Analytical performance and clinical application of a new rapid bedside assay for the detection of serum cardiac troponin I

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Detection of cardiac troponin I (cTnI) in patients suspected of having an acute coronary syndrome is highly predictive for an adverse outcome. We evaluated a bedside test for cTnI that uses a polyclonal capture antibody and two monoclonal indicator antibodies. Clinical studies were performed in patients with acute coronary syndrome and patients with chest pain but no evidence of acute myocardial injury. The whole-blood, 15-minute assay had a concordance of 98.9% with an ELISA for cTnI and a detection limit of 0.14 μg/L, and the device tolerated temperatures between 4 °C and 37 °C. Diagnostic sensitivity for myocardial infarction at arrival (3.5 ± 2.7 h after onset of symptoms) was 60% [creatine kinase isoenzyme MB (CK-MB) mass, 48%; CK activity, 36%; P < 0.01], and 4 h later, diagnostic sensitivity was 98% (CK-MB mass, 91%; CK activity, 61%; P < 0.01). In 38% of the patients with unstable angina, at least one positive cTnI test was found (CK-MB mass, 4%; CK activity, 2%). No false-positive test results were found in renal failure or injury of skeletal muscle. We conclude that the diagnostic efficacy of the cTnI rapid test was comparable with the cTnI ELISA and superior to CK-MB determination. Therefore, this device could facilitate decision-making in patients with chest pain at the point of care.

Acute myocardial infarction is highly probable in patients with typical anginal pain accompanied by marked ST-segment elevation. In these patients, thrombolytic therapy is usually administered independently of confirmation by biochemical markers (1). However, in up to 50% of the patients with acute myocardial infarction, the electrocardiogram is nondiagnostic, and the diagnosis depends on biochemical markers (2–5). About 4% of patients with developing myocardial infarction are inappropriately sent home (4–7), and electrocardiographic changes in high-risk patients with unstable angina are even less specific (8).

Measurements of creatine kinase isoenzyme MB (CK-MB) activity are rapid and inexpensive but suffer from a low cardiac specificity and poor sensitivity to detect minor myocardial injury (2, 4, 9). CK-MB measurements, even when determined immunologically, appear to be inadequate for clinical decision-making in the emergency room (9–12).

Cardiac troponins have recently been introduced as cardiac markers (12–19). Only cardiac troponin I (cTnI) has not yet been detected in skeletal muscle cells at any stage of development or pathological condition (15, 18, 19). cTnI can be distinguished by monoclonal antibodies recognizing the amino acid sequence distinct for cardiac muscle cells (17–20). cTnI determination promises higher diagnostic efficacy and potency for new diagnostic options (12–14):

- Wider diagnostic window of time with early appearance (attributed to release from cytosolic pool) and prolonged appearance (attributed to proteolysis of the contractile apparatus).
- High proportional rise of troponins (reflecting the low plasma cTnI concentrations in healthy persons), allowing detection of minor myocardial damage.
- No cross-reactivity of skeletal isoforms.
- Lack of cTnI in skeletal muscle tissue.

A recently developed rapid assay for cTnI can be performed at the point of care within 15 min by medical or paramedical staff and might facilitate risk stratification of patients in emergency rooms (12, 21). Therefore, we compared its analytical performance with two established ELISA systems.
**Materials and Methods**

**RAPID ASSAY**

The rapid assay system uses chromatographic solid-phase technology (Spectral Diagnostics) with a cocktail of two gold-labeled monoclonal mouse indicator antibodies (8I-7 and 2I-14) and a biotinylated polyclonal goat capture antibody. The test system contains at least 0.3 μg of each antibody (17, 21). The selected antibodies bind both free and complexed cTnI with high affinity (17, 22).

We added 200 μL of heparinized whole blood or centrifuged plasma to the device. After separation of cellular blood components from the plasma fraction by a glass fiber fleece, the migrating plasma dissolves a buffer and solubilizes the adsorbed antibodies. The antibodies and the patient’s cTn molecule form sandwich complexes, which migrate to the signal zone and accumulate in the reading window by means of biotin-streptavidin interaction. Positive results (cTnI ≥ 0.1 μg/L; manufacturer-defined discriminator) are indicated by a color line that appears within 15 min.

The unreacted indicator antibodies pass on and bind to the control line consisting of solid phase anti-mouse IgG antibodies (≥0.2 μg). The appearance of this control line distal to the signal line confirms impeccable test function, including unimpeded plasma flow.

