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Review

Analytical performance of cardiac troponin assays – Current status and future needs

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ABSTRACT

Concurrent with the introduction of cardiac troponin measurements into the diagnostic definition of myocardial infarction (MI), clinicians and laboratory professionals signaled a clear clinical need for improved analytical quality. This was an important precipitant for developing high-sensitivity cardiac troponin (hs-cTn) assays, currently used in rapid algorithms guiding investigations of patients presenting to the emergency department with possible MI. The hs-cTn assays were also important for the detection and monitoring of low-grade chronic myocardial injury, a condition that has been linked to increased long-term risk of cardiovascular morbidity and mortality. This review summarizes the general recommendations for defining analytical performance specifications while providing relevant clinical situations related to analytical performance. Importantly, outcome studies suggest analytical quality performance for hs-cTn is sufficient for early discharge of patients investigated for possible MI. However, bias due to change in calibrators or reagents may significantly affect the percentage of patients discharged. Biological variation data is suitable for defining performance specifications when hs-cTn measurements are used for diagnosing and monitoring chronic myocardial injury. Further improvement in analytical performance for hs-cTn testing may result in even faster decision making in the emergency setting; while also identifying those with chronic injury at risk for an adverse cardiac event.

1. Introduction

The hallmark of cardiomyocyte injury is the release of cardiac specific troponin T and troponin I into the systemic circulation. Commercial assays for cardiac troponins were introduced during the 1990s, and since then the steadily improvement in analytical performance advocated by cardiologists, emergency physicians and clinical chemists have shaped clinical practice and lead to the development of the current generations of high-sensitivity cardiac troponin assays. Throughout the evolution of the cardiac troponin tests, laboratory professions were deeply involved in the specification of the analytical goals for sensitivity, specificity (through proper antibody selection) and the standardization and harmonization that were needed for the creation of the next generation cardiac troponin assays.

Cardiac troponin testing was clinically endorsed in the consensus definition of myocardial infarction (MI) published in 2000 [1], one year after the National Academy of Clinical Biochemistry endorsed cardiac troponin in this setting [2]. Similar to the current 4th Universal Definition of Myocardial Infarction published in 2018 [3], MI was defined as a significant rise or fall in cardiac troponin concentrations together with one measured concentration above the 99th percentile upper reference limit (URL) from a healthy population of the assay, if the clinical setting was compatible with acute coronary syndrome (ACS). The 99th percentile URL was analytically a challenge to determine and monitor in the early 2000s since the available assays were not able to measure quantitative results in a large proportion of the healthy population. This finding coupled to the high analytical uncertainty at the 99th percentile concentration opted some sites to use another cutoff for the detection of injury. Most hospitals therefore used higher cut offs, and there were large inter-hospital variations in the applied cut offs [4,5]. However, clinical and laboratory advocates for improved analytical performance with outcome data supporting even minor myocardial injury being prognostically important spurred the diagnostic industry to further develop these assays, leading to the development of

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the current generation of high-sensitivity cardiac troponin (hs-cTn) assays.

2. Clinical implications of high-sensitivity cardiac troponin assays

The first publications on hs-cTn assays detailing analytical, clinical, and the diagnosis of MI were published in 2009, with the past decade leading to more assays being developed [6–8]. By definition, hs-cTn assays should measure quantitative concentrations in at least 50% of the healthy population and show a maximum 10% "guideline acceptable" analytical variation (CV_A) at the 99th percentile of the assay [9,10].

The advantage of the hs-cTn assays was an improved "signal to noise ratio", meaning that the assays could differentiate between a significantly increased cardiac troponin concentration ("signal") and random variation due to pre-analytical, analytical and biological uncertainty ("noise"), even at minor myocardial necrosis. The improved analytical certainty at low concentrations made it possible to detect myocardial injury at an earlier stage, reducing the observation time for patients with chest pain susceptive of non-ST elevation myocardial infarction (NSTEMI) from six to three hours to even earlier measurements [7,11], and further paved the road for early and rapid clinical decision making in the work-up of patients presenting to the emergency department (ED) with acute chest pain [12,13].

