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Assessing QT prolongation and electrocardiography restitution using a beat-to-beat method

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Abstract

Historically, the heart rate corrected QT (QTc) interval has been the standard method to assess for impaired ventricular repolarization, particularly for drug development. However, QTc does not reflect changes in autonomic state or QT-RR hysteresis which can also affect the interpretation of arrhythmogenic risk. With the advent of more accurate algorithms to automatically measure the QT interval from continuously collected digital ECG data, usage of heart rate corrected functions is no longer necessary. The dynamic beat-to-beat QT interval method compares the QT interval to individual cardiac cycles from all normal autonomic states at similar RR intervals, thus eliminating the need for correction functions. The upper 97.5% reference boundary of these beat-to-beat QT interval values is defined across the entire 24-hour RR interval range. Beats with QT intervals exceeding this limit are flagged as outlier beats for further arrhythmia vulnerability assessment. The same beat-to-beat technique can also be used to assess the QT-TQ interval relationship known as ECG restitution. This analysis potentially provides an additional means to quantify cardiac stress or arrhythmia vulnerability as the heart works more in relationship to each rest cycle. (Cardiol J 2010; 17, 3: 230–243)

Key words: QT prolongation, autonomic tone, restitution, electrocardiogram, beat-to-beat

Introduction

The QT interval varies with heart rate and mathematical formulas have been used since 1920 to normalize the interpretation of the QT interval [1, 2]. Bazett [1] described how the QT interval divided by the square root of the RR interval (in seconds) produced a constant value for QT, which he termed K. This K eventually was the basis for the correction term QTc, but even Bazett recognized that the QT interval/heart rate relationship is more complex. In his original paper he also described how K lessened with sympathetic, and increased with vagal, stimulation and different K values should be used for varying autonomic states such as standing, sitting or lying. Since then, more than 20 correction formulas have been proposed, but none have been demonstrated to be universally applicable [3].

The importance of proper assessment of the QT interval was emphasized in the 1990s after the withdrawal of several marketed drugs that were found to increase the incidence of sudden cardiac death related to the polymorphic ventricular tachycardia, torsades de pointes (TdP). Drugs such as terfenadine [4, 5] and cisapride [6] were shown to prolong the QT interval which later resulted in regulatory guidance for conduct of clinical studies from the International Conference of Harmonization known as ICH E14 [7]. The guidance outlines a highly-controlled methodology for examination of

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the QTc interval in drug development, known as the thorough QT/QTc study (TQT), that is designed to statistically detect an increase of at least 5 ms. With more TQT studies having been conducted, it is becoming evident that some drugs with very low incidences of reported arrhythmia can cause a positive signal (i.e. a false positive) [8–11]. More importantly, there is a risk that dangerous drugs may be inadvertently approved because of differing results obtained with QTc methodologies when changes in heart rate or autonomic state occur (i.e. a false negative). Both these issues have had a significant impact on the ability of pharmaceutical companies to bring new medications quickly to the marketplace. The Food and Drug Administration has recognized the problem with assessment of QT effects of drugs that also changes heart rate or autonomic tone and has initiated an effort with the Cardiovascular Safety Research Consortium [12] to suggest alternative methods.

The dynamic beat-to-beat method

One of the alternative methods under consideration is dynamic QT beat-to-beat (QTbtb) analysis. This has been reported to differentiate changes of QT interval duration due to heart rate or autonomic state from impaired repolarization [13]. QTbtb analysis compares QT intervals to individual cardiac cycles from all normal autonomic states at similar RR intervals, thereby eliminating potential sources of error from the use of correction functions. All sequential usable beats from continuously collected electrocardiography (ECGs) are used. The final dataset boundaries assume all normal hysteresis (defined as the lag in QT interval adaptation for changes in RR interval), sinus arrhythmia and QT-RR variability are included within the 24-hour dataset resulting from changes in autonomic state incurred during activities such as eating, sleeping and walking. Beats beyond the individual QT reference limit would be assumed to possess potential risk of arrhythmia for which further analyses would be necessary (these are described later). The upper confidence boundary of all beat-to-beat QT interval values is defined across the entire 24-hour RR interval range. Beats exceeding this limit are flagged as outlier beats for further arrhythmia vulnerability assessment.

The normal physiological boundary of the QT-RR relationship is defined from all beats acquired during a continuous 24-hour ECG recording under unstressed conditions of up to 24-hour duration. The upper (or lower if needed) reference bound can be defined from the beat-to-beat QT and immediately preceding RR interval dataset and plotted as a 'cloud' to designate the limit (solid black line in Fig. 1A). For normal healthy volunteers, we have used the upper 97.5% reference bound of QT intervals because the heterogeneity increases dramatically beyond this level. When viewing the plots, one should bear in mind that many of the roughly 100,000 data points (from a 24-hour recording) overlap. Therefore the true density distribution of QT-RR pairs is not immediately evident in a two-dimensional plot. This is apparent in the distribution heterogeneity of the 2.5% of plotted beats values that exist beyond these upper QT interval boundaries. The boundaries for RR interval are more well-defined. So in order to maximize the range for which QT assessment can occur, we have found that 99% of all RR intervals can be included in the analysis. Since automated algorithms for reading each cardiac cycle can be affected by artifacts, rigorous criteria are also employed for beat flagging of the dataset. All flagged beats are over-read by analysts to determine if they are usable.

Using the described 24-hour data as a baseline for all subsequent analyses, the effect of a drug, placebo and baseline-adjusted placebo-corrected value can be readily assessed. For any specific time point or timeframe, e.g. during the peak concentration of a drug and its time-matched placebo, all beats are analyzed. Figure 1B shows the five-minute cloud after dosing of placebo at the Cmax time-matched period depicted on top of the 24-hour background QT/RR cloud. The center of this 5-min cloud of data, or 'centroid', is calculated as the median QT and the median RR interval. This median QT value (QTbtb) for any nominal time point is compared to the centroid of all beats extracted within a similar RR interval range (e.g. ± 12 ms depicted as a rectangular slice) from the 24-hour baseline dataset to provide a delta-QTbtb value (Figure 1D–F). The beats used to calculate the delta-QTbtb for the nominal time points can also be used for calculating the corrected QTcB or QTcF values (Fig. 2B, C). The same procedure used to define the delta-QT--btb value for the placebo can then be applied for the on-drug treatment nominal time points (Fig. 2C). The placebo-adjusted time-matched values (delta-delta QTbtb) are simply calculated by subtraction of the time-matched placebo values from the same time-matched values on treatment from the same subject (Fig. 2C). For a typical drug study, these are usually five minute periods taken immediately before a pharmacokinetic (PK) determination so that standard delta-delta QTbtb/PK curves can be

