Evaluation of a Rapid Whole Blood ELISA for Quantification of Troponin I in Patients with Acute Chest Pain

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Background: Troponin I (cTnI) provides important prognostic information in patients with chest pain. We wished to evaluate a rapid, whole-blood analyzer for quantitative point-of-care testing.

Methods: A quantitative point-of-care test system (Stratus CS®; Dade-Behring) for cTnI with an incorporated centrifuge was evaluated in 412 patients with chest pain less than 12 h.

Results: Results were available within 15 min. CVs were 4.5% at 0.1 μg/L, 4.2% at 0.25 μg/L, and 6.5% at 0.82 μg/L. The detection limit was 0.01 μg/L. The 97.5% percentile in a healthy population was 0.08 μg/L. Based on ROC curve analysis, a threshold of 0.15 μg/L was calculated for the detection of acute myocardial infarction (AMI). With it, sensitivity for the detection of patients with AMI (n=62) was 63% at arrival and 98% after 4 h (Stratus II®, 48% and 85%, respectively; P <0.01). In 42% of patients with unstable angina (n=121), cTnI was ≥0.08 μg/L (Stratus II, 28%; P <0.01). During 30 days, death or AMI occurred in 25.5% of these cTnI-positive vs 2.9% of cTnI-negative patients (Stratus II, 29.4% vs 5.8%).

Conclusion: The Stratus CS provided better analytical performance and comparable or better prognostic information than the Stratus II.

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The acute coronary syndrome is a high-risk phase for patients with coronary heart disease (CHD) mainly defined by clinical symptoms (1). Associated electrocardiographic findings are rather heterogeneous, and traditional biochemical markers such as creatine kinase MB (CK-MB) suffer from low cardiac specificity and sensitivity (2–6). Test systems for cardiac troponin T and troponin I (cTnI) provide the highest cardiac specificity and analytical sensitivity for the detection of myocardial injury (7–12). These allow early identification of patients with acute myocardial infarction (AMI), and in one-third of patients with unstable angina (in spite of normal CK enzyme activity), a minor increase in troponin related to myocardial injury is detectable. Several studies have documented the superior prognostic value of the troponins for early and safe risk stratification of patients with acute chest pain (12–21). In addition, first evidence has been provided that troponin-positive patients with unstable angina particularly benefit from glycoprotein IIb/IIIa receptor blockers as well as prolonged treatment with low-molecular weight heparin (22, 23).

The first commercially available ELISA test system for determination of cTnI was presented in 1995 (Stratus II®). The Stratus II has been shown to provide sufficient analytical sensitivity for the detection of minor myocardial injury in patients with acute coronary syndrome (10, 24). In this prospective study, we evaluated the new Stratus CS® test system assumed to combine the advantage of point-of-care testing with the analytical performance of second-generation quantitative ELISA technology. We investigated this new test system in a routine setting of patients presenting with acute chest pain in the emergency room.
Materials and Methods

Determination of Cardiac Markers

cTnI was analyzed with both the established Stratus II and the new Stratus CS systems, both from Dade-Behring.