The improved analytical performance of hs-cTn assays also increased the ability to predict long-term cardiovascular (CV) risk in the general population and in high-risk patients with and without prior CV disease. Seminal papers have demonstrated the strong predictive power of stable low-grade increased cardiac troponin concentrations [14–16] and the condition was recently acknowledged as clinically important and termed chronic myocardial injury [3]. The observation that chest pain patients with chronic injury show similar long-term prognosis as those diagnosed with acute MI [17] has led to increased interest in and search for treatment possibilities in this population [18–20].

3. Define sufficient analytical performance

Strategies to define the necessary analytical performance for laboratory tests have recently been updated [21]. The current recommendations suggest three different models for defining performance specifications (PS) depending on the clinical use of the test; clinical outcome, biological uncertainty (usually termed "biological variation") and state of the art. Cardiac troponins are used in different clinical contexts and all three models may therefore be relevant. The optimal way to define PS based on clinical outcomes are by conducting a randomized clinical trial (RCT) measuring if patient outcomes are different based on different analytical performance. This strategy typically requires large patient cohorts and/or long follow-up periods so an alternative and simpler approach is to measure indirect outcomes. This may be done by registering changes in surrogate endpoint, usually changes in clinical classification [21,22]. It is important to acknowledge that this model evaluates how the total uncertainty affects the patient outcome. There are no separate recommendations related to within-subject biological or analytical uncertainty, so a biomarker with a very low within-subject biological variation and a very high analytical uncertainty could provide an excellent outcome if the total uncertainty is sufficiently low for clinical needs.

The second option is to use the biological variation, this is recommended for components that usually are in a steady-state when an individual is in good health, and may be less feasible for components that have large physiological variations. This concept implies that an increase or decrease from the steady state concentration may signal a clinically significant change and it is usually feasible to identify the smallest possible reliable delta values when clinical cut offs are defined. Analytical uncertainty should therefore only add minor changes to the unavoidable biological variation and typically, a CV_A being half the within-subject biological variation and a bias less than ¼ of the combined within-subject and between-subject variation, is regarded sufficient [23]. The last model is state of the art; and can be employed by all laboratories as precision goals and total uncertainty are provided [24].

4. Defining analytical performance specification for cardiac troponin based on patient outcomes

The largest study investigating the impact analytical performance and cutoffs for cardiac troponin has on clinical outcomes is the High-STEACS study; a stepped-wedge cluster-based RCT including 48 282 patients with possible ACS [25]. The somewhat disappointing result was that even if the hs-cTnI assay re-classified 17% of patients from unstable angina pectoris (UAP) to non-ST segment elevation MI (NSTEMI), this did not improve the long-term prognosis of the patients. The authors explained this unexpected observation as most of the re-classified patients were unavailable for treatment (i.e., type 2 MI or patients who were already maximally treated for coronary artery disease) or were less likely to receive it (i.e., female sex). Other endpoints came out positively, e.g. the overall length of hospital stay was reduced by a third since most patients without ACS could be discharged earlier. Other caveats for this study included no a prior criteria on what constituted a significant change in hs-cTnI concentrations (which is dependent on analytical performance) with only educational and implementation efforts focused on the change to a hs-cTnI assay and the sex-specific 99th percentile cutoffs. Another RCT trial, the RAPID trial, compared two emergency department algorithms for rapid follow-up of possible ACS, using different time frames and cut offs for lower reportable results (1 h observation and 5 ng/L vs. 3 h observation and 29 ng/L). This study showed similar diagnostic performance for both algorithms, but shorter duration of stay and higher discharge rate in the ED in the 1 h low troponin algorithm arm [26]. These studies underscores the complexity and high work-load when RCTs are used to define PS for laboratory tests, and also that even if a high analytical quality seems beneficial the definitive effects on "hard end-points" may be difficult to demonstrate.