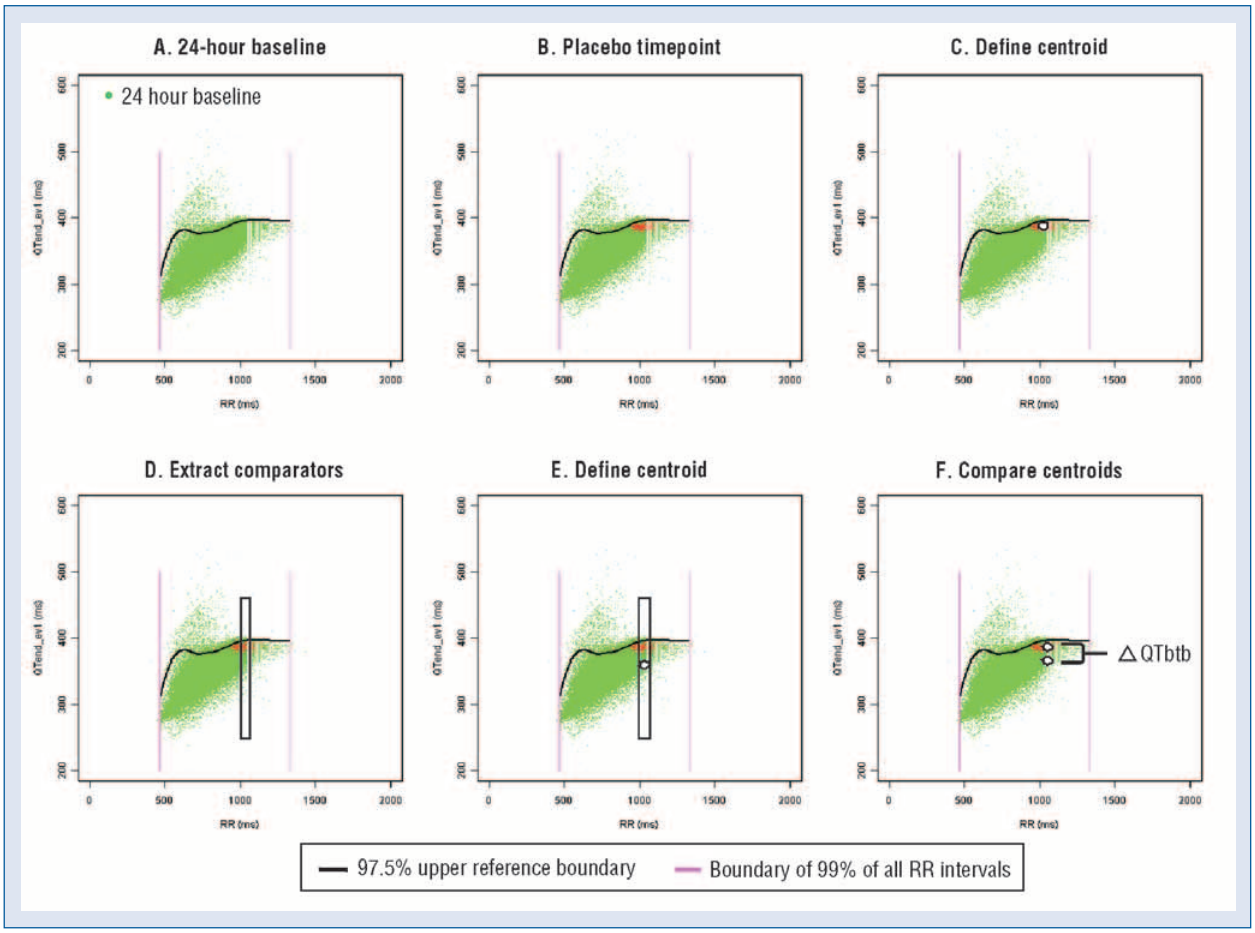

Figure 1. The beat-to-beat analysis process. **A.** For a single individual, the baseline for comparisons utilizes all QT and RR intervals collected from continuous ECG recordings over a 24-hour unstressed ambulatory period (● dots). From the dataset, the 97.5% upper reference bound is defined and plotted in relationship to the 24-hour data (irregular horizontal line). The baseline consists of all beats within 99% two-sided reference bounds of all RR intervals (vertical lines); **B.** Nominal time point values, i.e. from Cmax time-matched period, are collected. This is typically five minutes of sequential beats extracted from continuous ECG; **C.** Centroid of nominal time point is calculated by determining median QT and median RR interval values; **D.** All beats from 24-hour baseline cloud are extracted that have similar RR intervals (± 12 ms) compared to nominal time point centroid (represented by vertical rectangular area); **E.** Median QT determined from extracted beats of comparator 24-hour centroid data; **F.** Median centroid QT values of nominal time point and 24-hour baseline are compared to provide ΔQTbeat-to-beat (ΔQTbtb) for placebo baseline.

generated (not shown). However, one advantage of using continuous ECG collection with beat-to-beat analyses is that entire timeframes of data when on-drug can be compared to off-drug periods to quickly determine whether an effect is present (Fig. 2D). This can be further quantified as described below.

An essential component of the beat-to-beat method is to determine whether repolarization is significantly impaired beyond normal autonomic boundaries by applying quantile regression techniques [14] to define the upper 97.5% reference boundary of QT over RR intervals from the normal 24-hour data (from baseline day of the study). Figure 2B–D illustrates how the beats during the nominal time period on-drug compare against this baseline relationship. An outlier analysis examines the percentage of beats that exceed the upper 97.5% reference boundary of the baseline data during any period. By definition, for a drug with no effect, this percentage should be around 2.5% of beats exceeding the upper boundary. The percentage outlier values can be handled as described above for QTbtb values, and thus a time-matched placebo-adjusted value can be obtained for each time point. A lower 90% two-sided confidence interval can be determined for the mean of the percentage outlier values at any time point to determine whether there is a statistically significant increase in outliers.

