

Methodologies to characterize the QT/corrected QT interval in the presence of drug-induced heart rate changes or other autonomic effects

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This White Paper, written collaboratively by members of the Cardiac Safety Research Consortium from academia, industry, and regulatory agencies, discusses different methods to characterize the QT effects for drugs that have a substantial direct or indirect effect on heart rate. Descriptions and applications are provided for individualized QT-R-R correction, Holter bin, dynamic QT beat-to-beat, pharmacokinetic-pharmacodynamic modeling, and QT assessment at constant heart rate. Most of these techniques are optimally performed using continuous electrocardiogram data obtained in clinical studies designed to characterize a drug's effect on the QT interval. An important study design element is the collection of drug-free data over a range of heart rates seen on treatment. The range of heart rates is increased at baseline by using ambulatory electrocardiogram recordings in addition to those collected under semisupine, resting conditions. Discussions in this study summarize areas of emerging consensus and other areas in which consensus remains elusive and provide suggestions for additional research to further increase our knowledge and understanding of this topic. (Am Heart J 2012;163:912-30.)

In the last 15 years, several drugs found to increase the incidence of torsade de pointes and sudden cardiac death have been associated with prolongation of the QT interval.^{1,2}

To ensure public safety and provide consistent methodology toward decision making for new therapeutics, the International Conference of Harmonisation (ICH) implemented guidelines for clinical studies evaluating the QT interval known as ICH E14 (www.ich.org). The guidance provides recommendations on how to evaluate a drug's effect on cardiac repolarization as measured by QT interval on the surface electrocardiogram (ECG) that might potentially be the basis of drug-induced torsade de pointes; its main purpose is to prospectively exclude an

unacceptable degree of QT prolongation of an investigational drug, not to assess it definitively.

The E14 guidance document prescribes a highly controlled, rigorous clinical evaluation of the QT interval for new therapeutics, referred to as the “thorough QT/QTc” study. In these studies, QT interval data are typically obtained in replicate at multiple time points from subjects at rest (eg, supine for 10 minutes) using 12-lead ECGs from standard ECG machines or extracted from continuous 12-lead (Holter) recordings. Typically, the QT interval data are corrected for heart rate using fixed correction methods (eg, Fridericia) or using baseline-generated QT correction methods. The baseline methods can be derived for each individual in the study or from pooled study-specific QT-R-R interval data then fitted using linear or nonlinear regression models. It has been recommended to assess the ability of the QT correction method to remove the heart rate effect; one approach is to apply a linear mixed-effects model using on-treatment data.³ For drugs without a substantial effect on the heart rate, these correction methods work reasonably well and produce similar corrected QT interval (QTc) results. However, with more studies being conducted over a wide range of therapeutic classes, it has become apparent that QT interval correction by these methods using a narrow range of heart rates does not allow adequate evaluation when substantial changes in heart rate or autonomic state occur. In these cases, this may result in either an uninterrupted

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Submitted February 20, 2012; accepted February 20, 2012.

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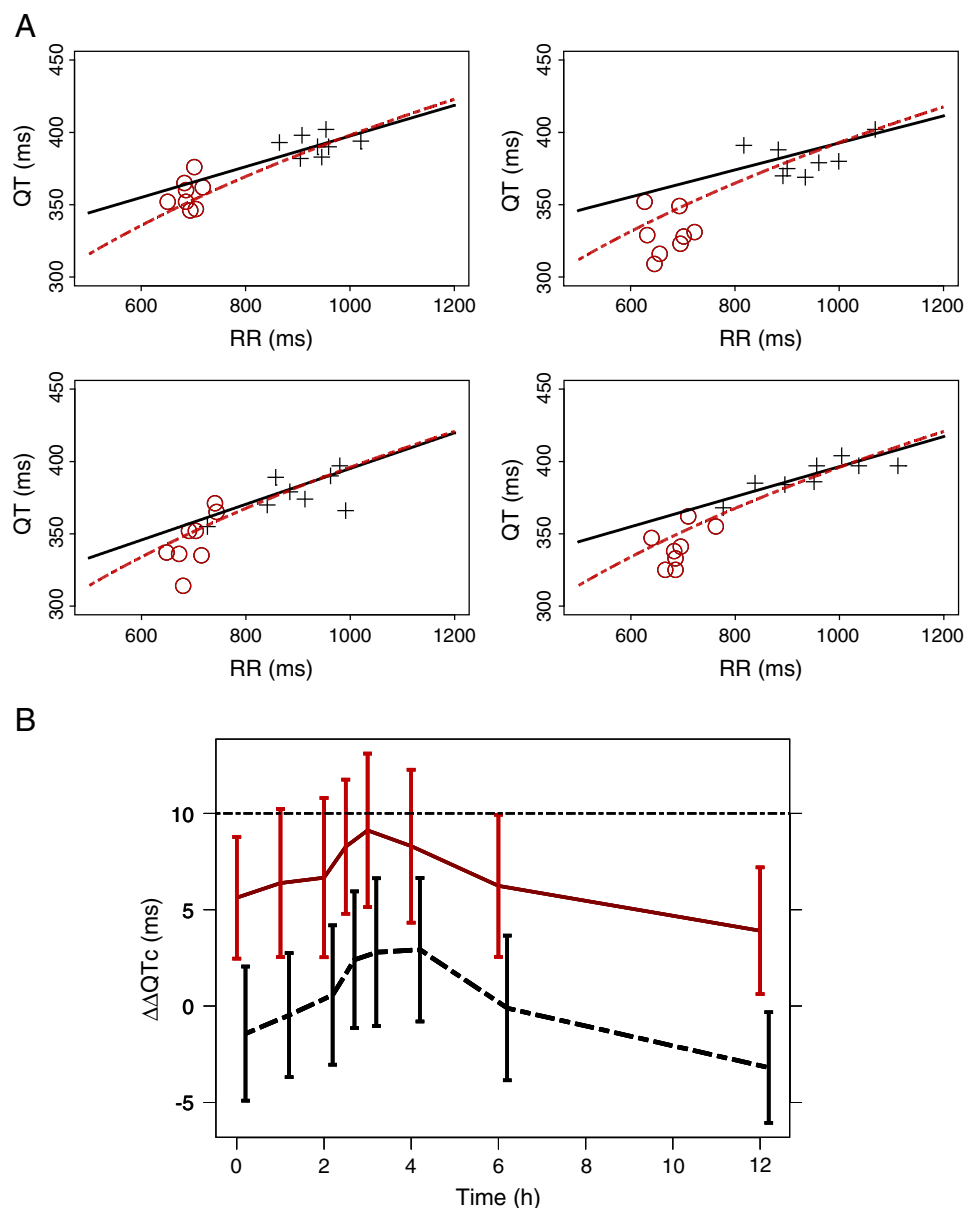
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0002-8703/\$ - see front matter

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doi:10.1016/j.ahj.2012.02.023

Figure 1



A, QT-R-R relationship for 4 representative subjects at baseline (+) and on treatment (o). $QTcI_{fixed}$ was computed using linear regression of baseline, resting QT, and R-R interval data (black solid line). Fridericia relationship (red dotted line) is also shown. **B**, Mean and 90% CIs for the difference in baseline-adjusted QTc between treatment and placebo: $QTcI_{fixed}$ in black and $QTcF$ in red.

study that would need to be repeated or, of more concern, a study in which the wrong conclusion is reached, as illustrated in the following example.

A thorough QT study was conducted for a drug that increased heart rate by a mean of 20 beats/min in healthy volunteers. Resting baseline QT-R-R data were used to compute a fixed individual-specific correction factor computed from linear regression on each individual's

data ($QTcI_{fixed}$), which is different from the $QTcI$ described later in this article.

As illustrated in Figure 1A, the QT-R-R relationship was not linear outside the range of resting heart rates. Relative to the drug-free data, $QTcI_{fixed}$ had a tendency to under-correct the QT interval during treatment. Compared with the fixed linear individual-specific correction, in this case, the Fridericia correction better described the QT-R-R

relationship off- and on-treatment, and individual QT-R-R relationships were not well described over the full range of heart rates. Therefore, the different correction methods gave conflicting results of drug effect (Figure 1B). QTcI_{fixed} inaccurately excluded a significant effect because the uppers of 2-sided 90% CIs for the difference in QTc between placebo and treatment at all times were below 10 ms, the regulatory threshold of concern. In contrast, the upper 90% CIs for QTcF Fridericia-corrected QT interval exceeded 10 ms at 5 time points, which corresponded to high drug concentrations.

Analyzing TQT studies can be problematic for therapeutics affecting heart rate or autonomic tone. Corrected QT interval usually focuses on correction for changes in heart rate; however, there are many other physiologic effects on the QT interval, such as autonomic tone, electrolyte changes, and metabolic state. Therefore, QT correction must be carefully considered in the design and analysis of a study when these and other factors may impact the QT-R-R interval relationship.

The Cardiovascular Safety Research Consortium is a public-private partnership developed to foster collaborations among academia, industry, and regulatory agencies with focus on cardiac safety issues of drugs in development (www.cardiac-safety.org). This article summarizes the current consensus regarding reasonable approaches to evaluate the QT or QTc interval for therapies that have effects on heart rate or autonomic tone. Although the essential concepts of the different methods are fairly similar, at present, we do not know which methodology or approach is the optimal one with respect to QT correction for drugs with a substantial effect on the heart rate or autonomic tone. Differences will likely depend on practicality, quality, and quantity of data available. Therefore, we describe methods that we believe can improve this assessment and would like to encourage further research in this area. The Cardiovascular Safety Research Consortium views expressed in this article are suggestions and do not represent new regulatory policy.

