Exploring machine learning techniques in the context of early-stage clinical research

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CHAPTER 2

NUMBER OF ECG REPLICATES I NFLUENCES THE ESTIMATED QT PROLONGING EFFECT OF A DRUG

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ABSTRACT

INTRODUCTION The present analysis addressed the effect of the number of ECG replicates extracted from a continuous ECG on estimated QT interval prolongation for different QT correction formulas.

METHODS For one hundred healthy volunteers, who received a compound prolonging the QT interval, 18 ECG replicates within a 3 minute window were extracted from 12-lead Holter ECGs. Ten QT correction formulas were deployed and the QT_c interval was controlled for baseline and placebo and averaged per dose level.

RESULTS The mean prolongation difference was >4 ms for single and > 2 ms for triplicate ECG measurements compared to the 18 ECG replicate mean value. The difference was <0.5ms after 14 replicates. In contrast, concentration-effect analysis was independent of replicate count and also of QT correction formula.

CONCLUSION The number of ECG replicates impacted the estimated QT interval prolongation for all deployed QT correction formulas. However, concentration-effect analysis was independent of both the replicate number and correction formula.

INTRODUCTION

Drugs can be associated with cardiac arrhythmias and subsequent sudden cardiac death.[1] Careful cardiac assessment of the drug's effect on the ventricular repolarization has therefore become mandatory.[2] The effect on the ventricular repolarization manifests itself as morphological changes in the ST segment of the surface ECG and a prolongation of the QT-interval.[3] The ICH E14 guideline[4] covers the regulator's requirements on the assessment of the compound's QT interval prolonging effect as a proxy for (polymorphic) ventricular arrhythmia, which includes a thorough QT (TQT) study. A TQT study is a study specifically designed to evaluate the QT interval prolonging effect of a novel compound and consists of a placebo-controlled, cross-over study with a positive control.[4] Although many of these have been performed since the introduction of the guideline,[5] the TQT study is still under debate. The scientific value of the TQT remains subject of discussion, as the study exposes additional healthy volunteers or patients to the novel compound, and the costs are high.[5–7]

Several studies have evaluated novel approaches to assess a QT prolonging effect of novel compounds. Dense ECG recording that was implemented into phase I single ascending dose and multiple ascending dose studies showed that is possible in this context to reliably assess QT interval prolonging effects.[8,9] In addition, implementation of a concentration-effect analysis may improve the assessment of the QT prolonging effect even further.[8,10]

However, several elements in current practice to measure a compound's QT prolonging effect are not underpinned by peer-reviewed scientific data. This includes the number of ECG replicates that are recorded, which is arbitrarily set at three or more by the regulators,[4,11] and the QT correction formula that is deployed.[12,13] Therefore, we performed an analysis on ECG recordings obtained in a placebo-controlled phase I single ascending dose trial with a compound that prolonged the QT interval.

Aim of the study

The aim of the present analysis was to demonstrate the feasibility of a novel approach in which several epochs extracted from a continuous

ECG recording were used to assess the compound's effect on the QT interval. The optimal number of ECG epochs (replicates) required to assess this effect was investigated with the FDA recommended approach and the concentration-effect analysis.

METHODS

The present analysis was performed on a placebo-controlled, double-blind, single ascending dose study that was conducted at our center in 2016. The analysis was performed on this study because of the implementation of a Holter ECG in the study and the dose-dependent QT interval prolonging effect of the investigated compound. The study consisted of 10 consecutive cohorts of 10 volunteers of whom, at each dose level, eight received the active compound and two volunteers matching placebo. The dose of the investigated compound increased with each cohort, as is typical for a phase I single ascending dose trial. All subjects consented to their data being registered and the study was performed in accordance to Dutch law on medical-scientific research.

Data acquisition

All subjects were equipped with a 12-lead Holter ECG (Holter H12+ recorder, Mortara instruments BV, Milwaukee, WI, USA), which was mounted just before the dose administration until 24 hours after the dose administration. Standard electrode positioning was used. Subjects were in a supine position and in a calm, relaxed state for at least 5 minutes before any 5 minute window of continuous ECG recording. The ECG recordings from the Holter ECG were extracted during the latter 5 minutes. The protocol was approved by the Dutch health authorities and by the local ethics committee, Foundation Beoordeling Ethiek Biomedisch Onderzoek. Extractions were performed on a single time point which was associated with the largest QT interval prolongation observed using standard 12-lead ECGs made in triplicate. The Holter ECG strips were analyzed by Intermark ECG Research Technology BV (Someren, the Netherlands), who were blinded to treatment, using LabChart v8.1.3 (ADInstruments, Sydney, Australia) with a validated algorithm (ECG analysis module v2.4; ADInstruments, Sydney, Australia)., Per subject, 18 ECG epochs could be extracted and optimized for signal quality from the 5 minute window. The QT and RR interval were measured with the algorithm and manually adjusted when necessary as recommended by the E14 R3 guideline.[11]

QT_c formulas

The corrected QT (QT_c) interval was calculated based on the QT and RR interval, in addition to patient characteristics for selected QT formulas.

