Evaluation of four rapid immunochromatographic tests for the detection of cardiac Troponin I

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ABSTRACT:

Cardiac troponin I (cTnI) is a sensitive and specific marker of acute coronary syndromes and myocardial damage. During the past few years, it has become the preferred biochemical marker of myocardial infarction. However, due to the sensitivity required for its detection, only automated systems can be used in developed countries. However, these are rather expensive and unaffordable for most laboratories in developing countries. Many manufacturers have therefore proposed rapid immunochromatographic tests to detect cTnI.

The aim of this study was to assess the limit of detection (LOD) and performance of four rapid immunochromatographic tests available in Madagascar. The four tests evaluated were Hexagon Troponin, Nadal troponin I cassette, Troponitest+®, and Amicheck-Trop. Amicheck-Trop had a sensitivity and negative predictive value of around 80% whereas, for the three others it was around 20%. The specificity of Amicheck-Trop of 87.3% was lower than that of the other tests (98%-100%). These differences were explained by the Limit of detection of the tests: 0.3-0.4ng/ml for Amicheck-Trop, but only 1.8 -2 ng/ml for the three other tests.

Conclusion: Amicheck-Trop could be useful in the management of ACI or myocarditis in developing countries in sparsely equipped laboratories.
Reperfusion therapy has improved the prognosis of acute myocardial infarction (AMI). Early accurate diagnosis of acute coronary syndrome (ACS) and rapid evaluation of its severity may influence the patient’s prognosis. However, in many patients with acute chest pain, the electrocardiogram (ECG) is often equivocal in the early hours after an event, even in cases of proven infarction. In such cases, the ECG may never show the classical features of ST elevation and new Q waves. Hence, in the early stages, there is not enough evidence in these patients for clear diagnosis and risk stratification. Cardiac troponin I (cTnI) is a sensitive and specific marker of acute coronary syndromes and myocardial damage. During the past few years, it has become the preferred biochemical marker of myocardial infarction (1, 3).

The introduction of very sensitive assays for cTnI, make it now possible to measure cTnI even in healthy subjects (9). It has been previously shown that minor elevations of cTnI are predictive of long-term fatal outcomes, not only in subjects with diagnosed cardiovascular disease (CVD), but also in subjects with no known CVD (10). The consensus of the AACC and the European Society of Cardiology is that the 99th percentile of the upper reference limit (URL) should be used as a cut-off for the diagnosis of myocardial infarction (2, 8); and that the analytical goal of the assay should be imprecision of 10% CV at the 99th URL percentile.

This strategy supposes that only quantitative tests using automated systems can be used. However, these are rather expensive and unaffordable for most laboratories in developing countries. Many manufacturers have therefore proposed rapid immunochromatographic tests. In spite of its cost, reperfusion therapy is being used in some developing countries to identify high risk patients as soon as possible and reduce the rate of death.

However, although the manufacturers of the tests give an indication of their limits of detection and performance, no independent evaluations can be found. The aim of this study was
therefore to assess the limit of detection (LOD) and performance of four rapid immunochromatographic tests available in Madagascar and to compare these with the architect automatic system (ABBOTT, Wiesbaden, Germany).

Material and Methods

Description of the Troponin I detection tests

All of the tests evaluated for the detection human cardiac troponin I (cTnI) were rapid immunochromatographic tests. The four tests evaluated were Hexagon Troponin (Human Diagnostics, Wiesbaden, Germany), Nadal troponin I cassette (Nal Von Minden, Regensburg, Germany), Troponitest+® (All Diag, Strasbourg, France), and Amicheck-Trop (Zephir Biomedicals, Goa, India). All these tests can be stored between 2-4°C to 25-30°C. They required 70µl of serum or plasma for Hexagon Troponin, 120µl of serum or plasma for Nadal troponin I cassette, 120µl of serum, plasma or whole blood for Troponitest+® and 160µl of serum, plasma or whole blood for Amicheck-Trop. As recommended by the manufacturers the test was rejected in the absence of the control bar, whereas the sample was considered to be negative in the absence of a red bar on the test line. For the first three tests when any red color was visible in the patient window, the sample was considered to be positive. The limit of detection given by the manufacturers is 1ng/ml for the 3 tests. For Amicheck-Trop, when visually the intensity of the test band was less than the reference band, the concentration of cTnI was considered to be between 0.3 and 1 ng/ml; when the intensity of the test band was equal or greater than the reference band, the cTnI concentration was considered to be >1ng/ml.

