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Analytical Considerations in Deriving 99th Percentile Upper Reference Limits for High-Sensitivity Cardiac Troponin Assays: Educational Recommendations from the IFCC Committee on Clinical Application of Cardiac Bio-Markers

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Running head: The 99th percentile URLs for hs-cardiac troponins

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Abbreviations

cTn: Cardiac troponin

hs-cTn: High-sensitivity cardiac troponin

UDMI: Universal definition of myocardial infarction

MI: Myocardial infarction

ED: Emergency department

URL: Upper reference limit

CI: Confidence interval

NSTEMI: Non-ST- elevation myocardial infarction

NT-proBNP: N terminal pro B-type natriuretic peptide

BNP: B-type natriuretic peptide

HbA1c: Hemoglobin a1c

eGFR: estimated glomerular filtration rate

Abstract

The International Federation of Clinical Chemistry Committee on Clinical Application of Cardiac Bio-Markers provides evidence-based educational documents to facilitate uniform interpretation and utilization of cardiac biomarkers in clinical laboratories and practice. The committee's goals are to improve the understanding of certain key analytical and clinical aspects of cardiac biomarkers and how these may interplay in clinical practice. Measurement of high-sensitivity cardiac troponin (hs-cTn) assays is a cornerstone in the clinical evaluation of patients with symptoms and/or signs of acute cardiac ischemia. To define myocardial infarction, the Universal Definition of Myocardial Infarction requires patients who manifest with features suggestive of acute myocardial ischemia to have at least one cTn concentration above the sex-specific 99th percentile upper reference limit (URL) for hs-cTn assays and a dynamic pattern of cTn concentrations to fulfill the diagnostic criteria for MI. This special report provides an overview of how hs-cTn 99th percentile URLs should be established, including recommendations about pre-screening and the number of individuals required in the reference cohort, how statistical analysis should be conducted, optimal pre-analytical and analytical protocols, and analytical/biological interferences or confounds that can affect accurate determination of the 99th percentile URLs. This document also provides guidance and solutions to many of the issues posed.

Keywords: cardiac troponin, high-sensitivity cardiac troponin, 99th percentile, chest pain, diagnosis, myocardial infarction, myocardial injury, prognosis, non-ischemic cardiovascular disease, normality, reference interval

Introduction

Cardiac troponin (cTn) measurements play a fundamental role in defining myocardial infarction (MI) per the Universal Definition of Myocardial Infarction (UDMI) guidelines *(1)*. High-sensitivity cardiac troponin (hs-cTn) assays are recommended. hs-cTn testing permits the early and rapid exclusion of MI in the emergency department (ED) in a substantial number of patients in addition to identifying those with a very high probability of MI *(2-4)*. hs-cTn testing also provides classification of those at short and long-term risk for major adverse cardiovascular events and mortality even in the absence of MI. The 99th percentile upper reference limit (URL) has been endorsed as the recommended hs-cTn threshold in the UDMI and the laboratory medicine community for over 20 years *(1, 5-7)*. Clinical diagnostic criteria include a rise and/or fall in hs-cTn concentrations with at least one value above the 99th percentile sex-specific URLs, all taken in conjunction with clinical findings thought to be indicative of acute ischemia *(1)*.

The 99th percentile is derived from cTn measurements in an apparently healthy cohort and is dependent on participant selection, statistical analyses and analytical and biological variability. These metrics are not standardized between studies, therefore different 99th percentiles may be recommended for the same hs-cTn assay *(8-10)*, which can potentially lead to non-conformity of how acute MI is diagnosed. This issue is accentuated by the fact that many centers do not even use the 99th percentile URL. This is problematic for the individual patient who may receive a different diagnosis depending on which URL the local hospital has implemented. It is also challenging from an epidemiological standpoint, making comparisons between hospitals in the same region difficult and potentially masking differences in treatment, follow-up and survival. To increase harmonization of an MI diagnosis, a common protocol for deriving 99th percentile URLs should be applied in similar ethnic/racial populations. This may be accomplished in several ways, but one feasible option is the use of large high-quality studies deriving 99th URLs that are applicable for different population regions. These should then be documented and communicated within the manufacturers' package inserts, and thereafter implemented by the local laboratories in the relevant region. This special report provides an