**QUANTITATIVE CARDIAC MARKER DETERMINATION**

The two identical indicator antibodies, but as Fab fragments, were used on a semiautomated one-step cTnI ELISA system on the basis of “big bead” technology performed on the Cobas EIA® (Roche Diagnostic Systems) (17, 23, 24). After incubation of the serum samples with 100 μL of conjugate and the coated bead, the exceeding conjugate was removed by radial elution. The second incubation period was performed with 250 μL of solution of 3,3′,5,5′-tetracarbethoxybenzidine and hydrogen peroxide converted into a colored product by horseradish peroxidase-labeled antibodies. The reaction was terminated by adding 1000 μL of stopping solution (50 mL/L sulfuric acid) to allow cTnI quantification photometrically. The lower detection limit of this assay system is 0.03 μg/L (17), and a discriminator value of 0.1 μg/L was used. For calibration, six calibrators containing purified recombinant cTnI (Spectral Diagnostics), ranging from 0 to 6.0 μg/L, were used. Interassay precision was 6.2% at 0.2 μg/L and 5.4% at 2.4 μg/L.

In parallel, cTnI and CK-MB mass were quantified by means of a commercially available analyzer system (Access® analyzer, Beckmann Diagnostics). It is based on chemiluminescence technology, and the capture antibody is fixed to magnetic microparticles. The turnaround time is 18 min. The detection limit for cTnI is 0.03 μg/L. Values ≥0.1 μg/L were considered to be positive (25), and day-to-day precision (CV) was 8.1% at 0.2 μg/L and 4.6% at 2.4 μg/L. The detection limit for CK-MB mass is 0.15 μg/L, and the upper reference is 5.0 μg/L (26). The interassay CVs were 8.4% at 8.2 μg/L and 7.2% at 14.7 μg/L.

Catalytic activity of total CK was measured colorimetrically in the emergency laboratory at room temperature (Hitachi 717, Boehringer Mannheim) and with different cutoffs for women (70 U/L) and men (80 U/L) according to the manufacturer’s specifications. All biochemical analyses were performed by technicians unaware of the patients’ histories.

**EVALUATION OF TEST PERFORMANCE**

Blood samples were obtained from 10 patients with acute myocardial infarction and 5 patients with minor myocardial injury (high frequency count ablation, unstable angina, and myocarditis). After quantitative determination of cTnI two times by Cobas EIA, the samples were diluted stepwise with human serum from healthy volunteers (cTnI concentration ≤ 0.03 μg/L) down to a cTnI concentration below the analytical sensitivity of the Cobas EIA system.

Testing of each dilution step was performed five times separately by blinded investigators (n = 6) who were trained in the assessment of the rapid test. The cutoff for the rapid assay was calculated as the cTnI concentration determined with the Cobas EIA (identical antibodies) detected in all trials by each of the blinded observers.

To evaluate the effects of temperature, rapid tests were duplicated on devices heated to 37 °C, cooled to 4 °C, and frozen to −20 °C for 7 days.

**CLINICAL EVALUATION**

The study population was recruited from the emergency room of the University Hospital Hamburg between September 1, 1995 and October 31, 1997. All of these patients had chest pain lasting 12 h or less and were stratified into four groups of consecutive patients: group 1, 159 patients with acute myocardial infarction, which was defined in the absence of ST-segment elevations as an increase of total CK activity within 24 h after onset of chest pain higher than twice the upper limit of normal associated with increased CK-MB; group 2, 321 patients with angina at rest associated with ST-T-segment aberrations. Patients had unstable angina according to the Braunwald classification III B (53%) + C (47%) (27); group 3, 37 patients with cardiac chest pain of nonischemic origin related to acute congestive heart failure, perimyocarditis, ventricular arrhythmia, or rejection after heart transplantation who had no marked coronary stenosis (≥70% diameter stenosis); and group 4, 120 patients with acute chest pain but no evidence of acute myocardial injury. These patients had no ST-segment changes and underwent treadmill testing, stress echocardiography, or angiogram, providing no abnormalities. During 30 days of follow-up, no cardiac event was documented in this group.

Furthermore, we investigated 27 asymptomatic patients (ages, 34 ± 12 years) with end-stage renal failure.
but no history of coronary heart disease recruited from the department of nephrology at the time of periodic hemodialysis.

**Statistical Analysis**

All results for continuous variables are expressed as means ± SDs (range), and for comparison between two groups, the Mann–Whitney U-test was used. Comparison of categorical variables (positive/negative) were generated by the McNemar test (two-sided) with appropriate degrees of freedom. The calculations were done with SPSS 6.1 (SPSS, Inc.), and $P$ values <0.05 were considered statistically significant.

Analytical results were considered concordant if the rapid assay was interpreted as positive and the quantitative determination by ELISA was above cutoff or if rapid assay was interpreted as negative and the ELISA result was below cutoff. Otherwise, the result was categorized as discordant (false-negative/false-positive).

The diagnostic sensitivity was calculated as the number of positive test results among all patients with acute myocardial infarction observed, and the diagnostic specificity was defined as the number of negative test results among all patients with excluded acute myocardial infarction.

