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MULTICENTER COMPARISON OF FOUR CONTEMPORARY SENSITIVE TROPONIN IMMUNOASSAYS

MULTICENTRIČNO POREĐENJE ČETIRI SAVREMENA IMUNOESEJA OSETLJIVA NA TROPONIN

Gian Luca Salvagno[1], Davide Giavarina[2], Moira Meneghello[1], Roberta Musa[3], Rosalia Aloe[3], Giorgio Da Rin[4], Giuseppe Lippi[3]

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,

Kratak sadržaj

Uvod: Radna grupa za kliničku primenu srčanih biomarkera Međunarodne federacije za kliničku hemiju trenutno preporučuje evaluaciju svih imunoeseja za troponin u okviru iste populacije, kako bi se uporedile njihove performanse. Zbog toga smo osmislili ovu multicentričnu studiju za poređenje četiri najčešće upotrebljavane savremene metode osetljive na troponin I (TnI).
Metode: Sedamdeset šest uzoraka seruma stavljeno je u centrifugu, razdvojeno i podeljeno u pet alikvota. Prvi alikvot je upotrebljen za kliničko merenje, dok su ostali poslati laboratorijama koje su učestvovale u istraživanju, gde su istovremeno otopljeni. Visokoosetljivi troponin T (HS-TnT) meren je na uređaju Roche Cobas, dok je TnI određen pomoću testova Ortho Vitros CtnI, Beckman Coulter DXI 800 Accu TnI, Siemens Vista cTnI i Abbott Achitect STAT cTnI.
Rezultati: Otkriveno je znatno odstupanje u vrednostima TnI i HSTnT. Iako je korelacija bila prihvatljiva i u rasponu od 0,86 do 0,89, slaganje dijagnostičkih vrednosti je bilo slabo, sa statističkom vrednošću »kappa« uvek manjom od 0,50. Iako je direktnim poređenjem četiri savremena imunoeseja osetljiva na TnI dobijeno više povoljnih rezultata, sa Pearsonovim korelacijama većim od 0,970 i statističkom vrednošću

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 cTnI versus DXI 800 AccuTnI).

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

»kappa« od 0,59 ili više, uočili smo velike 95% intervale poverenja, značajna odstupanja i veliko rasipanje vrednosti, uz jedan izuzetak (Vitros cTnI vs. DXI 800 AccuTnI).

Zaključak: Rezultati ove studije potvrđuju da još postoje značajna neslaganja između savremenih imunoeseja osetljivih na TnI. Kao glavni razlog nameće se prisustvo nasumičnih varijacija, pre nego konstantnih odstupanja, zbog čega ove metode nisu međusobno zamenjive a harmonizacija deluje kao cilj koji se neće brzo i lako dostići.

Ključne reči: infarkt miokarda, troponin, imunoeseji, poređenje

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 (TnI) 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 TnI and TnT are two structurally and

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

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

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 in our country, 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, as described elsewhere (11). The mean transportation time was 85±12 min. Upon arrival to the different centers, the samples were kept stored at –70 °C until all laboratories had received the shipment, thus allowing a simultaneous start of testing. Before analysis, all aliquots (thus including the sample for reassessment of HS-TnT) were thawed at room temperature and centrifuged. The analytical characteristics of the troponin immunoassays used in this study, as defined in previous investigations (12–16), are synthesized in *Table I*. The epitopes recognized by capture (C) and detection (D) monoclonal antibodies are C: 24–40, 41–49; D: 87–91 for Vitros cTnI ES, C: 41–49; D: 24–40 for DXI 800 AccuTnI, C: 27–32; D: 41–56 for Dimension Vista cTnI, and C: 87–91; 24–40; D: 41–49 for Architect STAT cTnI (17). Results of measurements were finally reported as mean and standard error of the mean (SEM). The statistical analysis of data was performed with Analyse-it for Microsoft Excel (Analyse-it Software Ltd, Leeds, UK), and data comparison was based on linear regression analysis, Spearman's correlation, agreement by kappa statistic and Bland & Altman plots with t-statistics. The study was based on pre-existing samples, the results were not reported and did not affect the clinical management of patients, so that ethical permission and informed consent were unnecessary according to our local ethical committee. The study was, however, performed in accordance with the Declaration of Helsinki and under the terms of all relevant local legislations.

