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Number of ECG Replicates and QT Correction Formula Influences the Estimated QT Prolonging Effect of a Drug

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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 100 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 QTc 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 with the 18 ECG replicate mean values. The difference was <0.5 ms after 14 replicates. By contrast, concentration–effect analysis was independent of replicate count and also of the 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.

Key Words: data science, ECG replicates, drug trials

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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 3 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, 8 received the active compound and 2 volunteers matching placebo. The dose of the investigated compound increased with each cohort, as is typical for a phase I single ascending dose trial. The compound was administered orally, and the mean Tmax was around 2 hours for all cohorts, with a half-life of about 7–8 hours. All subjects consented to their data being registered, and the study was performed in accordance with Dutch law on medical-scientific research.

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Data Acquiescence

All subjects were equipped with a 12-lead Holter ECG (Holter H12+ recorder; Mortara instruments BV, Milwaukee, WI), which was mounted just before the dose administration until 24 hours after the dose administration. All administrations were performed between 1 hour for the same dose level. Ingestion of food was prohibited up to 4 hours and 25 minutes after dose administration. Water was allowed ad libitum at all times except 1 hour before and 1 hour after any dosing, except that study drug was always given with 240 mL (8 ounces) of noncarbonated water. The ECG extractions were performed at 3 hours after dose administration, so ingestion of food was not expected to have an effect on the outcome of the analysis. Standard electrode positioning was used. Subjects were in a supine position and in a calm, relaxed state for at least 5 minutes before any 3-minute window of continuous ECG recording. The ECG recordings from the Holter ECG were extracted during the latter 3 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 that was associated with the largest QT interval prolongation based on the mean of 3 ECGs observed using standard 12-lead ECGs. This time point was found after the initial statistical analysis that was routinely performed at the end of the phase I study, based on

TABLE 1. (Randomized) Selection Pattern of ECG Windows Used for QT Analysis

No. 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								■			■	■	■	■	■	■	■	■
12									■	■	■			■	■	■	■	■
13				■	■		■	■		■	■		■		■	■	■	■
14		■				■						■		■	■	■	■	■
15			■				■	■	■			■	■	■	■	■	■	■
16						■			■	■		■	■	■	■	■	■	■
17					■						■		■	■		■	■	■
18				■		■	■	■	■		■	■	■		■	■	■	■

The main goal of the selection method was to mimic a time interval between recordings. Fields in gray 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.

triplicate ECGs, which showed that the peak QT prolongation occurred at 3 hours after dose administration. 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 3-minute window. Each ECG epoch was 7.5 seconds. The mean waveform from all complexes in lead II within the ECG was averaged, and the QT measurement was done on the average waveform. The time between the intake of the drug and the QT measurement after was the same for every subject, 3 hours. It was considered that the 3-minute epoch that the ECG extractions take would have negligible influence on the variation in plasma concentration, as the half-life of the investigated compound was >5 hours. The QT and RR interval were measured with the algorithm and manually adjusted when necessary as recommended by the E14 R3 guideline.[11]

QTc Formulas

The corrected QT (QTc) 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. 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.

For each of the evaluated correction formulation, the following calculations were performed:

$\Delta_{Baseline}$QTc Calculation

Per subject, the QTc interval for each number of ECG replicates that were extracted at 3 hours after dose administration was calculated. This generated 18 QTc intervals per subject. The subject's baseline (ie, predose) mean QTc value was then subtracted from each of the calculated QTc interval values, resulting in a QTc change from baseline (ΔQTc) for each number of replicates.

$\Delta_{placebo}\Delta_{Baseline}$QTc Calculation

The mean ΔQTc from the subjects in the placebo group was subtracted from the ΔQTc of the subjects who received the active compound, resulting in 18 placebo-corrected ΔQTc ($\Delta_{placebo}\Delta_{Baseline}$QTc, ΔΔQTc) per subject. The calculation for the ΔΔQTc was performed in accordance with the E14 guideline.[4]

$\Delta_{18\ replicates}\Delta_{placebo}\Delta_{baseline}$QTc Calculation

Because the true value of the ΔΔQTc is unknown, the best estimate of the ΔΔQTc for each formula was considered to be the mean ΔΔQTc of 18 ECG replicates. The difference between the mean ΔΔQTc of each replicate count (1–18) and the mean ΔΔQTc of 18 ECG replicates was calculated, and this results in a $\Delta_{18\ replicates}\Delta_{placebo}\Delta_{baseline}$QTc (ΔΔΔQTc). The results of this analysis were displayed as a heat map (Fig. 1).