ECG extraction within window

ECGs in the present analysis were extracted without a time interval between the ECGs. In order to simulate a clinical situation, ECG recordings for each replicate count were selected in such a way to mimic a time interval in between the recording of these ECGs, as would be the case in a clinical situation. Table 1 displays the scheme that was used for our analysis.

$\Delta Baseline QT_c$ calculation

Per subject the QT_c interval for all evaluated QT correction formulas and number of ECG replicates was calculated. This generated 180 QT_c intervals, with 10 different formulas and a total of 18 ECG replicates per subject. The subject's baseline mean QT_c value was then subtracted from all calculated QT_c interval values, resulting in a QT_c change form baseline (ΔQT_c) for all 10 QT_c formulas and the 18 ECG replicates.

$\Delta placebo\Delta Baseline QT_c$ calculation

The mean ΔQT_c from the subjects in the placebo group was subtracted from the ΔQT_c of the subjects who received the active compound, resulting in 180 placebo-corrected ΔQT_c ($\Delta placebo\Delta Baseline QT_c$, $\Delta\Delta QT_c$) per subject. The calculation for the $\Delta\Delta QT_c$ was performed in accordance with the E14 guideline.[4]

Table 1 *Table displaying the (randomized) selection pattern of ECG windows used for QT analysis. The main goal of the selection method was to mimic a time interval between recordings. Fields in grey are selected ECG replicates for a given experiment. For example, for experiments based on 3 ECG replicates, ECG replicates 1, 8, and 15 were used. And, ECG number 3 is used in the experiments based on 4, 6, 7, 10, 11, 12, 14, 15, 17, or 18 ECGs.*

Nr of replicates ECG	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1			■				■			■								
2					■			■				■						
3				■		■				■				■				
4									■									
5		■			■			■		■			■					
6						■				■				■				
7				■														
8		■		■					■				■					
9					■			■										
10						■			■			■		■				
11								■										
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13				■			■			■			■					
14		■				■						■						
15			■				■		■			■						
16						■			■			■						
17					■						■		■					
18				■		■			■		■							

Δ18 replicatesΔplaceboΔbaselineQT_c calculation

Since the true value of the $\Delta\Delta QT_c$ is unknown, the best estimate of the $\Delta\Delta QT_c$ for each formula was considered to be the mean $\Delta\Delta QT_c$ of 18 ECG replicates. The difference between the mean $\Delta\Delta QT_c$ of each replicate count (1 to 18) and the mean $\Delta\Delta QT_c$ of 18 ECG replicates was calculated, this results in a Δ18 replicatesΔplaceboΔbaselineQT_c ($\Delta\Delta\Delta QT_c$). The results of this analysis were displayed as a heat map (Figure 1).

Δ18 replicates 90% CI ΔbaselineQT$_c$ calculation

The difference between the range of the 90% CI of the ΔQT_c of each replicate count and the range of the 90% CI of the ΔQT_c of 18 ECG replicates was calculated and averaged per cohort and then averaged over all 10 cohorts (Δ18 replicates90%CI ΔbaselineQT_c), as displayed in Figure 2.

Concentration-effect analysis

The concentration of the drug at the time of the ECG recording was derived from the concentration time profile of the compound using the Logarithmic Trapezoidal method[14].

A concentration-effect analysis was performed as previously described by Darpo et al.[8]. In short, subjects were divided into 10 groups based on the drug estimated investigated medicinal product concentration. These were plotted against the mean $\Delta\Delta QT_c$ for all QT_c formulas and number of ECG replicates.

Statistical analysis

Data are depicted as mean ±their standard deviation or percentages where appropriate. Python v3.5.2 (Wilmington, DE, USA) was used for statistical analysis. For concentration-effect analysis, a linear regression was used.

