Clinical Chemistry 00:0
1–9 (2023)

Review

Lower Limits for Reporting High-Sensitivity Cardiac Troponin Assays and Impact of Analytical Performance on Patient Misclassification

Kristin M. Aakre [a,b,*] Fred S. Apple,[c,d] Nicolas L. Mills [e,f] Steven J.R. Meex,[g,h] and Paul O. Collinson [i,j] on behalf of the International Federation of Clinical Chemistry Committee on Clinical Applications of Cardiac Biomarkers (IFCC C-CB)

BACKGROUND: Cardiac troponin measurements are indispensable for the diagnosis of myocardial infarction and provide useful information for long-term risk prediction of cardiovascular disease. Accelerated diagnostic pathways prevent unnecessary hospital admission, but require reporting cardiac troponin concentrations at low concentrations that are sometimes below the limit of quantification. Whether analytical imprecision at these concentrations contributes to misclassification of patients is debated.

CONTENT: The International Federation of Clinical Chemistry Committee on Clinical Application of Cardiac Bio-Markers (IFCC C-CB) provides evidence-based educational statements on analytical and clinical aspects of cardiac biomarkers. This mini-review discusses how the reporting of low concentrations of cardiac troponins impacts on whether or not assays are classified as high-sensitivity and how analytical performance at low concentrations influences the utility of troponins in accelerated diagnostic pathways. Practical suggestions are made for laboratories regarding analytical quality assessment of cardiac troponin results at low cutoffs, with a particular focus on accelerated diagnostic pathways. The review also discusses how future use of cardiac troponins for long-term prediction or management of cardiovascular disease may require improvements in analytical quality.

SUMMARY: Clinical guidelines recommend using cardiac troponin concentrations as low as the limit of detection of the assay to guide patient care. Laboratories, manufacturers, researchers, and external quality assessment providers should extend analytical performance monitoring of cardiac troponin assays to include the concentration ranges applicable in these pathways.

Introduction

Acute coronary syndromes are common and present as unstable angina, acute myocardial infarction, or sudden cardiac death. Due to the risk of cardiac death, many patients with symptoms suggestive of acute coronary syndrome are admitted for investigation despite only 1 in 10 having a final diagnosis of myocardial infarction (MI) (1). In response to clinical requirements, manufacturers have improved the analytical sensitivity and precision of cardiac troponin (cTn) assays (2). This has enabled the use of accelerated diagnostic pathways that may predict low, intermediate, or high risk of MI within a shorter time frame using high-sensitivity assays, compared to conventional cTn assays (3). The cornerstone in these pathways is accurate measurements of cTn concentration below the upper reference limit of the assay and detection of small changes in cTn on serial testing (1, 4–9). Another benefit resulting from the improved analytical sensitivity, though not yet implemented in clinical practice, is the possibility of evaluating cardiovascular risk in stable patients or the general population (10–13).

Some accelerated diagnostic pathways use clinical cutoffs below the limit of quantification (LOQ) of the assay and may be ineffective if quantitative results are not reported down to the limit of detection (LOD) (14). This has challenged conventional laboratory

[a]Department of Medical Biochemistry and Pharmacology and Department of Heart Disease, Haukeland University Hospital, Bergen, Norway; [b]Department of Clinical Science, University of Bergen, Bergen, Norway; [c]Department of Laboratory Medicine and Pathology, Hennepin Healthcare/HCMC, Minneapolis, MN, United States; [d]Department of Laboratory Medicine and Pathology, University of Minnesota, Minneapolis, MN, United States; [e]BHF Centre for Cardiovascular Science, University of Edinburgh, Edinburgh, United Kingdom; [f]Usher Institute, University of Edinburgh, Edinburgh, United Kingdom; [g]Central Diagnostic Laboratory, Maastricht University Medical Center, Maastricht, the Netherlands; [h]CARIM School for Cardiovascular Diseases, Maastricht University, Maastricht, the Netherlands; [i]Department of Clinical Blood Sciences and Cardiology, St.George's University Hospitals NHS Foundation Trust, London, United Kingdom; [j]St.George's University of London, London, United Kingdom.
*Address correspondence to this author at: Department of Medical Biochemistry and Pharmacology, Haukeland University Hospital, Jonas Lies vei 65, 5021 Bergen, Norway. E-mail kristin.moberg.aakre@helse-bergen.no.
Received June 21, 2023; accepted September 27, 2023.
https://doi.org/10.1093/clinchem/hvad185

practice in relation to the lower cutoff for reporting cTn results, where the laboratory needs to balance the risk of misclassification due to higher levels of imprecision against improvements in efficacy of patient flow (14–18).

The International Federation of Clinical Chemistry Committee on Clinical Application of Cardiac Bio-Markers (IFCC C-CB) provides evidence-based educational statements to support standard interpretation and utilization of cardiac biomarkers in clinical laboratories and practice. This review focuses on the utility of very low cTn concentrations. It discusses how the classification of assays is influenced by the lower cutoff chosen for reporting, and how analytical performance at low concentrations could influence the clinical classification of patients as low or intermediate risk within accelerated diagnostic pathways for MI and future applications for cTn testing in the long-term prediction or management of cardiovascular diseases. Recommendations are provided on how to ensure sufficient analytical performance, together with reflections on how future clinical applications may change what is considered an acceptable analytical performance goal.