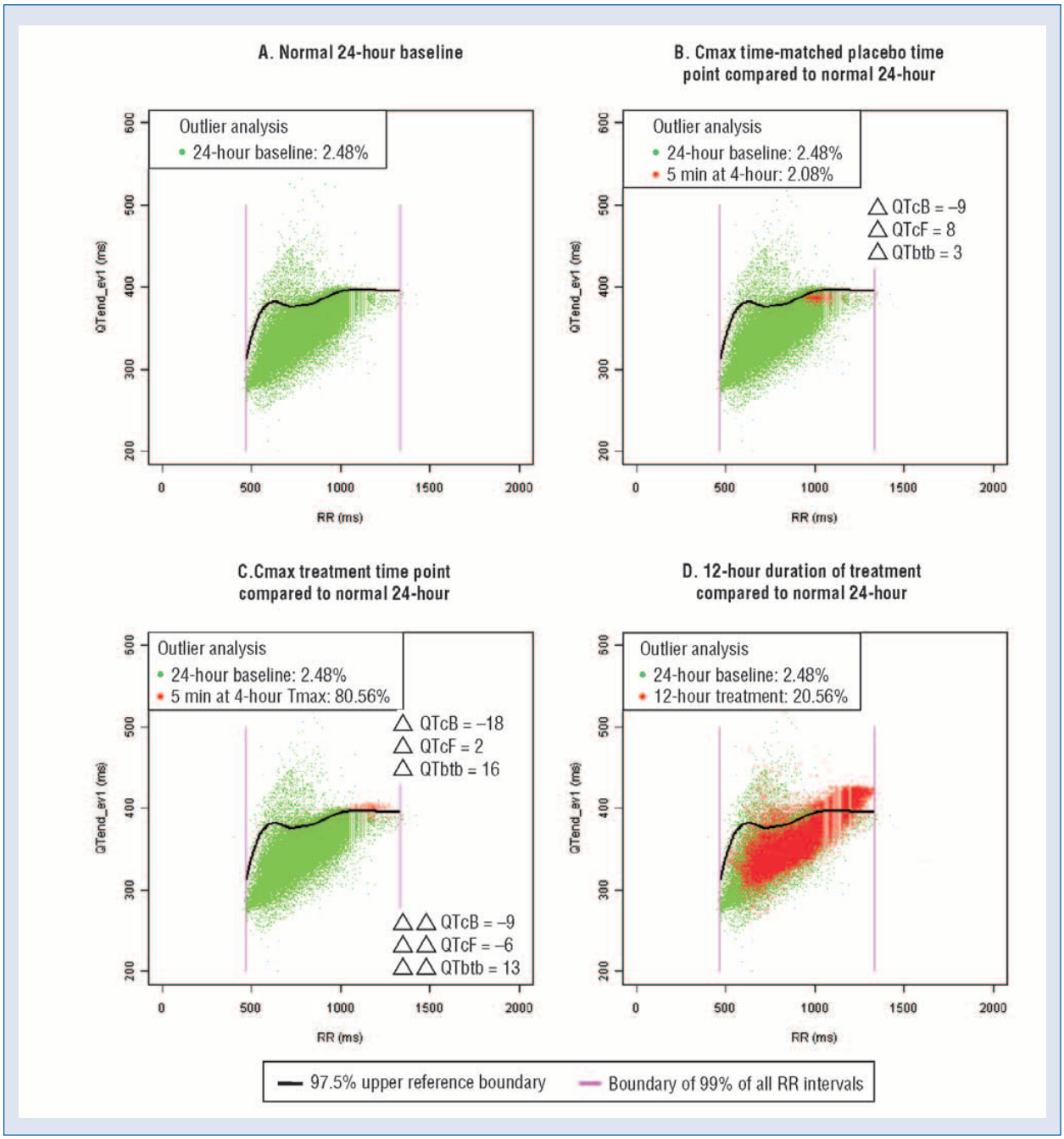

Figure 2. Comparison of QTbeat-to-beat (QTbtb) *vs* QTcB and QTcF values for the same beats during time-matched placebo and treatment periods in comparison to 24-hour normal boundary from a single subject QT-RR interval relationship; **A.** Baseline 24-hour dataset with upper 97.5% QT interval and 99% two-sided RR interval reference bounds (irregular horizontal line); **B.** Placebo time-matched nominal time period as calculated in Figure 2 for ΔQTbtb compared to same beats where Bazett and Fridericia corrections were used; **C.** Treatment (ΔQTbtb, ΔQTcB, ΔQTcF) and placebo-adjusted (ΔΔQTbtb, ΔΔQTcB, ΔΔQTcF) time-matched nominal time period calculated. Note that treatment period produces significant outlier beats above the upper 97.5% QT reference bound, yet QTcB and QTcF indicate no adverse effect; **D.** Same technique can be used for entire timeframe that subject is exposed to drug, to determine whether effect is present.

As mentioned above, this type of analysis can also be conducted for any period of time that the drug is used, including the entire time at efficacious concentrations to ascertain the net effect of drug *versus* normal QT/RR relationship (Fig. 2D).

One of the strengths of beat-to-beat analysis is the ability to assess changes in QT interval in varying autonomic states where QTc can cause false positive/negative indications of arrhythmia liability [15]. Figure 3 illustrates this concept and is demonstrated in Figure 4 after reflex tachycardia in a single individual induced during standing from a supine position (unpublished study data). When resting quietly in the supine position (Fig. 4A), the QT and RR intervals are increased with respect to the 24-hour baseline and the relationship is very flat, with reduced QT and increased RR variabilities. When the subject is asked to quickly stand up from this position, vagal influences lessen and sympathetic influences increase so the QT shortens and becomes more variable while the RR interval shortens during heart rate acceleration. This behavior creates a short-term hysteresis that deviates from the baseline correction fit and results in significant QTcB and QTcF prolongation of greater than 10 ms during heart rate acceleration (Fig. 4B). The QTbtb is less affected by the hysteresis and is only slightly prolonged. Almost all beats stay below the upper 97.5% reference bound (i.e. less than 2.5% outliers). These effects of altered autonomic state on QT-RR or QTbtb are within normal ranges for the individual and therefore very different from effects discussed below where prolonged repolarization of the QT interval is beyond the normal 24-hour boundary of RR intervals (see Fig. 5 for example).

Further quantifying arrhythmia vulnerability using the beat-to-beat method

When a significant increase in the number of outlier beats is observed, two subsequent analyses can be performed on the beat-to-beat dataset to determine the likelihood of increased arrhythmia vulnerability. The first procedure assesses the heterogeneity of just the outlier beats exceeding the upper 97.5% reference bounds for QT intervals. A second procedure performed on all the data, not just outliers, called ECG restitution, describes the stability of the beat-to-beat QT interval in relation to the amount of rest obtained during each cardiac cycle (TQ interval). ECG restitution also quantifies the extreme beats that represent increased cardiac stress (described in detail below).

The first procedure to describe heterogeneity uses a bootstrap analysis [16] and is applied to only beats that exceed the upper 97.5% reference bound. The median value during any time period on-drug is determined, so as to ascertain whether these beats are of greater magnitude in general compared to beats normally exceeding the upper 97.5% reference bound of QT intervals off-drug. Bootstrapping provides confidence intervals of the median value. The width of the confidence intervals is used as a measure of heterogeneity of the QT interval outlier beats, which has been associated with increased arrhythmia liability [17–19] and can be compared to the width of the confidence intervals at normal levels from the same individual when obtained off-drug.

The second procedure is to evaluate the cardiac ECG restitution. Restitution is the ability of the heart to recover from one beat to the next [20, 21]. This measures the QT interval (working phase of the heart) in relation to the previous TQ interval (resting phase of the heart). When the heart is not under stress, this ratio is less than 1, meaning the heart is resting more than it is working [22–24]. However, as stress increases on the heart, for example during exercise, the heart works more than it rests, increasing this ratio to greater than 1. An illustrated example is provided in Figure 6 as well as by data presented in discussion (Tables 1, 2). Sustained periods with inadequate recovery between beats would presumably lead to increased arrhythmia vulnerability, as occurs in extreme cases with salvos of non-sustained ventricular tachycardia or an R on T phenomenon [25, 26] where TQ interval equals zero. Arrhythmia liability not associated with QT prolongation may be more related to the TQ interval shortening or increase in the QT/TQ ratio of each beat. When QT prolongation is present along with increased QT and RR variability or increased heart rate during proarrhythmic states, the QT/TQ ratio can increase dramatically for transient periods of time, possibly leading to initiation of re-entry [21]. Thus, in addition to the median QT interval, the median TQ interval and median QT/TQ ratio are assessed in ECG restitution. To quantify effects on the entire restitution relationship across all heart rates, several parameters have been developed:

— **Lower 5% of TQ intervals:** It has been proposed that as the relative refractory period approaches zero, arrhythmia vulnerability may increase due to the likelihood of re-entry [27]. TQ interval is the ECG equivalent to the diastolic interval, and thus measuring the lower limit for 95% of the beats was utilized.

