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Pregledni članak

SENSITIVE CARDIAC TROPONIN ASSAYS: MYTH AND MAGIC OR A PRACTICAL WAY FORWARD?

OSETLJIVI TESTOVI ZA SRČANI TROPONIN: MIT I MAGIJA ILI PRAKTIČAN NAPREDAK?

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Summary: Cardiac troponins (cTn) are considered to be the 'gold standard' biomarkers for the diagnosis of acute coronary syndrome (ACS) a pathological spectrum which includes cardiac ischemia, angina, myocardial infarction and ultimately cardiac failure. The growing evidence base for the diagnostic and prognostic use of cTn in ACS has resulted in a universal redefinition of acute myocardial infarction (AMI). A diagnosis of AMI includes the detection of an elevated cTn (or CK-MB) with at least one measurement within 24 hours of the cardiac episode being >upper 99th percentile of a reference population, in conjunction with evidence of myocardial ischemia. A number of high sensitivity immunoassays with claims of superior imprecision and a definable 99th percentile have been produced. Clinically, these have two important impacts. First, there is a drive to change the values into whole numbers by the application of a unit change which carries the scope for confusion. Secondly, the near-normal Gaussian distribution of sensitive cTn in healthy subjects will increase the frequency of cTn positivity in the non-ACS population. The problem is to decipher if such minor elevations in cTn are of clinical concern. What is certain is that AMI remains a clinical not a biochemical diagnosis and the interpretation of cTn concentrations should be made according to the clinical context.

Keywords: cardiac troponins, biomarkers, acute myocardial infarction

Kratak sadržaj: Srčani troponini (cTn) smatraju se »zlatnim standardom« među biomarkerima za dijagnostikovanje akutnog koronarnog sindroma (ACS), patološkog spektra koji obuhvata srčanu ishemiju, anginu, infarkt miokarda i konačno prestanak rada srca. Sve veći broj dokaza koji idu u prilog dijagnostičkoj i prognostičkoj upotrebi cTn u ACS doveo je do opšteg ponovnog definisanja akutnog infarkta miokarda (AMI). Dijagnoza AMI uključuje detekciju povišenog cTn (ili CK-MB) – najmanje jednom u 24 časa od srčane epizode izmeren je nivo > gornjeg 99. procenta referentne populacije – uz dokaze o ishemiji miokarda. Izrađeno je nekoliko veoma osetljivih imunoeseja s navodno superiornom nepreciznošću i 99. procentilnom vrednošću koji se može definisati. U kliničkom smislu, oni imaju dvojaku važnost. Prvo, postoji težnja da se vrednosti promene u cele brojeve, menjanjem jedinice koja unosi zabunu. Drugo, gotovo normalna Gaussova raspodela osetljivog cTn kod zdravih subjekata povećaće učestalost pozitivnog cTn u populaciji bez ACS. Problem je kako utvrditi da li su ti blago povišeni nivoi cTn od kliničkog značaja. Ono što je sigurno jeste da AMI ostaje klinička a ne biohemijska dijagnoza i da se tumačenje koncentracije cTn mora izvoditi u skladu s kliničkim kontekstom.

Ključne reči: srčani troponini, biomarkeri, akutni infarkt miokarda

Introduction

Since their introduction in the late 1980's, cardiac troponin (cTn) T (cTnT) and I (cTnI) have demonstrated a high specificity and sensitivity for myocardial cell damage and play a central role in aiding the diagnosis of acute coronary syndrome (ACS). The superior clinical value of troponin measurement over classical markers such as creatine kinase (CK) and its MB (CK-MB) isoenzyme has led to the recommendation that it be adopted as the 'gold standard'

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cardiac biomarker following the universal definition of acute myocardial infarction (AMI) (1). Cardiac troponin measurement offers considerable benefit for risk stratification and interventional decision making in ACS patients (2).

A diagnosis of AMI includes the detection of an elevated cTn (or CK-MB if cTn is not available) with at least one measurement within 24 hours of the cardiac episode being greater than the upper 99th percentile of a reference population; in conjunction with evidence of myocardial ischemia (1). For adequate clinical confidence, a 10 % coefficient of variation (CV) is recommended at the 99th percentile concentration.

Cardiac Troponin assays

The measurement of cardiac troponin is done by automated immunoassay (3) and several generations of assays for both cTnT and cTnI have been produced. Immunoassay technology is widespread in clinical chemistry laboratories and offers high volume throughput at high speed. Smaller point of care devices principally based on lateral flow are also available for near patient testing. Immunoassay principles work on the signal-to-noise ratio to distinguish inherent non-specific background noise in the assay to signal generated by the antigen-antibody sandwich interaction. Troponin challenges immunoassay performance by the necessity to recognise low but meaningful signal (concentrations) of cTn from the noise. With increasing popularity due to ease of measurement, the presence of a tick box on a laboratory request form and a reduction in test price, cTn is a commonly requested test.

A number of manufacturers have reformulated or introduced so-called 'high sensitivity' cTn assays. With the aim of meeting the recommendations, the introduction of such assays provides challenges for both the laboratory and the clinician, both of which will be discussed here.