overview of how 99th percentile URLs should be derived and recommendations that may be useful for manufacturers, clinical laboratories, and research studies aimed at harmonizing the diagnosis of MI. We include some changes, modifications, and clarifications in comparison to previous recommendations that are intended to increase the robustness of future 99th percentile URL determinations, including the following areas:

a) pre-inclusion screening procedures needed to document and ensure all subjects in the reference cohort are healthy;
b) an increase in the number of male and female subjects, to allow for improved accuracy when calculating the sex-specific 95% CI for the 99th percentile;
c) investigation of biological interferences in specimens that demonstrate unexpected hs-cTn concentrations, based on clinical assessment or statistical outlier analyses;
d) standardization of pre-analytical factors and elimination of analytical interferences;
e) utilization of several reagent and calibrator lots to reduce influences in lot shifts when determining the hs-cTn 99th percentile.

Cardiac troponin 99th percentile URLs applied in the Universal Definition of Myocardial Infarction

Clinical laboratory reference intervals are fundamental tools utilized by the medical community and patients to interpret laboratory test results and distinguish between health conditions defined as ‘normal’ or ‘abnormal’. Categorization as ‘abnormal’ may imply disease but could also be an unintentional finding without any clinical implication, since a certain percentage of healthy individuals by definition will be measured outside the URL depending on the percentile used to differentiate between the two conditions. hs-cTn measurements differ from many other laboratory assays in that the assay-specific 99th percentile, not the 97th percentile, serves as

the URL and is used as a diagnostic cut-off for myocardial injury of any etiology, and in the proper clinical setting to support the diagnosis of acute MI.

Recommendation #1: High-sensitivity cardiac troponin concentrations above the sex-specific 99th percentile URL should be used as the diagnostic threshold for myocardial injury and MI, consistent with the Universal Definition of Myocardial Infarction.

The Fourth Universal Definition of Myocardial Infarction *(1)* defines cTn concentrations above the sex-specific 99th percentile of the assay as 'myocardial injury' (Fig. 1). Acute myocardial injury requires at least one cTn concentration above the 99th percentile in conjunction with dynamic changes *(1)*. Acute myocardial injury can be of ischemic or non-ischemic origin and occurs due to multiple pathological or even physiological causes *(1)*. An acute MI is diagnosed when acute myocardial injury and myocardial ischemia are simultaneously present, based on clinical, ECG or imaging findings and documentation. Several types of MI can be defined based on the pathophysiology. Type 1 MI is due to plaque disruption with coronary atherothrombosis while type 2 occurs in the absence of acute plaque disruption in a clinical setting with oxygen demand and supply imbalance *(1)*. The latter may be caused by multiple etiologies, e.g., coronary spasms, embolism or artery dissection, sustained brady or tachyarrhythmia, severe anemia, hypotension or respiratory failure *(1)*.

Acute myocardial injury due to non-ischemic causes may occur from non-coronary heart disease (e.g., heart failure, myocarditis, Takotsubo syndrome, cardiac procedures or contusion) or systemic causes such as sepsis, severe illness, chronic kidney disease, toxic agents, stroke, pulmonary embolism, or even intense physical exercise. hs-cTn concentrations increase rapidly during acute myocardial injury, and accordingly low baseline and 1- or 2-h delta values are used for early rule out and prediction of low risk of myocardial injury in patients presenting to the ED with symptoms suggestive of non-ST-elevation myocardial infarction (NSTEMI) (Fig. 1).

The 0/1h algorithm as well as the 0/2 hour algorithm, both recommended by the European Society of Cardiology, also include criteria to assist in the 'rule in' of patients at high

risk of MI based either upon a single high threshold (higher than the 99th percentile) or a delta observed on serial sampling at one hour. It is important to note that these 'rule in' criteria do not usurp the use of the 99th percentile URL as the standard cutoff for diagnosing myocardial injury and MI. Rather, the criteria specified in 0/1h and 0/2h algorithms can be used as a surrogate to rapidly identify patients with high probability of MI. However, the positive predictive value of such criteria is generally less than 80% *(11-14)* and in cohorts with low prevalence of NSTEMI it may be substantially lower. Therefore, the diagnosis of MI should still be confirmed based on the criteria specified in the UDMI.