RESULTS

A total of 100 subjects were included initially. One subject, who received active treatment in cohort 2, was omitted because of insufficient data quality and the final analysis was performed on data of 99 subjects. Twenty subjects received placebo and were pooled into the placebo cohort. Ten other cohorts, where the dose was increased in successive cohorts, consisted of eight healthy volunteers each on active treatment. Baseline characteristics are displayed in Table 2.

The mean QT interval and RR interval per cohort at baseline and at the time of the Cmax are displayed in Table 3.

Figure 1 *Average of the mean ΔΔQTc compared to the mean ΔΔQTc of 18 ECG replicates (mean ΔΔΔQTc) of all cohorts for every correction method in absolute values (milliseconds). The mean ΔΔQTc deviates with more than 0.5ms (10% of the safety limit) from the most accurate measurement when it is based on less than 14 ECG replicates and more than 1ms when it is based on less than 5 replicates.*

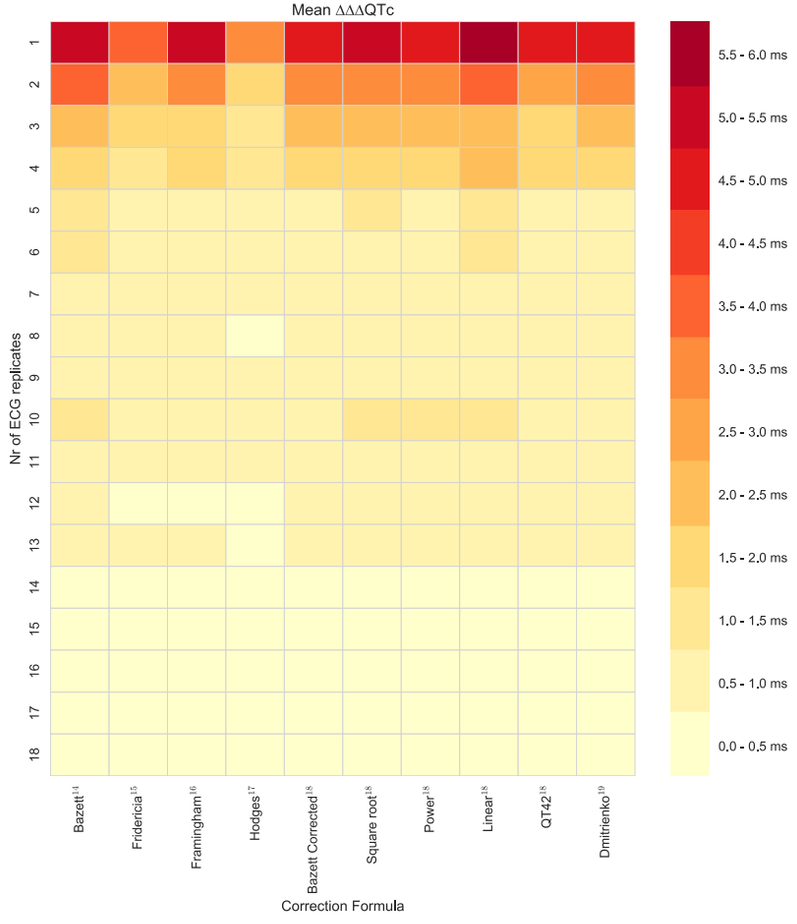


Figure 2 *Average upper limit of the 90% confidence interval of ΔΔQTc compared to the upper limit of the 90% confidence interval of ΔΔQTc of 18 ECG replicates (mean Δ18 replicates 90%CI ΔbaselineQTc) of all cohorts for every correction method in absolute values (milliseconds). For 7 out of 10 correction formulas, the 90% confidence interval of the ΔΔQTc within a cohort increases by more than 0.5 ms (10% of the safety limit) when it is based on less than 11 ECGs per subject compared to a ΔΔQTc based on 18 ECGs per subject.*

