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Electrocardiographic Method for Identifying Drug-induced Repolarization Abnormalities Associated with a Reduction of the Rapidly Activating Delayed Rectifier Potassium Current

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Abstract— **several important non-cardiac drugs have been removed from the market after revealing harmful effect that was not identified during prior safety-assessment studies. We developed a new technique for the measurements of repolarization abnormalities from surface ECGs; this method improves sensitivity and specificity of the current technique used to identify the presence of abnormal ion current kinetics in the myocardial cells namely a prolongation of the QT interval on the surface ECG signal. We described in this paper the method and preliminary results, revealing the superiority of our technique that may play a role in the future of drug-safety assessment.**

I. Introduction

A Prolongation of the QT interval from the surface electrocardiogram (ECG) is currently used as a surrogate marker of drug cardiotoxicity because it is associated with an increased risk of "torsade de pointes" (TdP), a rare and dangerous type of polymorphic ventricular tachycardia [1]. The US Food and Drug Agency (FDA) currently recommends to pharmaceutical companies to test the safety of all new compounds for their potential QT prolonging effect. This safety assessment is based on a clinical trial specifically designed for identifying drug-induced QT prolongation: such studies are called "thorough QT studies" (tQTS). Because there is no standard for the technique used in measuring the QT interval from the surface ECG [2], the Agency requires that the QT-measurement technique be validated on a positive control group consisting of groups of healthy individuals on an antibiotic drug called moxifloxacin that is known to prolong the QT interval of 5-10 msec [3].

Almost all drugs that have been associated with TdPs modify the same ion current of the myocardial cells: the rapidly activating delayed rectifier K^+ repolarization current (I_{kr}) [4]. However, while some drugs that have no history of cardiac events will prolong the QT interval, others will be associated with very small prolongation and torsadogenic properties. Consequently, the FDA faces a challenging issue related to the validity of their methodological strategy for drug-safety assessment and the Agency recognizes the need for other electrocardiographic markers than QT prolongation.

Preliminary results investigating the role of repolarization parameters other than QT interval in ECGs of individuals, in whom I_{Kr}-inhibition was either from the congenital or from the induced-form of the long QT syndrome (LQTS), revealed large changes in the morphology of T-wave [5-7]. In this study, we present a set of new ECG parameters designed to specifically quantify delay within the ventricular repolarization process of the heart measured on surface ECGs. We use a technique based on the singular value decomposition of the repolarization signal and measuring specific intervals within the resulting first two eigenvectors. We validate these new parameters, and compare their ability to identify the presence of moxifloxacin.

II. Method

A. Study Population

The Heart Research Follow-up program at University of Rochester was granted the access to a small subset of recordings from the FDA ECG Warehouse from a randomized placebo-controlled parallel study including 40 healthy individuals (18 females). Age was not different between placebo and moxifloxacin arms (27.5±7.9 vs. 26.5±7.9 yrs, p=0.38). One hundred and sixty ECGs from the baseline, placebo and moxifloxacin arms were analyzed.

B. ECG recordings

The recording equipment used in this study was not released. Nevertheless, technical specifications of the signal were available from the ECG file. The recordings were 180 Hz sampling frequency and an amplitude resolution of 6.25 μV/bit. The design of the study was partially released and the data we analyzed were as follow: each individual had two ECGs recorded per day for 2 days (Day 0 and Day 1).

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During each day, ECGs were acquired at hour 0 (H0) and hour 2 (H2). During Day 1, H2 corresponded to 2 hours after dosing (moxifloxacin or placebo). We will use ECGs recorded at H0 during Day 0 and Day 1 to estimate the stability of our measurements.

C. Scalar Measurements of the ECG signal

The measurements of the RR intervals and repolarization intervals were based on technology developed at the University of Rochester (COMPAS software) using c++ (Microsoft Visual Studio v6.0). The identification of the end of the T-wave on lead II is computed based on a technique identifying the crossing- point between the baseline and the descending slope of the T-wave (least-squares technique). The end of the T-wave is visually checked by technicians and manually adjusted using an on-screen caliper if the automatic algorithm failed to correctly identify the end of the T-wave. The QT interval measurements were done in all available cardiac beats of Lead II, and the average value was reported.

