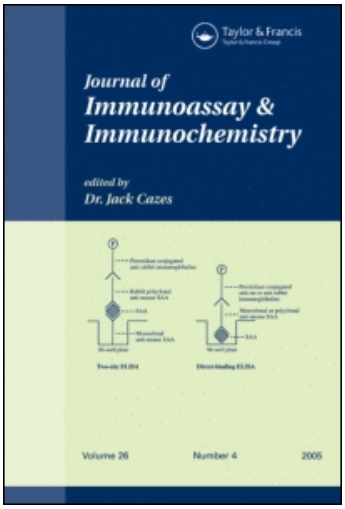

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EVALUATION OF A NEW TROPONIN I METHOD ON THE BAYER IMMUNO 1™ IMMUNOASSAY ANALYSER

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ABSTRACT

We have evaluated the analytical and clinical performance of an automated immunoassay for serum cardiac troponin I (Bayer Immuno 1™, Bayer Diagnostics, Tarrytown, NY). The between batch imprecision was found to be between 1.2 and 3.2% over the concentration range 2.5 – 34.0 μg/L. The analytical range obtained from duplicate analysis of patient samples and defined as a coefficient of variation of 10% or less was 0.3 – 200 μg/L. The detection limit was found to be less than 0.1 μg/L. A method comparison with the Dade Stratus method (Dade Behring, Wilmington, DE) yielded regression statistics with a slope of 0.705 and an intercept of –0.260. An analysis of samples from 40 patients with renal failure demonstrated six with detectable levels of troponin I (0.2 – 1.9 μg/L). Samples from patients with paraproteinaemia did not demonstrate detectable troponin I (from n = 30); however, two patients with elevated rheumatoid factor titers (from n = 20) demonstrated a detectable amount of troponin I (0.1 and 0.2 μg/L). In a study of 100 patients admitted with acute chest pain and a diagnosis of unstable angina, 6 were subsequently diagnosed as

having suffered a myocardial infarction. On admission the sensitivity and specificity of the troponin I results were 26.7% and 94.7%, respectively, moving to 100% and 83% 12 hours after admission.

Key words: troponins; immunoassay; myocardial infarction; clinical cut off values.

INTRODUCTION

Cardiac contractile proteins have been postulated as new markers of myocardial damage. Troponin I and troponin T exist in three different isoforms with unique structures, one for slow-twitch skeletal muscle, one for fast-twitch, and one for cardiac muscle (1). They are part of the troponin-tropomysin complex involved in the regulation of actin-myosin interactions in striated muscle. Cardiac troponin I is a 22.5 kDa polypeptide and it is the only TnI isotype present in the myocardium (2). Cardiac troponin I has an extra 30 amino acid residues at the N terminus and its amino acid sequence shows roughly 40% dissimilarity from the skeletal muscle isoforms (1).

The troponins are released from myocardium following myocardial injury (3). There appears to be two phases of release; initially from the cytosolic pool and then a more prolonged phase due to myofibrillar degradation (4, 5). A larger amount of troponin T (6-8%) than troponin I ($\approx$ 2.5%) is thought to be present in the cytosolic pool (6).

Cardiac troponin I is a sensitive marker for myocardial damage and shown to be an effective marker in the diagnosis of acute

myocardial infarction (7). The level of cardiac troponin I in the serum starts to increase 2 to 8 hours following onset of chest pain (1, 8); peak concentrations are seen about 12 hours after infarction depending on the occurrence of early reperfusion of the infarct-related coronary artery. The troponin I levels remain elevated for at least 4 days following acute myocardial infarction (1).

Increased troponin I concentrations are found in about 30% of patients with unstable angina (3). Several authors have shown that cardiac troponin I predicts a higher cardiac event rate during hospitalisation and in subsequent months and may, therefore, be used for risk stratification (6, 9-11). A recent meta analysis of eighteen publications indicated that troponin I and troponin T have a similar predictive value (12).