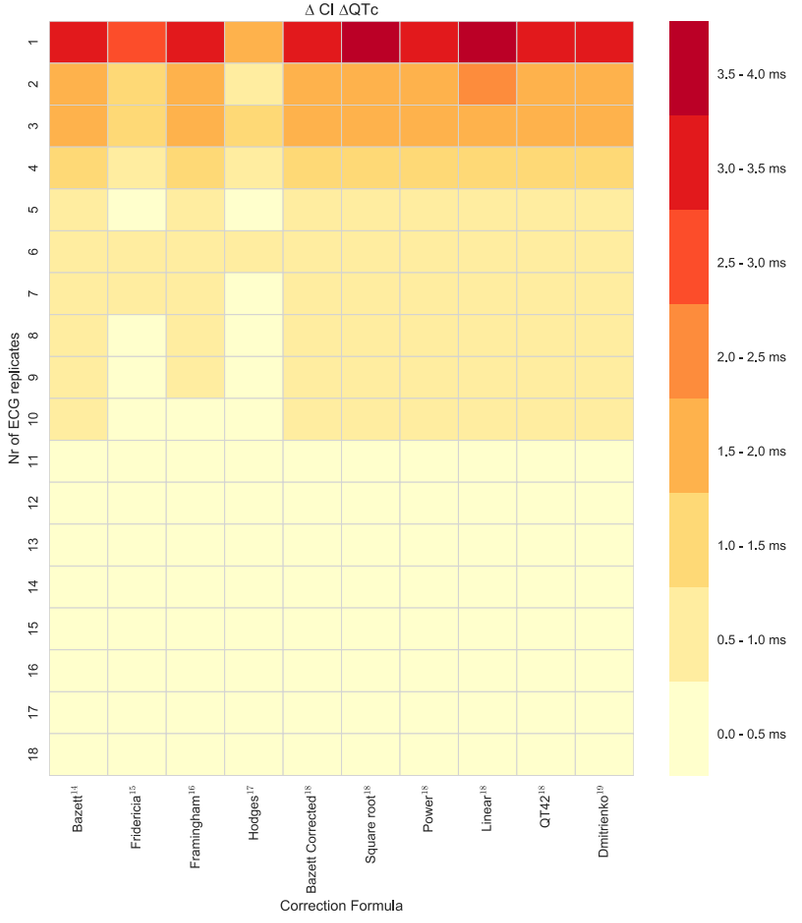


Table 2 *Baseline data. Average values with standard deviation or percentages where appropriate.*

Age (Years)	24.2 ± 4.8
Gender (Male)	100%
Systolic Blood Pressure (mmHg)	121.1 ± 9.2
Diastolic Blood Pressure (mmHg)	72.89 ± 8.05
Heart Rate (min^{-1})	59.9 ± 8.4
BMI (kg/m^2)	23.0 ± 2.9
Temperature (°C)	36.6 ± 0.36
Alcohol Usage (Units / Day)	1.1 ± 1.0
Smoking History (Cigarettes / Day)	0.0 ± 0.0
Cafeine Usage (Units / Day)	1.56 ± 1.16
HbA1c (%)	32.63 ± 2.6
ALAT (U / L)	25.84 ± 12.28
ASAT (U / L)	27.72 ± 7.16
Total Cholesterol (mmol / L)	4.2 ± 0.77
Creatinin (µmol / L)	81.03 ± 8.59
Glucose (mmol / L)	4.67 ± 0.45
PR Interval (ms)	149.13 ± 19.94
QRS Duration (ms)	101.0 ± 8.39
QT interval (ms)	405.89 ± 23.69

Mean and upper limit of 90%CI of $\Delta\Delta QT_c$

The variability of the mean $\Delta\Delta QT_c$ reduced substantially with each additional ECG replicate and remained within 0.5 ms (10 % of the safety limit of 5 ms) after 14 ECG replicates for all QT correction formulas. In Figure 1, the mean $\Delta\Delta\Delta QT_c$ for each number of ECG replicates for each QT correction formula is displayed. In addition, Figure 3 displays the results for a single cohort, with green squares that indicate a $\Delta\Delta QT_c$ prolongation <5 ms and red squares that indicate a $\Delta\Delta QT_c$ prolongation of >= 5 ms.

Table 3 *Estimated mean investigational medicinal compound concentration and the estimated QT prolongation using 3, 5 and 18 ECG replicates corrected with the Fridericia formula per decile with the standard deviation and with corresponding slope. The dose effect relation hardly changes with the increase in the number of ECG replicates measured.*