Physiology background

Physiologic aspects need to be addressed in the separation of primary and secondary QT interval changes in the presence of heart rate changes. The following 3 aspects seem important: (i) subject-specific QT interval relationship to heart rate, (ii) individuality of the speed of QT interval adaptation to heart rate, and (iii) heart rate-independent effects of autonomic changes on cardiac repolarization.

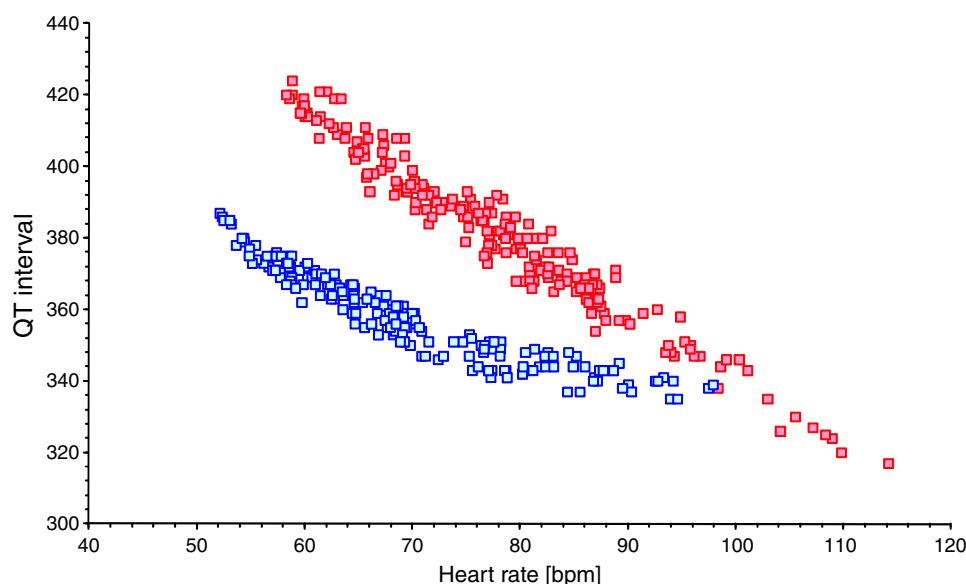
Of the physiologic factors influencing QT interval, heart rate has been studied most extensively. As heart rate increases and decreases, QT interval shortens and prolongs, respectively. Despite numerous suggestions proposing fixed correction methods to assess the QT and heart rate relationship,⁴⁻¹⁶ none was truly successful in

the presence of substantial heart rate changes. This is because the pattern of the relationship of QT interval to the underlying heart rate (ie, the slope, intercept, and the curvature of the dependency) differs largely between individuals (Figure 2) while being relatively stable within each subject,^{17,18} unless influenced by physiopathologic changes. Some of the proposed fixed descriptions of QT-heart rate relationship (eg, Fridericia formula) are closer to the center of the population distribution of the relationships than others and can be reasonably used if the heart rate changes are not substantial. Once the underlying heart rate changes are large, fixed correction methods commonly lead to both false-positive and false-negative conclusions.

The level of heart rate changes that precludes successful application of fixed correction methods is a matter of debate. Based on personal experiences of the authors, for heart rate changes not exceeding 5 beats/min, the difference in mean estimates of QTc changes provided by individual corrections is not usually very different from the better of the fixed correction methods. Once the underlying heart rate changes are substantial (eg, >5 beats/min), fixed correction methods cannot be used with confidence. For instance, β -blockers appear to increase QTc analyzed by the Fridericia formula, whereas with exact QT-R-R regression calculations, no QTc effect of β -blockade was found.¹⁹

The QT interval duration does not adapt to heart rate changes instantly. A lag time exists between heart rate changes and the stabilization of the QT and heart rate relationship, a phenomenon that is frequently called *QT-R-R hysteresis* (Figure 3).^{20,21} The speed with which QT interval adapts to heart rate changes has not been studied extensively, but it has been shown that it is also individual specific.²² On average, it takes approximately 2 minutes for the QT interval to adapt to a heart rate change,²⁰⁻²² although this time lag might be substantially prolonged in patients with cardiac disease²³ and may be altered by autonomic perturbations.²⁴ The hysteresis has implications for heart rate measurements. The underlying relationship of heart rate to QT interval depends on the heart rate history of >2 minutes. This information is not available when short (eg, 10-second) tracings are used only. In such cases, it is frequently believed that keeping subjects supine eliminates any heart rate fluctuations, which may not be true.²⁵ If only short tracings are recorded, increased variability in the data is expected (Figure 4). The QT-R-R hysteresis also causes the QT interval to be fairly stable during respiratory arrhythmia, a normal physiologic response in healthy subjects where the heart rate fluctuates with breathing cycle. Thus, in the presence of respiratory arrhythmia in healthy subjects, deriving a function relating the QT interval measurement to the preceding R-R interval duration only is physiologically unfounded and might lead to incorrect conclusions.²⁶

Figure 2



Example of 2 QT–heart rate profiles found in a healthy man aged 35 years (blue marks) and a healthy woman aged 22 years (red marks). Note that the patterns differ both in the slope and in the curvature. Any mathematical description of one of the patterns cannot be reasonably used for the other pattern.

In addition to heart rate, there are other covariates also influencing QT interval duration. As discussed elsewhere in this article, although autonomic changes lead to heart rate changes, there are also heart rate-independent influences on QT duration. Autonomic-mediated changes can be induced directly,^{27,28} indirectly through centrally mediated neural reflexes²⁹ (ie, baroreflex, Valsalva, etc), or chronically due to disease impact on the autonomic reactivity (eg, diabetes,³⁰ heart failure,³¹ and depression³²). Increases in vagal tone on the heart generally increase the QT interval. This is most evident with the normal changes in the QT–RR interval relationship while sleeping³³ or eating.³⁴ Whether short-term vagal dominance has the same effect is a matter of debate. Several studies have shown that during steady-state atrial pacing conditions, atropine decreases the QT interval.^{35–37} On the other hand, sympathetic influences on the QT interval are much more complex. Increases in sympathetic tone during exercise generally shorten the QT interval, but this is also influenced by the type of β - or α -adrenergic stimulation,^{38,39} the rate of heart rate acceleration,²⁰ and the health status of the individual.⁴⁰ Magnano et al⁴¹ showed that isoproterenol in healthy volunteers produced longer QT intervals than exercise or atropine at a given heart rate. However, during steady-state ventricular pacing, isoproterenol reduced the QT interval.²⁴

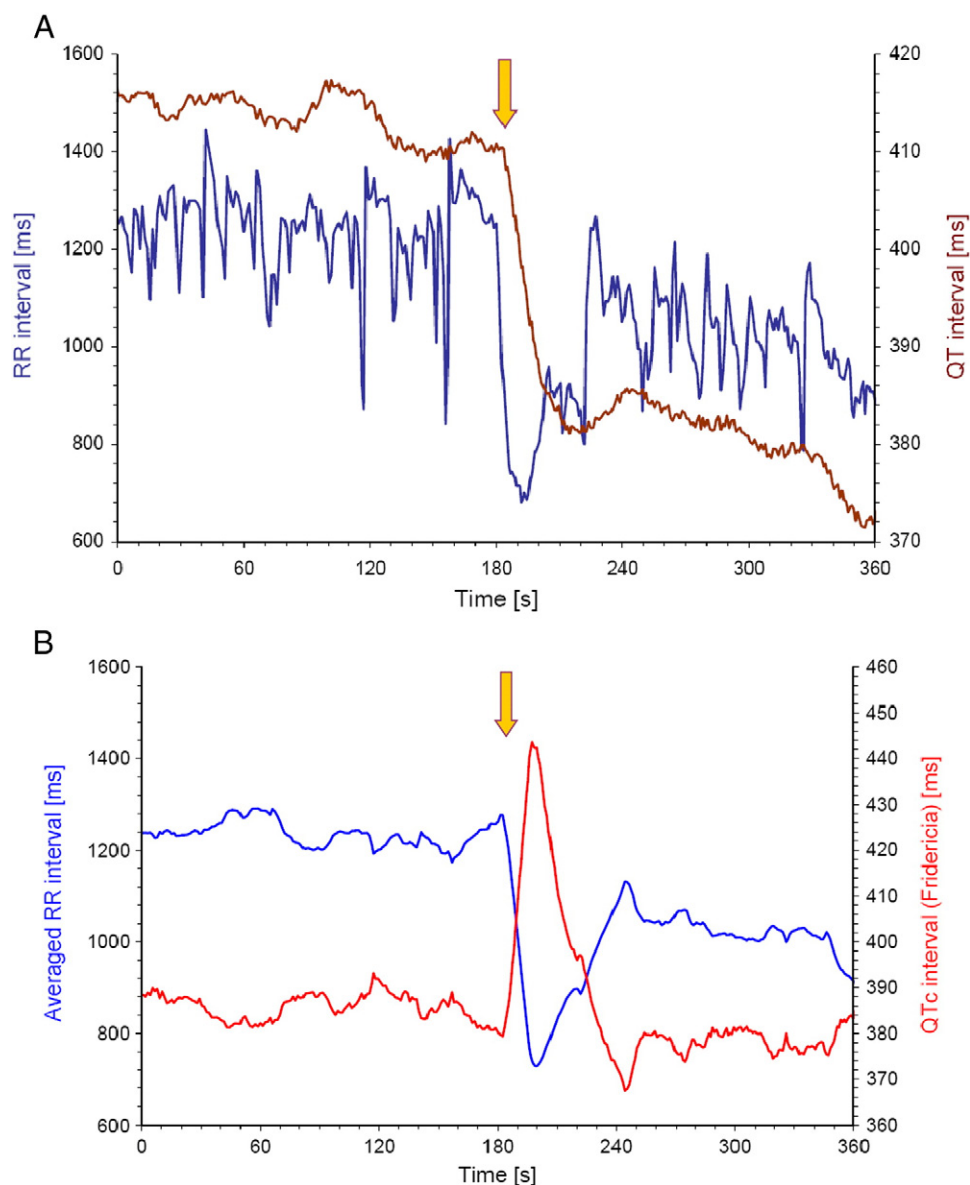
Having reviewed these 3 physiologic aspects discussed, the individuality of the relationship of QT interval to heart

rate is clearly the most important to consider when dealing with drugs that change heart rate profoundly. Consequently, this physiologic aspect is the core of all the methods described in the following section.

