard. Specifically, if hs-cTnI were considered to be correct in the same data set, there would be a substantial false-positive rate for hs-cTnT. More frequently detected hs-cTnI in normal subjects should have some positive relationship to clinical sensitivity for early AMI diagnosis.[4–6] Indeed, Cullen et al. reported equivalent sensitivities of hs-cTnT and hs-cTnI with a lower specificity of hs-cTnT for AMI diagnosis using cTnI as a laboratory standard biomarker measured with conventional cTnI assays.[51] However, in most studies, the generally used URLs provide comparable sensitivities of hs-cTnI and hs-cTnT for AMI diagnosis.[5,51,52] Another explanation would be that, in these published clinical studies, hs-cTnT release from chronically injured skeletal muscle may be more clinically relevant than suggested by the very low prevalence of these diseases in the usual populations, because mild chronic skeletal muscle abnormalities are easy to overlook and was not screened for in these studies. One common responses to this suggestion is that if some hs-cTnT elevations might be due to chronic skeletal muscle disease, hs-cTnT should not be so highly predictive of mortality. However, picking up another disease process might even increase the predictive capacity for total mortality, but might reduce its predictive impact on cardiovascular mortality and morbidity. The literature is vast, but there are data that would fit this pattern.[53]

However, the major flaws of all of the above-mentioned study conclusions are that these comparisons were mainly done in stored samples, and differences in the *in vitro* stabilities of troponins must be ruled out as the simplest reason for the observed discrepancies. In many studies, the samples were stored for months or even years before measurement. Definitively, more information, including immediate cTn measurements without sample storage and cardiac MRI as the most sensitive imaging modality currently available, is still needed in order to assess whether there are true differences in sensitivities and specificities of hs-cTnI and hs-cTnT, particularly – as outlined in detail above – in patients with chronic kidney or neuromuscular diseases.

Implications for clinical practice

Algorithms for the work-up of patients with increased cTn concentrations without overt myocardial ischaemia have been reported by our group and others before.[1,2,49,50] The concept of a significant change in serial testing is critical for the differentiation of acute from chronic myocardial injury.[1,2] A change of >20% in serial testing with baseline values >URL excludes analytical variation of hs-cTn assays as the underlying cause of the observed kinetics.[2] The algorithm shown in Figure 1 for the work-up of clinically unexpected or unexplained hs-cTn concentrations rules in or rules out acute or chronic myocardial injury and analytical interferences of hs-cTn assays. When cTn results and the clinical presentation are strikingly discordant, whenever

possible, the alternative cTn should be tested and the hospital's laboratory should be contacted in order to consider and rule out rare analytically false-positive cTn test results. Interdisciplinary assessment of cardiologists and laboratory specialists may prevent unnecessary and potentially harmful diagnostic and therapeutic procedures (e.g. invasive coronary angiography, heparin treatment or dual platelet inhibition). Stable or cTn elevations without significant dynamic changes, however, may also be markers of chronic structural heart disease. Currently, the most sensitive imaging modality for confirming minor myocardial injury is cardiac MRI,[54] but the sensitivity of this approach is still lower than that of hs-cTn assays for the detection of minor myocardial injury because it requires confluent areas of injury in order to generate a signal, and thus can miss more diffuse injury. Therefore, this costly and not always easily available imaging technology should be restricted to patients with markedly increased unexplained hs-cTn concentrations after previous use of other modes of imaging, such as echocardiography. As outlined above, based on increasing published data, patients with unexplained increased cTnT after following the algorithm shown in Figure 1 should also be evaluated for possible, clinically still asymptomatic chronic skeletal muscle (in particular myositis and muscular dystrophy) or chronic, as-yet undiagnosed kidney diseases.

Conclusion: critical clinical concepts

1. When cTn results and the clinical presentation are strikingly discordant, rare analytically false test results should also be considered by clinicians, and the laboratory should be contacted in order to rule out analytical interferences. No assay – including cTn – is perfect.
2. After AMI, cTnT tends to stay increased for somewhat longer than cTnI, which may explain test result discrepancies during the 1–2 weeks after an AMI.
3. Clinically, the most cardiac-specific marker in the rare population of patients with chronic skeletal muscle damage (e.g. muscular dystrophies or chronic myositis) appears to be cTnI, which is easier to interpret in case of suspected AMI in these patients.
4. In stable patients with chronic renal failure without cardiac symptoms, hs-cTnT increases above the usual URL are markedly more frequent than hs-cTnI increases.
5. The importance of the change in hs-cTn levels in serial testing for a diagnosis of acute myocardial injury must be emphasised. A changing pattern may be caused by pre-analytical problems, but this is inconsistent with analytical interferences with the assay. Most elevations of hs-cTnT in chronic skeletal muscle or renal diseases do not manifest with a changing pattern of values in short-term serial testing.
6. In patients without cardiac symptoms but with chronic neuromuscular or chronic kidney diseases and cTn mismatches, unnecessary and potentially harmful cardiac interventions should be avoided if not indicated for other reasons than an elevated cTn.

Conflict of interest

During recent years, Dr Mair received consulting fees from Philips Health Care Incubator and lecture fees from Roche Diagnostics. Dr Lindahl received research support from bioMerieux and Fiomi, as well as speaker/consulting honoraria from bioMerieux, Roche, Radiometer, Philips, ThermoFisher and Fiomi. Dr Giannitsis received honoraria for lecturers from Roche Diagnostics, BRAHMS ThermoFisher, AstraZeneca and Mitsubishi Chemical Europe. He has received an institutional research grant from Roche Diagnostics and serves as a consultant for Roche Diagnostics and BRAHMS ThermoFisher. Dr Huber received lecture fees from Novartis. Dr Plebani received consulting fees from Roche Diagnostics, Abbott Diagnostics and Siemens Health Care Diagnostics. Dr Möckel received lecture and consulting fees from Novartis, Roche Diagnostics, Bayer Healthcare, Boehringer Ingelheim, AstraZeneca and BRAHMS ThermoFisher, and research grants from Roche Diagnostics and BRAHMS ThermoFisher. Dr Müller received research grants from the Swiss National Science Foundation and the Swiss Heart Foundation, the European Union, the Cardiovascular Research Foundation Basel, 8sense, Abbott, AstraZeneca, Alere, BRAHMS ThermoFisher, Critical Diagnostics, Nanosphere, Roche, Siemens, Singulex, Sphingotec and the University Hospital Basel, as well as travel support or speaker/consulting honoraria from Abbott, ALERE, AstraZeneca, BG Medicine, bioMerieux, BRAHMS ThermoFisher, Cardiorentis, Daiichi Sankyo, Lilly, MSD, Novartis, Pfizer, Roche, Siemens and Singulex. Dr Jaffe has or presently consults for Abbott, Alere, Roche, Radiometer, Siemens, Beckman, Trinity, ET Healthcare, Dart Neurosciences, Lpath, Diadexus, Novartis and theheart.org. Dr Thygesen has received speaker/consulting honoraria from Roche Diagnostics, Abbott Diagnostics and Siemens Health Care Diagnostics.

Funding

This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors.

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