Definitions of Analytical Sensitivity Metrics

The limit of blank (LOB) is the concentration found when a blank sample is measured repeatedly. The LOD refers to the lowest concentration that can be reliably distinguished from measuring a blank sample with a reasonable level of certainty (LOB + $1.645_{(\text{SD low-concentration sample})}$). It signifies the presence of cTn albeit with a substantial analytical error of the precise concentration. The LOQ is conventionally defined as the lowest cTn concentration that can be measured and quantified with a defined level of precision, with an analytical variation of 20%. Laboratories commonly establish both the LOD and LOQ of their cTn assay, but the cutoff used as the lower limit for reporting results varies. Both scientific arguments and diagnostic testing regulations contribute to this heterogeneity. For example, in the United States, the Food and Drug Administration (FDA) mandates that the LOQ is the lowest reportable value. Although the difference between the LOD and LOQ for most assays is quantitatively small (1 to 4 ng/L), the choice for reporting has potentially important consequences for the safety and effectiveness of accelerated diagnostic pathways and furthermore for the application of cTn results for long-term risk prediction. It is important to recognize that the LOB, LOD, and LOQ are assay-specific parameters, similar to the 99th percentile, and vary between cTn assays and platforms.

Influence of Analytical Bias on Single-Sample Absolute Cutoffs

For consistent performance of guideline-recommended accelerated diagnostic pathways (19, 20) the analytical performance of high-sensitivity cTn (hs-cTn) assays must be consistent over time at the applicable cutoffs. Changes in the calibrator or reagent lots may cause shifts in a measured concentration of the assay from the previous lots. Even though such shifts are embedded in the long-term analytical variation they may affect the classification of patients as low or intermediate risk within these pathways, for the applicable time period in use (16). Low-risk patients are eligible for immediate discharge from the emergency department (ED), but those above this cutoff are classified as intermediate risk and undergo serial measurements and further clinical observation. An example of how assay shifts may influence clinical classification is shown in Fig. 1. In a hypothetical population of patients with cTn concentrations corresponding to the low-risk cutoff, it would be expected that on any given measurement around half will fall above and half below this value. If a calibrator or reagent shift occur, the proportion of patients above or below the cutoff will change and be directly related to the magnitude of the shift expressed as number of analytical standard deviations of the assay. If the shift was of 1 analytical standard deviation, then 84% of patients from this hypothetical population will be measured above or below the cutoff depending on the direction of the shift. A shift of 2 analytical standard deviations would result in 97.5% of patients falling on the skewed side. This situation applies to all laboratory tests utilizing absolute cutoffs but has the largest clinical impact when the cutoff is close to the median concentration or falls within the interquartile range (IQR) of concentrations typically measured in patients undergoing the test. This is particularly relevant for cTn as the median and IQR of cTn concentrations in patients without MI are very similar to the cutoffs used to classify patients by the European Society of Cardiology (ESC) guidelines as "very low" and "low" risk in the 0/1 and 0/2 h pathways or the single-sample rule-out cutoff used in the High-Sensitivity Troponin in the Evaluation of patients with Acute Coronary Syndrome (High-STEACS) pathway (1, 4–9, 19). Analytical drift in the assay of 1 to 2 ng/L upwards could substantially influence the efficacy of these accelerated diagnostic pathways (9), with one study suggesting that the percentage of ED patients being measured below the cutoff signalling low risk of MI might vary from 15% to 30%, depending on lot used (16).

Influence of Analytical Bias on Assay Classification and Long-Term Risk Prediction

When lot-to-lot variations are present, the percentage of healthy individuals with measurable concentrations may

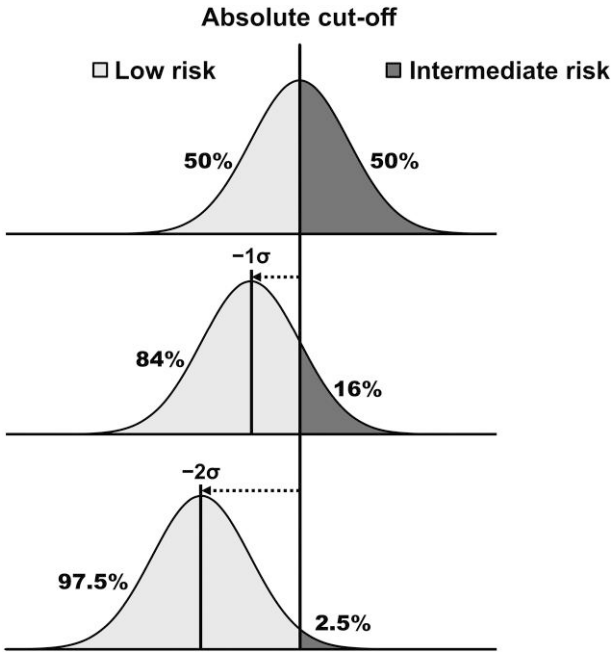

Fig. 1. The figure shows how assay shifts affect classification of patients who have an in vivo biomarker concentration similar to the lower limit for reporting results that are applied. Upper panel: no shift, 50% are measured below the cutoff and 50% are measured above the cutoff. Middle panel: the assay has shifted 1 analytical SD downward and 84% of patients are measured below the cutoff. Lower panel: the assay has shifted 2 analytical SDs downward and 97% of patients are measured below the cutoff. Light grey; rule-out, dark grey; rule-in/observe.

vary depending on the level of the reagent/calibrator lot used. Such variations could explain some of the variation observed between studies reporting measurable results in healthy individuals, e.g., 24% to 58% (cTnT) and 64% to 81% (cTnI [Abbott Architect]) (21). As a hs-cTn assay is defined as one showing results above the LOD in more than 50% of healthy individuals (22), which reagent lots are used in the applicable studies may in fact influence the classification of assays as high sensitivity either in a positive or negative manner. This is of particular concern if the study has used only one reagent/calibrator lot.