$\Delta_{18\ replicates}$ 90% CI $\Delta_{baseline}$QTc Calculation

The difference between the range of the 90% confidence interval (CI) of the ΔQTc of each replicate count and the range of the 90% CI of the ΔQTc of 18 ECG replicates was calculated and averaged per cohort and then averaged

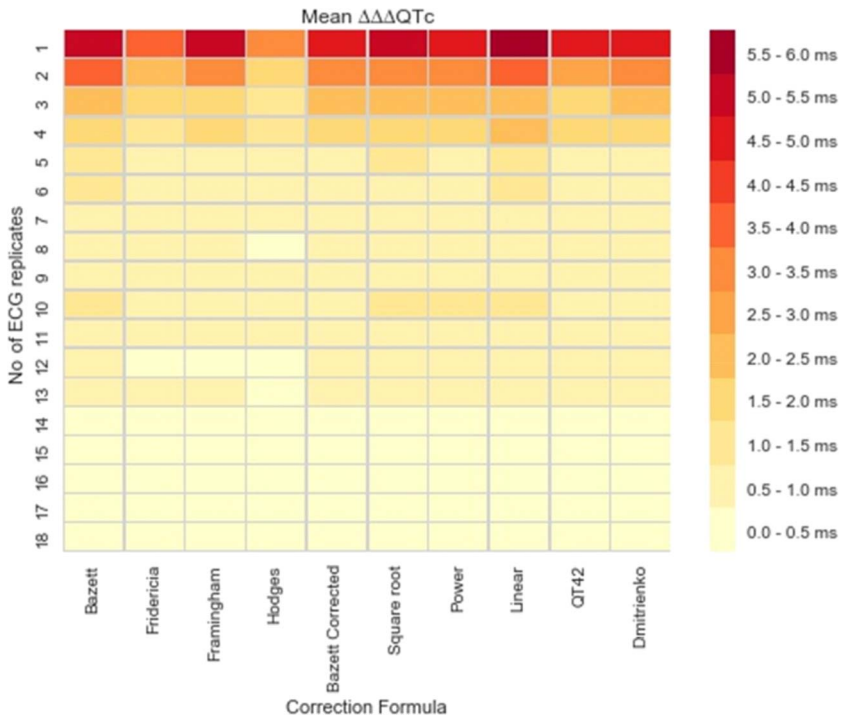

FIGURE 1. Average of the mean ΔΔQTc compared with the mean ΔΔQTc of 18 ECG replicates (mean ΔΔΔQTc) of all cohorts for the Bazett correction method in absolute values (milliseconds). The mean ΔΔQTc deviates with more than 0.5 ms (10% of the safety limit) from the most accurate measurement when it is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

