CHAPTER 6

Evaluation of the Stratus Cardiac Status and the Triage Cardiac Panel point- of- care testing devices for performing troponin I, CKMB-mass and myoglobin measurements.

Joost CJM Swaanenburg[1], Stan AJ van den Broek[2], Harry de Wit[1].

[1] Department of Pathology and Laboratory Medicine,
[2] Department of Cardiology, University Hospital Groningen.

Introduction

For many years the cardiac enzyme CKMB has been the gold standard for the detection of myocardial injury. However, CKMB is not heart-specific. As cardiac troponin I and cardiac troponin T are reported to be fully heart-specific (1,2), these parameters are more suited to diagnose myocardial necrosis. Moreover, these parameters are recently recommended by the National Academy of Clinical Biochemistry (NACB) (3) to be the new (biochemical) standard for the diagnosis of acute myocardial infarction (AMI) and thus replacing CKMB as one of the three WHO-criteria (4). Furthermore, the NACB recommended that two decision limits are needed for the optimum use of sensitive and specific cardiac markers such as cardiac troponin I and cardiac troponin T: a low abnormal value establishes the first presence of true myocardial injury, and a higher value is suggestive of injury to the extent that it qualifies as AMI. An other issue of which the NACB did some recommendations is the turn around times (TAT's) of laboratory testing of cardiac markers. It was recommended that results have to be reported within one hour after the collection of blood. If this requirement cannot be fulfilled consistently, point of care (POC)-testing should be considered.

Recently, it became possible to measure troponin I, CKMB-mass and myoglobin quantitatively as POC-test. The Stratus Cardiac Status analyser and the Triage Cardiac Panel are examples of these quantitative POC-testing devices.

The aim of this study was to investigate the performances of the Stratus CS™ analyser and the Triage CP™ in relation to the established central laboratory AxSYM™ analyser (5). We evaluated the correlations between the point of care testing devices and the central laboratory equipment for the cardiac markers cardiac troponin I, CKMB mass, and myoglobin. Furthermore, the analytical imprecisions of the POC-analysers were determined.

Patients and methods

Patients, who were admitted to the emergency department with complaints of acute chest pain, were included during a six weeks period for the evaluation of the POC testing devices.

After collection, blood was centrifuged at 1000x g and serum was separated from the cells in order to measure the cardiac troponin I, CKMB mass and myoglobin on the AxSYM analyser (Abbott Diagnostic Division, Hoofddorp, The Netherlands). The established cut off value of cardiac troponin I for acute myocardial infarction (AMI) has been reported to be 2.0 µg/l (5). At the same time Li-heparin anticoagulated blood was collected to measure all three biochemical parameters on the Stratus CS analyser (Dade-Behring, Leusden, The Netherlands) and the Triage CP (Biosite Diagnostics, manufactured by Merck, Amsterdam, The Netherlands).

The Stratus Cardiac Status is a fluorometric enzyme immunoassay based analyser for quantitative determination of cardiac troponin I, CKMB mass and myoglobin. Although preprocessed plasma specimens can also be measured, the system has been designed to analyse closed sample tubes containing Li-heparin anticoagulated whole blood. In order to separate plasma from the cells, a centrifugation step has been incorporated into the test system. Furthermore, a bar-coded test pack must be placed into the analyser for each particular test to be performed. Up to four test packs can be placed into the analyser at the same time. All required reagents have been enclosed within the test

pack. The test system utilises radial partition immunoassay technology which has been improved by the use of monoclonal capture antibody coupled to Starburst^{R} dendrimers (6). 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. Subsequently, centrifuged plasma is 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 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. The whole analytical procedure takes 14 minutes for one parameter analysis and 16 minutes for determining all three parameters of the same sample. The manufacturer recommended via a packet insert a cut off value for AMI of 1.5 µg/l (7).
The Triage Cardiac Panel is a self calibrating fluorescence immunoassay system for the quantitative determination of cardiac troponin I, CKMB mass, and myoglobin in heparin-treated whole blood and plasma specimens. After addition of the sample to the sample port, the cells are separated from the plasma via a filter which has been incorporated in the device. A predetermined quantity of plasma is allowed to react with fluorescent antibody conjugates within the reaction chamber. After incubation, the reaction mixture flows down the device detection lane. Complexes of the analytes and fluorescent antibody conjugates are captured on discrete zones, producing binding assays that are specific for each analyte. The concentration of each measured analyte is directly proportional to the detected fluorescence. Each testing device measures simultaneously cardiac troponin I, CKMB mass, and myoglobin. All results are available in 15 minutes. The cut off value for AMI is recommended by the manufacturer to be 1.0 µg/l (8).
The correlations between the cardiac marker test results of the Stratus CS, the Triage CP and the AxSYM were performed using Passing and Bablok regression analysis. The between day imprecision was determined using samples with different concentrations for all three analytes.