Evaluation of cardiac Troponin I detection tests

To evaluate the four rapid immunochromatographic tests, a collection of reference serum samples that had been stored at –80°C was used. Concentrations of cTnI had previously been
determined in these serum samples using one enzyme immunoassay (EIA) quantitative test: Architect® Troponin I (Abbott Laboratories, Wiesbaden, Germany) (5). Discordant samples (positive with the Architect test and negative with the rapid tests) were confirmed using Vidas® troponin I (BioMérieux, Marcy-l’Etoile, France). These quantitative automated tests were considered as the reference method for the evaluation of the rapid tests. One hundred positive sera and 110 negative sera, according to the architect test, were used for the study. Among the 110 negative sera, 10 were positive for rheumatoid factor. Among the 100 positive sera, 38 had a cTnI titre ≥ 0.1ng/ml and < 0.3ng/ml; 27 had a titre ≥ 0.3ng/ml and < 1.0 ng/ml; 17 had a titre ≥ 1.0 ng/ml and < 3.0 ng/ml; and 18 had a titre > 3.0 ng/ml.

The sensitivity, specificity, positive predictive values (PPVs), and negative predictive values (NPVs) of the rapid tests were calculated as follows: sensitivity = true positives × 100% / [true positives + false negatives]; specificity = true negatives × 100% / [false positives + true negatives]; positive predictive value (PPV) = true positives × 100% / [true positives + false positives]; negative predictive value (NPV) = true negatives × 100% / [true negatives + false negatives]; accuracy = [true positives + true negatives] × 100% / number of sera tested.

**Evaluation of the limit of detection**

To verify the limits of detection given by the manufacturers, different dilutions with cTnI concentrations of 3.98, 13.26, and 18.58 ng/ml were set up in sera that were negative for cTnI with Architect (< 0.1 ng/ml).

**Statistical analysis**

Statistical analyses were performed with R software (R Development Core Team (2009). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL [http://www.R-project.org](http://www.R-project.org)).
Results

Evaluation of the tests

As shown in table 1, Hexagon Troponin, Nadal troponin I cassette and Troponitest+®, gave positive results mainly in sera with cTnI titres > 3ng/ml, whereas Amicheck-Trop gave positive results in sera with cTnI titres > 0.3 ng/ml. The limit of positivity explains the poor sensitivity and NPV of the three former tests, even when we used the LOD given by the manufacturers (table 2). However, the specificity and PPV of these tests were rather good. In contrast, Amicheck Trop had a good sensitivity and NPV (even excellent when we used a cut-off value of 0.3ng/ml) but its specificity and PPV were lower than that of the other tests (table 3).

Evaluation of the limit of detection

Different sera at different concentrations were used to evaluate the LOD. The results are presented in table 3. Amicheck had the lowest LOD of around 0.3-0.4 ng/ml. The Nadal Troponine I cassette had a LOD between 1.6 and 1.86 ng/ml (a sera at 1.8ng/ml came up negative whereas a sera at 1.6ng/ml came up positive. These results have been controlled). Hexagon and Troponitest had a LOD of around 2.0 ng. These findings agreed with the performance evaluations and explain the sensitivity of the tests.

Discussion

This study did not assess the use of these tests in the diagnosis of AMI. However, it gives an indirect evaluation of the potential of these tests in AMI and other diseases through comparison with a fully recognized test. The main aim of the study was to verify the LOD and reliability of the tests. The LOD found for Amicheck was similar to that indicated by the manufacturers (0.3-0.4ng/ml). It was also the lowest LOD among the tests. For the other tests,
the LOD given by the manufacturers was 1ng/ml. The LOD found in our study was closer to 2ng/ml for hexagon and troponitest+, and around 1.8 ng/ml for Nadal Troponin.

