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Interval estimation of the upper and lower limits of the reference range of the QT interval in resting electrocardiograms by the bootstrap method

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Abstract

The upper and lower limits of the reference range of the *QT* interval (*QT*) of the electrocardiogram (ECG) were determined using 2529 healthy young males. The *QT* were classified into 10 classes by the value of the paired *RR* interval (*RR*) observed in the same ECG. In each class, the 2.5th and 97.5th percentiles of the conditional distribution of *QT* were determined as point and interval estimates by the bootstrap method. Using these estimates, the upper and lower limits of the reference range of *QT* in all *RR* ranges from 0.6 to 1.5 s were approximated as $QT_{upper\ limit} = 436 \times RR^{0.35}$ and $QT_{lower\ limit} = 375 \times RR^{0.28}$, respectively. In each class, the 95% confidence intervals (CI) of the upper and lower limits were also estimated. Low reliability was shown by the wide CI of the limits in both extremes of *RR* range when a sufficient sample size cannot be obtained.

Key words: bootstrap, *QT* interval, reference range, outliers, tails of distribution, interval estimation

1. Introduction

Reference values (footnote 1) for ECG parameters are usually presented as a set of point estimates of the mean, upper and lower limits. However, since the variance of these values are likely to change depending on the sample size used in the estimation, it is difficult to know the reliabilities of the limits only from the point estimates.

In our previous studies[1][2], we had considered that the reliabilities of the limits could be quantitatively described based on the length of the 95% confidence intervals (CI) of the limits, and had presented these limits not only as point estimates, but also as interval estimates. Since we could not prepare enough samples for non-parametric study, we assumed the normality of the conditional distribution of *QT* and approximated the 97.5th and 2.5th percentiles of the

1 We use the terms 'reference range' and 'outlier' in place of 'normal' and 'abnormal', respectively, in describing the *QT*. The reference range of the *QT* was established from the 2.5th (lower limit) and 97.5th percentiles (upper limit) of measurements in healthy subjects. Measurements that exceeded the upper limit or fell below the lower limit of this range are referred to as outliers.

distribution as the mean ±1.96 sigma of the distribution. Following these studies, we[3] added new cases from the same institution as that in the previous studies and we had estimated the upper and lower limits with CIs, without assuming the normality of the conditional distribution of *QT*. In the present study, we summarized the series of our previous reports and presented the exponential equation approximating the upper and lower limits of the *QT* interval (footnote 2) to be useful for clinical trials. We, also showed how to set the reference value with confidence interval for clinical tests by the bootstrap method.

2. Subjects and Methods

A 12-lead electrocardiogram (ECG) was recorded for 2609 healthy Japanese men aged 20 to 35 years old as a part of screening for candidacy in a phase 1 clinical trial. The screening was performed from March 2006 to March 2009. The *RR* for each case was determined by averaging the *RR*s of all leads measured from normal and noise-free beats of the 10-s ECG recording. *QT* was measured using *QT* analysis software (FCP-7431 Version S) provided by Fukuda Denshi. After excluding 80 cases who did not meet the enrollment criteria (see Appendix), 2529 cases were used for the study. The mean and standard deviation of the age of the 2529 subjects were 24.3 and 3.8 years old, respectively.

The joint distribution of *RR* and *QT* was used to determine a linear regression equation for *QT* in terms of *RR*:

$$QT_L = \alpha \times RR + \beta \qquad (2.1)$$

and an exponential regression equation for *QT*:

$$QT_E = \gamma \times RR^{\delta}. \qquad (2.2)$$

The coefficients, α and γ, intercept β, and exponent δ of these equations were determined using the least squares method.

The *RR* range (0.600 to 1.500 s) corresponding to the enrollment criteria for the clinical trial was divided into 10 classes. To compensate for the fewer samples at both ends of the *RR*, the class lengths at both ends were broadened. Thus, the lower and upper limits of the lowest *RR* class were set at 0.600 and 0.7375 s, respectively, and the limits of the highest *RR* class were set at 1.3375 and 1.500 s, respectively. The limits of the other 8 intervals were graduated in 0.075-s intervals from the upper limit of the lowest interval. $RR = 1$ s occurred in the interval between 0.9625 and 1.0375 s. The lower and upper limits of the 10 intervals are shown in **Table 1**. Each subject was classified into one of the 10 classes based on their *RR*. The value of *RR* for each case