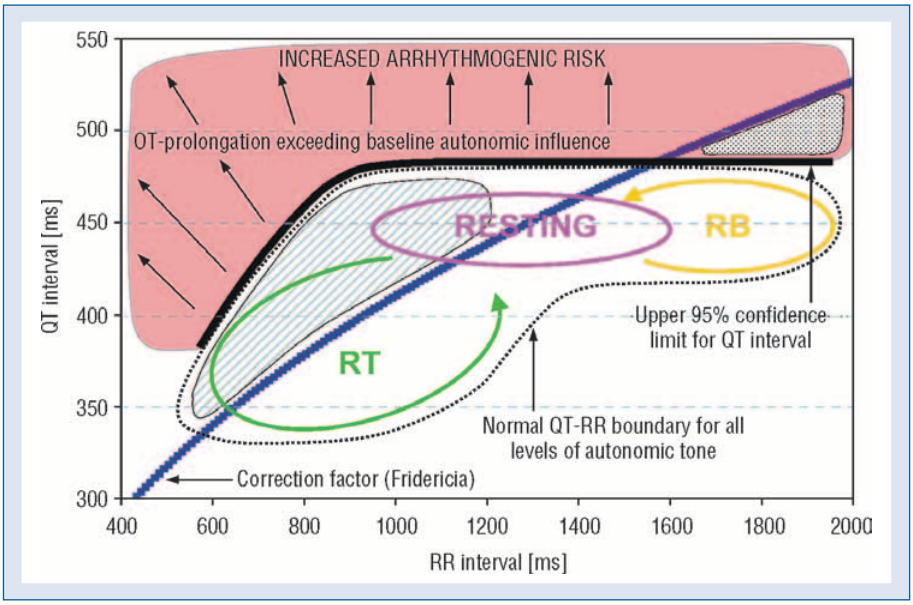

Figure 3. The normal dynamic QT-RR interval relationship (dotted-line forming asymmetric cloud) encompasses autonomic reflex responses such as tachycardia (RT) and bradycardia (RB) with hysteresis. The statistical outer boundary of the normal cloud is defined as the upper 95% confidence bounds. The Fridericia correction factor applied to the resting QT-RR interval relationship overcorrects dynamic responses in the normal range (striped area above correction line and below 95% confidence bounds i.e. false positive) or underestimates QT prolongation at slow heart rates (shaded area above 95% confidence bounds but below Fridericia correction i.e. false negative). QT prolongation of undefined arrhythmogenic risk (dark shaded area) occurs when exceeding the 95% confidence bounds of QT intervals during unstressed autonomic influence. From: Fossa et al. J Pharmacol Exp Ther, 2005; 312: 1–11.

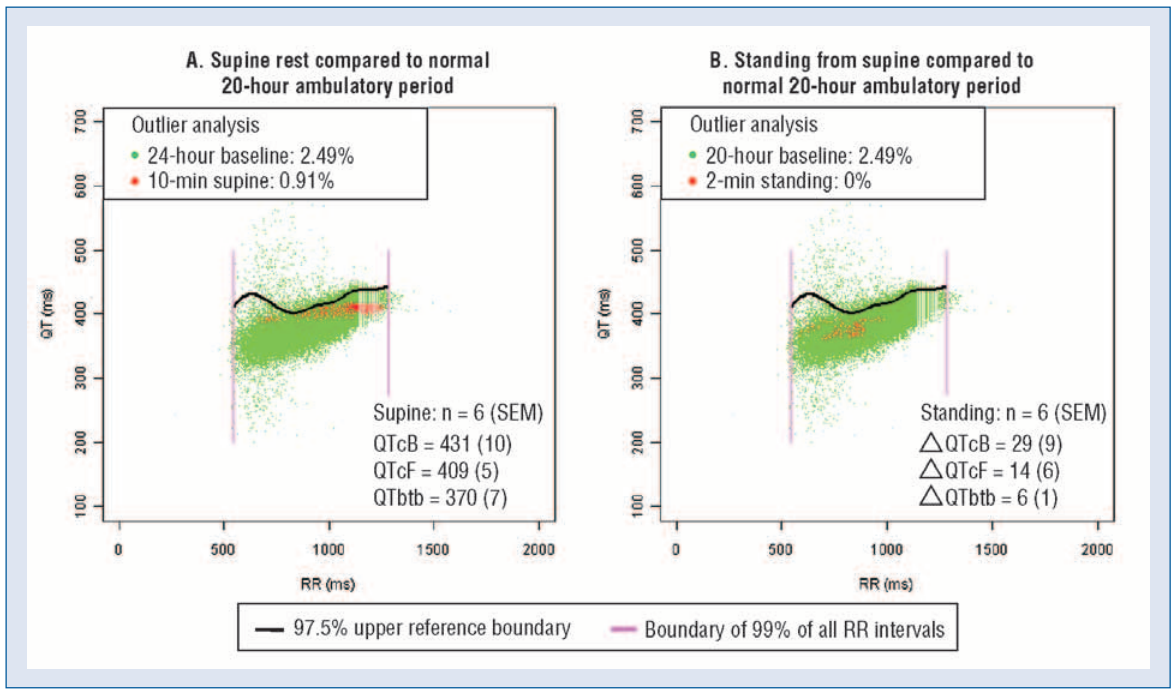

Figure 4. Effect of standing from supine position on QTcB, QTcF and QTbtb to normal 20-hour ambulatory QT-RR interval relationship. Panels **A** and **B** represent effect on beat-to-beat relationship from single subject with results for entire study (n = 6 normal male volunteers). Note that even though standing response does not produce beats outside normal range, QTcF and QTcB may reflect impaired repolarization beyond ICH-E14 safety standard.