The presence of cardiac troponin I in the serum is highly specific for myocardial injury (1, 3). Assays show no cross-reactivity with skeletal troponin I (4, 6). Cardiac troponin I is not present in foetal skeletal muscle and is not present in skeletal muscle after severe injury (7). Troponin I can, therefore, be used as a specific marker of myocardial injury post-operatively (13), after traumatic injury and in patients with skeletal myopathies (2, 8, 14, 15).

Raised serum levels of troponin I and troponin T have been reported in chronic renal failure (16-18). Because troponin I is

recognised as a highly specific marker for cardiac tissue damage, speculation exists that the increased troponin I results seen in renal failure may indicate minor myocardial damage and that patients showing such increases are at a higher risk for adverse outcomes (19).

Troponin I exists in serum in different forms. Troponin I has two adjacent phosphoserine residues which are substrates for cAMP and cGMP dependent protein kinases (20). Troponin I may also be phosphorylated by protein kinase A and C (21). Phosphorylation decreases myofilament sensitivity to calcium (22) and changes the conformation of the troponin I molecule (23). Troponin C has been shown to inhibit the phosphorylation of troponin I (24). Troponin I also contains cysteine residues which may be oxidised. Oxidation of sulfhydyl groups of troponin I affects the interactions with other troponin components (25). Troponin I is released into the blood stream following myocardial infarction mainly as a complex with troponin C (24, 26). Wu et al (5) also found that troponin I was released into blood as a complex with troponin T and troponin C, and as a complex with troponin C alone, with only a small proportion of the protein being released in a free form. The proportion of free to complexed troponin I has been shown to change with time following myocardial infarction and to be different in different patients (24). All these factors contribute to the difference in cardiac troponin I results shown by different assays due to

heterogeneity in the cross-reactivities of antibodies to the various forms of troponin I.

Several different assays are now available for troponin I. We have evaluated a new troponin I method for the Bayer Immuno 1™ immunoassay analyser.

SUBJECTS AND METHODS

Blood samples were collected from patients admitted with acute chest pain and suspected of having suffered a myocardial infarction. Three independent studies were undertaken: i) samples were saved from 38 patients with clinical evidence of myocardial infarction where the specimens had initially been submitted to the laboratory for routine analysis of creatine kinase; ii) four serial samples were obtained from each of 10 patients who had suffered a myocardial infarction. Specimens were collected on admission and on the mornings of the following day, and on the mornings of day 2 and day 3. The timing of the second sample was within 12h of the first sample in most cases; iii) admission and a second sample on 100 consecutive patients admitted with non ST segment elevation acute coronary syndrome. Patients who subsequently developed Q waves were excluded. Patients who had a CK rise >400 I.U./L without Q wave development on the ECG were

diagnosed as non Q wave myocardial infarction, the remaining patients were diagnosed as unstable angina.

Specimens were also collected from 40 patients with renal failure, 30 patients with paraproteinaemia and 20 patients with a positive rheumatoid factor titer. All samples were stored at -20°C for up to two months before analysis.

The diagnosis of acute myocardial infarction was based on WHO criteria involving two out of three positives from presentation with crushing chest pain of greater than 30 minutes duration, typical ECG changes and increases in the level of serum creatine kinase. The diagnosis was made by a cardiologist without knowledge of the troponin I concentration; analysis of serum troponin I was made without knowledge of the final diagnosis.

The troponin I method on the Bayer Immuno 1 system (Bayer Corporation, Tarrytown, NY, USA) is an enzyme-labelled immunoassay. Reagent 1 contains a monoclonal antibody to troponin I labelled with fluorescein. Reagent 2 contains an affinity purified goat antibody to troponin I labelled with alkaline phosphatase. Sample is dispensed into a cuvette with a suspension of magnetic particles covalently coated with monoclonal antibodies to fluorescein. Reagent 1 and 2 are then added and the mixture is incubated for 13 minutes. The magnetic particles are washed and buffered p-nitrophenylphosphate added. Particle bound

alkaline phosphatase activity is measured by the rate of increase in absorbance at 405 nm. The rate is proportional to the troponin I concentration. Time to the first test result is 23 minutes. Successive results are produced at 30 s intervals. The detection limit of the assay was claimed to be 0.1 μg/L. The cut-off level quoted for myocardial infarction was quoted as 0.9 μg/L.