**Results**

**Detection Limit of the Rapid Device**

Under blinded conditions, 1198 blood samples were analyzed with the rapid device by two trained observers and with both ELISA systems, the Cobas EIA and the Access analyzer. The concordance of the rapid device (29% positive test results) with the Cobas EIA (26% positive results) was 98.9% (Fig. 1), and with the Access analyzer (23% positive results) was 97.2% at predefined cutoff values of 0.1 μg/L.

All cTnI values $\leq 0.14$ μg/L (Cobas EIA; $n = 302$) were categorized as positive rapid test results. For cTnI values between 0.06 and 0.14 μg/L, an increasing rate of positive test results was found. Only two samples with cTnI values <0.06 μg/L showed a positive rapid test result (0.03 and 0.04 μg/L) and have to be considered as false-positive. One test kit indicated malfunction by absence of a control signal, despite obviously adequate plasma migration.

Intensity and speed of color development were apparently proportional to the cTnI concentration. In samples with cTnI amounts $\leq 4$ μg/L, a positive test signal developed within 5 min, and samples with cTnI concentrations $\leq 0.4$ μg/L could not be rated earlier than 15 min after serum application (Fig. 2). To prevent false-positive test results, the time interval from blood application to test assessment should not exceed 15 min.

**Interobserver Variability**

The rapid test results of a stepwise dilution series revealed from patients with myocardial injury were categorized by six blinded observers who were trained but varied in experience (50–1000 test assessments). In the range from 0.06 to 0.14 μg/L, significant, ($P = 0.02$) interobserver variability was observed (Fig. 3). At a cTnI concentration of 0.1 μg/L, four observers rated the test as positive and two as negative. The discordance correlated with the observer’s experience in assessment of rapid assays. Experienced observers rated all tests at cTnI concentrations of 0.08 μg/L as positive, whereas trained but unexperienced observers rated all of these tests as...
negative ($P < 0.01$). Additionally, insufficient artificial illumination and hemolytic or lipemic samples increased the incidence of discrepant classifications. All test results with cTnI concentrations above 0.14 μg/L were considered positive by all observers, independent of experience.

**TEMPERATURE STABILITY**
Incubation of 15 test devices at 37°C for 7 days did not influence the analytical sensitivity of the test system (0.10 vs 0.11 μg/L for incubated devices). Similarly, lowering the temperature of 20 tests devices down to 4°C was not associated with a decrease of analytical sensitivity (0.10 vs 0.10 μg/L for cooled devices). However, the freezing of 20 test devices at −20°C for 7 days did affect the sensitivity (0.10 vs 0.45 μg/L for frozen devices; $P < 0.01$).

**CLINICAL EVALUATION**

*Patients with acute myocardial infarction (n = 159).* In 159 patients with acute myocardial infarction, the first determination of the cardiac markers was performed 3.5 ± 2.7 (0.5–12) h after onset of symptoms. A positive cTnI bedside test was found in 60% of the patients, with increased cTnI concentrations with the Cobas EIA in 58% and with the Access analyzer in 54% of the patients (not significant for both).

Four hours later, at least one positive bedside test was obtained in 98%. Increased quantitative cTnI concentrations with the Cobas EIA were found in 94% and in 92% of the patients with the Access analyzer (not significant for both).

Immunoassay determination of CK-MB revealed increased values at arrival in 48% ($P = 0.004$ compared with cTnI rapid assay) and for conventional CK activity in 36% of the patients ($P < 0.001$). Four hours later, in 91% and 61% of the patients, CK-MB mass ($P = 0.03$) and CK enzyme activity ($P < 0.001$), respectively, were increased, which was equal to the diagnostic sensitivity of the cTnI rapid device at the time of arrival (60%) (Fig. 4).

*Patients with unstable angina (n = 321).* Among 321 patients with unstable angina, 122 patients (38%) had at least one positive cTnI test result within 4 h after arrival and 7.4 ± 2.9 h after onset of symptoms, respectively (Fig. 5). Positive results were confirmed by quantitative determination in 92% of the patients for Cobas EIA. Only one patient with a negative cTnI rapid assay had a cTnI value of 0.12 μg/L (Cobas EIA) and 0.09 μg/L (Access). In 98% of the cTnI-positive patients, a substantial coronary stenosis could be detected by angiogram. Increased CK-MB mass was found in 13 patients (4%), and in 7 patients (2%), CK enzyme activity was increased (81–134 U/L).

