Study of the 99th-percentile reference value for sensitive cardiac troponin I assays in Egyptian population

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Received 21 April 2020
Accepted 16 September 2020
Published 03 August 2022

The Egyptian Journal of Laboratory Medicine
2020, 2:33–39

Background
To establish the 99th-percentile cutoff for sensitive cardiac troponin-I (cTnI) assay in Egyptian population.

Patients and methods
A sample size of 400 volunteers were divided into two groups [group A, including 200 participants without cardiovascular risk factors (CVRF), and group B, including 200 participants with more than 1 CVRF]. These two groups were used to create cardiac troponin-I (cTnI) 99th-percentile cutoffs, which were implied later on a third group of 100 patients suspected as myocardial infarction (MI) to illustrate the effect of the calculated values and to assess their precision in the diagnosis of MI.

Results
After calculation of the 99th percentile of each group, the present data revealed that the exclusion of CVRFs resulted in lowering of the calculated cutoffs from 0.05 to 0.038 ng/ml for group B and group A, respectively. Additionally, it has been clear that sex and age are important factors influencing the cTn concentrations. These lower values have predicted more patients at risk of having acute MI. There were correlations of troponin I with age, sex, and traditional risk factors.

Conclusion
Troponin-I concentrations in apparently healthy individuals rely on cardiac risk factors, sex, and age. Using the lower cutoff values after exclusion of risk factors led to prediction of more patients at risk compared with higher thresholds at the expense of specificity.

Keywords:
99th-percentile value, chemiluminescence, immunoassays, myocardial infarction, sensitive cardiac troponin I

Egyptian Journal of Laboratory Medicine 2:33–39

Introduction

Acute myocardial infarction (AMI) is defined as cardiac cells' necrosis due to acute myocardial ischemia [1]. Symptoms of AMI include acute chest pain, dyspnea, nausea, fatigue, or a combination of these symptoms. If an acute coronary syndrome is considered, a 12-lead ECG is obtained and evaluated for ischemic changes and blood is sent for cardiac marker testing [2]. Rapid and accurate diagnosis of this killing disease is of great importance to deliver early effective treatment to these patients. So, both certain diagnosis and precise exclusion of AMI are very important to establish a comprehensible management system for acute coronary syndrome patients coming to the emergency room. This, in turn, would result in the optimal benefit of the resources [3]. The universal definition of MI has consolidated the role of the markers of myocardial necrosis, positioning cardiac troponins (cTns) as the more preferable marker in diagnosing AMI [1,4,5]. The 99th-percentile upper-reference limit (URL), calculated in a healthy reference population, has been assigned as the decision threshold for the diagnosis of MI with coefficient of variation of 10% at this concentration [1,5]. Newer more sensitive assays were innovated to help clinicians to make their decision as measurable cTn concentrations in a proportion of apparently normal population can be potent indicator for future cardiac insults [6]. Consequently, the convenient identification of the diagnostic cutoff values is of particular importance. The criteria of the reference population used to settle the 99th-percentile URL for cardiac troponin-I (cTnI) have not been fully determined [7]. In addition, the influence of cardiac diseases and cardiovascular risk factors (CVRF) as well as sex on troponin-I concentrations has not been assessed in large representative population-based studies [8]. This study aimed at the selection of healthy population to establish the 99th-percentile cutoffs for sensitive cTnI assay in Egyptian population. Also, we aim at clarifying the effect of traditional risk factors, age, and sex on the 99th-percentile values of cTnI and

how these different cutoff values affect the real-world setting of chest-pain patients.

