Highly sensitive troponin assays — a two-edged sword?

Lower specificity and lower positive predictive value necessitate a cautious approach

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The advent of cardiac troponin (cTn) assays has redefined acute myocardial infarction (AMI) and revolutionised the care of patients with suspected AMI presenting to emergency departments (EDs).[1] So central has cTn measurement become to the diagnosis of AMI that, since 2000, the formal criteria start with detection of rise and/or fall in serum troponin levels (with at least one value above the 99th percentile of the value distribution of a reference population for an assay with optimal precision at this level, defined as a coefficient of variation ⩽ 10%), to which clinical evidence of myocardial ischaemia is added in regard to symptoms, electrocardiogram (ECG) changes or findings on cardiac imaging.[2] This revised definition of AMI, with cTn assays using 99th percentile cut-off values, has altered the epidemiology of the disease. Data from Western Australia suggest that in the two decades before the advent of cTn testing in 1998, age-specific hospitalisation rates for AMI had decreased by an average of 30%, but this downward trend was abolished between 1998 and 2004.[3]

Progressive lowering of diagnostic thresholds for AMI

Using the logic that detection of even lower levels of cTn may assist in earlier AMI diagnosis and improved risk stratification, each new generation of cTn assays has been developed with the aim of greater sensitivity. As the limit of detection has progressively decreased, so has the 99th percentile threshold for labelling a given cTn value as abnormal and potentially diagnostic of AMI. This has occurred in tandem with improved ability to detect small changes in cTn levels at these lower concentrations. While first-generation assays identified 99th percentile concentrations at 0.5 μg/L, new fifth-generation highly sensitive (hs) cardiac troponin T (cTnT) immunoassays can identify concentrations as low as 14 ng/L with reasonable precision.[4] Indeed, cTnT levels as low as 3 ng/L can now be detected in up to 66% of seemingly healthy individuals, compared with detection of cTnT in 10% to 20% of the same individuals using assays that are currently available for clinical use.[5]

The promise of hs-cTn assays

It is assumed that increasingly sensitive cTn assays will result in higher diagnostic rates for AMI and that, as a consequence of providing appropriate therapies to patients with newly diagnosed AMI, risk of death and recurrent cardiac events will fall. A recent before–after study involving 2092 patients with suspected acute coronary syndrome (ACS) has provided early support for this assumption — lowering the 99th percentile cut-off value from 200 ng/L to 50 ng/L for a sensitive assay led to an 8 percentage point increase in the number of patients diagnosed with AMI, while incidence of death and recurrent AMI at 12 months among patients with cTn cut-off values between 51 ng/L and 199 ng/L significantly decreased from 39% to 21%.[6] In other studies, the use of hs-cTn assays compared with previous-generation assays has resulted in between 9% and 27% of patients with chest pain being recategorised to AMI on the basis of hs-cTnT results alone.[7-9]

The advent of hs-cTn assays also holds the promise of detecting or ruling out AMI earlier as a result of improved sensitivity. In a study of 718 patients that compared three sensitive cardiac troponin I (cTnI) assays and one hs-cTnT assay with the standard fourth-generation cTnT assay,[10] the sensitivity and negative predictive value of the hs-cTnT assay were superior to those of the standard assay among patients who presented within 3 hours of symptom onset (Box 1). In another study, use of hs-cTnT assays reduced the average time to confirm or exclude AMI after presentation from 4 hours to 71 minutes.[11] However, a more recent study comparing a hs-cTnI assay with a contemporary cTnI assay, using a diagnostic cut-off value at the 99th percentile of 30 and 32 ng/L respectively, revealed identical negative predictive values on admission (94.7% v 94.0%) and at 3 hours after admission (99.4% for both).[12]

Where hs-cTn may be superior as a rule-out criterion relates to whether troponin can be detected at all on presentation to the ED. In a prospective evaluation of two patient cohorts totalling 1618 patients presenting with chest pain, only one of 355 patients (0.3%) who had undetectable cTnT according to a hs-cTnT assay at presentation to the ED (< 3 ng/L) subsequently had elevated levels consistent with AMI.[13] This equated to sensitivity ranging from 99.8% to 100% and negative predictive value ranging from 99.4% to 100%.

