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Comparison of Cardiac Troponin I Measurements on Whole Blood and Plasma on the Stratus CS Analyzer and Comparison with AxSYM

To the Editor:

Assays for cardiac troponin T and troponin I (cTnI) are powerful tools for early identification of patients

with acute myocardial infarction (AMI) and for pointing out subjects at highest risk among patients with unstable angina pectoris (1–3). These applications require increasingly rapid and more sensitive tests. To this end, one of the most commonly used automated assays for cTnI (Stratus[®] II; Dade) has undergone extensive revision (4, 5). A second-generation quantitative radial partition immunoassay is now used in combination with the Stratus CS fluorometric analyzer, a system designed to meet the needs of STAT and point-of-care testing. The Stratus CS yields cTnI results that are clinically consistent with those obtained with the Stratus II (6).

The Stratus CS uses closed routine sample tubes containing anticoagulated whole blood (lithium heparin). The system takes ≤ 14 min to centrifuge the tube and perform the analysis. The Stratus CS can also manage precentrifuged plasma samples. In the present study, the Stratus CS was used to determine cTnI on whole blood and on preprocessed plasma samples drawn from the same patients. Our purpose was to check the similarity of the results obtained for both samples in the entire measuring range. We also aimed at comparing results from the Stratus CS with cTnI results yielded by the Abbott AxSYM analyzer, with special attention to concentrations below the threshold for AMI.

The Stratus CS is a fluorometric enzyme immunoassay analyzer for quantitative determination of the cardiac markers creatine kinase-MB mass, myoglobin, and cTnI (Dade Behring). The test system uses radial partition immunoassay technology, which has been enhanced through the use of a monoclonal capture antibody coupled to Starburst[®] dendrimers (7). According to the insert for the cTnI method, coronary risk increases at cTnI concentrations >0.4 $\mu\text{g/L}$, and this value was considered as the cutoff for AMI in the present study. Reference values are <0.08 $\mu\text{g/L}$ (6). The two monoclonal antibodies used in the method recognize both free and complexed cTnI. The results were compared with those

obtained by the AxSYM cTnI micro-particle enzyme immunoassay (Abbott Laboratories). This system also allows for automated quantification of cTnI in serum or plasma (lithium heparin) (8, 9). According to the manufacturer's package insert, cTnI values >0.4 $\mu\text{g/L}$ are increased above the reference values established in blood donors, whereas values >2.0 $\mu\text{g/L}$ are indicative of AMI.

Blood samples were obtained from 85 patients hospitalized at the University Hospital of Liège, Belgium. Twenty-three patients were admitted for suspected AMI; diagnosis was confirmed in 16 cases (14 Q-wave AMI and 2 non-Q-wave AMI), and a myocardial lesion was excluded in 7 cases. Twelve patients had unstable angina pectoris, 9 had stable angina, and 19 had undergone cardiac surgery. In the remaining patients were 5 patients with polytrauma, 1 non-cardiac surgery patient, and 16 chronically hemodialyzed patients.

In this study, we used two different types of samples: one tube (tube I; Hemogard lithium heparin, Vacutainer; Becton Dickinson) to be analyzed without further treatment on the Stratus CS, and one (tube II; Venoject-II lithium heparin + separator; Terumo Europe) to provide the plasma to be analyzed on the two analyzers. A sample was collected into tube II from each of the 85 above-mentioned patients. In a subgroup of 53 patients, tube I was taken in addition to tube II. Both tubes were taken within periods of time not exceeding 2 min. The order in which the tubes were sampled (tube I first or tube II first) was randomized in each category of patients. Tube I was kept at room temperature and analyzed within 2 h. No manual treatment of the tube was required prior to the assay.

cTnI results obtained for whole blood were compared with the corresponding results obtained for plasma aliquots from tube II, also analyzed by the Stratus CS using the special design for plasma samples (comparison A1). For 15 patients, selected at random among the patients with cTnI results <0.15 $\mu\text{g/L}$ on the Stratus CS, tube I was removed from

the Stratus CS after completion of the analysis, decapped, and centrifuged manually (8000g for 10 min). The supernatant was transferred to a cup, kept at 4 °C, and reanalyzed on the Stratus CS. Each plasma sample was analyzed within 1 h after the corresponding whole blood sample, and the results were used for a second comparison of whole blood with plasma on the Stratus CS (comparison A2). Tube II was kept at room temperature and centrifuged at 3000g within 1 h. The supernatant was divided into two aliquots and kept at 4 °C. One aliquot was analyzed on the Stratus CS, the other on the AxSYM; both plasma samples were analyzed simultaneously on the two analyzers within 2 h after blood collection (comparison B1). We also compared the cTnI results obtained on the Stratus CS (whole blood) with those obtained on the AxSYM, using an aliquot of plasma from tube II (comparison B2).