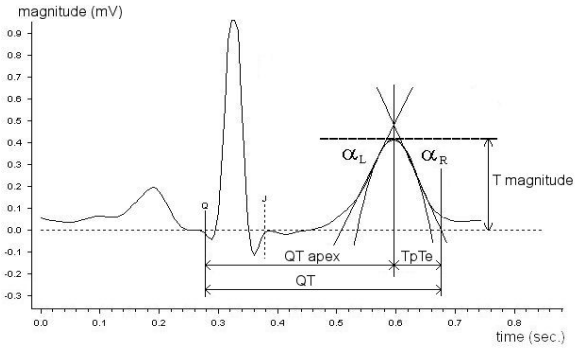

Fig. 1. Definition of the scalar measurements from a cardiac beat. They are computed and manually adjusted on the scalar ECG (lead II). Six measurements were implemented including the QT, QT apex , TpTe interval (all in msec), the magnitude of the T-wave (in mV) and the right and left slope of the T-wave (μV/msec).

The least-squares technique was used to measure the right and left slope of the T-wave. The apex of the T-wave relied on a method using a parabola fitting the T-wave where the maximum of the parabola identified the location of the apex. QT apex and TpTe intervals were such as QT=QTapex+TpTe (see Fig. 1). The amplitude of the ECG signal at the apex of the T-wave was defined as the T-wave magnitude.

D. Vectocardiographic Measurements

The vectorcardiographic measurements were based on a principal component analysis of the repolarization segment defined between the J point and the point located 220 ms before the next R peak. This ensures that the transformation encompasses only the components of the ventricular repolarization signal. The method relies on the computation of the singular value decomposition (SVD) [8], in which any matrix **A** (MxN) can be written as:

$$A = USV^T$$

where **U** is a (M x N) column-orthogonal matrix, **S** a diagonal matrix with elements equal to or greater than zero (the eigenvalues: $\lambda_1 ... \lambda_N$), and **V** an (N x N) orthogonal matrix containing the singular vectors.

The matrix **A** is constructed from the 12-lead standard ECG signal following a beat-to-beat approach. **A** is an M x N matrix where N is the number of leads available in the recordings and M is the number of samples defining the duration of the repolarization signal for a given cardiac beat. The product **A.V** provides the projection of the original data onto the principal components. The repolarization signal within the space defined by the three first components (ev_1, ev_2, ev_3) is called the T-loop. The ev_n signals are the eigenleads. The plane ($ev_1 \llcorner ev_2$) defines the preferential plane of the T-loop (Fig. 2). Following the dipolar theory of electrocardiography, the T-loop represents the path followed by the cardiac vector (VECG) during the repolarization process of the heart ventricles.

1) Measurements of T-loop Morphology

The T-loop morphology is assessed by computing the ratio of the two first eigenvalues (λ_2/λ_1). This ratio is proportional to the roundness of the T-loop. The planarity of this loop is quantified by λ_3 [9].

2) Measurements of Delayed Repolarization on T-loop

The measurements of the QT interval lack precision because they rely on the identification of the end of the T-wave which cannot be accurately defined due to its intrinsic morphology. In this work, we define new repolarization measurements independent from the localization of the end of the T-wave, they are based on the heart vector. We define it as the vector describing T-loop path across time in the preferential plane. The magnitude of the heart vector represents the distance between the isoelectric point and the repolarization potential in $ev_1 \llcorner ev_2$ plan. We have arbitrary chosen the point in time when the heart vector reaches its maximum value (MV) as the reference point for our measurement. MV is detected at time, $t = T_{MV}$, where equation 1 is fulfilled.