Chronic myocardial injury is characterized by increased serial hs-cTn concentrations that do not change acutely (≤ ±20% variation as suggested by UDMI *(1)*), frequently caused by structural heart disease like hypertension or left ventricular dysfunction *(15)*, long-term cardiac exposure to multiple metabolic risk factors *(16)* or toxic substances including drugs *(17)*. Chronic increases in hs-cTn often signal a poor long-term prognosis, including an increased risk for cardiovascular diseases and mortality. Large observational studies have demonstrated an association between long-term prognosis and hs-cTn concentrations even lower than the 99th percentile and it appears that there is a continuous relationship from the limit of detection of an assay, including all normal values up to the 99th percentile URL *(18)*. Accordingly, even hs-cTn concentrations between the limit of detection concentration and the 99th percentile URL are associated with increased risk in patients who presented to the ED but never demonstrate a hs-cTn > 99th percentile during their hospital presentation *(19)* (Fig. 1) and those in whom hs-cTn is obtained for primary or secondary prevention.

The majority of analyte reference intervals are derived statistically using the central 95th percentile distribution of results from a presumably healthy cohort *(20)*. Although the 97.5th percentile has been proposed by some as the appropriate diagnostic cut-off for MI using hs-cTn assays, the 99th percentile remains the reference standard for diagnosis of myocardial injury and MI and has been embedded within UDMI, IFCC and AACC guidelines *(1, 5-7)* since 2000. The 99th percentile was initially recommended to avoid large imprecision associated with the first generations of cTn assays which would influence clinical interpretation *(21)*. Use of the

97.5th percentile would have resulted in a significantly higher misclassification rate of healthy individuals (2.5% versus 1.0%). Previously, diagnosis of myocardial infarction using creatine kinase MB utilized twice the URL. Contemporary clinical data indicate replacement of the 99th percentile with the 97.5th percentile would lead to more patients being classified as having chronic myocardial injury, whilst the increase in NSTEMI would be minor *(22)*. It has been argued, and we agree, that there is minimal evidence to support this change. Use of the 97.5th percentile would likely cause clinical confusion, encourage use of non-standardized definitions for MI diagnosis, and reduce the validity of data collected from large epidemiology and therapeutic trials that have been used to derive treatment protocols for patients with MI. At present, laboratory medicine, cardiology and emergency medicine guidelines continue to support the 99th percentile *(7)*.

Selecting an appropriate reference cohort to derive the 99th percentile

Currently, there are 2 hs-cTnT assays and multiple hs-cTnI assays that are globally commercially available *(23)*. All have different performance characteristics depending on the instrumentation platform used to measure cTn and each assay utilizes different capture and detection antibodies. Thus, assays are not standardized or harmonized. Accordingly, the 99th percentile URLs must be determined for each individual assay and platform.

Recommendation #2: The hs-cTn 99th percentile URL should be derived from a reference cohort of healthy individuals, approximately 50% male and 50% female, with an age range from 18 and up to 80 years. All relevant ethnic/racial groups should be incorporated.

Reference cohorts should exclude subjects with comorbidities or chronic conditions that potentially affect the heart, nor should hospitalized patients be included in the applicable reference cohort for determining 99th percentiles. Including individuals with such comorbidities is not acceptable and will change the distribution of measured hs-cTn concentrations *(1, 6)*, substantially influencing both the length and distortion of the upper tail, which significantly

affects statistical calculations. Cohorts that exclude individuals who are prescribed medications related to cardiovascular disease or risk factors (e.g., aspirin, anti-hypertensive, anti-diabetes drugs, or lipid lowering drugs) have lower 99th percentiles compared to a less rigorously screened population; hence, these individuals should be excluded *(8-10)*. Health status of the reference population should be initially screened using questionnaires *(20)* or a clinical visit, where participants are queried about comorbidities, chronic conditions and medication use. Pre-inclusion screening criteria should also encompass surrogate biomarker testing to exclude undiagnosed subclinical disease, primarily diabetes, renal dysfunction, or myocardial dysfunction. We recommend a standardized and conservative approach towards exclusion of individuals treated for or diagnosed with any conditions which may influence and increase the hs-cTn 99th percentile URL (Table 1).

The relationship between age and cTn concentrations is complex, with some studies suggesting a direct relationship between age and cTn and others suggesting that with appropriate rigorous patient selection, this relationship disappears. For example, age has been shown to be a significant predictor of cTn concentrations but this effect is substantially minimized if echocardiography screening is undertaken to eliminate cardiac pathology *(9)*. Ideally, all age strata should be equally represented in the reference population, but recruitment of a large cohort of completely healthy >60-year-olds within a reasonable timeframe may be problematic; accordingly, age stratified 99th percentile URLs are not recommended. A reasonable age distribution should still be attempted, with all age strata represented fairly within the reference cohort.

