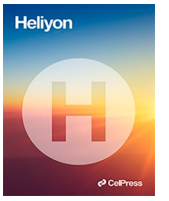
Heliyon 10 (2024) e23788

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



Review article

Investigating outlier rates of cardiac troponin I and troponin T assays: A systematic review

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ARTICLE INFO

Keywords:
Troponin
Molecular biomarker
Immunological assay
Outlier

ABSTRACT

Objectives: This review aimed to harmoniously summarize and compare outlier rates for various cardiac troponin (cTn) assays, including high-sensitivity-cTn (hs-cTn) assays and contemporary cTn (generation of assays prior to hs-cTn ones) assays, from the published studies.
Methods: The PRISMA guidelines were utilized to perform this systematic review. Five databases, including PubMed, Scopus, Embase, Cochrane Library, and Web of Science, were searched using specific keywords up to June 30th, 2023. Studies reporting specifically calculated outlier rates for cTn assays when conducting in-vitro diagnosis in human samples were included. Selected studies were then further assessed using the GRADE tool.
Results: Thirteen studies were included. The data from the studies were summarized statistically in this review. The results showed substantial evidence of improved analytical robustness or reduced respective mean rates of outliers, critical outliers, and analytical outliers for hs-cTn assays (0.14 %, 0.18 %, and 0.18 %) compared to contemporary cTn assays (0.63 %, 0.71 %, and 0.50 %).
Conclusion: The findings offer promisingly provide a comprehensive reference for laboratory scientists and clinical staff in choosing the most suitable cTn assay for patient care regrading outlier rates. Besides, this review reveals the advancements of hs-cTn assays with lower outlier rates than contemporary cTn assays. The emerging challenges for continuously improving analytical robustness of cTn assays are also elaborated.

1. Introduction

Over the past two decades, cardiac troponin I (cTnI) and cardiac troponin T (cTnT) have established themselves as the gold standard biomarkers for diagnosing acute myocardial infarction (AMI). The diagnosis relies on detecting a concentration change, i.e., rise or fall, in cardiac troponin (cTn) with at least one result exceeding the 99th percentile using cTn assays [1,2]. Since the 1990s, multiple generations of commercial cTn assays have been introduced and developed for the AMI diagnosis and prognostic assessment in patients with or without acute coronary syndrome [3,4]. One of the most critical parameters for evaluating the assays in the

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https://doi.org/10.1016/j.heliyon.2023.e23788
Received 3 July 2023; Received in revised form 12 December 2023; Accepted 13 December 2023
Available online 17 December 2023

marketplace is the total imprecision [coefficient of variation (CV), %] at the 99th percentile of an individual cTn assay [5]. In 2007, the first Universal Definition of Myocardial Infarction recommended that the total imprecision for each cTn assay should be less than 10 % at the 99th percentile upper reference limit (URL) [6]. Alternatively, assays with an intermediate CV of 10 %–20 % at the 99th URL are unlikely to negatively affect clinical decisions during serial cTn monitoring and deemed as clinical useable [7,8]. Another significant parameter for a cTn assay is the measurable number of healthy subjects below the 99th percentile [5]. A 2-tier system as a scorecard was then proposed in 2009 to categorize various cTn assays using total imprecision (CV, %) at the 99th percentile and measurable normal values (%) below the 99th percentile [5]. The 2-tier system showed significance in verifying different cTn assays regardless of manufacturer's claims. The total imprecision of cTn assays at the 99th percentile of $\leq$10 %, >10 % to $\leq$20 %, and >20 %, are assigned to evaluate the acceptance by guideline acceptable, clinically useable, and not acceptable, respectively. Highly-sensitivity (hs) and contemporary (generation of assays pre-hs assays) cTn assays are able to be distinguished on measurable normal values below the 99th percentile by <50 % (labeled as level 1), and $\geq$50 % (labeled as level 2 to level 4), with level 2 to level 4 defined specifically as follows, 50 % to <75 %, 75 % to <95 %, and >95 % for level 2, level 3, and level 4, respectively [5,9,10]. The total imprecision at the 99th percentile of the majority of contemporary cTn assays has been demonstrated to be clinically useable and acceptable [5,8]. Hs-cTn assays, by contrast, meet the guideline's acceptable acceptance [8]. Thanks to the efforts of the manufacturers, the transition from contemporary to hs-cTn assays has been accomplished and hs-cTn assays started to enter the marketplace in the past decade [5,8].