Eq. 1) $$MV = \max\left\langle \vec{VECG}(t) - \vec{VECG}(T_Q) \right\rangle,$$

where: $$\vec{VECG}(t) = \vec{ev_1}(t) + \vec{ev_2}(t),$$

and T_Q is the time coinciding with the beginning of the QRS complex. So, in conclusion Eq. 1 can be re-written as:

$$MV = \max\left\langle \sqrt{\{ev_1(t) - ev_1(T_Q)\}^2 + \{ev_2(t) - ev_2(T_Q)\}^2} \right\rangle$$

We defined novel intervals called 30% of Early, Late and Total Repolarization Durations: $ERD_{30\%}$, $LRD_{30\%}$ and $TRD_{30\%}$ (see Fig. 2).
These intervals are centered on the time of MV. Specifically,

$$ERD_{30\%} = T_{MV} - T_E$$

where T_E is the value for $t < T_{MV}$, such that:

Eq. 2) $$\left\| \vec{VECG}(t) - \vec{VECG}(T_{MV}) \right\| = MV \cdot 30\%$$

and

$$ERD_{30\%} = T_L - T_{MV},$$

where T_L is the value for $t > T_{MV}$, where Eq. 2 is fulfilled.

Finally, $TRD_{30\%} = ERD_{30\%} + LRD_{30\%}$

The choice for 30% of the MV value was an *a-priori* choice.

E. Statistical Analysis

The statistical analysis was done using the Statistical Analysis System software (SAS Institute, Cary, NC, USA). We used binary logistic regression to identify the ECGs of individuals recorded after moxifloxacin dosing. We strategically adopted three models in order to assess the gain of using our novel repolarization measurements in comparison with models based on existing technology. For designing each model, a best subsets regression model was selected based on the Akaike information criterion (AIC) which penalizes the log-Partial likelihood of the model for each additional parameter [10]. Then, the comparison between the three models was done using receiver operating characteristics curves (ROC). The area under the ROC curve was used to compare the discriminant power of the designed models.

We estimated the intra and inter-patient variability of the parameters using a linear mixed effect model and then computed the intra-class correlation coefficient (ICC) (representing intra-patient variability) to compare the stability of repolarization parameters.

III. RESULTS

A. Repolarization Measurements and Heart Rate Correction.

QT interval prolongation is used as a reference in our study and thus one might demonstrate the ability of our technology to identify the presence of moxifloxacin-induced QT prolongation. In addition, the QT interval is dependent on the heart rate of prior beats. Thus, it needs to be corrected in order to do a fair comparison of QT interval duration between ECGs recorded at different heart rate. The correction formulae readily available (Bazett and Fridericia)

TABLE I
ANALYSIS OF CENTRAL TENDENCIES (UNIVARIATE ANALYSIS)

	Mean difference vs. placebo	95% CI		P value
T mag (mV)	**-0.026**	**-0.054**	**0.002**	**0.034**
QT apex (ms)	**10.7**	**1.9**	**19.5**	**0.009**
QT offset (ms)	**12.1**	**-1.1**	**25.1**	**0.037**
αL (μV/ms)	**-0.36**	**-0.66**	**-0.06**	**0.009**
αR (μV/ms)	**0.58**	**0.08**	**1.08**	**0.012**
TpTe (ms)	-0.5	-5.9	4.9	0.43
λ_2/λ_1	0.01	-0.07	0.11	0.37
Planarity - λ_3 (mV)	-0.01	-0.03	0.01	0.26
$LRD_{30\%}$ (ms)	0.07	-5.10	5.24	0.48
$ERD_{30\%}$ (ms)	**10.3**	**2.9**	**17.7**	**0.003**

The values in bold are highlighting the parameters (corrected for heart rate) that show a moxifloxacin effect (|Δ|>0).