Patients and methods

Clinical patients

This study was conducted on sample size, which was calculated by the Community Medicine Department of Faculty of Medicine of Alexandria using EPI Info 7 program (This is an Internet site for calculation: CDC.gov/epiinfo/index.html). Four hundred participants were included and divided into two groups: the first one (group A) included 200 participants who had no CVRF, the second group B included 200 participants with CVRFs, including obesity, diabetes mellitus, hypertension, dyslipidemia, smoking, history of AMI in first-degree relatives, and low estimated glomerular-filtration rate (eGFR) (≥1 CVRF). These two groups were used to settle cTnI 99th-percentile cutoffs that were implied later on a third group of 100 patients suspected to have MI to clarify the effect of these previous cutoffs and to assess their sensitivity and specificity in diagnosis of MI. The study was performed after taking informed consent from the patients and after permission from the ethical committee of Faculty of Medicine of Alexandria University.

Methods

All participants in group A and group B were submitted to full history taking, clinical examination, including blood pressure and anthropometric measurements, and laboratory investigations. Blood samples were withdrawn under complete aseptic technique for routine laboratory investigations (fasting blood glucose, cholesterol, triglycerides, urea, and creatinine) using fully automated chemistry analyzer Dimension RXL Max. eGFR was then calculated using MDRD formula[9] and for measuring the sensitive cTnI using Advia centaur automated chemiluminescence immunoassay analyzer. After measuring cTnI, the 99th-percentile cutoffs were calculated for the overall participants and for the two studied groups [group A (no CVRF) and group B (≥1 CVRF)]. A third group of 100 patients presenting to the emergency room with acute pain or equivalent symptoms (suspected for AMI) were used to detect the effect of various troponin-I cutoffs that have been calculated earlier. Blood samples for cTnI were taken at the Chest Pain Unit on admission and were analyzed. The diagnostic value of the different cutoffs was evaluated after calculating the number of true/false positives/negatives. A group of criteria is required to confirm the diagnosis of AMI (true positive), namely, the detection of an increase in cTnI results on serial measurements and at least one of the following: (a) symptoms of ischemia, (b) new or presumed new significant ST-T-wave changes or left-bundle branch block on 12-lead ECG, (c) development of pathological Q waves on ECG, (d) imaging evidence of new or presumed new loss of viable myocardium or regional wall motion abnormality, and (e) intracoronary thrombus detected on angiography or autopsy.

Statistical analysis

Data were fed to the computer and analyzed using IBM SPSS software package, version 20.0 (IBM Corp., Armonk, New York, USA). Qualitative data were described using numbers and percent. The Kolmogorov–Smirnov test was used to verify the normality of distribution. Quantitative data were described using range (minimum and maximum), mean, SD, and median. Significance of the obtained results was judged at the 5% level. χ^2 test was used to compare between different groups regarding categorical variables. Student *t* test was used to compare between two studied groups regarding normally distributed quantitative variables. Mann–Whitney test was used to compare between two studied groups regarding abnormally distributed quantitative variables. Pearson coefficient was used to correlate between two normally distributed quantitative variables. Regression study was done to detect the most independent affecting factor for cTnI.

Results

Demographic data and cardiovascular risk factors

Table 1 shows a comparison between the two studied groups (no CVRF and ≥ 1 CVRF) according to demographic data and CVRFs. It was demonstrated that group A includes 105 (52.5%) males and 95 (47.5%) females, while group B includes 125 (62.5%) males and 75 (37.5%) females. According to age, the mean was 50.29 ± 8.51 and 52.35 ± 7.96 years for group A and group B, respectively. In addition, regarding BMI, group A had a mean of 25.49 ± 1.03 kg/m^2, while group B had a mean of 32.25 ± 3.09 kg/m^2 and the two groups were significantly different (P < 0.001). As regards the CVRFs, group A with no CVRF. In contrast, group B includes diabetes mellitus 136 (68%), hypertension 116 (58%), dyslipidemia 95 (47.5%), smokers 90 (45%), cardiac diseases 15 (7.5%), and history of AMI in first-degree relatives 130 (65%))Table 2).