Unanswered questions

However, despite their potential to facilitate decision making and their early adoption by many laboratories, hs-cTn assays have amplified some longstanding questions regarding all cTn assays. To determine the utility of hs-cTn assays in routine clinical practice, these questions need to be answered.

1 Diagnostic performance of hs-cTnT versus fourth-generation cTnT assays in patients who present within 3 hours of symptom onset (n = 222)*

	Sensitivity (95% CI)	Specificity (95% CI)	NPV (95% CI)	PPV (95% CI)
hs-cTnT assay (limit of detection, 0.002 µg/L)	85% (66%–96%)	84% (78%–89%)	98% (94%–99%)	42% (29%–56%)
Fourth-generation cTnT assay (limit of detection, 0.010 µg/L)	44% (26%–65%)	99% (96%–100%)	93% (88%–96%)	80% (52%–95%)

hs = highly sensitive. cTnT = cardiac troponin T. NPV = negative predictive value. PPV = positive predictive value. * Table adapted from Reichlin and colleagues[10] (supplementary appendix, available at http://www.nejm.org/doi/suppl/10.1056/NEJMoa0900428/suppl_file/nejm_reichlin_858sa1.pdf), and data represent Roche assays. Values listed correspond to 10% coefficient of variation. ◆

Defining cTn changes that are diagnostic of AMI, and timing of serial tests

The change, or delta, in cTn level which distinguishes an elevation due to AMI from an elevation due to non-ACS causes must be defined. The delta can be expressed as a percentage increase, a change in absolute value, or a rate of change over a specified period. Currently there is no universal consensus regarding the delta for hs-cTn assays that defines AMI. Some experts recommend a 20%[14] or 30%[15] increment from baseline — a degree of change that reflects statistical significance. Recently updated Australian guidelines suggest a >50% change from baseline over a 24-hour period,[16] which lends greater specificity to the test for an acute event.[11] Other investigators advise that absolute changes in baseline cTn levels, rather than relative changes, provide greater diagnostic accuracy.[17] However, none of these criteria has been validated and recommendations are largely based on expert opinion.

Second, the optimal timing for taking a second blood sample to distinguish between prolonged and transient elevation of cTn is unclear. Australian guidelines state that, when using hs-cTn assays, repeat cTn testing *might* occur at a minimum of 3 hours after presentation to ED *and* at least 6 hours after onset of chest pain.[16] Current US guidelines recommend an interval of at least 6 hours between samples, whereas a study of 258 patients presenting to an ED showed that the prevalence of AMI was the same regardless of whether second samples were taken 3, 4 or 5 hours after the first sample.[18] Other studies involving sensitive cTn assays suggest that serial testing after 3 hours following admission does not improve overall diagnostic accuracy.[17,19]

Finally, positive serial cTn results (ie, >99th percentile and >50% change from baseline) must differentiate very transient cTn elevation due to myocardial ischaemia (which may result from non-ACS causes)[20] from more prolonged elevation due to myocardial necrosis (ie, infarction). The more sensitive hs-cTn assays are likely to detect more cases of the former, which may be misinterpreted as ACS.

> “Use hs-cTn assays to rule out rather than rule in AMI”

Biological variability

Biological variation in low baseline cTn values must be considered when defining diagnostically significant deltas. Serial testing of healthy volunteers has revealed short-term (0–4-hour) biological variation in hs-cTnT values — 64% to 90% increases and 39% to 47% decreases.[21] Low-level non-specific binding of cTn to other proteins, especially fibrin and various heterophile antibodies, can cause false-positive and false-negative results, as can haemolysis of even modest extent.

Defining normal cTn levels in reference populations and 99th percentile cut-off values

Given their ability to precisely detect cTn levels well below 99th percentile values for reference populations previously defined by standard assays,[22] hs-cTn assays will redefine the cTn distribution for a “normal” population, which will in turn redefine the 99th percentile cut-off value for an “elevated” test result. However, this work is yet to be completed. Historically, there has been little standardisation of cTn assays; the 99th percentile cut-off values vary according to demographics and the screening methods used to select “healthy” individuals, and they are specific to individual assays. It has been suggested that sex- and age-specific 99th percentile values should be applied,[23] which would necessitate frequent recalibration of cTn cut-off values using reference populations that reflect wider demographic trends.

