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Evaluation of a new ultrasensitive assay for cardiac troponin I

Gregori Casals, Xavier Filella *, Josep Lluis Bedini

Department of Biochemistry and Molecular Genetics and Core Laboratory (CDB), Hospital Clínic, C/Villaroel, 170, 08036 Barcelona, Catalonia, Spain

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Abstract

Objectives: We evaluated the analytical and clinical performance of a new ultrasensitive cardiac troponin I assay (cTnI) on the ADVIA Centaur® system (TnI-Ultra™).

Design and methods: The evaluation included the determination of detection limit, within-assay and between-assay variation and comparison with two other non-ultrasensitive methods. Moreover, cTnI was determined in 120 patients with acute chest pain with three methods. To evaluate the ability of the new method to detect MI earlier, it was assayed in 8 MI patients who first tested negative then positive by the other methods.

Results: The detection limit was 0.009 μg/L and imprecision was <10% at all concentrations evaluated. In comparison with two other methods, 10% of the anginas diagnosed were recategorized to MI.

Conclusions: The ADVIA Centaur® TnI-Ultra™ assay presented high reproducibility and high sensitivity. The use of the recommended lower cutpoint (0.044 μg/L) implied an increased and earlier identification of MI.

Keywords: Troponin I; Myocardial infarction; Acute coronary syndrome; Chest pain; Cardiac markers

Introduction

The role of Laboratory Medicine in the management and diagnosis of myocardial infarction (MI) has become increasingly significant. The first biochemical markers of cardiac necrosis used for the diagnosis of MI were the enzymatic methods AST (Aspartate Aminotransferase) and CK (Creatine Kinase). Afterwards, the electrophoresis methods of CK and LDH (Lactate Dehydrogenase) isoenzymes along with a myoglobin assay improved the diagnostic accuracy. Most recently, highly sensitive and specific immunoassay methods such as CK-MB and cardiac troponins have become available, playing a central role in the evaluation of acute coronary syndrome. In this context, the rise and fall of biochemical markers of myocardial necrosis (cardiac troponins or CK-MB) have become necessary criteria for the diagnosis of acute MI in the American College of Cardiology (ACC) and European Society of Cardiology (ESC) definition of MI [1]. The preferred cardiac marker is troponin because of its high specificity for myocardial damage [1]. In the consensus document, elevation of troponin is defined as a value exceeding the 99th percentile of a reference control group. This implies lower cutoff values for MI than currently used in most laboratories. This recommendation is based on the consideration that any elevation in cardiac troponin is indicative of myocardial injury in the clinical setting of ischemic MI [1–4]. Accordingly, clinical decisions may be made on the basis of small elevations in cardiac troponin. This recommendation demands highly sensitive, precise and specific troponin assays. A coefficient of variation of <10% at the 99th percentile of the reference control group is recommended [1]. Two cardiac troponin assays are commercially available for use in clinical laboratories: cardiac troponin T (cTnT) and cardiac troponin I (cTnI). Both cTnT and cTnI are released similarly during MI. The first commercially available cardiac troponin assay was a cTnT immunoassay, but it is currently available from only one manufacturer (Roche Diagnostics). There are several manufacturers of cTnI immunoassays.

In this study, we evaluated the analytical and clinical performance of a new cTnI method on the ADVIA Centaur® system (TnI-Ultra™). The study included a comparison of the ADVIA Centaur TnI-Ultra assay with two other methods: the ADVIA Centaur cTnI and Beckman Coulter Access 2 AccuTnI

* Corresponding author. Fax: +34 3 227 93 76.
E-mail address: xfilella@clinic.ub.es (X. Filella).

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(AccuTnI) assays. We also evaluated whether or not the ADVIA Centaur TnI-Ultra assay allows for earlier identification of MI relative to the other two assays.

Methods

Assay principle

The ADVIA Centaur TnI-Ultra assay is a three-site sandwich immunoassay using direct chemiluminometric technology. An ancillary reagent is included to reduce nonspecific binding. The assay includes a polyclonal goat anti-troponin I antibody labeled with acridinium ester and 2 biotinylated mouse monoclonal anti-troponin I antibodies. The capture monoclonal antibodies recognize amino acid sequences 87–91 and 41–49 located in the stable region of the TnI molecule; the signal antibody recognizes amino acids 27–39. Magnetic latex particles conjugated with streptavidin are the solid phase reagent. Troponin I in the sample is bound to the antibodies in the reagent. The biotin contained in the immune complex is then bound to the streptavidin-labeled magnetic particles. Time to first result is 18 min, with successive results in 20-second increments. The sample volume required is 100 μL. The detection limit provided by the manufacturer is 0.006 μg/L, and the assay range is 0.006 to 50 μg/L. Patient samples with troponin I levels above 50 μg/L must be diluted.