Outliers, defined as erroneous and irreproducible results, are hardly explained by analytical imprecision [11]. The erroneous outcomes of outliers may induce adverse assessments and critical misdiagnosis in clinical management for patients with or without AMI from laboratory technicians and clinicians [11,12]. Two cases have been reported for the occurrence of outliers in cTn assays and nearly lead to unneeded invasive procedures for the patients [13]. Therefore, the outlier rate of an assay, in addition, becomes a crucial parameter to weight the analytical robustness of assays [11].

Outliers occur erratically in a much larger magnitude of results and reveal insufficient testing reliability in various cTn assays [11, 13]. Over the past decade, some peer-reviewed literature has been published and contributed to evaluating outlier rates for leading marketshare cTn assays, mainly from the manufacturers of Abbott, Beckman Coulter, Roche, and Siemens. However, in previous, endeavors were rarely involved in systematical reviewing and comparing outlier rates for the assays, especially in a perspective of the transition from contemporary to hs-cTn assays. In this review, we aim to report a comprehensive investigation of outlier rates of the abovementioned manufacturers from published studies in the last decade. Most notably, this work provides promisingly evidence-based summaries, particularly in terms of outlier rates. The summaries would be used as references for laboratory technicians and clinical staff when choosing appropriate cTn assays to avoid clinical misdiagnosis caused by outliers or even unneeded invasive strategies. Moreover, we attempt to discuss emerging challenges of present cTn assays with analytical robustness as well.

2. Materials and methods

The systematic review and the abstract in the review were conducted following the PRISMA checklist and the PRISMA abstract checklist, respectively [14].

2.1. Search strategy

Investigations on cTn assay outliers before 2010 have limited relevance to the current generations of troponin diagnosis. The literature search was conducted during June 2023. To compile a comprehensive dataset, electronic databases including PubMed, Scopus, Embase, Cochrane Library, and Web of Science were used to search literature published from January 1st, 2010, up to June 30th, 2023. Additionally, a separate search was performed in the Google Scholar database to ensure that all relevant studies are identified during the search in the three electronic databases. The keywords used for searching were "outlier", "nonreproducible result", "flier", or "flyer", in combination with "troponin assay". Outlier, nonreproducible result, flier, or flyer shares the same meaning for nonreproducible false positive or negative measurements [13,15]. Endnote's (Endnote 20, Clarivate) duplicate identification strategy was manually performed to remove all duplicates. Our search strategy in the review is described below: firstly, we identified studies of involving human populations measured with troponin assays that reported outliers during the measurements; next, we collect data on outlier rates for various assays; and finally, we systematically evaluated and compared the different outlier rates for all the assays under investigated.

2.2. Eligibility criteria

The review aims to provide a solid and comprehensive reference for laboratory scientists and clinical staff in choosing appropriate cTn assays. The eligibility criteria were established for this purpose. The literature inclusion criteria used in this review included (1) studies involved with human clinical diagnosis; (2) human serum or plasma samples measured with cTn assays; (3) in-vitro studies, and (4) outlier rates specifically calculated and reported.

Studies then were excluded by following the exclusion criteria: (1) reviews, opinion articles, and case reports; (2) studies written in non-English language; and (3) and studies that did not mention the z values for outlier definitions.

2.3. Selection of studies

The study selection process consists of two main stages. In the first stage, the titles and abstracts were analyzed and evaluated

separately by L.Z., S.Z. and J.Z. The inclusion criteria were used to select the items. Then H.F. resolved any disagreement among the initial two authors. In the second stage, H.F. reviewed the entire text of the studies and excluded studies those failed to meet the inclusion criteria. Finally, the remaining items were reviewed by all authors with H.F. conducting the final evaluation.

2.4. Data collection and extraction

All the studies identified and included in this review were extracted by L.Z. and H.F. The data were extracted into Microsoft Excel 2013 from each included article for further statistical analysis. Three authors (J.Z., S.Z., and H.F.) checked the collected data from the texts of the original studies. A final discussion was held to resolve any divergence among all authors until a consensus was reached among all authors. In this review, data were collected based on different characteristics that are critical for specialists in choosing cTn assays, including the type of analyzer, assay, sample type, z value, SD value, cut-off, difference value, and various outlier rates and n values (total sample volumes).