In the future, cTn measurements may be used for risk stratification in the general population or in the follow-up of patients with chronic coronary syndromes or structural heart disease. Current data suggest that the absolute difference between the lower quartile (low risk) and upper quartile (high risk) in cohorts used for evaluating long-term risk of cardiovascular events may be, depending on assay, as little as 2 to 6 ng/L (10–13). Within-laboratory lot variation or between-instrument variation of ±2 ng/L is commonly observed (9, 14, 16, 23, 24) and an assay shift of 4 ng/L may reclassify a substantial proportion of patients, or give a false signal of improvement or deterioration in those with chronic cardiac conditions. Recent data indicate that the total error across several laboratories, reagent, and calibrator lots is even larger (±3 ng/L) (25), and may overlap with the absolute concentration differences that classify individuals in the general population or with stable cardiac conditions as at low or high risk (10–13). These future applications of hs-cTn testing highlight the need for rigorous long-term monitoring of assay performance and stability at low concentrations in routine clinical practice, both within and between laboratories. Another option could be inclusion of cTn results into clinical calculators or artificial intelligence tools providing long-term risk estimates. Multivariable risk estimates may be less affected by analytical variation compared to single laboratory results. The acceptable analytical variation for cTn in such tools is likely to be algorithm-specific and remains to be tested and validated in future studies.

Influence of Analytical Imprecision on Absolute Cutoffs and Delta Values

As illustrated in Fig. 1, analytical imprecision has a gaussian distribution and the coefficient of variation of the assay determines the distribution of a result about

the true value. For a single measurement this will be n ± $Z^*SD_A^*n$ where SD_A is the analytical standard deviation, n is the analyte concentration, and Z is selected for the appropriate probability of the standard deviation. cTn should be reported as a whole number, therefore a value of, for example, 3 ng/L needs to lie in the range 2.5 to 3.4 ng/L to be reported as 3 ng/L. Accordingly, it is possible to estimate the impact of analytical imprecision on the risk of misclassification of patients. This may only occur when the analytical error causes rounding to above or below the applicable cutoff. For a cutoff of <3 ng/L, patients with a true value of less than 3 ng/L, such as 2 ng/L, could be reclassified to intermediate risk due to assay imprecision if cTn measures 2.5 ng/L or greater. To avoid this in 95% of patients (the analytical variation is considered as a one-tailed test; Z = 1.64 for 95% probability), the analytical variation should be less than 15.2% around this cutoff [0.5/(2.0*1.64)]*100. Similarly, reclassification to low risk may occur if a cTn concentration of 3 ng/L is measured as 2.4 ng/L or lower, due to analytical imprecision. This can occur in 5% of patients when the analytical variation is 12.2%, [0.6/(3.0*1.64)]*100. Recommended single-sample cutoffs to identify patients as low risk vary by assay (currently from 1 to 5 ng/L) (19). Table 1 shows the analytical variation required to keep the combined rate of misclassifications below 2.5% to 20% for a range of cTn concentrations used within accelerated diagnostic pathways. According to this, assays need to have an LOQ at around 3 ng/L and an analytical variation of 10% below 7 ng/L for these imprecision goals to be achievable in routine laboratory practice. This performance may be achievable for within-series imprecision (26), even though higher analytical variation has been reported (15).

When delta changes are included in accelerated diagnostic pathways, they are always combined with an absolute cutoff value. The acceptable imprecision for the rule-out delta will be based on the corresponding absolute cutoff. Using the case scenario of a one-sided change (change up) the analytical variation goal will be (delta/1.64*$\sqrt{2}$)/absolute value assuming that intra-individual biological variation is minimal over 1 h, as it is typically much lower compared to analytical variation (27, 28). For a delta value of <3 ng/L at an absolute concentration of 12 ng/L, the analytical variation needs to be less than 7.2%, {[2/(1.64*1.414)/12]}*100 to avoid misclassification in more than 5% of patients. Here the analytical imprecision estimate should also include the effect of different instruments, as serial measurements may be performed on different instruments within the same laboratory. This needs close monitoring as studies have shown that the analytical variation across instruments may indeed produce imprecision exceeding the delta value (29, 30). The analytical performance goals are summarized for different delta values with corresponding absolute cutoffs in Table 2. Cutoff values with deltas are for the 0/1 h and 0/2 h pathway as suggested by the ESC and the High-STEACs early rule-out pathway (4, 19).

Preanalytical Performance

Laboratories should consider how their preanalytical handling of samples may affect the total error of reported results and be particularly aware that small differences can be critical when using approaches such as single-sample rule-out or evaluation of small deltas. This includes sample matrix and tubes chosen, timing and speed of centrifugation, and storage stability. Hemolysis is problematic for many cTn assays (26, 30, 31), and obtaining blood via line draws from peripheral veins (typically done in the ED for practical reasons) will often cause hemolysis (32). This may be exacerbated by inadequate filling of the blood tubes and may be further exaggerated if sample transport is not gentle. In routine practice, preanalytical errors may typically occur as random errors affecting single samples and if frequent may influence the so-called "flier" rate of the laboratory. Fliers are defined as a measured concentration that cannot be confirmed on re-sampling the patient or re-analyzing the sample.

Systematic preanalytical errors may occur if the laboratory does not follow the instructions in the package insert of the assay, e.g., for practical purposes. If plasma or serum, tubes with different additives (or from different manufacturers) or several storage options are used interchangeably it will be very difficult to predict and monitor the total error of the results and such practice should be avoided.

Patient-related factors may also influence cTn concentrations. cTnT manifests circadian rhythm with variations up to ±10% (28, 33) resulting in lower or higher concentrations at different times of the day. This may affect classification of patients whose homeostatic set point is close to applicable cutoffs. There are no options to correct for this in the ED, but if testing is performed for risk stratification in the general population or for chronic disease monitoring, the use of a fixed time of the day for testing might be helpful.

Diagnostic investigations may also influence cTn measurements. Dobutamine, which is often used for stress testing or moderate- to high-intensity exercise can cause increases in cTn even in healthy individuals (34, 35). Finally, smoking, for reasons that are unclear, can lower cTn concentrations (36). Thus, all these issues must be considered to reduce preanalytical variability of cTn measurements.