Differences in hs-cTn concentrations have been reported between ethnic groups, thus the reference cohort should also include a representative distribution reflecting the regional ethnic composition in the applicable geographical area. Presently, some of the 99th percentile URLs for hs-cTn assays available in the United States are different compared to those utilized in the European Union and/or globally (depending on assay) *(23)*, which may in part reflect these differences but also likely indicate variability in the enrollment criteria used since some manufacturers use convenience specimens rather than prospectively rigorously screened

cohorts. It has not been determined if region-specific hs-cTn 99th percentile URLs should be implemented in other parts of the world (e.g., Asia or Africa), but this needs to be explored further.

Myocardial imaging may further differentiate between myocardial healthy and diseased, and imaging criteria have been shown to further lower the 99th percentile estimate *(9)*. Use of imaging procedures to screen participants comes with a substantial increase in cost, therefore is not required as part of the routine pre-screening of individuals in the reference cohort. However, data from population-based cohort studies that used imaging could be part of a larger dataset to determine the 99th percentile URL, and in such cases normal cardiac findings may be documented.

Recommendation #3: Sex-specific hs-cTn 99th percentile URLs should be determined and reported.

Health and diagnostic disparities in women with cardiovascular disease are apparent, and outcomes are worse in females compared to males with cardiovascular disease *(24)*. Sex-specific differences are also evident for hs-cTn assays, with lower 99th percentiles in females *(23)*. This is believed to be largely due to differences in cardiovascular physiology (females have smaller cardiac mass by weight), but also a higher incidence of subclinical coronary artery disease in men at an earlier age *(1)*. Due to varying analytical sensitivities of hs-cTn assays, 99th percentile differences may be subtle or significant, and assays that show measurable concentrations in larger percentages of a healthy cohort are seemingly more sensitive to detecting a difference between females and males. As for other analytes, such as creatinine or creatine kinase, if sex-specific reference intervals are determined to be statistically significant then clinical laboratories should report them. In this regard, hs-cTn should not be an outlier. Sex-specific URLs lead to greater recognition of disease and possible also cardiovascular risk, and the long-term prognostic power for hs-cTnI in particular is higher in women *(25, 26)*. Whether attention or treatment to increased hs-cTn concentrations in females will impact outcomes is the focus of an ongoing randomized clinical trial called “hs-cTn

Optimizing the Diagnosis of Acute Myocardial Infarction/Injury in Women", or CODE-MI (NCT03819894).

Statistical recommendations related to the 99th percentile

Statistical techniques and methods utilized for outlier exclusion greatly influence calculation of the 99th percentile. Appropriate statistical handling of data generated is as critical as selection of the reference population.

Recommendation #4: The 99th percentile URL should be determined using the non-parametric method or a method with similar statistical capability. The cohort should preferably include a minimum of 400 males and 400 healthy females, a total of 800 subjects, to derive 99th percentiles with sufficient statistical power allowing calculation of the 95% confidence interval (CI).

We recommend using the non-parametric statistical method (or the Harrell-Davis method) as opposed to the Robust method *(27)*. The Robust method was designed to establish a central 95% reference interval and not the 99th percentile, and is therefore less accurate when the number of subjects is greater than 120 and for biomarkers showing a skewed distribution. A minimum of 300 subjects per partition generates sufficient statistical uncertainties (confidence intervals) of 90% at the 99th percentile *(28)*. However, we are revising the recommendation to allow confidence intervals of 95% to be utilized in order to minimize influence from outliers and increase reproducibility between cohorts, which will in turn increase the minimum number of subjects to 400 males and 400 females (minimum of 800 total subjects) *(28)*. According to this method, the appropriate cut-off concentration corresponding to the hs-cTn 99th percentile URL is determined by the four highest observations. If n=400, then the low and high 95% CIs will be determined by persons ranged as number 391 and 400, respectively, and the 99th percentile will be similar to the concentration between observation number 395 and 396 of the reference cohort. A rigid clinical selection of participants in the healthy cohort should minimize the risk of outliers due to unrecognized cardiac disease and outlier exclusion should therefore be

conservative. The Reed/Dixon criteria may be preferred as it will exclude fewer subjects than the Tukey method *(27)*. Bootstrapping methods may be useful for calculation of confidence intervals as this will increase certainty of the estimated limits. Confidence intervals should not be reported clinically but should be disclosed in clinical research trials and studies reported in peer-reviewed journals.