2 The *QT* interval represents electrical depolarization and repolarization of the left and right ventricles. On the ECG recording, the *QT* interval (*QT*) is defined as the time interval from the onset of one *QRS* complex to the end of the T wave of the same *QRS* complex. Since the *QT* interval is dependent on the heart rate (the faster the heart rate the shorter the *QT* interval), the reference value of *QT* was given as a function of *RR*, explained below. The *RR* interval (*RR*) is defined as the time interval from the onset of one *QRS* complex to the onset of the next *QRS* complex.

in the same class was replaced with the class value of the interval, whereas the actual values of *QT* were used in the analysis. Using the bootstrap method[4], the median, lower limit and upper limit of the reference range of *QT* in each class were estimated as follows:

Step 1: The samples corresponding to the sample size in the i-th class (n_i; $i=1$, 10) were repeatedly sampled from the same class and a set of *QT* populations consisting of n_i cases was constructed. The same procedure was repeated j times for the same subjects in the i-th class to produce j sets of *QT* populations consisting of n_i cases (BS_{ik}: $k=1,..,$j). The number of bootstrap samples (j) was set at 1000 in this study.

Step 2: The 2.5th percentile (L_{ik}; $k=1,..,$j) was determined in each of the j sets of BS_{ik} for each i.

Step 3: The 2.5th ($L2.5_i$), 50th ($L50_i$), and 97.5th ($L97.5_i$) percentiles of j samples of L_{ik} were determined in each class. $L50_i$ was defined as the estimated lower limit of the reference range of *QT* in the i-th class. $L2.5_i$ and $L97.5_i$ were defined as the lower and upper limits of the CI of $L50_i$, respectively.

Step 4: Similarly to step 2, the 50th (M_{ik}; $i=1,..,10$, $k=1,..,$j) and 97.5th (U_{ik}; $i=1,..,10$, $k=1,..,$j) percentiles were determined in each of the j sets of BS_{ik}.

Step 5: Similarly to step 3, the 2.5th ($M2.5_i$), 50th ($M50_i$), and 97.5th ($M97.5_i$) percentiles of j samples of M_{ik} were determined in each class. $M50_i$ was defined as the median reference value of *QT* in the i-th class, and $M2.5_i$ and $M97.5_i$ were defined as the lower and upper limits of the CI of $M50_i$, respectively. Similarly, the 2.5th ($U2.5_i$), 50th ($U50_i$), and 97.5th ($U97.5_i$) percentiles of j samples of U_{ik} were determined in each class. $U50_i$ was defined as the upper limit of the reference value of *QT* in the i-th class, and $U2.5_i$ and $U97.5_i$ were defined as the lower and upper limits of the CI of $U50_i$, respectively.

Step 6: Using 10 pairs of the class value from the i-th class of the *RR* and $U50_i$ ($i=1,..,10$), an exponential regression equation for the upper limit of the reference range of *QT* was estimated in terms of *RR* as follows:

$$QT_{upper\ limit} = c \times RR^d \qquad (2.3)$$

The coefficient c and exponent d of the equation were determined using the weighted least squares method.

Step 7: Similarly to step 6, exponential regression equation for the median;

$$QT_{median} = e \times RR^f \qquad (2.4)$$

and lower limit of the reference range of *QT*;

$$QT_{lower\ limit} = g \times RR^h \qquad (2.5)$$

were estimated in terms of *RR*.

We compared the detection rate at both extremes with the anticipated rate of 2.5% after identifying outliers in the subject population using the criteria.

The study was approved by the institutional ethics committee of the clinic and all subjects gave written informed consent before starting the study. IBM SPSS Statistics (ver. 19) and Microsoft Excel 2003 SP2 were used for statistical analysis.

3. Results

3.1 Joint distribution of RR and QT

The *RR* and *QT* intervals ranged from 0.60 to 1.50 s and from 326 to 530 ms, respectively. Hereafter, the units of *QT* and *RR* are ms and s, respectively. The joint distribution of *RR* and *QT* in the 2529 cases is shown in Figure 1. The mean of the conditional distribution of *QT* for a given *RR* increased curvilinearly with the increase of *RR*. The correlation coefficient between *RR* and *QT* was 0.78. A linear regression equation for *QT* in terms of *RR* corresponding to Eq. (2.1);

$$QT_L = 126.5 \times RR + 276.0 \qquad (3.1)$$

was obtained with a root mean square error (RMSE) of 16.36 ms over all subjects. Similarly, an exponential regression equation for *QT* in terms of *RR* corresponding to Eq. (2.2);

$$QT_E = 403.3 \times RR^{0.32} \qquad (3.2)$$

was obtained with an RMSE of 16.32 ms.

