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Distribution of cardiac troponin I in the Japanese general population and factors influencing its concentrations

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Background: The 99th percentile of cardiac troponin I level in the general population is accepted as the cut-off for the diagnosis of acute myocardial infarction (AMI). However, it is not clear whether the cut-offs derived in racially and geographically different populations are applicable in Japan.

Methods: Troponin I was determined using the Abbott ARCHITECT STAT high-sensitive troponin I immunoassay in 698 apparently healthy individuals who visited the Japanese Red Cross Medical Center for a health checkup.

Results: The 99th percentile of the hsTnI in the overall population was 22.5 (95% confidence interval (CI), 16.8-36.6) pg/mL, 17.7 (95% CI 12.0-22.8) pg/mL for females and 30.6 (95% CI 17.1-53.4) pg/mL for males. The median of the hsTnI in the overall population was 3.2 (95% CI, 3.0-3.3) pg/mL, 2.6 (95% CI 2.4-2.8) pg/mL for females and 4.0 (95% CI 3.8-4.3) pg/mL for males. The age and gender had a significant influence on these values. The troponin I level also showed significant associations with the body mass index (BMI), the gamma glutamyl transferase (GGT), lactate dehydrogenase (LDH), estimated glomerular filtration rate (eGFR), and cardiac abnormalities by electrocardiography (ECG) but not with the high-sensitive C-reactive protein (hsCRP) level.

Conclusions: The 99th percentiles of the troponin I measured in the general population in Japan were comparable as the ones derived in the US, Germany, and Singapore. The troponin I level was dependent on the gender, age, BMI, and cardiac abnormalities found by ECG but not by the hsCRP level.

KEYWORDS
99th percentile, cardiovascular risk, general population, health checkup, high-sensitive troponin

1 | INTRODUCTION

Cardiovascular diseases are the second most common cause of death in Japan, and the number of new cases is continuously growing.[1] Among the cardiovascular diseases, one-third is attributed to ischemic heart diseases including acute myocardial infarction (AMI).[2] For the better management and prognosis of AMI, prompt and accurate diagnosis is crucial.

Cardiac troponin I is a protein that is specifically expressed in cardiomyocytes and is eluted in the blood when cardiomyocytes are injured such as by ischemia.[3] Due to its specificity, cardiac troponin I has become one of the most reliable biomarkers for the diagnosis of AMI.[3] In several

guidelines including "Third Universal Definition of Myocardial Infarction [4]" and "2014 AHA/ACC Guideline for the Management of Patients With Non–ST-elevation Acute Coronary Syndromes,[5]" the 99th percentile cardiac troponin level in the normal population is accepted as the cut-off for AMI. It is not clear, however, whether the cut-offs determined in certain areas could be applied to racially and geographically different populations. One high-sensitivity troponin I (hsTnI) assay (ARCHITECT *STAT* high-sensitive troponin I, Abbott Laboratories, Chicago, IL, USA) is one of the most reliable troponin I assays on the Japanese market, which is reported to measure 80%-90% of the general population.[6,7] The overall 99th percentile of this assay were reported to be 26.2 pg/mL in the population in the US as described in the package insert,[8] 25.6 pg/mL in the multi-ethnic Asian cohort[6] and 27.0 pg/mL in the Gutenberg Health Study.[7] They also reported the 99th percentiles were different by gender[6-8] and age.[6,7] We think it is important to confirm whether the cut-offs are comparable in the Japanese population and whether the gender and age differences are present as reported above.

In the Gutenberg Health Study, they also showed exclusion of subjects with functional and/or structural cardiac abnormalities from the general population lowered the 99th percentile from 27.0 pg/mL to 21.3 pg/mL.[7] In the MORGAN Biomarker Project Scottish Cohort study, they suggested 4.7 pg/mL in women and 7.0 pg/mL in men as the threshold to detect individuals at high risk for future cardiovascular events.[9] Considering this, we speculated that individuals with clinical/sub-clinical cardiac abnormalities were included in the general population to a certain extent, so that the 99th percentiles determined in each study could have been influenced by the percentage of those individuals in the population. We, therefore, think it is important to assess factors that influence hsTnI level to clarify the characteristics of the population used for the determination of the 99th percentiles.