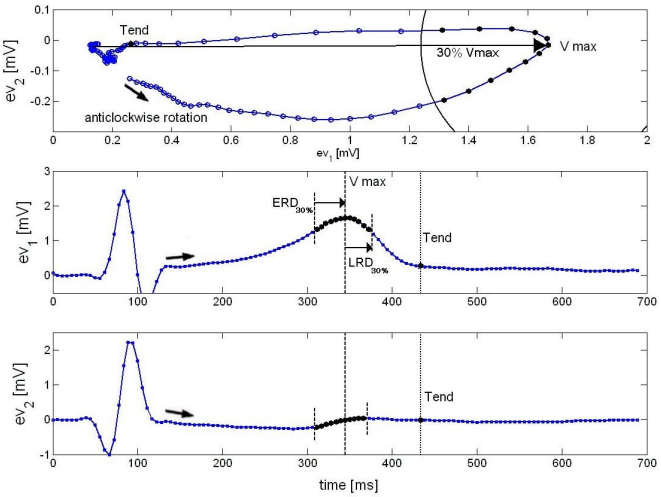

Fig. 2. Definition of the $ERD_{30\%}$, $LRD_{30\%}$ and $TRD_{30\%}$ parameters based on a geometric threshold (circle perimeter) in the preferential plane of the T-loop. The two intervals identified in the T-loop are represented within the signals of the two first eigenvectors (ev_1 and

are imperfect because the QT-RR relationship is different between individuals. An alternative strategy to the pre-defined formulae is to use a pooled-formula. It is defined from baseline recordings (off-drug and off-placebo). Each QT interval measurement is associated with the RR intervals from the immediately preceding beat. Then, QT-RR relationship is defined using a linear regression modeling technique. The correction is done by normalizing the QT measurements to a heart rate of 60 bpm (or RR=1000msec). One may define this correction method as follow: the linear regression model provides the coefficients describing the relationship between RR and a parameter called P:

$$P = \beta \times RR + \alpha,$$

by simple mathematical transformation, we can express the heart rate corrected value of the parameter P (Pc) such as:

$$Pc = P + \beta \times (1 - RR),$$

where RR is expressed in seconds, and β is dimensionless. All results presented in the following paragraphs have been

corrected for heart rate using this technique.

B. I_{kr} Abnormalities from Surface ECGs: Univariate Analysis.

We computed the Δ values for all heart-rate corrected parameters described in the previous paragraphs. The Δ values are baseline corrected changes in ECG after dosing; Δm for moxifloxacin and Δp for placebo dosing. The final Δ values reported in this section were adjusted for placebo, and they are computed as Δ=Δm-Δp. Table I describes the results of the central tendency analysis: TpTe, λ_2/λ_1 and T-loop planarity (λ_3) did not reveal any significant changes due to moxifloxacin. However, all the other parameters showed significant changes: T-wave magnitude, the scalar repolarization intervals including QT, QTapex, the right and left slopes of the T-wave, and the $ERD_{30\%}$ parameter. The T-wave magnitude was significantly reduced by the drug (-0.026±0.014 mV). Because repolarization intervals were also increased, it was expected that the left slope of the T-wave would decreased (-0.36±0.15 μV/ms) and its right slope increased (0.58±0.26 μV/ms). This last one was initially negative. Our results indicate also that QTapex and QToffset were both increased by a very similar value (10.7±4.5 ms vs 12.0±6.7), and TpTe interval was not changed (-0.5±2.8 ms).

This could indicate that the increased duration of the repolarization mainly occurs before the apex of the T-wave when induced by moxifloxacin. This result is consistent with the findings from the vectocardiographic analysis in which $ERD_{30\%}$ was primarily increased by the drug while $LRD_{30\%}$ was not. In addition, it is noteworthy that QT apex, right and left slopes of T-wave and $ERD_{30\%}$ are associated with a much lower p-value than QT interval, the latter being dependent on a precise location of the end of the T-wave.

C. I_{kr} Abnormalities from Surface ECGs: Multivariate Analysis.

Binary logistic regression was used to build statistical models combining repolarization parameters in order to identify the ECG tracings of individual who received a dose of moxifloxacin. First, we defined a model relying exclusively on "classic" parameters namely QTc, RR, gender and age. Again, QTc was corrected using the previously described pooled formula. This model, called the clinical model, performed poorly and QT was the only parameters significantly contributing to the model. Fig. 3 describes the ROC curve of the clinical model; the optimal threshold provided a sensitivity and specificity of 58% and 82%, respectively.