2.5. Risk of bias in individual studies

The quality of evidence in the selected studies was assessed using the GRADE tool [16]. Since there was no other specific methodology for analysis of quality [17], the GRADE tool was adapted for in-vitro studies. The initial three authors separately evaluated the studies as high, moderate, low, and very low based on the overall quality. When any divergence appeared among the three authors, H. F. was responsible for resolving them.

2.6. Definition of an outlier and various outlier rates

To evaluate the robustness of cTn assays, outliers were determined by defining a critical difference (CD) between duplicate results of analyses using the following equation: $CD = z \times \sqrt{2} \times \sqrt{SD^2_{analytical}}$ [11,13,18–22], where z is a defined probability and SD is testing standard deviation of assays.

In most cases, a predefined probability of $z = 3.48$ or 3.5 was used for duplicate analysis, corresponding to a probability of 0.0005 [11,13,18–22]. Therefore, the CD value was calculated, to indicate an expected difference greater than the CD between two results less than five times in ten thousand events. Various z values, such as 1.96 [23] and 3.3 [24] (corresponding to predefined probabilities of 0.05 and 0.001, respectively), were also employed in outlier evaluations in various studies as well. Meanwhile, a triplicate analysis, including the initial and two replicates was also performed with a predefined probability of z of 0.0005, where $z = 3.29$ [25].

The value of SD was obtained by linear interpolation and extrapolation from a plot of overall SD versus concentration from Quality Control (QC) data [11,19]. To compare different platforms with identical criteria [19] and overcome dependence upon measured precision [25], a fixed 10 % or 20 % CV was likewise assumed to acquire equivalent fixed SD values during the analysis [13,25].

Once a CD was determined by acquiring z and SD values separately, outliers could be identified if the difference between the duplicated results exceeded the CD. Critical outliers were defined as individual outliers that could result in potential adverse or clinically-risk outcomes [13,18,20,23–25]. For instance, if the two duplicate results were on different sides of the clinical cut-offs or decision levels, such as 99th percentile URLs [8,23]. Notably, sex-specific differences were taking into account, as men tend to have higher concentrations than women at the 99th percentiles, which correlates with men having larger left ventricular mass than women [26]. Contemporary cTn assays lack the analytical sensitivity to differentiate the 99th percentile by sex [8]; that is, sex-specific cut-offs for the hs-cTn assays were recommended and used [27]. Additionally, the concentration unit of ng/L was adopted to avoid confusion and unnecessary zeros for hs-cTn assays [8]. Analytical outliers were also included in the works [28–30]. An analytical outlier was identified by evaluating the difference in absolute values or relative percentage between initial and repeat results. Furthermore, singlet measurement outliers were determined as outliers occurring exclusively in the initial result of the pair [11,19].

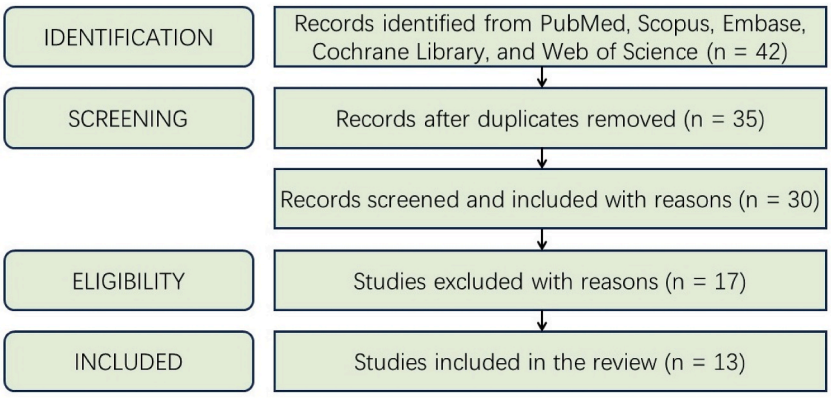

Fig. 1. PRISMA flow diagram showing the results of the literature search.

Table 1
Summary of the descriptive characteristics of the included studies (N = 13).