Notwithstanding these study findings, the clear diagnostic definition and diagnostic cut off established for myocardial infarction allows one to use the alternative approach and calculate the number of re-classified patients depending on different analytical quality criteria (i.e., a more feasible strategy). International recommendations suggest that patients presenting with possible NSTEMI should be investigated using specific cardiac troponin-based rule-out and rule-in algorithms. Based on hs-cTn results patients are eligible for early discharge or further investigations or treatment for ACS [12,13]. These algorithms typically encompass an initial review of the admission sample in patients who present more than 2–3 h after symptom onset. If the concentration is below the limit of detection (LoD) of the hs-cTn assay the patient is eligible for "rule-out", meaning they may be discharged if the ECG and/or the clinical symptoms are less likely of ACS. Remaining patients are subjected to serial sampling on 1 to 3 h intervals depending on the algorithm. Based on baseline and delta values obtained patients are allocated to "rule-out", "observation" or "rule-in" for NSTEMI. Ruled-out patients may be discharged if the clinical suspicion of ACS is low, ruled-in may go directly to cardiac angiography and eventually invasive treatment, while those in the observation group undergo diagnostic follow-up and eventually a minor fraction is diagnosed with NSTEMI. These algorithms have reasonable high accuracy for NSTEMI, still the implementation have been slow through Europe and Northern America [5]. Laboratory reluctance due to uncertainty about analytical performance of hs-cTn assays at low concentrations and government restrictions (e.g. in the USA) regarding the lower limit of reportable results may provide an obstacle to implementation. A much cited study surveyed clinical expectations and found that most emergency physicians would accept a miss-rate for NSTEMI of less than 1% [27]. As absolute concentrations are used as cut offs both in the admission sample rule-

out and several times in the serial sampling protocols it is obvious that intermittent bias due to change in calibrators or reagents may influence the percentage of patients allocated into different categories and false rule-outs and rule-in could potentially occur [28–30]. If the combined pre-analytical uncertainty and CV_A is high this may also cause erroneous delta values for individual patients, and result in incorrect follow-up strategy [31–33]. In spite of this it is reassuring that most of the algorithms have been extensively validated through a large number of clinical cohorts demonstrating a performance in line with or close to the 1% clinical criteria [34], leading to a growing consensus that these algorithms are clinically safe. Yet, the challenge for the laboratory to maintain objective acceptable performance may still be difficult [35,36], and all novel assays/algorithms need rigorous clinical validation before implementation into daily practice.

Even if safe, different studies show large variations in the efficiency of an algorithm (ability to rule-out patients as non-NSTEMI and rule-in patients as NSTEMI) [37,38]. This variation may relate to clinical difference between study cohorts but is also likely explained by the use of different reagent and calibrator lots, as a slight bias in assay accuracy may have significant impact of percentage of patients presenting with concentrations above or below the limit of detection (LoD) or the cut offs used in the serial protocols [28,29,39]. A recent simulation study done by Lyon et al showed that bias would lead to misclassification of 5–10% of patients when very low concentrations were applied as thresholds [40], and another study showed percentages of concentrations below the LoD measured in the ED could vary between 15% and 30%, depending on lot used [41]. The reason why these minor biases, do not translate into a reduced sensitivity for NSTEMI is most likely due to the fact that the majority of NSTEMI patients rapidly develop myocardial necrosis which by far exceeds the amount needed to increase the cardiac troponin concentration above the LoD or the suggested delta values in serial protocols [42]. As shown in Fig. 1, minor biases related to different reagent lots mainly affect classification of the large number of patients exhibiting low concentrations, these are usually patients with non-coronary chest pain. Accordingly, the effect of intermittent bias most likely influence the percentages of true negatives who are eligible for rule-out, e.g. affecting the efficiency, but not the safety, of the algorithm.