The measuring range for cTnI on the Stratus CS is 0–50 µg/L, similar to that of the AxSYM. One sample had to be diluted on the two instruments (72.8 µg/L on Stratus CS and 369 µg/L on AxSYM). Seven additional samples (10.2–33.5 µg/L on Stratus CS) were above the measuring range on the AxSYM and had to be diluted. The calculated results (56.0–369.0 µg/L) were included in the comparisons.

In the 53 patients for whom tubes I and II were available, we compared (comparison A1) the cTnI values obtained by the Stratus CS on whole blood and plasma; the values were related by the equation:

$$\begin{aligned} \text{Whole blood} &= & (1) \\ 0.991 (0.981-1.01) \\ \text{plasma} &- 0.075 \\ &(-0.20 \text{ to } 0.05); r^2 = 0.998 \end{aligned}$$

When only cTnI values <0.15 µg/L (n = 29) were selected for the comparison, the regression equation was:

$$\begin{aligned} \text{Whole blood} &= & (2) \\ 0.921 (0.815-1.03) \text{ plasma} &- 0.002 \\ &(-0.008 \text{ to } 0.003); r^2 = 0.923 \end{aligned}$$

For 15 patients of the latter subgroup, the results obtained by the Stratus CS on whole blood were compared with those obtained for the plasma in the same system after subsequent centrifugation of the tubes (comparison A2). There was no statistically significant difference between the initial values and results obtained after manual centrifugation (Student *t*-test for paired observations).

The comparison of measurements obtained for plasma samples (n = 85) analyzed on the Stratus CS and AxSYM (comparison B1) indicated that the cTnI values on the two analyzers were related by the equation:

$$\begin{aligned} \text{Stratus CS} &= & (3) \\ 0.171 (0.166-0.176) \text{ AxSYM} &- \\ 0.06 (-0.37 \text{ to } 0.25); \\ r^2 &= 0.981 \end{aligned}$$

The regression equation between results obtained for whole blood on the Stratus CS and plasma on the AxSYM (comparison B2) was close to Eq. 3. When the comparison was performed for results obtained for the 64 patients with cTnI values <2.0 µg/L on the AxSYM (comparison of plasma samples), the regression equation was:

$$\begin{aligned} \text{Stratus CS} &= & (4) \\ 0.202 (0.175-0.229) \text{ AxSYM} &+ \\ 0.015 (0.013-0.017); r^2 &= 0.789 \end{aligned}$$

To analyze the discrepancies between the results obtained by the two methods in this clinically important concentration range between the upper reference value (URL) and the cutoff for AMI, the cTnI results were expressed in relation to the respective URL: results obtained on the Stratus CS were divided by 0.08 and those obtained on the AxSYM were divided by 0.4. Fig. 1 shows the distribution of the differences between each pair of transformed results (value on AxSYM minus value on Stratus CS) in relation to their mean value. The mean of the differences (\bar{d}) was not statistically different from zero, indicating that there was no systematic bias toward one method. Nevertheless, based on the threshold values of the methods (0.08 and 0.4 µg/L for Stratus CS, 0.4 µg/L and 2.0 µg/L for AxSYM), nine patients were classified in different diagnostic categories according to the method used. In four patients for whom unstable angina or AMI was excluded and who did not show increased cardiac markers in the following hours or days, cTnI results on

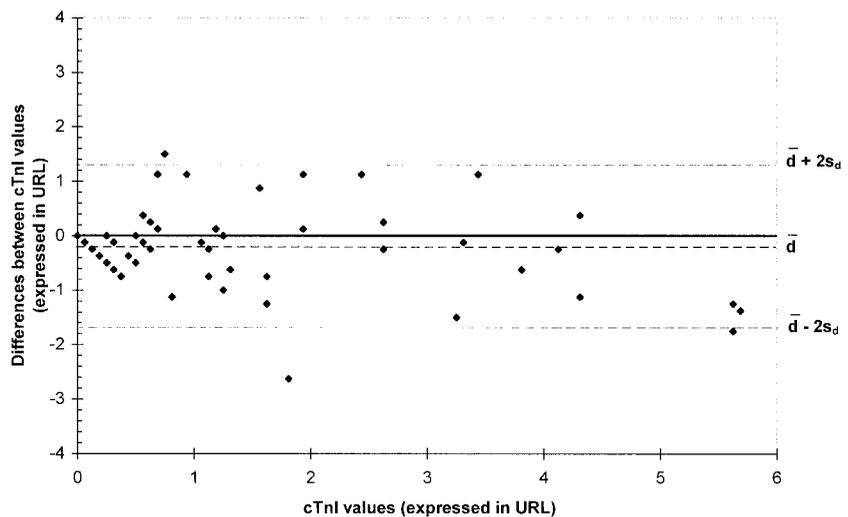


Fig. 1. Differences between results for plasma cTnI by Stratus CS and AxSYM according to the cTnI concentrations of the samples.