The comparison method used was the troponin I method on the Stratus II (Dade Behring, Wilmington DE, USA) immunoassay analyser described elsewhere (8). The detection limit of the assay was quoted as 0.35 μg/L and the cut-off level for myocardial infarction was quoted as 1.5 μg/L.

EXPERIMENTAL PROCEDURES AND RESULTS

Imprecision

The between run method imprecision was assessed by analysis of aliquots of three quality control materials (Bayer Corporation, Dade Behring) in 20 analytical runs. For the purposes of this part of the study the calibration curve was established according to the manufacturers instructions and all subsequent analyses read off against this stored calibration curve. The data are shown in Table 1. In addition, the calibrators were analysed as samples in each of the analytical runs.

TABLE 1

Between Run Imprecision of Bayer Immuno 1 Troponin I Assay

Mean troponin I (μg/L)	SD (μg/L)	CV (%)
2.5	0.08	3.2
6.5	0.11	1.8
34.0	0.51	1.2

The coefficient of variations were found to be 2.3%, 1.5%, 1.0% and 0.83% for the 5, 10, 20 and 60 μg/L calibrators, respectively.

Detection Limit

Twenty replicates of the zero calibrator gave a mean signal of 3.7×10^{-3} absorbance unit with a coefficient of variation of 7.3%. The signal equated to a troponin I concentration of less than 0.1 μg/L when the original calibration data was used to manually generate a calibration curve.

The functional sensitivity of the method was also assessed from the analysis of duplicates using patient samples. Using a cut off of 10% the lower limit of the analytical range was found to be 0.3 μg/L (see Figure 1).

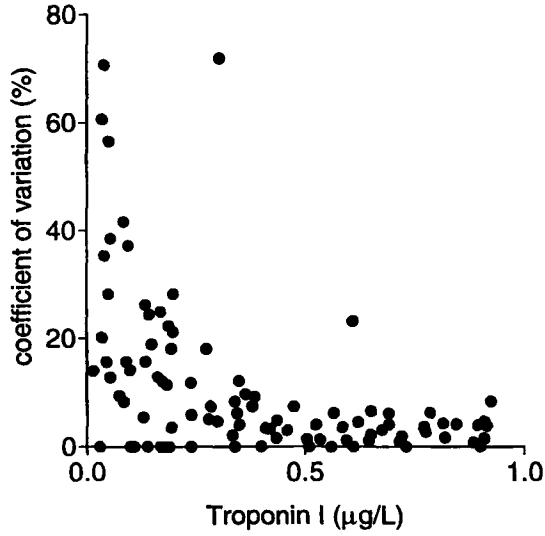

FIGURE 1 Precision profile obtained from duplicate analysis of patient samples for troponin I; data restricted to 0-1.0 µg/L range

Method Comparison

A total of 128 samples were analysed by the Bayer Immuno 1 System and Stratus methods; the data are shown in Figure 2. Regression analysis was performed according to the method of Passing and Bablock (27); this analysis yielded a slope of 0.705 (95% confidence intervals 0.667-0.760) and an intercept of –0.260 (95% confidence intervals –0.756 – 0.126) (n = 62; 66 samples yielded results of <0.1 µg/L).