Dealing with non-ACS causes of elevated cTn levels

The downside of the ability of hs-cTn assays to detect lower levels of cTn is decreased specificity and low positive predictive value for AMI if the chosen delta is too small. In patients with a low pretest probability of ACS, most elevated cTn levels will not be attributable to AMI. As assay sensitivity has increased, the list of non-ACS causes of an abnormally elevated cTn level (transient or prolonged) has expanded. Conditions associated with

2 Additional cTnT values meeting or not meeting AMI definition with hs-cTnT assay, compared with a standard cTnT assay, across differing AMI probabilities in the target population*

AMI probability	Positive tests with standard assay, per 1000 patients	Positive tests with hs assay, per 1000 patients	Additional positive tests with hs assay v standard assay meeting AMI definition, per 1000 patients	Additional positive tests with hs assay v standard assay not meeting AMI definition, per 1000 patients
17%	199	328	21	108
10%	146	275	12	117
5%	108	237	8	121
3%	93	222	3	126

cTnT = cardiac troponin T. AMI = acute myocardial infarction. hs = highly sensitive. * Table reproduced with permission from the American Association for Clinical Chemistry.[28] Data from Reichlin and colleagues[10] were used for the base-case AMI prevalence (17%) and for sensitivity and specificity for the assays. These sensitivity and specificity data at presentation were used to calculate the positive test rate at various AMI probabilities. The threshold values used to define a positive test result were the limit of detection for the standard assay and the 99th percentile value for the hs-cTnT assay. ◆

3 Interpreting hs-cTn assay results with caution

Understand analytical considerations

- Know the 99th percentile value for the assay in use locally.
- Suspect non-AMI diagnoses in patients with elevated cTn levels that do not change over time (except patients with AMI who present late, when peak cTn levels may have already been reached and are not subject to change).
- Ask laboratory staff to report analytical conditions associated with greater likelihood of erroneous measurement (eg, presence of haemolysed sample, circulating antibodies, other interfering substances).

Diagnose AMI based on the clinical scenario and cTn result

- Estimate the pretest probability that a given patient has AMI on the basis of clinical criteria and/or clinical prediction rules.
  - ➢ If the patient's pretest probability is intermediate to high and cTn level is elevated and showing dynamic change, the diagnosis of AMI can be confirmed (although dynamic changes can also occur in acute pulmonary thromboembolism).
- Consider non-ACS causes of elevated cTn in patients with a low pretest probability of AMI, particularly when there is no dynamic change.
- Consider acute illnesses that cause myocardial oxygen supply–demand imbalance (type 2 AMI) in patients who are at risk of such conditions.

Use hs-cTn assays to rule out rather than rule in AMI

- If cTn is not elevated within 6 hours of symptom onset according to a hs-cTn assay, the patient is highly unlikely to have AMI and/or be at risk of short-term adverse outcomes.
  - ➢ If further investigations are warranted to assess for stable coronary artery disease, consideration should be given to doing these in a timely manner in an outpatient setting.

hs = highly sensitive. cTn = cardiac troponin. AMI = acute myocardial infarction. ACS = acute coronary syndrome. ◆

myocardial damage and/or decreased clearance of cTn — such as sepsis, hypovolaemia, atrial fibrillation, congestive heart failure, pulmonary embolism, myocarditis, myocardial contusion and renal failure — may increase cTn levels.[24] cTn levels can also be elevated by certain cardiotoxic drugs, such as doxorubicin and trastuzumab; carbon monoxide poisoning; and prolonged strenuous exercise, such as marathon running.[25]