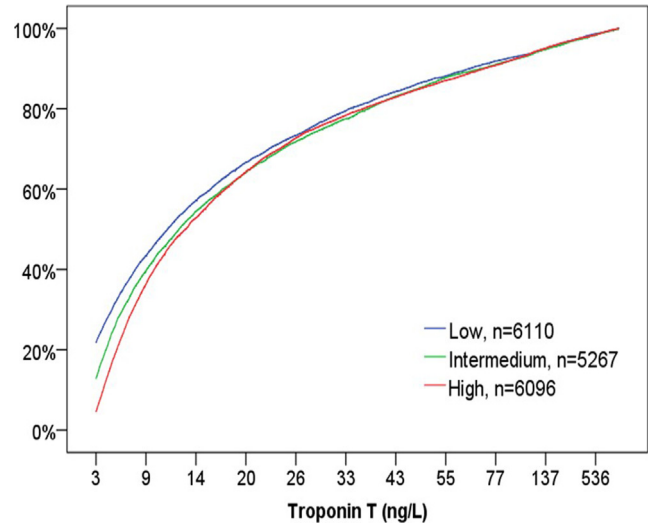

Fig. 1. Cumulative percentages of troponin results obtained from the emergency department at Haukeland University Hospital using three different reagent lots. The median concentrations of results in the low range (≤20 ng/L) were 5.53 ng/L (low lot), 6.52 ng/l (intermedium lot) and 7.60 (high lot). Lot variation mainly affected low concentrations as percentage below 5 ng/L (LoD) ranged from 18% to 31% while percentage below 52 ng/L (cut off for rule-in) ranged from 86% to 88%.

5. Defining analytical performance specification for hs-cTn assays based on biological variation

Chronic myocardial injury is a condition were cardiac troponin concentrations are expected to be stable and even a slight deviation from baseline could be clinically relevant. Biological variation data are therefore feasible for making PS when cardiac troponins are used in the diagnosis or monitoring of chronic myocardial injury. Herein, chronic myocardial injury is defined as stable cardiac troponin concentrations above the 99th percentile of the assay, but the risk of future cardiovascular events or death increases constantly from lower to higher concentrations starting even below the LoD of the hs-cTn assay [41]. Studies have demonstrated that medical treatment (statins), physical activity programs or even surgery in the morbidly obese may stabilize or slightly reduce cardiac troponin at low concentrations [18–20]. Such minor changes in concentrations are commonly interpreted as surrogate markers of prognosis and PS based on long-term biological variation would facilitate early identification of clinically significant changes.

A *meta*-analysis of available biological variation data for cardiac troponins will be undertaken by the EFLM and eventually be available on the EFLM Biological Variation Database webpage (personal communication Aasne K Aarsand), including tools for calculating the recommended CV_A and bias. Until then an overview of biological variation studies for hs-cTn assays are provided in Table 1. Most studies suggest a long-term within-subject variation below 15%. Between – subject variation are dependent on clinical condition with low values (below 30%) in healthy and substantially larger variations in patients with chronic disease. Based on this a desirable analytical variation for hs-cTn assays of approximately 7% and a desirable bias (e.g. useful for evaluating intermittent bias due to changes in calibrators and reagent lots) below 9% may be suggested for cardiac troponin concentrations below the 99th percentile concentration. Assay specific analytical performance data as stated by the manufacturer are reported from the IFCC Committee on Clinical Applications of Cardiac Bio-Markers [43] and illustrates that most assays have slightly higher CV_A at low concentrations. Even though the manufacturer information provided is useful for analytical specifications, the IFCC table does not include data on total uncertainty or bias due to changes in calibrators or reagent lots, which may be of interest when chronic injury is being monitored. Studies demonstrate a long-term total analytical uncertainty for hs-cTn assays in routine laboratories of < 3.5 ng/L with within-series CV_A of less than 1 ng/L [28] for concentrations ≤10 ng/L. Of note, these estimates are slightly higher compared to the most conservative PS that may be calculated based of biological variation.

6. Is further improvement in analytical performance of hs-cTn assays clinically useful?

Assays with further improvement in analytical performance, which are able to measure cardiac troponin concentrations in all healthy individuals with high precision, may have a potential for further improvement in clinical practice. First, such assays will have an even better signal to noise ratio, making it possible to detect very small increases in cardiac troponin concentrations and potentially reduce the 2–3 h time lag that is currently recommended between symptom onset and cardiac troponin measurements when NSTEMI is being investigated. Depending on the country, 15–40% of chest pain patients are early presenters [44,45], and an assay that could identify myocardial injury after a shorter time-lag would have a potential for streamlining the work-flow of chest pain patients in the ED compared to todays practice. Few studies have tested this but there are indications that the hs-cTnI$_{(sgx)}$ assay from Singulex Clairety System (currently unavailable on the commercial market) that could measure quantitative troponin concentrations in nearly 100% of healthy individuals with high precision, may be able to identify myocardial necrosis with a shorter time – lag compared to todays assays [46].

