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Clinical laboratory practice recommendations for high-sensitivity cardiac troponin testing

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Introduction

The role of cardiac troponins (cTn) have become increasingly important in diagnosing myocardial infarction (MI), especially in patients without electrocardiogram abnormalities (1). Since the introduction of high-sensitivity (hs-)cTn immunoassays, there has been extensive clinical guidance on utilizing these biomarkers in patients with acute coronary syndromes (2). However, recommendations from a laboratory perspective were lacking until the recently reported consensus recommendation from the Academy of the American Association for Clinical Chemistry and the Task Force on Clinical Applications of Cardiac Bio-Markers of the International Federation of Clinical Chemistry and Laboratory Medicine by Wu and colleagues (3). This globally relevant expert opinion provided ten clinical laboratory practice recommendations associated with hs-cTn testing (3). These important consensus perspectives were developed to provide global consistency and knowledge in areas where formal guidance and/or data evidence was incomplete. In this Editorial, we not only acknowledge multiple recommendations as defined by Wu and co-workers, but also highlight several specific key aspects from our perspective.

Required hs-cTn guidance for clinicians

Since the launch of the first hs-cTn immunoassay (hs-cTnT, Roche), which was regulatory approved (CE Mark) outside the United States (OUS) in 2010, we recognize that the role of laboratory specialists in educating clinicians, both primary care physicians and clinical specialists, increased significantly. Guidance is predominantly required for patients with a hs-cTn concentration exceeding the MI cut-off threshold, with a rise or fall that is not that obvious, or with a negative coronary angiography. In addition, numerous (pre-)analytical factors and biological variability can lead to hs-cTn results that require guidance from clinical laboratory specialists. Although a lot of research focuses on (patho)physiological properties of cTn and its future potentials, in current clinical practice cTn are biomarkers for MI mandated by guidelines to be evaluated following a serial sampling protocol (4). As Wu *et al.* appropriately described, laboratory specialists should educate clinicians on the importance of specific metrics to differentiate clinically relevant hs-cTn concentration changes from analytical and biological variation. As minor hs-cTn changes can have significant clinical impact at a patient level, validating daily quality control (QC), especially

at the lower analytical measuring range, is essential. These should preferably be worldwide commutable QC materials for harmonization of the different hs-cTn immunoassays leading to reduction of interassay bias.

hs-cTn cut-off values

The third universal definition of MI recommends cTn testing with a defined cut-off value based on the 99th percentile upper reference limit (URL) of a healthy population (2). Due to significant sensitivity increases in the most recent generation hs-cTn immunoassays, very low hs-cTn concentrations can be measured with excellent reproducibility [coefficient of variation (CV) smaller than 10%] (5). Unfortunately, this significant increase in assay sensitivity led to decreased clinical specificity as detectable hs-cTn concentrations can now be measured in other (non-)pathological conditions in absence of MI (6-8). In addition to multiple co-morbidities, also age and sex influence hs-cTn concentrations (9,10). Consequently, the population used to determine the 99th percentile URL should be carefully selected. Sandoval *et al.* provided several key recommendations and proposed that multiple surrogate biomarkers should be evaluated to define a healthy population without co-morbidities that influence hs-cTn results (11). In addition, medical history and medication usage should be taken into account and the population should be diverse with gender, age and ethnicity appropriately distributed (11). Although we acknowledge their proposal, there is thus far no global consensus on how to define the population used to determine the 99th percentile URL specifically for hs-cTn testing. A perfectly healthy population without hs-cTn influencing co-morbidities and medications will not be a representative population of patients presenting with suspected MI to the emergency department (ED). From our experience, and also reflected by variable MI cut-off values reported in literature, this resulted in a rather heterogeneous implementation of 99th percentiles across clinical laboratories, especially for cTnI (11,12). We therefore discourage clinical laboratories to individually determine their own 99th percentile cut-off threshold for MI and recommend them to adapt cut-off values derived from large cohorts in peer-reviewed literature (13-15).

Comparison of hs-cTnI and hs-cTnT

Both hs-cTnT and hs-cTnI provide high diagnostic and prognostic accuracy in patients presenting to the ED with

acute chest pain (16). Therefore, both assays are considered equivalent and laboratories usually implement one hs-cTn immunoassay, which in practice predominantly depends on the clinical chemistry analyzer series used within the clinical laboratory. Nevertheless, it appeared that hs-cTnI seemed to be more prone to outliers compared to hs-cTnT (10,11,13). In addition, harmonization of hs-cTnI assays (currently strictly regulatory cleared OUS; CE Mark) is still an issue due to the heterogeneity of multiple available assays (6). Apart from analytical heterogeneity, studies conducting hs-cTn assays also highlighted possible biological differences between cTnI and cTnT (16-18). These include the diurnal rhythm of cTnT versus random fluctuation of cTnI, subtle differences in diagnostic performance and clinical decision limits that are not biologically equivalent for cTnT and cTnI (16-18).