Author	Analyzer	Assay	Sample type	Z value	SD value	Cut-off	Difference	Outlier type	Rate	Sample volume
Favresse et al. [13]	Roche Cobas e801	hs-cTnT	Serum	3.48	Fixed 10 % CV	NN	NN	Outlier	3.22 % 0 %	1243 1160
						14 ng/L U		Critical outlier	1.37 % 0 %	1243 1160
Karon et al. [25]	Abbott ARCHITECT	STAT cTnI	Serum	3.29	Fixed 10 % CV	0.028 μg/L		Critical outlier	3.66 %	3008
	i2000SR	STAT hs-cTnI				26 ng/L U			0.47 %	3008
Klose et al. [30]	Abbott	STAT cTnI	Plasma Serum	NN	NN	NN	20 %	Analytical outlier	0.3 % 0.2 % 0.7 %	1000 1000 1000
		STAT hs-cTnI	Plasma Serum						0 % 0.1 % 0.1 %	1000 1000 1000
Lee et al. [23]	Abbott ARCHITECT	STAT cTnI	Plasma	1.96	From QC data	0.03 μg/L	NN	Critical outlier	0.97 %	1239
	i2000SR	STAT hs-cTnI				25 ng/L U			0.091 %	1239
Morgan et al. [24]	Abbott ARCHITECT	STAT cTnI	Plasma	3.3	NP	NN	NN	Outlier	0.58 %	3797
Pretorius et al. [19]	Abbott ARCHITECT i2000SR	STAT cTnI	Serum	3.48	From QC data	NN	NN	Outlier	0.1 %	2391
	Beckman Coulter Access2	Access Enhanced AccuTnI	Serum	3.48	From QC data				0.44 %	2391
	Roche Cobas e801	hs-cTnT	Serum	3.48	From QC data				0.06 %	2391
	Siemens ADVIA Centaur XP	TnI-Ultra	Serum	3.48	From QC data				0.1 %	2391
Author	Analyzer	Assay	Sample type	Z value	SD value	Cut-off	Difference	Outlier type	Rate	Sample volume
Pretorius et al. [21]	Beckman Coulter DxI600	Access hs-cTnI	Serum	3.5	From QC data	NN	NN	Outlier	0.046 %	4336
Ryan et al. [20]	Abbott ARCHITECT	STAT cTnI STAT hs-cTnI	Plasma	3.5	From QC data	NN	NN	Outlier	0.22 % 0.18 %	4009 3878
	i2000SR	STAT cTnI STAT hs-cTnI				34 ng/L M 16 ng/L F		Critical outlier	0.13 % 0.1 %	3878 3878
Sawyer et al. [18]	Abbott ARCHITECT	STAT cTnI	Plasma	3.5	From QC data	NN	NN	Outlier	1.95 % 0.48 %	7011 7089
	i2000SR	STAT hs-cTnI STAT cTnI STAT hs-cTnI				0.04 μg/L 34.2 ng/L M 15.6 ng/L F		Critical outlier	0.59 % 0.51 % 0.37 % 0 %	1522 7011 7089 1522
Ungerer et al. [11]	Beckman Coulter DxI800 and Access2	Access AccuTnI	Plasma and serum	3.5	From QC data	NN	NN	Outlier	0.55 %	13,100
Ungerer et al. [22]	Beckman Coulter DxI800	Access AccuTnI+3	Serum	3.5	From QC data	NN	NN	Outlier	0.025 %	4010
Wockenfus et al. [29]	Roche Cobas e411	hs-cTnT	Plasma	NN	NN	NN	5 ng/mL or 5 %	Analytical outlier	0.11 %	17,154
Wockenfus et al. [28]	Roche Cobas e411	4th generation STAT cTnT hs-cTnT	Plasma Serum Plasma Serum	NN	NN	NN	0.03 μg/L or 20 % 10 ng/mL or 10 %	Analytical outlier	0.7 % 0.6 % 0.8 % 0.11 %	1185 1185 1185 1185

U = unisex; QC = quality control; NP = not provided in the study; NN = no need to obtain for calculation of the specific type of outlier rate.

3. Results

3.1. Study selection

A total of 42 items were originally identified in PubMed, Scopus, Embase, Cochrane Library, and Web of Science. Endnote's duplicate identification strategy was manually performed by the initial two authors to remove all duplicates, resulting in 35 studies remaining. We did not use any automation tools to exclude studies in this review. Any individual study was double-checked on the Google Scholar platform. After removing studies for which full text could not accessed and obtained, 30 studies remained. Applying the inclusion and exclusion criteria, we further refined our selections, resulting in the final set of 13 studies for this review. The excluded studies, long with their perspective reasons for exclusion, are detailed in Table S1. To provide a visual representation of the study selection process, we have included a flow diagram shown in Fig. 1.

