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LETTER TO THE EDITOR

Troponin I concentrations in heparinized plasma and serum differ when measured with the Advia Centaur TnI-Ultra assay

Daan van de Kerkhof, Berry Peters and Volkher Scharnhorst

Cardiac troponins I (TnI) and T (TnT) are the preferred diagnostic markers of acute myocardial infarction (AMI) [1]. Most laboratories prefer heparinized plasma over serum for the analysis in order to minimize the turnaround time. Siemens Medical Solutions Diagnostics recently marketed a novel TnI-Ultra assay with increased analytical sensitivity. Siemens recommends the use of serum, heparinized plasma or EDTA plasma for the determination of the TnI concentration. The product sleeve [2] of the kit reports a 1 % lower TnI concentration in heparinized plasma than in serum, based on a comparison of serum and heparinized plasma samples obtained from 53 subjects. The Passing–Bablok equation reported was: [heparinized plasma TnI] = $0.99 \times [\text{serum TnI}] + 0.03 \mu\text{g/L}$. No details of the group of subjects are provided, and the heparin concentration and brand of tubes used are not reported.

During the routine method validation performed at our laboratory, we compared the TnI analysis in heparinized plasma with serum analysis. Blood from 34 patients who had undergone coronary artery bypass grafting (CABG) surgery was drawn into plain gel serum tubes and heparin tubes. The samples were collected within the first 8 h after surgery. A maximum of two samples were collected per patient at different time-points and a total of 58 samples were collected. Subjects volunteering for the study were recruited according to hospital guidelines.

Blood samples were collected in vacuum polypropylene tubes containing lithium heparin (BD Vacutainer, 4 mL, LH 68 IU Plus, BD Vacutainer Systems Preanalytical Solutions). The estimated heparin concentration in the sample is 17 IU/mL of whole blood, corresponding to 28 IU/mL in plasma based on a hematocrit of 0.40. Serum samples were collected in plain gel tubes (BD Vacutainer SST II Advance, 4 mL, BD Vacutainer Systems Preanalytical Solutions). Samples were centrifuged at 3000g for 10 min. Plasma was analysed within 4 h

of storage at room temperature. The assay was calibrated in accordance with the manufacturer's instructions. Data was analysed with Analyze-It version 1.72 (Analyze-It Software Ltd, UK). The 99th percentile limit of a selected reference population was $0.06 \mu\text{g/L}$ and a concentration $\geq 0.05 \mu\text{g/L}$ can be determined with $\leq 10 \%$ total precision [3].

As shown in Figure 1, TnI results of heparinized plasma correlated well with the serum values, but were relevantly lower. The median concentration in heparin was $0.65 \mu\text{g/L}$ and in serum $0.75 \mu\text{g/L}$. The difference between the median values was $0.10 \mu\text{g/L}$ (13 % of the median serum value), which is statistically significant ($p < 0.0001$) as determined with the Wilcoxon signed-rank test. As is also shown in Figure 1, the negative bias of TnI in heparinized plasma as compared to serum was less in the concentration range $< 0.3 \mu\text{g/L}$. Four samples showed a lower concentration in serum than in plasma. The concentration range of $< 0.3 \mu\text{g/L}$ represents the high-sensitivity range of the TnI-Ultra assay, which could not be determined with adequate precision by the predecessor cTnI assay of Siemens [3].

Several studies have reported significantly reduced TnI concentrations in heparinized plasma compared to serum. The Liaison cTnI assay (DiaSorin, Italy) showed a difference of 27 % (in 124 paired samples; 95 % confidence interval 24–29 %), for which the heparin concentration was not described [4]. For the Immulite cTnI assay (Siemens Medical Solutions Diagnostics, Tarrytown, USA) a difference of 14 % was found (in 20 paired samples; 95 % confidence interval 9–19 %), using a heparin concentration of 60 IU/mL full blood [5]. Another study, however, showed an insignificant difference for the ACS:180 cTnI assay (Bayer Diagnostics, currently Siemens) [6].

The heparin concentration used in our validation is relatively low, i.e. 17 IU/mL in full blood. Relevant