High-sensitive C-reactive protein (hsCRP) is a biomarker which is elevated in blood by general inflammation. Several cohort studies have previously shown the ability of hsCRP to stratify risks of myocardial infarction, ischemic stroke and cardiovascular death.[10] Even at the hsCRP level below the upper limit of normal (3 mg/L), the relative cardiovascular risk of the group of hsCRP level between 1-3 mg/L was higher than the group with less than 1 mg/L hsCRP. Similarly, Zeller et al.[9] showed in the MORGAN Biomarker Project Scottish Cohort study that the risk of cardiovascular events or coronary death could be stratified by hsTnI below the 99th percentile level, applying 1.9, 4.8, 12.7 pg/mL as the cut-offs. Everett et al.[11] also reported that tertiles of the general population grouped by the baseline hsTnI level (cut-offs for men: 3.0 and 4.6 pg/mL, cut-offs for women: 2.6 and 3.9 pg/mL) showed that the risk of vascular events and all-cause mortality was higher in the groups with higher hsTnI levels.

Both hsCRP and hsTnI being the predictive markers for cardiovascular diseases as mentioned above while each of them is elevated by the different mechanisms, it is important to assess, together with the other factors, whether the hsTnI level is directly influenced by the hsCRP level.

Therefore, the objectives of this study were to determine the 99th percentile hsTnI cut-offs (overall, female and male) in the general population in Japan, to confirm the differences by gender and age, and to assess the other factors influencing the hsTnI concentrations.

2 | MATERIALS AND METHODS

2.1 | Subjects

A total of 698 apparently healthy individuals (385 females and 313 males) between the ages of 23 and 86 who visited the Japanese Red Cross Medical Center (Shibuya-ku, Tokyo, Japan) for a health checkup from January through April in 2014 were included in this study. This study was conducted based on the guidelines of Clinical and Laboratory Standards Institute (CLSI) document C28-A3c.[12]

TABLE 1 Characteristics of the study population

		Female (N=385)			Male (N=313)			Gender difference[a]
			Quartiles			Quartiles		
	Unit	Median	25%	75%	Median	25%	75%	*P*-value
Age	Y	48.0	41.0	58.0	51.0	40.0	62.0	.069
BMI	Kg/m^2	21.1	19.4	22.7	23.2	21.2	25.1	<.001
SBP	mmHg	115.0	105.0	127.0	124.5	113.0	137.0	<.001
DBP	mmHg	71.0	64.0	78.0	77.0	69.0	86.0	<.001
AST	U/L	20.0	17.0	23.0	21.5	18.0	25.0	<.001
ALT	U/L	15.0	12.0	20.0	21.0	15.0	28.0	<.001
GGT	U/L	173.5	154.0	192.3	170.0	153.0	186.0	<.001
LDL-C	mg/dL	17.0	13.0	23.0	28.0	21.0	48.8	.014
HDL-C	mg/dL	109.0	92.0	131.0	116.0	96.3	135.8	<.001
eGFR	mL/min/1.73 m^2	69.0	60.0	80.0	56.0	48.0	67.0	.074
HbA1c	% (NGSP)	78.6	70.1	87.7	76.6	66.5	85.5	.038
LDH	U/L	5.6	5.4	5.8	5.5	5.3	5.8	.228
hsCRP	mg/L	0.218	0.111	0.667	0.340	0.150	0.653	<.001

[a]The differences by gender were assessed by the Wilcoxon signed-rank test.

2.2 | Clinical examinations

Clinical examinations included body mass index (BMI), systolic and diastolic blood pressures (SBP and DBP), and electrocardiography (ECG). Information about gender and age were collected from all subjects in interviews.

2.3 | Laboratory tests

Routine biochemistry tests were performed on BioMajesty JCA-BM6070 (JEOL Ltd., Tokyo, Japan) that included the assays for aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma glutamyl transferase (GGT), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), creatinine (Cre), hemoglobin A1c (HbA1c), and lactate dehydrogenase (LDH).

hsTnI (STAT hgh-sensitive troponin I, Abbott Laboratories) were performed for the measurement of cardiac troponin I on Architect i2000 (Abbott Laboratories). According to the package insert, the limit of detection (LoD) of the assay was 1.9 pg/mL, the lowest concentration where 10% coefficient of variation (CV) was supported was 4.7 pg/mL and the overall 99th percentile was 26.2 pg/mL.[8]

hsCRP (CardioPhase hsCRP, Siemens Healthcare Diagnostics, Inc., Tarrytown, NY, USA) tests were performed for the measurement of CRP. According to the package insert, the limit of detection (LoD) of the assay was 0.175 mg/L, the CV was 7.6% at 0.410 mg/L and the reference value was 3 mg/L.[13]

Serum samples were used for the measurement of all the above assays except for HbA1c, which was measured with whole-blood samples.