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 signif-

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.

	Roche HS-cTnT	Vitros cTnI ES	DXI 800 AccuTnI	Dimension Vista cTnI	Architect STAT cTnI
Mean ± SEM (μg/L)	0.15±0.03	0.54±0.15	0.57±0.16	0.70±0.20	0.59±0.29
Values >99th percentile	54/76 (71%)	30/76 (39%)	29/76 (38%)	33/76 (43%)	45/76 (59%)

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).

	Vitros cTnI ES	DXI 800 AccuTnI	Dimension Vista cTnI	Architect STAT cTnI
Roche HS-cTnT				
Correlation	r=0.882; p<0.001	r=0.861; p<0.001	r=0.881; p<0.001	r=0.887; p<0.001
Kappa statistic	0.42 (0.26–0.58); p<0.001	0.40 (0.25–0.56); p<0.001	0.48 (0.31–0.64); p<0.001	0.23 (0.01–0.45); p=0.038

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 r=0.995; p<0.001 Kappa statistic, 0.97 (0.92–1.03); p<0.001	y=1.32x–0.01 r=0.995; p<0.001 Kappa statistic, 0.86 (0.75–0.98); p<0.001	y=0.95x + 0.07 r=0.980; p<0.001 Kappa statistic, 0.62 (0.46–0.78); p<0.001
DXI 800 AccuTnI	–	y=1.25x r=0.986; p<0.001 Kappa statistic, 0.89 (0.79–0.99); p<0.001	y=0.90x + 0.07 r=0.977; p<0.001 Kappa statistic, 0.60 (0.44–0.76); p<0.001
Dimension Vista cTnI	–	–	y = 0.71x + 0.09 r=0.970; p<0.001 Kappa statistic, 0.59 (0.42–0.76); p<0.001

icant 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 cTnI (*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 TnI 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