3.2. Study characteristics

The key characteristics of each study are descriptively summarized in Table 1 All the studies have been published since 2010 and are written in English. Each study was evaluated and categorized to present outlier, critical outlier, or analytical outlier rates for various cTn assays. Eight of the studies were involved with the reports of outlier rates for the cTn assays [11,13,18–22,24]. Five of the studies reported the critical outlier rates for the assays [13,18,20,23,25]. Moreover, three of the studies presented the analytical outlier rates for the assays [28–30]. The analysis of three types of outlier rates would benefit the laboratory staff and clinicians during the assay choosing process for different purposes.

Several studies reported the outlier rates for both contemporary and hs-cTn assays on the same analyzer [18,20,23,25,28,30]. The reports are particular important when evaluating the improvements in analytical robustness or reduction in outlier rates for cTn assays.

3.3. Outlier rates of contemporary and hs-cTn assays

Comparisons of different outlier rates between the contemporary and hs-cTn assays for identical cohorts on the same platforms from the manufacturers, i.e., Abbott or Roche, were carried out in these studies [18,20,23,25,28,30]. The hs-cTnI assays have been demonstrated with substantially lower critical outlier rates [18,23,25] and analytical outlier rates [30] than contemporary cTnI assays of Abbott (STAT cTnI). By contrast, there was no significant reduction in the proportion of outliers between the contemporary and hs-cTnI assays from Abbott [20]. However, it is worth noting that this study used two instruments or analyzers of Abbott were utilized for CD calculation together with larger whole imprecision data for the two assays, potentially resulting in unevenly-reduced determinations of outliers. Therefore, the outlier rate using hs-cTnI assay (0.77 %, 30/3878) was found significantly lower than the contemporary assay (2.24 %, 90/4009) when an absolute cut-off was applied to harmonize the assay imprecision between assays [20]. Compared to the contemporary cTnT assay of Roche (4th generation STAT cTnT), the hs-cTnT assays did not show a lower level of analytical outlier rates [28]. This discrepancy could be explained by a more rigorous definition of analytical outliers for the hs-cTnT assay in the study. Outlier rates between the contemporary (Access AccuTnI, Access Enhanced AccuTnI, and Access AccuTnI+3) and hs-cTnI assays of Beckman Coulter were likewise compared, although in separately-single studies [11,21,22]. A significantly lower outlier rate for the hs-cTnI assay than the Access AccuTnI was claimed [21]. The Access AccuTnI+3 showed a similar outlier rate to the hs-cTnI assay [21], probably due to the mechanical characteristics of analyzers rather than the assay formulations [21]. However,

Table 2
A summary of reported outlier rates of contemporary and hs-cTn assays.

Company/analyzer	Assays	Sample types	z values	SD values	Outlier rates	References
Abbott ARCHITECT[a]	STAT cTnI	Plasma	3.3	NP[b]	0.58 % (n = 3797)	[24]
Abbott ARCHITECT i2000SR	STAT cTnI	Plasma	3.5	From QC data	1.95 % (n = 7011)	[18]
					0.48 % (n = 7089)	
	STAT hs-cTnI				0.59 % (n = 1522)	
Abbott ARCHITECT i2000SR	STAT cTnI	Serum	3.48	From QC data	0.10 %[c] (n = 2391)	[19]
Abbott ARCHITECT i2000SR	STAT cTnI	Plasma	3.5	From QC data	0.22 %[d] (n = 4009)	[20]
	STAT hs-cTnI				0.18 %[d] (n = 3878)	
Beckman Coulter DxI800 and Access2	Access AccuTnI	Plasma and serum	3.5	From QC data	0.55 % (n = 13,100)	[11]
Beckman Coulter Access2	Access Enhanced AccuTnI	Serum	3.48	From QC data	0.44 %[c] (n = 2391)	[19]
Beckman Coulter DxI800	Access AccuTnI+3	Serum	3.5	From QC data	0.025 % (n = 4010)	[21,22]
Beckman Coulter DxI600	Access hs-cTnI	Serum	3.5	From QC data	0.046 % (n = 4336)	[21]
Roche Cobas e801	hs-cTnT	Serum	3.48	Fixed 10 % CV	3.22 %[c] (n = 1243)	[13]
					0 %[c] (n = 1160)	
Roche Cobas e801	hs-cTnT	Serum	3.48	From QC data	0.06 %[c] (n = 2391)	[19]
Siemens ADVIA Centaur XP	TnI-Ultra	Serum	3.48	From QC data	0.10 %[c] (n = 2391)	[19]

[a] No specific analyzer of Abbott ARCHITECT was claimed in the study.
[b] NP, not provided; QC, quality control; CV, coefficient of variation.
[c] Singlet measurement outliers were identified in the studies.
[d] Only elevated values up to 0.3 μg/L were calculated.

there was no feasible comparison of outliers between the contemporary and hs-cTn assays of Siemens [19].