Table 1
Short and long term biological variation and reference change values (95% CI) for high-sensitivity troponin assays.

Publication	Condition	Subject (n)	Sampling interval	Assay	Mean/median [a] concentration (ng/L)	CV_A	CV_I	CV_G	RCV
Short-term variation Hs-cTnT									
Vasile et al 2010 [55]	Healthy	20	60 min	Hs-cTnT	1.53[a]	53.5	48.2	85.9	+84.6 (80% CI)
Frankenstein et al 2011 [56]	Healthy	20	60 min	Hs-cTnT	NR	7.8	15	NR*	−39 /+64
Frankenstein et al 2011 [56]	Healthy	20	60 min	Hs-cTnT	NR	9.7	21	NR	−47 /+90
Dupuy et al 2013 [57]	Healthy	24	60 min	Hs-cTnT	12.5[a]	2.4	6.3	56.9	−10 /+12
Aakre et al 2014 [58]	Healthy	15	90 min	Hs-cTnT	5.1	9.9	1.2	32.6	−27 / +29
Dupuy et al 2013 [57]	Healthy	24	3 h	Hs-cTnT	12.5[a]	2.4	6.2	55.6	−16 /+19
Aakre et al 2014 [58]	ESRD	18	90 min	Hs-cTnT	86.1	1.4	1.9	110.0	−8 / +5
Nordenskjöld et al 2013 [59]	Stable CAD	16	4 h	Hs-cTnT	12.5	4.0	7.3	70.0	−21 /+26
Short-term variation Hs-cTnI									
van der Linden 2017 [60]	Healthy	18	60 min	Hs-cTnI$_{(Abbott)}$	BL: 4.7[a]	10.0	8.6	49.4	−30.6 /+44.0
Aakre et al 2014 [58]	Healthy	17	90 min	Hs- cTnI$_{(Abbott)}$	3.9	17.3	5.0	37.7	−39 / +64
van der Linden 2017 [60]	Healthy	18	3 h	Hs-cTnI$_{(Abbott)}$	BL: 4.7[a]	9.9	9.4	48.0	−31.4 /+45.8
van der Linden 2017 [60]	Healthy	18	6 h	Hs-cTnI$_{(Abbott)}$	BL: 4.7[a]	10.1	9.2	48.6	−31.4 /+45.7
van der Linden 2017 [60]	CKD	18	60 min	Hs-cTnI$_{(Abbott)}$	BL: 6.8[a]	5.6	7.7	62.4	–23.2 /+30.2
Aakre et al 2014 [58]	ESRD/Dialysis	15	90 min	Hs- cTnI$_{(Abbott)}$	41.5	6.2	3.3	148.1	−18 / +21
van der Linden 2017 [60]	CKD	18	3 h	Hs-cTnI$_{(Abbott)}$	BL: 6.8[a]	5.6	8.7	61.6	−24.8 /+33.0
van der Linden 2017 [60]	CKD	18	6 h	Hs-cTnI$_{(Abbott)}$	BL: 6.8[a]	5.9	8.9	61.6	−25.5 /+34.2
Nordenskjöld et al 2013 [59]	Stable CAD	23	4 h	Hs-cTnI$_{(Abbott)}$	11.7	8.0	13.5	187	−35 /+54
Simpson et al 2014 [61]	Acute chest pain	283	1.5–17 h	Hs-cTnI$_{(Abbott)}$	NR	8.5	14	84	−36 /+56
Zaninotto et al 2020 [62]	Healthy	35	0 h, 1 h, 2 h, 3 h, 7 h	Hs-cTnI$_{(Access)}$	2.35[a]	8.4	4.1	17.3	–22.4 /+29.5
Wu et al 2009 [63]	Healthy	12	60 min	Hs-cTnI$_{(Singulex)}$	Range 1 – 5 (mean value)	8.3	9.7	57	–32 / +46
Long-term variation Hs-cTnT									
Vasile et al 2010 [55]	Healthy	20	Weekly	Hs-cTnT	1.67[a]	98	94	94	+315