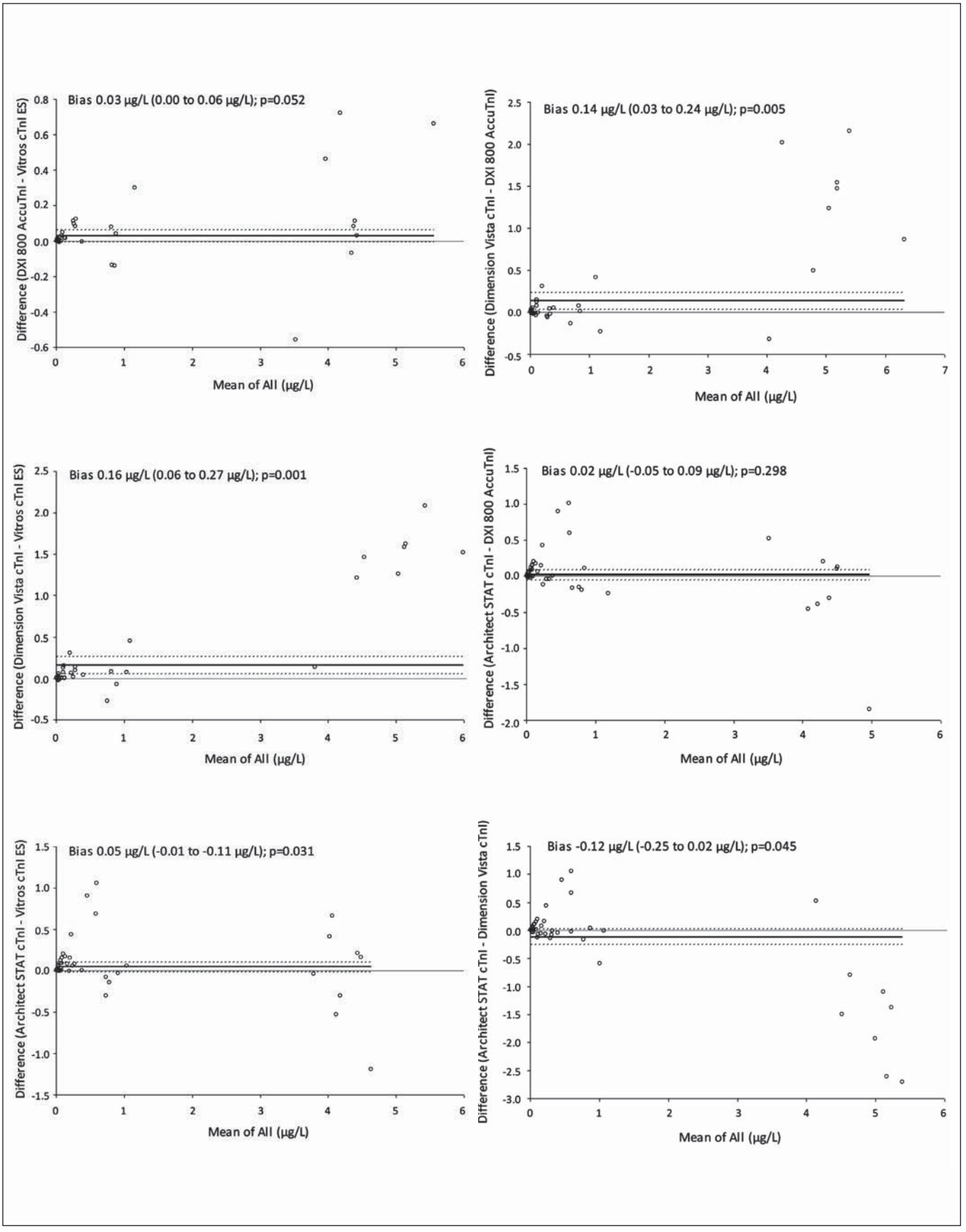

Figure 1 Bland & Altman plots (mean and 95% CI bias) of the different troponin I (TnI) immunoassays.

(*Table I*). Therefore, according to a genuine analytical perspective, this means that test results obtained with these two immunoassays may be potentially interchangeable, thus allowing longitudinal comparison of patient data using either technique in different laboratories within the same network (22). The comparison of data obtained with DXI 800 AccuTnI and Architect STAT cTnI also yielded a non-significant bias ($p=0.298$), but the agreement of diagnostic values was modest (kappa statistic of 0.60). The comparability of results among the other immunoassays was overall less favourable, in particular for the presence of a clinically meaningful bias and much greater dispersion of values in Bland and Altman plot analysis (*Figure 1*), which would clearly preclude interchangeability of data in clinical practice. The worst agreement was found by comparing Architect STAT cTnI with each of the other three cTnI immunoassays, with kappa values always below 0.60 and 95% CIs sufficiently wide that one can surmise that about half the data may be incorrectly classified. This discrepancy may be at least partially attributed to the unique combination of C antibodies of this assay (i.e., C: 87–91; 24–40), which differs widely from that of the others (i.e., C: 24–40, 41–49 for Vitros cTnI ES, C: 41–49 for DXI 800 AccuTnI, and C: 27–32 for Dimension Vista cTnI, respectively).

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 cTnI 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