*Patients with cardiac chest pain of nonischemic origin (n = 37).* Patients had acute congestive heart failure (n = 15), perimyocarditis (n = 11), ventricular arrhythmia (n = 8), or rejection after heart transplantation (n = 3). In patients with congestive heart failure (73% ischemic), eight positive rapid test results (53%) were documented, but none of them had marked coronary stenosis. In seven patients with perimyocarditis (58%), a positive test result was documented, and all patients with ventricular arrhythmia and rejection of transplanted heart had increased cTnI concentrations (Fig. 5). All positive test results were confirmed by Cobas EIA and in 95% by the Access analyzer.

*Patients with chest pain of noncardiac origin (n = 120).* Patients had acute pulmonary embolism (n = 5), acute pleuritis (n = 7), pneumonia (n = 12), musculoskeletal syndrome (n = 51), or traumatic chest pain (n = 45). Positive cTnI tests were found in two patients with fulminant pulmonary embolism and echocardiographic signs of right ventricular overloading (Fig. 5). Both were confirmed by Cobas EIA and Access analyzer. No cTnI was detected in the other patients, both by rapid device and ELISA. CK-MB mass was increased in 32 (71%) patients with traumatic chest pain, in 3 (6%) patients with musculoskeletal syndrome, and in 4 (33%) patients with pneumonia.

*Patients with end-stage renal failure (n = 27).* In 27 patients with end-stage renal failure but no history of coronary heart disease, 53 blood samples were collected before and after planned hemodialysis. No positive bedside tests were detected, and likewise no increased cTnI concentrations were determined by ELISA (Fig. 5). CK-MB mass (9.5 ± 7.2 μg/L) was increased in 8 patients (30%), and CK activity (128 ± 56 U/L) was increased in 12 patients (44%).

**Discussion**
Our results indicate that the new qualitative bedside test is a reliable, convenient, and rapid method for the qualitative determination of cTnI. The device was easy to handle and not affected by temperature fluctuations between 4 and 37°C. However, increasing analytical sensitivity was documented after storage at −20°C for 7 days.
The rapid test system provided a high test accuracy compared with both ELISA systems. The major advantage of a rapid test system is the applicability of whole blood anticoagulated by heparin so that additional time-consuming preparations such as clotting and centrifugation can be omitted. The performance of the rapid test system at the point of care by medical or paramedical personnel avoids additional time delay and possible sample damage or confusion. But the absence of a quantitative test result represents a disadvantage because positive correlation between the amount of troponin release and cardiac risk has been shown (8, 28). Although the speed of color development and the intensity of the test signal apparently depends on the cTnI concentration in the blood sample, the rapid device provides at best a semiquantitative test result. However, no different therapeutic strategies according to the troponin concentration have been established thus far (12, 28), favoring cTnI rapid testing for rapid decision-making and in facilities without access to quantitative troponin measurements.

The analytical sensitivity of the rapid assay is influenced by factors other than the analytical characteristics of the test device, as well. The variability of visual assessment as to the presence or absence of the signal line at low troponin concentrations represents a critical factor in clinical routine. The cutoff defined in our analysis as the cTnI concentration leading to positive test results by all observers in all repetitive samples was settled at 0.14 μg/L. However, we could demonstrate that between 0.10 and 0.14 μg/L, the sensitivity depends on the users’ training status. Therefore, a careful acquaintance with training samples is recommended. The highly experienced personnel achieved a detection limit of 0.08 μg/L cTnI without an increase in false-positive test results.

The rapid test device revealed a distinct improvement (60% vs 48%; 3.5 ± 2.7 h after onset of pain; P < 0.01). The cTnI determination is expected to reveal absolute myocardial specificity because cTnI is not expressed in fetal and healthy or diseased adult human skeletal muscle tissue, promising no false-positive test results in patients with substantial skeletal muscle damage or renal failure (29–33). The investigated cTnI test systems produced no positive results in patients with end-stage renal failure and acute or chronic skeletal muscle injury, whereas 30% and 71% of the patients, respectively, had increased CK-MB mass concentrations.

Diagnostic specificity, however, for the detection of acute myocardial infarction according to WHO criteria was lower than that of CK-MB because of a marked rate of positive cTnI test results in patients with unstable angina (38% vs 4% for CK-MB mass; P < 0.001). The low incidence of patients with increased CK-MB concentrations might be related to strict exclusion of all patients with non-Q-wave acute myocardial infarction. Additional positive cTnI test results were documented in patients with acute congestive heart failure, myocarditis, ventricular arrhythmia, and rejection of transplanted heart. Recent studies indicate that, independent of the diagnosis of myocardial infarction, the detection of troponins is associated with increased subsequent cardiac risk; therefore, these positive test results are not to be rated as false-positive (12–14).

In conclusion, the use of this cTnI rapid device could improve efficacy and safety of decision-making in patients with chest pain that might produce more cost-effective use of intensive care facilities. Future prospective studies must define the role of the new diagnostic marker in patient management to improve their adverse prognosis (12, 34).

References


