MULTICENTER COMPARISON OF FOUR CONTEMPORARY SENSITIVE TROPONIN IMMUNOASSAYS

MULTICENTRIČNO POREĐENJE ČETIRI SAVREMENA IMUNOESEJA OSETLJIVA NA TROPONIN

Gian Luca Salvagno¹, Davide Giavarina², Moira Meneghello¹, Roberta Musa³, Rosalia Aloe³, Giorgio Da Rin⁴, Giuseppe Lippi³

1. Sezione di Chimica Clinica, Dipartimento di Scienze della Vita e della Riproduzione, Università degli Studi di Verona, Verona, Italy
2. Laboratorio di Chimica Clinica ed Ematologia, Ospedale San Bortolo, Vicenza, Italy
3. Unità Operativa Diagnostica Ematochimica, Azienda Ospedaliero – Universitaria di Parma, Parma, Italy
4. Struttura Complessa di Medicina di Laboratorio, Ospedale di Bassano del Grappa, Bassano del Grappa (VI), Italy

Summary

Background: The IFCC Task Force on Clinical Applications of Cardiac Biomarkers currently recommends evaluation of all troponin immunoassays within the same population to compare their performance. Hence, we planned a multicenter study to compare the four most widespread contemporary sensitive troponin I (TnI) methods.

Methods: Seventy-six serum samples were centrifuged, separated and divided in 5 aliquots. The first aliquot was used for clinical measurement, whereas the rest were shipped to participating laboratories, where they were simultaneously thawed. High-sensitivity troponin T (HS-TnT) was measured on a Roche Cobas, whereas TnI was assessed with the Ortho Vitros cTnI, Beckman Coulter DXI 800 AccuTnI, Siemens Vista cTnI and Abbott Architect STAT cTnI.

Results: A substantial bias was found between TnI and HS-TnT values. Although the correlation was acceptable and comprised between 0.86–0.89, the agreement of diagnostic values was poor, with the kappa statistic always lower than 0.50. Although the direct comparison between the four contemporary sensitive TnI immunoassays generated more favourable results, with Pearson’s correlations greater than 0.970 and the kappa statistic equal to or higher than 0.59,

Address for correspondence:
Prof. Giuseppe Lippi
U.O. Diagnostica Ematochimica,
Azienda Ospedaliero – Universitaria di Parma,
Via Gramsci, 14,
43126 – Parma, Italy
Tel. 0039-0521-703050
Fax. 0039-0521-703791
e-mail: glippi@ao.pr.it, ulippi@tin.it
we observed wide 95% confidence intervals, significant bias and large dispersion of values, with a single notable exception (i.e., Vitros cTnl versus DXI 800 AccuTnl).

**Conclusions:** The results of this study attest that substantial discrepancies still exist among contemporary sensitive TnI immunoassays. The presence of random variation rather than constant bias appears to be the major contributor to this variance, thus precluding the interchangeability of methods and making the objective of harmonization a rather long and challenging enterprise.

**Keywords:** myocardial infarction, troponin, immunoassays, comparison

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**Introduction**

Acute myocardial infarction (AMI) is the leading cause of death and morbidity worldwide (1). According to the most recent guidelines, the diagnostic workup of patients with suspected AMI is strongly dependent upon laboratory testing, wherein diagnostic values of cardiospecific troponin I (Tnl) or troponin T (TnT) are essential to establish a diagnosis of ST-elevation MI (STEMI), and especially of non-ST-elevation MI (NSTEMI) (2). In this latter condition, detection of an increased troponin value with at least one measurement within 3 to 6 hours from the onset of symptoms exceeding the 99th percentile upper limit of a reference population (URL) in association with clinical evidence of myocardial ischemia is the only evidence needed for achieving the final diagnosis (3).

Beside an improper use of the terminology that designates some commercial immunoassays, including improbable definitions such as «ultra-sensitive», «extra-sensitive» or «modified-sensitive» among others, the current classification of troponin methods is based upon the number of measurable values (i.e., exceeding the limit of detection [LOD] of the method) attainable in a (presumably) healthy population. When this value is lower than 50%, the method is classified as «contemporary sensitive», whereas the assay can be designated as «high-sensitivity» (HS) when this value exceeds 50% (4, 5). According to a clinical perspective, the methods are then classified as «guideline acceptable» when the 99th URL value is associated with ≤ 10% coefficient of variation (CV), «clinically usable» when the 99th URL value has a CV comprised between 10% and 20%, and «not acceptable» when the 99th URL value is associated with > 20% CV (5).

