

The upper and lower limits of the reference range of the QT interval in resting electrocardiograms of healthy young Japanese men

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The study was performed to examine RR and QT intervals in resting 12-lead electrocardiograms in healthy Japanese men. The subjects were 2529 men aged 20 to 35 years old. Upper and lower limits of the reference range for QT were estimated using the bootstrap method. Cases were classified into 10 classes based on the RR interval and 1000 groups of bootstrap samples of QT per class were generated. The 2.5th, 50th and 97.5th percentiles and the confidence intervals of the conditional distribution of QT per class were estimated. Exponential regression equations approximating the upper and lower limits for QT were derived. The upper and lower limits were approximated by $QT_{\text{upper limit}} = 436 \times RR^{0.35}$ and $QT_{\text{lower limit}} = 375 \times RR^{0.28}$, respectively. Sixty-two cases (2.45%) exceeded the upper limit and 61 (2.41%) fell below the lower limit. These percentages agree with the definition that 2.5% of the population is outliers in the QT distribution. Exponential equations approximating the upper and lower limits of the QT interval may be useful in enrollment of healthy Japanese men in phase 1 clinical trials.

Key words: QT interval, reference value, bootstrap method

Introduction

A phase 1 clinical trial is the first stage in testing of new drugs in human subjects. This makes the safety of volunteers of primary importance and many tests are performed before enrollment of subjects in phase 1 trials. These tests include measurement of the QT interval (QT) before and during trials, since prolongation of QT by drug administration is a risk factor for fatal cardiac arrhythmia⁽¹⁾. An extremely short QT has also recently been associated with fatal arrhythmia⁽²⁾. Many institutions performing clinical trials in Japan use conventional QT criteria such as Bazett's⁽³⁾ or Fridericia's formula⁽⁴⁾ for selection of subjects, but the validity of these criteria in healthy young Japanese men has not been proven. Therefore, there is a need to establish defined criteria based on QT intervals in this target population to ensure that appropriate subjects are enrolled in clinical trials.

In this study, we use the terms 'reference range' and 'outlier' in place of 'normal' and 'abnormal', respectively, in describing the QT interval. The reference range of laboratory data was established from the 2.5th and 97.5th percentiles of measurements in a control group. Measurements that exceeded the upper limit or fell below the lower limit of this range are referred to as outliers.

In 2008, we proposed a new criterion for the upper limit of the reference range for QT in healthy young Japanese men, using the bootstrap method⁽⁵⁾. In 2011, we added an equation for the lower limit of the reference range to enable identification of outliers at both extremes⁽⁶⁾. From these results, we

found that the exponent of the regression equation of QT in terms of RR did not always agree with those of equations representing the upper and lower limit of the reference range. However, because of a shortage of cases, we were unable to achieve the accuracy we had hoped for, especially for the longer and shorter ends of the RR interval. We also assumed the normality of the conditional distribution of QT in these studies and approximated the 97.5th and 2.5th percentiles of the distribution as the mean ± 1.96 sigma of the distribution.

In the present study, addition of new cases from the same institution as that in the previous studies permitted estimation of the upper and lower limits with 95% confidence intervals, without assuming the normality of the conditional distribution of QT. Based on this analysis, we propose updated criteria for QT to identify subjects who should be excluded from clinical trials.

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. All ECGs were recorded for 10 s with the subjects in the resting supine position from about 1 p.m. to 3 p.m. and after at least 60 minutes of rest. The RR interval (RR) for each case was determined by averaging the RR intervals 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) (12) provided by Fukuda Denshi. This software determines the end of the T wave by the gradient method and includes an algorithm that uses first-order differential and threshold equations. QT was measured for all 12 ECG leads and the average of all QT's, excluding leads that were impossible to measure due to noise or baseline drift, was used in the subsequent analysis.

For all ECGs, the computer measurements of RR and QT were verified by comparison of the automated measurements with manual measurements made by a single physician using the printed ECG waveform. Some cases showed a U-wave or flat T-wave that might have affected computer recognition of the end of the T wave, but in all cases the difference between the automated and manual measurements was within 20 ms. Therefore, the automated measurements were used in all cases.