Recommendation #5: Biological interferences should be investigated in specimens with hs-cTn results that are outliers without a valid clinical explanation.

Even though a strict clinical screening will be been undertaken, biological confounders should be taken into consideration when deriving the 99th percentile URLs for hs-cTn assays. Like all immunoassays, cTn-assays may infrequently be affected by antibodies that bind cTn or components of the assay. These complexation effects can sometimes result in stable increased cTn concentrations up to 10-times the URL, and have been designated 'macrotroponin'. The presence of macrotroponin can be determined by reasonable, easy methods involving removal of the immunoglobulins in the sample *(29)*, and should be considered a concern when apparently healthy individuals show unexpectedly high concentrations. Interferences from heterophile antibodies (endogenous antibodies that may interfere with different clinical immunoassays) can also be present, whereby the heterophile antibodies cross-react with the cTn antibodies and provide a false positive or negative result. Circulating anti-cTn antibodies can also result in false low cTn concentrations in rare cases, but since the 99th URL is determined using the highest cTn values, the concerns are focused primarily around macrotroponin. We recognize the challenge of assay design to minimize both macrotroponin, heterophile, and/or autoantibody interferences. Clinically, patients with these interferences routinely undergo extensive and unnecessary clinical investigation, with additional investigation using a different hs-cTn assay and/or imaging tests revealing normal results. If healthy subjects with macrotroponin, heterophile or autoantibody antibodies incidentally are enrolled in the reference population, this could artificially shift the 99th percentile

to a higher hs-cTn concentration *(29)*. Accordingly, these individuals should be excluded based on outlier testing or clinical suspicion if diagnosed by relevant analytical techniques *(29)*.

Laboratory variables that may affect the 99th percentile

In addition to identification of biological confounders, stringent pre-analytical and analytical criteria are critical to accurately calculate the 99th percentile URL.

Recommendation #6: Pre-analytical protocols and analytical interferences should be standardized and optimized for accurate determination of 99th percentile URLs.

There are numerous important pre-analytical factors that may influence accurate determination of hs-cTn results, including body position, time of day, centrifugation speed, storage time before analyzing, interfering substances and collection tubes. hs-cTnT demonstrates diurnal variation *(30, 31)*, thus timing for specimen collection should be standardized. Hemolysis may cause false negative (hs-cTnT) or false positive (hs-cTnI, assay dependent) results *(32-34)*, and specimens may be compromised due to icterus or turbidity/lipemia (hs-cTnI) *(32)*. Hemolysis, icterus and lipemia should be verified in all specimens obtained when determining 99th percentiles and excluded based on the applicable assay specific cutoffs for interferences greater than 10%. Individuals who consume biotin daily may have false negative results with some hs-cTn assays that are dependent on biotin/streptavidin binding properties *(34)*. Different additives used in plasma collection tubes (e.g. heparin or EDTA) may also affect hs-cTn concentrations *(23, 35)*. Specimen type, collection tubes and additives should be evaluated and if significant differences exist, then different hs-cTn 99th URLs should be derived for each tube/additive as applicable.

Recommendation #7: Unavoidable lot-to-lot analytical uncertainty should be integrated in determination of the 99th percentile URL.

Studies have reported instrument dependent lot-to-lot variability in hs-cTn reagents and/or calibrators, shifting concentrations ± 1-2 ng/L over time, with lower concentrations near the

limit of detection being affected the greatest *(36, 37)*. Therefore, if all specimens from the reference population are measured using the same reagent and/or calibrator lot, the hs-cTn concentration defined as the 99th percentile may deviate upward or downward in accordance with the concentration value of that particular lot. It is uncommon for clinical laboratories to use multiple reagent or calibrator lots simultaneously. Thus, to mitigate this risk, specimens from the reference population could be analyzed across different laboratories or over a longer period of time. Several modules of the same instrument should be used within the applicable laboratory to ensure that reagent and calibrator lot-to-lot variability, as well as intra- and inter-laboratory differences and uncertainties, are incorporated into the dataset to increase the overall robustness of the statistical calculations. This could be done by either: 1) performing a multicenter study, 2) merging data from healthy individuals included in population-based cohort studies, or 3) using bio-banked specimens that are analyzed across multiple laboratories, with the included sites/cohorts/laboratories utilizing different reagent and calibrator lots (Fig. 2).