Thanks to the efforts of scientific researchers, various outlier rates of the contemporary and hs-cTn assays have been explored. The reported outlier, critical outlier, and analytical outlier rates of the assays from literature are summarized in Table 2, Table 3, and Table 4, respectively. Then, the arithmetic mean rates of outliers, critical outliers, and analytical outliers can be obtained from the tables. The outcomes exhibited evidence of reduced respective mean rates of outliers, critical outliers, and analytical outliers for hs-cTn assays (0.14 %, 0.18 %, and 0.18 %) compared to contemporary cTn assays (0.63 %, 0.71 %, and 0.50 %).

3.4. Risk of bias

The studies selected in the review were assessed using the GRADE tool, and the results of this assessment are presented in Table S2. During the assessment process, two studies were graded as low quality, which are further elaborated as follows. Klose et al. performed the measurements across five laboratories that may induce a high degree of operation deviation and did not specify the analyzer model [30], leading to serious study limitations and inconsistency. Similarly, the study by Morgan et al. did not show information about the analyzer model for the measurements [24]. The lack of reporting analyzer models may provide less certain references for laboratory and clinical staff. Eight studies were rated as moderate quality, while three were rated as high quality. The studies were scored as inconsistent owing to missing the process of statistical analysis of the data. The studies, being rated as indirect, failed to exhibit calculated outlier rates in the study texts directly.

4. Discussion

Outliers may induce reverse determination for clinical care, while the exact nature of outliers still remains unclear [18–20]. The causes of false-positive or negative results are associated with the interferences of heterophilic antibodies [31], cTnI autoantibodies [32], rheumatoid factor [33], or biotins [34]. However, these interferences are consistent with reproducible results that differ from irreproducible results of outliers [13,18]. No outlier was found using QC materials, suggesting outliers possibly occurred with patient specimens and sample-related factors [18,20]. The presence of fibrin in serum or plasma samples is one of the possible explanations for outliers [12,25,28]. More specifically for serum, an incomplete clotting of serum in an expedited processing leads to the fibrin interference [30]. Slower centrifugation speed could have led to more outliers owing to fibrin generation during the processing [25]. Yet there was no statistical difference in outlier rates between various centrifugation speeds and sample types, i.e., serum and heparin plasma [11,19]. The explanation of fibrin for inducing outliers was still speculative and solid evidence is needed [13,19,20].

The occurrence of outliers may be associated with reagents as well. Contamination with magnetic/paramagnetic particles in a specific reagent lot (429,178) of hs-cTnI from Roche caused an abnormal outlier rate of 3.22 % and an abnormal critical outlier rate of 1.37 % [13]. After a replacement with a new reagent lot (460,113), the outlier and critical outlier rates returned back to normal at zero. However, both sample samples and QC materials were affected by the reagent lot of 429,178; thus, the QC enabled the detection of outliers in this situation. Besides, a larger reagent pack size of a 500-test reagent lot also probably resulted in a higher outlier rate [19,

Table 3
A summary of reported critical outlier rates of contemporary and hs-cTn assays.

