Introduction

The high-sensitivity immunoassays for cardiac troponin I (hs-cTnI) and cardiac troponin T (hs-cTnT) are actually recommended by all the most recent guidelines as gold standard laboratory methods for the detection of myocardial injury and the diagnosis of acute myocardial infarction (AMI) [1–8]. In particular, the Fourth Universal Definition of Myocardial Infarction [1] defines myocardial injury as a distinct condition characterized by at least one hs-cTnI or hs-cTnT value above the 99th percentile of the biomarker distribution values evaluated in a reference “healthy” adult population (99th reference upper reference limit URL value).

The use of hs-cTnI and hs-cTnT methods has allowed to progressively reduce the time to diagnosis of NSTEMI (Non-ST-Elevation Myocardial Infarction) from 6-12 h to less than 3 h in patients admitted to Emergency Department (ED) [2–8]. In particular, the most recent 2020 and 2023 European Society of Cardiology (ESC) guidelines recommend the fastest clinical algorithms with blood sampling on admission and after 1 or 2 h (0–1 h or 0–2 h) [4, 8]. This recommendation is based on data suggesting that these algorithms (especially the 0–1 h algorithm) allow to rule-in and rule-out NSTEMI in the shortest possible time [4, 8]. According to all the most recent guidelines [4–8], the use of accelerated diagnostic protocols can take advantage from the improved analytical performances of hs-cTnI and hs-cTnT methods in order to reduce the length of stay and crowding of patients in ED, and also the global cost related to the management of patients with acute chest pain.

In this issue of the Journal, Li et al. [9] are reporting the evaluation of the analytical performances and clinical results of a new chemiluminescent (CLIA) method for hs-cTnI (Mindray, Shenzhen, China) measurement in comparison with those of the well-established ARCHITECT hs-cTnI method (Abbott Diagnostics). The conclusions of Authors are that this new CLIA hs-cTnI method has high precision, sensitivity and specificity and also clinical diagnostic performances comparable to the ARCHITECT hs-cTnI assay [9]. Accordingly, the Authors suggest that this new CLIA hs-cTnI method is an attractive alternative for the diagnosis of myocardial infarction with a high level of accuracy and safety [9].

Of course, a new hs-cTnI method with an excellent analytical performance is a great news for all the clinical laboratories interested in the rule-in and rule-out of NSTEMI in patients admitted to ED. However, the accurate evaluation of the analytical performance of an immunoassay method for the cTnI is a very difficult task [10, 11], and therefore some further considerations are needed.

Evaluation of analytical performance of hs-cTnI and hs-cTnT assays

Li et al. [9] assessed the analytical performance of the CLIA method by evaluating the imprecision at sex-specific URLs and the detectable results above limit of detection (LoD) in a cohort of healthy population. Moreover, the sex-specific 99th percentile upper reference limits (99th percentile URL value) of the
CLIA hs-cTnI assay were determined from a healthy population of 424 males and 408 females, and the diagnostic performance was validated in a population of 934 patients with suspected acute coronary syndrome (ACS) [9]. Authors reported that sex-specific 99th percentile URL values were 15.3 ng/L for female, 31.3 ng/L for male and 24.2 ng/L for overall population, respectively. Moreover, the interferences with triglycerides, conjugated and unconjugated bilirubin, antinuclear antibodies, and rheumatoid factors were also evaluated. Finally, the diagnostic sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and area under curve (AUC) of hs-cTnI CLIA assay were 97.97 %, 90.70 %, 79.02 %, 99.21 % and 0.9885, respectively, which are comparable to the ARCHITECT hs-cTnI assay [9]. According to the authors conclusions, therefore, this hs-cTnI CLIA assay is a method with clinical diagnostic performance comparable to that of the ARCHITECT hs-cTnI assay [9].

The diagnostic accuracy of the rapid rule-in and rule-out protocols for AMI is closely linked to the improved analytical performance of hs-cTnI and hs-cTnT methods at low concentrations range compared to previous methods with contemporary analytical characteristics [6,10,11]. Indeed, the most popular commercially available hs-cTnI and hs-cTnT assays in European and North American countries show LoD values <5 ng/L and also similar imprecision profiles, when evaluated in the same clinical laboratories using standardized analytical protocols (Figure 1) [10–12].