The Stratus CS is a fluorometric enzyme immunoassay analyzer for quantitative determination of the cardiac marker CK-MB mass, myoglobin, and troponin I only. The test system is designed to analyze closed routine sample tubes containing anticoagulated whole blood [lithium-heparin (Li-heparin)]. Alternatively, this test system can analyze preprocessed plasma specimens placed into sample cups. For separation of the plasma from whole blood samples, the centrifugation step is incorporated into the test system. Tests to be performed are selected by introducing unitized, bar-coded test packs. Up to four test packs can be introduced for each sample. All required reagents are enclosed within the test packs, which are transferred by disposable pipette tips. Dilutions can be performed automatically by adding test-specific dilution packs. The test system utilizes radial partition immunooassay technology, which has been enhanced through the use of monoclonal capture antibody coupled to Starburst® dendrimers. These dendrimers are well-defined polymers composed of polyamidoamine groups, distinguished by uniformity in molecular size and shape (25). The cTnI-specific antibodies used are identical to those used on the Stratus II system (26) and likewise are capable of detecting both free and complexed cTnI. The dendrimer technology provides for better presentation and functionality of the capture antibody on the glass fiber solid phase surface used in the assay. This in turn leads to more efficient capture of the target antigen. The assay process is initiated by applying the dendrimer-antibody reagent onto the glass fiber matrix to form a reaction zone, which serves to capture the analyte of interest. Centrifuged plasma is then added, followed by the first incubation period. Thereafter, the alkaline phosphatase-labeled second antibody is applied to the matrix, followed by a second incubation period. The unbound, labeled antibody fraction is removed from the reaction zone by radial elution using the substrate-wash reagent. Captured phosphatase-labeled antibodies convert the included enzyme-substrate into a fluorescent product; this permits quantification of the cardiac marker by front surface fluorescence measurement. Analysis on the Stratus CS was performed by six specially trained medical and paramedical collaborators in the emergency room. For the evaluation of analytical performance, centrifuged plasma samples were used; in the clinical study, whole blood samples were analyzed at the point of care.

The Stratus II, a two-step ELISA with a detection limit of 0.35 μg/L for cTnI, has been described elsewhere (10, 24). According to previous studies, values above 0.7 μg/L were considered positive, and a diagnostic threshold of 1.5 μg/L for the diagnosis of myocardial infarction was used (27, 28).

In parallel CK-MB mass and myoglobin were quantified by means of the Stratus II test system. Their upper limits of normal are 5.0 μg/L and 110 μg/L, respectively. All biochemical analyses were conducted on the Stratus II. In addition, CK and CK-MB enzyme activity were measured routinely in the emergency laboratory (Hitachi 717; Boehringer, Mannheim; diagnostic threshold value for CK, 80 U/L in males and 70 U/L in females).

Evaluation of Analytical Performance

The Stratus CS was evaluated relative to the Stratus II regarding:

- Interassay precision over 60 assays run on 30 consecutive days with the same lot and unchanged calibration [by using plasma controls with low (1.5 μg/L) and medium (2.5 μg/L) cTnI concentrations from patients with “minor myocardial injury” after catheter ablation of ventricular arrhythmia (CK-MB mass within the reference interval; aliquots kept at –80 °C)];
- Detection limit [defined as the lowest concentration that can be differentiated from zero (mean + 3 SD) and limit of quantification with acceptable precision (CV ≤20%) by a stepwise dilution series of plasma samples from patients with minor myocardial injury;]
- Upper limit of normal by determination of plasma samples from patients with non-cardiac chest pain. The diagnostic threshold value was defined as the mean concentration + 2 SD;
- Correlation between plasma samples anticoagulated with EDTA, Li-heparin, and sodium heparin (Na-heparin).

Clinical Evaluation in the Emergency Room

From August to December 1998, 412 consecutive patients (143 females and 269 males; ages, 59.4 ± 9.1 year) were enrolled at the emergency room of the University Hospital Hamburg, Germany. The study inclusion criterion was chest pain lasting <12 h before arrival, as possibly associated with an acute coronary syndrome. The mean duration of symptoms at arrival was 4.8 ± 3.5 h; chest pain continued in 48% of the patients when enrolled in the study.

The study exclusion criterion was evidence of characteristic ST-segment elevations on the baseline electrocardiogram. Patients having myocardial infarction or coronary revascularization during the preceding 14 days were also excluded because of the possible continuous release of troponins after myocardial infarction or potential myocardial injury during revascularization.

Plasma samples anticoagulated with Li-heparin, Na-heparin, and EDTA, respectively, were collected within 15 min after the patient’s arrival in the emergency room and additionally 4 h later as a supplement to the routine blood collection. For patients arriving within 2 h after onset of...
symptoms, the second blood sample was shifted to 6 h after arrival. Whole blood samples were analyzed immediately for cTnI with the Stratus CS system at the point of care. Additional plasma samples were centrifuged within 15 min after collection and immediately analyzed for cTnI, CK-MB mass, and myoglobin with the Stratus II system.