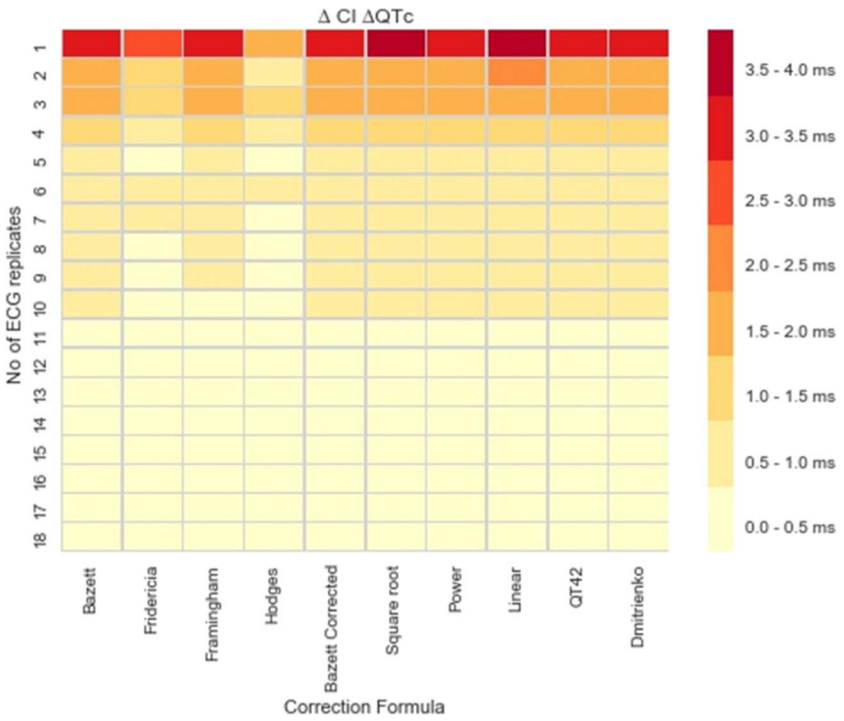

FIGURE 2. Average upper limit of the 90% CI of ΔΔQTc compared with the upper limit of the 90% CI of ΔΔQTc of 18 ECG replicates (mean Δ18 replicates 90% CI ΔbaselineQTc) of all cohorts for the Bazett correction method in absolute values (milliseconds). The 90% CI of the ΔΔQTc within a cohort increases by more than 0.5 ms (10% of the safety limit) when it is based on less than 11 ECGs per subject compared with a ΔΔQTc based on 18 ECGs per subject.

over all 10 cohorts ($\Delta_{18\ \text{replicates}}$ 90% CI Δ_{baseline}QTc), 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 investigational medicinal product (IMP) concentration. These were plotted against the mean ΔΔQTc for all QTc formulas and number of ECG replicates.

Statistical Analysis

Data are depicted as mean ± their SD or percentages where appropriate. Python v3.5.2 (Wilmington, DE) 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 8 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.

Mean and Upper Limit of 90% CI of ΔΔQTc

The variability of the mean ΔΔQTc 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 ΔΔΔQTc for each number of ECG replicates for each QT

TABLE 2. Baseline Data

Age (yr)	24.2 ± 4.8
Gender (Male)	100%
Systolic blood pressure (mm Hg)	121.1 ± 9.2
Diastolic blood pressure (mm Hg)	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/d)	1.1 ± 1.0
Smoking history (cigarettes/d)	0.0 ± 0.0
Caffeine usage (units/d)	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
Creatinine (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

Average values with SD or percentages where appropriate.
ALAT, alanine aminotransferase; ASAT, aspartate aminotransferase; BMI, body mass index.

TABLE 3. Estimated Mean Investigational Medicinal Compound Concentration, Mean RR, and the Estimated QT Prolongation Using 3, 5, and 18 ECG Replicates Corrected With the Fridericia Formula per Decile With the SD and With the Corresponding Slope

Decile	Estimated Mean ± SD Investigational Medicinal Compound Concentration at Time of ECG extraction (ng/mL)	Mean ± SD RR Prolongation (ms)	Mean ± SD QT Prolongation (ms) Using 3 ECG Replicates	Mean ± SD QT Prolongation (ms) Using 5 ECG Replicates	Mean ± SD QT Prolongation (ms) Using 18 ECG Replicates
1	7.6 ± 2.5	−7.5 ± 79.6	6.51 ± 16.59	5.21 ± 12.47	4.84 ± 11.54
2	23.2 ± 3.1	32.4 ± 116.3	6.08 ± 7.13	8.37 ± 5.63	7.31 ± 5.2
3	59.6 ± 10.7	32.6 ± 86.5	−1.04 ± 10.79	0.45 ± 14.15	0.83 ± 13.11
4	119.6 ± 18.8	−29.2 ± 91.7	5.93 ± 11.59	8.78 ± 10.08	6.53 ± 9.6
5	181.3 ± 12.8	−1.3 ± 64.4	0.81 ± 9.06	2.82 ± 6.54	3.55 ± 7.93
6	238.5 ± 22.7	47.2 ± 122.3	9.74 ± 13.30	9.01 ± 11.84	9.28 ± 12.15
7	335.3 ± 30.2	−16.8 ± 136.9	16.61 ± 13.63	15.65 ± 12.52	15.11 ± 11.96
8	397.9 ± 16.2	−43.5 ± 77.5	16.12 ± 18.56	14.56 ± 13.02	15.42 ± 12.72
9	485.3 ± 32.0	−62.3 ± 127.5	5.06 ± 13.22	7.46 ± 13.38	6.77 ± 13.71
10	616.1 ± 55.5	−123.2 ± 125.4	19.40 ± 13.37	20.17 ± 9.01	19.78 ± 10.98
Slope (mL × ng^{-1} × ms)		−0.198116	0.022492	0.021380	0.022055
R^2		0.644797	0.462857	0.539141	0.583485
P		0.005156	0.030387	0.015601	0.010115

The dose–effect relation hardly changes with the increase in the number of ECG replicates measured.

correction formula is displayed. In addition, Figure 3 displays the results for a single cohort, with green squares indicate a ΔΔQTc prolongation of <5 ms and red squares indicate a ΔΔQTc prolongation of ≥5 ms.