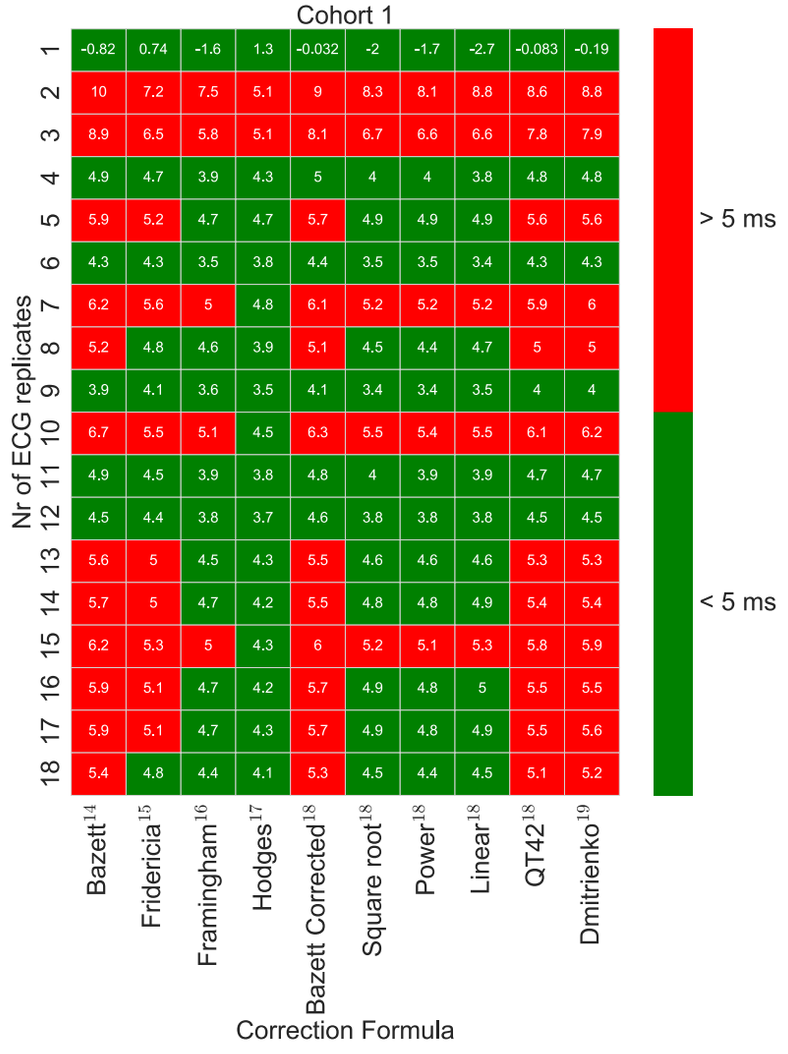
Decile	Estimated mean ± SD investigational medicinal compound concentration (ng/mL)	Mean ± SD QT prolongation (ms) using 3 ECG wreplicates	Mean ± SD QT prolongation (ms) using 5 ECG replicates	Mean ± SD QT prolongation (ms) using 18 ECG replicates
1	7.6 ± 2.5	6.51 ± 16.59	5.21 ± 12.47	4.84 ± 11.54
2	23.2 ± 3.1	6.08 ± 7.13	8.37 ± 5.63	7.31 ± 5.2
3	59.6 ± 10.7	-1.04 ± 10.79	0.45 ± 14.15	0.83 ± 13.11
4	119.6 ± 18.8	5.93 ± 11.59	8.78 ± 10.08	6.53 ± 9.6
5	181.3 ± 12.8	0.81 ± 9.06	2.82 ± 6.54	3.55 ± 7.93
6	238.5 ± 22.7	9.74 ± 13.30	9.01 ± 11.84	9.28 ± 12.15
7	335.3 ± 30.2	16.61 ± 13.63	15.65 ± 12.52	15.11 ± 11.96
8	397.9 ± 16.2	16.12 ± 18.56	14.56 ± 13.02	15.42 ± 12.72
9	485.3 ± 32.0	5.06 ± 13.22	7.46 ± 13.38	6.77 ± 13.71
10	616.1 ± 55.5	19.40 ± 13.37	20.17 ± 9.01	19.78 ± 10.98
Slope (ml*ng-1*ms)		0.022492	0.021380	0.022055
R^2		0.462857	0.539141	0.583485
p-value		0.030387	0.015601	0.010115

The variability of the range of the 90% CI of the $\Delta\Delta QT_c$ also reduced substantially with additional (>1) ECG replicates and remained within 0.5 ms after 11 ECG replicates for all QT correction formulas. Different QT correction formulas and the ECG replicates are displayed in Figure 2 for the range of the 90% CI of the $\Delta\Delta QT_c$.

Concentration-effect analysis of $\Delta\Delta QT_c$

The result of the assessment of the effect of the number of ECG replicates on the concentration-effect analysis is shown in Table 3.