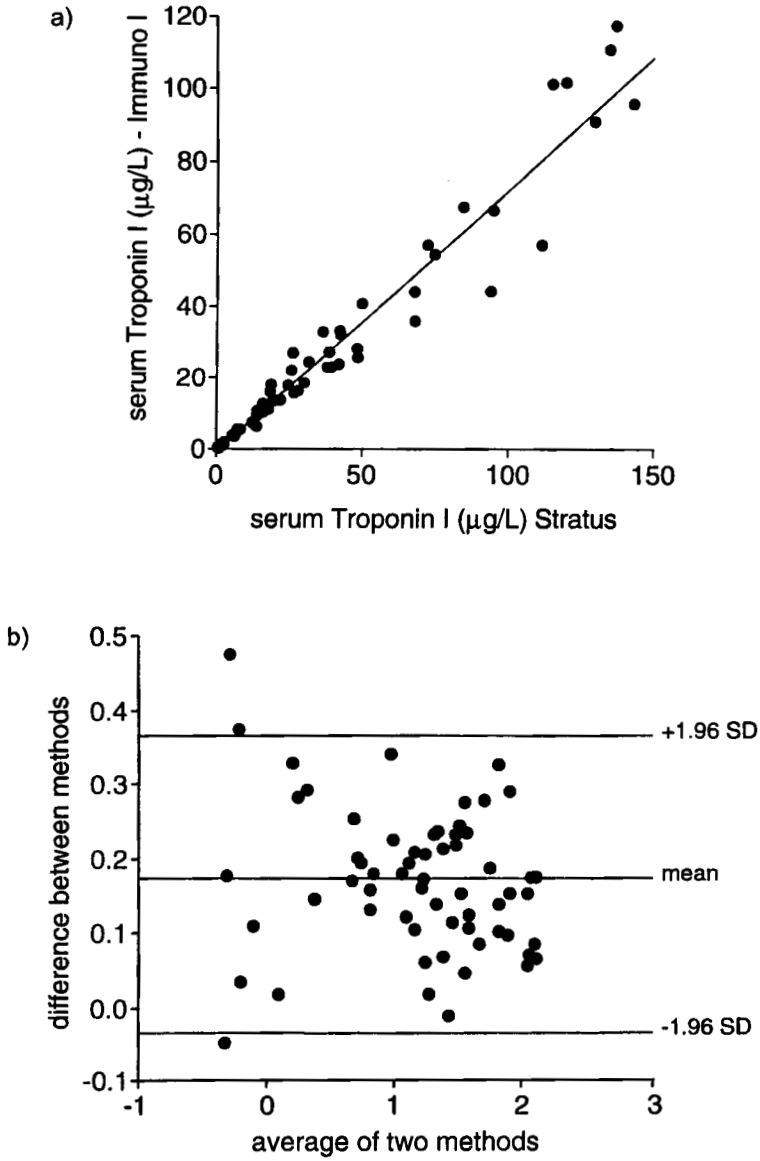

FIGURE 2 a) A direct comparison of results from serum cardiac troponin I by the Immuno 1 and Stratus methods
b) Altman and Bland plot

Serial Samples from Patients with Acute Myocardial Infarction

Eight of the admission samples from the 10 patients suffering an acute myocardial infarction had troponin I values of ≤ 0.1 μg/L; the samples collected on day 1 showed levels ranging from 13.5 to 225 μg/L. The distribution of troponin I results in the ten patients over the first four days after admission are shown in Figure 3.

Patients with Unstable Angina

Of the 100 patients admitted with acute chest pain and a diagnosis of unstable angina, 6 were subsequently diagnosed as having suffered a non Q wave myocardial infarction (prevalence 0.06). The range of troponin I results by the Immuno 1 method in this group was <0.1 – 3.20 μg/L (median 2.0 μg/L) on admission, and 1.80 – 47.18 μg/L (median 14.0 μg/L) at 12h. One of the 6 had a value above the cut off level quoted by the manufacturer of 0.9 μg/L on admission. One of the 6 had a value above the cut off level quoted by the manufacturer of 0.9 μg/L on admission. All 6 were above the cut off at 12h. In the 94 remaining patients the range of troponin I values was <0.1 – 10.3 μg/L with a median value of 1.8 μg/L at 12h. Of the 94 patients who had not had a myocardial infarction, a total of 69 patients had troponin I values of <0.1 μg/L, 9 patients had values of <0.9 μg/L and 16 patients had