Results

Evaluation Stratus CS. One-hundred-thirty-seven patients (91 men, mean age 56.5 (standard deviation (sd) 11.6) y; 46 women mean age 66.2 (sd 11.8) y) were included for the analytical evaluation. From these patients 197 measurements were performed. The correlations between cardiac troponin I, CKMB mass, and myoglobin measurements performed on the Stratus CS and the AxSYM analysers are shown in figure 1. From this figure it can be concluded that for both CKMB mass and myoglobin there is a reliable correlation (slope is 0.73 (confidence interval (CI) 0.67-0.77), intercept is -0.25 (CI -0.34- -0.10) and coefficient of correlation (r) is 0.993 for CKMB mass, and for myoglobin slope is 0.68 (CI 0.63-0.74), intercept is 7.3 (CI 4.6-10.2) and r is 0.988 respectively), whereas there is a moderate correlation for cardiac troponin I (slope is 0.25 (0.23-0.28), intercept is 0.01 (CI 0.01-0.02), and r is 0.943). Furthermore, figure 2 shows that the concentration 2.0 µg/l for the AxSYM (the established cut off value for AMI) corresponds with a concentration 0.5 µg/l for the Stratus CS. In table 1A the frequency distribution is shown of the combinations whether or not the corresponding patient results of the Stratus CS and AxSYM are below or beyond various cut off values. In this table also the efficiencies are shown which have been

calculated from these data. The efficiency for the combination troponin I cut off value 1.5 µg/l (Stratus CS, recommended by the manufacturer) and 2.0 µg/l (AxSYM) is 87.3%, whereas the efficiency is 95.9%, if for the Stratus CS 0.5 µg/l is used as cut off value. In our hospital a concentration 0.5 µg/l for the AxSYM is used as low cut off value for the detection of minimal myocardial injury. This corresponds to a concentration 0.15 µg/l for the Stratus CS (resulting in an efficiency of 92.9%, see table 1A).

The between day imprecision was tested by measuring three samples with different concentrations during forteen consecutive days. The troponin I concentrations of these samples were 0.61 µg/l, 8.3 µg/l, and 16.2 µg/l respectively and the corresponding coefficients of variation 4.5%, 3.5% and 2.7% respectively. For CKMB mass the between day imprecisions were 3.2% for the low concentration (3.6 µg/l), 1.7% for the elevated concentration (18.1 µg/l) and 4.4% for the high concentration (53.0 µg/l). Myoglobin showed imprecisions of 2.6% for the low concentration (46 µg/l), 1.2% for the medium concentration (228 µg/l), and 3.0% for the high concentration (477 µg/l).

Evaluation Triage CP. For the correlation between the AxSYM and the Triage CP seventy-eight patients were included. From these patients 112 measurements were performed. The correlations between cardiac troponin I, CKMB mass, and myoglobin measurements performed on the Triage CP and AxSYM analysers are also shown in figure 1. From this figure it can be concluded that there is a reliable correlation for CKMB mass (slope is 1.01 (CI 0.84-1.05), intercept is -0.21 (CI -0.43- -0.08), and r is 0.979), whereas there are moderate correlations for cardiac troponin I (slope is 0.15 (CI 0.12-0.21), intercept is 0.0, r is 0.922) and myoglobin (slope is 1.31 (CI 1.13-1.59), intercept is 16.8 (CI 5.3-24.6), and r is 0.877 respectively). From figure 2 it can be deducted that the troponin I concentration of 2.0 µg/l for the AxSYM analyser corresponds with a concentration of 0.4 µg/l for the Triage. In table 1B the frequency

