The Evaluation of Cardiac Troponin I Assays Measured Radiometer AQT90 Flex and ReLIA Analyzers and Determination of Heterophilic Antibody Positivity Rate

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Abstract

Measurement of cardiac troponin I (cTnI) is important for the diagnosis of myocardial infarction and for risk stratification. The aim of this study was to compare the diagnostic performance of AQT90 Flex cTnI and ReLIA cTnI assays as based Vidas Ultra cTnI results, and also determine rate of the heterophilic antibody (HAs) positivity. A total of 246 cTnI and HAs assays were performed from the blood and serum samples. The diagnostic cut-off value for cTnI assays was accepted as 0.01 µg/L, 0.023 ng/mL and 0.15 ng/mL for Vidas Ultra, AQT90Flex and ReLIA analyzers, respectively. Sensitivity, specificity, predictive value of positive and negative test, false positive and negative rate and efficiency were calculated. The sensitivity, negative predictive value, false positivity rate and accuracy of cTnI assay performed on AQT90 Flex analyzer were higher than cTnI assay performed on ReLIA analyzer. However, cTnI assay performed on ReLIA analyzer have higher specificity and false negativity rate as compared to cTnI test performed on AQT90 Flex analyzer. The agreement of cTnI results were calculated using of kappa analysis. The analysis revealed a substantial agreement between cTnI assays performed on Vidas Ultra and AQT90 Flex analyzer (p<0.001, κ=0.618), and fair agreement between cTnI assays performed on Vidas Ultra and ReLIA analyzer (p<0.001, κ=0.373).The positivity rate of HAs was found as 4.87%. The results of the study showed that AQT90Flex cTnI assay has higher sensitivity and accuracy, lower specificity and false negativity rate as compared to ReLIA cTnI assay. However, fairly lower heterophilic antibody positivity rate is found

Key words: diagnostic performance, troponin I, AQT90 Flex, ReLIA, Vidas Ultra.

INTRODUCTION

Myocardial infarction (MI) is defined by the demonstration of myocardial cell necrosis due to significant and sustained ischemia. Cardiac troponin I (cTnI) in blood is the most sensitive and specific biochemical marker of myocardial infarction (Braunwald et al., 2002). The assessment of acute MI as formally established by the World Health Organization (WHO) was diagnosed in the presence of one of the following criteria: ECG showing unequivocal pathological Q waves and/or ST segment elevation or depression in serial recordings or history of typical or atypical angina pectoris, together with equivocal changes on the ECG and elevated enzymes or history of typical angina pectoris and elevated enzymes with no changes on the ECG or not available or fatal cases, whether sudden or not, with naked eye appearances of fresh MI and/or recent coronary occlusion at necropsy (ante mortem thrombus, hemorrhage into an atheromatous plaque or embolism (Mendis et al., 2011).
Several well-designed studies have shown that cTnl and cTnT to be the most diagnostically sensitive and specific biomarker of myocardial injury (Moe and Wong, 2010). Moreover, on the basis of improved sensitivity and superior tissue specificity compared to other biomarkers of necrosis, cTnl is recommended for the diagnosis of AMI by the National Academy of Clinical Biochemistry (NACB) Laboratory Medicine Practice Guidelines and the International Committee of Experts in Epidemiology, Pathology, Clinical, and Laboratory Medicine (Alpert et al., 2000). Recent advances in assay technology have lead to a refinement in cardiac troponin assays that have had a profound impact on clinical practice. High-sensitive cTnl assays have two differentiating features from contemporary cTnl assays: 1) detection of cTnl in healthy persons and 2) a precise definition of what is “normal” (= the 99th percentile). Recent multicenter studies have shown that high-sensitive cTnl assays improve the early diagnosis of acute MI (Twerenbold et al., 2011). Although there is no consensus regarding when the term “sensitive” and when the term “high-sensitive” should be applied in the description of cTnl assays, it is important to note that there are substantial analytical differences among the new assays. It is still unclear whether these analytical differences impact on their clinical performance (Twerenbold et al., 2011; Apple, 2009).