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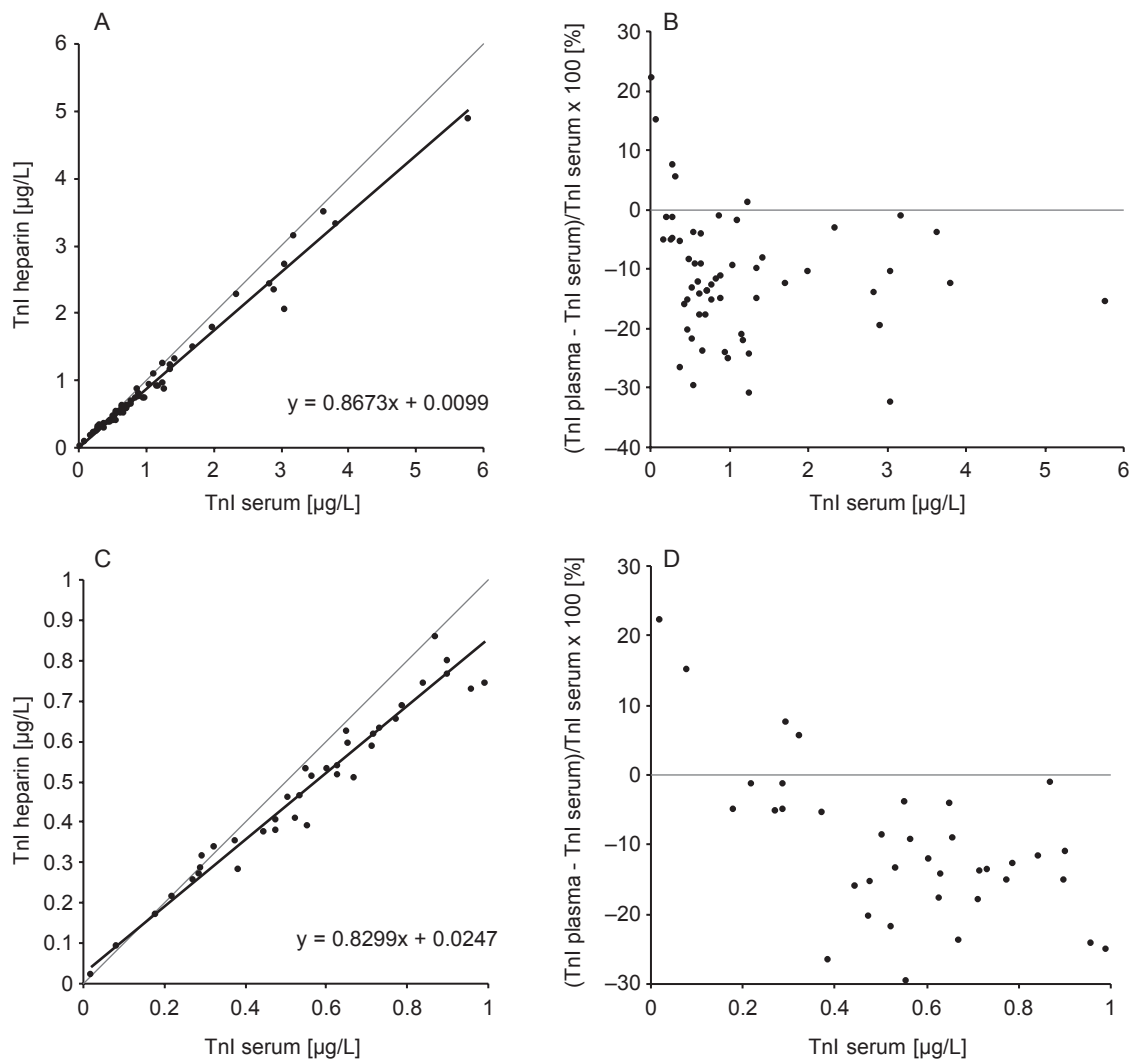


Figure 1. Passing-Bablok agreement and bias analysis show lower results for TnI when analysed in heparinized plasma compared to serum. In the low concentration range ($<0.3 \mu\text{g/L}$), the difference between heparin and serum is smaller. Figure A shows Passing-Bablok agreement analysis of all patient results ($n=58$). Figure B shows the bias analysis of all patient results. Figure C+D show Passing-Bablok agreement and bias analysis in the concentration range $<1 \mu\text{g/L}$ ($n=37$). Bias is defined as: $(\text{TnI concentration in plasma} - \text{TnI concentration in serum})/(\text{TnI in serum}) \times 100 \%$.

interference of heparin on the TnI assay is therefore observed at a lower heparin concentration than the 60 IU/mL studied by Gerhardt et al. [5]. The effect of heparin is also more extensive than that observed by Stiegler et al. [7], who studied the effect of 15 IU/mL heparin in full blood with TnI assays of Abbott AxSYM and Bayer ACS:Centaur. It is clear that the bias caused by heparin not only depends on the assay used, but also on the brand of tubes, concentration of heparin and concentration of TnI in the plasma.

It is assumed that the negatively charged heparin molecules bind to the positively charged troponin complexes, reducing the immunoreactivity [8]. The

product sleeve of the TnI-Ultra assay suggests that the antibodies used in the assay are unaffected by binding of heparin to the troponin molecule. Our study shows that this suggestion is not true for the heparinized tubes that we used and that the manufacturer's product sleeves should be interpreted with care and confirmed experimentally. Furthermore, our results emphasize that serum and heparinized plasma samples cannot be used interchangeably in the same patients. It remains to be determined whether the diagnostic outcome in the low concentration range of the assay will be significantly different when using heparinized plasma instead of serum.

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