Table 1 shows the limit of blank (LoB) LoD and limit of quantitation (LoQ) at 10 ng/L values of the CLIA hs-cTnI reported by Li et al., by the manufacturer (Mindray, Shenzhen, China), and also of the analytical ARCHITECT hs-cTnI method, evaluated by a reference laboratory of a multicenter study using a standardized protocol. The analytical results of the CLIA hs-cTnI methods reported by Li et al. [9] are significantly lower than those reported for the same method by the manufacturer and also than those reported by a reference laboratory for the ARCHITECT hs-cTnI method [10–12]. In particular, the calculated LoB value by Li et al. [9] is not significant different to 0 ng/L, and so also the LoD value, which is calculated from the LoB value, is significantly lower than that reported by the manufacturer for the CLIA hs-cTnI method and by other Authors for the ARCHITECT hs-cTnI method [10–12]. Accordingly, the data reported by Li et al. [9] on the analytical performance of the CLIA hs-cTnI method are impressive and unexpected and deserve a more detailed discussion.

Li et al. [9] reported that the analytical parameters of the CLIA hs-cTnI method were calculated according to the reference CLSI documents EP05-A3 2014 and EP17-A2 2012, published before the development of high-sensitivity immunometric assays for cTnI and cTnT [13, 14]. These CLSI documents recommend some general analytical procedures for all laboratory test methods (not only for assay of cardiac troponins). Accordingly, these protocols should be specifically adapted for the accurate evaluation of the very low LoB values showed by the hs-cTnI and hs-cTnT immunometric assays [10, 11]. The LoB value is usually defined as the highest apparent analyte concentration expected to be...
found when replicates of a blank sample containing no analyte are tested [10, 11]. According to Armbruster and Pry [15], although the samples tested to evaluate the LoB values do not contain the biomarker (i.e., a zero concentration is expected), a blank (zero) sample can produce an analytical signal on the clinical laboratory instrumentation used for hs-cTnI and hs-cTnT assay, that might otherwise be consistent with a low estimated concentration of the biomarker. In particular, the accurate evaluation of the LoB value for an immuno-metric assay method strictly depends on both curvilinear shape and inter-assays variation of biomarker concentrations measured by the automated laboratory instrumentation close to the 0 point of the calibration curve of the hs-cTnI and hs-cTnT methods (usually fitted using some regression models, such as a 4-parameter logistic function) [10, 11].

As theoretically expected, for each immunometric method, the variations close to the 0 point of the calibration curve is strongly dependent to the number of measured curve replicates and especially to the number of different lots of reagents and standard calibrators used for evaluation of the LoB value with the automated laboratory instrumentation [10, 11]. As previously discussed in detail [10, 16], an accurate evaluation of the LoB value of a hs-cTnI and hs-cTnT immunometric assay is a very expensive and difficult task because the experimental protocol usually requires several curve replicates performed during two or more months using at least two lots of reagents and calibrators, as well as a specific analytical skills practice.

The lower LoB and LoD values of the CLIA hs-cTnI method reported by Li et al. [9] compared to the respective values suggested by manufacturer (Table 1) are probably due to a lower number of experimental tests performed in their study. This explanation is also confirmed by the data reported in this study [9] about the imprecision profile of the CLIA hs-cTnI method. Recent studies performed in the same laboratory using standardized protocols have demonstrated that imprecision profile of the most popular hs-cTnI methods and even of the hs-cTnT method are very similar [10–12, 16], while the data related to the imprecision profile of the CLIA method reported by Li et al. [9] are significantly lower (Figure 1), suggesting that inter-assays variability of the CLIA hs-cTnI (expressed as CV%) may be underestimated in this study. Indeed, in Figure 1 are reported the imprecision profiles calculated by a reference laboratory using a standardized protocol for the most popular hs-cTnI methods and the hs-cTnT method compared to that reported by Li et al. [9]. In particular, it is evident that the 10 % CV of the biomarker concentration of the CLIA (i.e., 0.21 ng/L), reported by Li et al. [9] is significantly lower than those reported by all other hs-cTnI methods and even the hs-cTnT method (i.e., about 4–10 ng/L) [10–12, 16].

