

# Evaluation of cardiac troponin I stability in blood sample using the AccuTnI+3 assay

M. NOWOCZYN<sup>1,2,3</sup>, C. LEFEVRE<sup>1</sup>, F. GRANDHOMME<sup>1</sup>, J. ALEXANDRE<sup>2,3,4</sup>, S. FRADIN<sup>1</sup>, S. ALLOUCHE<sup>1,2,3</sup>

<sup>1</sup>Department of Biochemistry, CHU de Caen, Caen, France

<sup>2</sup>Caen Normandie Université, Medical School, Caen, France

<sup>3</sup>Signalisation, Electrophysiologie et Imagerie Des Lésions D'ischémie-Reperfusion Myocardique Normandie Univ, Unicaen, Signalisation, Electrophysiologie et Imagerie Des Lésions D'ischémie-Reperfusion Myocardique, Caen, France

<sup>4</sup>Department of Pharmacology, CHU de Caen, Caen, France

**Abstract. – OBJECTIVE:** Troponins are considered as the biomarkers of choice to highlight cardiac injury in emergency departments, but are also valuable to detect cardiac injury in a non-emergency setting. In this latter case, transport of blood samples to laboratories often exceeds the manufacturer's recommendations (<2 h between vein puncture and analysis for the Beckman Coulter AccuTnI+3 assay). We aim to evaluate *in vitro* the stability of troponin I (cTnI) at two intervals (<2 h and at 4 h) over a wide range of concentrations using the Beckman Coulter AccuTnI+3 assay.

**PATIENTS AND METHODS:** For each of the 95 patients included in this study, we analyzed the first blood sample with a time of transport <2 h, and the second sample after 4 h from vein puncture. We then calculated the correlation between the two periods of analysis and evaluated the bias by a Bland-Altman test.

**RESULTS:** Taking into account of our analytical reproducibility, we did not observe any significant differences in cTnI values between <2 h and 4 h.

**CONCLUSIONS:** The time between vein puncture and analysis of cTnI can be extended to 4 h.

Key Words:

Cardiac troponin I, *In vitro* stability, Immunoassay.

## Introduction

Cardiac troponins I or T (cTnI or cTnT) are specific biomarkers of cardiac damages. They are widely used in an emergency context for diagnosis of acute myocardial infarction (AMI) following international<sup>1</sup> and European<sup>2</sup> guidelines. Beside AMI, cardiac troponins can be used as predicti-

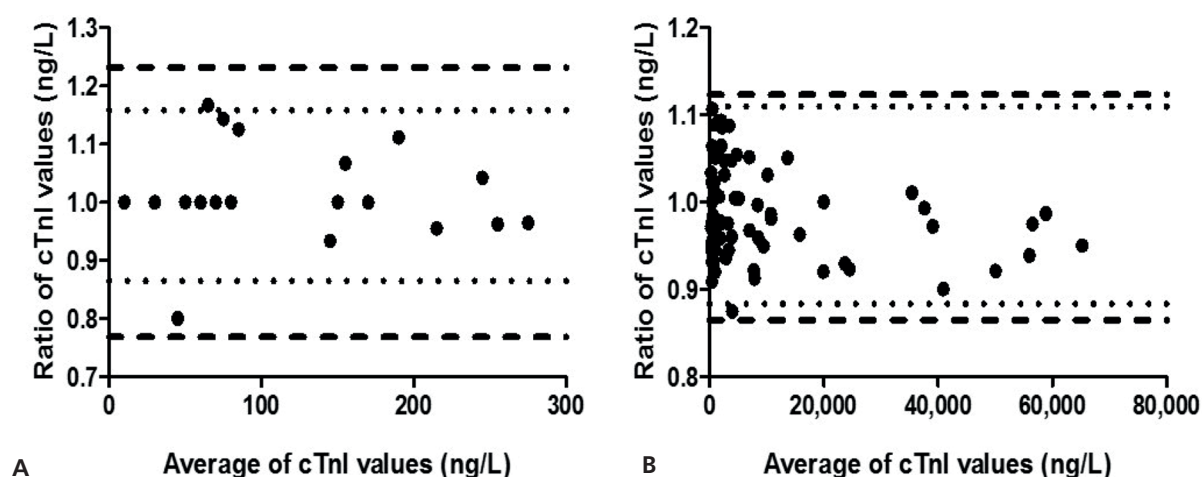
ve markers of cardiovascular mortality, notably in patients undergoing vascular surgery<sup>3</sup>, non-cardiac surgery<sup>4</sup>, traumatic cardiac injury<sup>5</sup> or in pregnant women suffering from pre-eclampsia<sup>6</sup>. Cardiac troponins are thus extensively prescribed in various clinical situations, and their analysis must be carried in appropriate conditions to reach the highest possible accuracy.