The median of the *QT* distribution (= $M50_i$) was compared with the arithmetic mean (Mean) for each of the 10 classes (Table 1). $M50_i$ was less than Mean in each class, which suggests that the conditional distribution of *QT* slightly leaned toward the shorter end. In 9 of 10 classes excepting class 5, the length of the upper half of the range of the *QT* ($U50_i - M50_i$) was slightly longer than that of the lower half of the range ($M50_i - L50_i$). In class 5, the length of the upper half was equal to that of the lower one.

However, the difference between *M50* and Mean in each class was minimal and the values agreed to two significant figures. The ranges of the CI of the median ($M97.5_i - M2.5_i$) were from 3 to 10 ms and did not increase with *RR*, but with the inverse of the sample size. QT_{median} was estimated as

$$QT_{median} = 403.03 \times RR^{0.31} \qquad (3.3)$$

The coefficient of 403.03 and exponent of 0.31 were very close to the respective values for QT_E.

3.2 Point and interval estimation of the upper and lower limits of the QT reference value

The upper limit of the reference value of *QT* in the i-th class ($U50_i$) and the upper ($U97.5_i$) and lower ($U2.5_i$) limits of the CI of $U50_i$ ($i = 1, .., 10$) estimated for each of the 10 classes using the bootstrap method are shown in Table 2. In the 5th class (class value 1 s, sample size 491), $U2.5_i$, $U50_i$, and $U97.5_i$ were 429.0, 432.0 and 436.3 ms, respectively, and the length of the CI was 7.28 ms. Similarly to $M50_i$, $U50_i$ increased along with *RR*. The lengths of the CIs broadened in the shorter and longer *RR* classes.

In Step 6 above, the equation;

$$QT_{upper\ limit} = 436 \times RR^{0.35} \qquad (3.4)$$

was obtained for the upper limit of the reference range of the *QT* population. The RMSE of this equation was 5.98 ms. The upper and lower limits of the CI of $QT_{upper\ limit}$ in the i-th class were approximated using $U97.5_i$ and $U2.5_i$, respectively. The value of $QT_{upper\ limit}$ exceeded $U97.5_{i=4}$ by 0.2 ms in the 4th class, but remained within the CIs of $U50_i$ in all other classes.

Using the same procedure for the upper limit, $L50_i$ and the upper ($L97.5_i$) and lower ($L2.5_i$) limits of the CI of $L50_i$ ($i = 1, .., 10$) were estimated for each of the 10 classes (Table 2). Similarly to $M50_i$ and $U50_i$, $L50_i$ increased along with *RR*. In the 5th class, $L2.5_{i=5}$, $L50_{i=5}$ and $L97.5_{i=5}$ were 373.3, 376 and 378 ms, respectively, and the length of the CI was 4.75 ms. The lengths of the CIs broadened to 12 ms in the shorter and longer *RR* classes, but showed no increasing or decreasing trend with respect to *RR*. Using similar steps to estimate $QT_{upper\ limit}$, the exponential equation;

$$QT_{lower\ limit} = 375 \times RR^{0.28} \qquad (3.5)$$

was obtained for the lower limit of the reference range of the *QT* population. The RMSE of this equation was 1.63 ms. The upper and lower limits of the CI of $QT_{lower\ limit}$ in the i-th class were approximated using $L97.5_i$ and $L2.5_i$, respectively. The value of $QT_{lower\ limit}$ remained within the CIs of $L50_i$ in all 10 classes. Curves representing Eq. (3.4) and Eq. (3.5) and the CIs of the upper and lower limits of the reference range are shown with the plot of the joint distribution of *RR* and *QT* in Figure 2.

The length of the CI of the upper limit ($U97.5_i - U2.5_i$) was longer than that of the lower limit ($L97.5_i - L2.5_i$) in 9 of 10 classes excepting class 4. In class 4, the length of CI of $U50_{i=4}$ was exceptionally short (4ms) and shorter than that of $L50_{i=4}$ (Table 2).

3.3 Detection of outliers in the target population

Using Eq. (3.4), 62 of the 2529 cases (2.45%) with *QT* exceeding the limit were identified as outliers with longer *QT*. Similarly, using Eq. (3.5), 61 cases (2.41%) fell below the limit and were identified as outliers with shorter *QT*. The other 2406 cases were located between the two limits and thus had *QT* within the reference range.