Although the ongoing introduction of novel HS methods carries some unquestionable technical advantages due to the lower analytical sensitivity and improved imprecision at the diagnostic threshold (6), there is ongoing debate around the fact that the clinical performance of some contemporary sensitive immunoassays may be comparable to, or even better than that of HS methods, especially in health-care settings such as the emergency department (ED) where a greater diagnostic specificity is essential in order to prevent overcrowding caused by the larger number of patients with troponin values above the 99th URL (4). Several laboratories are, hence, delaying the introduction of HS methods on the grounds that the former contemporary sensitive immunoassays may be better suited for AMI diagnostics in short-stay units such as the ED.

All that said, two leading problems still remain with the use of contemporary sensitive assays. First, cardiospecific Tnl and TnT are two structurally and

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**Table I** Analytical characteristics of the five contemporary sensitive troponin I (Tnl) and the high-sensitivity troponin T (HS-TnT) immunoassays used in this study.

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Company</th>
<th>Method</th>
<th>LOD</th>
<th>CV 10%</th>
<th>99th percentile</th>
</tr>
</thead>
<tbody>
<tr>
<td>Academic Hospital of Verona, Verona, Italy</td>
<td>Roche Diagnostics, Basel, Switzerland</td>
<td>Cobas HS-TnT</td>
<td>0.005</td>
<td>0.013</td>
<td>0.014 µg/L</td>
</tr>
<tr>
<td>Academic Hospital of Parma, Parma, Italy</td>
<td>Beckman Coulter, Brea CA, USA</td>
<td>DXI 800 AccuTnl</td>
<td>0.011</td>
<td>0.058</td>
<td>0.034 µg/L</td>
</tr>
<tr>
<td>Academic Hospital of Parma, Parma, Italy</td>
<td>Ortho-Clinical Diagnostics, Rochester, NY, USA</td>
<td>Vitros cTnl ES</td>
<td>0.003</td>
<td>0.028</td>
<td>0.021 µg/L</td>
</tr>
<tr>
<td>General Hospital of Vicenza, Vicenza, Italy</td>
<td>Siemens Healthcare Diagnostics, Tarrytown, NY, USA</td>
<td>Dimension Vista cTnl</td>
<td>0.015</td>
<td>0.036</td>
<td>0.022 µg/L</td>
</tr>
<tr>
<td>General Hospital of Bassano del Grappa, Bassano del Grappa (VI), Italy</td>
<td>Abbott Diagnostics, Lake Forest, IL, USA</td>
<td>Architect STAT cTnl</td>
<td>0.010</td>
<td>0.076</td>
<td>0.020 µg/L</td>
</tr>
</tbody>
</table>

LOD, Limit of detection; CV 10%, 10% coefficient of variation.
biologically distinct proteins. Their kinetics after myocardial injury is notably different and test results are hence inherently barely commutable (7). It is also noteworthy that the various TnI methods available in the diagnostic market have been developed with different cocktails of antibodies, which display heterogeneous reactivity against the various molecular isoforms and degradation products of TnI (8). Last but not least, global standardization of TnI immunoassays is still an unmet target (9).

Since the IFCC Task Force on Clinical Applications of Cardiac Biomarkers currently recommends comparison of all contemporary sensitive and/or HS assays within the same population to establish whether the different methods exhibit comparable analytical performance (10), we planned a multicenter study using the four most widespread contemporary sensitive TnI immunoassays currently available on the diagnostic market, and thus including the Ortho-Clinical Diagnostics Vitros cTnI ES, Beckman Coulter DXI 800 AccuTnI, Siemens Healthcare Diagnostics Dimension Vista cTnI, and Abbott Diagnostics Architect STAT cTnI (Table I).