### Interferences in hs-cTnI and hs-cTnT methods

Another important analytical problem related to hs-cTnI methods is the presence of some interfering substances in the measured sample which may affect the measurement of the biomarker [11]. Li et al. [9] excluded the interferences related to triglycerides, unconjugated and conjugated bilirubin and hemoglobin. Moreover, 300 samples positive for Anti-Nuclear Autoantibodies (ANA) and other 300 positive for rheumatoid factor (RF) were also tested to exclude a possible interference. In the already cited study, no false positive or false negative sample was observed for ANA, while only one sample was found positive for RF [9].

However, the presence in blood samples of circulating cTnI autoantibodies or macrotraponin complex is currently considered the most common cause of interference in hs-cTnI methods [11, 17–24]. The presence of the interfering substances has a progressively greater effect as the true TnI concentration in the specimen tends toward zero [11]. Accordingly, interferences strongly increase the error in measurement at very low analyte concentrations and consequently it can significantly increase the measured hs-cTnI concentration above the cut-off value suggesting to clinicians a false diagnosis of myocardial injury [1]. Another important point is that the hs-cTnI methods may be differently affected by the presence of macro-complexes [1]. In particular, a study by Kavساκ et al. [22] reports that the Access hs-cTnI method (Beckman Coulter Diagnostics) was found less affected by this type of interference than the ARCHITECT hs-cTnI method (Abbott Diagnostics). Accordingly, the presence of macro-complexes may, at least in part, explain the difference in biomarker measurement among the hs-cTnI methods [10, 11, 16, 22].

### Table 1: Comparison between the sensitivity parameters of the new CLIA hs-cTnI method, reported by Li et al. and the manufacturer and those of ARCHITECT hs-cTnI method reported by a reference laboratory [10].

<table>
<thead>
<tr>
<th>hs-cTnI method</th>
<th>Study [Reference]</th>
<th>LoB, ng/L</th>
<th>LoD, ng/L</th>
<th>LoQ 10 %, ng/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>CLIA hs-cTnI</td>
<td></td>
<td>0.07</td>
<td>0.21</td>
<td>0.51</td>
</tr>
<tr>
<td>Li et al. [9]</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CLIA hs-cTnI</td>
<td>Manufacturer (Mindray, Shenzhen, China) [9]</td>
<td>0.1–0.5</td>
<td>0.5–0.7</td>
<td>1.1–2.4</td>
</tr>
<tr>
<td>Clérıco et al. [10]</td>
<td></td>
<td>0.7</td>
<td>1.3</td>
<td>4.7</td>
</tr>
</tbody>
</table>

[1]: Armbruster and Pry [15]
[10]: Li et al. [9]
[11]: Li et al. [9]
[12]: Li et al. [9]
[13]: Li et al. [9]
[14]: Li et al. [9]
[15]: Li et al. [9]
[16]: Li et al. [9]
[17]: Li et al. [9]
[18]: Li et al. [9]
[19]: Li et al. [9]
[20]: Li et al. [9]
[21]: Li et al. [9]
[22]: Li et al. [9]
[23]: Li et al. [9]
[24]: Li et al. [9]
Discussion and conclusions

It is conceivable that a validation protocol using more different lots of reagents and standards as well as also several replicates in the same and different working days should improve the accuracy of sensitivity and reproducibility of hs-cTnI methods, but a more accurate validation protocol is also more demanding and expensive for individual clinical laboratories [10, 11, 15].

Despite that all international guidelines recommend the use of hs-cTnI and hs-cTnT methods for the diagnosis of AMI [4–8], at present time there is no agreement about the experimental protocols required to accurately assess the analytical parameters of these methods and also on how these results should be reported in scientific articles [10, 11]. Furthermore, there are some differences even between the FDA and EMA guidelines, which makes it difficult to validate hs-cTnI methods that are compliant to recommendations of both agencies [25–27]. As also observed by some international guidelines [25, 26], this dis-homogeneity in the validation and reporting performances of different methods may induce some difficulties in the implementation of hs-cTnI and hs-cTnT in clinical practice.

We could agree with the conclusions made by Li et al. [9] that the CLIA hs-cTnI method evaluated in their study shows analytical sensitivity and diagnostic performance comparable to those of the Architect hs-cTnI method. However, the excellent analytical performance of this CLIA hs-cTnI assay should be confirmed by other experimental studies using a standardized protocol [10, 11, 14].

References