Hs-cTnT assay characteristics

In January 2017, the United States (US) Food and Drug Administration (FDA) cleared the fifth generation cTnT assay by Roche Diagnostics and reported it to be an hs-cTnT assay. Interestingly, the US FDA prescribed assay limits that are not identical to those recommended in OUS CE marked countries. This was mainly due to the fact that different populations were used to determine their respective 99th percentile URL. The limit of blank (LoB) and limit of detection (LoD), on the other hand, were based on an identical protocol (EP17-A2) and resulted in comparable cut-offs (*Table 1*) (19). Additionally, the limit of quantification (LoQ) in the US is 6 ng/L as determined by FDA, while this is 13 ng/L in CE marked countries. This is explained by the fact that the US FDA defined the LoQ at the lowest concentration with a CV $\leq 20\%$ in contrast to a CV $\leq 10\%$ in OUS countries.

From a reporting perspective, US clinical laboratories are mandated by the FDA to apply the LoQ (CV $\leq 20\%$) as the lowest reportable value, while this is less strictly regulated for OUS clinical laboratories. Thus, a very important characteristic for OUS clinical laboratories is to define their lowest reportable hs-cTnT concentration (*Table 2*). Applying the LoQ would ensure that all reported results are precise, but since serial sampling is advised in European guidelines (4), we believe that especially a change in hs-cTnT is utterly relevant and therefore recommend to use the LoD (3 ng/L; e601/2) as the lowest reportable hs-cTnT concentration.

Table 1 US and OUS hs-cTnT assay limits as described in the package inserts (Roche Diagnostics)

hs-cTnT assay limits	Module	US hs-cTnT concentration (ng/L)	OUS hs-cTnT concentration (ng/L)
Limit of blank	e411	3	2.57
	e601/2	2.5	2.26
Limit of detection	e411	5	4.88
	e601/2	3	2.85
Limit of quantification	e411	6 (CV 20%)	13 (CV 10%)
	e601/2		

Package insert versions: US; 2018-02, V1.0–OUS; 2017-03, V9.0. US, United States; OUS, outside the United States; CV, coefficient of variation.

Table 2 Lowest reportable hs-cTnT concentration scenarios per region and analyzer as described in the package inserts (Roche Diagnostics)

Region	Module	Lowest reportable hs-cTnT concentration (ng/L)
US	e411, e601/2	6 (LoQ at CV 20%)
OUS	e411	5 (LoD)
		13 (LoQ at CV 10%)
	e601/2	3 (LoD)
		13 (LoQ at CV 10%)

Package insert versions: US; 2018-02, V1.0–OUS; 2017-03, V9.0. US, United States; OUS, outside the United States; LoQ, limit of quantification; CV, coefficient of variation; LoD, limit of detection.

The importance of blood matrices and cTnT degradation

Solely lithium heparinized (LH) plasma is approved to be used for the hs-cTnT immunoassay in the US while several blood matrices were allowed for the fourth-generation immunoassay. Clinical centers in the US should take this into consideration when implementing or transferring to the hs-cTnT assay. Outside the US, multiple blood matrices are allowed but comparing hs-cTnT concentrations across blood matrices is discouraged when applying observation algorithms in suspected MI patients. Recent studies demonstrated altered molecular cTnT form compositions in MI patients between blood matrices with smaller molecules in serum compared to LH plasma (20). This could lead to altered assay immunoreactivity that potentially influences hs-cTnT results.

In addition to pre-analytical cTnT proteolysis, *in vivo* cTnT fragmentation was also observed in patients suffering

from MI and ESRD patients with distinctive molecular compositions (21,22). Future research should be performed to investigate the immunoreactivity of these fragments towards the current hs-cTnT assay, but even more importantly, investigate whether specific cTnT fragments could be a target for enhanced assay specificity for MI. This was also recently recognized and suggested by other experts in the field (23,24).

Thus, although the effect of pre-analytical and/or *in vivo* cTnT degradation on the hs-cTnT immunoassay and their direct impact on clinical decisions still remains to be investigated, we recommend OUS clinical laboratories to standardize the blood matrix for hs-cTnT testing. In addition, we advise LH plasma to be used for the most efficient turnaround times promoting clinical decision making. Furthermore, we agree with Wu and colleagues regarding extensive documentation of (pre-)analytical variables when reporting hs-cTn values (3). This applies both in a clinical and research setting where hs-cTn values are reported.

Conclusions

The introduction of hs-cTn immunoassays allowed accurate assessment of hs-cTn concentrations at very low concentrations with excellent precision. Despite its outstanding diagnostic and prognostic value in MI diagnoses, the increase in assay sensitivity led to decreased clinical specificity due to (pre-)analytical and/or (patho-) physiological influences. Guidance, education, and support of clinicians by laboratory specialists will remain essential until hs-cTn specificity for MI is enhanced.

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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