Materials and Methods

The collection of samples was centralized at the Academic Hospital of Verona, Italy. In brief, all serum samples referred to the local clinical chemistry laboratory with a request for troponin testing over the same working day were centrifuged, separated and divided in 5 aliquots of 0.5 mL each immediately after receipt. Insufficient samples and those containing visible interference (i.e., hemolysis, turbidity and icterus) were not included in this study. The first aliquot was used for clinical measurement of HS-TnT as for local protocol, whereas the remaining 4 aliquots were stored at –70 °C for further testing. After one week of storage, the samples were transported to the participating laboratories using certified transport boxes, under controlled conditions of temperature and humidity.

Table II Values (mean ± standard error of the mean; SEM) obtained with four commercial contemporary sensitive troponin I (TnI) and one high-sensitivity troponin T (HS-TnT) immunoassay.

<table>
<thead>
<tr>
<th></th>
<th>Roche HS-cTnT</th>
<th>Vitros cTnI ES</th>
<th>DXI 800 AccuTnI</th>
<th>Dimension Vista cTnI</th>
<th>Architect STAT cTnI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± SEM (µg/L)</td>
<td>0.15±0.03</td>
<td>0.54±0.15</td>
<td>0.57±0.16</td>
<td>0.70±0.20</td>
<td>0.59±0.29</td>
</tr>
<tr>
<td>Values &gt;99th percentile</td>
<td>54/76 (71%)</td>
<td>50/76 (39%)</td>
<td>29/76 (38%)</td>
<td>33/76 (43%)</td>
<td>45/76 (59%)</td>
</tr>
</tbody>
</table>

Table III Person’s correlation (r) between values and agreement (kappa statistic and 95% CI) of data exceeding the 99th percentile of the upper limit of the reference range of each troponin I (TnI) immunoassay as compared with Roche high-sensitivity troponin T (HS-TnT).

<table>
<thead>
<tr>
<th></th>
<th>Vitros cTnI ES</th>
<th>DXI 800 AccuTnI</th>
<th>Dimension Vista cTnI</th>
<th>Architect STAT cTnI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Correlation</td>
<td>r=0.882; p&lt;0.001</td>
<td>r=0.861; p&lt;0.001</td>
<td>r=0.881; p&lt;0.001</td>
<td>r=0.887; p&lt;0.001</td>
</tr>
<tr>
<td>Kappa statistic</td>
<td>0.42 (0.26–0.58); p&lt;0.001</td>
<td>0.40 (0.25–0.56); p&lt;0.001</td>
<td>0.48 (0.31–0.64); p&lt;0.001</td>
<td>0.23 (0.01–0.45); p=0.038</td>
</tr>
</tbody>
</table>

Results

Seventy-six serum samples were finally collected throughout the study period. The concentration of HS-TnT and TnI in the different samples, along with the frequency of values above the relative 99th percentile URLs, are shown in Table II. Although a signifi-
significant correlation was found when comparing the results of HS-TnT with those obtained using the four contemporary sensitive TnI immunoassays (correlations ranging from 0.861 to 0.887; all p<0.001), the agreement of values exceeding the 99th percentile URL was very modest, with kappa coefficients comprised between 0.23 and 0.48 (Table III). A much better agreement was observed when values obtained with the different contemporary sensitive TnI assays were compared among each other, with correlations ranging from 0.970 to 0.995 (all p<0.001), and agreement for values exceeding the 99th percentile URL comprised between 0.59 and 0.97 (Table IV). Although a relatively modest bias was observed among the different methods, always comprised between −0.12 μg/L and 0.16 μg/L (Figure 1), the t-statistic revealed significant differences for all comparisons except between Vitros cTnI ES and DXI AccuTnI, and between DXI AccuTnI and Architect STAT cTnl (Figure 1).

Discussion

The current recommendations of the IFCC Task Force on Clinical Applications of Cardiac Biomarkers contain an explicit suggestion that all contemporary sensitive and/or HS troponin immunoassays should be compared within the same population to establish whether or not some analytical and/or clinical differences may exist (10). This recommendation has, however, been mostly overlooked in the current scientific literature, since there are only two studies that have compared different TnI and TnT methods for establishing the 99th percentile values from a common, presumably healthy population (17–19). Even more importantly, no previous study has directly compared Tnl values obtained with four of the most frequently used contemporary sensitive immunoassays in the same population, to the best of our knowledge. This is noteworthy, considering that acquisition of troponin immunoassays is often part of large tenders for automated instrumentation, and the choice of immunochemistry analyzers specifically dedicated to the measurement of cardiac biomarkers is improbable and virtually unrealistic in a world of limited resources (8). The logical consequence is that critical patients might have their troponin tested with different methods between peripheral facilities and reference hospitals, where they are usually admitted for intensive therapeutic management according to a typical »hub and spoke« network that has been implemented in several countries (20), including our area of Northern Italy (21).