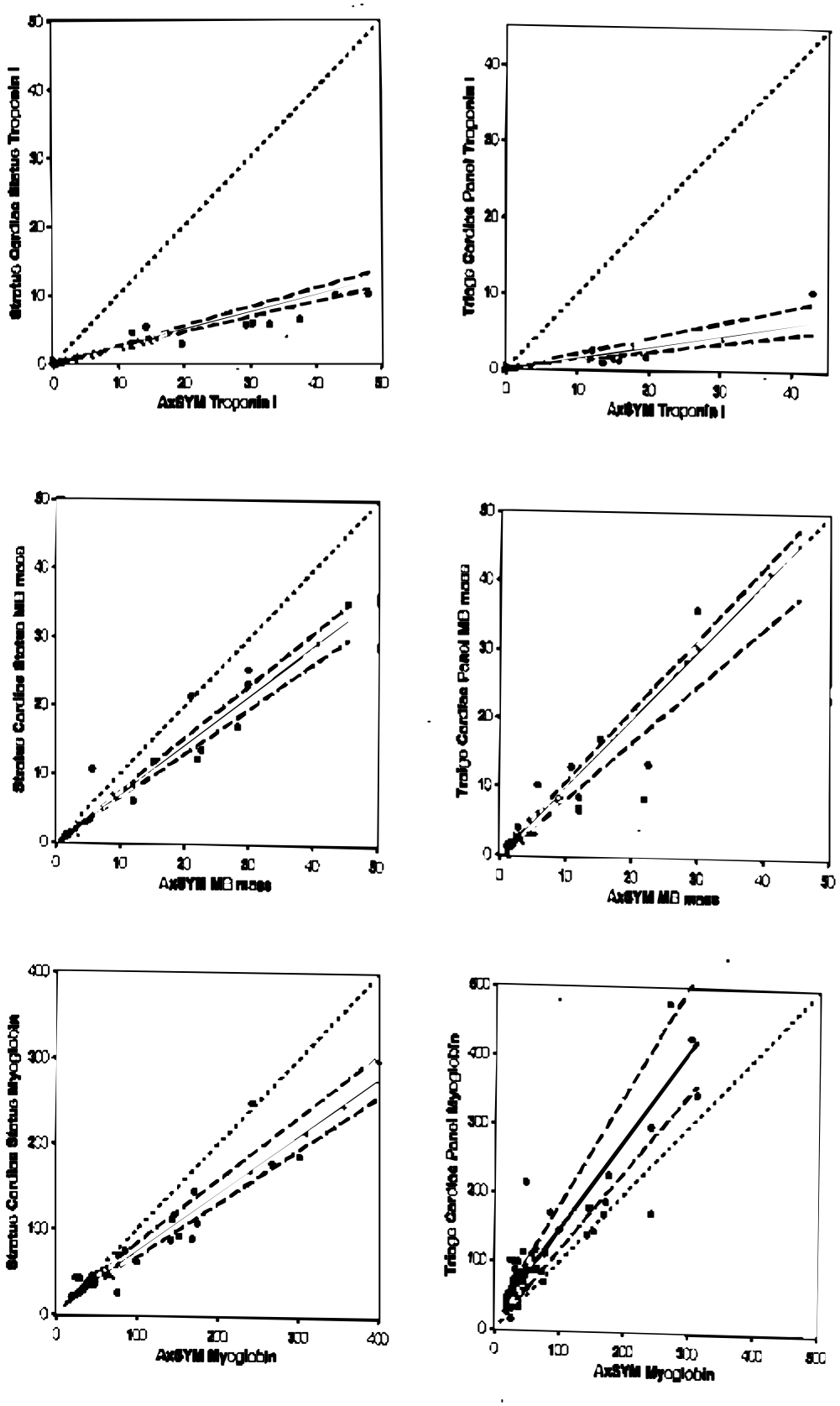

Figure 1. Regression analysis performed according to Passing and Bablok is shown between the AxSYM and Stratus CS and between the AxSYM and the Triage CP for the cardiac markers troponin I, CKMB mass and myoglobin (all units µg/L).

