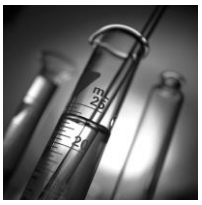
pathlab



August 2018

Introduction of a High Sensitivity Troponin I Assay

CLINICAL UPDATE

Evolving expectations for cardiac troponin assays

The performance expectations for cardiac troponin (cTn) assays have changed significantly with recent improvements to sensitivity and precision. These expectations are reflected in the Third Universal Definition of Myocardial Infarction (MI), and in recent updates to the European Society of Cardiology (ESC) guidelines for management of MI in patients without ST-segment elevation.[1,2]

These guideline updates reinforce the importance of using the 99th percentile Upper Reference Limit (URL) of healthy individuals as the cut-off to aid in diagnosis of MI. This shift toward using the 99th percentile URL cut-offs has increased the number of patients that are monitored for MI and helped identify patients with elevated cTn due to other conditions.[3,4] This has led to a necessary adjustment in patient management strategies that, ultimately, improve patient care. The updated guidelines also advocate for earlier serial sampling and the ESC makes recommendations for early rule-in and rule-out protocols. These protocols may reduce unnecessary delays to diagnosis, shorten emergency department (ED) stays and help identify patients who are eligible for early discharge with outpatient management.

High sensitivity troponin assays

Compared to contemporary troponin assays, high sensitivity assays demonstrate significantly improved precision at and below the 99th percentile URL. This allows for better discrimination of small differences in cTn values between serial measurements.[5] A more precise determination of the 99th percentile URL also facilitates reporting distinct reference interval for male and female subjects.[6] Multiple studies confirm that high sensitivity assays detect cTn release earlier and increase sensitivity for MI diagnosis at presentation, which may facilitate earlier rule-in and rule-out of MI.[2,5,7]

Reference population study and the 99th percentile URL

Methodology and results

A multi-centre prospective study was conducted to establish the 99th percentile URL for Access hsTnI in a population of apparently healthy adults. Serum and lithium heparin plasma samples were evaluated. Subjects ranging from 21 to 99 years of age were enrolled at five geographically diverse locations throughout the United States. A total of 494 males and 595 females were included with 45% ?60 years of age. Subjects were surveyed and were excluded if they met any of the following criteria:

- Disease(s) of/or affecting the cardiovascular system
- Currently taking a medication for cardiovascular disease
- Diabetes
- Chronic kidney disease
- Other serious chronic disease(s) (e.g. cancer, COPD, HIV, lupus erythematosus, etc.)
- Acute bacterial or viral infection
- Pregnancy

The observed 99th percentile URL in 1,089 lithium heparin plasma samples measured using the non-parametric method is 17.5 (ng/L) (95% CI: 12.6–20.7). No quantitative differences in results were observed between serum and lithium heparin plasma samples.

Table 1: 99th percentile URL of a healthy population

		99th percentile URL (95% CL)	
Population	N	New Access hsTNI (ng/L)	Current Access TNI (ng/L)
Females	595	11.6 (8.4 to 18.3)	N/A
Males	494	19.8 (14.0 to 42.9)	N/A
Overall	1,089	17.5 (12.6 to 20.7)	US population: 20 (10 to 50) EU population: 40 (30 to 90)

Current guidance from the IFCC states high sensitivity assays must have analytical imprecision ≤10% CV at the 99th percentile URL of a healthy population.[8] For Access hsTnI, the 10% CV limit of quantitation (LoQ) was measured to be 5.6 (ng/L). In addition, IFCC states that a high sensitivity assay must be able to measure cTn above the limit of detection (LoD) in >50% of a healthy population. In the study presented above, >50% of subjects had cTnI levels above the observed limit of detection.

Table 2: Imprecision at the established 99th percentile URLs

Population	99th percentile value (ng/L)	% CV based on LoQ imprecision profile
Females	11.6	4.2
Males	19.8	3.6
Overall	17.5	3.7

Discussion

Sex-specific reference intervals Women, on average, have less myocardial mass than men. Accordingly, any ischemic event damages a smaller absolute quantity of myocardium, which may lead to lower levels of circulating troponin. For this reason, it has been hypothesized that sex-specific reference intervals may improve the diagnostic accuracy of high sensitivity assays for women.[9] Beckman Coulter has reported sex-specific 99th percentile values for Access hsTnI. Clinicians and researchers can use these values as an aid for determining whether separate cut-offs are beneficial. The diagnostic accuracy of sex-specific cut-off values is discussed further in the pivotal clinical trial overview.

