CLINICAL AND VACCINE IMMUNOLOGY, Mar. 2011, p. 414–417 Vol. 18, No. 3
1556-6811/11/$12.00 doi:10.1128/CVI.00502-10

Evaluation of Four Rapid Immunochromatographic Tests for the Detection of Cardiac Troponin I▽

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Received 19 November 2010/Returned for modification 27 December 2010/Accepted 4 January 2011

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 about 80%, whereas for the three others, they were about 20%. The specificity of Amicheck-Trop of 87.3% was lower than the specificities of the other tests (98% to 100%). These differences were explained by the limits of detection of the tests: 0.3 to 0.4 ng/ml for Amicheck-Trop but only 1.8 to 2 ng/ml for the three other tests. It was concluded that Amicheck-Trop could be useful in the management of acute myocardial infarction or myocarditis in sparsely equipped laboratories in developing countries.

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) findings are 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 now make it possible to measure cTnI even in healthy subjects (10). It has previously been 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 (11). 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 cutoff for the diagnosis of myocardial infarction (2, 9) and that the analytical goal of the assay should be imprecision of a 10% coefficient of variation 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 (LODs) and performance, no independent evaluations can be found. The aim of this study was therefore to assess the LODs and performance of four rapid immunochromatographic tests available in Madagascar and to compare these with the Architect automatic system (Abbott Laboratories, Wiesbaden, Germany).

MATERIALS AND METHODS

Description of troponin I detection tests. All of the tests evaluated for the detection of human 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 at between 2 to 4°C and 25 to 30°C. They required 70 μl of serum or plasma for Hexagon Troponin, 120 μl of serum or plasma for Nadal troponin I cassette, 120 μl of serum, plasma, or whole blood for Troponitest+, and 160 μl 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 positive. The limits of detection given by the manufacturers are 1 ng/ml for the three tests. For Amicheck-Trop, when the intensity of the test band was visually less than that of 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 to or greater than that of the reference band, the cTnI concentration was considered to be >1 ng/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. The concentrations of cTnI in these serum

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▽ Published ahead of print on 12 January 2011.

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

Test	Result	No. of serum samples with the following cTnI serum concn by Architect test (ng/ml):					
		<0.1	0.1–0.3	0.3–1.0	1.0–3.0	>3.0	Total
Hexagon Troponin	Positive	0	2	1	1	17	21
	Negative	110	36	26	16	1	189
Nadal troponin I cassette	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-Trop	0.3–1[a]	14	17	26	16	4	77
	>1[a]	0	0	0	1	14	15
	Negative	96	21	1	0	0	118
Total		110	38	27	17	18	210

[a] cTnI concentration (in ng/ml), according to the intensity of the test band.

samples had previously been determined using one enzyme immunoassay (EIA) quantitative test: Architect Troponin I (Abbott Laboratories) (5). Results for discordant samples (positive with the Architect test and negative with the rapid tests) were confirmed using the Vidas troponin I assay (bioMérieux, Marcy l'Etoile, France). These quantitative automated tests were considered the reference methods for the evaluation of the rapid tests. One hundred positive and 110 negative serum specimens, according to the Architect test, were used for the study. Among the 110 negative serum specimens, 10 were positive for rheumatoid factor. Among the 100 positive serum specimens, 38 had cTnI titers of ≥0.1 ng/ml and <0.3 ng/ml, 27 had titers of ≥0.3 ng/ml and <1.0 ng/ml, 17 had titers of ≥1.0 ng/ml and <3.0 ng/ml, and 18 had titers of >3.0 ng/ml.

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

Evaluation of 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 the Architect test (<0. 1 ng/ml).

Statistical analysis. Statistical analyses were performed with R software (7).

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 titers of >3 ng/ml, whereas Amicheck-Trop gave positive results in sera with cTnI titers of >0.3 ng/ml. The limit of positivity explains the poor sensitivities and NPVs of the three former tests, even when we used the LOD given by the manufacturers (Table 2). However, the specificities and PPVs of these tests were rather good. In contrast, Amicheck-Trop had a good sensitivity and NPV (even excellent, when we used a cutoff value of 0.3 ng/ml), but its specificity and PPV were lower than those of the other tests (Table 3).