Age, heart rate, RR and QT were obtained for all cases that met the enrollment criteria for ECG findings. Eighty cases were excluded based on these criteria, leaving 2529 cases in the study (**Table 1**). The joint distribution of RR and QT was used to determine a linear regression equation for QT in terms of RR: $QT_L = \alpha \times RR + \beta$; and an exponential regression equation for QT: $QT_E = \gamma \times RR^\delta$. 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 interval, the class widths at both ends were broadened. Thus, the lower and upper limits of the lowest RR were set at 0.600 and 0.7375 s, respectively (width 0.1375 s), and the limits of the highest RR were set at 1.3375 and 1.500 s, respectively (width 0.1625 s). 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 2**. Each subject was classified into one of the 10 classes based on their RR interval. The value of RR for each case 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⁽⁷⁾, the median, lower limit and upper limit of the reference range of QT in each class were estimated as follows:

Step 1: The number of 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} ; $i = 1, \dots, 10$, $k = 1, \dots, j$). The number of bootstrap samples (j) was set at 1000.

Step 2: The 2.5th percentile (L_{ik} ; $k = 1, \dots, j$) 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 value of QT in the i -th class. $L2.5_i$ and $L97.5_i$ were defined as the lower and upper limits of the 95% confidence interval (CI) of $L50_i$, respectively.

Step 4: Similarly to step 2, the 50th (M_{ik} ; $i = 1, \dots, 10$, $k = 1, \dots, j$) and 97.5th (U_{ik} ; $i = 1, \dots, 10$, $k = 1, \dots, j$) 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 95% 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 95% CI of $U50_i$, respectively.

Step 6: Using 10 pairs of the class value from the i -th class of the RR and $U50_i$ ($i = 1, \dots, 10$), an exponential regression equation for the upper limit of the reference value of QT was estimated in terms of RR ($QT_{\text{upper limit}} = c \times RR^d$). The coefficient c and exponent d of the equation were determined using the weighted least squares method.

Step 7: Similarly to step 6, regression equations for the median ($QT_{\text{median}} = e \times RR^f$) and lower limit ($QT_{\text{lower limit}} = g \times RR^h$) of the reference value of QT were estimated in terms of RR.

After drawing the curves for the upper and lower limits of the reference range on the plots of the joint distribution of RR and QT, the validity of the pair of equations was confirmed by the naked eye. More precisely, 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 Review Board of the institution where all ECGs were recorded and all subjects gave written informed consent before starting the study. Microsoft Excel 2003 SP2 was used for statistical analysis.

Results

Joint distribution of RR and QT

The mean age of the 2529 subjects was 24.3 years old. The RR and QT intervals ranged from 0.60 to 1.50 s and from 326 to 530 ms, 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 ($QT_L = 126.5 \times RR + 276.0$) 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 ($QT_E = 403.3 \times RR^{0.32}$) was obtained with an RMSE of 16.32 ms.

The median of the QT distribution was compared with the arithmetic mean (Mean) for each of the 10 classes (**Table 2**). $M50_i$ was less than Mean in each class, which suggests that the conditional distribution of QT slightly leaned toward the shorter end. However, the differences between $M50$ and Mean in each class were minimal and the values agreed to two significant figures. The ranges of the 95% 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 $QT_{\text{median}} = 403.03 \times RR^{0.308}$. The coefficient of 403.03 and exponent of 0.31 were very close to the respective values for QT_E . The exponent of 0.32 in QT_E was closer to the value of 1/3 proposed by Fridericia⁽⁴⁾ than to that of 1/2 proposed by Bazett⁽³⁾.