4. Discussion

Since the definition of the reference value was generally established by the range between the 2.5th and 97.5th percentiles of measurements, it was preferable to determine both limits directly from the sufficient numbers of samples.

Pavlov et al [5] compared the parametric and nonparametric approaches for determining the reference value and observed deterioration of the reliability with a decreasing sample size in the nonparametric approach. Linnet[6] also suggested that nonparametric reference interval estimation at small to moderate sample sizes (<120) was associated with a large degree of uncertainty.

We had shown that the marginal distribution of *RR* of the healthy population could be approximated by a normal distribution[1]. So, we expected that the sample size at both the shorter and longer ends of *RR* would be much less than those in the intermediate classes. In the previous study using 1276 cases[2], the number of cases for the *RR* range less than 0.737 s was 30, whereas that for the *RR* range longer than 1.337 s was 57. To avoid the deterioration of the reliability, we assumed the normality of the conditional distribution of *QT*, and approximated the 97th and 2.5th percentiles of the distribution as the mean ±1.96 sigma of the distribution.

In the present study, we doubled the number of cases which were from the same institution as that in the previous studies[2][7][8]. As a result, the number of cases for the *RR* range less than 0.737 s increased from 30 to 66, whereas that for the *RR* range longer than 1.337 s increased from 57 to 107. To keep the sufficient sample size, we had expanded the class width in both ends of the *RR* range twice as compared with our previous studies. However, the sample size was still not enough to fulfill the Linnet's requirement[6].

In clinical medicine, many laboratory data had been approximated by normal or log-normal distribution to estimate their reference ranges.

Since p-value of Shapiro-Wilk test was 0.27, we had assumed normality of the data in class 5, and estimated mean 404.13 and standard deviation 15.22 ms. The upper and lower limits of the reference range defined as the mean ±1.96 sigma were thus 433.96 and 374.30 ms. Provided normal assumptions hold, we also calculated CI for these limits. The CIs of upper and lower limits were from 431.63 to 436.29 and 371.97 to 376.63 ms.

As an alternative approach, assuming the normality of the log-transformed data in the same class 5 (p-value of Shapiro-Wilk test was 0.46), we had estimated mean 2.01 and standard deviation 0.016. The lower limit in the transformed data was 2.57, corresponding to a *QT* of 375.12 ms, and the upper limit was 2.64, corresponding to 434.76 ms. The CIs in linear scale were 432.22 to 437.22 ms for the upper limit and 372.99 to 377.31 ms for the lower limit.

However, since there was no reason to suppose that all variables followed a normal distribution, we estimated the limits of the reference range non-parametrically.

Using the bootstrap method, we directly estimated the upper and lower limits. They were 432 and 376. Both limits estimated non-parametrically well agreed with those estimated parametrically.

The CIs of upper and lower limits estimated by the non-parametric approach were 429.00 to 436.28 and 373.25 to 378.00 ms, respectively. The length of CI of upper limit was 2.53 ms longer than that of lower limit.

It must be noticed that the length of the CI of upper and lower limits were always identical if we assumed the normality of the data. On the other hand, it is known that the length of CI of upper limit is longer than that of lower limit under the assumption of the log-normality of the data. In fact, we found that the length of CI of the upper limit was 0.69 ms longer CI than that of the lower limit. However, the difference was much shorter than we obtained using non-parametric approach.

From these results, we found that we could estimate the limits of reference range using the parametric approach if the data were well approximated by the assumed distribution. However, we also found that the assumed distribution could not be applied to data around the limits even if the distribution well fitted to the samples in the central region. Samples in the tails might have a unique characteristics from those in the central region, and we had to estimate the variance of the limits independently.

The bootstrap method can be easily applied to such a purpose without assuming any type of the distribution. We could estimate the length of the CI of the limits of reference range and find the difference of the length of the CI between upper and lower limit. Using these CIs, we could rate the reliability of the limits.

It is needed further studies to elucidate the reasons why the CI of the upper limit tended to lengthen than lower one.