Table 1. Tabulated cutoffs with corresponding maximum allowable analytical coefficient of variation from 2.5% to 20% significance that will produce reclassification of patient to intermediate risk of MI (0.5 ng/L above the decision cutoff) or low risk of MI (0.6 ng/L below the decision cutoff plus an increment of 1 ng/L).

	Reclassification to intermediate risk due to analytical variation					Reclassification to low risk due to analytical variation				
		Allowable CV (%) according to percentage misclassifications (z value, one-tailed)					Allowable CV (%) according to percentage misclassifications (z value, one-tailed)			
Rule-out cut-off, ng/L	Rounded up concentration, ng/L	2.5% (1.96)	5% (1.64)	10% (1.28)	20% (0.84)	Rounded down concentration, ng/L	2.5% (1.96)	5% (1.64)	10% (1.28)	20% (0.84)
<1	NA[a]	NA	NA	NA	NA	0.4	30.6	36.6	46.9	71.4
<2	1.5	25.5	30.5	39.1	59.5	1.4	15.3	18.3	23.4	35.7
<3	2.5	12.8	15.2	19.5	29.8	2.4	10.2	12.2	15.6	23.8
<4	3.5	8.5	10.2	13.0	19.8	3.4	7.7	9.1	11.7	17.9
<5	4.5	6.4	7.6	9.8	14.9	4.4	6.1	7.3	9.4	14.3
<6	5.5	5.1	6.1	7.8	11.9	5.4	5.1	6.1	7.8	11.9
<7	6.5	4.3	5.1	6.5	9.9	6.4	4.4	5.2	6.7	10.2
<8	7.5	3.6	4.4	5.6	8.5	7.4	3.8	4.6	5.9	8.9
<9	8.5	3.2	3.8	4.9	7.4	8.4	3.4	4.1	5.2	7.9

[a]Abbreviation: NA, non applicable.

Monitoring the Analytical Performance of cTn Assays in the Routine Laboratory

Laboratories that provide cTn results used for rapid rule-out of MI should monitor analytical performance carefully, with a particular focus on the concentrations corresponding to those used in these pathways. On standard central analytical platforms, this typically requires daily internal quality assessment at concentrations slightly above the cutoff that identifies patients as low risk (single-sample rule-out) and with a second intermediate control sample corresponding to the 99th percentile upper reference limit. Commercial material for internal quality assessment at such low concentrations is commonly not available, but the laboratory could use in-house plasma or serum pools at the applicable concentrations. If these are measured every day, and on different instruments, both the short-term, long-term, and between-instrument analytical variation may be calculated. External quality assessment should be performed regularly. If the laboratory wishes to monitor shifts across reagent/calibrator lots, the laboratory may establish up to 10 serum or plasma pools over the total measuring range of the assay, prioritizing the creation of pools at concentrations used for clinical decisions. The pools should be aliquoted and frozen and one series, including all concentrations measured for every new reagent or calibrator lot, should be analyzed and compared to earlier measurements, obtained from the same pool. Over time these data will demonstrate the total error of the assay and give the laboratory an accurate measure of the expected differences between different reagent lots (16). The laboratory will also be able to detect clinically important lot shifts with high certainty, immediately upon receiving a new batch of reagents or calibrators.

Analytical Performance during Development of Accelerated Diagnostic Protocols

Total analytical imprecision includes short- (hours, days, weeks) and long-term (months, years) between-instrument analytical variation, with the latter including the impact of reagent/calibrator lot variation. If the cTn data used for developing accelerated diagnostic pathways are collected continuously and measured in fresh samples over longer time periods (including several different instruments, reagent, and calibrator lots) the data will reflect the total analytical variation and hence diagnostic sensitivity, specificity, and predictive values are estimated based on results reflecting total preanalytical and analytical error (9). This means that overall error is more likely to be randomly distributed and the clinical data obtained will be more robust and transferable to other clinical laboratories using the same assay and instruments. This was the case for the High-STEACS early rule-out pathway, where the cutoff used to identify low-risk patients was derived and validated in consecutive patients using fresh samples measured in real-time across

Table 2. Summarizing the analytical imprecision (percentages) necessary to determine delta values with sufficient certainty at different absolute baseline concentrations.

Delta, ng/L	<2	<3	<4	<5	<6	<7	<8
Baseline troponin, ng/L							
1	43.1	86.2	129.3	172.5	215.6	258.7	301.8
2	21.6	43.1	64.7	86.2	107.8	129.3	150.9
3	14.4	28.7	43.1	57.5	71.9	86.2	100.6
4	10.8	21.6	32.3	43.1	53.9	64.7	75.5
5	8.6	17.2[a]	25.9	34.5	43.1	51.7	60.4
6	7.2	14.4	21.6	28.7	35.9	43.1	50.3
7	6.2	12.3	18.5	24.6	30.8	37.0	43.1
8	5.4	10.8	16.2	21.6	26.9	32.3	37.7
9	4.8	9.6	14.4	19.2	24.0	28.7	33.5
10	4.3	8.6	12.9	17.2	21.6	25.9	30.2
11	3.9	7.8	11.8	15.7	19.6	23.5	27.4
12	3.6	7.2[b]	10.8	14.4	18.0	21.6	25.2
13	3.3	6.6	9.9	13.3	16.6	19.9	23.2
14	3.1	6.2	9.2	12.3	15.4	18.5	21.6

Analytical quality necessary for using the 0/1 h High-Sensitivity Troponin in the Evaluation of patients with Acute Coronary Syndrome algorithm for hs-cTnI (Abbott); baseline cTnI < 5 ng/L and Δ < 3 ng/L): 17%[a]. Analytical quality necessary for using the 0/1 h European Society of Cardiology algorithm for hs-cTnT; baseline cTnT < 12 ng/L and Δ < 3 ng/L: 7%[b]

multiple hospitals and instruments (7). Furthermore, for patients with intermediate cTn concentrations on presentation who require serial measurements, the pathway uses a delta of less than 3 ng/L to identify those with stable intermediate values who could be considered for discharge. This delta value was based on an analytical performance evaluation using values in the intermediate range (17), so that the chances of a change of 3 ng/L or more on serial measurements being due to analytical imprecision is less than 5% (4). In contrast, if samples are measured in one run using one lot of reagents and calibrators, as is typically done for biobank samples, only short-term analytical variation will be embedded in the data (9), and extra caution related to the long-term imprecision should be taken. Total analytical error should be considered when rule-out pathways are suggested, and if the cutoffs are poorly validated (e.g., based on one study using biobank samples measured with one reagent/calibrator lot) special safety precautions should be considered until the data has been validated in a sufficient number of studies, ensuring assay stability.