Estimation of the upper limit of the QT reference value

The median ($U50_i$) and the upper ($U97.5_i$) and lower ($U2.5_i$) limits of the 95% CI of $U50_i$ ($i = 1, \dots, 10$) estimated for each of the 10 classes using the bootstrap method are shown in **Table 3**. Similarly to $M50_i$, $U50_i$ increased along with RR. 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 width of the 95% CI was 7.28 ms. The widths of the 95% CI broadened in the shorter and longer RR classes, but showed no increasing or decreasing trend with respect to RR (**Table 3**).

In steps 6 and 7 above, the equation $QT_{\text{upper limit}} = 436 \times RR^{0.35}$ was obtained for the upper limit of the reference value of the QT population. The RMSE of this equation was 5.98 ms. The upper and lower limits of the 95% CI of $QT_{\text{upper limit}}$ in the i -th class were approximated using $U97.5_i$ and $U2.5_i$, respectively (**Table 3**). In the RR range between 0.812 and 1.263 s the width of the 95% CIs ranged from 4 to 14.4 ms, whereas for the RR ranges <0.812 s and >1.263 s the 95% CIs ranged from 10 to 32.4 ms. The value of $QT_{\text{upper limit}}$ exceeded $U97.5_4$ by 0.2 ms in the 4th class, but remained within the 95% CIs of $U50_i$ in all other classes.

Estimation of the lower limit of the QT reference value

The median ($L50_i$) and the upper ($L97.5_i$) and lower ($L2.5_i$) limits of the 95% CI of $L50_i$ ($i = 1, \dots, 10$) were estimated for each of the 10 classes using the bootstrap method (**Table 3**). Similarly to $M50_i$ and $U50_i$, $L50_i$ increased along with RR. In the 5th class, $L2.5_i$, $L50_i$ and $L97.5_i$ were 373.3, 376 and 378 ms, respectively, and the width of the 95% CI was 4.75 ms. The widths of the 95% CIs broadened to 12 ms in the shorter and longer RR classes, but showed no increasing or decreasing trend with respect to RR (**Table 3**). Using similar steps to estimate $QT_{\text{upper limit}}$, the exponential equation $QT_{\text{lower limit}} = 375 \times RR^{0.28}$ was obtained for the upper limit of the reference value of the QT population. The RMSE of this equation was 1.63 ms. The upper and lower limits of the 95% CI of $QT_{\text{lower limit}}$ in the i -th class were approximated using $L97.5_i$ and $L2.5_i$, respectively (**Table 3**). In the RR range between 0.812 and 1.263 s the width of the 95% CIs ranged from 4.8 to 9.8 ms, whereas for the RR ranges <0.812 s and >1.263 s the 95% CIs ranged from 8 to 12.3 ms. The value of $QT_{\text{lower limit}}$ remained within the 95% CIs of $L50_i$ in all 10 classes.

Curves representing $QT_{\text{upper limit}} = 436 \times RR^{0.35}$ and $QT_{\text{lower limit}} = 375 \times RR^{0.28}$ are shown with the plot of the joint distribution of RR and QT in **Figure 2**.

Detection of outliers in the target population

Using $QT_{\text{upper limit}} = 436 \times RR^{0.35}$, 62 of the 2529 cases (2.45%) with QT exceeding the limit were identified as outliers with longer QT. Similarly, using $QT_{\text{lower limit}} = 375 \times RR^{0.28}$, 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. The accuracy of the upper and lower limits of the QT distribution was clearer when the subjects were divided into two groups based on RR intervals of <1 s and >1 s. At the upper extremes for RR intervals <1 s and >1 s, 20 of 971 cases (2.45%) and 42 of 1558 cases (2.70%) were identified as outliers, respectively. At the lower extremes for RR intervals <1 s and >1 s, 25 (2.57%) and 36 cases (2.31%) were identified as outliers. These detection rates were within 0.26%, which suggested that the detection rate of 2.5% was satisfactory for both upper and lower extremes of RR.