Methods

Different methods to assess QT effects with drugs that also affect the heart rate are described in this section. Superficially, they might be seen as very different from each other. However, they all share a common principle: To characterize possible drug-induced QT changes in the presence of heart rate alteration, baseline drug-free QT data are collected in each study subject over a broad range of heart rates so that the drug-free QT–RR profile can be described with sufficient precision. For example, the current practice occasionally requires QT interval comparisons from completely nonoverlapping data when on-treatment heart rates increase to around 80 to 90 beats/min from baseline drug-free, resting, and placebo heart rates of only around 50 to 60 beats/min. Under such circumstances, improved methodologies are necessary to decide whether the treatment leads to QTc interval prolongation or shortening. (Figure 5) Irrespective of the technology used, such a decision requires drug-free data that allow measuring or estimating QT interval duration at heart rates seen on treatment. This common principle is much more important than the methodologic differences among the different techniques described further.

The methods differ in assumptions made about the influence of varying physiologic facets of the core problem of comparing on-treatment and off-treatment (or placebo) ECG measurements. Some of the assumptions made by the

Figure 3

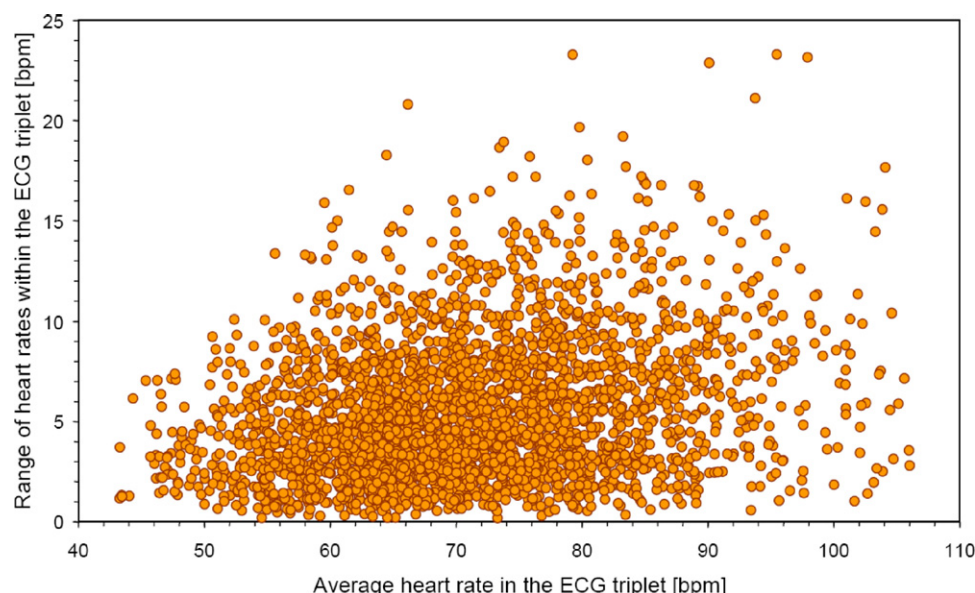
Example of the effects of QT-R-R hysteresis. **A**, Beat-to-beat measurement of R-R (dark blue line, right axis) and QT intervals (brown line, left axis) in a 22-year-old healthy man. At a time of 180 seconds (arrow), the subject changed position abruptly from supine to unsupported sitting. The heart rate accelerated almost instantly approximately from 50 to 85 beats/min for the next 20 to 30 seconds. During this time, the QT interval remained initially under the influence of the preceding slow heart rate and, only later, more gradually shortened to the new level of a heart rate of approximately 60 beats/min. **B**, Sequence of 20 averaged preceding R-R intervals at each cycle (light blue line, right axis). When using the Fridericia formula to calculate the QTc interval from the measured QT intervals and the 20-cycle R-R averages (red line, left axis), a brief QTc "prolongation" in excess of 60 ms was seen. This prolongation is entirely artificial because the calculated R-R interval did not reflect the true duration of QT-R-R hysteresis. Such inaccuracies occur, unless the QT-R-R hysteresis is eliminated by studying episodes of stable heart rate or by using heart rates corrected by calculating the true hysteresis profiles.

different methods have already been sufficiently validated, whereas others are still a matter of debate. Clearly, the fewer and more validated the assumptions, the less likely a method is to become inappropriate and misleading. The core

assumptions made by the different methods are summarized in Table 1.

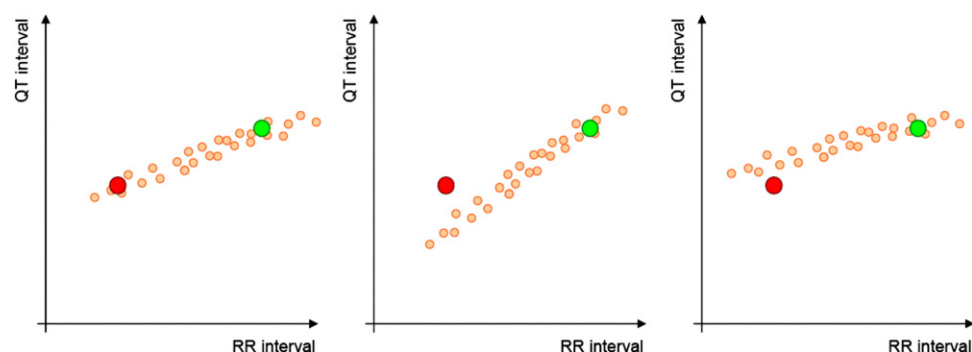
One feature that most methods, traditional and the ones discussed here, have in common is that they generate QTc

Figure 4



An example of heart rate variability in an ECG study involving approximately 8,000 individual 10-second ECG tracings. Per protocol, each subject was repeatedly placed in a supine position for 10 minutes, after which 3 individual 10-second ECGs were obtained within 2 minutes. The panel shows averages of heart rates in these ECG triplets with ranges of heart rates within the triplets. Placing the subjects into a supine position did not eliminate heart rate fluctuations because, although physical reasons for heart rate changes may have been eliminated, other reasons (eg, psychologic) were not.

Figure 5



Schematic representation of the influence of individual QT-R-R patterns. In all 3 panels, the red and green marks represent QT and R-R interval readings on active treatment and on placebo, respectively. The positions of these readings are the same in all 3 panels. The small orange marks represent drug-free readings at different heart rates that define the drug-free QT-R-R relationship in different individuals. Although the active drug-placebo readings are both on the curve of the individual relationship in the left panel (and, therefore, mean no drug-related change in heart rate QTc), the same readings mean drug-related QTc prolongation in the middle panel and drug-related QTc shortening in the right panel.

values, allowing for conclusions about the magnitude of QTc prolongation at specific doses or drug exposures. The absolute magnitude of QTc prolongation, as obtained in TQT studies, does not directly relate to the proarrhythmic risk of the drug, but it is generally accepted that a QTc prolongation exceeding, for example, 20 ms is more concerning than smaller changes. To

view the QTc prolongation this way is, however, an oversimplification of the proarrhythmic risk and does not take into account that the heart rate itself plays an important role. Most proarrhythmic events related to delayed cardiac repolarization occur at slow heart rates; a QTc prolongation of around 10 ms is unlikely to carry the same risk at a heart rate of 90 beats/min

Table 1. Differences in methodologic assumptions about the influence of different physiologic facets of comparing on- and off-treatment ECG measurements

Assumptions	Methodology				
	Individual QT-R-R correction	Holter bin comparisons	Beat-to-beat comparisons	“One stage” methodology	Fixed heart rate methodology
QT-R-R relationship at baseline	That it can be mathematically modeled	Separated into R-R bins	The upper and low 95% confidence bounds of the QT interval across all R-R range from 24-h data are considered physiologically normal	Can be mathematically modeled	Fixed heart rate methodology
QT-R-R relationship on treatment	No assumption	Equal number of R-R bins will be populated.	Any effect on QT not exceeding the upper or lower 95% confidence bounds is considered normal (ie, no increase in outlier beats)	That it can be mathematically described including quantification of drug influence	Studied at fixed heart rates on- and off-drug
QT-R-R hysteresis	That it can be mathematically modeled or ignored if studying only recordings preceding by stable heart rate	That it can be ignored if studying only recordings preceding by stable heart rate	Contained within normality defined by upper and lower 95% confidence bounds of baseline	That it can be mathematically modeled including quantification of drug influence	Studied at fixed heart rates on- and off-drug
Heart rate overlap between on- and off-treatment recordings	That reasonable extrapolation beyond baseline data is possible	That there is a full overlap between on- and off-treatment recordings	That there is a full overlap between median on-treatment effect and 24-h baseline recordings	That reasonable extrapolation beyond baseline data is possible	Studied at fixed heart rates on- and off-drug
Difference of drug effects at different heart rates	No assumption	No assumptions	No assumption	That it can be mathematically described including quantification of drug influence	Artificially ensuring no differences
Accounting for changes in treatment-related autonomic tone	No changes from baseline	No changes from baseline	Changes compared with normal autonomic boundary of 24-h baseline	Can be mathematically described	That it can be ignored and/or restricted to preset heart rates
Importance of time-matched comparisons	Can be performed	That it can be ignored	Can be performed	Can be performed	Only to the extent that it affects the heart rate
Adaptability to PK-PD modeling	Yes	Yes	Yes	Built-in	Yes
Mathematical apparatus	That a regression formula can be found expressing QT-R-R relationship at baseline	None required	That reference regions for upper 97.5% (and lower if needed) QT interval can be defined and calculation of % outlier beats	That formulae can be found expressing (a) QT-R-R relationship at baseline, (b) QT-R-R relationship on treatment, (c) changes between QT-R-R relationships at different drug levels. That the dependency (eg, linear) of the changes on different drug levels is known	None required