Kidney-function tests

Fig. 1 showed a comparison between the two defined groups according to renal-function tests. Regarding

Table 1 Baseline characteristics of overall study population and the two defined groups and the comparison between the two groups according to age, sex, and other cardiovascular risk factors

	Total (*N*=400) [*n* (%)]	Group A (*N*=200) [*n* (%)]	Group B (*N*=200) [*n* (%)]	Test of significance	*P*
Sex					
Male	213 (53.3)	105 (52.5)	125 (62.5)	χ^2=4.092	0.043*
Female	187 (46.8)	95 (47.5)	75 (37.5)		
Age (years)					
Minimum–maximum	35.0-65.0	35.0-64.0	33.0-65.0	*t*=1.603	0.110
Mean±SD	50.95±8.31	50.29±8.51	51.61±8.08		
Median	51.0	50.0	52.0		
BMI (kg/m^2)					
Minimum–maximum	20.40-40.0	23.0-28.0	20.40-40.0	*t*=29.368	<0.001*
Mean±SD	28.87±4.09	25.49±1.03	32.25±3.09		
Median	26.95	25.60	32.25		

χ^2, *P*, χ^2 and *P* values for χ^2 test for comparing between the two groups; *t*, *P*, *t* and *P* values for Student *t* test for comparing between the two groups. *Statistically significant at *P* value less than or equal to 0.05.

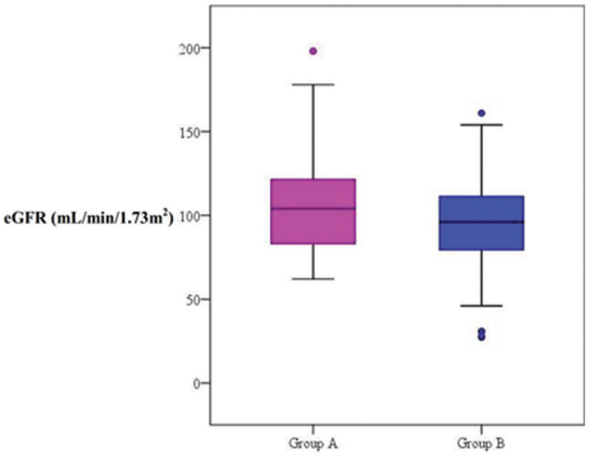
Table 2 Descriptive analysis for group B for different risk factors

	n (%)
DM	136 (68.0)
HTN	116 (58.0)
Dyslipidemia	95 (47.5)
Smoking	90 (45.0)
Cardiac diseases and medications	15 (7.5)
Family history	
Positive	130 (65.0)

DM, diabetes mellitus; HTN, hypertension.

the eGFR, the mean was 105.52 ± 27.43 and 95.16 ± 24.93 ml/min/1.73m^2 for group A and group B, respectively. There was a significant difference between the two studied groups regarding eGFR (P = 0.002).

Figure 1



Comparison between the two studied groups according to eGFR. eGFR, estimated glomerular-filtration rate.

Sex-stratified data of troponin-I concentrations in the overall participants and according to the groups (no cardiovascular risk factor and ≥1 cardiovascular risk factor)

Table 3 shows the distribution of sex-stratified data of cTnI (mean, median, and concentration of the 99th percentile) for the overall participants and the two studied groups (no CVRF and ≥1 CVRF). Females had lower troponin-I levels, which ranged from 0.0340 to 0.0450 ng/ml compared with males with cTnI values that ranged from 0.0380 to 0.0500 ng/ml. cTn concentrations were significantly higher in men than in women (P < 0.001). Exclusion of individuals with CVRFs in group A resulted in lowering cTnI 99th-percentile cutoffs for both sexes, male and female.

Distribution of cardiac troponin-I concentrations according to different age ranges in the two studied groups

Fig. 2 compares between the two mentioned groups according to different age ranges. The cTn concentrations are less in the age group less than 45 years old, increasing in the age group 45–60 years old, with further increase in the age group more than 60 years. There was a statistically significant difference between the two groups according to age (P < 0.001).