Clinical Implications of Increasing the Lower Limits for Reporting cTn Results

The lower limit for reporting quantitative cTn results differs between countries as some regions are able to report and use the LOD whilst others use the LOQ. The higher cutoff is chosen due to fear of misclassification based on the larger analytical error below the LOQ. On the contrary, it may be argued that increasing the cutoff above the LOD will decrease the clinical sensitivity for MI and hence also the safety of these pathways. What is considered to be an unacceptable miss rate for MI is debated, with 1% to 3% or less (sensitivity of 97% to 99%) usually accepted (37, 38). Analytical imprecision will have less of a clinical impact when the disease is less prevalent. Increases in cTn within the reference range are associated with higher risk of MI or cardiac death, but in consecutive patients presenting to the ED with concentrations below the 99th percentile the prevalence of MI or cardiac death at 30 days is as low as 4% (8). The number of affected patients may be calculated based on disease prevalence. As example, an analytical variation of 36% at a cutoff for rule-out of 2 ng/L would yield a probability of misclassifying an intermediate risk patient to rule-out of 20% (Table 1). This might seem like a high probability, but if the prevalence of MI is 4% (8), the overall probability of misclassifying a patient with MI would be 0.8% (4×0.20). It should be noted that the distribution of troponin concentrations amongst MI patients presenting with a baseline concentration below the 99th percentile will be left skewed, meaning that the prevalence of MI amongst patients with a baseline concentration around the 2 ng/L threshold is likely to be lower than 4%,

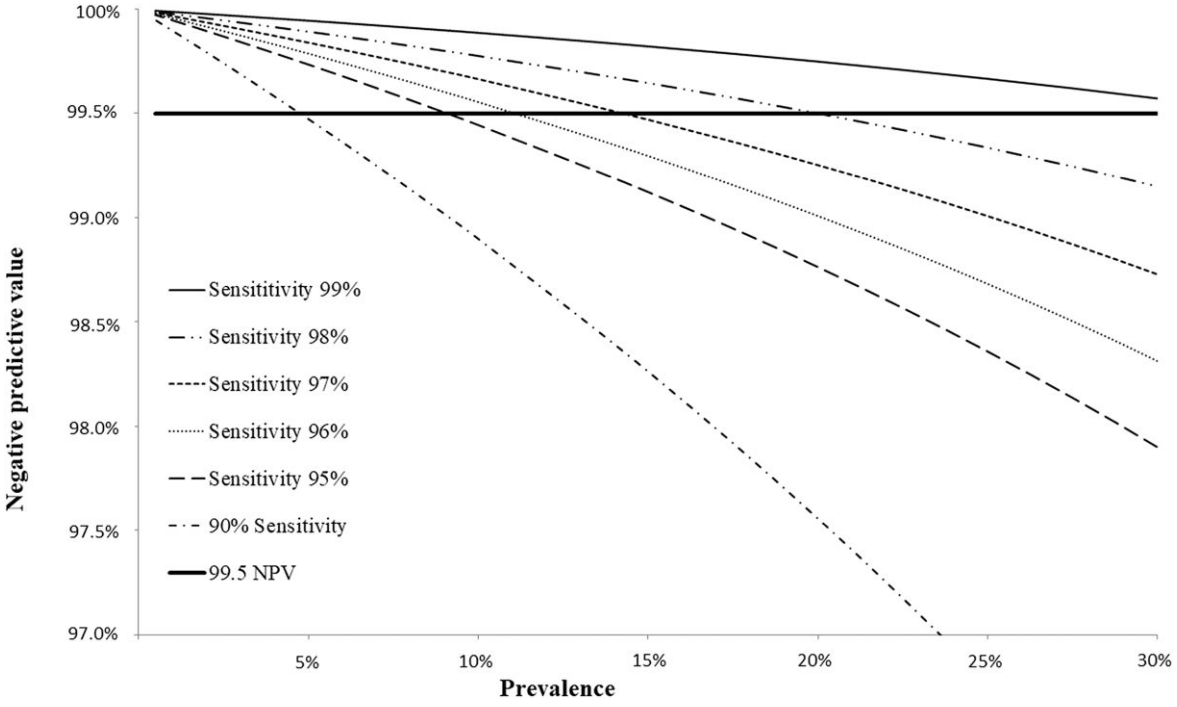

Fig. 2. Effect of disease prevalence on negative predictive value for different values of test sensitivity. When test sensitivity is reduced due to imprecision the net effect will be lower when disease prevalence is low.

suggesting that the real-life risk of missing an MI is even lower than the estimate of 0.8%. This assumption is supported by large clinical observation studies demonstrating a 30-day risk of MI or death varying from 0.2% to 0.7% for accelerated diagnostic protocols using low concentrations of hs-cTn (1).