Evaluation of the limit of detection. Different serum samples with different concentrations were used to evaluate the LOD. The results are presented in Table 3. Amicheck-Trop had the lowest LOD of about 0.3 to 0.4 ng/ml. The Nadal troponin I cassette had an LOD of between 1.6 and 1.86 ng/ml (a serum sample with cTnI at 1.8 ng/ml came up negative, whereas a serum sample with cTnI at 1.6 ng/ml came up positive; these results have been controlled for). Hexagon Troponin and Troponitest+ had LODs of about 2.0 ng. These findings agreed with the performance evaluations and explain the sensitivities 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 the diagnosis of AMI and other diseases through comparison with the results of a fully recognized test. The main aim of the study was to verify the LODs and reliabilities of the tests. The LOD found for Amicheck-Trop was similar to that indicated by the manufacturers (0.3 to 0.4 ng/ml). It also had the lowest LOD among the tests. For the other tests, the LOD given by the manufacturers was 1 ng/ml. The LOD found in our study was closer to 2 ng/ml for Hexagon Troponin and Troponitest+ and about 1.8 ng/ml for the Nadal troponin I cassette.

No interference with rheumatoid factor was found for any test. Amicheck-Trop had superior sensitivity and NPV by far, but it also had the lowest specificity and PPV. The specificities of the Hexagon Troponin, Nadal troponin I cassette, and Troponitest+ tests were excellent, but their high LODs explain the poor sensitivities 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 a serum sample with cTnI at 1.8 ng/ml testing negative with the Nadal troponin I cassette, while another serum sample with cTnI at 1.6 ng/ml testing positive with the same test. However, in regard to the number of serum samples tested and the assays for the limit of detection, these results seem quite reliable.

With the most sensitive tests, low levels of troponin, but levels above the 99th percentile for 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

TABLE 2. Evaluation of the four rapid tests for detection of cardiac troponin I with a panel of reference serum samples

Assay	Cutoff value	No. of serum samples with the following results:					Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)	Accuracy (%)
		Total	True positive	False positive	True negative	False negative					
Hexagon Troponin	0.1 ng/ml[a]	210	21	0	110	79	21.0 (13.5–30.3)[b]	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[a]	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+	0.1 ng/ml[a]	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	0.1 ng/ml[a]	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[c]	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	1 ng/ml[c]	210	19	0	175	16	54.3 (36.6–71.2)	100 (96.8–100)	100 (75.1–100)	91.6 (86.7–95.1)	92.4 (87.9–95.6)
Troponitest+	1 ng/ml[c]	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[c]	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 (80.6–90.1)

[a] Determined by the Architect assay.
[b] Values in parentheses are 95% confidence intervals.
[c] According to the manufacturer.

TABLE 3. Evaluation of the limit of detection of cTnI for the four rapid immunochromatographic tests using different dilutions of sera

Serum cTnI concn (ng/ml)	Test result			
	Nadal troponine I cassette	Hexagon Troponine	Troponitest+	Amicheck-Trop
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	Negative
0.21	Negative	Negative	Negative	Negative
0.15	Negative	Negative	Negative	Negative

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-Trop, the most sensitive test. Indeed, WHO estimates that the decisional threshold for cTnI varies from 0.4 to 1.5 ng/ml for most quantitative tests. Only Amicheck-Trop 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, a test for troponin should be performed, and if this is positive, medical treatment should be started. If the test for troponin is negative, retesting should be performed 6 h later. If the result is still negative, a cardiac stress test should be performed to provoke ischemia (8, 9).

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; the patient is positive for troponin in the case of myocarditis.

In conclusion, despite their obvious limitations, these rapid tests can be useful in a sparsely equipped laboratory. However, the sensitivities of these tests still need to be improved and semiquantitative tests should be favored.

ACKNOWLEDGMENT

This work was supported by a grant from the Institut Pasteur de Madagascar.

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