DEFINITION OF CLINICAL DIAGNOSES
AMI at the time of arrival was defined according to the WHO criteria (29). We included patients with a final diagnosis of AMI, defined by typical increase of CK enzyme activity greater than twice the upper limit of normal associated with CK-MB activity ≥6% of CK activity during the first 24 h after onset of symptoms. Unstable angina was defined according to the Braunwald classification (30). Of 121 patients with a clinical diagnosis of unstable angina, 69 patients had a history of CHD. For 62 of these patients, coronary angiography was performed during the index hospital stay. All remaining 52 patients without history of CHD underwent coronary angiography; functional CHD was documented in 98% of this subgroup.

Control patients were those without evidence of CHD. These patients had no ST-segment changes during serial electrocardiographic recordings in the emergency room and a negative treadmill test, stress echocardiography, or angiogram (42%). Additionally, during a 30-day follow-up period, the control patients had no occurrences of major cardiac events (death, nonfatal AMI, urgent revascularization). None of these control patients returned to the hospital because of a recurrence of chest pain. Thus, these control patients represent a subgroup with very low cardiac risk (15).

STATISTICAL ANALYSIS
All results for continuous variables are expressed as means ± SD; comparison between two subgroups was performed by the Mann–Whitney U-test (two-sided). A comparison of categorical variables was generated by the Fisher exact test. Linear regression analysis was performed on a single test from each sample over the dynamic range of the assays. ROC analysis was used for clinical test evaluation; results are expressed as areas under the individual ROC curves, including 95% confidence intervals. Significance was set at $P$ values <0.05. Measured concentrations of cardiac markers were classified as positive in the case of results above or equal to the chosen threshold value and as negative for all samples values below threshold.

Results
ANALYTICAL PERFORMANCE OF cTnI TEST SYSTEMS
Precision. Interassay imprecision (CV) for the Stratus CS was 4.5% at 0.1 $\mu$g/L, 4.2% at 0.25 $\mu$g/L, and 6.5% at 0.82 $\mu$g/L cTnI (n = 60 assays over 30 days). For the Stratus II, within-run imprecision was 14% at 0.8 $\mu$g/L, 9.2% at 1.5 $\mu$g/L, and 7.4% at 2.5 $\mu$g/L cTnI. During the 30-day period, there were no changes in mean values.

The lowest cTnI concentration as measured with acceptable precision (CV ≤20%), was 0.8 $\mu$g/L for the Stratus II and 0.03 $\mu$g/L for the Stratus CS.

Detection limit. The detection limit (defined as the lowest cTnI concentration creating a test signal higher than the mean $+$ 2 SD of 20 replicates of the zero calibrator) for the Stratus II was 0.5 $\mu$g/L (one-third of the diagnostic threshold value) and for the Stratus CS was 0.01 $\mu$g/L (one-twentieth of the diagnostic threshold value).

ANALYTICAL EFFECT OF DELAYED cTnI DETERMINATION
Serial blood samples were analyzed for cTnI with delays measuring up to 12 h after blood collection. For each of five patients providing different concentrations of cTnI (Stratus CS, 0.05–0.5 $\mu$g/L), a total of seven plasma samples anticoagulated with Li-heparin plasma were collected. Determination of cTnI in duplicate was performed at each time point (1, 2, 3, 4, 6, 8, and 12 h), with samples maintained at room temperature. No differences were observed for delays of up to 2 h, whereas significantly lower values were obtained after 8 h. At 12 h, the measured cTnI values were ~50% of the original cTnI values for samples with ≥0.1 $\mu$g/L cTnI (Fig. 1). For samples with cTnI values <0.1 $\mu$g/L, after 6 h, the cTnI concentration was below the detection limit.