Comparison between new Access hsTNI and Current TNI assay

The overall URL established for the new Access hsTnI assay (17.5 ng/L) is similar to the current AccuTnI+3 assay URL (20 ng/L) established in the U.S. population study; the 95% confidence intervals of the cut-offs for both assays overlap. This is an expected outcome for two reasons:

> The new Access hsTnI calibrators are traceable to Beckman Coulter's internal reference calibrators

> Subjects for these two reference interval population studies were enrolled using protocols with similar inclusion and exclusion criteria

A second reference interval population study was also undertaken. This study was conducted in a European cardiac-healthy population, and established a different 99th percentile value for AccuTnI+3: 40 ng/L. This value diverges from the 99th percentile URL value established for the new Access hsTnI assay. It is widely recognized that the inclusion and exclusion criteria used for selecting the reference population influences observed URL values.[6,9] The European population study for AccuTnI+3 employed different selection criteria and the median age of the population in this study was older. These factors play a significant role in the difference between the established URL values. There is still debate about selecting the most appropriate reference population for troponin assays. Some contend that subjects should be young and apparently healthy, with no potential cardiovascular disease or cardiac risk factors. Others believe it is more appropriate to use a population that represents the intended use for troponin: patients whose demographics are similar to subjects who present to the emergency department and are ruled-in for MI. Ultimately, each laboratory should confirm the URLs reported for Access hsTnI in order to ensure the specific population they serve is properly represented, and to support current practice at their institutions. The Access hsTnI URL values that were established align with industry guideline and expert consensus recommendations.[10] The following sections provide further detail regarding the expected diagnostic accuracy of Access hsTnI using the new 99th percentile values.

Clinical trial—diagnostic accuracy of Access hsTnI

Methodology and results

A multicenter prospective study was conducted to evaluate the diagnostic accuracy of the Access hsTnI assay using the established 99th percentile URLs. The study was designed to establish the clinical performance of Access hsTnI as an aid in the diagnosis of MI. The study included 1,851 evaluable subjects from ED patients presenting with chest pain or equivalent ischemic symptoms suggestive of Acute Coronary Syndromes (ACS). A total of 14 geographically diverse, primary care hospital-associated emergency departments participated, reflecting regional, urban, suburban, and rural patient populations. True MI statuses of all subjects were adjudicated by an independent panel of expert physicians using criteria consistent with the Universal Definition of Myocardial Infarction.[11] Adjudicators were blinded to the Beckman Coulter assay results, and the attending physicians' diagnosis. All results presented below were based on the adjudicated diagnoses. The MI incidence was 13% (238/1851). Samples were tested at three independent clinical laboratories on multiple Access Immunoassay systems. Testing was performed using serum and lithium heparin plasma samples. Study results for lithium heparin plasma are shown in Table 3. Results are presented for the following time intervals between ED presentation and specimen collection:

- Baseline, ≥1 to 3 hours, ≥3 to 6 hours and ≥6 to 9 hours after admission
- Sensitivity, specificity and predictive values were calculated per CLSI Guideline I/LA21-A2[12] and can be defined as follows:
- **Sensitivity:** % MI correctly diagnosed
- **Specificity:** % non-MI correctly diagnosed
- **Positive predictive value (PPV):** probability of MI diagnosis in patients with cTnI >99th percentile URL
- **Negative predictive value (NPV):** probability of non-MI diagnosis in patients with cTnI ≤99th percentile URL

Table 3: clinical performance of Access hsTnI using the established 99th percentile URLs

		Sensitivity		Specificity		PPV		NPV	
99th percentile URL cut-off (ng/L)	**Time after admission to ED**	**%**	**95%CL**	**%**	**95%CL**	**%**	**95%CL**	**%**	**95%CL**
Overall: 17.5	Baseline	90	86 to 94	90	89 to 92	57	52 to 62	99	98 to 99
	≥1 to 3 hrs	97	92 to 99	90	88 to 92	55	48 to 61	100	99 to 100
	≥3 to 6 hrs	94	89 to 98	88	86 to 90	56	49 to 62	99	98 to 100
	≥6 to 9 hrs	94	81 to 99	88	83 to 92	58	44 to 70	99	96 to 100
Females: 11.6	Baseline	94	86 to 98	90	88 to 92	48	39 to 57	99	99 to 100
	≥1 to 3 hrs	98	87 to 100	90	87 to 93	45	34 to 56	100	99 to 100
	≥3 to 6 hrs	100	92 to 100	88	84 to 91	46	36 to 57	100	99 to 100
	≥6 to 9 hrs	93	66 to 100	84	75 to 90	45	26 to 64	99	93 to 100
Males: 19.8	Baseline	91	86 to 95	88	86 to 90	59	53 to 66	98	97 to 99
	≥1 to 3 hrs	96	89 to 99	88	85 to 91	58	49 to 66	99	98 to 100
	≥3 to 6 hrs	93	85 to 97	86	83 to 89	57	49 to 65	98	97 to 99
	≥6 to 9 hrs	96	77 to 100	87	79 to 92	58	41 to 75	99	94 to 100