Correlation of troponin I

Correlation analysis showed a number of significant relationships between cTnI and demographic variables, as well as different risk factors. In the overall group, there were significant positive correlations between cTnI and age, BMI, diabetes mellitus, hypertension, dyslipidemia, smoking, family history, cardiac diseases, and medications (P < 0.001, respectively), while there were negative significant correlations between cTnI and female sex as well as eGFR (P < 0.001, respectively). In group A, there were associations only between cTnI and age, sex, BMI, as well as eGFR (P < 0.001, P < 0.001, P = 0.016, and P = 0.037, respectively). In group B, there were only correlations between cTnI and age, sex, smoking, and eGFR (P < 0.001, P < 0.001, P = 0.001, and P = 0.042, respectively) (Table 4).

Table 3 Sex stratified data of cardiac troponin-I concentrations in the overall participants and according to the groups (no cardiovascular risk factor) and (≥1 cardiovascular risk factor)

Troponins	Overall	Group A	Group B	*U*	*P*
Both sex	*N*=400	*N*=200	*N*=200		
99th percentile (ng/ml)	0.0490	0.0380	0.0500		
Minimum–maximum	0.001-0.0500	0.001-0.0380	0.0060-0.0500		
Mean±SD	0.0199±0.0134	0.0111±0.0085	0.0286±0.0115	4429.0*	<0.001*
Median	0.0170	0.009	0.0280		
IQR	0.0090-0.0300	0.0040-0.0158	0.0180-0.0380		
Male	*N*=230	*N*=105	*N*=125		
99th percentile (ng/ml)	0.0500	0.0380	0.0500		
Minimum–maximum	0.0010-0.0500	0.0010-0.0380	0.0060-0.0500		
Mean±SD	0.0242±0.0139	0.0139±0.0093	0.0328±0.0110	1379.5*	<0.001*
Median	0.0230	0.0130	0.0350		
IQR	0.0130-0.0370	0.0070-0.0190	0.0250-0.0410		
Female	*N*=170	*N*=95	*N*=75		
99th percentile (ng/ml)	0.0450	0.0340	0.0450		
Minimum–maximum	0.001-0.0450	0.001-0.0340	0.0090-0.0450		
Mean±SD	0.0140±0.0100	0.0081±0.0063	0.0215±0.0087	585.5*	<0.001*
Median	0.0120	0.0060	0.0190		
IQR	0.0060-0.0190	0.0040-0.0110	0.0150-0.0270		

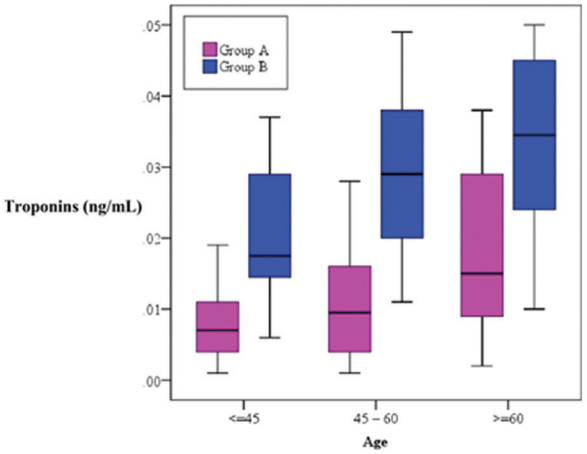
IQR, interquartile range. *U*, *P*, *U* and *P* values for Mann–Whitney test for comparing between the two groups. *Statistically significant at *P* value less than or equal to 0.05