Discussion

The mean of the conditional distribution of the QT interval for a given RR is well known to increase curvilinearly along with the increase of the RR interval, and many criteria and equations have been proposed to approximate this relationship⁽⁸⁾. Two of the most widely used are Bazett's and Fridericia's formulas. In a previous study, we obtained an exponent that differed greatly from the value proposed by Bazett, which could not be used to approximate our target population. In contrast, the exponent of RR in Fridericia's formula was very close to the exponent of the equation that approximated the upper limit of our subject population. However, we were unable to obtain information on another important parameter, the coefficient of RR in Fridericia's formula. We were also unable to use other criteria since the subjects were examined for potential enrollment in studies with different purposes, including epidemiological studies treating a broad range of populations using diverse recording devices.

Since the variance of the conditional distribution of QT increased along with the increase of RR, we expected that the exponent of RR in the equation for the upper limit would differ from that for the lower limit. In fact, the exponents of RR for $QT_{\text{upper limit}}$, QT_{median} , and $QT_{\text{lower limit}}$ were estimated to be 0.35, 0.31, and 0.28, respectively. These values were obtained directly by the bootstrap method, but are consistent with the values of 1/3 proposed by Fridericia (who first obtained an exponent of 0.3558 by the least squares method) and 0.308 (0.298–0.318) proposed by Yoshinaga⁽⁹⁾, which were both obtained indirectly using the mean and standard deviation of the distribution.

To show the advantage of our criteria, we used similar criteria consisting of two equations for the upper and lower limits with a common exponent of RR and compared the accuracy for detection of outliers. Keeping the coefficient of RR unchanged, the exponents of RR for $QT_{\text{upper limit}}$ and $QT_{\text{lower limit}}$ were replaced with a common exponent of 0.31, corresponding to the exponent of RR for QT_{median} , to give the equations $QT_{\text{C upper limit}} = 436 \times RR^{0.31}$ (instead of $QT_{\text{upper limit}} = 436 \times RR^{0.35}$) and $QT_{\text{C lower limit}} = 375 \times RR^{0.31}$ (instead of $QT_{\text{lower limit}} = 375 \times RR^{0.28}$). Using the new equations for $QT_{\text{C upper limit}}$ and $QT_{\text{C lower limit}}$, 64 cases (2.53%) exceeded $QT_{\text{C upper limit}}$ and 65 cases (2.84%) fell below $QT_{\text{C lower limit}}$ and were identified as the outliers. The detection rates with these equations were consistent with the rates of 2.45% and 2.41% at the long and short QT ends, respectively, obtained using the original equations.

We also investigated the detection rate by dividing the subjects into two groups based on RR intervals of ≤ 1 s and > 1 s. For $RR \leq 1$ s, 6 of 20 outliers based on $QT_{upper\ limit}$ and 6 of 25 outliers based on $QT_{lower\ limit}$ shifted to the reference range; thus, the detection rate of outliers by $QT_{C\ upper\ limit}$ and $QT_{C\ lower\ limit}$ decreased from 2.45% (original equation) to 1.44% and from 2.57% to 1.96%, respectively. For $RR > 1$ s, 8 cases classified in the reference range by $QT_{upper\ limit}$ exceeded $QT_{C\ upper\ limit}$ and 10 cases classified in the reference range by $QT_{lower\ limit}$ fell below $QT_{C\ lower\ limit}$. As a result, the detection rate of outliers by $QT_{C\ upper\ limit}$ and $QT_{C\ lower\ limit}$ increased from 2.70% to 3.21% and from 2.31% to 2.90%, respectively. Collectively, these results show that, compared with use of the equations for $QT_{upper\ limit}$ and $QT_{lower\ limit}$ as standards, 12 false negative cases occurred for subjects with $RR \leq 1$ s and 18 false positive cases occurred for subjects with $RR > 1$ s using $QT_{C\ upper\ limit}$ and $QT_{C\ lower\ limit}$. The results of these two types of misclassifications cancelled each other out, which gave the apparently valid detection rate of around 2.5% with $QT_{C\ upper\ limit}$ and $QT_{C\ lower\ limit}$.