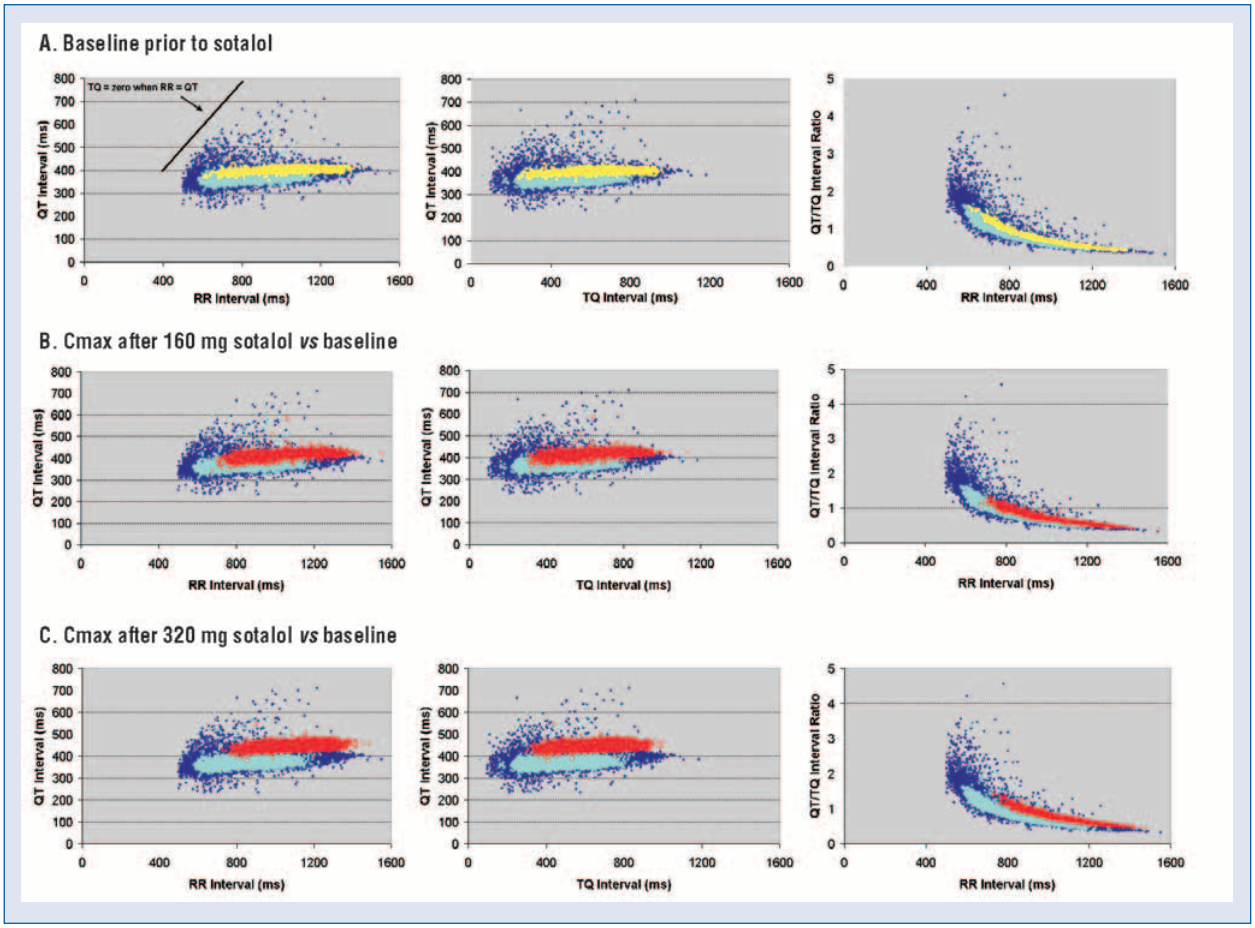

Figure 5. Beat-to-beat QT, RR and TQ interval relationships from a single healthy male subject; **A.** Relationship of baseline 24-hour (◆ diamonds), time-matched i.e. no drug given, baseline response at the Cmax period (● circles; hours 10am–noon), and nocturnal (■ squares; hours 3am–5am) sequential beats showing the QT *vs* RR intervals, QT *vs* TQ intervals and QT/TQ interval ratio *vs* RR interval; **B.** Sequential beats occurring during Cmax (● circles) after 160 mg of sotalol; **C.** 320 mg of sotalol compared to the baseline 22.5-hour and Cmax time-matched responses with no drug. From: Fossa et al. ANE, 2007; 12: 338–348.

— **Percentage of beats with QT/TQ ratio greater than 1:** As the ventricle spends more time working (QT interval or action potential duration) per cycle of rest (TQ or diastolic interval), cardiac instability may ensue, theoretically leading to increased arrhythmia vulnerability. This relationship has been associated with transition of ventricular tachycardia to fibrillation by the steepness of the restitution relationship [23, 28]. Assessment of the QT/TQ slope from normal sinus rhythm data would not take into account the density of beats occurring at any one point, and would be further complicated by hysteresis at a particular heart rate. Therefore, the percentage of beats with a QT/TQ ratio greater than 1 reflects the relative time spent on the restitution curve where stability is not as certain.

— **Upper 98% quantile of the QT/TQ ratio:** This measure reflects the magnitude of the steepness of the restitution relationship. The 98% quantile takes into account the most extreme beats with the highest likelihood of leading to arrhythmia.

A demonstration of this methodology was reported in healthy volunteers following oral doses of either 160 or 320 mg of sotalol, the IKr and beta-adrenergic blocker [21] Even though sotalol produced a dose-dependent increase in the QT interval between 71 and 194 ms, the number of beats with a QT/TQ ratio greater than 1 was reduced by 25% over the entire day on drug (Fig. 5). This can be explained by the fact that heart rate slowed in these individuals due to normal beta-receptor function blockade with sotalol, thereby allowing the TQ interval increase to more than offset the increase

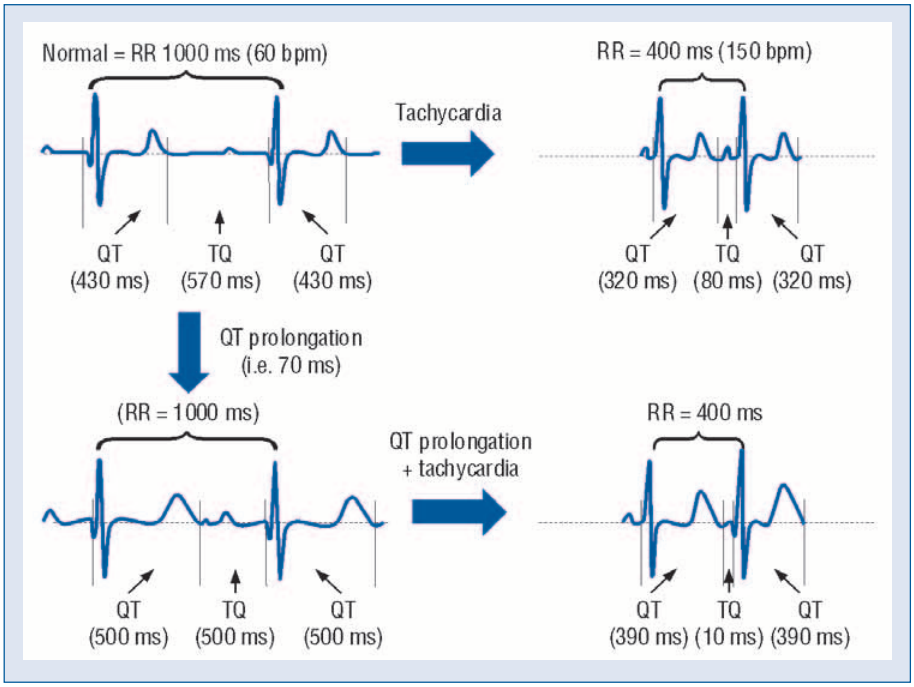

Figure 6. Relationship between heart rate (RR interval), QT interval and diastolic period (TQ interval) during rest and tachycardia in the presence and absence of QT prolongation. Please note that this is a hand-drawn illustration for conceptual purposes and does not accurately reflect interval measurements; **Top left complex:** Normal resting heart rate of 60 bpm provides a TQ interval of 570 ms; **Top right complex:** Tachycardia of 150 bpm reduces TQ interval approximately seven-fold to 80 ms; **Bottom left complex**: QT prolongation of 70 ms at resting heart rate of 60 bpm has relatively little effect on TQ interval (from 570 ms to 500 ms); **Bottom right complex**: QT prolongation of 70 ms is combined with tachycardia of 150 bpm to cause dramatic decrease of TQ interval to 10 ms (57-fold reduction from rest) thus providing little time for oxygenation and return of ion kinetics to normal state for next beat. From: Fossa et al. J Pharmacol Exp Ther, 2006; 316: 498–506.

Table 1. Baseline ECG and restitution values.