It is also important to consider the negative predictive value (NPV). The NPV can be calculated as (specificity × [1 − prevalence])/(specificity × [1 − prevalence]) + ([1 − sensitivity] × prevalence). The specificity of cTn for the exclusion of myocardial injury or MI is effectively 100% so this equation reduces to (1 − prevalence)/([1 − prevalence] + [1 − sensitivity] × prevalence). As can be appreciated from Fig. 2, at a MI prevalence of 4%, a drop in clinical sensitivity to 90% as a consequence of analytical error will still yield a NPV of >99.5%.

A consequence of increasing the lower limit for reportable results above the cutoff suggested by clinical studies for safe single-sample rule-out of MI would be that all patients would need serial measurements. This may increase the risk of overcrowding in the ED with subsequent risk of mortality and increased costs (39, 40). As outlined above, it is unlikely that this disadvantage will be compensated by greater precision in identification of patients with possible MI. Accordingly, it could be argued that the reduced efficacy associated with the stipulation that higher cutoffs are used for reportable cTn results to avoid assay imprecision may increase risk to the overall ED population, although this has not yet been evaluated in any scientific study.

Conclusion

Clinical guidelines suggest that using single-sample rule-out based on cTn cutoffs as low as the LOD of the assay is safe and efficient when investigating patients with possible MI. Laboratories and external quality assessment providers serving hospitals using such pathways should align their analytical performance goals and assessment to the applicable protocol and measure the long- and short-term stability of cTn assays carefully. Developers of guidelines, manufacturers, and regulatory bodies should be aware of how analytical quality at low concentrations affects the clinical utility of assays. If long-term monitoring of risk based on cTn measurements is realized, analytical performance goals should be reconsidered as even stricter guidance may apply.

Nonstandard Abbreviations: MI, myocardial infarction; cTn, cardiac troponin; LOQ, limit of quantification; LOD, limit of detection; hs-cTn, high-sensitivity cTn; ED, emergency department.

Author Contributions: *The corresponding author takes full responsibility that all authors on this publication have met the following required criteria of eligibility for authorship: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; (c) final approval of the published article; and (d) agreement to be accountable for all aspects of the article thus ensuring that questions related to the accuracy or integrity of any part of the article are appropriately investigated and resolved. Nobody who qualifies for authorship has been omitted from the list.*

Kristin Aakre (Conceptualization-Lead, Methodology-Equal, Project administration-Lead, Resources-Equal, Writing—original draft-Lead), Fred Apple (Conceptualization-Equal, Methodology-Equal, Resources-Equal, Writing—review & editing-Equal), Nicholas Mills (Conceptualization-Equal, Methodology-Equal, Resources-Equal, Writing—review & editing-Equal), Steven Meex (Conceptualization-Equal, Methodology-Equal, Resources-Equal, Writing—review & editing-Equal), Paul Collinson (Conceptualization-Equal, Methodology-Equal, Resources-Equal, Writing—original draft-Equal).

Authors' Disclosures or Potential Conflicts of Interest: *Upon manuscript submission, all authors completed the author disclosure form*.

Research Funding: None declared.

Disclosures: K.M. Aakre, Associate Editor of *Clinical Biochemistry*, Chair of the International Federation of Clinical Chemistry Committee on Clinical Application of Cardiac Bio-Markers, served on advisory boards for Roche Diagnostics, Siemens Healthineers, and SpinChip, has received honorarium for consultancy from CardiNor, honorarium for lecturing from Siemens Healthineers and Snibe Diagnostics, and research grants from Siemens Healthineers and Roche Diagnostics. S.J.R. Meex, research grants from Abbott Diagnostics and Roche Diagnostics, and consultancy and honorarium for lecturing from Roche Diagnostics. N.L. Mills is supported by a Chair Award (CH/F/21/90010), a Programme Grant (RG/20/10/34966), and a Research Excellence Award (RE/18/5/34216) from the British Heart Foundation; reports research grants awarded to the University of Edinburgh from Abbott Diagnostics, Roche Diagnostics, and Siemens Healthineers; is on the advisory board and receives honoraria from Abbott Diagnostics, Siemens Healthineers, Roche Diagnostics, and LumiraDx; Associate Editor of *Circulation*. F.S. Apple, consultant for HyTest Ltd and AWE Medical Group; Associate Editor for *Clinical Chemistry* (Association for Diagnostics & Laboratory Medicine [ADLM]); on advisory boards for Werfen, Siemens Healthineers, Qorvo, and Abbott Vascular; honorarium for speaking at industry conferences from Siemens Healthineers; principal investigator on Industry Funded Grants (non-salaried) on cardiac biomarkers through Hennepin Healthcare Research Institute from Abbott Diagnostics, Abbott POC, BD, Beckman Coulter, Quidel/Ortho, Roche Diagnostics, and Siemens Healthineers. P.O. Collinson, Associate Editor of *The Journal of Applied Laboratory Medicine,* ADLM; received honoraria for lectures from Siemens Healthineers; on the advisory board of Psyros Diagnostics and Radiometer and has previously advised Siemens Healthineers and LumiraDx; consultant to IFCC Committee on Clinical Applications of Cardiac Bio-Markers (C-CB) (unpaid).