Frankenstein et al 2011 [56]	Healthy	17	Weekly	Hs-cTnT	NR	7.8	31	NR	−58 /+138
Frankenstein et al 2011 [56]	Healthy	17	Weekly	Hs-cTnT	NR	9.7	30	NR	−58 /+135
Aakre et al 2014 [58]	Healthy	14	Weekly	Hs-cTnT$_{(}$	5.4	9.7	8.3	26.8	−30 / +42
Corte et al 2015 [64]	Healthy	11	Weekly	Hs-cTnT	7.8[a]	5.1	5.9	30.4	−26.2 /+35.5
Jones et al 2020 [65]	Moderate CKD	18	Weekly	Hs-cTnT	11.1[a]	3.1	7.4	78.4	−20 /+25
Aakre et al 2014 [58]	ESRD	15	Weekly	Hs-cTnT	70.9	1.7	8.3	101.5	−21 / +26
Mbagaya et al 2015 [66]	ESRD	16	Weekly	Hs-cTnT	range 23.5–208 (mean values)	2.1	10.5	64.2	−25.6 /+34.4
Fahim et al 2015 [67]	ESRD	42	Weekly	Hs-cTnT	34[a]	3.1	7.9	83	−17 /+20 (90% CI)
Fahim et al 2015 [67]	ESRD	39	Monthly	Hs-cTnT	34[a]	2.4	12.6	79	−25 /+34 (90% CI)
Corte et al 2015 [64]	ESRD	18	Monthly	Hs-cTnT	43.2[a]	6.0	14.7	77.8	−43.4 /+76.6
Nordenskjöld et al 2013 [59]	Stable CAD	16	23 days (range 4–58)[b]	Hs-cTnT	12.7	4.0	10.7	65.0	−27 /+37
Frankenstein et al 2011 [68]	Heart failure (IHD n = 17; cDMP n = 24)	41	four intervals from 14 to 90 days	Hs-cTnT	BL IHD: 18 [a] BL cDMP: 8.5[a]		2.7 – 3.5	NR	7.6 – 9.7
Täger et al 2019 [69]	Cardiovascular disease	169	1 year	Hs-cTnT	11-12[a] [d]	NR	15.9	NR	44.2
Peeters et al 2019 [70]	AVS	16	1 year[c]	Hs-cTnT	9.2	3.7	11.2	57.2	−30.1 /+43.1
Long-term variation Hs-cTnI									
Aakre et al 2014 [58]	Healthy	15	Weekly	Hs- cTnI$_{(Abbott)}$	3.2	13.8	15.6	25.9	−44 / +77
Ceriotti et al 2020 [71]	Healthy	89	Weekly	Hs-cTnI$_{(Singulex)}$	0.78	11.6	15.0–17.8[e]	36.8 – 65.3[e]	NR
Ceriotti et al 2020 [71]	Healthy	66	Weekly	Hs-cTnI$_{(Siemens)}$	2.85	10.7	12.8 – 14.7[e]	28.8–36.3[e]	NR
Jones et al 2020 [65]	Moderate CKD	18	Weekly	Hs-cTnI$_{(Abbott)}$	4.3[a]	8.3	15	105.6	−38 /+60
Aakre et al 2014 [58]	ESRD	14	Weekly	Hs- cTnI$_{(Abbott)}$	27.7	5.8	14.3	126.7	−35 / +53
Nordenskjöld et al 2013 [59]	Stable CAD	23	23 days (range 4–58)[b]	Hs-cTnI$_{(Abbott)}$	7.8	8.0	23.6	163.0	−49 /+97
Peeters et al 2019 [70]	AVS	16	1 year[c]	Hs-cTnI$_{(Abbott)}$	3.6	14.9	9.2	35.0	−42.2 /+73.1
Wu et al 2009 [63]	Healthy	17	2 weeks	Hs-cTnI$_{(Singulex)}$	Range 1.5 – 10.3 (mean value)	15	14	63	−45 /+81
Wu et al 2012 [72]	CV high-risk patients	17	Range 5–15 months	Hs-cTnI$_{(Singulex)}$	1.72	15	28	71	−45 /+98