The estimated glomerular filtration rate (eGFR) was calculated according to the revised equations for the Japanese population as shown below[14]:
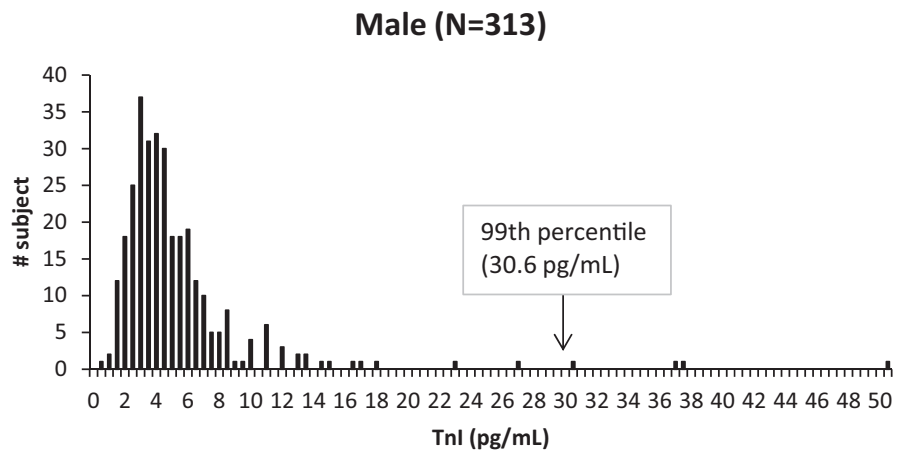
$$194 \times [Cre]^{-1.094} \times [age]^{-0.287} (male)$$

$$194 \times [Cre]^{-1.094} \times [age]^{-0.287} \times 0.739 (female)$$

(A) Distribution of the hsTnI level in females
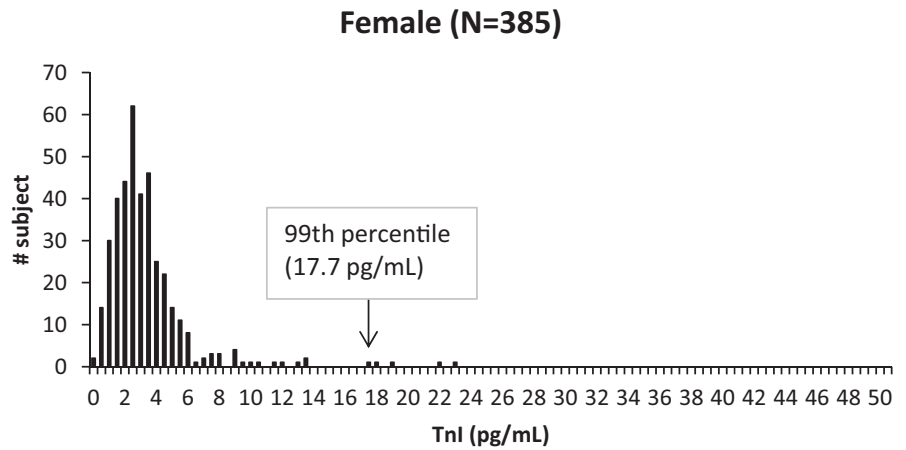
(B) Distribution of the hsTnI level in males

FIGURE 1 Distribution of the hsTnI level in (A) females, (B) males

TABLE 2 One-way ANOVA of log(hsTnI) with female 1 age groups

	N	Mean	SE	95% CI Lower	95% CI Upper
20's	21	0.214	0.064	0.089	0.340
30's	59	0.246	0.038	0.171	0.321
40's	130	0.278	0.026	0.227	0.328
50's	95	0.466	0.030	0.407	0.525
60's	59	0.595	0.038	0.520	0.670
70's	21	0.643	0.064	0.517	0.768
	df	Sum of squares	Mean square	F	P-value
Between groups	5	7.877	1.575	18.434	<.001
Within groups	379	32.390	0.085	0.000	
Total	384	40.266	0.000	0.000	

CI, confidence interval; SE, standard error; df, degree of freedom.