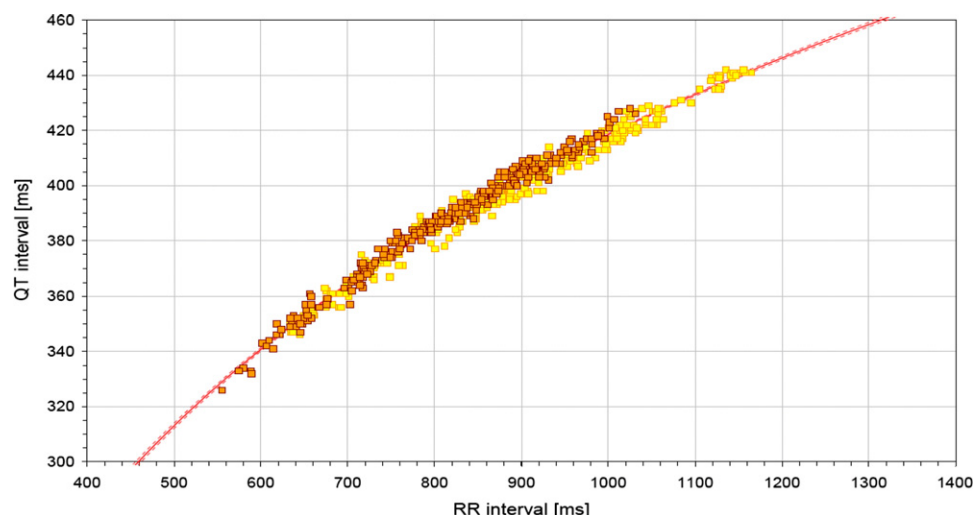
PK-PD, Pharmacokinetic pharmacodynamic.

compared with 50 beats/min. On the other hand, a drug that causes an increase in heart rate of 20 beats/min might cause other cardiac adverse events. These considerations illustrate the importance of characterizing further how ECG effects identified in a TQT study might translate into cardiovascular events. This topic is, however, outside the scope of this study.

Individualized heart rate correction using baseline QT and R-R data

When a drug changes heart rate substantially, the individual-specific QT-R-R relationship must be sufficiently defined (Figure 6), using multiple ECG readings covering a broad heart rate range.^{42,43}

Figure 6



Example of a drug-free data used to define individual QT–R–R relationship. The data have been obtained from 2 different drug-free days (distinguished by different colors). At each drug-free day, full day-time 12-lead Holter recording was obtained and scanned for all different periods preceded by stable heart rates. Among these, selections were made during each day, ranging from the slowest to the fastest heart rate. In each selection, the QT interval corresponding to the underlying heart rate (expressed as the R–R interval value) was measured. The data were subsequently used to model the QT–R–R pattern mathematically. The curve-linear regression line (solid red line) is shown together with its 95% confidence interval (dashed pink lines).

The stability of the heart rate needs to be verified, and individual R–R intervals should be obtained for a sufficient time before the QT measurement.²⁵ When the heart rate preceding the QT measurement is not stable, QT–R–R hysteresis needs to be taken into account. Available data show that because of QT–R–R hysteresis, QT interval duration is influenced by the preceding R–R interval history of no less than 2 minutes and that the effects of the hysteresis manifest when the preceding heart rate fluctuates by as little as ± 2 beats/min.^{21–23} An equivalent stable heart rate can be estimated based on the drug-free estimates of QT–R–R hysteresis using the R–R interval history profile.^{44,45} This method then provides the hysteresis-corrected heart rate value for which the QT interval duration can be corrected.

Once a sufficient number of drug-free ECG readings are obtained at different heart rates, the individual QT–R–R profile can be described mathematically to be subsequently used for the QTc calculation purposes.^{17,46} Between individuals, not only are the QT–R–R intercept and slope variable, but so too is the curvature of the patterns.⁴⁶ Thus, one proposal is to consider differently curved regression models and to select the optimum model for each subject based on the least-square residuals.^{42,43} However, there is no consensus regarding the best approach, and criteria for the most appropriate methodology need further discussion. Likewise, the research is presently ongoing on how to test whether the QT–R–R regression has been properly fitted to the available baseline data and whether the baseline data are sufficient for accurate QT–R–R modeling.

The curve of the individual QT–R–R profile is derived from the baseline data and does not necessarily require measure-

ments at exactly the same heart rates as are encountered on the investigational drug. Some extrapolations beyond the baseline readings at slower and faster heart rates are methodologically permissible, providing that the defined pattern is sufficiently broad (in the heart rate terms), but these extrapolations should be kept as small as possible. The goal of the individualized heart rate correction formulae is to straighten the distribution of the uncorrected QT interval readings for an individual subject so that, in the QTc–R–R plot, the baseline pattern becomes a horizontal line, whereas all vertical distances are preserved (Figure 7). If a correction formula can be expressed in the form of $QT_c = QT + g(RR)$ (eg, most formulae of linear and nonlinear regression models), this property holds. Because the correction formula is derived from baseline data, no assumptions on the QT–R–R pattern on treatment are needed, and the method is applicable to treatment data irrespective of whether the QT–R–R pattern is changed by treatment or not. This is because the principle of individualized heart rate correction depends on eliminating heart rate effects in the baseline data, but no such elimination is required for the on-treatment data. Specifically, studying whether QTc data on treatment are or are not related to heart rate is inappropriate in the design of individual corrections.

The main characteristics of the baseline-derived individualized QT–R–R correction methods are as follows:

- Corresponds closely to the present physiologic understanding of intrasubject stability of QT–R–R relationships
- Allows combination with individual QT–R–R hysteresis correction