In France, to reach optimal quality, accreditation procedures must be followed by all medical laboratories according to ISO 15189:2012<sup>7</sup>. Among the pre-analytical requirements, laboratories must have a documented procedure to ensure that samples are analyzed within appropriated time and conditions.

According to the manufacturer's recommendations for the AccuTnI+3 assay (Beckman Coulter, Brea, CA, USA), cTnI must be measured within the 2 h following blood collection. If this pre-analytical condition is not respected, the cTnI assay is canceled, nurses must be notified and a second blood sample has to be drawn. This is a time wasted for both clinical departments and laboratories and a strong inconvenience for patients, notably for those who present poor peripheral venous access. We evaluated in our laboratory the *in vitro* stability of cTnI until 4 h after sample collection, which will be more suited to clinical practice in medicine and surgical departments.

## Patients and Methods

We performed a prospective study on 95 patients from the Cardiology and Cardiac Surgery Departments of our University Hospital Center



**Figure 1.** Bland-Altman plots for cTnI values. *A*, Values under 280 ng/L (n=24); *B*, Values over 280 ng/L (n=71) X-axis represents mean of cTnI values ( $(cTnI_{4h} + cTnI_{2h})/2$ ) and Y-axis represents the ratio of cTnI values ( $cTnI_{4h}/cTnI_{2h}$ ). Each dot corresponds to a couple of values, dotted lines represent the 95% confidence interval of bias determined with Bland-Altman test and dash lines represent ACL.

(CHU) of Caen, between July and December 2015, following Ethics Committee approval. We chose these wards to obtain in our study both normal (<40 ng/L) and elevated (>40 ng/L) cTnI values. For each prescription, two blood samples were collected in lithium heparin tubes containing gel separator (Vacutainer PST™II Becton-Dickinson (BD), Franklin Lakes, NJ, USA) and carried to our biochemistry laboratory; one was analyzed within the 2 h following collection, the other one was kept at room temperature before centrifugation and analyzed 4 h after collection.

Samples were centrifuged according to BD recommendations, and plasma was analyzed either on an Access Immunoassay System or a DxI (Beckman Coulter), using Access AccuTnI+3 reagent with calibrations and quality controls performed as described by validated quality procedures. We used 3 levels of control solution containing respectively 40 ng/L, 520 ng/L and 2.540 ng/L of cTnI to calculate intra-laboratory reproducibility. Plasma cTnI is measured using a two-site sandwich enzyme immunoassay with monoclonal antibodies conjugated to alkaline phosphatase and paramagnetic particles, with a chemiluminescence-based detection. We collected results for each pair of samples and excluded cTnI values <10 ng/L as ratios between values <2 h and 4 h cannot be calculated for them.

### Statistical Analysis

Statistical analysis of data was performed using GraphPad software. We calculated the acceptable

change limits (ACL)<sup>8</sup> according to the formula defined in ISO 5725-6:  $ACL = 2.77 \times CVa$ , where CVa represents the analytical imprecision. We checked the assumptions of normality of ratios using the D'Agostino and Pearson normality test. Correlation between the two periods of analysis (<2 h and 4 h) was tested by the Spearman's rank correlation coefficient ( $r^2$ ), which is a non-parametric test because distribution of raw cTnI values is not Gaussian. We also performed a Bland-Altman analysis between <2 and 4 h to evaluate the bias, expressed as ratio vs. the average of cTnI values.