Future needs

Increasing clinical knowledge related to the diagnostic and prognostic performance of hs-cTn assays allow for improvement in patient triage and clinical care strategies. Future developments for increasing the analytical sensitivity and imprecision of cTn assays is likely to further improve categorization of patients in the ED. Improved technology in artificial intelligence systems and machine learning algorithms could potentially integrate information regarding age, sex, ethnicity, co-morbidity and hs-cTn results that are available in electronic medical records, making it possible to automatically identify presence of acute or chronic myocardial injury or risk scores both for short and long-term major adverse cardiovascular events. This ultimately could be reported clinically in electronic health records to improve prognosis and outcomes. High quality studies based on reliable hs-cTn data measurements are key for future research studies.

Conclusion

The hs-cTn 99th percentile URL is a key threshold in the UDMI and throughout routine clinical and laboratory practice. To increase harmonization of acute MI diagnosis, the 99th percentile URL should be determined in a standardized/harmonized manner, including rigorous clinical and analytical screening procedures, sufficient number of included participants, acknowledging of all pre-analytical, analytical and biological factors affecting the cTn assay and adequate statistical handling, that may affect the estimates.

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Table 1. Conditions that should be excluded from the reference population.

CONDITION	SCREENING TOOL
All known cardiovascular or cardiac diseases	Reported in questionnaire
Treatment for hyperlipidemia	Medication reported in questionnaire
Treatment for hypertension	Medication reported in questionnaire
Subclinical heart disease	Exclude if NT-proBNP > 125 ng/L or BNP > 35 ng/L *(38)*
Diabetes	Treatment (including diet) reported in questionnaire Exclude if HbA1c ≥ 48 mmol/mol (≥ 6.5%), fasting glucose ≥ 7.1 mmol/L (126 mg/dL), 2 hour plasma glucose during oral tolerance test (100 g) or a randomly measured glucose ≥ 11.1 mmol/L (200 mg/dL) *(39)*
Chronic renal disease	eGFR < 60 mL/min/1.73m^2 or urine albumin/creatinine ratio > 3 mg/mmol (> 30 mg/g) *(40)*
Abnormal BMI	< 18 m^2/kg or > 35 m^2/kg
Smoking	Reported in questionnaire
Chronic disease that could affect the heart (cancer, lung, liver, unstable or non-treated thyroid disease, autoimmune diseases)	Reported in questionnaire
Recent acute hospitalization (within the last 3 months)	Reported in questionnaire
Pregnancy	Reported in questionnaire
For biotin sensitive assays only: Ongoing treatment with biotin (within one week)	Reported in questionnaire

NT-proBNP, N terminal pro brain natriuretic peptide; BNP, Brain natriuretic peptide; HbA1c: Hemoglobin a1c; eGFR, estimated glomerular filtration rate.

Figure legends:

Figure 1. Different hs-cTn cut-offs used for diagnosing MI (sex-specific 99th percentile URLs), early rule-out, observation or suggestive of rule-in of MI in the ED, and for risk assessment of patients.

Figure 2. Determination of the hs-cTn 99th percentile URL using different reagent and calibrator lots. Data obtained, using e.g. four different lots, should be merged before statistical analysis is performed. All specimens could be analyzed within the same laboratory if access to different lots is provided. The number of four different lots is a suggestion and arbitrary chosen.

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Disclosures/Conflicts of interests

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AKS has received consulting fees from Radiometer.

RB has received grants from Siemens Healthineers, Abbott Point of Care, National Institute of Health Research, Asthma UK; British Lung Foundation. He has received consulting fees from Roche, Aptamer Group, Abbott, Psyros, Siemens Heltineers, Beckman Coulter, Radiometer. He has received lecturing fees from EMCREG (Emergency Medicine Cardiac Research & Education Group) International and participated in advisory boards for FORCE Trial (NIHR funded), REWIRE trial (Queen Mary University, London), PRONTO trial (NIHR funded), LumiraDx (advisory board). He has a fiduciary role in Royal College of Emergency Medicine Research Committee and has received service from My110, andox, Avacta, LumiraDx, Chronomics, iXensor, BD, Horiba.

AJ has received consultant fees from Abbott, siemens, radiometer, Ortho Diagnostics, Beckman Coulter, Sphingotec, Roche, ET Healthcare, Amgen, Novartis. He has stock options with RCE Technologies.