Hence, to avoid inappropriate treatment and unnecessary investigations for presumed ACS, it is important to interpret elevated cTn levels in the context of the clinical presentation and to consider alternative causes. Several studies have shown that between 70% and 90% of patients who test negative using fourth-generation assays but positive using hs-cTnT assays had non-ACS conditions.[7-10] Although even low elevations in cTnT levels (measured using hs-cTnT assays) that lack a specific diagnosis are a marker of worse prognosis, an effective clinical strategy is yet to be determined for patients who have such elevations but do not have ACS. A recent study indicates that higher values of hs-cTn at presentation and higher changes within the first hour, combined with ECG changes, can accurately distinguish ACS from non-ACS causes of chest pain.[26] These findings have been incorporated into an algorithm that can rule AMI out or in within 1 hour of presentation according to baseline and 1-hour delta values of hs-cTnT.[27]

Managing patients who test positive using hs-cTn assays only

A final challenge relates to the current lack of evidence regarding optimal management of patients with ACS who test positive for cTn using hs-cTn assays but negative using less sensitive assays. Any cTn assay will have little impact on the early management of patients presenting with classical symptoms and signs, including unequivocal ECG changes, that indicate spontaneous AMI secondary to plaque rupture (type 1 AMI). The value of cTn assays lies more in the early detection of increasingly more common non-ST-elevation AMI in patients whose presentations are often more atypical, frequently induced by acute non-cardiac disease processes (type 2 AMI), and associated with more equivocal ECG changes and a smaller extent of myocardial necrosis.

It is currently unclear whether all such patients, if otherwise eligible, would benefit from aggressive treatments such as anticoagulation and percutaneous coronary intervention. Clinical trials which established the efficacy of such treatments in ACS predated the use of hs-cTn as the biomarker of myocardial necrosis. Hence, studies are needed to assess treatment effects in patients who test positive using hs-cTn assays but negative using less sensitive assays.

Potential implications for clinical practice

While the only hs-cTn assay which is currently commercially available in Australia has already been adopted by many laboratories, its use is by no means universal, although the shift to hs-cTn assays is likely to accelerate with the introduction of one or more hs-cTnI assays in 2012. However, hs-cTn assays have the potential to be a two-edged sword if they are not used carefully — for example, without monitoring for potentially deleterious effects. There is little doubt that positivity rates for hs-cTn assays will be considerably greater than for current assays. For example, an Italian study reported a 2.4-fold increase in the proportion of ED patients with positive test results — from 19% to 45% — following introduction of hs-cTnT assays, which led to an 85% increase in the number of patients hospitalised (based on a comparison of the first 3 months after assay introduction and the same period in the previous year).[28] Positivity rates in a New Zealand study more than doubled — from 22% to 50% of patients (based on assessment of patient blood samples using both hs-cTnT and fourth-generation assays).[9]

This increased positivity rate includes many false positives for AMI as a result of the lower specificity and lower positive predictive value of hs-cTn assays compared with fourth-generation assays (Box 1). Among 1000 patients with an AMI probability of 10%, it has been estimated that hs-cTn assays (compared with standard assays) would produce 12 additional positive results that meet the definition of AMI and 117 that do not meet the definition; with an AMI probability of 17%, hs-cTn assays would produce 21 additional positive results that meet the definition and 108 that do not (Box 2).[29] In low-risk patients (AMI probability, ⩽5%), additional numbers of positive results meeting the AMI definition would be eight or fewer, and those not meeting the definition would be up to 126.[29]

These estimates pose a considerable logistical challenge for cardiologists, general physicians and ED physicians as the vastly increased numbers of patients who test positive for cTn using hs-cTn assays could invoke more serial cTn testing and further cardiac investigations, resulting in

longer hospital stays, ED overcrowding, and more admissions to acute medical assessment and chest pain units. This increased resource utilisation may not be offset by more rapid discharge and avoidance of testing in the no more than 25% of patients with chest pain who test negative for cTn using hs-cTn assays at presentation.

More before–after studies involving hospitals that have introduced hs-cTn assays would provide clarification about real-world effects. The opportunity to conduct prospective studies that assess clinical outcomes and cost-effectiveness of hs-cTn assays in comparison to existing cTn assays should be seized as these new assays are introduced into sites where they have not previously been used. At the very least, we advise clinicians to interpret hs-cTn assay results with caution (Box 3) while awaiting results of future studies.

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