Company/analyzer	Assays	Sample types	z values	SD values	Cut-offs	Critical outlier rates	References
Abbott ARCHITECT i2000SR	STAT cTnI STAT hs-cTnI	Plasma	1.96	From QC[a] data	0.03 μg/L 25 ng/L (Unisex)	0.97 % (n = 1239) 0.091 % (n = 1239)	[23]
Abbott ARCHITECT[b]	STAT cTnI	Plasma	3.3	NP	0.028 μg/L	0.16 % (n = 3797)	[24]
Abbott ARCHITECT i2000SR	STAT cTnI STAT hs-cTnI	Serum	3.29	Fixed 10 % CV	0.028 μg/L 26 ng/L (Unisex)	3.66 % (n = 3008) 0.47 % (n = 3008)	[25]
Abbott ARCHITECT i2000SR	STAT cTnI	Plasma	3.5	From QC data	0.04 μg/L	0.51 % (n = 7011) 0.37 % (n = 7089)	[18]
	STAT hs-cTnI				34.2 ng/L (Male) 15.6 ng/L (Female)	0 % (n = 1522)	
Abbott ARCHITECT i2000SR	STAT cTnI	Plasma	3.5	From QC data	34 ng/L (Male)	0.13 %[c] (n = 3878)	[20]
	STAT hs-cTnI				16 ng/L (Female)	0.10 %[c] (n = 3878)	
Roche Cobas e411	4th generation STAT cTnT	Serum	3.29	Fixed 20 % CV	0.01 μg/L	0.33 % (n = 3008)	[25]
Roche Cobas e801	hs-cTnT	Serum	3.48	Fixed 10 % CV	14 ng/L (Unisex)	1.37 %[d] (n = 1243) 0 %[d] (n = 1160)	[13]

[a] QC, quality control; NP, not provided; CV, coefficient of variation.
[b] No specific analyzer of Abbott ARCHITECT was claimed in the study.
[c] Only elevated values up to 0.3 μg/L were calculated.
[d] Singlet measurement outliers were identified in the study.

Table 4
A summary of reported analytical outlier rates of contemporary and hs-cTn assays.

Company/ analyzer	Assays	Sample types	Definition of analytical outliers	Analytical outlier rates	References
Abbott[a]	STAT cTnI	Plasma Serum	Initial and repeat results differed by >20 %.	0.3 %[b] (n = 1000) 0.2 %[b,c] (n = 1000) 0.7 %[b,d] (n = 1000)	[30]
	STAT hs-cTnI	Plasma Serum		0 %[b] (n = 1000) 0.1 %[b,c] (n = 1000) 0.1 %[b,d] (n = 1000)	
Roche Cobas e411	4th generation STAT cTnT	Plasma Serum	Initial and repeat results differed by > 0.03 μg/L for results <0.2 μg/L or ≥20 % for results ≥0.2 μg/L.	0.7 % (n = 1185) 0.6 % (n = 1185)	[28]
	hs-cTnT	Plasma Serum	Initial and repeat results differed by > 10 ng/L for results <100 ng/L or ≥10 % for results ≥100 ng/L.	0.8 % (n = 1185) 0.8 % (n = 1185)	
Roche Cobas e411	hs-cTnT	Plasma	Initial and repeat results differed by > 5 ng/L for results <100 ng/L or ≥5 % for results ≥100 ng/L.	0.11 % (n = 17,154)	[29]

[a] No specific analyzer of Abbott was claimed in the study.
[b] Singlet measurement outliers were identified in the study.
[c] BD Vacutainer Serum Separation Tubes were used in the group.
[d] BD Vacutainer Rapid Serum Tubes were used in the group.

23,35].

Analyzer-related factors are other potential reasons for resulting in outliers. Either of two Abbott ARCHITECT i2000SR analyzers (iSR6055 and iSR6041) was used to measure clinical human plasma samples with the STAT cTnI reagent in the two single studies (Study 1 and Study 2) [18]. The outlier rates are significantly higher for measurement performed on the analyzer of iSR6055 than the analyzer of iSR6041 in Study 1 (3.43 % compared with 0.38 %), while the reversed in observed in Study 2 (0.24 % compared with 0.74 %). Each analyzer was replaced with six wash valves for enhanced maintenance protocols prior to Study 2. Yet there was no statistical change on QC dataset in two months no matter pre or post the maintenance and valve change. It was still not clear if the replacement of valves influenced outlier occurrence. Furthermore, variation among analyzers (e.g., hardware and mechanical characteristics) and analyzer inactivity may also affect the outlier rate [19,21,35].

The certain cause of outliers is still not known, but the spurious results need to be detected and excluded to eliminate the risk of adverse patient outcomes. QC is designated to monitor the day-to-day precision and accuracy of a given assay, and realize early recognition of assay or analyzer errors [36]. However, outliers were not detectable in QC materials for impression analysis [18,20]; besides, QC processing was carried out to evaluate intra- and inter-assay imprecision tests and calculate values of SDs in the studies [11,19,21–23], but it failed to predict the occurrence of outliers in the patient samples.