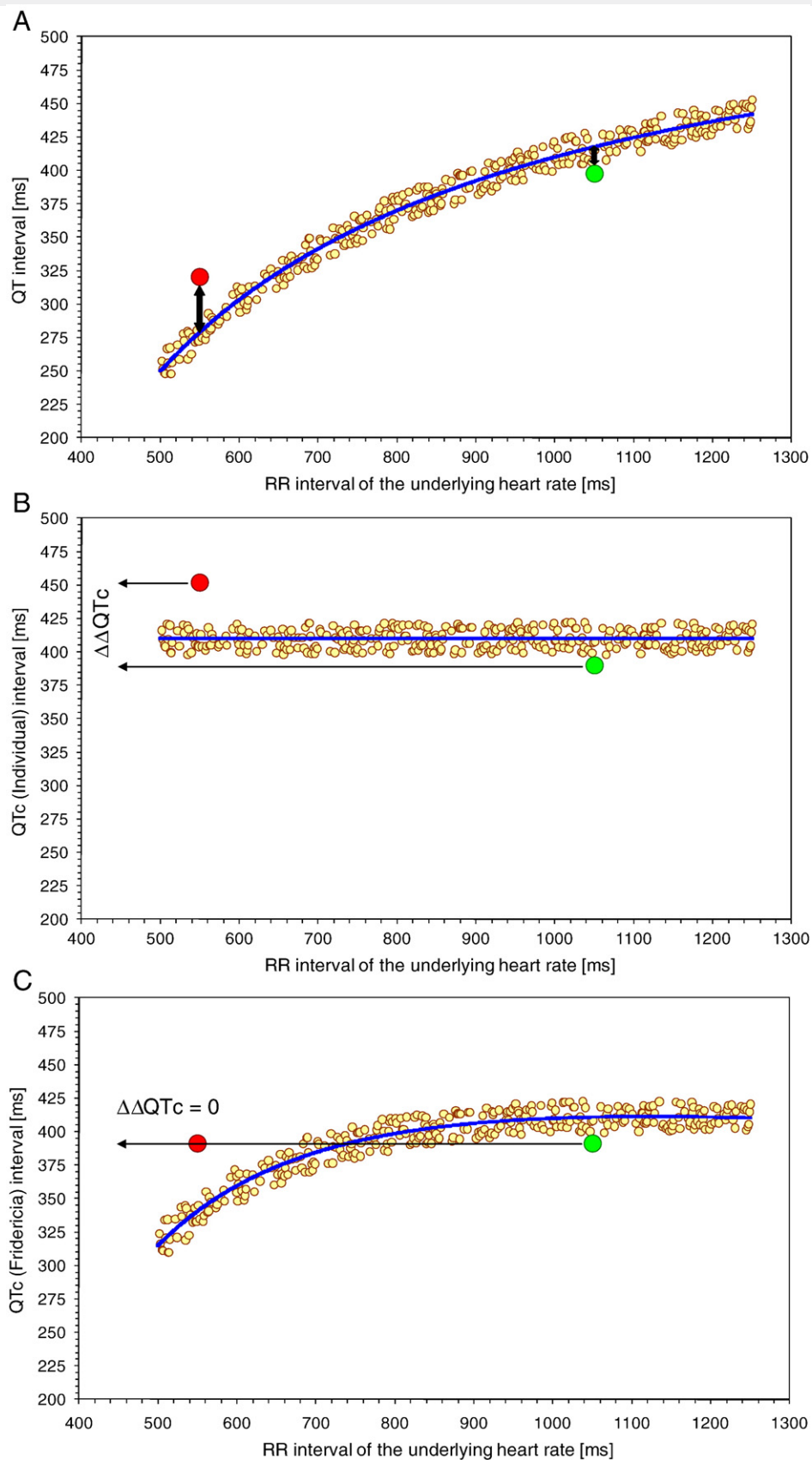
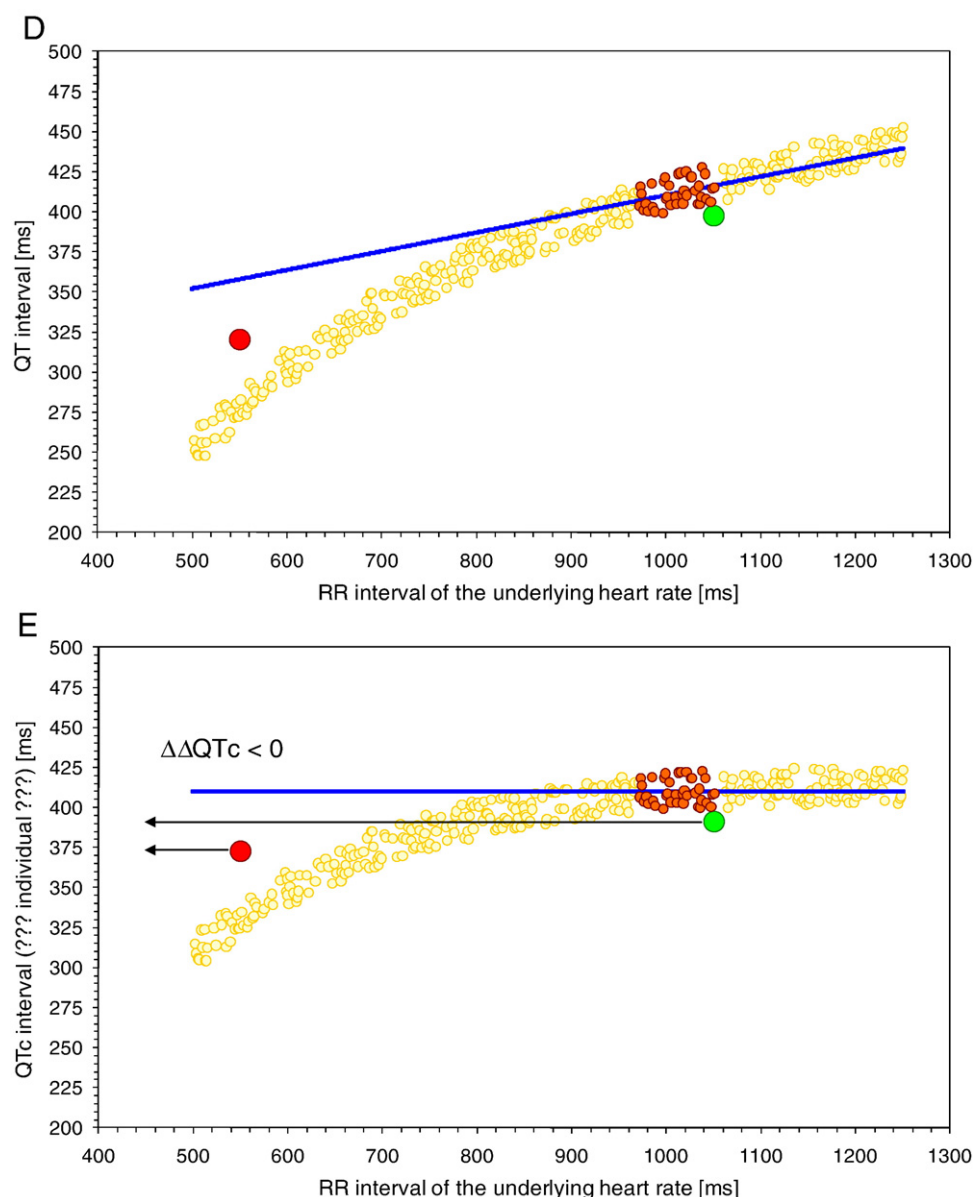
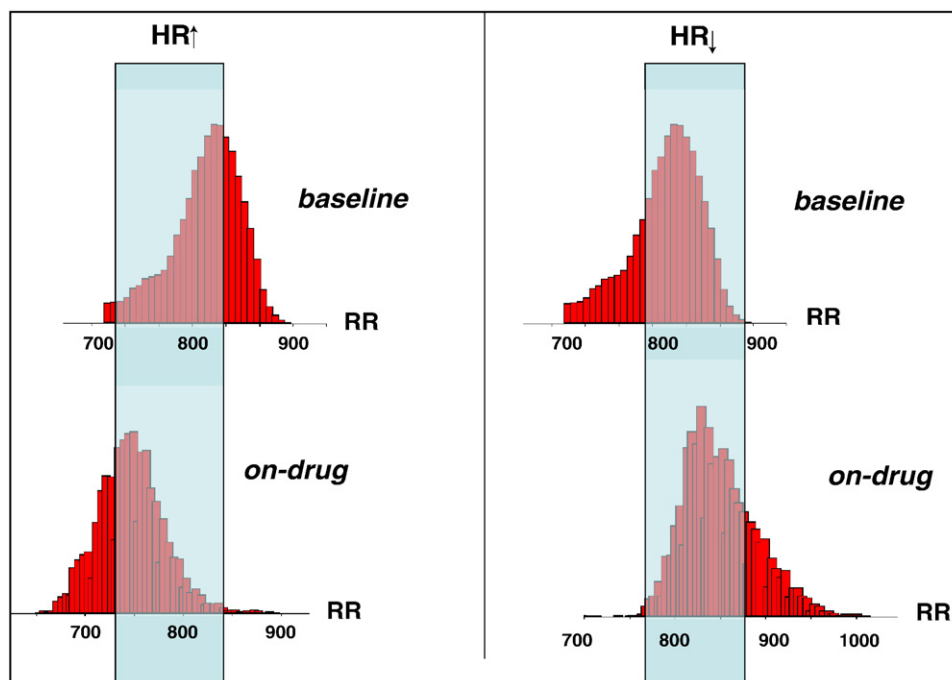
Figure 7

Figure 7 (continued)



A, Schematic distribution of baseline QT–R–R readings (brown-yellow circles) with their mathematical curvilinear regression (blue line). The panel also shows schematic QT readings on drug (large red circle) and placebo (large green circle). The true QT/QTc drug effect is the vector difference of the vertical distances between the on-drug and placebo readings and the baseline pattern (ie, between the on-drug or placebo QT reading and the baseline QT value at the very same heart rate). In this example, the QT reading on drug is 40 ms above, and the QT reading on placebo is 20 ms below the baseline pattern (the black arrows). When the baseline pattern is converted to a heart rate correction formula that makes the baseline pattern straight while preserving vertical distances (**B**), the difference between the QTc values on drug and on placebo is exactly 60 ms (as it should be because of the vertical QT differences from the baseline pattern shown in panel **A**). When a correction formula does not make the baseline pattern straight (eg, Fridericia correction applied to the data of this example [**C**]; the blue line is the baseline regression shown in panel **A** processed by Fridericia formula), incorrect conclusions about the QTc effect of the drug are possible. In particular, when a restricted range of baseline data are used to describe baseline QT–R–R distributions (panel **D**—linear regression was derived from a narrow range of baseline values marked in dark brown), and this baseline QT–R–R distribution is converted into a heart rate correction formula, nonsensical results may be obtained (panel **E**—the heart rate correction derived from the linear regression shown in panel **D** incorrectly identified on-drug QTc shortening; the blue line in panel **E** is the “narrow” linear regression of panel **D** processed by the correction formula derived from this narrow regression).

Figure 8

Example of R-R distributions with drugs that increase (left-hand side) and decrease (right-hand side) heart rate. The shaded area represents the data used for comparison.

- Allows reasonable extrapolation beyond the range of drug-free QT-R-R data
- Reduces QTc variability and, thus, improves power of thorough QT studies

Holter bin analysis

Holter bin is a method designed to compare the uncorrected on- and off-drug QT interval at the same heart rates. The basic concept behind this method is that inside a predefined window of interest, individual cardiac beats characterized by the same heart rate are pooled and placed into separate bins.⁴⁷

In its original implementation, the individual cardiac beats pooled inside each bin are signal averaged to form a single representative waveform.⁴⁸ Because of signal averaging, the quality of the representative waveforms is generally very good, and the number of waveforms to be reviewed is highly reduced (1 set of measurements per R-R bin), leading to the possibility of using highly automated analyses. On the other hand, signal-averaged binning is challenged by the poor resolution of some commercial Holter systems and requires adequate signal processing to avoid distorted measurements. As an alternative, instead of averaging the individual beats inside the bins, one can also average the individual measurements.