As mentioned above, cTnl is the important biochemical marker of choice for assessing patients presenting to the Emergency Department with cardiac symptoms. Given the urgent nature of such requests, a rapid turn-around-time (TAT) is an important prerequisite for troponin assays and one which makes point-of-care testing attractive to clinicians. The currently available cTnl assays produced differing results. One important reason is that the assays may differ in their responses to the various isoforms of cTnl present in circulation or in biochemical preparations (Datta et al., 1999; Wu et al., 1998). Troponin assays has continually been plagued by interferences caused by heterophilic antibodies (HAs). HAs can cause interference in immunoassays, but the estimated incidence varies tremendously, from 0.2% to 40% (Kim et al., 2002). The presence of HA interferences in immunoassays has led manufacturers to indicate this possibility in package inserts and to add “blocking” agents to their immunoassay formulations. Despite these measures, the problem persists (Yeo et al., 2000; Fitzmaurice et al., 1998). The purpose of this preliminary study was to compare the performances of Radiometer cTnl assays performed on AQT90 Flex and ReLIA analyzers, as based on the cTnl assay performed on Vidas Ultra analyzer, and also determine the rate of interfering antibodies in all subjects.

MATERIAL AND METHODS

This is a clinical investigation study. Approval for the study was obtained from the Ethics Committee of Ordu University School of Medicine. This study was conducted between January 2012 and December 2012 at Ministry of Health Training and Research Hospital, Biochemistry Laboratory. The samples from 246 patients (130 male, 116 female) were submitted for routine cTnl analysis by the three different cTnl assays. All assays were performed according to manufacturers’ instructions. For determination of the cTnl level, serum sample was used for Vidas Ultra and ReLIA analyzers, and also EDTA whole blood sample for AQT90 Flex analyzer. The diagnosis of MI was performed to increased cTnl levels that obtained from Vidas Ultra analyzer and other clinical findings (Mendis et al., 2011). The descriptive specifications for different cTnl assays are presented in Table 1. Diagnostic specifications for different assays in the prediction of MI are presented in Table 2.

Assay Principle

The VIDAS troponin I Ultra assay (bioMerieux, Marcy L'Etoil, France) combines a one-step immunoassay sandwich method with a final fluorescent detection. The sample is transferred into the wells containing anti-cTnl Ab labeled with ALP. The conjugated enzyme catalyzes the hydrolysis of the substrate into a fluorescent product of which is measured at 450 nm. The intensity of the fluorescence is proportional to the concentration of cTnl present in the sample. The limit of detection has been determined to be 0.01 μg/L. The reportable range of the assay is 0.01–30 μg/L. The upper 99th percentile URL has been determined to be <0.01 μg/L.

AQT90 FLEX cTnl immunoassay (Radiometer Medical ApS, Denmark). cTnl assay is a one-step sandwich immunofluorometric assay based on the use of three monoclonal antibodies, two for capture and one for detection. The capture antibodies are targeting epitopes 41–49 in the stable mid-fragment, and 190–196 in the C-terminal end. The tracer antibody targets epitopes 137–149. During 15 min of incubation time, the tracer and capture antibodies form a complex with the analyte present in the sample. The measured signal is converted to a concentration using the calibration curve stored in the memory of the instrument. The limit of detection has been determined to be 0.0095 μg/L. The reportable range of the assay is 0.010–50 μg/L. The upper 99th percentile URL has been determined to be ≤0.023 μg/L ReLIA (ReLIA Diagnostic Systems, Inc, San Francisco, CA, USA) cTnl test is a bi-directional lateral flow immunoassay utulizing a double- antibody sandwich format. Analyte in the sample reacts with anti-cTnl immunogold
conjugate and forms an immune complex. The immunogold complex reacts with the anti-cTnI antibody pre-coated on the nitrocellulose. Then, the DNP-BSA gold reacts with the anti-DNP antibody coated on the nitrocellulose and in the presence of cTnI colloidal gold shows as red color. When the reaction is complete, ReLIA instrument analyses density of reflectance of the bands, and the relative intensity of the test and control bands. Then the instrument determined the concentration of analyte based on the standard curve programmed into the instrument and display the results as ng/mL. The limit of detection has been determined to be 0.15 ng/mL. The reportable range of the assay is 0–16 ng/mL. The upper 99th percentile URL has been determined to be <0.15 ng/mL.