Members of the International Federation of Clinical Chemistry (IFCC) Committee on Clinical Applications of Cardiac Biomarkers (C-CB): Kristin M Aakre, MD PhD,[a,b] Fred S Apple, PhD,[c,d] Paul O Collinson, MD PhD,[e,f] Ola Hammarsten, PhD,[g] Allan S Jaffe, MD PhD,[h] Steven JR Meex, PhD,[i,j] Nicholas L Mills, MD PhD,[k,l] Torbjørn Omland, MD PhD,[m,n] Yader Sandoval, MD,[o] Louise Cullen[p,q,r]

[a]Department of Medical Biochemistry and Pharmacology & Department of Heart Disease, Haukeland University Hospital, Bergen, Norway
[b]Department of Clinical Science, University of Bergen, Bergen, Norway
[c]Department of Laboratory Medicine and Pathology, Hennepin Healthcare/HCMC, Minneapolis, MN, United States
[d]Department of Laboratory Medicine and Pathology, University of Minnesota, Minneapolis, MN, United States
[e]Departments of Clinical Blood Sciences and Cardiology, St. George's University Hospitals NHS Foundation Trust, London, United Kingdom
[f]St. George's University of London, London, United Kingdom
[g]Department of Clinical Chemistry and Transfusion Medicine, University of Gothenburg, Gothenburg, Sweden
[h]Departments of Cardiology and Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN, United States
[i]Central Diagnostic Laboratory, Maastricht University Medical Center, Maastricht, the Netherlands
[j]CARIM School for Cardiovascular Diseases, Maastricht University, Maastricht, the Netherlands
[k]BHF Centre for Cardiovascular Science, University of Edinburgh, Edinburgh, United Kingdom
[l]Usher Institute, University of Edinburgh, Edinburgh, United Kingdom
[m]Department of Cardiology, Akershus University Hospital, Lørenskog, Norway
[n]Institute of Clinical Medicine, University of Oslo, Oslo, Norway
[o]Minneapolis Heart Institute, Abbott Northwestern Hospital and Minneapolis Heart Institute Foundation, Minneapolis, MN, United States
[p]Department of Emergency Medicine, Royal Brisbane and Women's Hospital, Brisbane, Australia
[q]School of Medicine, University of Queensland, Brisbane, Australia
[r]Faculty of Health, Queensland University of Technology, Brisbane, Australia.

References

1. Neumann JT, Twerenbold R, Ojeda F, Sorensen NA, Chapman AR, Shah ASV, et al. Application of high-sensitivity troponin in suspected myocardial infarction. N Engl J Med 2019;380:2529–40.
2. Jaffe AS, Ravkilde J, Roberts R, Naslund U, Apple FS, Galvani M, Katus H. It's time for a change to a troponin standard. Circulation 2000;102:1216–20.
3. Jaffe AS, Body R, Mills NL, Aakre KM, Collinson P, Saenger A, et al. Single sample rule out for myocardial infarction—recommendations from the IFCC committee on cardiac bio-markers. J Am Coll Cardiol 2023;82:60–9.
4. Chapman AR, Anand A, Boeddinghaus J, Ferry AV, Sandeman D, Adamson PD, et al. Comparison of the efficacy and safety of early rule-out pathways for acute myocardial infarction. Circulation 2017;135:1586–96.
5. Shah ASV, Anand A, Strachan FE, Ferry AV, Lee KK, Chapman AR, et al. High-sensitivity troponin in the evaluation of patients with suspected acute coronary syndrome: a stepped-wedge, cluster-randomised controlled trial. Lancet 2018;392:919–28.