Table 1. Two by two tables for various cut off values of cardiac troponin I depicting frequencies of non-elevated and elevated values of the AxSYM versus the Stratus CS (A) and versus the Triage CP (B) analysers.

A

		AxSYM	
		# 2.0	> 2.0
Stratus CS	# 1.5	149	24
	> 1.5	1	23

Efficiency 87.31%

		AxSYM	
		# 2.0	> 2.0
	# 0.5	146	4
	> 0.5	4	43

Efficiency 95.94%

		AxSYM	
		# 0.5	> 0.5
	# 0.15	129	7
	>0.15	7	54

Efficiency 92.89%

B

		AxSYM	
		# 2.0	> 2.0
Triage	# 1.0	88	12
	> 1.0	0	8

Efficiency 88.89%

		AxSYM	
		# 2.0	> 2.0
	# 0.4	85	2
	> 0.4	3	18

Efficiency 95.37%

		AxSYM	
		# 0.5	> 0.5
	# 0.2	76	5
	> 0.2	3	24

Efficiency 92.59%

Efficiency: (frequency of both non-elevated + both elevated values) / total frequency.

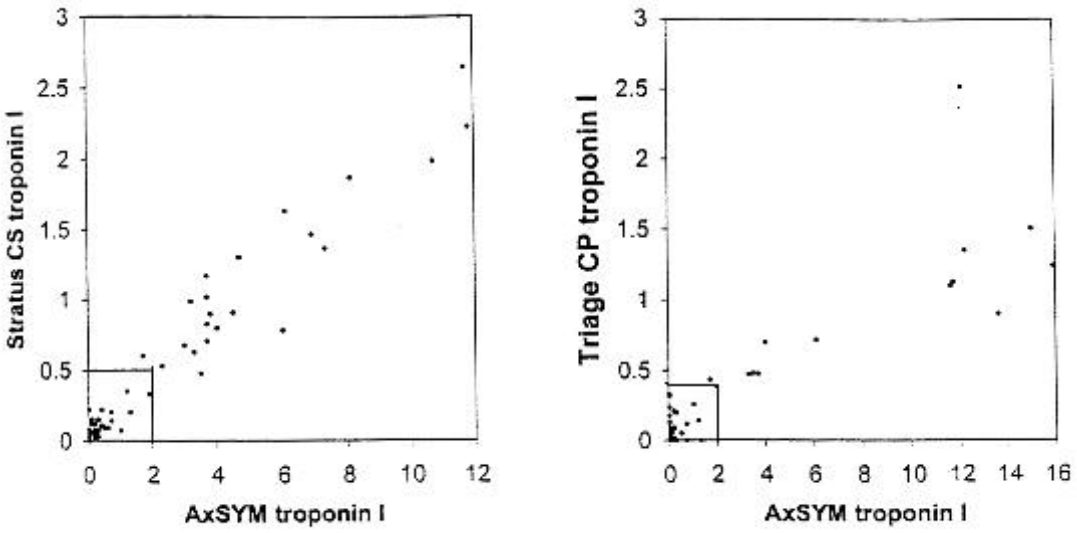

*Figure 2.*The correlation between the AxSYM and the Stratus CS, and between the AxSYM and Triage CP in the low concentration area. The cut off values for AMI of the Stratus CS and the Triage CP are related to the established AxSYM cut off value 2.0 µg/L.

distribution is shown of the combinations whether or not the corresponding patient results of the Triage CP and AxSYM are below or beyond various cut off values. From this table it can be seen that the efficiency for the cut off values 2.0 µg/l (AxSYM) and 0.4 µg/l (Triage) is 95.4%, whereas this is 88.9%, if for the Triage the manufacturer's recommended cut off value of 1.0 µg/l is used. To a low cut off value 0.5 µg/l for the AxSYM analyser corresponds a value 0.2 µg/l for the Triage CP. This combination of Triage CP and AxSYM low cut off values results in an efficiency of 92.6% (see table 1B). The imprecision of the Triage CP was between 3 - 6% for the three analytes at low, moderate and high concentration levels, respectively.