STRATUS CS—CORRELATION WITH DIFFERENT ANALYTICAL MATERIALS
As the clinically most important range, increasing concentrations of cTnI between 0.05 and 5 $\mu$g/L (Stratus CS) in plasma samples with different anticoagulants were compared. Linear regression analysis (including 95% confidence intervals for slope and intercept) indicated no
To calculate a diagnostic threshold value that would distinguish those patients with myocardial injury from those without, we drew fresh plasma samples from 161 patients with non-cardiac chest pain. Additionally, thawed plasma samples collected from 218 patients meeting an identical inclusion criterion were also analyzed. The cTnI concentration obtained with the Stratus CS (0.024 ± 0.029 μg/L) was significantly lower than the value obtained with the Stratus II (0.51 ± 0.34 μg/L; P = 0.002; Fig. 5). Accordingly, for clinical decision-making regarding patients with acute chest pain, cTnI values ≥0.08 μg/L (mean ± 2 SD) as analyzed by the Stratus CS were set as positive.

**Clinical Evaluation in Patients with Acute Chest Pain**

Of 412 consecutive patients presenting with acute chest pain, 15.1% had AMI (as based on a typical increase of CK enzyme activity within 24 h after arrival), 29.3% had unstable angina, and 9.0% had stable angina. In 1.0% of the patients (n = 4), pulmonary embolism was diagnosed, in 2.2% acute heart failure (n = 9) was diagnosed, and in 0.7% myocarditis (n = 3) was diagnosed. No evidence for CHD was found in 39.1% of the patients.

In 30.6% of these patients with chest pain, at least one blood sample was positive with the Stratus CS (≥0.08 μg/L). In 24.8% of the patients, cTnI was above 0.7 μg/L for the Stratus II. Initial testing on admission identified only 65% and 60%, respectively, of the positive cTnI test results. CK-MB mass (as analyzed by the Stratus II) was increased in 13.6% of the patients on arrival and in 19.2% of the patients 4 h later. In 14.6% of the patients on arrival...
and in 19.4% of the patients after 4 h, myoglobin was above the upper limit of normal.

**Diagnostic Sensitivity for the Detection of AMI**

The ROC curves for cTnI, as analyzed by both the Stratus CS and the Stratus II, are shown in Fig. 6. Analysis was based on all of the 412 patients with chest pain for $<12$ h. For cTnI test results at the time of arrival as analyzed by the Stratus CS, the area under the curve (0.859) was significantly greater than that of cTnI on the Stratus II (0.813; $P < 0.001$), CK-MB mass (0.726; $P < 0.001$), and myoglobin (0.771; $P < 0.001$). At a diagnostic threshold value of 0.15 $\mu$g/L, 63% of the patients with myocardial infarction (39 of 62) were identified at the time of arrival ($3.2 \pm 2.9$ h after onset of symptoms regarding AMI patients). Taking into account those patients with unstable angina and “false positive” findings of increased cTnI, the corresponding clinical specificity was 92.6%. With the sample collected 4 h after a patient’s arrival, cTnI was above this diagnostic threshold in 98% of the patients (and specificity was 89.5%). Only one patient had a cTnI concentration below 0.15 $\mu$g/L. The measured cTnI concentration of 0.10 $\mu$g/L, however, was already above the calculated threshold value of 0.08 $\mu$g/L for the detection of minor myocardial injury. For the Stratus II, 48% of patients on arrival and 85% of patients within 4 h after arrival had cTnI concentrations above the established diagnostic threshold value of 1.5 $\mu$g/L, with corresponding specificities of 95.2% and 93.3%, respectively. For a threshold value of $\geq 0.7$ $\mu$g/L, the diagnostic sensitivity increased to 52% and 90%, respectively, (not significant...
for both) and specificity decreased to 93.6% and 91.5%, respectively (not significant for both).

CK-MB mass and myoglobin were positive on arrival in 48% and 65% of the patients, respectively. For the second sample, CK-MB mass and myoglobin were increased in 90% and 97% of the patients, respectively. However, two patients with a negative test for myoglobin in the second blood collection had been positive for myoglobin in the first blood sample, yielding a 100% cumulative sensitivity.