Parameter	Mean baseline values from Holter (SD)					
	Resting awake (7–9am)		Nocturnal (3–5am)		20 h — unstressed ambulatory	
	Day 1	Day 2	Day 1	Day 2	Day 1	Day 2
Traditional						
Median QT	415 (16)	406 (19)	416 (28)	415 (21)	383 (16)	380 (15)
Median RR	1100 (135)	1058 (144)	1072 (155)	1058 (125)	912 (84)	909 (87)
Median HR	55 (7)	58 (8)	57 (8)	57 (7)	66 (6)	67 (6)
Median QTcB	397 (21)	396 (21)	401 (24)	405 (17)	404 (19)	402 (20)
Median QTcF	403 (16)	399 (16)	406 (21)	408 (15)	397 (16)	395 (17)
Reference regions						
Area of discordance	54 (10)	45 (18)	40 (19)	45 (18)	See Fig. 8	See Fig. 8
Area of 95% region	22404 (6283)	21244 (4025)	31729 (11803)	23759 (11472)	41387 (9511)	40445 (12261)
Restitution						
Median QT	685 (126)	653 (132)	660 (133)	644 (110)	531 (76)	531 (80)
Lower 5% TQ	440 (82)	421 (77)	420 (83)	447 (38)	327 (38)	308 (30)
Median QT/TQ ratio	0.62 (0.11)	0.64 (0.12)	0.64 (0.11)	0.66 (0.10)	0.73 (0.10)	0.73 (0.12)
% QT/TQ ratio > 1	4.36 (4.27)	5.19 (5.41)	4.47 (3.04)	2.75 (1.51)	11.92 (9.79)	12.98 (12.53)
Upper 98% QT/TQ	1.09 (0.20)	1.13 (0.20)	1.18 (0.17)	1.05 (0.08)	1.28 (0.14)	1.34 (0.16)

Table 2. ECG and restitution responses after autonomic challenges on two separate days.

Parameter	Mean Holter biomarkers from automatic challenges (SD)									
	Standing		Burst exercise		Isoproterenol				Phenylephrine	
					Acceleration		Deceleration			
	Day 1	Day 2	Day 1	Day 2	Day 1	Day 2	Day 1	Day 2	Day 1	Day 2
Traditional										
Median QT	380 (20)	379 (15)	307 (29)	302 (32)	355 (16)	365 (35)	351 (10)	339 (21)	396 (11)	387 (10)
Median RR	801 (141)	742 (127)	466 (76)	440 (49)	565 (39)	584 (53)	609 (69)	556 (32)	1044 (67)	1005 (124)
Median HR	77 (16)	83 (16)	131 (19)	138 (15)	107 (8)	103 (9)	100 (11)	108 (6)	58 (4)	61 (8)
Median QTcB	430 (28)	442 (26)	450 (24)	452 (28)	471 (19)	478 (49)	454 (25)	455 (30)	389 (18)	388 (25)
Median QTcF	413 (16)	421 (11)	397 (23)	395 (28)	428 (17)	435 (42)	417 (17)	413 (25)	392 (15)	387 (17)
Reference regions										
% of beats as outliers	27 (38)	16 (23)	83 (23)	84 (12)	74 (14)	62 (31)	52 (32)	66 (23)	22 (9)	16 (8)
Area of 95% ellipse	13878 (5698)	15754 (7500)	27733 (31305)	22122 (9868)	11228 (6236)	12633 (8238)	8018 (2850)	7611 (3185)	22002 (17369)	12583 (5480)
Restitution										
Median TQ	420 (128)	363 (117)	158 (54)	142 (33)	210 (32)	218 (66)	256 (66)	216 (39)	648 (67)	617 (121)
Lower 5% TQ	238 (42)	240 (54)	134 (48)	101 (35)	162 (24)	147 (45)	162 (33)	155 (33)	460 (89)	446 (57)
Median QT/TQ ratio	1.01 (0.41)	1.17 (0.49)	2.07 (0.49)	2.20 (0.52)	1.73 (0.32)	1.85 (0.72)	1.46 (0.40)	1.62 (0.39)	0.62 (0.08)	0.65 (0.16)
% QT/TQ ratio > 1	44.00 (26.98)	60.67 (26.55)	94.33 (6.62)	93.00 (2.10)	93.95 (11.82)	92.21 (13.74)	86.73 (16.92)	95.77 (4.35)	2.42 (4.74)	6.83 (15.77)
Upper 98% QT/TQ	1.88 (0.42)	1.73 (0.38)	2.77 (0.92)	3.74 (1.52)	2.37 (0.45)	2.90 (1.20)	2.31 (0.43)	2.43 (0.69)	0.94 (0.26)	0.91 (0.18)

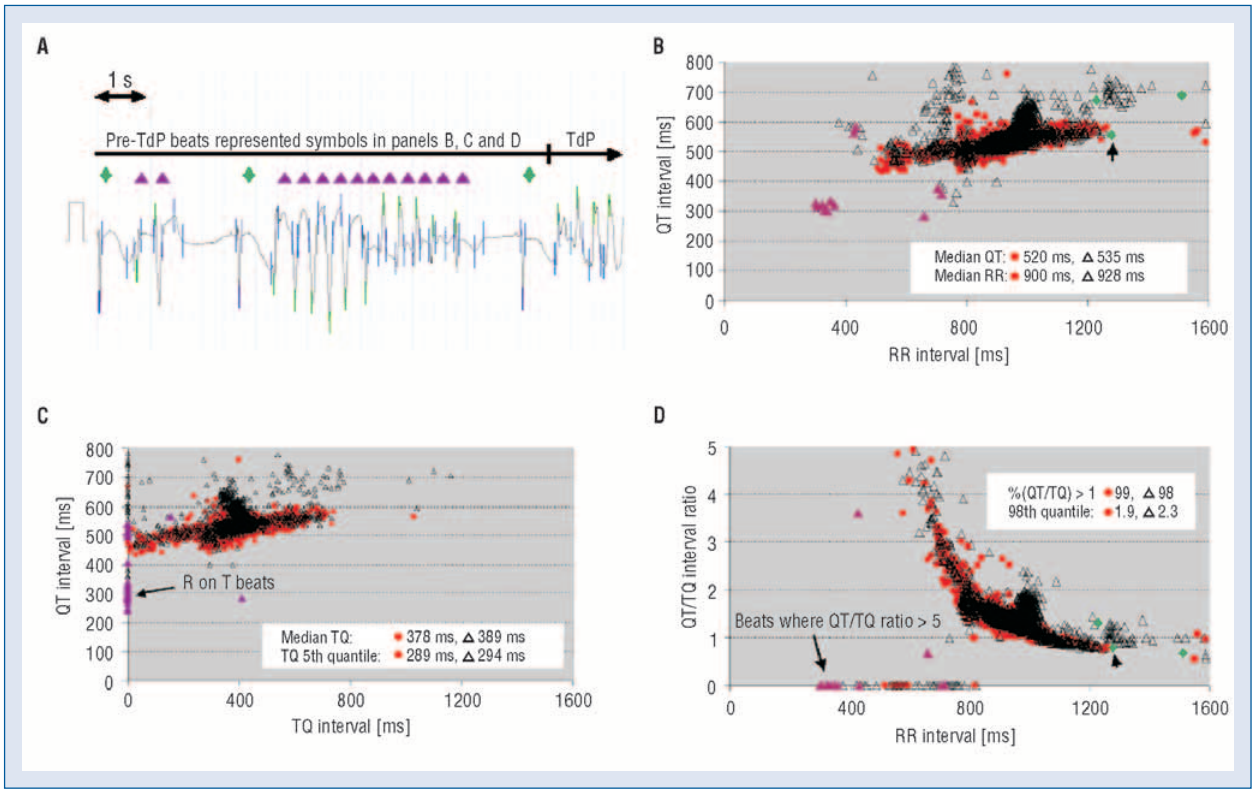

Figure 7. Restitution prior to torsades de pointes. ECG prior to torsades de points (TdP) event from a 66 year-old female subject with coronary artery disease given sotalol (2 mg/kg iv over 20 min) and beat-to-beat QT, RR and TQ interval relationships; **A.** 10 s of ECG beats prior to TdP showing mix of sinus (◆green filled diamonds) and ventricular (▲ filled triangles) beats. Relationship of time-matched response to Cmax (● filled circles; hours 10am–noon), and 105 minute period prior to TdP event (△black hollow triangles; hours noon–1:45pm) showing the **B**: QT *vs* RR intervals; **C.** QT *vs* TQ intervals and **D.** QT/TQ interval ratio *vs* RR interval; Additionally, the last 10 s of beats from ECG (**A**) are shown prior to TdP with the arrow pointing to the last sinus beat. From: Fossa et al. ANE, 2007; 12: 338–348.

