Letter to the Editor

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Evaluation of reference change values for a hs-cTnI immunoassay using both plasma samples of healthy subjects and patients and quality control samples

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To the Editor,

In 2016, the guidelines of European Society of Cardiology (ESC) on the management of acute coronary syndromes (ACS) in patients presenting without persistent ST-segment elevation (NSTEMI) suggested 0 h/1 h rule-in and rule-out algorithms when measurement of cardiac troponins is performed using high-sensitivity immunoassay methods [1]. These guidelines recommend the use of absolute change (i.e. expressed as difference in cardiac troponins (cTn) concentrations, ng/L) rather than percentage variation for the assessment of the rise and/or fall of cTn values [1]. In particular, these guidelines suggest that very small absolute differences in cTnI concentrations (such as 2, 5 and 6 ng/L) may be used for the rule-in or rule-out of NSTEMI with Architect hs-cTnI method [1]. Although the guidelines state that the 0 h/1 h algorithms have been previously validated [1], to the best of our knowledge, there are no data available in the literature on the reference changing values (RCV) concerning the Architect hs-cTnI method. Therefore, the aim of this study was to evaluate the RCV for the hs-cTnI Architect method, especially for the range of cTnI concentrations below the 99th percentile of the reference population (URL).

The analytical characteristics and performance of the hs-cTnI method using the i1000SR platform (ARCHITECT STAT High Sensitive Troponin-I, Abbott Diagnostics Division, Ireland) were previously evaluated and compared with those of other hs-cTnI immunoassay methods in our laboratory using standardized protocols [2–4]. The limit of blank (LoB) and limit of detection (LoD) for the Architect hs-method were 0.7 ng/L and 1.3 ng/L, respectively [2–4]. The reference values (i.e. the 99th percentile upper limit values, URL), suggested by the manufacturer, for men, women and total population are 15.6 ng/L (90% CI: 13.8–17.5 ng/L), 34.2 ng/L (28.9–39.2 ng/L) and 26.2 ng/L (23.3–29.7 ng/L), respectively.

The imprecision profile of the hs-cTnI Architect method is reported in Figure 1. Seven plasma samples of healthy subjects and patients with cardiac diseases with mean cTnI concentrations from 1.3 ng/L to 100.9 ng/L were used for the calculation of the imprecision profile [2–4]; these plasma pools were measured in 39 different runs with three different lots of reagent materials and calibrators throughout 2 months. Five control samples were also used for the calculation of the imprecision profile. One of these (mean cTnI concentration: 19.7 ng/L) was the low control value, distributed by the manufacturer. The other four quality controls were distributed in an external quality assessment (EQA) program (mean cTnI concentrations: 34.9 ng/L, 45.9 ng/L, 107.9 ng/L, and 136.2 ng/L, respectively) (Qualimedlab, Pisa Italy). The five control samples were used for the laboratory internal quality control for 1 year (from January to December 2018). According to the imprecision profile reported in Figure 1, the limits of quantitation (LoQ) at 20% and 10% were 1.8 ng/L and 4.8 ng/L, respectively.

According to Fraser [5], the bidirectional Z-score RCV between two results (95% CI) can be calculated by considering both the analytical variability of the method (CV_A) and the intra-individual variability (CV_I), using the Equation 1:

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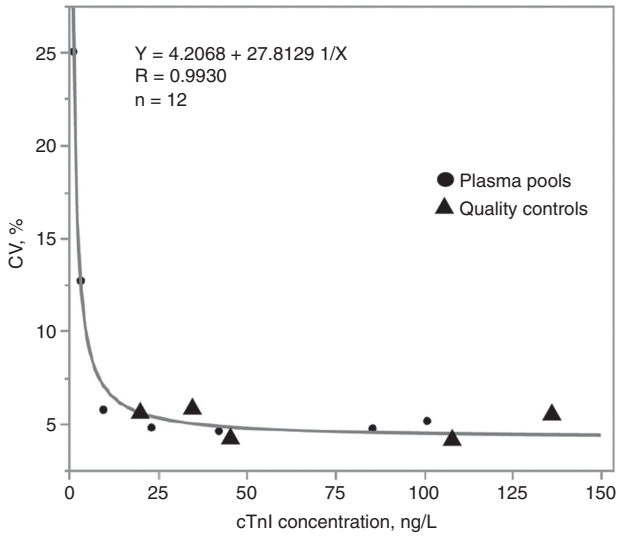

Figure 1: Imprecision profile of the hs-cTnI method using ARCHITECT i1000SR platform (STAT Architect high-sensitivity TnI method, Abbott Diagnostics, Ref. B3P250).
For the calculation of this imprecision profile, seven plasma pools and five quality control samples were assayed. The seven plasma pools were collected from healthy subjects and cardiac patients and measured in 40 different runs throughout 60 working days using three different lots of reagents and calibrators [2, 3].

$$RCV = 1.96[2(CV_A^2 + CV_I^2)]^{1/2} \quad (1)$$

Van der Linden et al. [6] recently reported that the CV_I of cTnI measured with the Architect method is on average 8.6% (95%CI 7.6%–9.7%) in adult healthy subjects. Therefore, considering the CV_A values calculated from the imprecision profile (Figure 1) and rounding the CV_I value to 9%, it is possible to calculate, according to the Eq. 1, the RCV and Δ change values for the range of cTnI concentrations from 2 ng/L to 40 ng/L, as reported in Table 1.