The presence of heterophil antibody was determined by Virapid (Vircell, S.L, Santa Fe, Granada, Spain) immunochromatographic test. Briefly, the colloidal gold is solubilized and the first immunological reaction between the specific antibodies of the serum and the protein coupled to the gold particles took place. These proteins reacted with the Paul-Bunnel heterophile antibodies. Then, these complexes moved along the membrane to the corresponding test lines. In order to perform the reading of the test and to determine the positivity of the samples, when the intensity is higher than or equal to 0.5, the result is considered positive.

**Statistical Analysis**

Statistical analysis was carried out using SPSS program. Sensitivity, specificity, predictive value of positive and negative test, false positive and negative rate and efficiency were calculated. Sensitivities, specificities, NPV and PPV were determined as follows: sensitivity = TP/TP + FN, specificity = TN/TN + FP, and NPV=TN/FN+FP, PPV= TP/TP+FP. The agreement of results were calculated using kappa analysis.

**RESULTS**

The diagnostic performance of cTnI obtained from AQT90 Flex as following; sensitivity 94.1%, specificity 71%, predictive value of positive test 69.3%, predictive value of negative test 94.5%, false positivity rate 29%, false negativity rate 5.9%, accuracy of the test 80.5%.

The diagnostic performance of cTnI obtained from ReLIA as following; sensitivity 44.6%, specificity 90.3%, predictive value of positive test 76.3%, predictive value of negative test 70.1%, false positivity rate 9.7%, false negativity rate 55.4%, accuracy of the test 71.5%.

The scatter diagrams of cTnI assays on Vidas Ultra and AQT90 Flex, and also Vidas Ultra and ReLIA are shown in Figure 1 and 2, respectively.

The agreement of cTnI results were calculated using of the kappa analysis. The concordance between Vidas Ultra and other assays of cTnI are shown in Table 3. According to this analysis, it was found substantial agreement between Vidas Ultra and AQT90 Flex (p<0.001, κ=0.618), and fair agreement between Vidas Ultra and ReLIA (p<0.001, κ=0.373).

The positive rate of heterophilic antibodies (HAs) was found as 4.87%, and totally 12 patients have HAs positivity.

The limit of blank (LOB), the highest measurement result that indicates analyte is not present in the sample, determined from 60 measurements of the zero calibrator. The LOB values of Vidas Ultra, AQT90 Flex and ReLIA as following; 0.01 μg/L, 0.001 μg/L, and 0.01 ng/mL, respectively.

The limit of detection (LOD), the lowest amount of analyte in a sample that can be detected with (stated) probability, determined by measuring five low level cTnI pools in replicates of 5. The LOD values of Vidas Ultra, AQT90 Flex and ReLIA as following; 0.014 μg/L, 0.006 μg/L, and 0.01 ng/mL, respectively.

The limit of quantitation (LOQ), the lowest amount of analyte in a sample that can be quantitatively determined with acceptable precision, under stated experimental conditions-10%CV - determined by measuring 10 low level cTnI pools in duplicate; twice a day for 10 days. The LOQ values of Vidas Ultra, AQT90 Flex and ReLIA as following; 0.01 μg/L, 0.029 μg/L, and 0.01 ng/mL, respectively.

**DISCUSSION**

Through the introduction of quality specifications for cardiac troponin measurement, clinical testing laboratories have become aware of the requirements for robust and reliable cTnI assays and the need for both analytical and clinical validation of methods introduced into routine patient testing for cTnI (Tate, 2008; Panteghini et al., 2001). There is need to standardize the clinical use of such methods in the diagnosis and management of acute coronary syndromes and to clearly define the decision thresholds (Chien et al., 2010). Laboratories require information on the pre-analytical, analytical and post-analytical performance of cTnI assays that can be obtained from manufacturer’s package inserts,
local and international method evaluations, and through collaborative laboratory and clinical studies carried out locally or internationally by way of trials (Tate, 2008; Thygesen et al., 2007). Assays of cTnI have undergone substantial developments in recent years, and the addition of new antibodies directed against epitopes in the heart-specific region of the cTnI molecule has led to improved characteristics early in MI (Hjortshøj et al., 2011).
Table 1. The descriptive specifications for different cTnI assays.