MINOR MYOCARDIAL INJURY
Among 121 patients with unstable angina, the measured cTnI concentrations were $1.18 \pm 1.34 \mu g/L$ (range, 0.01–14.18 μg/L) for the Stratus CS, $1.4 \pm 3.32 \mu g/L$ (0.4–16.5 μg/L) for the Stratus II, $62.2 \pm 49.5 \mu g/L$ (0.01–1.03 μg/L) for myoglobin, and $2.21 \pm 3.75 \mu g/L$ (0.3–9.12 μg/L) for CK-MB mass. We used the mean cTnI value + 2 SD of a healthy population as the threshold value for minor myocardial injury. Accordingly, cTnI was $\approx 0.08 \mu g/L$ for 45% of the patients with the Stratus CS; cTnI was $\approx 0.7 \mu g/L$ for 28% with the Stratus II. CK-MB mass was increased in only 8.3% of patients; myoglobin was increased in 5.0% of the patients with unstable angina. Furthermore, serial blood samples during an extended period of 24 h were collected for two particular patients suffering from angina pectoris at rest and arriving within 2 h after onset of symptoms. The measured concentrations of cTnI and CK-MB mass for these two patients are depicted in Fig. 7. Serial measurements of CK-MB enzyme activity remained within the reference interval, ruling out non-Q-wave myocardial infarction in these patients.

During the 30-day follow-up period, death or nonfatal AMI was documented in 25.9% of the patients with unstable angina and a positive cTnI test result within 4 h after arrival (as analyzed by Stratus CS); with the Stratus II, cardiac events were observed in 29.4% of the patients. In contrast, for patients with all negative cTnI test results, only 1.5% (n = 1) sustained a major cardiac event during the 30-day follow-up period, whereas for 5.8% of the cTnI-negative patients (n = 5) as analyzed by the Stratus II suffered death or myocardial infarction. The one patient with a negative cTnI test (as analyzed by the Stratus CS) had an AMI on the 27th day after presentation with chest pain in the emergency room.

Other patients with a positive cTnI test result had pulmonary embolism (Stratus CS, n = 1; Stratus II, n = 1), congestive heart failure (n = 2; n = 0), suspected myocarditis (n = 2; n = 1), or unexplained increases in cTnI (n = 1; n = 1).

In 11 patients, CK-MB mass was increased without cTnI release (as analyzed by both cTnI test systems); in 14 patients, myoglobin was increased without detectable cTnI. There was no evidence in any of these patients of CHD, nonischemic myocardial injury, or cardiac event (death, AMI, revascularization) during the 30-day follow-up period.

Discussion

ANALYTICAL PERFORMANCE
In this study, the novel Stratus CS test system had excellent analytical characteristics: high precision, a low detection limit, and a low limit of quantification with acceptable precision. Because identical antibodies and calibration are used in both the Stratus II and the Stratus CS, a high correlation between the test results was observed for higher cTnI values ($\approx 4.0 \mu g/L$). Furthermore, this study documents that in terms of lower cTnI concentrations (<4.0 μg/L), the Stratus CS presents major advantages in analytical performance. The CV for the lowest internal control (0.1 μg/L) was 4.5% as analyzed by the Stratus CS but >10% as analyzed by the Stratus II. The detection limit for the Stratus II was 0.5 μg/L compared with 0.01 μg/L for the Stratus CS. These striking differences might be related to the improved ELISA design of
the Stratus CS, which uses dendrimer technology. In spite of using antibodies identical to those used by the Stratus II, the Stratus CS produces an enhanced test signal and a consistently lower background signal (25). Accordingly, for lower cTnI concentrations (<4.0 µg/L), the correlation between the Stratus II and the Stratus CS was poor ($r^2 = 0.62$). It should be emphasized that the observed differences in analytical sensitivity between the Stratus CS and the Stratus II are presumably related less to the instruments themselves than to improved ELISA technology. Other assays for cTnI capable of such improved sensitivity might provide comparable results.