TABLE 3 One-way ANOVA of log(hsTnI) with male age groups

	N	Mean	SE	95% CI Lower	95% CI Upper
20's	17	0.506	0.067	0.374	0.638
30's	58	0.550	0.036	0.478	0.622
40's	73	0.571	0.032	0.507	0.635
50's	68	0.572	0.034	0.506	0.638
60's	64	0.697	0.035	0.629	0.765
70's	31	0.761	0.050	0.663	0.859
	df	Sum of squares	Mean square	F	P-value
Between groups	5	1.793	0.359	4.675	<.001
Within groups	305	23.394	0.077	0.000	
Total	310	25.187	0.000	0.000	

CI, confidence interval; SE, standard error; df, degree of freedom.

2.4 | Statistical analyses

JMP 11.0.0 (SAS, Cary, NC, USA) was used for statistical analyses. There was no outliers of hsTnI values confirmed by the Dixon's method described in the CLSI document C28-A3c.[12] Values of hsTnI below the LoD were set to an arbitrary constant below the LoD which did not influence the estimation of the 99th percentiles. For the statistical analyses, we coded the gender as 0 for females and 1 for males. We coded the ECG results as 0 for the results "no abnormalities," "no treatment necessary" and "observation," and 1 for the results "treatment necessary" and "further examination necessary." The variables including age, BMI, SBP, DBP, AST, ALT, GGT, LDL-C, HDL-C, eGFR, HbA1c, LDH and hsCRP were non-Gaussian when confirmed by the Shapiro-Wilk test. The 99th percentiles and medians of hsTnI distributions were estimated based on smoothed empirical likelihood and the 95% confidence intervals of the 99th percentiles and medians were obtained from "Custom Quantiles" function in the JMP software. To assess the difference of the basic characteristics and the hsTnI levels by gender, we performed the Wilcoxon signed-rank test. To assess the difference of hsTnI distribution by age, we performed a one-way ANOVA. To assess factors influencing hsTnI, we performed single and multiple linear regression analyses. Stepwise method was used for the multiple linear regression analysis. Except for the gender and ECG, we log-transformed all the variables for the linear regression analyses because they were non-Gaussian as stated above. Because some routine test data were lacking, 454 subjects with complete test results were used for the single and multiple linear regression analyses.

3 | RESULTS

3.1 | Basic characteristics of the subjects

The characteristics of the subjects by gender is provided in Table 1. All the listed variables except for age, eGFR, and LDH were significantly different by gender.

3.2 | Distribution of hsTnI

Histograms of hsTnI distribution by gender are shown in Figure 1A,B. The 99th percentile of the hsTnI in the overall population was 22.5 (95% CI 16.8-36.6) pg/mL, 17.7 (95% CI 12.0-22.8) pg/mL for females and 30.6 (95% CI 17.1-53.4) pg/mL for males. The median of the hsTnI

TABLE 4 Results of a multivariable linear regression for the association of various factors with the log(hsTnI)

	Single linear regression			95% CI			Multiple linear regression[a]			95% CI		
	Intercept	Coefficient	SE of coefficient	Lower	Upper	P-value		Coefficient	SE of coefficient	Lower	Upper	P-value
Gender (Female=0, Male=1)	0.384	0.240	0.029	0.184	0.296	<.001	Intercept	-2.640	0.538	-3.694	-1.585	<.001
Log(Age)	-1.320	1.067	0.129	0.814	1.321	<.001		0.170	0.029	0.112	0.227	<.001
Log(BMI)	-1.369	1.379	0.177	1.032	1.726	<.001		0.634	0.125	0.389	0.879	<.001
Log(SBP)	-2.457	1.417	0.178	1.068	1.767	<.001						
Log(DBP)	-1.949	1.305	0.173	0.965	1.644	<.001						
Log(AST)	-0.485	0.741	0.133	0.480	1.002	<.001						
Log(ALT)	-0.052	0.430	0.072	0.289	0.571	<.001						
Log(GGT)	-0.068	0.403	0.050	0.304	0.502	<.001		0.181	0.051	0.080	0.282	<.001
Log(LDL-C)	-1.106	0.781	0.132	0.522	1.041	<.001						
Log(HDL-C)	1.515	-0.563	0.131	-0.820	-0.305	<.001						
Log(eGFR)	1.936	-0.762	0.137	-1.032	-0.493	<.001		-0.355	0.123	-0.597	-0.113	.004
Log(HbA1c)	-0.333	1.124	0.273	0.588	1.660	<.001						
Log(LDH)	-1.691	0.981	0.196	0.597	1.365	<.001		0.625	0.172	0.289	0.962	<.001
Log(hsCRP)	0.559	0.121	0.028	0.067	0.175	<.001		0.166	0.076	0.017	0.315	.029
ECG	0.483	0.189	0.050	0.090	0.287	<.001						