6. Wildi K, Boeddinghaus J, Nestelberger T, Twerenbold R, Badertscher P, Wussler D, et al. Comparison of fourteen rule-out strategies for acute myocardial infarction. Int J Cardiol 2019;283:41–7.
7. Shah AS, Anand A, Sandoval Y, Lee KK, Smith SW, Adamson PD, et al. High-sensitivity cardiac troponin I at presentation in patients with suspected acute coronary syndrome: a cohort study. Lancet 2015;386:2481–8.
8. Chapman AR, Shah AS, Mills NL. Appropriate use of high-sensitivity cardiac troponin levels in patients with suspected acute myocardial infarction. JAMA Cardiol 2017;2:228.
9. Tjora HL, Steiro OT, Langorgen J, Bjorneklett RO, Skadberg O, Bonarjee VVS, et al. Diagnostic performance of novel troponin algorithms for the rule-out of non-ST-elevation acute coronary syndrome. Clin Chem 2022;68:291–302.
10. Omland T, de Lemos JA, Sabatine MS, Christophi CA, Rice MM, Jablonski KA, et al. A sensitive cardiac troponin T assay in stable coronary artery disease [eng]. N Engl J Med 2009;361:2538–47.
11. Willeit P, Welsh P, Evans JDW, Tschiderer L, Boachie C, Jukema JW, et al. High-Sensitivity cardiac troponin concentration and risk of first-ever cardiovascular outcomes in 154,052 participants. J Am Coll Cardiol 2017;70:558–68.
12. Blankenberg S, Salomaa V, Makarova N, Ojeda F, Wild P, Lackner KJ, et al. Troponin I and cardiovascular risk prediction in the general population: the BiomarCaRE consortium. Eur Heart J 2016;37:2428–37.
13. Ford I, Shah AS, Zhang R, McAllister DA, Strachan FE, Caslake M, et al. High-Sensitivity cardiac troponin, statin therapy, and risk of coronary heart disease. J Am Coll Cardiol 2016;68:2719–28.
14. Wu AHB, Kavsak PA, Aakre KM, Christenson RH, Greene DN, Apple FS, et al. Lot-to-Lot variation for commercial high-sensitivity cardiac troponin: can we realistically report down to the assay's limit of detection? Clin Chem 2020;66:1146–9.
15. Kavsak PA, Clark L, Jaffe AS. Effect of repeat measurements of high-sensitivity cardiac troponin on the same sample using the European society of cardiology 0-hour/1-hour or 2-hour algorithms for early rule-out and rule-in for myocardial infarction. Clin Chem 2017;63:1163–5.
16. Haagensen K, Collinson P, Asberg A, Aakre KM. How does the analytical quality of the high-sensitivity cardiac troponin T assay affect the ESC rule out algorithm for NSTEMI? Clin Chem 2019;65:494–6.
17. Kavsak PA, Don-Wauchope AC, Hill SA, Worster A. Acceptable analytical variation may exceed high-sensitivity cardiac troponin I cutoffs in early rule-out and rule-in acute myocardial infarction algorithms. Clin Chem 2016;62:887–9.
18. Wu AHB, Christenson RH, Greene DN, Jaffe AS, Kavsak PA, Ordonez-Llanos J, Apple FS. Clinical laboratory practice recommendations for the use of cardiac troponin in acute coronary syndrome: expert opinion 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. Clin Chem 2018;64:645–55.
19. Collet JP, Thiele H, Barbato E, Barthelemy O, Bauersachs J, Bhatt DL, et al. 2020 ESC guidelines for the management of acute coronary syndromes in patients presenting without persistent ST-segment elevation. Eur Heart J 2021;42:1289–367.
20. Gulati M, Levy PD, Mukherjee D, Amsterdam E, Bhatt DL, Birtcher KK, et al. 2021 AHA/ACC/ASE/CHEST/SAEM/SCCT/SCMR guideline for the evaluation and diagnosis of chest pain: A report of the American college of cardiology/American heart association joint committee on clinical practice guidelines. Circulation 2021;144:e368–454.
21. Kimenai DM, Henry RM, van der Kallen CJ, Dagnelie PC, Schram MT, Stehouwer CD, et al. Direct comparison of clinical decision limits for cardiac troponin T and I. Heart 2016;102:610–6.
22. Apple FS. A new season for cardiac troponin assays: it's time to keep a scorecard. Clin Chem 2009;55:1303–6.
23. Kavsak PA, Jaffe AS, Greene DN, Christenson RH, Apple FS, Wu AHB. Total analytic error for low cardiac troponin concentrations (</=10 ng/L) by use of a high-sensitivity cardiac troponin assay. Clin Chem 2017;63:1043–5.
24. Kavsak PA, Petryayeva E, Clark L. Analytical variation and Abbott diagnostics high-sensitivity cardiac troponin I risk categories in asymptomatic individuals. Can J Cardiol 2019;35:1605 e7– e8.
25. Kavsak PA, Clark L, Arnoldo S, Lou A, Shea JL, Eintracht S, et al. Analytic result variation for high-sensitivity cardiac troponin: interpretation and consequences. Can J Cardiol 2023;39:947–51.
26. IFCC Committee on Clinical Applications of Cardiac Bio-Markers (C-CB). High-Sensitivity* Cardiac Troponin I and T Assay Analytical Characteristics Designated by Manufacturer. v012019. https://ifcc.org/ifcc-education-division/emd-committees/committee-on-clinical-applications-of-cardiac-bio-markers-c-cb/biomarkers-reference-tables/ (Accessed May 2023).
27. Zaninotto M, Padoan A, Mion MM, Marinova M, Plebani M. Short-term biological variation and diurnal rhythm of cardiac troponin I (access hs-TnI) in healthy subjects. Clin Chim Acta 2020;504:163–7.
28. Aakre KM, Roraas T, Petersen PH, Svarstad E, Sellevoll H, Skadberg O, et al. Weekly and 90-Minute biological variations in cardiac troponin T and cardiac troponin I in hemodialysis patients and healthy controls. Clin Chem 2014;60:838–47.
29. Flowers KC, Hunt C, Collinson PO. Interanalyzer analytical variation of a high-sensitivity cardiac troponin T assay can exceed the cutoff of the European society of cardiology 1-hour algorithm for ruling out non-ST-segment elevated myocardial infarction. Clin Chem 2020;66:495–6.
30. Donato LJ, Wockenfus AM, Katzman BM, Baumann NA, Jaffe AS, Karon BS. Analytical and clinical considerations in implementing the Roche Elecsys Troponin T Gen 5 STAT Assay. Am J Clin Pathol 2021;156:1121–9.
31. Bais R. The effect of sample hemolysis on cardiac troponin I and T assays. Clin Chem 2010;56:1357–9.
32. Lippi G, Avanzini P, Aloe R, Cervellin G. Blood collection from intravenous lines: is one drawing site better than others? Lab Med 2014;45:172–5.
33. Klinkenberg LJ, van Dijk JW, Tan FE, van Loon LJ, van Dieijen-Visser MP, Meex SJ. Circulating cardiac troponin T exhibits a diurnal rhythm. J Am Coll Cardiol 2014;63:1788–95.
34. Kleiven O, Omland T, Skadberg O, Melberg TH, Bjorkavoll-Bergseth MF, Auestad B, et al. Race duration and blood pressure are major predictors of exercise-induced cardiac troponin elevation. Int J Cardiol 2019;283:1–8.
35. Lee G, Twerenbold R, Tanglay Y, Reichlin T, Honegger U, Wagener M, et al. Clinical benefit of high-sensitivity cardiac troponin I in the detection of exercise-induced myocardial ischemia. Am Heart J 2016;173:8–17.
36. Lyngbakken MN, Skranes JB, de Lemos JA, Nygard S, Dalen H, Hveem K, et al. Impact of smoking on circulating cardiac troponin I concentrations and cardiovascular events in the general population: the HUNT study (Nord-Trondelag Health Study). Circulation 2016;134:1962–72.
37. Than M, Herbert M, Flaws D, Cullen L, Hess E, Hollander JE, et al. What is an acceptable risk of major adverse cardiac event in chest pain patients soon after discharge from the emergency department? : a clinical survey. Int J Cardiol 2013;166:752–4.
38. National Institute for Health and Care Excellence. High-sensitivity tests for the early rule-out of NSTEMI, DG40. 2020. https://www.nice.org.uk/guidance/dg40 (Accessed December 2023).
39. Sun BC, Hsia RY, Weiss RE, Zingmond D, Liang LJ, Han W, et al. Effect of emergency department crowding on outcomes of admitted patients. Ann Emerg Med 2013;61:605–11 e6.
40. Jones S, Moulton C, Swift S, Molyneux P, Black S, Mason N, et al. Association between delays to patient admission from the emergency department and all-cause 30-day mortality. Emerg Med J 2022;39:168–73.