No interference was found with rheumatoid factor for any test. Amicheck troponine had by far superior sensitivity and NPV but also had the lowest specificity and PPV. The specificity of the hexagon troponine, Nadal troponine, and Troponitest tests was excellent but their high LOD explains the poor sensitivity of these tests. These results could have benefitted from replicate testing which was performed only in case of discordant results between the tests and in case of abnormal results such as in the cases of the sera at 1.8ng/ml negative with the Nadal Troponine I cassette while another sera at 1.6ng/ml came up positive with the same tests. However in regard of the number of sera tested and the assays for the limit of detection these results seem quite reliable.

With the most sensitive tests, low levels of troponin, but above the 99th percentile of a healthy population, are detected in different clinical situations such as cardiac trauma, myocarditis, pulmonary embolism, postcardiac surgery, cardioversion, sepsis, arrhythmias, critically ill patients in intensive care, end-stage renal failure, stroke, and epileptic seizures (4). However, the high LODs of the immunochromatographic tests limit their use to only a few indications, mainly AMI and myocarditis. For the management of AMI, we propose to use Amicheck Troponin, the most sensitive test. Indeed, WHO estimates that the decisional threshold for cTNI varies between 0.4 to 1.5 ng/ml for most quantitative tests. Only Amicheck troponin can detect these serum levels of cTnI, whereas the three other tests would give a negative result.

In cases of ST fragment elevation on ECG, reperfusion therapy should be initiated as soon as possible. If there is no ST fragment elevation, troponin should be tested and if this is positive, medical treatment should be started. If troponin is negative, it should be retested 6 h later. If still negative a cardiac stress test should be performed to provoke ischemia (7.8).
The test can also be used to diagnose an AMI retrospectively, since troponin remains at high levels for more than 7 days after AMI (6). However these tests cannot be used to evaluate the prognosis as they are not quantitative.

These rapid tests may also be used to differentiate between pericarditis and myocarditis, which require different treatments; troponin is positive in the case of myocarditis.

In conclusion, despite their obvious limitations, these rapid tests can be useful in a sparsely equipped laboratory. However the sensitivity of these tests still needs to be improved and semi-quantitative tests should be favoured.

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Table 1: Crude results of the evaluation of the four rapid tests with a panel of reference serum samples.

| Test       | cTnI Serum concentration with Architect (ng/ml) | < 0.1 | 0.1-0.3 | 0.3-1.0 | 1.0-3.0 | > 3.0 | Total |
|------------|-----------------------------------------------|-------|---------|---------|---------|-------|-------|
| Hexagon    | Positive                                      | 0     | 2       | 1       | 1       | 17    | 21    |
|            | Negative                                      | 110   | 36      | 26      | 16      | 1     | 189   |
| Nadal      | Positive                                      | 0     | 0       | 0       | 1       | 18    | 19    |
|            | Negative                                      | 110   | 38      | 27      | 16      | 0     | 191   |
| Troponitest| Positive                                      | 2     | 0       | 0       | 0       | 14    | 16    |
|            | Negative                                      | 108   | 38      | 27      | 17      | 4     | 194   |
| Amicheck   | 0.3-1                                         | 14    | 17      | 26      | 16      | 4     | 77    |
|            | >1                                             | 0     | 0       | 0       | 1       | 14    | 15    |
|            | Negative                                      | 96    | 21      | 1       | 0       | 0     | 118   |
|            | Total                                          | 110   | 38      | 27      | 17      | 18    | 210   |
Table 2: Evaluation of the four rapid tests for detection of cardiac troponin I with a panel of reference serum samples