Table 4 Correlation between cardiac troponin-I and demographic data as well as different risk factors in overall sample and the two defined groups

	cTnI					
	Overall		Group A		Group B	
	r	*P*	*r*	*P*	*r*	*P*
Age (years)	0.323*	<0.001*	0.376*	<0.001*	0.355*	<0.001*
Female sex	−0.377*	<0.001*	−0.344*	<0.001*	−0.476*	<0.001*
BMI (kg/m^2)	0.537*	<0.001*	0.170*	0.016*	−0.051	0.471
DM	0.496*	<0.001*	–	–	0.064	0.368
HTN	0.410*	<0.001*	–	–	−0.016	0.820
Dyslipidemia	0.354*	<0.001*	–	–	−0.022	0.760
Smoking	0.469*	<0.001*	–	–	0.228*	0.001*
Family history	0.442*	<0.001*	–	–	−0.027	0.704
Cardiac diseases and medications	0.202*	<0.001*	–	–	0.122	0.085
eGFR (ml/min/1.73 m^2)	−0.234*	<0.001*	−0.150*	0.034*	−0.144*	0.042*

cTnI, cardiac troponin-I; DM: diabetes mellitus; eGFR, estimated glomerular-filtration rate; HTN, hypertension. *r*, Pearson coefficient. *Statistically significant at *P* value less than or equal to 0.05.

Figure 2



Comparison between the two studied groups according to cTnI in different age groups. cTnI, cardiac troponin-I.

A multivariate analysis in this cohort is given in Table 5, showing the independent association between troponin I and age, sex, BMI, diabetes mellitus, dyslipidemia, family history, and eGFR.

Cutoff applications

A third group of 100 patients presenting to the emergency room with chest pain or equivalent symptoms (suspected as AMI) was used to determine the impact of different troponin-I cutoffs that have been calculated earlier. Irrespective of sex specificity, Table 6 shows that the 99th-percentile value of the overall participants (0.049 ng/ml) had less sensitivity (81.16%) and more specificity (74.19%) compared with the 99th-percentile value of group A (no CVRF) (0.038 ng/ml). Moreover, the male-specific cutoff value of cTnI for group A (0.038 ng/ml) was

Table 5 Multivariate linear regression for cardiac troponin-I

	B	SE	Beta	*t*	*P*
Age (years)	0.0004	0.0001	0.2600	7.5609	<0.001*
Female sex	−0.0097	0.0010	−0.3581	9.8144	<0.001*
BMI (kg/m^2)	0.0005	0.0002	0.1581	2.6493	0.008
DM	0.0052	0.0014	0.1835	3.6937	<0.001*
HTN	0.0011	0.0013	0.0363	0.8231	0.4109
Dyslipidemia	0.0035	0.0012	0.1102	2.7686	0.006*
Smoking	0.0024	0.0014	0.0759	1.7577	0.0796
Family history	0.0026	0.0013	0.0903	2.0035	0.046*
Cardiac diseases and medications	0.0012	0.0024	0.0170	0.4891	0.625
eGFR	−0.0001	0.0000	−0.1568	4.4507	<0.001*
R^2=0.553, adjusted R^2=0.553, SE 0.009, F=50.424*, P<0.001*					

DM, diabetes mellitus; eGFR, estimated glomerular-filtration rate; HTN, hypertension. *B*, unstandardized coefficients; Beta, standardized coefficients. *t*, *t* test of significance. *Statistically significant at *P* value less than or equal to 0.05.

Table 6 Diagnostic precision (the green cells true negative/the red cells true positive)

	Troponins (cohort used for cutoff)	Cutoff (ng/ml)	Negative	Positive	Sensitivity	Specificity	PPV	NPV
	Overall	0.0490	23	13	81.16	74.19	87.50	63.89
			8	56				
Total cases	Sex-specific male	0.0500	16	8	82.98	88.89	95.12	66.67
			2	39				
	Sex-specific female	0.0450	7	5	77.27	53.85	73.91	58.33
			6	17				
Group A	Group A no CVRF	0.0380	22	8	88.41	70.97	87.14	73.33
			9	61				
	Sex-specific male	0.0380	15	5	89.36	83.33	93.33	75.0
			3	42				
	Sex-specific female	0.0340	7	3	86.36	53.85	76.0	70.0
			6	19				

CVRF, cardiovascular risk factor; NPV, negative predictive value; PPV, positive predictive value.

lower than the corresponding male-specific cutoff of the overall participants (0.05 ng/ml). Therefore, the male-specific cutoff of group A had higher sensitivity (89.36%) and a lower specificity (83.33%) compared with the corresponding one of the overall participant (Table 6).