The variability of the range of the 90% CI of the ΔΔQTc 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 ΔΔQTc.

Concentration–Effect Analysis of ΔΔQTc

The result of the assessment of the effect of the number of ECG replicates on the concentration–effect analysis is shown in Table 3. 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 QTc formulas. We observed an effect on the mean QTc interval prolongation and on the range of the 90% CI of the QTc interval prolongation—parameters that are required by the regulators.

For accurate assessment of the QT interval, triplicate ECGs are currently used as the industry standard, although evidence for this is limited. The specified cutoff for a positive TQT is 5 ms for mean ΔΔQTc prolongation. The present analysis showed that all QT correction formulas have a mean difference of 1 ms when triplicate ECGs were extracted compared with 18 ECG replicate extraction. This implies that triplicate ECG extractions are likely to result in inaccurate QT estimation and can only be used as an 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 because 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 SDs, 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. The observed association between the IMP concentration and the RR interval is not expected to affect the conclusions of this analysis because the QTc interval is already corrected for changes in the RR interval.

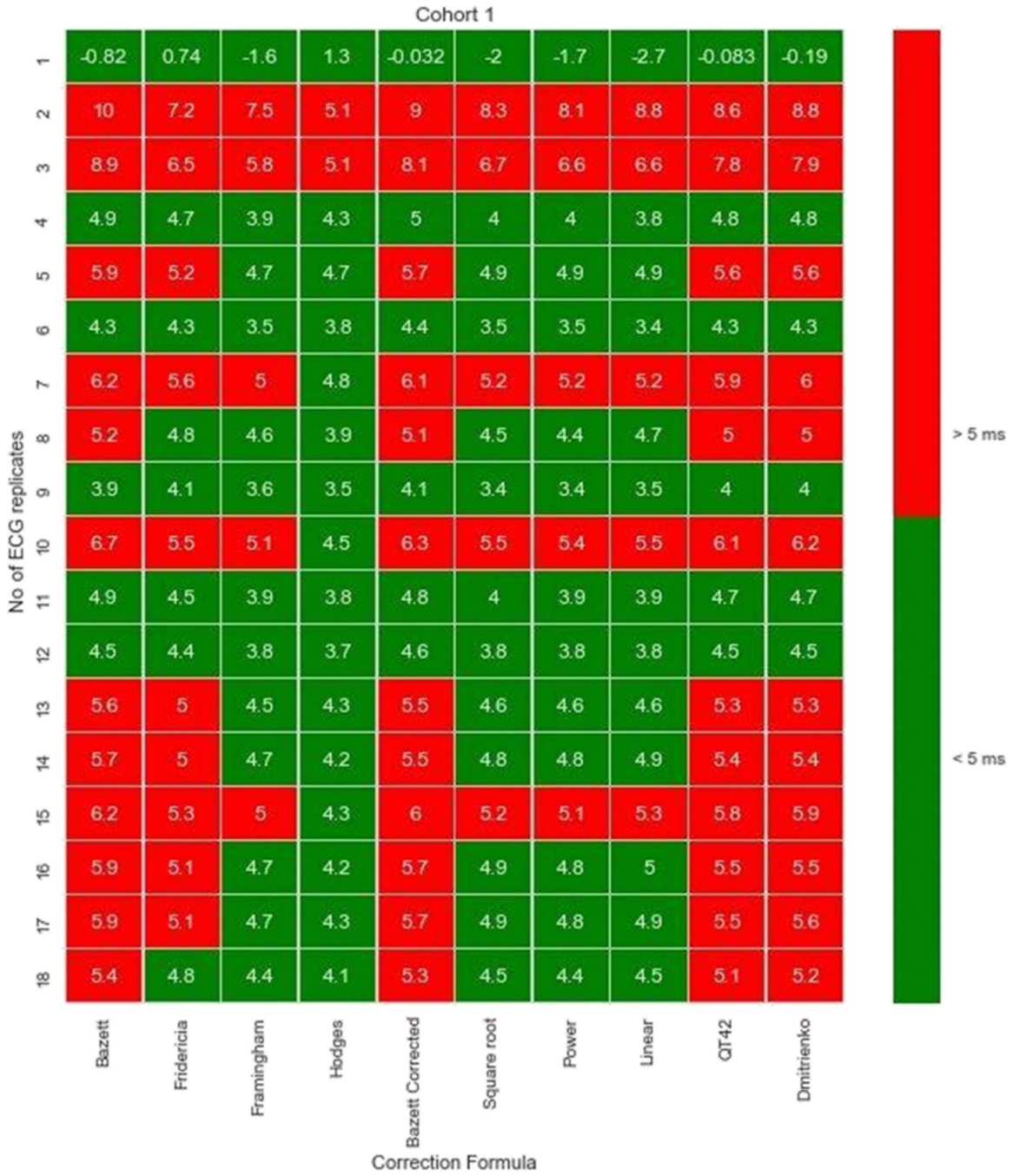

FIGURE 3. Mean ΔΔQTc in milliseconds of an example cohort (cohort 1) for each number of ECG replicates for every correction method. In this figure, the variation between the number of ECG replicates and between the correction formulas can be clearly seen.

Several studies have compared the agreement of multiple QT correction formulas in large data sets that were collected in healthy volunteers.[13,14] 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 2 main issues with QT correction for RR interval are (1) the intrinsic variability of QTc 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.