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<th></th>
<th>Minimum</th>
<th>Maximum</th>
<th>Median</th>
<th>SD</th>
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<tbody>
<tr>
<td>Vidas Ultra (µg/L)</td>
<td>0.01</td>
<td>13.69</td>
<td>0.42</td>
<td>1.74</td>
</tr>
<tr>
<td>AQT90 Flex (ng/mL)</td>
<td>0.01</td>
<td>15.0</td>
<td>0.02</td>
<td>1.94</td>
</tr>
<tr>
<td>ReLIA (ng/mL)</td>
<td>0.01</td>
<td>16.0</td>
<td>0.01</td>
<td>1.77</td>
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Table 2. Diagnostic specifications for different assays in the prediction of myocardial infarction

<table>
<thead>
<tr>
<th></th>
<th>AQT90 Flex</th>
<th>ReLIA</th>
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<tr>
<td>Sensitivity (%)</td>
<td>94.1</td>
<td>44.6</td>
</tr>
<tr>
<td>Specificity (%)</td>
<td>71</td>
<td>90.3</td>
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<tr>
<td>Positive predictive value (%)</td>
<td>69.3</td>
<td>76.3</td>
</tr>
<tr>
<td>Negative predictive value (%)</td>
<td>94.5</td>
<td>70.1</td>
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<tr>
<td>False positivity rate (%)</td>
<td>29</td>
<td>9.7</td>
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<tr>
<td>False negative rate (%)</td>
<td>5.9</td>
<td>55.4</td>
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<tr>
<td>Accuracy (%)</td>
<td>80.5</td>
<td>71.5</td>
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Table 3. The concordance between Vidas Ultra and other assays of cTnI.

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<tr>
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<tr>
<td>Vidas Ultra-AQT90 Flex</td>
<td>0.618</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>Vidas Ultra-ReLIA</td>
<td>0.373</td>
<td>p&lt;0.001</td>
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The purpose of the current study was to investigate the clinical performance of new point-of-care cTnI assays performed on AQT90 Flex and ReLIA analyzers as based on the cTnI results performed on Vidas Ultra analyzer. The previous studies reported that VIDAS Ultra cTnI method provides an excellent tool to aid in the early diagnosis of MI, with very high clinical sensitivity at presentation in patients presenting with symptoms suggestive of acute coronary syndrome (Chien et al., 2010; Apple and Collinson, 2012; Di Serio et al., 2003; Apple et al., 2008). We assessed sensitivity, specificity, and positive and negative predictive values, accuracy for cTnI assays performed on AQT90 Flex and ReLIA analyzers. To our results, cTnI assay performed on AQT90 Flex analyzer had higher sensitivity, negative predictive value, false positivity rate, and accuracy. However cTnI assay performed on ReLIA analyzer had higher specificity, positive predictive value, and false negativity value. In addition, we observed substantial agreement between results of cTnI that measured Vidas Ultra and AQT90 Flex analyzer, but fair agreement results of cTnI that measured between Vidas Ultra and ReLIA. To our knowledge, our study was a preliminary study reporting clinical performance of cTnI assay that measured on ReLIA analyzer. ReLIA cTnI test is an in vitro diagnostic cassette test which is intended to measure the concentration of cTnI in human whole blood/serum/plasma, as an aid in the diagnosis of acute myocardial infarction. ReLIA cTnI assay has 20 minute (min) turnaround time (TAT). The National Academy of Clinical Biochemistry (NACB) and the International Federation of Clinical Chemistry (IFCC) recommend that TATs for cardiac markers should be < 60 min (Panteghini et al., 1999; Wu et al., 1999). The American Heart Association (AHA)/ ACC also recommend a TAT of 60 min, but states that a 30 min TAT is preferable (Braunwald et al., 2000). Therefore TAT for ReLIA cTnI is preferable. There is no comparable study to ReLIA cTnI results obtained in our study. The concept of highly specific cTnI assays, with the biomarker detectable at pathologic concentrations shortly after onset of symptoms, could make them attractive in the POCT setting (Hjortshøj et al., 2011). The results of our study showed ReLIA cTnI test is lower sensitivity and higher false negativity rate as compared to AQT90 Flex cTnI test. These findings suggest that in terms of the possibility of wrong diagnosis in patients with suspected MI could occur. Thus, additional assays must be applied before it is possible to exclude MI by the ReLIA cTnI assay alone.