The impact of sensitive cTn assays on the laboratory

Until recently many commercial assays did not meet the guideline requirement of a 10 % CV at the 99th percentile (*Table I*), compromising the clinical sensitivity of the assay (4). Many laboratories derive their own 10 % cut-off concentration for the clinical discriminant of a positive cTn and adopt a number of methods to construct this concentration. Whilst a 99th percentile is advantageous to increase the diagnostic sensitivity, laboratorians still favour the 10 % coefficient of variation cut-off value over the 99th percentile, presumably as this is within a margin of safety they are both familiar and comfortable with and is established for other immunoassay tests (5).

There is a drive to change units from values currently reported in μg/L to ng/L. As an example, the cTnT detection limit of 0.01 μg/L would become 10 ng/L. This alone is scope for clinical confusion, especially when looking at a change in absolute cTn concentration to adequately describe a rise and fall of cTn. Clinically, a delta change of 0.01 to 0.02 μg/L would be considered insignificant, yet could be mistaken as a significant change when reporting the same results as 10 to 20 ng/L. More importantly, those who favour still the WHO criteria (6) for AMI would need to adjust their cut-off from 0.1 μg/L to 100 ng/L.

The laboratorians will also be faced with an increased number of low level cTn positive samples in patients who do not have a final diagnosis of ACS. It is well established that cTn is not a biomarker for AMI but for cardiac cell damage; a number of secondary ischemic and non-ischemic cardiac injuries are known to be associated with elevated cTn (*Table II*). The majority of comorbid pathologies (except those performing extreme exercise) confer a poor prognostic risk when using the 99th percentile.

Clinical utility of high sensitivity cardiac troponin assays

High sensitive cTn will affect the clinical interpretation of positive results. Not only will there be greater positives outside the remit of ACS, but within the ACS population, it may be possible to diagnose AMI earlier.

Using a contemporary assay (Centaur TnI-Ultra, Siemens Healthcare Diagnostics) Collinson and colleagues have demonstrated a 99th percentile of 0.039 μg/L (39 ng/L) based on a population of 309 (41 % male) apparently healthy individuals screened for risk factors. These included no history of vascular disease, diabetes mellitus, hypertension, or heavy alcohol intake, no cardiac medication, a mean blood pressure <160/90 mm Hg (2 readings), fasting blood glucose <6.0 mmol/L, eGFR >60 mL/min/1.73 m^2, no significant valvular heart disease, LVH, diastolic heart failure, LVEF <50 % or regional wall motion abnormalities on echocardiography (7). Within the reference population, cTnI was completely undetectable in 25 subjects and considered negative in 53 %. There was no correlation between cTnI and age and there were no significant differences between gender (7). Using the same population, the researchers have defined the 99th percentile of the hs-cTnT assay (Roche Diagnostics) to be 15.5 ng/L (Collinson et al., unpublished data) and for the Enhanced AccuTnI (Beckman Coulter, Inc.) to be 42.0 ng/L (Gaze et al., unpublished data) with 96 % of subjects having a definable cTnI below the 99th percentile with this assay. What is interesting to note are the differences between the numbers of detectable cTn by assay within the reference population (*Figure 1*).

The prognostic value of the AccuTnI assay has been demonstrated in the use of Orbofiban in Patients with Unstable Coronary Syndromes (OPUS)-Thrombolysis in Myocardial Infarction (TIMI) 16 (OPUS-TIMI 16) clinical trial (8). A cut-point of >0.04 μg/L (>40 ng/L) was an independent predictor of the 30-day risk of death odds ratio (OR), 4.1; (95 % CI 1.2–13.8), death and AMI (OR, 3.4; 95 % CI, 1.8–6.7), and death, MI, or need for urgent revascularisation (OR, 2.3; 95 % CI, 1.5–3.6) and was also associated with risk of death or development of a further AMI at 10 months.

Using the TnI-Ultra compared to the previous Centaur assay, Melanson and colleagues (9) compared the rates of positivity obtained between the two assays over a 24 hour period in 103 patients who presented initially with a negative cTnI but converted to cTnI positive. TnI-Ultra was positive before cTnI in 66 (64.1%) of cases demonstrating superior sensitivity (*Figure 2*). Furthermore, a single admission cTn measurement using hs-cTnI may be a useful rule-out test irrespective of the length of chest pain (10, 11). Reichlin and colleagues (12) also demonstrated excellent diagnostic performance of sensitive cTn assays at presentation.

There are two points of clinical note from these *New England Journal* papers. First, both studies found no superior diagnostic value in measuring a delta change in cTn compared with an absolute value as demonstrated by receiver operator characteristic curve analysis. Secondly, the so-called hs-cTn assays performed diagnostically as well as the contemporary assays (5, 12–14) only when using the 99th percentile

Table I Manufacturer performance claims for laboratory based cardiac troponin assays (adapted from reference 4).