in QT prolongation for each heart beat, thus stabilizing restitution. This may explain how drugs with significant prolongation can still be used safely in some therapeutic populations. However, when ECG restitution was examined in a patient with heart disease given sotalol, heart rate did not decrease. Presumably, this was because of a lack of normal beta-receptor blockade that resulted in a dramatic increase in the QT/TQ ratio to the point where several R on T beats were observed before the onset of TdP (Fig. 7). In this particular case, 98% of all beats had a QT/TQ ratio greater than 1 even at rest hours before the onset of arrhythmia.

Discussion

QTc has long been recognized to be a poor surrogate for assessing arrhythmia liability of drugs and clinical prognoses. Because of the lack of a better surrogate, regulatory emphasis focused on the precision of the QTc measurement. Since these studies can be very large due to the number of subjects needed for the statistical design, cost savings over manual measures were sought. This accelerated the improvements in automated algorithms for QT measurement [29]. Many of these new automated algorithms have focused on morphological changes in the multi-lead ECG signal, most importantly to quantify changes related to spatial heterogeneities of the heart [30, 31]. With the use of precise automated ECG interval measures from larger volumes of data, greater utilization of this software can now be applied to new continuous ECG methodologies that allow beat-to-beat analyses and perhaps further improvements in arrhythmia liability assessment.

The QTbtb methodology was originally developed to detect subtle cardiovascular effects in drug discovery using trained conscious dogs with low resting heart rates. In that model it has undergone extensive validation with drugs that cause arrhyth-

mia in man [32, 33]. Since dogs have a more profound sinus arrhythmia than humans, few correction factors work well across a large heart rate range, particularly if RR interval measures are averaged at baseline [34]. Hysteresis, or lag in adaptation of the QT interval for a given change in RR interval, plays such an important role in the normal physiological relationship of the QT-RR intervals, particularly during sinus arrhythmia [35, 36]. In humans, this same hysteresis is an important factor for assessing the QT interval during changes in heart rate [37] or physiologically induced changes in hemodynamics or central control of autonomic tone with or without disease state conditions. Hysteresis of the QT interval during heart rate accelerations has been shown to increase in patients with long QT syndromes, and thus may be important in assessing arrhythmia liability [15, 38, 39]. However, correcting for hysteresis or utilizing QTc may prove difficult to quantify relative to arrhythmia effects because hysteresis is not constant [35] and may depend on the degree of heart rate acceleration and the absolute heart rate from which it accelerates. To speculate, this may be why TdP is associated with a long pause, possibly leading to larger hysteresis on the subsequent QT interval triggering arrhythmia.

Future needs for beat-to-beat methodology

In order to fully utilize quantified measures of beat-to-beat and ECG restitution analyses, the range of normality in healthy physiological conditions needs to be defined. In studies where these methods have been used so far, control or placebo treatment groups have served the purpose of assessing comparative changes. However, if this method is to be used in patients with pre-existing conditions, studies related to known clinical outcomes are necessary to understand the importance of absolute values. The QT-RR interval functionality has been reported to be quite reproducible within the same individual but highly variable between subjects [40]. This is consistent with findings from the unpublished data where reference regions of normality for continuous 24-hour QT-RR data from six healthy volunteers were shown to have approximately 3% discordance from day to day in the same individuals (Fig. 8). Larger studies to confirm these results and quantify similar reference regions in specific disease states are now needed.

Lastly, since autonomic changes do not always result in benign outcomes [41–43], normal limits must be defined for robust perturbations of the beat-to-beat dynamics that may be encountered in everyday life but do not result in arrhythmia. This also will help define the limits for changes observed in patients at risk for arrhythmia. Tables 1 and 2, extracted from the above-mentioned study of reference regions defining normality, show the quantified beat-to-beat ECG restitution at rest and during four different autonomic challenges, respectively. For a representative individual (Fig. 9), the QT/TQ relationship across the range of RR intervals is extremely well defined. As heart rate increases, the TQ interval diminishes rapidly resulting in a very robust increase in the QT/TQ ratio beyond 1, with limits that can be quantified with different autonomic challenges. These preliminary data in combination with other results reported [21] indicate that healthy individuals have approximately no more than 20–25% of their cardiac cycles with QT/TQ ratios greater than 1, with an upper 98% bounds of approximately 1.3. It is interesting that even though phenylephrine-induced reflex bradycardia reduced the upper limits of the QT/TQ ratio, the median QT/TQ value was increased by approximately 4% from supine rest, suggesting that hypertension may have measurable effects on the ECG. Excessive stress, even in normal healthy individuals with burst exercise, can increase these limits temporarily by 300–400%. This obviously would be unsustainable. Further studies from patients experiencing arrhythmia could provide information as to what level or extent can trigger an arrhythmogenic outcome.

In summary, the strength of beat-to-beat analysis is that it uses all raw QT-RR interval data, therefore incorporating QT hysteresis, beat-to-beat temporal variability and sinus arrhythmia within the overall upper (or lower if needed) reference bound for all cardiac cycles. This not only avoids the complexities and inaccuracies of QT correction algorithms, but also allows comparisons under more extreme autonomic changes because no data averaging of the ECG signal or of the QT measurements is used. This same data can also be used for ECG restitution analyses to potentially quantify the arrhythmogenic vulnerability due to temporal irregularities.