[16,17] 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 ΔΔQTc for all QT formulas exceeds 0.5 ms until 14 ECGs have been recorded and included in the analysis. This finding indicates that on average, the mean ΔΔQTc deviates by more than 10% of the safety limit from the best measured mean ΔΔQTc (based on 18 replicates per subject), when based on fewer than 14 replicates per subject. As can be seen in Figure 1, the mean QT prolongation with 3 used ECG replicates differs on average more than 2 ms from the mean QT prolongation with 18 used ECG replicates for all correction formulas except for the Fridericia, Hodges, and QT42 formulas. A difference of more than 2 ms could have a great impact on drug evaluation considering that safety limits are generally set to 5 ms.

The results of the current analysis confirm the findings by Natekar et al,[22] in which the within- and between-subject variance with QTcF and individual-specific corrected QT interval (QTcI) declined with increasing replicates. In the current study, similar results were found in the analysis of other commonly used correction formulas such as Bazett's formula. The individual correction formula was not included in the current study because a variation in heart rate before dose administration must be available for such an analysis, which was not the case for the data that were used to perform the present analysis. The results also confirm the results of Zang et al in which it is shown that increasing the number of ECG replicates would require a lower subject sample size for achieving the same power.[23]

The results appear to be in contrast to the analysis by Lester et al who reported that more than 3 ECGs would no longer contribute to reducing the SD of QT prolongation.[24] However, this does not contradict the results found in this study because this does not mean that the accuracy does not improve with more measurements. The standard error still decreases with the number of replicates. No more than 3 ECGs are needed for insight into the variation of QT prolongation within one subject, while the present analysis suggests that 18 ECGs per subject provide a more accurate estimation of QT prolongation in the general population, which is important for safety issues of pharmaceutical drugs.

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,[9,10] 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, because any overestimation or underestimation of the compound concentration will be similar for all subjects, 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. By contrast, concentration–effect analysis provides robust data on QT interval prolongation independent of the formula and number of replicates.