The same tendency was found in detection using the exponent of 1/3 proposed by Fridericia. Using $QT_{F\ upper\ limit} = 436 \times RR^{0.33}$ instead of $QT_{upper\ limit} = 436 \times RR^{0.35}$ and $QT_{F\ lower\ limit} = 375 \times RR^{0.33}$ instead of $QT_{lower\ limit} = 375 \times RR^{0.28}$, 64 cases (2.53%) exceeded $QT_{F\ upper\ limit}$ and 73 cases (2.89%) fell below $QT_{F\ lower\ limit}$. These results appeared consistent with the classification using the original equations. However, with division of the subjects into two RR intervals, as described above, 11 false negative cases occurred for the shorter RR interval and 25 false positive cases occurred for the longer RR interval.

From these results, we conclude that use of individual exponents of RR in the equations for the upper and lower limits of QT, in place of a common exponent, is necessary to achieve a better approximation for the broad range of the RR interval.

Limitations of the study

The results of the study are valid for resting ECGs of healthy young Japanese men with heart rate between 40 and 100 bpm, with QT measured by Fukuda analysis software. The applicability of the results to the general population under different recording conditions has yet to be proven.

Summary

We investigated the relationship between the RR and QT intervals in resting ECGs of 2529 healthy young Japanese men, and found that the upper and lower limits of the reference range of QT 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. Of the 2529 cases, 62 (2.45%) surpassing the upper limit were diagnosed as cases of QT prolongation, and 61 (2.41%) with QT below the lower limit were diagnosed as cases of short QT. The detection rates of these outliers agreed well with the expected value of 2.5% at both ends of the broad range of the RR interval. From these results, we conclude that the two exponential equations approximating the upper and lower limits of the QT reference range coincide well with the QT distribution on ECGs of healthy Japanese men, and therefore can be used in enrollment of volunteers in phase I trials.

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Figure Legends

Figure 1. Relationship between QT and RR intervals in resting ECGs of 2529 healthy young Japanese men. QT_E (bold line) and QT_{median} (thin line).

Figure 2. $U50_i$ (■) and $L50_i$ (▲) obtained in each RR class using the bootstrap method were plotted and connected with a dotted line. The exponential regression equations for $QT_{upper\ limit}$, QT_{median} and $QT_{lower\ limit}$ are shown as a standard, bold and thin lines, respectively.