(*continued on next page*)

Table 1 (*continued*)

Publication	Condition	Subject (n)	Sampling interval	Assay	Mean/median [a] concentration (ng/L)	CV_A	CV_I	CV_G	RCV
Mbagaya et al 2015 [66]	ESRD	15	Weekly	$cTnI_{(siemens)}$	range 11.5–168 (mean values)	7.1	20.2	100.5	−44.4 / +79.8

CV_A: Analytical variation. CV_I: Witin-subject biological variation. CV_G: Between-subject biological variation. RCV: Reference change values. NR: not reported. BL: Baseline. ESRD: End stage renal disease. AVS: Aorta valve stenosis. IHD: ischemic heart disease. dCMP: dilated cardiomyopathy. a: median. b: average between inclusion and admission samples. c: blood samples were taken on seven occasions during a 1-year period. d: two consecutive measurements. e: depending on gender.

Second, UAP and chest pain of non - cardiac origin may be differentiated by clinical judgement or the use of advanced investigations, e.g. coronary computer tomography angiography. UAP is myocardial ischemia with stable troponin concentrations as the coronary arteries are not completely occluded and no biochemically detectible necrosis occur. UAP requires similar treatment as NSTEMI while patients with chest pain of non - cardiac origin may be discharged without further follow-up. The hourly biological variation of cardiac troponins are quite low, median within-subject biological variation are 6% and 9% for cardiac troponin T and I, respectively (Table 1). The delta values currently determining a significant troponin release in the 0/1 and 0/3 h protocols (e.g. 3–6 ng/L) are consequently largely decided based on the assays CV_A at low concentration. Assays with improved precision will diminish the “noise” zone for delta values (see Fig. 2), and possible identify patients with presently biochemical undetectable myocardial injury. This could open a possibility to differentiate UAP from patients with non-cardiac chest pain, which would be of large clinical importance. However, a recent study did investigate if the more sensitive $hs\text{-}cTnI_{(sgx)}$ assay could identify reversible ischemia in patients with stable coronary artery disease, unfortunately showing disappointing results [47].

Even if more sensitive assays become available the troponin amount release during UAP will always be low, as this condition is dominated by reversible myocardial injury. It is not fully understood how troponins are released during cardiac injury and it is still debated if reversible injury can lead to significant troponin increase [48]. Different troponin fragments have been detected after acute MI [49,50] but it is unclear if fragments are released directly from injured cells (before necrosis) or results from systemic metabolized troponin molecules. Multiple studies have shown that troponin concentrations may increase after physical activity. Few studies have characterize the post-exercise troponin molecules, but one study could not detect intact troponin molecules after physical activity, only fragments were present [51]. As physical activity is associated with clear health benefits a common interpretation is that troponin release after activity is physiological and possible related to reversible injury. The troponin fragments detected after exercise could therefore be a potential biomarker of reversible injury. However, similar fragments have also been detected in patients with end stage renal disease, who have chronic myocardial injury and an expected poor prognosis [52]. The utility of troponin fragments to detect or diagnose reversible ischemia occurring during UAP is therefore unclear and should be elaborated in future studies.

Third, hs-cTn assays that give measurable concentrations in all individuals and show low analytical uncertainty may improve our ability to diagnose and monitor subclinical low-grade myocardial injury even below the current cut of the 99th percentile of the assay [53,54]. This would require further improvement aligning analytical performance closer to conservative estimates for biological variation.

Finally, assays with improved analytical performance will produce excellent research tools, to the betterment of patient care via an expansion of our knowledge and the treatment options for acute and chronic myocardial injury.