Note: The Access hsTnI assay is not intended to be used in isolation; results should be interpreted in conjunction with other diagnostic tests and clinical information.

Discussion

The Access hsTnI assay demonstrates excellent clinical sensitivity. Sensitivity results show that Access hsTnI, using the 99th percentile URL cut-off (17.5 ng/L), correctly diagnosed approximately 97% of MI-positive patients between 1–3 hours after they were admitted to the ED. High sensitivity provides confidence that MIs are less likely to be missed. NPV results show that nearly 100% of subjects with a cTnI value below the 99th percentile URL (17.5 ng/L) at 1–3 hours after admission to the ED had a non-MI diagnosis. High NPV supports rapid rule-out decisions.

The diagnostic accuracy of sex-specific cut-off values is similar to the accuracy of the overall 99th percentile cut-off. The female cut-off value exhibits lower PPVs and slightly improved sensitivity in some sampling timeframes when compared to the overall cut-off. It should be noted that PPV and NPV depend upon prevalence and will vary by facility and region.

Delta analysis—establishing an absolute delta value

Methodology and results

Delta values indicate a significant rise or fall among serial cTnI measurements. The use of delta values may improve clinical specificity and PPV for acute MI compared to an evaluation based upon the 99th percentile cut-off alone.[13,14] When serial samples are obtained and cTnI deltas are considered in the clinical context of each patient, acute MI may be more rapidly distinguished from other conditions causing myocardial injury. Deltas must be defined specifically for each manufacturer's assay, and there must be clear criteria around the calculation method.[8] An analysis was performed to evaluate diagnostic accuracy of potential delta values for Access hsTnI, when used in conjunction with the 99th percentile URL; 1,721 subjects from the multicentre prospective study were included. Lithium heparin plasma samples were used in the analysis. Two groups were assessed:

> Subjects with a cTnI value >the 99th percentile URL and maximum observed cTnI change ≥delta value between time points (positive result)

> Subjects who did not have a cTnI value >the 99th percentile URL, or did not have a cTnI change ≥delta value between time points, or both (negative result)

Results were compared to true MI statuses of all subjects, as determined by the independent adjudication panel. Sensitivity, specificity, PPV, and NPV are reported in Table 4. Delta values were also assessed in conjunction with the sex-specific 99th percentiles, evaluating males and females separately; there was not found to be a significant impact to diagnostic accuracy.

Table 4: Diagnostic accuracy of delta change values using the overall 99th percentile URL cut-off (17.5 (ng/L))

Delta change value (≥)	Time	Sensitivity %	Specificity %	PPV %	NPV %
3 (ng/L)	Baseline vs 1 to 3 hrs	76	95	66	97
	Baseline vs 3 to 6 hrs	87	92	62	98
5 (ng/L)	Baseline vs 1 to 3 hrs	71	97	76	86
	Baseline vs 3 to 6 hrs	78	95	72	97
11 (ng/L)	Baseline vs 1 to 3 hrs	61	99	83	95
	Baseline vs 3 to 6 hrs	60	98	78	94
22 (ng/L)	Baseline vs 1 to 3 hrs	50	99	90	94
	Baseline vs 3 to 6 hrs	54	99	88	93

Note: PPV and NPV values are prevalence dependent and results will vary by facility and region. Each laboratory should validate these data or establish its own delta values to assure proper representation of specific populations.

Discussion

The results in Table 4 demonstrate that using a delta value can improve the clinical specificity and PPV for Access hsTnI. Specificity, using the 99th percentile alone, ranged from 84–90%. When a delta value was added to the analysis specificity ranged from 92–99%, depending upon the magnitude of the delta value considered. PPV using the 99th percentile alone ranged from 45–58%. When a delta value was added to the analysis PPV ranged from 62–90%, depending upon the magnitude of the delta value considered. With the use of delta values, the observed improvements to specificity and PPV were not significantly different between the 1–3 hour and 3–6 hour sampling timeframes. In this study, a patient with at least one value above the 99th percentile URL (17.5) and a change of 22 between baseline and three hours demonstrated a 90% probability of acute MI.