Hysteresis can be controlled by considering the stable preceding heart rate as the “binning” criterion by using an adjusted R-R interval that takes into account the history of each cardiac beat using any model for heart rate stability from the literature. Like other methods, Holter bin is sensitive to hysteresis, and using only the immediately preceding R-R interval vs the stable heart rate would lead to different results.⁴⁸

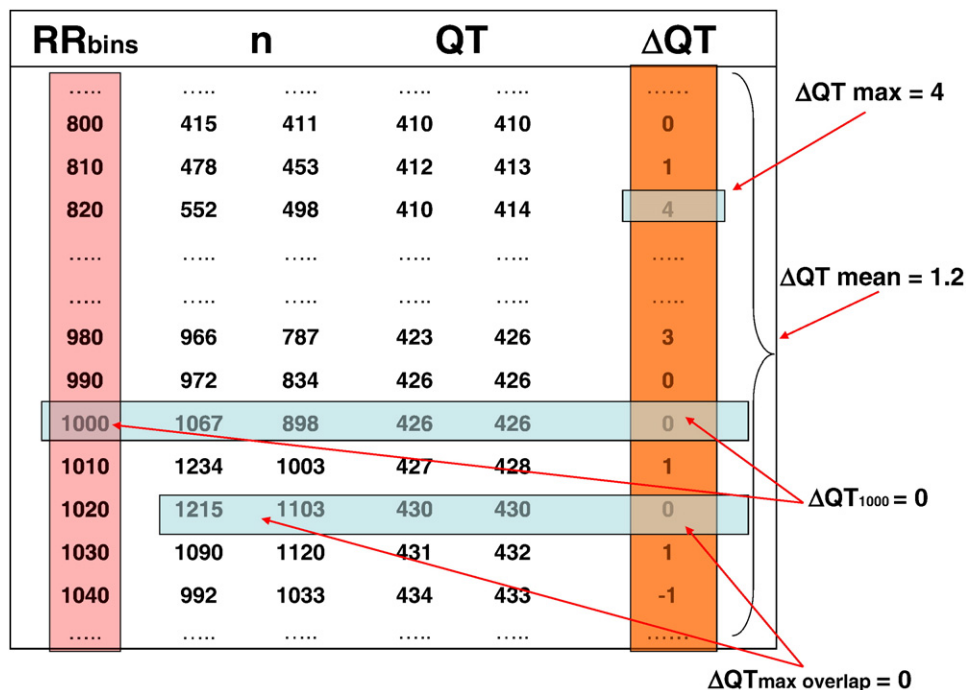
Holter bin is particularly suited to study metrics for comparing conditions or maneuvers that affect heart rate.⁴⁹ Rather than enforcing 1 or more QT-R-R models, the approach assesses on-drug vs off-drug changes by comparing the uncorrected QT intervals belonging to the same R-R bin (ie, ensuring a comparison at identical heart rate). Figure 8 is a schematic example with 2 drug experiments that modify heart rate.

In the first example (left-hand side), the experiment has increased heart rate (the on-drug R-R histogram is shifted to the left), whereas in the second example (right-hand side), the experiment has lowered heart rate (the on-drug R-R histogram is shifted to the right). In both cases, the data used for comparison (shaded areas) are limited to the common R-R regions.

It is important to ensure similar experimental conditions during the full length of the exploring time window at baseline and on-drug. For example, day and night data as well as periods with different autonomic tone (eg, resting vs exercise) should not be mixed within the same analysis. Similarly, on-drug periods should be centered on peak plasma concentration time. There is no ideal duration for the on-drug exploring window, although it should be long enough to capture a sufficient number of beats and to reach an acceptable level of signal-to-noise ratio. Typically, a duration of 2 to 4 hours for the on-drug exploring window is used,⁵⁰ but shorter durations can also be considered to assess compounds with faster concentration peaks.

Results of the Holter bin analysis are provided in a tabular form, with each row representing one specific R-R bin comparison (eg, active treatment vs off-drug). Figure 9 is an example of a bin-by-bin table for one subject where, in addition to the QT intervals,

Figure 9



Numerical representation of Holter bin output. For each R-R interval (bin), the QT intervals associated with 2 compared groups (eg, on-drug vs baseline) are considered. The length of this table (number of rows) depends on the degree of R-R overlap between the explored conditions. A number of outcome variables are typically generated (see text for description).

the number of individual beats pooled in each bin (second and third columns) and the difference in the QT interval between the 2 treatment groups (ΔQT) are also given.

Another important aspect of the method is to explore for a significant rate-dependence effect on ΔQT (as typically observed, eg, with IKr blockers). In the absence of any rate dependency (as typically observed, eg, with moxifloxacin), the bin-by-bin ΔQT values could be averaged to produce an overall (across bins) mean ΔQT . Other parameters that can be extrapolated by the table are the min/max ΔQT , or the ΔQT at some specific bins (ΔQT at R-R = 1,000).

Some variants of Holter bin have been recently proposed. In one example, a set of pooled cardiac beats, instead of being averaged to generate representative waveforms, were all measured individually, and summary statistics of the measurements are presented.⁵¹ Another approach using signal-averaged waveforms from telemetry data has also been proposed.⁵²

A scientific comparison between these variants on the same set of data has never been conducted; thus, any related claim on one or the other method would be speculative. Nonetheless, the original algorithm that implements Holter bin⁴⁸ has been used extensively in several pharmaceutical studies (inclusive of compounds that modify heart rate) and has systematically confirmed both on-drug effects and assay sensitivity findings from traditional methods.⁵⁰

Holter bin is not strictly compliant with ICH E14 guidelines because the effect of the drug is captured and pooled within a time window of 2 to 4 hours and is not assessed at individual

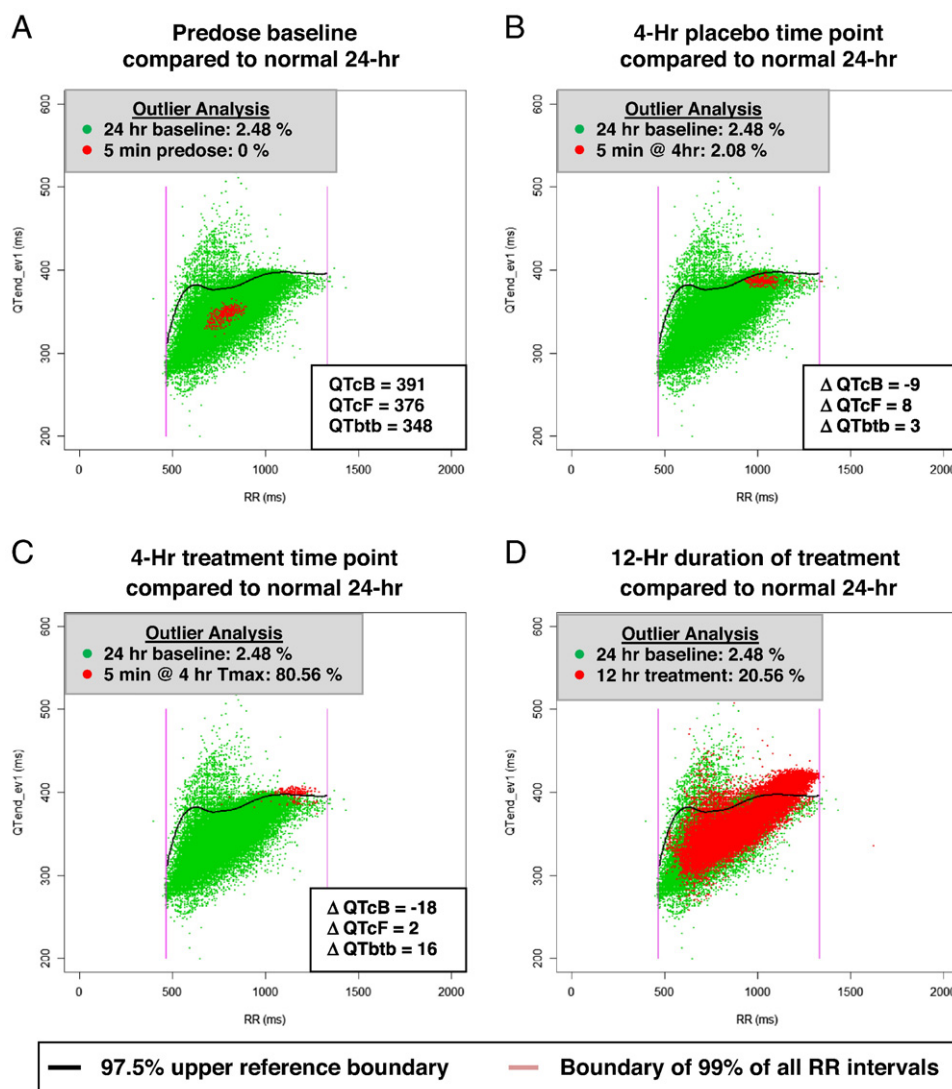
postdosing time points. However, some modifications of the method aimed to limit the Holter bin session to the baseline recording are being explored.

The main characteristics of the Holter bin analysis are as follows:

- There is no need to implement correction models.
- QT hysteresis can be controlled, either capturing only the beats preceded by stable heart rate or considering the hysteresis-free R-R interval of each beat before bin inclusion. It is very efficient in assessing QT effect associated with moderate heart rate changes.
- When heart rate changes are too large (typically >10 beats/min), the R-R overlap between on- and off-drug data can be highly reduced, and the method may become difficult to apply.
- It assumes no autonomic-mediated changes from baseline QT-R-R functionality.