Summary

The current investigation of the relationship between the *RR* and *QT* intervals in resting ECGs of 2529 healthy young Japanese men showed that the upper and lower limits of the reference range of *QT* in the *RR* ranges from 0.8 s to 1.3 s were well approximated by the pair of exponential equations $QT_{upper\ limit} = 436 \times RR^{0.35}$ and $QT_{lower\ limit} = 375 \times RR^{0.28}$, respectively. In this study, we did not assume the normality of the conditional distribution of *QT* in each class, and estimated the upper and lower limits of the reference range of *QT* as 97.5th and 2.5th percentiles, respectively. We also estimated confidence intervals of these border points non-parametrically. These intervals could be used for evaluating the reliability of the limits of the reference range. Using non-parametric approach, we found that the dispersion of the upper limit of *QT* was larger than that of the lower limit.

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Appendix

Exclusion criteria of the institution and number of cases excluded from the study.

ECG findings	Exclusion criteria of institution	Number of cases
Sinus tachycardia	＞100 beats/m	5
Sinus bradycardia	＜40 beats/m	2
Left axis deviation	＜30 degree	13
Right axis deviation	＞110 degree	12
Myocardial ischemia		2
Complete right bundle blanch block	Pattern + QRS duration ＞135 ms	5
Intraventricular conduction disturbance	QRS duration ＞135 ms	7
Short PR interval	PR interval ＜80 ms	1
First degree atrioventricular block	PR interval ＞260 ms	4
Atrioventricular junctional rhythm		15
Premature ventricular contraction		5
Premature supraventricular contraction		3
Escaped beat		3
Second degree atrioventricular block		1
Third degree atrioventricular block		1
WPW		1
	Total	80

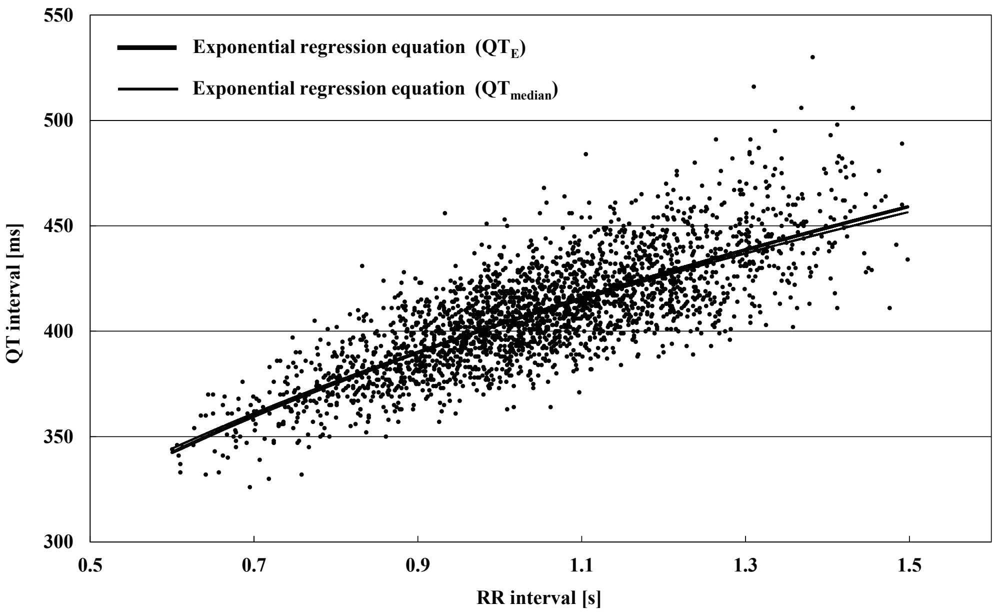

Figure 1. Relationship between QT and RR intervals in resting ECGs of 2529 healthy young Japanese men. Curves representing the exponential regression equations for QT (QT_E) and the median of QT (QT_{median}) are shown as bold and thin lines, respectively.