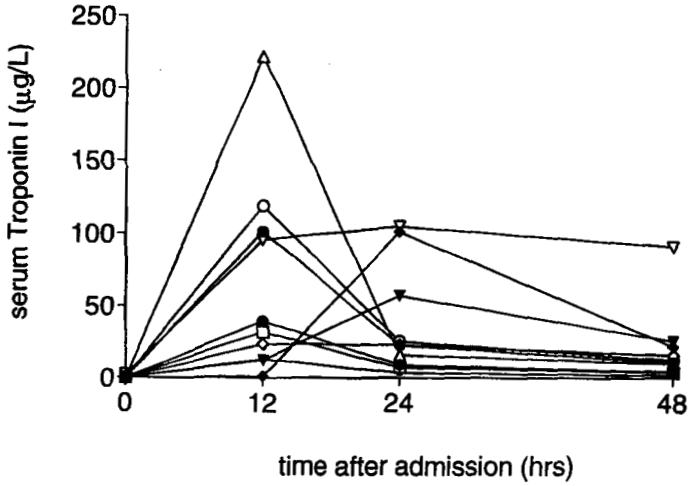

FIGURE 3 Distribution of troponin I results in 10 patients following myocardial infarction

values between 0.9 and 6.0 µg/L at 12h. The diagnostic performance is summarised in Table 2.

Patients with Chronic Renal Failure

Of the 40 patients studied 6 had detectable levels of troponin I (0.2 – 1.9 µg/L on the Bayer Immuno 1 system and 0.3 – 1.9 µg/L on the Stratus). In addition, 3 patients had detectable levels of troponin I by the Bayer Immuno 1 method alone (0.2 – 0.4 µg/L). The serum creatinine values in these patients ranged between 250 and 1200 µmol/L.

TABLE 2

Summary of diagnostic performance of troponin I assay for 100 patients admitted with acute chest pain and a working diagnosis of unstable angina using the cut-off value of 0.9 μg/L

	Time 0h.	**Time 12h.**
true positive	1	6
false positive	5	16
true negative	89	78
false negative	5	0
sensitivity (%)	16.7	100
specificity (%)	94.7	83
positive predictive value (%)	16.7	27.0
negative predictive value (%)	94.7	100
diagnostic efficiency (%)	90.0	84.0

Potential Protein Interferents

All of the samples containing a paraprotein had troponin I levels below the detection limit. In two of the samples containing rheumatoid factor a detectable response was observed (0.1 – 0.2 μg/L); in the case of the Stratus method only one of the samples gave a detectable response (1.4 μg/L).

DISCUSSION

The Bayer Immuno 1 system demonstrated excellent imprecision over the period of this study, the reproducibility was also reflected in the

low detection limit. This level of imprecision is superior to that reported for most of the troponin I methods available and has important implications for the use of this method in the early reliable detection of patients with acute myocardial infarction and in the risk stratification of patients with chest pain. Thus the imprecision at the quoted cut off level for myocardial infarction is less than 5% according to the precision profile and less than 3% at three times that value. Comparable data for other methods has been reported as follows: 2.4 – 5.1% for the ACCESS (19), 4.4 – 9.8% for the Stratus (8), 5.6 - 13.0% for the OPUS (28), 6.9 – 8.7% for the Axsym (29) and 4.2 – 5.5% for the ACS 180 (30) all over comparable concentration ranges.

Comparison of the numerical results between the Bayer Immuno1 system and Stratus system indicates that the latter produces results approximately one third higher. This may be due to a variety of reasons, including differences in the approach to assay calibration, differences in the specificity of the antibodies and also in differences with the detection of free and complexed troponin I. Apple (30) in a review of troponin I suggested that although there were problems with the standardisation of the various assays the relative changes of the marker with respect to the upper reference limit should allow comparability of findings. Figure 4 shows data plotted as multiples of the cut-off limit for detection of myocardial infarction. Whilst broadly