When adding the vectorial and scalar measurements to the list of parameters entered into the binary logistic regression model $ERD_{30\%}$, $TRD_{30\%}$ and αR (AlphaR in Fig. 3) were the first three selected parameters. QTc did not enter the model. This new model provided a better identification of ECGs from individual on moxifloxacin. The sensitivity was 73.7%, and the specificity was 89.5%. The area under the ROC curve was increased from 0.71 to 0.85.

The third model was based on two additional parameters, QTapex and TpTe. This new model is associated with an area under the ROC curve increase of 0.20 and an optimal discrimination between placebo and moxifloxacin ECGs of 95% sensitivity and 79% specificity (see Fig. 3).

D. Comparison of repolarization-parameter stability.

The variability of each parameter was investigated using the two baseline recordings (Ho) collected on Day 0 and Day1. The intra- and inter-patient variability of our repolarization measurements were quantified using a linear mixed effects model [11] taking the form: $Y_{ij} = \upsilon + \delta_i + \varepsilon_{ij}$, where Y_{ij} denotes the observation on day j (j is either Day 0 or Day 1) measured on the ith patient, υ is the average value of the investigated parameter (both across patients and time points), δ_i is a random effect representing the average departure of the ith individual from the population mean υ, and ε_{ij} is the variation of Y_{ij} around the individual mean $\upsilon + \delta_i$. Both ε_{ij} and δ_i are assumed normally distributed so that $\delta_i \sim N(0,d)$ and $\varepsilon_{ij} \sim N(0,\sigma^2)$. The variance components d and σ^2 denotes the inter- and intra-patient variations, respectively.
The intra-patient variability can be quantified using either the intra-individual variance σ^2 or by computing the intra-class correlation coefficient (ICC) defined as $ICC = d/(d+\sigma^2)$. The latter provides an adjustment of the intra-patient variability for the inter-patient variability. The closer the ICC to 1, the higher the stability of the repolarization measurements is. Table II reports the values of d, σ^2 and ICC for all investigated parameters. These results reveal a higher stability of $ERD_{30\%}$ in comparison to QT offset measurements even if this measure is fully automatic.

TABLE II
STABILITY OF REPOLARIZATION MEASUREMENT

	d	σ^2	ICC
T mag (mV)	0.003	0.001	0.74
QT apex (ms)	318.9	100.5	0.76
QT offset (ms)	289.9	143.6	0.68
αL (μV/ms)	0.27	0.12	0.69
αR (μV/ms)	0.75	0.34	0.69
TpTe (ms)	90.4	91.1	0.50
λ_2/λ_1	0.0009	0.0056	0.14
Planarity - λ_3 (mV)	0.0002	0.0004	0.33
$LRD_{30\%}$ (ms)	23.7	15.8	0.60
$ERD_{30\%}$ (ms)	109.4	31.9	0.77

The values of *d* and σ^2 are variances, their unit is the square of the unit reported in the first column. ICC has no unit.

It is noteworthy that all the measurements relying on the end of the T-wave (TpTe, QT offset) have low ICC values. Such

a result indicates the challenging aspect of the identification of the end of the T-wave. Moreover, one may note the very low ICC values of λ_2/λ_1 and λ_3. These parameters are extremely unstable with an intra-patient variability higher than the inter-patient variability.

IV. Discussion

The QT prolongation is recognized as an imperfect surrogate marker of drug cardiotoxicity. Some drugs are known to be associated with QT prolongation without inducing TdPs [12]. However, there is an accepted and widely-held belief that the I_{kr}-inhibition is a common ionic mechanism of drug associated with cardiotoxicity related to a delayed repolarization process. If the tested drug interacts with more than one ion current then it is likely that this interaction may mitigate or exacerbate the effect on the ventricular repolarization process. This observation defines the fundamental problematic assessment of drug-safety when measuring repolarization abnormalities from the surface ECG signal. Can we identify ECG abnormalities evidencing ion-specific inhibition? And can we assess the presence of ion interactions and their resulting impact on the predisposition for arrhythmic events?