Assay	LoD [μg/L]	99th percentile [μg/L]	% CV at 99th percentile	10 % CV [μg/L]
Abbott AxSYM ADV	0.02	0.04	15.0	0.16
Abbott ARCHITECT	<0.01	0.028	15.0	0.032
Abbott i-STAT*	0.02	0.08	16.5	0.10
Beckman Coulter Access AccuTnI	0.01	0.04	14.0	0.06
bioMerieux Vidas Ultra	0.01	0.01	27.7	0.11
Inverness Biosite Triage	0.01	0.056	17.0	NA
Ortho Vitros ECi ES	0.012	0.034	10.0	0.034
Roche E170	0.01	<0.01	18.0	0.03
Roche Elecsys 2010	0.01	<0.01	18.0	0.030
Roche Elecsys hs-cTnT	0.001	0.013	8.0	0.012
Siemens Centaur Ultra	0.006	0.04	10.0	0.03
Siemens Dimension RxL	0.04	0.07	20.0	0.14
Siemens Immulite 2500 STAT	0.1	0.2	NA	0.42
Siemens Immulite 1000 Turbo	0.15	NA	NA	0.64
Siemens VISTA	0.015	0.045	10.0	0.04
Tosoh AIA II	0.06	<0.06	8.5	0.09
Research High Sensitive Assays				
Beckman Coulter Access hs-cTnI	0.0020	0.0086	10.0	0.0086
Nanosphere hs-cTnI	0.0002	0.0028	9.5	0.0005
Singulex hs-cTnI	0.00009	0.0101	9.0	0.00088

Table II Secondary ischemic and non-ischemic causes of elevated cardiac troponin.

Secondary Ischemic Cardiac Injury
Percutaneous coronary intervention/Coronary artery bypass grafting
Cardiac contusion
Pulmonary embolus
Coronary artery spasm
Heart failure
Vasculitis (Kawasaki disease, Churg-Strauss syndrome)
Connective tissue disorders
Renal failure
Rhabdomyolysis
Cerebrovascular accident/subarachnoid haemorrhage
Autoimmune diseases (systemic lupus erythematosus, polymyositis, scleroderma)
Glycogen storage disease (Type II / Pompe's disease)
Non-ischaemic Cardiac Injury
Viral or bacterial infection
Therapeutic drugs (5-flurouracil, anthracyclines, cyclophosphamide)
Recreational drugs (alcohol, cocaine)
Toxic substances (paraquat)
Animal envenomation
Extreme exertion
Heat stroke
Direct cardiac trauma (stabbing)
Multi-organ failure

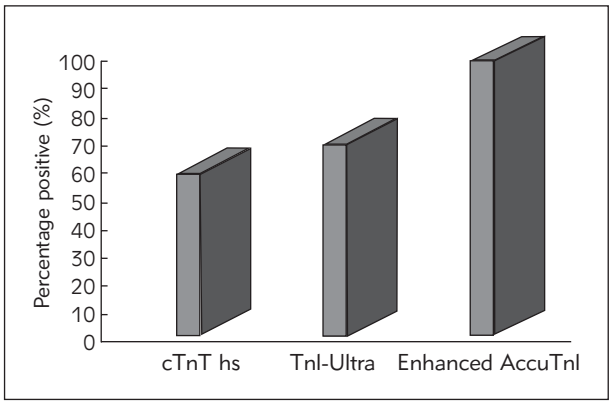

Figure 1 Percentage of positive samples identified in a fully characterised reference population by three different high sensitivity cTn assays (cTnT hs, 57.8%; TnI-Ultra, 68.4%; enhanced AccuTnI, 97.7%). Subjects (n=309, 41% male) free from cardiovascular risk factors or cardiovascular medication use.

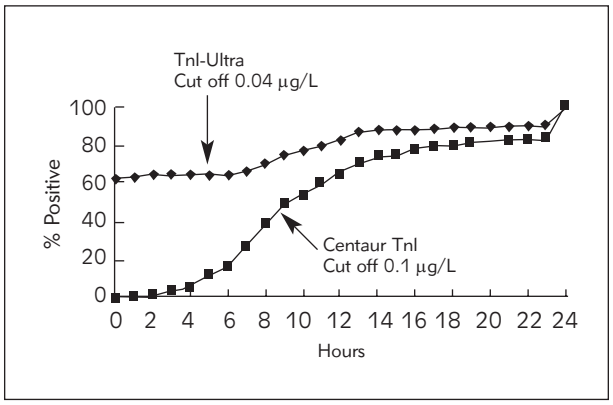

Figure 2 Detection of positive cTnI using a standard and 'high sensitivity' method according to time. Adapted from reference 9.

rather than the 10 % CV as the cut-off, questioning the true sensitivity of the hs-cTn assays. The drive to use the 99th percentile is warranted as demonstrated by the two *New England Journal* studies, however the real challenge is to adequately assess the clinical sensitivity and specificity in prospective studies of unselected chest pain presentations.

The future for troponin immunoassay

In order to achieve sensible low level cTn detection separated from background noise, newer detection mechanisms for immunoassay are needed. One such potential methodology is single molecule counting (15). In its current format, this assay is not adapted for high throughput at high speed. This is an attractive alternative if it can be adapted for routine immunoassay without compromising both analytical and clinical performance.

Conflict of interest statement

The authors stated that there are no conflicts of interest regarding the publication of this article.

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