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OH has stock options with https://www.alignedbio.com/

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Laboratories. McMaster University has also filed patents with Dr. Kavsak listed as an inventor on Quality Control Materials for Cardiac Troponin Testing and Identifying pregnant women at increased risk for hypertension and future cardiovascular disease. McMaster University has filed a patent with Dr. Kavsak listed as an inventor in the acute cardiovascular biomarker field, in particular, a patent has been awarded in Europe (EP 3 341 723 B1) on a Method of determining risk of an adverse cardiac event.

PC has participated in advisory board for Psyros Diagnostics and has a fiduciary role with radiometer. He is associate editor in The Journal of Applied Laboratory Medicine.

TO has received consultant fees from Roche, Bayer and CardiNor. He has received honoraria from Roche, has a patent pending with Roche, has participated in advisory board for Bayer and Roche, has a fiduciary role in CardiNor, has stocks in CardiNor, received equipment/material from Novartis and Abbott.

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All disclosures are given in the ICMJE firms attached.

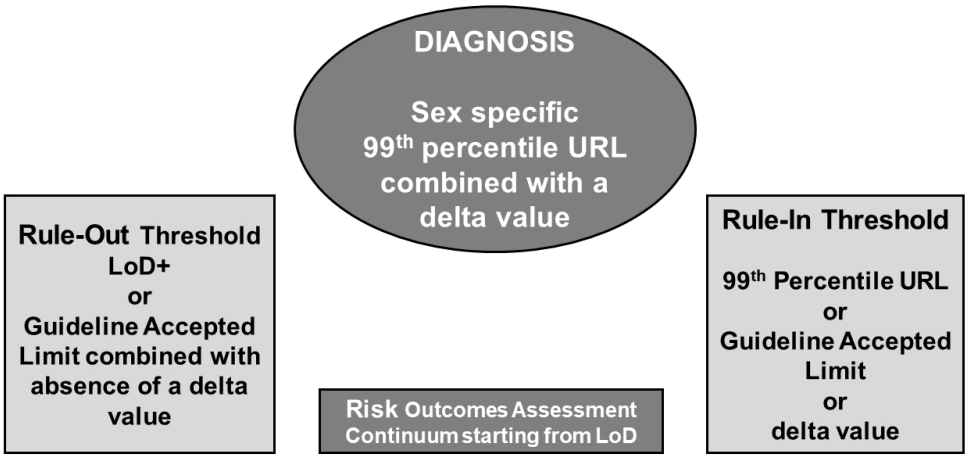

Figure 1. Different hs-cTn cut-offs used for diagnosing MI (sex-specific 99th percentile URLs), early rule-out, observation or suggestive of rule-in of MI in the ED, and for risk assessment of patients.

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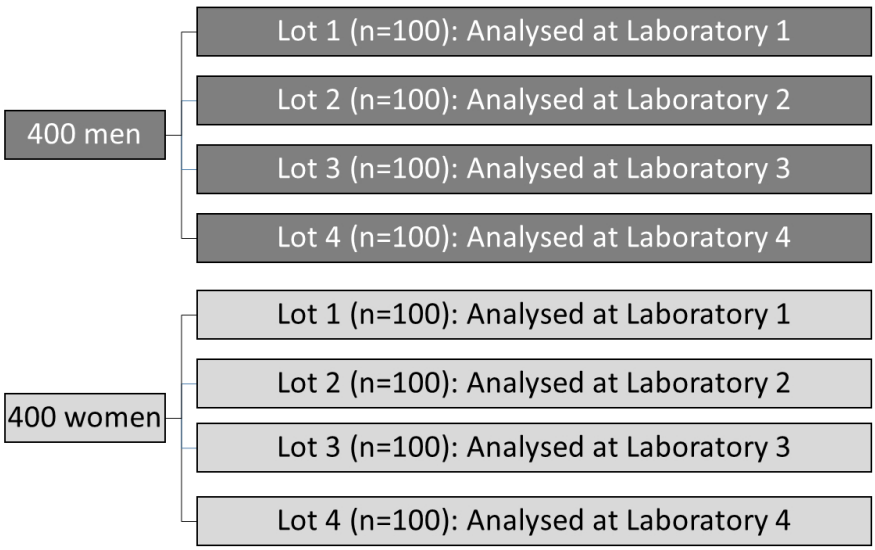

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