| Assays                      | Cut-off value | No. of samples with the following results | % sensitivity (95 % CI) | % specificity (95 % CI) | % positive predictive value (95 % CI) | % negative predictive value (95 % CI) | % accuracy (95 % CI) |
|-----------------------------|---------------|------------------------------------------|------------------------|------------------------|--------------------------------------|---------------------------------------|----------------------|
| Hexagon Troponin            |               | 210 21 0 110 79                          | 21.0 (13.5-30.3)       | 100 (95.1-100)         | 100 (77.1-100)                       | 58.2 (50.8-65.3)                   | 62.4 (55.4-68.9)     |
| Nadal troponin I cassette   | 0.1 ng/ml     | 210 19 0 110 81                          | 19.0 (11.8-28.1)       | 100 (95.1-100)         | 100 (75.1-100)                       | 57.6 (50.2-64.7)                   | 61.4 (54.5-68.1)     |
| Troponitest+®               |               | 210 14 2 108 86                          | 14.0 (7.8-22.4)        | 98.2 (93.6-99.8)       | 87.5 (61.6-98.4)                     | 55.7 (48.4-62.8)                   | 58.1 (51.1-64.8)     |
| Amicheck-Trop               |               | 210 78 14 96 22                          | 78.0 (68.6-85.7)       | 87.3 (79.5-92.8)       | 84.8 (75.8-91.4)                     | 81.4 (73.1-87.9)                   | 82.9 (77.1-87.7)     |
| Hexagon Troponin            | 1 ng/ml       | 210 18 3 172 17                          | 51.4 (33.9-68.6)       | 98.3 (95.1-99.6)       | 85.7 (63.6-96.9)                     | 91.0 (85.9-94.7)                   | 90.5 (85.6-94.1)     |
| Nadal troponin I cassette   | (according to manufacturers) | 210 19 0 175 16                          | 54.3 (36.6-71.2)       | 100 (96.8-100)         | 100 (75.1-100)                       | 91.6 (88.7-95.1)                   | 92.4 (87.9-95.6)     |
| Troponitest+®               |               | 210 14 2 173 21                          | 40.0 (23.8-57.9)       | 98.9 (95.9-99.8)       | 87.5 (61.6-98.4)                     | 89.2 (83.9-93.2)                   | 89.0 (84.0-92.9)     |
| Amicheck-Trop               | 0.3 ng/ml     | 210 46 31 148 1                          | 97.9 (88.7-99.9)       | 82.7 (76.3-87.9)       | 59.7 (47.9-70.7)                     | 99.3 (96.3-99.9)                   | 92.4 (90.6-91.0)     |

*95% confidence interval
Table 3: Evaluation of the limit of detection of cardiac troponin I (cTnI) for the four rapid immunochromatographic tests using different dilutions of sera

| Serum cTnI concentrations (ng/ml) | Nadal troponine | Hexagon troponine | Troponitest Alldiag | Amicheck Troponine |
|----------------------------------|-----------------|------------------|---------------------|-------------------|
| 6.63                             | Positive        | Positive         | Positive            | Positive          |
| 3.31                             | Positive        | Positive         | Positive            | Positive          |
| 2.48                             | Positive        | Positive         | Positive            | Positive          |
| 2.12                             | Positive        | Positive         | Positive            | Positive          |
| 1.86                             | Positive        | Negative         | Negative            | Positive          |
| 1.8                              | Negative        | Negative         | Negative            | Positive          |
| 1.66                             | Positive        | Negative         | Negative            | Positive          |
| 1.59                             | Negative        | Negative         | Negative            | Positive          |
| 1.32                             | Negative        | Negative         | Negative            | Positive          |
| 1.06                             | Negative        | Negative         | Negative            | Positive          |
| 0.93                             | Negative        | Negative         | Negative            | Positive          |
| 0.46                             | Negative        | Negative         | Negative            | Positive          |
| 0.41                             | Negative        | Negative         | Negative            | Positive          |
| 0.31                             | Negative        | Negative         | Negative            | Positive          |
| 0.21                             | Negative        | Negative         | Negative            | Negative          |
| 0.15                             | Negative        | Negative         | Negative            | Negative          |