7. Conclusion

The analytical performance of hs-cTn assays should be based on the clinical use of the test and different PS are necessary for diagnosis of NSTEMI and chronic myocardial injury. PS for NSTEMI may be based on clinical outcome studies, and current data show that the performance for most assays are sufficient for early and rapid identification of NSTEMI. The efficiency of the protocols, the number of non-coronary chest pain patients eligible for rule-out, is affected by even slight biases in calibrator and reagent lots. When hs-cTn assays are used for risk estimation, long-term biological variation data is most useful for determining the analytical performance needed. A further improvement in analytical performance of the troponin assays may open new

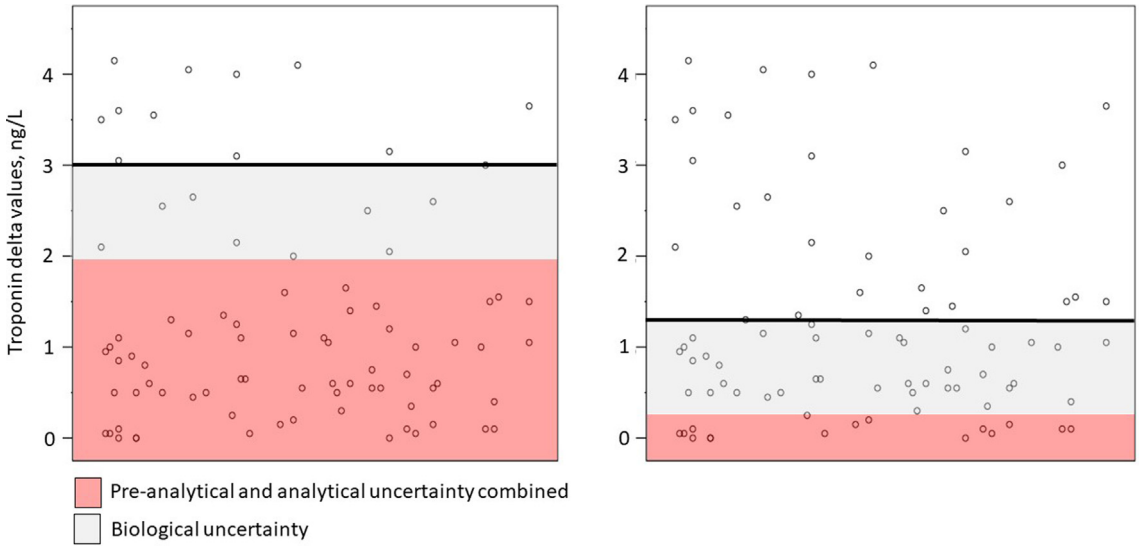

Fig. 2. This figure models how pre-analytical, analytical and biological uncertainty influence the magnitude of detectable troponin deltas. Both panels show the same distribution of 88 different troponin delta values. The gray area denotes the within-subject biological variation, i.e. physiological variations around a homeostatic set point, which are similar regardless of analytical perfomance. The red area shows the combined pre-analytical and analytical uncertainty and the horizontal line represent the reference change value above which a delta value may be measured with a certain level of confidence. In this particular example the percentage of delta values above the limit improves from 13% (panel on left side) to 32% (panel on right side) when the pre-analytical and analytical uncertainty is reduced. All numbers are examples. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

opportunities for improved efficiency in the ED, better risk estimation and long-term monitoring of chronic myocardial injury, with significant research possibilities in various populations.

8. Disclosures

Dr Aakre has received honorarium from Roche Diagnostics and Siemens Healthcare Diagnostics. Mr Saeeda has no disclosures. Dr Wu received grants/reagents/consultant/advisor/ honoraria from Abbott, Siemens, Roche, ET Healthcare, and Konica Minolta. UCSF has a patent with DR. Wu on high sensitivity troponin assays. Dr. Kavsak has received grants/reagents/consultant/advisor/ honoraria from several diagnostic companies who manufacture cardiac troponin assays, including Abbott Laboratories, Abbott Point of Care, Beckman Coulter, Ortho Clinical Diagnostics, Randox Laboratories, Roche Diagnostics and Siemens Healthcare Diagnostics. McMaster University has filed patents with Dr. Kavsak listed as an inventor in the acute cardiovascular biomarker field.

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