Between-laboratory variability (expressed as CV%) was also evaluated using 26 quality control samples distributed in an EQA program (Qualimedlab, Pisa, Italy) [7]. In the years 2017 and 2018, 19 Italian clinical laboratories participated in this EQA and produced a total of 450 cTnI results using the Architect method (i.e. 221 in the 2017 and 229 in the 2018, respectively). The cTnI mean concentrations of these control samples ranged from 22.5 ng/L to 3406.8 ng/L, and the mean between-laboratory variability was 5.8% (SD = 1.4%, minimum value 2.6%, maximum value 9%). These data demonstrate that the imprecision for cTnI concentrations above the 99th percentile URL value is nearly constant around a CV value of 5%–6%, confirming the results found by the imprecision profile (Figure 1). This observation implies that the contribution of biological/physiological variance is the major contribution (i.e. CV_I approximately 9%) to the total variance in an individual with elevated cTnI (CV_A approximately 5%–6%). However, at the present time, there are no hs-cTnI data supporting a proportional increase of the physiological (CV_I) contribution to variance in different pathological situations. Therefore, specific clinical studies are needed to evaluate this important issue.

Table 1: Reference change value (RCV) and absolute critical change (Δ change) of the hs-cTnI Access method using Architect platform in the range of cTnI concentration from 2 ng/L to 40 ng/L.

cTnI concentration, ng/L	CV_A, %	RCV 95% CI, %	Δ Change 95% CI, ng/L	RCV 99% CI, %	Δ Change 99% CI, ng/L
2	18.1	56.1	1.1	73.8	1.5
5	9.8	36.9	1.8	48.6	2.4
10	7.0	31.6	3.2	41.6	4.2
15	6.1	30.1	4.5	39.7	6.0
20	5.6	29.4	5.9	38.6	7.7
40	4.9	28.4	11.4	37.3	14.9

CV_A, Analytical variability evaluated according to the imprecision profile (Figure 1). RCV 95% and 99%, RCVs calculated at the probability of 95% and 99% CI, respectively. Δ Change 95% and 99%, Absolute critical change calculated at the probability of 95% and 99%, respectively. These change values were calculated assuming an intra-individual variability (CV_I) in healthy adult subjects of 9%, as reported by Van der Linden et al. [6]. According to the results reported in Figure 1, the analytical variability per cTnI values ≥40 ng/L is constant and equal to about 5%, and so the RCV 95% and 99% CI are also constant and equal to about 28% and 37%, respectively.

Our results are well in agreement with the absolute delta change values recommended by the 2016 ESC guidelines for the Architect cTnI method concerning the 0 h/1 h rule-out algorithm for the diagnosis of non-ST segment elevation MI [1]. Indeed, the ESC guidelines suggest for the rule-out of ACS-STEMI a hs-cTnI value measured at 0 h (admission to Emergence Department, ED) lower than 2 ng/L, or a 0 h value lower than 5 ng/L with a difference (Δ) 0 h/1 h less than 2 ng/L [1]. According to the data reported in Table 1, a cTnI value of 2 ng/L is not significantly (99%CI) different to the LoB (0.7 ng/L) and LoD (1.3 ng/L) of the Architect hs-cTnI method [2, 3]. For the rule-in of ACS-STEMI, ESC guidelines suggest a Δ change between cTnI values at admission and after 1 h of 6 ng/L [1], which is a cTnI value significantly higher than the LoD (Table 1).

It is important to note that our results may also be useful for the evaluation of cardiovascular risk in

the general population. Several studies demonstrated that the cardiovascular risk in the general population increases continuously and progressively from very low cTnI values, measured with high sensitivity methods. As an example, in the North-Trøndelag Health (HUNT) study [8] hs-TnI was measured with the Architect method in a cohort of 9005 participants free from known cardiovascular disease at baseline. During a median follow-up period of 13.9 years, 733 participants reached the composite end point of hospitalization for MI, heart failure, or cardiovascular death. Adding hs-TnI to established cardiovascular risk prediction models led to a net reclassification improvement higher than that obtained by including only the classic cardiovascular risk factors [8]. It is important to note that the range values for tertiles with the intermediate risk were 4–10 ng/L for women and 6–12 ng/L for men [8], respectively. These cTnI values are slightly higher than the upper 99%CI value of the LoD for the hs-cTnI Architect method (Table 1). Furthermore, the results of this study confirmed that the combined mortality and cardiovascular risk significantly increases even for cTnI values well below the 99th percentile URL values, divided for sex, as suggested by the manufacturer (i.e. 15.6 ng/L for women and 34.2 ng/L for men) [8]. According to these data, the observation of an increment in hs-cTnI levels, even of only 3–5 ng/L over some months in a patient with a suspect of cardiopathy should suggest an ongoing myocardial remodeling, potentially leading to the development of symptomatic heart failure [9, 10].

The Fourth Universal Definition of Myocardial Infarction [11] has recently stressed the fundamental role of cTnI and cTnT assay in the detection of myocardial injury and consequently in the diagnosis of MI. High-sensitivity cTn are able to promptly identify patients with asymptomatic myocardial injury, who are at highest risk of heart failure development. The early detection of patients at high risk should improve an early diagnosis, thereby also possibly warranting a better prognosis.

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