1. Lippi G, Plebani M. Biomarker research and leading causes of death worldwide: a rather feeble relationship. Clin Chem Lab Med 2013; 51: 1691–3.
2. Lippi G, Franchini M, Cervellin G. Diagnosis and management of ischemic heart disease. Semin Thromb Hemost 2013; 39: 202–13.
3. Casagranda I, Cavazza M, Clerico A, Galvani M, Ottani F, Zaninotto M, Biasucci LM, Cervellin G, Lenzi T, Lippi G, Plebani M, Tubaro M. Proposal for the use in emergency departments of cardiac troponins measured with the latest generation methods in patients with suspected acute coronary syndrome without persistent ST-segment elevation. Clin Chem Lab Med 2013; 51: 1727–37.
4. Lippi G, Cervellin G. Do we really need high-sensitivity troponin immunoassays in the emergency department? Maybe not. Clin Chem Lab Med 2013 Jul 30: 1–8. doi: 10.1515/cclm-2013–0524. [Epub ahead of print]
5. Apple FS. A new season for cardiac troponin assays: it's time to keep a scorecard. Clin Chem 2009; 55: 1303–6.
6. Gaze D. Sensitive cardiac troponin assays: Myth and magic or a practical way forward? J Med Biochem 2010; 29: 270–3.
7. Lippi G, Cervellin G, Aloe R, Montagnana M, Salvagno GL, Guidi GC. Non-commutability of results of highly sensitive troponin I and T immunoassays. Biochem Med (Zagreb) 2012; 22: 127–9.
8. Lippi G, Cervellin G. Choosing troponin immunoassays in a world of limited resources. J Am Coll Cardiol 2013; 62: 647–8.
9. Apple FS. Counterpoint: Standardization of cardiac troponin I assays will not occur in my lifetime. Clin Chem 2012; 58: 169–71.
10. Apple FS, Collinson PO; IFCC Task Force on Clinical Applications of Cardiac Biomarkers. Analytical characteristics of high-sensitivity cardiac troponin assays. Clin Chem 2012; 58: 54–61.
11. Lippi G, Lima-Oliveira G, Nazer SC, Moreira ML, Souza RF, Salvagno GL, et al. Suitability of a transport box for blood sample shipment over a long period. Clin Biochem 2011; 44: 1028–9.
12. Giannitsis E, Kurz K, Hallermayer K, Jarausch J, Jaffe AS, Katus HA. Analytical validation of a high-sensitivity cardiac troponin T assay. Clin Chem 2010; 56: 254–61.
13. Zaninotto M, Vernocchi A, Di Serio F, Viloria Mdel M, Hurtado JM, Perez-Guerrero JJ, Plebani M. Assay performance improved, but which »scorecard« designation for Vitros Troponin I? Clin Chim Acta 2012; 413: 826–8.
14. Zaninotto M, Mion MM, Novello E, Moretti M, Delprete E, Rocchi MB, Sisti D, Plebani M. Precision performance at low levels and 99th percentile concentration of the Access AccuTnI assay on two different platforms. Clin Chem Lab Med 2009; 47: 367–71.

15. Arrebola MM, Lillo JA, Diez De Los Ríos MJ, Rodríguez M, Dayaldasani A, Yahyaoui R, Pérez V. Analytical performance of a sensitive assay for cardiac troponin I with loci technology. Clin Biochem 2010; 43: 998–1002.

16. La'ulu SL, Roberts WL. Performance characteristics of five cardiac Troponin I assays. Clin Chim Acta 2010; 411: 1095–101.

17. Apple FS, Ler R, Murakami MM. Determination of 19 cardiac troponin I and T assay 99th percentile values from a common presumably healthy population. Clin Chem 2012; 58: 1574–81.

18. Petersmann A, Ittermann T, Fries C, Lubenow N, Kohlmann T, Kallner A, Greinacher A, Nauck M. Comparison of the 99th percentiles of three troponin I assays in a large reference population. Clin Chem Lab Med 2013 Jul 12: 1–6. doi: 10.1515/cclm-2013-0113. [Epub ahead of print]

19. Koraćević G, Ćosić V, Stojanović I. False positive troponin – a true problem. J Med Biochem 2013; 32: 197–206.

20. Birkemeyer R, Dauch A, Müller A, Beck M, Schneider H, Ince H, Jung W, Wahler S. Short term cost effectiveness of a regional myocardial infarction network. Health Econ Rev 2013; 3: 10.

21. Nobilio L, Ugolini C. Selective referrals in a 'hub and spoke' institutional setting: the case of coronary angioplasty procedures. Health Policy 2003; 63: 95–107.

22. Lippi G, Simundic AM. Laboratory networking and sample quality: a still relevant issue for patient safety. Clin Chem Lab Med 2012; 50: 1703–5.

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