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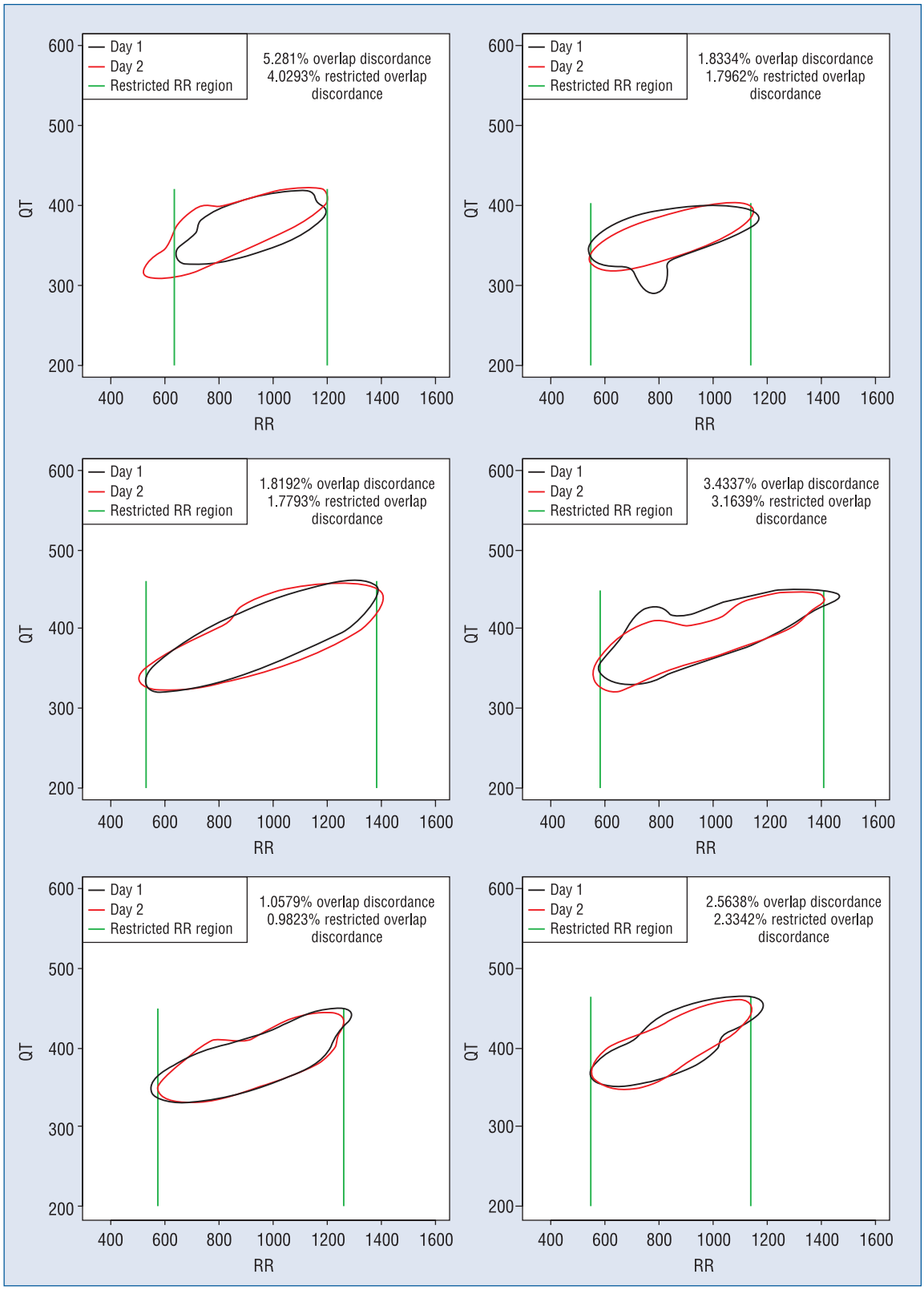

Figure 8. The 95% confidence reference regions (ellipses) for all cardiac cycles in the 20-hour period for six individuals repeated on separate days, at least one week apart. The area of discordance (i.e. number of non-overlapping beats with respect to all beats) averaged 2.66% (range 1.05–5.28) for unrestricted area and 2.34 (range 0.98–4.03) for RR restricted areas (vertical lines). From: Fossa et al. Gordon Research Conference on Cardiac Arrhythmia Mechanisms, Barga, Italy, 2009.

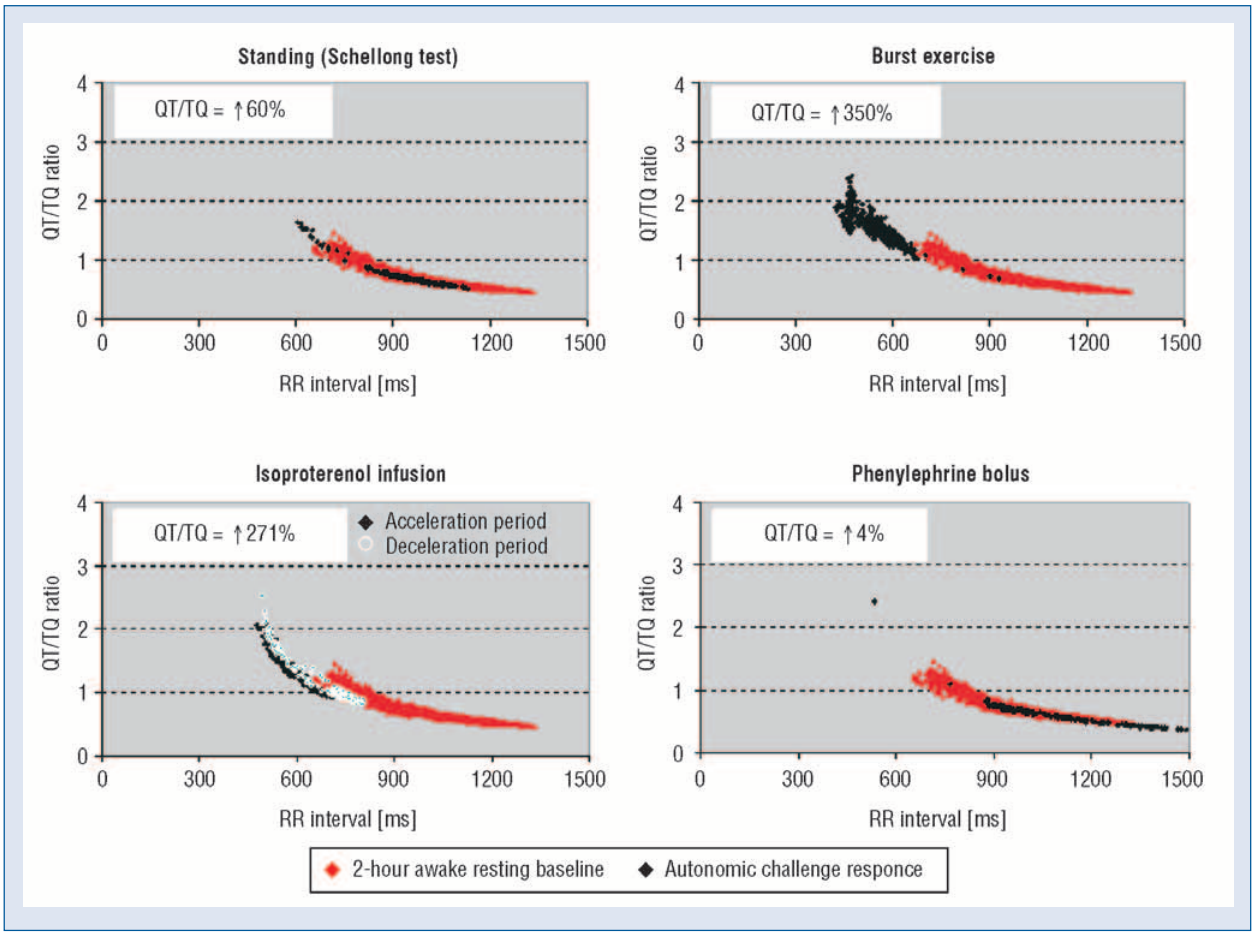

Figure 9. Comparison of autonomic challenge responses in the QT/TQ ratio *vs* RR interval relationship compared to resting baseline from a single subject. Two-hour (7–9am) resting baseline and responses after rapid standing, burst exercise on stationary bike for 1 min at fixed resistance, isoproterenol infusion to heart rate of 110 bpm, and phenylephrine bolus (150 μg) to increase systolic blood pressure by 20–30 mm Hg above baseline. From: Fossa et al. Gordon Research Conference on Cardiac Arrhythmia Mechanisms, Barga, Italy, 2009.

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