Dynamic QT beat-to-beat analysis

The dynamic QT beat-to-beat (QTbtb) analysis is a method reported to differentiate changes of QT interval duration due to heart rate or autonomic state from impaired repolarization.⁵³ The method does not use any rate correction factors but relies on the uncorrected QT interval during the influence of a maneuver or a drug and compares this to what is defined as normal for the same heart rate in the same study subject. The range of “normality” is defined based on QT-R-R data

Figure 10

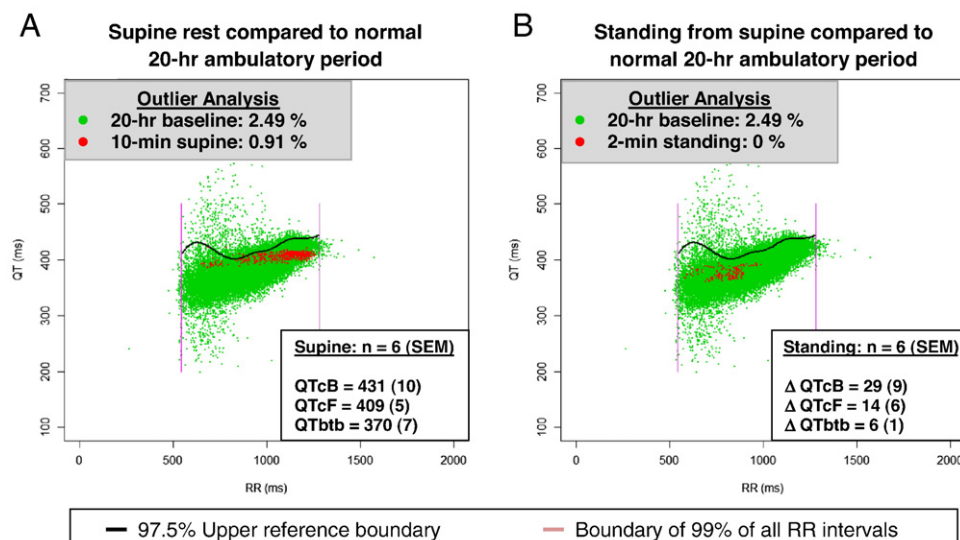
Comparison of QTbtb vs QTcB and QTcF values for the same beats during baseline, time-matched placebo, and treatment periods in comparison with a 24-hour normal boundary from a single-subject QT-R-R interval relationship. See details provided in the text (adapted from Fossa and Zhou⁵⁴).

derived from all baseline beats from a continuous ECG recording of up to 24 hours (referred to as “clouds” when displayed as a scatterplot; Figure 10A). The upper (or lower if needed) reference bound(s) of the QT-R-R relationship can be defined and designate the limit (solid black line in Figure 10A) for the overall continuous ECG measurements that include all sources of physiologic variability, such as hysteresis, sinus arrhythmia, autonomic tone during sleep, and everyday activities such as eating and moving around. Because up to 24 hours are analyzed at baseline, QT and R-R intervals in approximately 70,000 to 100,000 beats are measured. Continuous ECG data, with encompassing baseline autonomic states, have not been used traditionally for assessment and, thus, not often considered in the design of a study. Clearly,

this amount of data can only be handled effectively only if carefully quality-controlled, computer-based highly automated methods are used.

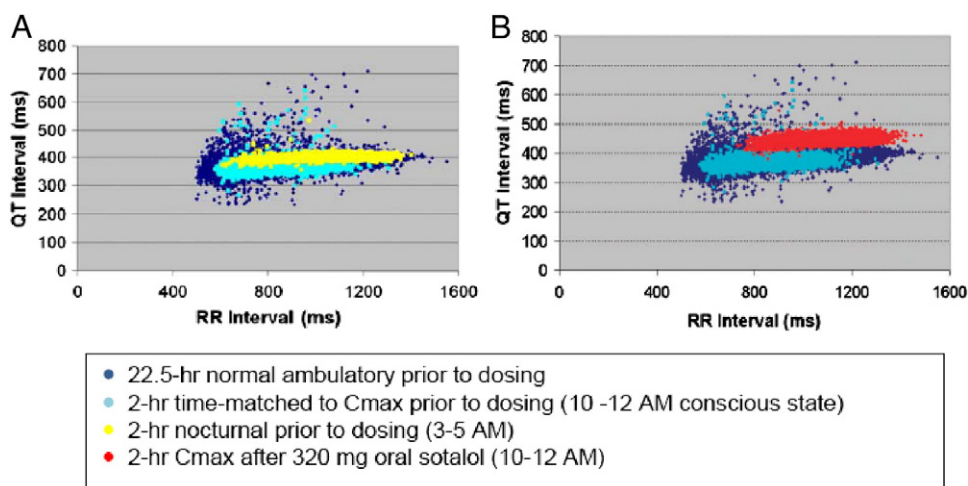
Drug effect can be assessed against the background of this normal QT-R-R data set. For a specific time point (eg, 4 hours after dosing of drug or placebo), all beats within a limited time window (normally 5 minutes) are analyzed. Figure 10B shows the 5-minute cloud (in red) 4 hours after dosing of placebo depicted on top of the 24-hour background QT-R-R data set cloud (in green). The center of this 5-minute cloud of data or centroid is calculated as the median QT at the median R-R interval. This QTbtb value for any nominal time point is compared with the centroid of all beats extracted within the same R-R interval range (± 12 ms) from the 24-hour baseline data

Figure 11



Effect of standing from supine position on QTcB, QTcF, and QTbtb compared with normal 20-hour ambulatory QT–R–R interval relationship. **A** and **B**, Effect on beat-to-beat relationship from a single subject with results for the entire study (n = 6 healthy male volunteers; adapted from Fossa and Zhou⁵⁴).

Figure 12



Differences in conscious, nocturnal, and impaired repolarization states on the QT–R–R interval relationship. **A**, Baseline 22.5-hour normal-ambulatory QT–R–R interval from a single individual with the 2-hour vehicle period corresponding to Cmax maximum concentration before dosing and a 2-hour period while asleep. **B**, Same individual's response after receiving 320 mg of sotalolol compared with baseline predose periods. See discussion provided in text; adapted from Fossa et al⁵⁶).

set to provide a Δ -QTbtb value (ie, 3-ms Δ -QTbtb for the 4-hour placebo period in Figure 10B). The same procedure is used to define the Δ -QTbtb value for the on-drug treatment nominal time points (ie, 16-ms Δ -QTbtb for the 4-hour treatment period in Figure 10C). The placebo-adjusted time-matched values (Δ - Δ QTbtb) are simply calculated by subtracting the time-matched

placebo values from the same time-matched values on-treatment from the same subject (ie, the 4-hour Δ - Δ QTbtb equals 13 ms in Figure 10C, B).

An important part of this method is to determine whether repolarization is significantly impaired beyond normal autonomic boundaries by applying statistical techniques to define the

upper 97.5% reference boundary of QT-R-R interval relationship from the normal 24-hour data (from baseline/placebo day of the study).^{53,54} Figure 10B-D illustrates how the beats during the nominal period on-drug compare with those at baseline. An outlier analyses 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 equal to or less than approximately 2.5% of beats exceeding the upper boundary (Figure 10A).⁵⁵

This same type of analysis can also be conducted for any period that the drug is used, including the entire time at therapeutic concentrations, to ascertain the net effect of drug vs normal QT-R-R relationship (Figure 10D). When a significant effect on outliers is observed, another analysis to compare the heterogeneity of the outlier beats can be conducted. This method uses a bootstrap analysis of only the beats that exceed the upper reference bounds to determine the magnitude and 95% confidence bounds of the on-drug beats compared with the beats exceeding the upper boundary under normal baseline conditions.

Figure 11 shows the effect of autonomic reflex tachycardia on the QT-R-R interval relationship induced by standing from a supine position. When resting quietly in the supine position (Figure 11A), the QT and R-R intervals are increased and the relationship is flat, with reduced QT variability and increased R-R variability (similar to sleep as shown by the yellow cloud in Figure 12A). When the subject is asked to stand up quickly from this position, vagal influences lessen and sympathetic influences increase, so the QT shortens and becomes more variable, whereas the R-R interval shortens during heart rate acceleration. This behavior creates a short-term hysteresis from the baseline correction fit and results in a significant QTc prolongation of greater than 10 ms (Figure 11B). The QTb_{tb} is less affected by the hysteresis and is only slightly prolonged. These effects of alteration of autonomic state on QT-R-R or QTb_{tb} are within normality for the individual and, therefore, very different from effects during prolonged repolarization where the QT interval is increased above the normal 24-hour boundary of R-R intervals (Figure 12B).⁵⁶

The main characteristics of the dynamic beat-to-beat method are as follows:

- It uses no correction and compares QT data at similar heart rates between nominal time points postdosing and a full 24-hour baseline.
- It requires no adjustments for hysteresis, sinus arrhythmia, and autonomic tone because all beats under normal physiologic conditions are contained within baseline reference bounds.
- Because there are no averaging of data and the 24-hour baseline encompasses a wide range of heart rates, comparison of QT intervals at the same heart rate on- and off-drug is possible.
- Because the method uses a full 24-hour baseline period during which all QT and R-R intervals are measured, computer-assisted technology is required to manage these data.

One-stage approach to analyze QT, R-R, and drug data simultaneously

In evaluating QT/QTc interval prolongation for a drug that changes heart rate, one potential question is whether the drug impacts the QTc-related parameter or the curvature-related parameter for the off-drug QT-R-R relationship. For a simple Bazett or Fridericia type of heart rate correction model for the

off-drug QT-R-R relationship, $QT = \alpha \times R-R^\beta$; this is equivalent to asking whether the drug changes the proportional constant ($QTc = \alpha$) or the exponent (the correction factor β) of the relationship, or whether the drug changes the intercept ($\log_{10}(\alpha)$) or slope (the correction factor β) in the log scale: $\log(QT) = \log(\alpha) + \beta \times R-R$. This has been described during treadmill exercise testing where the mean QT-R-R correction factor increased from 0.27 at rest to 0.40 during exercise, with a mean heart rate of 120 beats/min.⁵⁷ Whether and to what extent drugs that change heart rate also can change the QT-R-R curvature (ie, correction factor) have not been extensively studied. Another potential scenario is a compound that changes not only heart rate but also the QT-R-R relationship through changes in autonomic tone or other mechanisms. In this case, the previously proposed 1-stage approach for simultaneously analyzing QT, R-R, and the drug effect on QT and R-R⁵⁸⁻⁶⁰ could potentially be extended to address the QTc prolongation in the clinical setting of a drug-induced change in heart rate. This method analyzes the QT and R-R data with an appropriate statistical model using both the off- and on-drug data simultaneously, instead of deriving a correction factor from the off-drug QT and R-R data first and analyzing the corrected on-drug QT interval data based on the derived correction factor later. For the 1-stage approach, a common correction factor for all data or separate correction factors for the on-drug and off-drug treatment arms, respectively, can potentially be estimated by mixed-effects modeling and justified by appropriate model diagnostic plots and statistical criteria. For example, if including different correction factors for the off- and on-drug treatment QT-R-R relationships improves the goodness of fit to the observed QT interval data, the evaluation of QT interval prolongation should be based on the estimated impact of the on-drug treatment on the QTc-related parameter (the α for the power relationship: $QT = \alpha \times R-R^\beta$), with such different correction factors between off- and on-treatment being considered.