This data indicates a larger magnitude of change between serial measurements correlates to increased likelihood of diagnosing acute MI. It is important to note that the addition of delta values decreased sensitivity considerably. Not every patient presenting with an MI demonstrates a significant change in cTnI between serial measurements. For this reason, delta values should be interpreted with caution; results should always be considered within the broader clinical context. Current ESC guidelines recommend a three-hour rule-in/rule-out algorithm that may substantially reduce the time to diagnosis for patients presenting with acute MI (Figure 1). This algorithm requires using a delta change value, in addition to an assessment based upon the 99th percentile URL.2 A delta value from Table 4 may be used to support implementing this early rule-in/rule-out pathway. The delta value chosen should reflect the requirements for diagnostic accuracy specified by each institution's emergency physicians and cardiologists. With the use of delta values, the observed improvements to specificity and PPV were not significantly different between the 1–3 hour and 3–6 hour sampling timeframes.

References

1. Thygesen K. et al. Third universal definition of myocardial infarction. Circulation 2012; 126: 2020-2035.
2. Roffi M, Patrono C, Collet JP, et al. 2015 ESC Guidelines for the management of acute coronary syndromes in patients presenting without persistent ST-segment elevation: Task Force for the Management of Acute Coronary Syndromes in Patients Presenting without Persistent ST-Segment Elevation of the European Society of Cardiology (ESC). Eur Heart J. 2016 Jan 14; 37(3): 267-315.
3. Mills NL, Lee KK, McAllister DA, Churchhouse AMD, MacLeod M, Stoddart M, Walker S, Denvir MA, Fox KAA, Newby DE. Implications of lowering threshold of plasma troponin concentration in diagnosis of myocardial infarction: cohort study. BMJ 2012; 344: e1533.
4. Jeremias A, Gibson CM. Narrative review: alternative causes for elevated cardiac troponin levels when acute coronary syndromes are excluded. Ann Intern Med 2005; 142: 786-91.
5. Thygesen K, Mair J, Giannitsis E, Mueller C, Lindahl B, Blankenberg S, et al. How to use high-sensitivity cardiac troponins in acute cardiac care. Eur Heart J 2012; 33: 2252-7.
6. Apple F, Ler R, Murakami M. Determination of 19 Cardiac Troponin I and T Assay 99th Percentile Values from a Common Presumably Healthy Population. Clinical Chemistry 58:11, 1574-1581 (2012).
7. Korley FK, Jaffe AS. Preparing the United States for high-sensitivity cardiac troponin assays. J Am Coll Cardiol 2013; 61: 1753-8.
8. Clinical Applications of Cardiac Bio-markers. IFCC: International Federation of Clinical Chemistry and Laboratory Medicine, 26 July 2014. Web. 14 Feb. 2017.
9. Collinson P. et al. Influence of population selection of the 99th percentile reference value for cardiac troponin assays. Clinical Chemistry 2012; 58:1: 219-225.
10. Apple FS. et al. Cardiac troponin assays: guide to understanding analytical characteristics and teir impact on clinical care. Clinical Chemistry 2017; 63:1: 73-81.
11. Thygesen K, Alpert JS, White HD; Joint ESC/ACC/AHA/WHF Task Force for the Redefinition of Myocardial Infarction. Universal definition of myocardial infarction. Eur Heart J 2007; 28: 2525-38. J Am Coll Cardiol 2007; 50: 2173-95. Circulation 2007; 116: 2634-53.
12. CLSI. Clinical Evaluation of Immunoassays; Approved Guideline-Second Edition. CLSI document I/LA21-A2. Wayne, PA. Clinical Laboratory Standards Institute; 2008.
13. Cullen L, Parsonage WA, Greenslade J, et al. Delta troponin for the early diagnosis of AMI in emergency patients with chest pain. Int J Cardiol. 2013 Oct 3; 168(3): 2602-8.
14. Morrow DA, Bonaca MP. Real World Application of "Delta" Troponin. JACC Vol 62, No. 14, 2013.

John Woodford, Biochemistry and Specimen Services Lead of Speciality
07 858 0795 ext. 6828