The first finding of this investigation confirms that a substantial bias exists between TnI and HS-TnT values obtained on an identical study population. Although the correlation between values was globally acceptable, the general agreement of diagnostic values (i.e., those exceeding the 99th percentile URL) between Roche HS-cTnT and the various contemporary sensitive TnI immunoassays was poor, with kappa statistics always lower than 0.50. Regardless of the current lack of standardization, direct comparison between the four most widespread contemporary sensitive TnI immunoassays generated more favourable results, with Pearson’s correlations greater than 0.970 and agreement of diagnostic values (i.e., kappa statistic) always equal to or higher than 0.59. In particular, an excellent agreement was found between Vitros cTnI ES and DXI 800 AccuTnI, with the correlation of 0.995, kappa statistic for diagnostic values of 0.97, optimal values of slope (1.04) and intercept (0.01) of the linear regression analysis and, even more importantly, clinically negligible bias (0.03 μg/L; 95% CI, 0.00–0.06 μg/L; p=0.052) and limited dispersion of values (Figure 1). This is impressive, considering that the two immunoassays use different cocktails of antibodies

| Table IV Linear regression analysis (LR) and Pearson’s correlation (r) for troponin I (TnI) values, agreement (kappa statistic and 95% CI) of TnI data exceeding the 99th percentile of the upper limit of the reference range of each immunoassay. |
|----------------|----------------|----------------|
|                | DXI 800 AccuTnI | Dimension Vista cTnI | Architect STAT cTnI |
| Vitros cTnI ES | y=1.04x + 0.01  | y=1.32x–0.01  | y=0.95x + 0.07 |
|                | r=0.995; p<0.001| r=0.995; p<0.001| r=0.980; p<0.001 |
|                | Kappa statistic, 0.97 | Kappa statistic, 0.86 | Kappa statistic, 0.62 |
|                | (0.92–1.03); p<0.001| (0.75–0.98); p<0.001| (0.46–0.78); p<0.001|
| DXI 800 AccuTnI| – | y=1.25x | y=0.90x + 0.07 |
|                | r=0.986; p<0.001| r=0.977; p<0.001| r=0.970; p<0.001 |
|                | Kappa statistic, 0.89 | Kappa statistic, 0.60 | Kappa statistic, 0.59 |
|                | (0.79–0.99); p<0.001| (0.44–0.76); p<0.001| (0.42–0.76); p<0.001|
| Dimension Vista cTnI | – | – | y = 0.71x + 0.09 |
|                | r=0.956; r<0.001| r=0.977; p<0.001| r=0.970; p<0.001 |
|                | Kappa statistic, 0.60 | Kappa statistic, 0.59 | Kappa statistic, 0.59 |
|                | (0.42–0.76); p<0.001| (0.42–0.76); p<0.001| (0.42–0.76); p<0.001|
Figure 1 Bland & Altman plots (mean and 95% CI bias) of the different troponin I (TnI) immunoassays.
It is also noteworthy that a predictable trend could not be observed in most cases, which means that the use of common calibration will not be effective to harmonize test results, and the underlying reason for such differences – as previously hypothesized – may be attributed to the heterogeneous cocktails of antibodies, that recognize different epitopes and molecular forms of TnI.

Although this study is limited by a lack of clinical outcomes for interpretation of individual discordant data, the results clearly attest that – beside a notable exception (i.e., Vitros TnI ES versus DXI 800 AccuTnI) – substantial discrepancies still exist among contemporary sensitive TnI immunoassays. The presence of random variation rather than constant bias among the different methods has been identified as the major contributor to this variance, making the objective of harmonization a very long and challenging enterprise.

Conflict of interest statement

The authors stated that there are no conflicts of interest regarding the publication of this article.

References


Received: August 16, 2013
Accepted: September 4, 2013