The AQT90 FLEX cTnI assay has fast TAT with an analytical run time of approximately 18 minutes. Previous some studies have reported diagnostic performance of AQT90 Flex cTnI assay and other sensitive assays (Hjortshøj et al.,
In the present study, there is a high concordance between the AQT90 FLEX and Vidas Ultra cTnI results, and also AQT90 Flex has higher sensitivity, negative predictive value and accuracy as compared to ReLIA cTnI assay. However, AQT90 Flex cTnI assay showed higher false positive rate as compared to ReLIA cTnI assay. Similar to our study, Hjortshoj et al. (Hjortshoj et al., 2011) reported that AQT90 FLEX cTnI assay has higher negative predictive value. It is known that cTnI measurements are influenced by multiple factors, among which are the aforementioned posttranslational modifications proteolytic degradation, phosphorylation and complexing with other molecules (e.g., TnC, heparin, heterophilic or human antihouse antibodies, and cTnI specific autoantibodies circulating in patients' blood). Different mono- and polyclonal antibodies used in assays are sensitive to these factors to varying degrees (Apple and Collinson, 2012). The falsely increased or decreased cTnI results might cause presence of HAs. We found HAs positive rate s 4.87%, in the current study. In the clinical laboratory, HAs that cross-react with immunoglobulin's of different species are of particular interest because they can cross-link the solid phase and soluble antibodies in two-site “sandwich” immunoassays. Because many in vitro diagnostic immunoassays use murine monoclonal antibodies, heterophilic human anti-mouse immunoglobulin antibodies (HAMAs) are of particular concern. Development of HAMAs can follow the use of murine monoclonal antibodies for therapeutic and diagnostic imaging purposes (Kim et al., 2002; Carey et al., 1995). Although the incidence of interfering HA is low, clinically discrepant results in immunoassays continue to occur. Because of this, it is important that manufacturers continue to address this issue. One example is the revised Dimension cTnI assay, which reduces the effects of interfering HAs that were recently described for the original version of this method (Kim et al., 2002). The interfering factor had the greatest effect on cTnI measurements when there was only small amount of cTnI present in the sample, as in the early hours of an MI or unstable angina event (Hedberg et al., 2006).

In conclusion, although the rate of interfering HA is low in the present study, we observed clinically discrepant cTnI results that were measured by AQT90 Flex and ReLIA analysers. ReLIA cTnI assay has fairly lower sensitivity and higher false negative rate. This is first study that reported diagnostic performance of ReLIA cTnI assay. Therefore, we think further studies will be needed to determine the clinical diagnostic for ReLIA cTnI assay in rutin laboratory assay.

Author Contributions

All Authors confirmed they have contributed to the intellectual content of this paper and have met the following requirements: significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; drafting or revising the article for intellectual content; and final approval of the published article.

Author’s Disclosures or Potential Conflicts of Interest

Upon manuscript submission, all authors completed the authors’ disclosure form. Disclosures and/or potential conflicts of interest:

Employment of Leadership: None declared
Consultant or advisory role: None declared
Stock Ownership: None declared.
Honoria: None declared.
Research Funding: Research Fund of the University of Ordu (Project number: AR-1209).
Expert Testimony: None declared.
Role of Sponsor: No sponsor was declared.

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