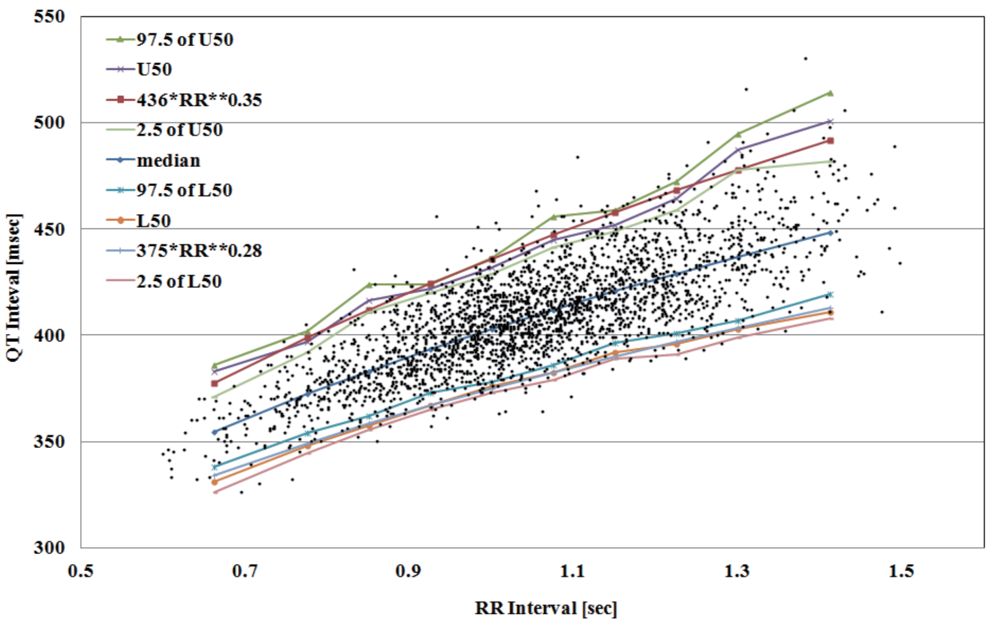

Figure 2. CIs of the upper and lower limits of the reference range. $U50_i$ (×) and $L50_i$ (●) obtained in each RR class using the bootstrap method are shown with an intermediate line. Curves representing the exponential regression equations for $QT_{upper\ limit}$, QT_{median} and $QT_{lower\ limit}$ are shown as a thick, intermediate and thin lines, respectively.

Table 1. Class value, range, sample size, arithmetic mean, SE of mean, median, and range of CI of median, and QT_{median} corresponding to each RR class.

		RR interval		QT interval				
Class no.	No. of cases	Class value (ms)	Range (s)	Mean (ms)	SE of mean (ms)	Median (ms)	Range of 95% CI of Median (ms)	QT_{median} (ms)
1	66	662.5	0.5995--0.7375	356.05	1.64	356	8.5	354.68
2	120	775	0.7375--0.8125	373.08	1.17	372	5.5	372.31
3	215	850	0.8125--0.8875	384.07	1.04	382	3.0	383.10
4	321	925	0.8875--0.9625	394.05	0.81	393	4	393.26
5	491	1000	0.9625--1.0375	404.13	0.69	404	3	402.85
6	458	1075	1.0375--1.1125	412.36	0.78	411	3	411.97
7	335	1150	1.1125--1.1875	421.50	0.88	420	5	420.65
8	257	1225	1.1875--1.2625	428.47	1.14	426	5	428.95
9	159	1300	1.2625--1.3375	440.63	1.74	439	6	436.91
10	107	1412.5	1.3375--1.4665	450.38	2.21	450	10	448.27

Table 2. Upper and lower limits of the reference value of QT in each class estimated using the bootstrap method. The values of $QT_{upper\ limit}$ and $QT_{lower\ limit}$ for the class value of each RR were also included.

Class no.	Class value (ms)	*L50* (ms)	95% CI of *L50* (*L2.5--L97.5*) (ms)	$375^{*}RR^{0.28}$ (ms)	*U50* (ms)	95% CI of *U50* (*U2.5--U97.5*) (ms)	$436^{*}RR^{0.35}$ (ms)
1	662.5	331.3	12.3 (326--338.3)	334.2	383	15 (371--386)	377.5
2	775	348	9.2 (344.7--353.9)	349.2	397	10.1 (392--402.1)	398.8
3	850	357.7	6.6 (355.4--362)	358.3	416.7	13 (411--424)	411.9
4	925	367	8 (365--373)	366.9	422	4 (420--424)	424.3
5	1000	376	4.7 (373.3--378)	375	432	7.3 (429--436.3)	436
6	1075	382.4	7 (379--386)	382.7	445	14.4 (441.6--456)	447.2
7	1150	392	7.4 (389--396.4)	390	451.7	10.3 (448.7--459)	457.9
8	1225	396	9.8 (391.2--401)	396.9	464.6	13.4 (459--472.4)	468.1
9	1300	402.9	8 (399--407)	403.6	487.2	17 (478--495)	477.9
10	1412.5	411	11.6 (407.9--419.5)	413.1	500.8	32.4 (482--514.4)	492