A comparison was made between the TnI-Ultra method and two other methods: AccuTnI and ADVIA Centaur cTnI. AccuTnI is based on a chemiluminescence method on an Access 2 Immunoassay system (Beckman-Coulter, Brea, CA, USA). This assay has a detection limit of 0.01 μg/L and the assay range is 0.01–100 μg/L. The ADVIA Centaur cTnI assay is based on a chemiluminescence method on an ADVIA Centaur Analyzer (Siemens Medical Solutions Diagnostics, Tarrytown, NY, USA). It is a two-site sandwich assay employing direct chemiluminometric technology using a combination of rabbit monoclonal antibodies and a polyclonal goat anti-troponin I antibody. The affinity support represents the human cTnI sequence aa 27–39, -RAYATEPHAKKKS. This assay has a detection limit of 0.01 μg/L and the assay range is 0.01–50 μg/L.

Detection limit

The TnI-Ultra detection limit was calculated as the lowest cTnI concentration corresponding to a signal 2 SD above the mean of 20 replicates of the zero calibrator in a single run.

Imprecision

The imprecision of the TnI-Ultra method was also evaluated. Six levels of quality control material were analyzed in duplicate once per day for 7 days, and two runs of quality control material were performed in duplicate for 8 days, for a total of 23 runs. The time interval between runs analyzed on the same day was at least 2 h. The control materials assayed were Bayer Cardiac Markers Controls (2 levels) and BioRad Liquicheck Cardiac Marker Controls (4 levels). Total precision was calculated to include within-assay and between-assay variation. The same reagent lot of TnI-Ultra was used.

Method comparison

The TnI-Ultra assay was compared with the ADVIA Centaur cTnI and AccuTnI assays using lithium heparin plasma samples. One hundred fifty unique patient samples were assayed in duplicate for each method. Samples were assayed with the AccuTnI assay on the Beckman Access 2 during regular daily clinical testing and then were tested with the ADVIA Centaur TnI-Ultra and ADVIA Centaur cTnI assays within 4 h on the same day. When testing was not possible within 4 h, plasma was refrigerated at 4 °C for analysis within 24 h. Samples were obtained from patients presenting with acute chest pain. In order to represent the range of values likely to be encountered in clinical practice, samples were selected as follows: 45 samples with AccuTnI <0.1 μg/L, 45 with AccuTnI between 0.1 and 1.5 μg/L, 45 with AccuTnI between 1.5 and 30 μg/L and 15 samples with AccuTnI >30 μg/L.

Clinical performance evaluation

An evaluation of the clinical concordance between the TnI-Ultra, cTnI and AccuTnI results was performed. Fresh lithium heparin samples for a total of 120 patients presenting with acute chest pain were included in the study. All samples were analyzed in duplicate. In this portion of our study, only AccuTnI values were used for the clinical diagnosis of MI. TnI-Ultra and ADVIA Centaur cTnI results were not used clinically. All patients were also evaluated by electrocardiography.

Table 1
Analytical imprecision of TnI-Ultra, Advia Centaur cTnI and AccuTnI assays

(a) Within-assay, between-assay and total imprecision of TnI-Ultra assay

Control	TnI (μg/L)	Within assay			Between assays			Total		
	Mean	SD	CV (%)	*n*	SD	CV (%)	*n*	SD	CV (%)	*n*
A	0.033	0.0024	7.2	23	0.0008	8.4	23	0.0032	9.6	46
B	0.063	0.0038	6.0	23	0.0044	7.1	23	0.0058	9.3	46
C	0.095	0.0048	5.0	23	0.0068	7.2	23	0.0083	8.7	46
D	0.681	0.0266	3.9	23	0.0337	5.0	23	0.0430	6.3	46
E	3.072	0.0986	3.2	23	0.1821	5.9	23	0.2256	7.3	46
F	14.626	0.5294	3.6	23	0.8889	6.1	23	1.0346	7.1	46

(b) Between-assay imprecision of Advia Centaur cTnI and AccuTnI

Control	Advia Centaur cTnI			AccuTnI		
	Mean (μg/L)	CV (%)	*n*	Mean (μg/L)	CV (%)	*n*
A	1.059	3.4	15	0.02	16.7	15
B	0.445	7.1	15	0.04	10.0	15
C	1.517	5.2	15	0.07	8.0	15
D	2.077	4.8	15	0.49	4.1	15
E	4.042	2.7	15	1.46	5.5	15
F	15.518	4.3	15	10.7	4.9	15

A: BioRad LT Low Control, B: Bayer Cardiac Marker Control 1, C: BioRad LT Control 1, D: BioRad LT Control 2, E: BioRad LT Control 3, F: Bayer Cardiac Marker Control 2.