Figure 1

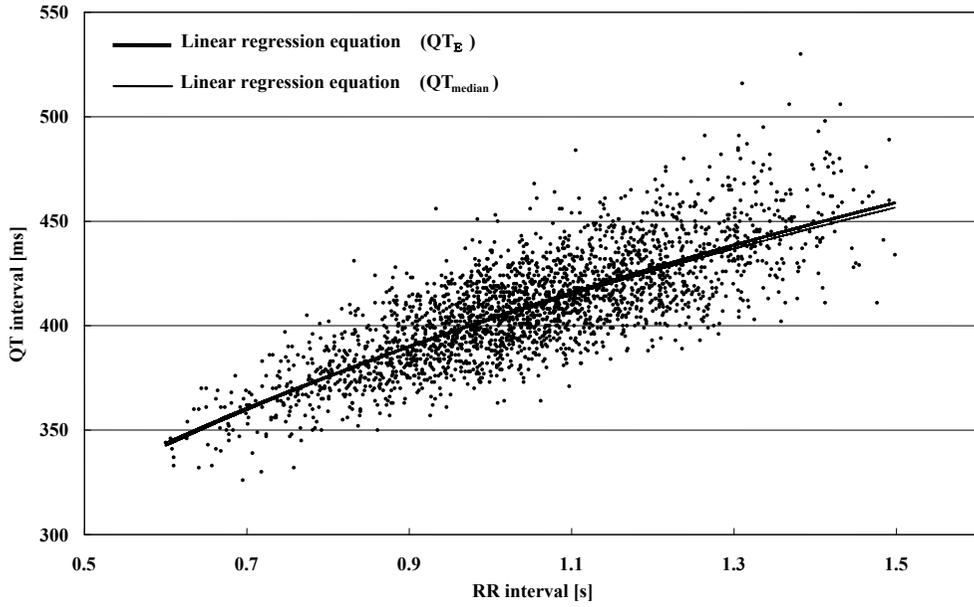


Figure 2

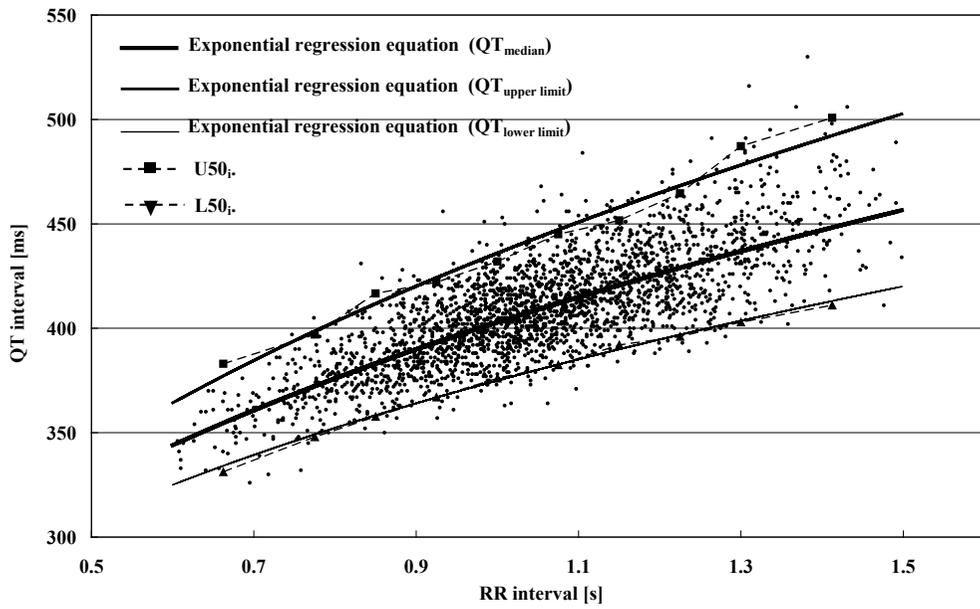


Table 1. Cases excluded from the study.

ECG findings	Exclusion criterion	Number of cases
Sinus tachycardia		5
Sinus bradycardia		2
Left axis deviation	<-30	13
Right axis deviation	>110	12
Myocardial ischemia		2
Complete right bundle branch block	>135	5
Intraventricular conduction disturbance	>135	7
Short PR interval	<80	1
First degree atrioventricular block	>260	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

Table 2. Class value, range, sample size, arithmetic mean, SE of mean, median, range of 95% CI of median, and QT_{median} corresponding to class value of each RR class.

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

Table 3. Upper and lower limits of the reference value of QT in each class estimated using the bootstrap method. Values of $QT_{\text{upper limit}}$ and $QT_{\text{lower limit}}$ for each RR class are also shown.

Class no.	Class value	2.5 th of L50	L50	$375 \times RR^{0.28}$	97.5 th of L50	2.5 th of U50	U50	$436 \times RR^{0.35}$	97.5 th of U50
1	662.5	326	331.25	334.17	338.25	371	383	377.49	386
2	775	344.68	347.98	349.17	353.93	392	397	398.79	402.08
3	850	355.35	357.7	358.32	362	411	416.65	411.89	424
4	925	365	367	366.90	373	420	422	424.26	424
5	1000	373.25	376	375	378	429	432	436	436.28
6	1075	378.98	382.43	382.67	386	441.58	445	447.18	456
7	1150	389	392	389.97	396.35	448.65	451.65	457.86	459
8	1225	391.2	396	396.93	401	459	464.6	468.10	472.4
9	1300	399	402.9	403.59	407	478	487.2	477.93	495
10	1412.5	407.85	411	413.07	419.5	482	500.8	492.02	514.4