Discussion

In this study we investigated the performance of two recently introduced POC testing devices. We found that for CKMB mass and myoglobin measured with a Stratus CS analyser there is an acceptable correlation with the established AxSYM analyser. However, the results of the Stratus CS are 25% lower than those of the AxSYM. Furthermore, cardiac troponin I showed a moderate correlation because of the lack of a world wide accepted cardiac troponin I standardisation. The comparison between the Triage CP and the AxSYM analysers showed less acceptable correlations. Especially, the myoglobin correlation data showed more discrepancy between the Triage CP and AxSYM than between Stratus CS and AxSYM. In contrast to the comparison between the Stratus CS and the AxSYM analysers, we found no systematic differences between the Triage CP and the AxSYM.

The recommended cut off value for AMI of the Stratus CS manufacturer (1.5 µg/l) is too high, as we found a lower value (0.5 µg/l), which correlates better to the AxSYM (fig 2) resulting also in a better efficiency (87.3% versus 95.9%). Moreover, for those patients for who the troponin I AxSYM

values were elevated in contrast to the troponin I Stratus CS values, the clinical history was more in agreement with the elevated troponin I AxSYM values. Moreover, subsequent cardiac troponin I measurements of these patients resulted in elevated concentrations for both methodologies. The same applies for the Triage CP: the cut off value for AMI recommended by the Triage CP manufacturer (1.0 µg/l) appeared to be too high compared with the AxSYM. A cut off value 0.4 µg/l is more in agreement with the established AxSYM value of 2.0 µg/l. This is also illustrated by the better efficiency (88.9% compared with 95.4% respectively). Evenso, the clinical histories of the patients with elevated troponin I AxSYM values and non-elevated troponin I Triage CP values were more according to the elevated troponin I AxSYM values.

For the low cut off values for the detection of minor myocardial injury we found for the Stratus CS analyser 0.15 µg/l, whereas this was 0.2 µg/l for the Triage CP. The lower cut off values which we report for both POC testing devices are in agreement with reported findings from other investigators (7,8).

Since it has been reported that elevated levels of cardiac troponin I and cardiac troponin T are prognostic factors for patients with acute chest pain complaints, the turn around time for these determinations are recommended to be within one hour. If the central laboratory cannot fulfill this requirement, POC testing must be considered (3). For several years it has been possible to measure the cardiac troponin I or cardiac troponin T contents in blood as POC-test using 'strip'-technology (9,10). These 'strip'- testing devices require to put 150-200 µl whole blood on a strip containing a capture-antibody to bind the troponin and a label-antibody to measure the intensity of the capture bound troponin. The results of these methodologies are qualitative, because test results are only reported as 'negative'or 'positive' (10). However, for reliable trend analysis quantitative results are required. Recently, a measuring device has been developed to quantitate the troponin T results of the strip-test technology (11). To measure quantitatively troponin I as POC test both evaluated testing devices seem to be useful alternatives, as the way of performing of these POC testing devices is such simple that non analytically educated personnel can reliably perform these tests.

After the analytical evaluation period the Stratus CS analyser was located at the Coronary Care Unit (CCU). During this period it turned out that this testing device is suitable for decentralised testing in daily clinical practice. Indeed, we found an excellent performance regarding the convenience and reliability when the analyses were carried out by non analytically educated personnel. These people slightly prefer the Stratus CS, because of the closed tube system. Because of this aspect, there is no risk for contamination with blood of the patient. Furthermore, in contrast to the Triage CP, where always all three parameters are simultaneously measured, it is possible to choose with the Stratus CS which parameter has to be determined. In favour of the Triage CP are the low costs of investment and the compactness of the device allowing easy reallocation near by the individual patient.

From this study we conclude that the Stratus CS analyser shows good correlations with the AxSYM analyser for CKMB mass and myoglobin, whereas the correlation for cardiac troponin I is moderate. The cardiac troponin I low cut off value for the detection of minor myocardial injury appears to be 0.15 µg/l, whereas the cut off value for AMI is 0.5 µg/l. The Triage Cardiac Panel shows a good correlation for CKMB mass, whereas the correlation data for cardiac troponin I and myoglobin are moderate. The cardiac troponin I low cut off value appears to be 0.2 µg/l, and the cut off value for AMI 0.4 µg/l. Furthermore, both POC testing devices are suited for point of care testing performed by non-analytically educated personnel.

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