The current protocol to identify outliers is a duplicate analysis of patient samples [18]. After samples are collected as serum or plasma and promptly centrifugated, an initial testing was immediately followed by a repeating test in a consecutive analysis way without any manual intervention and recentrifugation [11,18,20,23,24]. Alternatively, within 24 h of an initial analysis, the samples were stored at 2–8 °C, warmed to room temperature, and re-centrifuged before a repeat analysis [25,28]. While in a routine practice, cTn is not measured in duplicate [11]. As a consequence, the detection of an outlier needs an additional measurement, which is associated with increased reagent costs and lengthened turnaround time [20]. Re-centrifuging add-on cTn tests (defined as a delay in being stored and refrigerated of samples for a certain length of time prior to assay) may be a potential suboptimal sample handling management for clinical practice [20]. As discussed above, although a slower centrifugation speed was claimed to possibly lead to outliers due to fibrin interference, outlier rates were demonstrated to be having no significantly different between various centrifugation speeds. Unless the exact cause of outliers is uncovered, there may be no precise and specific optimization for sample processing, including sample type (i.e., serum or plasma) choosing, centrifugation speeds, sample storage conditions, reagent pack sizes, or analyzer statuses.

More importantly, improving the analytical robustness of cTn assays by the manufacturers may be the primary root solution to be compatible with presently-used sample processing and avoid the occurrence of outliers. In a general perspective, hs-cTn assays exhibited reduced or eliminated outliers than contemporary cTn assays. Currently, less analytical noise and improved diagnostic accuracy of the hs-cTn assays contribute to a % CV $\leq$ 10 % at the 99th percentile following the 2-tier system recommendation, whereas most the contemporary assays support a % CV at the 99th percentile between 10 % and 20 % [8]. The optimization and advancement of assay formulations for the hs-cTn assays by the manufacturers may contribute to the diagnostic accuracy and assay robustness. Finally, these improvements would benefit outlier rates for the hs-cTn assays.

5. Limitations

A comprehensive evaluation and comparison of various outlier rates for the cTn assays were performed in the review. Nevertheless, some limitations need further consideration. First, only six of all the studies investigated and compared outlier rates for both

contemporary and hs-cTn assays on the same analyzer. Four of the six studies showed that the hs-cTn assays had fewer outlier rates compared to the contemporary cTn assays. These comparisons promisingly revealed enhanced robustness in analytical performance for the hs-cTn assays and approached a limited conclusion. Furthermore, there are only three studies scored as high quality. Some risk bias exists in most of the studies, possibly leading to an insufficient exhibition of outlier rates for cTn assays.

6. Conclusions

This work provides a systematic summary of outliers in contemporary and hs-cTn assays in a harmonized comparison. The review serves as a convenient resource for laboratory technicians and clinical staff when choosing an appropriate analyzer and cTn assay for measuring cTn level to avoid unnecessary event rates of adverse outcomes or unneeded invasive procedures due to the occurrence of outliers.

The common way to identify and exclude outliers is to carry out a duplicate test after the initial measurement, although it leads to extra reagent costs and prolonged turnaround time. The exact cause of outliers remains unproven. Sample-, reagent-, or analyzer-related factors potentially induce outliers in cTn assays. Notably, processing protocols before cTn measurements, including choosing an appropriate sample type (serum or plasma), centrifugation speed, and sample storage condition are recommended to be optimized to reduce outlier rates for the assays.

Currently, a substantial reduction in outlier rates in hs-cTn assays compared to contemporary cTn assays. Therefore, hs-cTn assays are recommended to be utilized for cTn testing regarding outlier rates, while low-magnitude outlier rates are still detectable in hs-cTn assays. Based on the successful transition from contemporary cTn assays to hs-cTn assays with reduced outlier rates or improved analytical robustness, the accomplishments undoubtedly offer a possibility to eliminate outliers in the future generations of cTn assays. Meanwhile, continuous endeavors from scientists and manufacturers are eager to be achieved to uncover the nature of outliers and these may also be significant for further improvements on analytical robustness in cTn assays.

Data availability statement

Data included in article/supplementary material/referenced in article.

CRediT authorship contribution statement

Litao Zhang: Conceptualization, Writing – original draft. **Jia Zhu:** Data curation, Formal analysis, Writing – review & editing. **Shiqiang Zhang:** Data curation, Formal analysis. **Hao Fu:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors sincerely thank Dr. Yanting Jin for the assistance in the article preparation.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e23788.

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