Discussion

To diagnose AMI, there should be an excess in troponin I or T above a predefined diagnostic cutoff. The 99th percentile of a presumbly healthy individual has been settled by the Universal Definition of MI to be such a threshold [8]. The approach for selecting a reference population for calculating the 99th-percentile value for cTn assays has not yet been adequately defined [10]. In theory, 99th URL values strongly depend not only on demographic and physiological variables (i.e. criteria for considering the reference population 'healthy'), but also on the analytical performance of cTn methods and mathematical algorithms used for the calculation [11].

Regarding the sample size used to determine reference intervals, our study was conducted on a sample size of 200 participants in each group. General guidelines for determining reference intervals for laboratory parameters are set out in the Clinical Laboratory Standards Institute document. According to these recommendations, 120 reference individuals are required as a minimum sample size to dependly set reference intervals. In contrast, experts of the IFCC Task Force on Clinical Application of Cardiac Bio-Markers in accordance with Sandoval and Apple, propose substantially higher numbers of presumably healthy individuals (300 males and 300 females) to establish the cTn cutoff values with an appropriate statistical power and method [12,13].

The present data show that exclusion of individuals with CVRFs leads to lowering of the calculated cutoffs

from 0.05 ng/ml (group A) to 0.038 ng/ml (group B), although we employed the same assay, analytical platform, statistical analysis, and similar sample size. This was incongruent with several previous studies that showed reduction of cTn concentrations approximately by a half after exclusion of CVRFs and cadiac diseases. This brings into prominence that the most challenging issue in the derivation of the 99th-percentile values from healthy reference population is how to exclude those who are affected by cardiac, metabolic, and renal disorders [7,14,15].

In our study, female participants had lower cTn levels with 99th percentile that ranged from 0.034 to 0.045 ng/ml compared with males with 99th percentile that ranged from 0.038 to 0.05 ng/ml. This is in agreement with McKie *et al.*[16] and other studies, which reported sex-specific cutoffs for cTn [16,17], although in a study by Koerbin *et al.* [18], the sex difference was less pronounced.

In our study, the impact of age is obvious on the 99th-percentile values through the different age groups, as the cTn concentrations are less in the age group less than 45 years old, increasing in the age group 45–60 years old, with further increase in the age group more than 60 years. An increase in TnI concentrations in elderly patients has been previously demonstrated, but there is no agreement regarding the application of age-specific 99th percentiles [10,19,20].

There were significant relationships between cTnI and sex as well as various risk factors. This was in agreement with the study of Keller *et al.* [8].

The application of the lower cutoff values of group A after exclusion of CVRFs was able to detect more patients at risk compared with higher thresholds with a downside of reduced specificity. This is in agreement with the study of Keller *et al.* [8], which applied the calculated values on real-world 1818 chest-pain patients and resulted in increased sensitivity with reduced specificity of the lower cutoffs. To overcome this limitation of reduced specificity in the setting of early diagnosis of MI, the use of troponin kinetics has been proposed [21–23].

To conclude, our study clearly indicated that the selection of reference population has a critical role on the 99th-percentile value of cTnI as troponin-I concentration in apparently healthy individuals is dependent on traditional risk factors, sex, and age. Additionally, it underscores the need for further clinical studies aimed to establish the optimal protocol for the selection of the reference population and the optimal cutpoint for diagnosis of MI. Until such studies are completed, we suggest in everyday clinical decision making, particularly in patients with borderline cTn concentrations, to integrate the clinical presentation and ECG findings rather with the dynamics of cTn concentrations than with the absolute results of cTn measurement.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

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