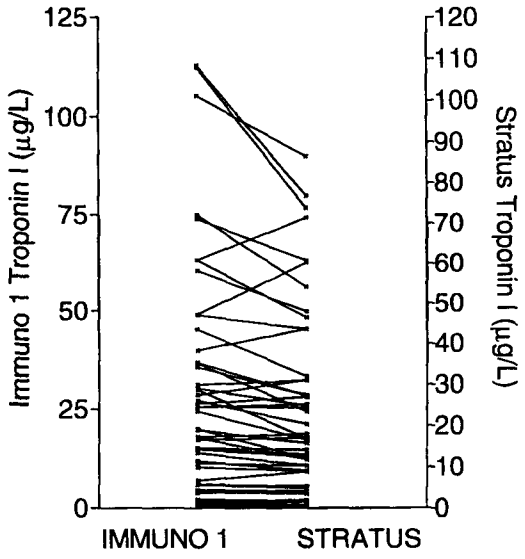

FIGURE 4 *Comparison of results obtained by the Immuno 1 and Stratus methods on individual patients plotted as multiples of cut-off limit for detection of myocardial infarction*

comparable at lower troponin I concentrations, at higher concentrations there are large differences between the two methods which must be due to factors other than standardisation. However, this is not necessarily the case if the imprecision of the assay has an impact on the definition of the detection limit and also of the reference range. Thus if one compares the detection limits of the Dade Stratus and Immuno 1 systems there is a 3.5 fold difference (0.35 and 0.1 µg/L, respectively whilst there is at least a 6 fold difference in the upper limit of the reference range (≤ 0.6 and ≤ 0.1 µg/L, respectively). The lower

limit quoted for the Immuno 1 system is presumably a reflection of superior method imprecision at this concentration of analyte. Apple in his review (31) quotes the detection limits for several assays and although it is a rather meaningless parameter the ratio of the upper reference limit to the detection limit provides a comparative indication of the imprecision at this low concentration range. Thus if one chooses the Stratus cut off of 1.5 μg/L and respective cut offs for the other systems based on their equivalence to the Stratus result (using slopes of the regression equations identified in reference 32), eg Beckman Access slope = 0.10, therefore cut off 0.15 μg/L, etc) the ratios of cut off to detection limit are as follows: Behring Stratus = 4.28, Behring Opus = 4.77, Beckman Access = 5.0, Abbott AxSym = 7.0, Bayer Immuno 1 = 10.65. Apple (32) makes the point that the wide variation in the numerical results obtained is partially explained by the epitope specificity of antibodies chosen for the various assay (33, 34), as well as the choice of calibrator. However, the discrimination at the clinical cut off point will also depend on the imprecision of the assay. The imprecision of the assay will also determine in part the upper limit of the reference range and here Apple in his review (31) reports them all as less than or equal to a given value – indicating the imprecision was not good enough to define a value. As yet no analytical method has

demonstrated sufficient methodological sensitivity to detect a measurable amount of the protein in the circulation of healthy subjects.

The data on serial samples from 10 patients with AMI indicated that the admission sample did not show an elevation of the troponin I except in one patient; however, in the case of the second sample collection the day after admission all of the levels were over 100 times the detection limit. The highest result (222 μg/L) was found in a patient who had received thrombolysis treatment. The pattern of troponin I values in the 10 patients was consistent with that seen in previous studies (30).

The diagnostic performance of the assay on patients admitted with acute chest pain and a working diagnosis of unstable angina indicated a sensitivity of 16.7% and a specificity of 94.7% with positive and negative predictive values of 16.7% and 94.7%, respectively. By 12h the sensitivity was 100% and the specificity was 83%, and positive and negative predictive values were 27.0% and 100%, respectively. Mair et al (1) found a sensitivity of 23% and specificity of 94% for the diagnosis of AMI when troponin I was measured on admission samples from patients presenting with acute chest pain.

Previous reports have offered a confused picture of the changes seen in troponin I in patients with chronic renal failure, some reports

indicating no increase (2) whilst others have found an increase (15-17). In this study 6 patients (15%) with renal failure demonstrated detectable levels of the protein. It is well known that patients with chronic renal failure develop a cardiomyopathy and a significant cause of mortality in these patients is due to cardiac related illness. There is insufficient data at this stage to ascertain whether the findings of a detectable level of troponin I is an indication of a low level of myocyte damage or due to leakage of free troponin from metabolically compromised myocytes.

The imprecision and sensitivity of the Bayer Immuno 1 assay should therefore enable a more accurate definition of the reference limit, earlier detection of myocyte damage in patients admitted to hospital with chest pain and furthermore provide a more sensitive predictive test of subsequent cardiac related events.

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