For each method, cTnI results are expressed as multiples of the URL: 0.08 µg/L for Stratus CS and 0.4 µg/L for AxSYM. The *x* axis represents, for each pair of results, the mean of the transformed data; the *y* axis represents the difference between the transformed data (AxSYM – Stratus CS). \bar{d} , mean of the differences; s_d , standard deviation of their distribution.

AxSYM were above reference values (0.5 and 0.6 $\mu\text{g/L}$), whereas the Stratus CS yielded negative results ($<0.08 \mu\text{g/L}$). On the other hand, in contrast to the negative results on AxSYM, values between 0.08 and 0.4 $\mu\text{g/L}$ on the Stratus CS were found in five samples from patients with myocardial damage. One of these patients was in the early phase of AMI (the two methods indicated positive results 2 h later), two had unstable angina, and two others were cardiac surgery patients (early postoperative period). Consequently, for all discordant results, the Stratus CS was in agreement with the clinical diagnosis. There was no discrepancy between the methods for results $>2.0 \mu\text{g/L}$ on the AxSYM.

In conclusion, when performed on samples deriving from two different tubes (whole blood and heparinized plasma) drawn from the same patients at the same time, cTnI values obtained by the Stratus CS in the entire measuring range, including values $\leq 0.15 \mu\text{g/L}$, were statistically indistinguishable. This attests not only to the excellent precision of the method, as already stated by others (6), particularly in the low concentration range, but also to the quality of the preanalytical phase automatically performed by the system. The agreement of the results allows the system to be used on different specimens in the same patients.

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Real-Time PCR Assay with Fluorescent Hybridization Probes for Rapid Genotyping of the CD14 Promotor Polymorphism

To the Editor:

A soluble form of CD14 (1) activates endothelium and smooth muscle (2). CD14 binds lipopolysaccharide, the cell wall component of gram-negative bacteria. Upon lipopolysaccharide binding, monocytes produce pro-inflammatory cytokines and pro-coagulant activity. In view of the growing evidence for a role of infection with gram-negative bacteria (3), inflammation, and hypercoagulability in the onset of atherosclerosis, two independent studies evaluated the frequency of a genetic polymorphism within the promotor of the CD14 gene in patients with myocardial infarction (MI) (4, 5). This polymorphism consists of a single base exchange (C \rightarrow T) at position -260 (4) [corresponding to position -159 in study by Unkelbach et al. (5)], with C introducing a *Hae*III restriction site.

The polymorphic site is located

near the Sp 1 binding site of the promotor (4). An increased risk for MI in patients homozygous for the T allele was found (4, 5). Moreover, Unkelbach et al. (5) observed an even stronger association between the TT genotype and the risk for MI in patients without other risk factors such as smoking and hypertension. The odds ratio for MI in normotensive nonsmoking TT homozygotes older than 62 years was 3.8 (5).

Because perioperative MI remains a major complication in surgical patients (6), genotyping for the CD14 promotor polymorphism could become a part of preoperative risk classification of surgical patients.

The techniques reported for CD14 genotyping (restriction fragment length polymorphism and single-strand conformation polymorphism analysis) are time-consuming and require multiple manual steps. Because a high throughput of samples is desirable for future studies, we suggest a rapid-cycle PCR with fluorescently labeled oligonucleotide hybridization probes on the LightCyclerTM instrument (Roche Diagnostics) and subsequent fluorescent probe melting point analysis, which allows genotyping within 60 min.

Genomic DNA samples from 100 healthy blood donors were extracted from whole blood according to standard procedures. The reliability of the proposed assay was confirmed by restriction enzyme digestion with *Hae*III.

PCR was performed in disposable capillaries (Roche Diagnostics) in a reaction volume of 10 μL containing 1 μL of DNA (20–80 ng), 0.5 $\mu\text{mol/L}$ each of the primers (sense, 5'-GGTGCCAACAGATGAGGTTAC-3'; antisense, 5'-CTTCGCTGCCTCTGACAGTT-3'), 1 μL of reaction buffer [LightCycler DNA master hybridization probes 10 \times buffer (1 \times = 1.75 mmol/L); Roche Diagnostics], and 0.2 $\mu\text{mol/L}$ each of the probes. The detection probe specific for the T allele (5'-TTCCTGTTACGGCCCCCT-3') was labeled at the 3' end with fluorescein. The anchor probe (5'-GGAGACACAGAACCCTAGATGCCCTGCA-3') was labeled with LightCycler Red 640 at the 5' end