REFERENCES

1. Straus SM, Sturkenboom MC, Bleumink GS, et al. Non-cardiac QTc-prolonging drugs and the risk of sudden cardiac death. *Eur Heart J.* 2005;26:2007–2012.
2. Darpo B, Nebout T, Sager PT. Clinical evaluation of QT/QTc prolongation and proarrhythmic potential for nonantiarrhythmic drugs: the International Conference on harmonization of technical requirements for registration of pharmaceuticals for human use E14 guideline. *J Clin Pharmacol.* 2006;46:498–507.
3. Clancy CE, Kurokawa J, Tateyama M, et al. K+ channel structure-activity relationships and mechanisms of drug-induced QT prolongation. *Annu Rev Pharmacol Toxicol.* 2003;43:441–461.
4. CHMP, C.f.M.P.f.H.U. *ICH E14: The Clinical Evaluation of QT/QTc Interval Prolongation and Proarrhythmic Potential for Nonantiarrhythmic Drugs*. 2005. Available at: https://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Efficacy/E14/E14_Guideline.pdf. Accessed February 9, 2018.
5. Darpo B, Garnett C. Early QT assessment—how can our confidence in the data be improved?. *Br J Clin Pharmacol.* 2013;76:642–648.
6. Guideline, ICH Harmonised Tripartite. The clinical evaluation of QT/QTc interval prolongation and proarrhythmic potential for non-antiarrhythmic drugs E14. Recommended for adoption at step 4. 2006.
7. Taubel J, Wong AH, Naseem A, et al. Shortening of the QT interval after food can be used to demonstrate assay sensitivity in thorough QT studies. *J Clin Pharmacol.* 2012;52:1558–1565.
8. Mehrotra DV, Fan L, Liu F, et al. Enabling robust assessment of QTc prolongation in early phase clinical trials. *Pharm Stat.* 2017;16:218–227.
9. Darpo B, Benson C, Dota C, et al. Results from the IQ-CSRC prospective study support replacement of the thorough QT study by QT assessment in the early clinical phase. *Clin Pharmacol Ther.* 2015;97:326–335.
10. Ferber G, Zhou M, Darpo B. Detection of QTc effects in small studies—implications for replacing the thorough QT study. *Ann Noninvasive Electrocardiol.* 2015;20:368–377.
11. Shah RR, Morganroth J, Kleiman RB. ICH E14 Q&A(R2) document: commentary on the further updated recommendations on thorough QT studies. *Br J Clin Pharmacol.* 79, 456–464, 2015.
12. (CHMP), C.f.M.P.f.H.U. *ICH Guideline E14: The Clinical Evaluation of QT/QTc Interval Prolongation and Proarrhythmic Potential for Nonantiarrhythmic Drugs (R3)—Questions and Answers*. 2016. Available at: http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500002878.pdf. Accessed February 9, 2018.
13. Vandenberk B, Vandeal E, Robyns T, et al. Which QT correction formulae to use for QT monitoring? *J Am Heart Assoc.* 2016;5:e003264.
14. Luo S, Michler K, Johnston P, et al. A comparison of commonly used QT correction formulae: the effect of heart rate on the QTc of normal ECGs. *J Electrocardiol.* 2004;37(suppl):81–90.
15. Yeh KC, Kwan KC. A comparison of numerical integrating algorithms by trapezoidal, Lagrange, and spline approximation. *J Pharmacokinet Biopharm.* 1978;6:79–98.
16. Bazett HC. The time relations of the blood-pressure changes after excision of the adrenal glands, with some observations on blood volume changes. *J Physiol.* 1920;53:320–339.

17. Fridericia LS. Die systolendauer im elektrocardiogramm bei normalen menschen und bei herzkranken. *Acta Med Scand.* 1927;53:469–486.
18. Sagie A, Larson MG, Goldberg RJ, et al. An improved method for adjusting the QT interval for heart rate (the Framingham Heart Study). *Am J Cardiol.* 1992;70:797–801.
19. Hodges ML. Bazett's correction formula reviewed: evidence that a linear QT correction method is better. *J Am Coll Cardiol.* 1983;1:694.
20. Rautaharju PM, Zhang ZM. Linearly scaled, rate-invariant normal limits for QT interval: eight decades of incorrect application of power functions. *J Cardiovasc Electrophysiol.* 2002;13:1211–1218.
21. Dmitrienko AA, Sides GD, Winters KJ, et al. Electrocardiogram reference range derived from a standardized clinical trial population. *Drug Inf J.* 2005;39:395–405.
22. Natekar M, Hingorani P, Gupta P, et al. Effect of number of replicate electrocardiograms recorded at each time point in a thorough QT study on sample size and study cost. *J Clin Pharmacol.* 2011;51:908–914.
23. Zhang L, Dmitrienko A, Luta G. Sample size calculations in thorough QT studies. *J Biopharm Stat.* 2008;18:468–482.
24. Lester RM, Azzam SM, Erskine C, et al. Triplicate ECGs are sufficient in obtaining precise estimates of QTcF. *Clin Pharmacol Ther.* 2016;99:S87.
25. Chow SC, Cheng B, Cosmatos D. On power and sample size calculation for QT studies with recording replicates at given time point. *J Biopharm Stat.* 2008;18:483–493.
26. Malik M, Camm AJ. Evaluation of drug-induced QT interval prolongation. *Drug Saf.* 2001;24:323–351.