The clinical evaluation of the Stratus CS in patients presenting with acute chest pain at the emergency room confirmed its higher sensitivity for the detection of myocardial infarction. A diagnostic threshold of 0.15 µg/L for the Stratus CS was calculated on the basis of 412 patients with chest pain. With the threshold set on this value, diagnostic sensitivity for the detection of AMI was superior to that of cTnI determination and CK-MB mass measurements, both analyzed by the Stratus II. In our study population, the diagnostic sensitivity was comparable to that of myoglobin. However, it should be noted that patients with AMI arrived in the emergency room 3.2 ± 2.9 h after onset of chest pain. Only 18% of the patients arrived within 3 h after onset of symptoms. A higher rate of early arriving patients with AMI might have produced significant differences between the two markers, myoglobin and cTnI. Four hours after arrival, for 98% of the patients with a final diagnosis of myocardial infarction, cTnI values were above the threshold value of 0.15 µg/L as analyzed by the Stratus CS. For myoglobin, at least one positive test result was obtained in these patients during this observation period of 4 h.

Only 8% of patients with unstable angina had increased CK-MB concentrations according to the predefined threshold value of 5 µg/L; in 5% of these patients, myoglobin was increased. By using a threshold value of 0.08 µg/L for cTnI (as analyzed by the Stratus CS) as based on the mean cTnI value + 2 SD of a healthy population, we found increased cTnI in 45% of patients with unstable angina; for the Stratus II, in 28% of the patients cTnI was ≥0.7 µg/L. This higher rate of positive tests for the Stratus CS was connected with a higher negative predictive value during the 30-day follow-up period (98.5% vs 94.2%; $P < 0.01$). No cardiac events occurred during the initial 3 weeks after presentation in these patients with <0.08 µg/L cTnI release. It is important to emphasize that the evaluation of different threshold values was performed in patients with non-cardiac chest pain. Several previous studies have shown that the troponin values in these patients are higher than those in healthy volunteers. With respect to the excellent sensitivity of the Stratus CS, it should be noted that all analyses were performed with fresh blood samples immediately after sample collection, which might have increased sensitivity for the detection of minor increases of troponin I.

### POINT-OF-CARE TESTING

Troponins can be determined both by qualitative rapid bedside tests and by quantitative ELISA test systems. Off-site quantitative determination of troponins has the disadvantages of delayed availability of test results and higher fixed costs. Therefore, rapid test systems have been developed based on monoclonal antibodies and chromatographic immunologic solid-phase technology (11, 26). These bedside tests can be performed at the point of care by medical or paramedical staff without additional equipment. The tests provide qualitative test results (positive/ negative) within 15–20 min without time-consuming preparations such as clotting and centrifugation. Rapid testing for troponins has been shown to provide high prognostic value (15).

However, recent bedside tests pose limitations for cardiac marker determination: qualitative rapid testing for troponins remains subject to reading errors, with substantial interobserver variability. The utilization of rapid tests requires considerable familiarity with training samples. The rapid tests must be read within an adequate time interval and under adequate illumination (11, 26). To overcome these disadvantages, quantitative whole blood test systems for point-of-care testing have been introduced. Recently, a quantitative whole blood system for cTnI, CK-MB mass, and myoglobin was shown to provide sensitivity and specificity for the diagnosis of AMI comparable to established quantitative test systems (31). Determination of cTnI provided highest diagnostic efficacy, and neither serial nor parallel analysis of the three markers increased sensitivity or specificity for the detection of myocardial infarction. In the current study, we were able to show similarly that the new Stratus CS (including a centrifugation step for whole blood samples) is easy to handle and provides reliable cTnI test results while preserving the advantages of a rapid test system. For both qualitative rapid tests and the Stratus CS, test results are available within 15 min after blood collection. With respect to reliability and maintenance of a test system, it might be preferable on a long-term basis to perform cTnI measurements in the laboratory with a large-scale analyzer that provides cost-effective analysis of multiple analytes. Although point-of-care testing with the Stratus CS was uncomplicated during the study period of 5 months, the long-term usage of point-of-care test systems remains controversial.

### References