## Results

cTnI values ranged from 10 ng/L to 66.890 ng/L, with a median at 1.010 ng/L. During the year 2015, coefficients of variation (CVs) were 8.36%, 4.47% and 4.42% for each control level, respectively. Since the CVs between low and medium levels were notably different, we calculated the mean between values of these levels, 280 ng/L, which was defined as the threshold to separate our troponin values into two distinct groups (<280 ng/L n=24 and >280 ng/L n=71). When cTnI values were lower than 280 ng/L, the ACL was 23.15% and 12.32% for values higher than this threshold (Table I).

For both groups, we also showed high correlations ( $r^2$ : 0.9886 for cTnI <280 ng/L and 0.9964 for the other group) between values obtained for blood samples <2 h and at 4 h (Table I). However,

**Table I.** Statistical analysis of cTnI values.

cTnI values	<280 ng/L	>280 ng/L
n	24	71
Regression equation	$y = 0.9858 + 2.3570$	$y = 0.9547 + 106.3$
Spearman correlation coefficient ( $r^2$ )	0.9886	0.9964
Bias of ratio evaluated by Bland-Altman method	1.0110	0.9892
SD of bias	0.0748	0.0540
95% confidence interval of agreement limits	0.8646 to 1.158	0.8834 to 1.1095
Acceptable Change Limit	23.15%	12.32%

a high correlation does not imply that there is a good agreement between pairs of results, so we also evaluated the bias (Table I). As shown in Figures 1 A and B, we can observe for both groups that even if some values are over the calculated bias, they all fit within the ACL.

## Discussion

Between July and December 2015, we performed in our laboratory ~13.000 cTnI assays, with about half of prescriptions requested in an emergency context (40.6% for the emergency unit, 12.3% for the vascular and thoracic surgery departments). The third main prescriber is the orthopedic and traumatology ward (10.7%), where cTnI is used both in emergency to evidence cardiac lesions and in a routine practice to evaluate long-term mortality<sup>9</sup>. The inclusion of a relatively low number of patients from cardiology and the vascular and thoracic surgery departments compared to a total number of cTnI assays performed for these two wards (95 patients included in this study vs. 2.280 tests performed) results from a low compliance of practitioners and nurses to our study.

As a majority of laboratory errors appears in the pre-analytical phase<sup>10</sup>, precise and strict conditions must be respected to improve reliability of laboratory tests. The *in vitro* stability of troponins, mainly linked to the time of blood samples transport from medical wards to laboratories, is one of the various pre-analytical factors that could influence the cardiac biomarkers testing (see for review<sup>11</sup>). Troponins were shown to undergo a rapid degradation<sup>12</sup> with a preferential cleavage localized at the C-terminus region<sup>13</sup>. To better evaluate *in vitro* stability of cTnI, we selected plasma from patients without and with cardiac injuries in which proteolytic enzymes are released from damaged tissues. Even if it's highly difficult to transpose resul-

ts obtained with one immunoassay to another, Peake et al<sup>14</sup> showed no significant variation of troponin levels until 4 h post-vein puncture using the ADVIA Centaur XPw TnI-Ultra assay (Siemens Healthcare Diagnostics, Deerfield, IL, USA). Those results are in good agreement with ours. Similar data were also reported by Wu et al<sup>15</sup> using the ultrasensitive Erenna<sup>®</sup> cTnI immunoassay (Singulex, Alameda, CA, USA) at 6 h where the CVs were less than 3%; furthermore, a publication summarizing data published in literature indicates that cTnI is stable in whole blood at room temperature for 8 h<sup>16</sup>. However, Brinkmann et al<sup>17</sup> in 2004 evidenced heterogeneous concentrations of cTnI before and after 24 h incubation at 37°C depending on the commercial immunoassays used. This indicates that *in vitro* degradation of troponins may result for some immunoassay in underestimating cTnI concentration. However, it is noteworthy that in this latter study the difference between t0 h and t24 h was not statistically analyzed and compared to analytical imprecision and ACL. All those data emphasize that *in vitro* stability of cTnI should be determined by each commercial immunoassay separately as different antibodies are used; this is even truer as high sensitivity or ultrasensitive assays allow measurements of very low troponins concentrations.

## Conclusions

To the best of our knowledge, such *in vitro* stability for cTnI using the AccuTnI+3 assay hasn't been evaluated yet and our data indicate that the time between vein puncture and the analysis can be extended until 4 h.

### Conflict of interest

The authors declare no conflicts of interest.

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