The 1-stage mixed-effects approach is in alignment with the pharmacokinetic-pharmacodynamic (PK-PD) modeling approach in that the heart rate can be treated as a covariate in the simultaneous analysis of QT, R-R, and drug concentration data.^{61,62} This approach was applied to sibenadet in a preliminary analysis. In a clinical ECG study, baseline-derived individualized QT-R-R corrections obtained from exercise or rest did not adequately correct QT for heart rate during sibenadet treatment.⁵⁷ Standard E14-statistical and PK-PD analyses based on either a fixed- or baseline-derived individual QT-R-R correction suggested that sibenadet prolonged the QTc interval. In contrast, an analysis using 1-stage mixed-effects PK-PD model allowing for a different correction factor for on- and off-treatment data showed that sibenadet does not prolong QT interval,⁶³ which is in agreement with the known mechanism of sibenadet and the nonclinical cardiovascular safety tests.⁶⁴ This method deserves further exploration in other data sets.

The 1-stage PK-PD modeling approach, with heart rate being treated as a covariate, quantifies the drug concentration, heart rate, and QT interval relationship simultaneously. This approach can be used to address QT interval prolongation in combination with a PK model for unstudied doses for the cases in which the drug product changes heart rate. The PK-PD modeling has been used to model QTc interval prolongation and drug concentration.⁶⁵

The main characteristics of the 1-stage method of simultaneously analyzing the QT, R-R, and dose/concentration data are as follows:

- It allows for estimation of different model parameters for QT-R-R relationship between on- and off-drug treatments in the presence of drug-induced change in heart rate.
- It allows for incorporation of the individuality of QT-R-R relationship into the data analysis
- PK-PD model can be used to prospectively evaluate QT interval prolongation at doses not directly studied.
- There is limited experience in terms of drugs that have an effect on the heart rate and also change the QT-R-R relationship.
- There is limited experience in using this approach to differentiate drug effects on QT interval or heart rate.

QT assessment during constant heart rate

Examination of a drug's effect on the QT interval during constant (or relatively stable) heart rate can be attained by 2 methods: pacing, by which constant heart rates exceeding the underlying sinus rhythm rate can be attained ("overdrive pacing"), and maneuvers, which produce a reproducible effect on the spontaneous (sinus rhythm) heart rate. Both approaches can generate heart rates that "override" the chronotropic effect of the drug, thereby enabling QT assessment at the same heart rate before and after drug administration.

Pacing studies. Assessment of electrocardiographic effect at different pacing rates has long been the standard practice in invasive electrophysiology (EP) testing. During these procedures, effects on cardiac repolarization can be studied on the surface ECG QT interval during sinus rhythm, during atrial or (rarely) ventricular pacing, and through the evaluation of effects on ventricular refractoriness or on the monophasic action potentials. Invasive EP procedures have been widely used in the testing of antiarrhythmic drugs, often in patients with a clinical indication for the procedure,^{66,67} as an add-on during cardiac catheterization for other reasons^{68,69} or occasionally in healthy volunteers.⁷⁰ To a more limited extent, atrial pacing during an EP procedure in patients has also been used to study electrocardiographic effects of nonantiarrhythmic drugs⁷¹ or other interventions.^{35,72} These studies confirmed the heart rate as the main determinant of the QT interval duration and also demonstrated a small QT shortening after pharmacologic, autonomic blockade.

More than 20 years ago, Milne and coworkers⁶⁸ introduced atrial pacing outside the EP laboratory as a method for assessing drug effects on the QT interval. A technique simpler than invasively placed electrodes is that of transesophageal atrial stimulation by which stable, atrial pacing can be achieved; the technique has mainly been used to study patients with supraventricular, reentrant tachycardias.^{73,74} The method has never gained wide acceptance, partially because it is uncomfortable or even painful for the patient. To a limited extent, this technique has also been used to study drug effects on the QT interval.^{75,76}

Occasionally, patients with permanent atrial or dual-chamber pacemakers have also been subjects for the study of drug and other effects on cardiac repolarization. The clear advantage of this technique is the avoidance of an invasive

procedure and that atrial pacing using the permanent pacemaker has no significant discomfort. Recently, such a study was completed in 20 patients.⁷⁷ The effect of 400 mg oral moxifloxacin on the surface ECG QTcF interval was assessed during sinus rhythm and atrial pacing at 70 and 100 beats/min, before and after autonomic blockade. The effect of moxifloxacin during sinus rhythm was in the same range as reported for similar studies: QTcF increased by 12 ms (90% CI 8.2-15.8 ms). During atrial pacing, the QT interval increased by 10 ms (6.4-13.0 ms) at 70 beats/min and by 7 ms (-0.2 to 14.6 ms) at 100 beats/min.

Maneuvers that produce an effect on the spontaneous heart rate. Exercise testing can be used reproducibly to generate heart rates exceeding the positive chronotropic effect of a drug. In theory, this method could be used to study a drug's effect at stable heart rates by achieving a steady state with submaximal exercise. Exercise testing has, however, rarely been used this way. Studies from mainly one investigational site used exercise testing to widen the range of heart rates for which a monoexponential formula was applied to calculate a QTc (QT1000).⁷⁸⁻⁸⁴ The methodology was similar across the studies: subjects were studied at supine, quiet rest; in the sitting position; and during exercise testing at baseline and on drug. Using this methodology, moxifloxacin, for example, caused a 15-ms QT1000 prolongation, an effect size similar to that observed in thorough QT studies.^{85,86}

A different approach was used by Frederiks and coworkers,⁸⁷ who studied the impact of autonomic maneuvers on the QT interval during sinus rhythm, assessed at the same heart rate. Thirteen healthy volunteers were investigated in the sitting position; autonomic balance was changed through leg lowering and handgrip, which enabled the study of ECG effects at similar heart rates with different autonomic balance between sympathetic and vagal tone. During handgrip, the heart rate increased from 65 to 72 beats/min, and the effect on the QT interval was compared with leg lowering at this heart rate (72 beats/min). The QT interval was prolonged by handgrip compared with leg lowering (Bazett-corrected QT 435 ± 21 vs 418 ± 15 ms, $P < .01$). If QTcF was calculated using group data, handgrip caused a small QTcF prolongation (from 413 to 422 ms), whereas a small shortening was observed during leg lowering (to 406 ms). However, the effect of hysteresis was not taken into account when using this approach.

The main characteristics of controlling heart rate by pacing or exercise are as follows:

- Because drug effects on the QT interval are assessed at a constant heart rate, the main confounder for QT assessment is removed and a correction algorithm is unnecessary.
- Pacing can only be achieved with overdrive, that is, at heart rates exceeding the spontaneous sinus node rate. Many drugs that delay cardiac repolarization have a more pronounced effect at slow heart rates, and QT prolongation at a relatively higher rate will, therefore, underestimate the effect during sinus rhythm.
- Exercise alters the autonomic balance with a predominance of sympathetic tone, which, in itself, may shorten the QT interval. The effect of exercise on drug-induced QT prolongation requires further study.

- **Feasibility:** the greatest constraint with pacing during an EP procedure or in patients with a pacemaker is access to patients; invasive add-on studies in patients with a clinical indication for a procedure are not without ethical concerns. It seems unrealistic that patients scheduled for an invasive procedure or with a permanent pacemaker would be routinely enrolled in these studies.

Special attention of a TQT study for drugs that affect the heart rate

The methodologies discussed throughout this White Paper include individualized QT-R-R correction, Holter bin, dynamic QTbtb, and PK-PD modeling. Currently, it is unclear which methodology is optimal in regard to QT assessment for drugs with a substantial direct or indirect (eg, via vasodilatation or autonomic actions) effect on heart rate. When evaluating a drug that impacts heart rate in a TQT study, a meticulous approach to data collection is critical. Importantly, there is a need to collect drug-free data that allow measuring or estimating QT interval duration at heart rates seen on treatment. The range of heart rates can be increased at baseline by collecting ambulatory ECG recordings in addition to those collected under semisupine, resting conditions. Because of limited experience using submaximal exercise to increase heart rates, there is no consensus for suggesting this methodology in TQT studies. Most of these techniques require (or are optimally performed using) continuous ECG data, in contrast to ECGs recorded at specific time points. Such data are readily obtained from high-fidelity 12-lead continuous ECG recorders (Holters). Careful technique is required to obtain optimal ECG signals. To perform robust PK-PD modeling, it is necessary to have a sufficient number of PK samples collected during the study interval. Given the rapidly evolving science in this area, publishing the results of TQT studies of drugs that affect the heart rate is strongly encouraged.

Acknowledgements

We would like to thank members of the Cardiovascular Safety Research Consortium that provided literary and scientific commentary to aid in the development of this paper: Charles Benson, MD, PhD; Glenn Carlson, MD; Corina Dana Dota, MD; Christer Gottfridsson, MD, PhD; David E. Gutstein, MD; Karen A. Hicks, MD; Robert B. Kleiman, MD; Peter Kowey, MD; Jay W. Mason, MD; Eric L. Michelson, MD, FACC; Lawrence Satin, MD, FACC; Norman Stockbridge, MD, PhD; David Svensson, PhD; Thomas Todaro, MD, FACC; and Tobjörn Vik, MD.

Disclosures

Note: Dr Garnett was chairing the writing committee; other authors are listed in the order of their contributions to each section.

Disclaimer: The opinions and conclusions expressed in this article are solely the views of the authors and

do not necessarily represent regulations and policies of the Food and Drug Administration or the authors' affiliated organizations.

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