Figure 3 *Mean $\Delta\Delta QT_c$ in milliseconds of an example cohort (Cohort 1) for each number of ECG replicates for every correction method. In this Figure the variation between the number of ECG replicates and between the correction formulas can be clearly seen.*



The mean IMP concentration per decile is displayed together with the estimated QT prolongation measured using 3, 5 and 18 ECG replicates corrected with the Fridericia formula and corresponding slope. For all QT correction formulas, a significant association was found in the concentration-effect analysis. This was also observed for all numbers of ECG replicates.

DISCUSSION

Based on our analysis we showed that the number of ECG replicates in QT studies has a substantial effect on the interpretation of a compound's QT interval prolonging potential for all deployed QT_c formulas. We observed an effect on the mean QT_c interval prolongation and on the range of the 90% confidence interval of the QT_c interval prolongation – parameters that are required by the regulators. To the best of our knowledge this is the first study to address the influence of the number of ECG replicates on the QT prolongation.

The ICH E14 document[4] dictates that, for accurate assessment of the QT interval, at least triplicate ECGs are implemented although evidence for this is limited. The specified cut-off for a positive TQT is 5 ms for mean $\Delta\Delta QT_c$ prolongation. The present analysis showed that all QT correction formulas have a mean difference of 1 ms when triplicate ECGs were extracted compared to 18 ECG replicate extraction. This implies that triplicate ECG extractions are likely to results in inaccurate QT-estimation and can only be used as exploratory method, but not to unambiguously quantify a QT prolonging effect.

The concentration-effect analysis has recently gained more attention in assessing the QT prolonging effect of a compound.[8] The present analysis corroborates these observations, as the concentration-effect analysis was substantially more robust in detecting a QT prolonging effect of the investigated compound as it was independent from the QT correction formula that was used and the number of ECG replicates. It is shown also here that the difference in QT prolongation between subjects becomes less when more QT replicates are measured. This can be deduced from the standard

deviations, the R^2 and the p-values. However, despite the decrease in variance in QT prolongation with an increase in the number of ECG replicates, the dose-effect relationship (slope) hardly changes. Noteworthy, applying Hodges' QT correction formula underestimated the drug plasma concentration that would result in a 10 ms QT interval prolongation.

Several studies have compared the agreement of multiple QT correction formulas in large datasets that were collected in healthy volunteers.[12,13] In those studies it was reported that the agreement between the most frequently deployed QT correction formulas is limited (Bazett's and Fridericia's correction formulas). The two main issues with QT correction for RR interval are 1) the intrinsic variability of QT_C interval due to the beat-to-beat RR interval variation, and 2) the absence of a gold standard – which makes complete validation of QT correction formulas virtually impossible. Other studies have suggested that an individual QT/RR interval calculation may provide the best RR correction of the QT interval.[15,16] Unfortunately we could not confirm this in the current work due to limitations of the data set, requiring a wider range of RR intervals to be available for analysis.

The present analysis shows that the variability of mean $\Delta\Delta QT_C$ for all QT formulas exceeds 0.5ms until 14 ECGs have been recorded and included in the analysis. This finding indicates that on average, the mean $\Delta\Delta QT_C$ deviates by more than 10% of the safety limit from the best measured mean $\Delta\Delta QT_C$ (based on 18 replicates per subject), when based on fewer than 14 replicates per subject. This underlines the previously identified issues with correction of QT for the RR interval, but also indicates that the performance of these QT correction formulas is comparable. The present analysis, in line with previous studies, confirms the suitability of a phase I SAD study as replacement for a TQT.[8,9] in particular with implementation of a 24 hour 12-lead Holter ECG. This provides optimal flexibility to accurately assess the effect of a compound on the QT interval. Furthermore, the analysis on a large volume of ECG replicates can be performed after the compound's development has been moved into a later stage and can be cancelled in case the development of the compound is abandoned, thereby saving resources.

Limitations

The current analysis is a retrospective analysis with its inherent limitations. In addition, the concentration of the investigational compound was not assessed at the same time point as the ECGs were extracted. It was therefore necessary to estimate the compound concentration at the time point the ECGs were extracted. However, since any overestimation or underestimation of the compound concentration will be similar for all subject, the presented slopes will deviate very little from the actual slopes.

CONCLUSION

The number of ECG replicates impacted the estimated QT interval prolongation for all deployed QT correction formulas. In contrast, concentration-effect analysis provides robust data on QT interval prolongation independent of the formula and number of replicates.

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