CI, confidence interval; SE, standard error

[a]Stepwise method was used for the multiple linear regression analysis.

in the overall population was 3.2 (95% CI 3.0-3.3) pg/mL, 2.6 (95% CI 2.4-2.8) pg/mL for females and 4.0 (95% CI 3.8-4.3) pg/mL for males.

3.3 | Statistical significance of hsTnI levels by gender and age

By the Wilcoxon signed-rank test, log(hsTnI) in males (N=313) was significantly higher than that in females (N=385, *P*<.001).

By the one-way ANOVA, log(hsTnI) values were significantly higher in the groups of higher age in males (*P*<.001, Table 2) and in females (*P*<.001, Table 3).

3.4 | Other factors influencing hsTnI levels

By the single linear regression analysis, all the examined variables were significantly associated with the log(hsTnI), but only the log(GGT), log(eGFR), log(LDH), and ECG as well as the gender and log(age) were significant by the stepwise multiple linear regression analysis (Table 4).

4 | DISCUSSION

In this study, we assessed the distribution of troponin I measured by the hsTnI assay in 698 apparently healthy subjects (385 females and 313 males) who visited the Japan Red Cross Medical Center for a health checkup. The patients ranged in age from their early 20s to late 80s. The 99th percentile in the overall population was 22.5 (95% CI 16.8-36.6) pg/mL, 17.7 (95% CI 12.0-22.8) pg/mL for females (Figure 1A) and 30.6 (95% CI 17.1-53.4) pg/mL for males (Figure 1B). All the 99th percentiles of the hsTnI distribution from the studies with 1531 US individuals,[8] with 1120 multi-ethnic Asian individuals [6] and with 4138 individuals in the Gutenberg Health Study[7] were within the 95% confidence intervals of the 99th percentiles we determined here. From this result, the influence of racial and geographical differences was not observed in the 99th percentiles we derived in this study.

We also confirmed here that the distributions of hsTnI are different by gender and age, which were consistent as previously reported.[6-8] To explain why males show the higher 99th percentiles, several reasons have been proposed; cardio-protective effect of estrogen, the smaller heart mass in females, for example.[15] As shown in Table 1, the male group showed significantly higher risk factors including BMI, SBP, DBP, and LDL-C. These factors may contribute in elevating the hsTnI levels in males in addition to the influence of estrogen and heart mass mentioned above.

By the multiple linear regression analysis, the hsCRP level was not significantly associated with hsTnI. This is of interest from two aspects; (i) hsCRP does not affect the selection process of the population for the evaluation of hsTnI distribution, and (ii) hsCRP and hsTnI are independent predictors for cardiovascular events. It is also of note that the cardiac abnormalities by ECG was significantly associated with the hsTnI level, which is consistent with the findings by Zeller et al.[7] that cardiac abnormalities, defined by clinical and echocardiographic data, alter the hsTnI levels in the general population.

The limitation of this study is that the subjects in this study were from Tokyo area. Although the influence of the geographical as well as racial difference was not observed in this study, we cannot exclude the possibility of the population bias. As shown in the association of the hsTnI level with ECG abnormalities, our population may include individuals with potential cardiac abnormalities. Therefore, there is a possibility that some other populations in Japan could show different 99th percentile cut-off depending on the percentage of those individuals. The impact of including those individuals in determining the hsTnI reference values for the diagnosis of AMI remains to be seen.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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