In prior investigations, we evidenced that the morphology of the T-wave may be a potential alternative to QT interval prolongation for the identification of drug-induced repolarization abnormalities [5;6;9]. In this study, we report two preliminary findings: 1) the QT interval was not the best repolarization parameter for the quantification of presence of moxifloxacin which is a drug specifically associated with I_{Kr}-inhibition, and 2) using parameters other than QT interval measurements, better identification of moxifloxacin-induced repolarization abnormalities could be obtained.

Consequently, we evidenced repolarization abnormalities from surface ECGs that are directly associated with I_{Kr} reduction, but independent from the identification of the end of the T-wave and the QT interval. Being independent from the precise location of the T-wave endpoint is an important methodological decision for our repolarization-measurement strategy, it increases the stability of our measurements. We demonstrated that all measurements relying on T offset have lower stability than the novel measurements. Also, we demonstrated that the results based on eigenvalues are characterized by low stability.

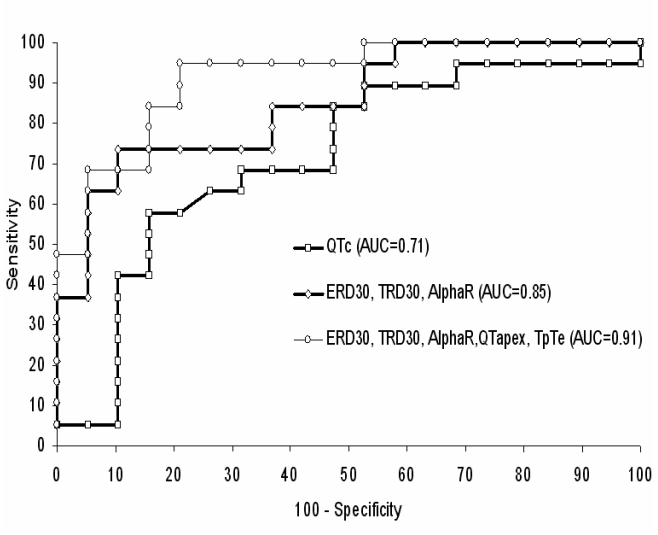

Fig. 3. The figure describes the three ROC curves for the logistic models implemented in this study. AUC: Area under the ROC curve. AlphaR is αR.

But providing a new more stable measurement is not enough, the role of new ECG measurement technique in the future of drug-safety assessment will depend on the understanding of the underlying mechanism leading I_{Kr}-inhibition to the occurrence of TdPs. Moxifloxacin is very rarely associated with such a type of polymorphic tachycardia. Consequently, one may wonder whether identifying moxifloxacin-induced repolarization abnormalities may be the right target compound to design a simple technique providing a precise assessment of the torsadogenic properties of a drug. In a study involving erythromycin, another antibiotic drug with dose-dependent I_{Kr}-inhibition properties, Antzelevitch et al. demonstrated the presence of "a prominent dispersion of repolarization across the ventricular wall, setting the stage for induction of TdP-like tachyarrhythmias displaying characteristics typical of reentry" in a canine model and at higher dose than those measured in human plasma (10- 100 μg/ml) [13]. This group demonstrated that QT prolongation is often associated with an increased heterogeneity of cardiac repolarization across the cardiac ventricle wall due to a prolongation of the action potential of the middle cells of the myocardium (so-called M cells) but not in the epicardial and endocardial cells. Thus, such a heterogeneously delaying process may be the mechanism triggering the TdPs. In a more recent work from Chen et al., moxifloxacin was studied and hyperdose (~18 fold above the typical unbound maximum-concentration exposure in clinical settings) was associated with an increased risk of inducing TdPs [14]. This group also reported a concentration-dependent prolongation of the QT interval and of the TpTe interval (potential surrogate marker of transmural repolarization dispersion). The lack of TdP reports for moxifloxacin is attributable to "its predicable peak concentration profile and other dose-limiting effects". Therefore it is unlikely to find large variation of plasma concentration of the drug in humans or the occurrence of

other side effects such as dizziness, headache and diarrhea limiting the possibility of finding high-level drug exposure.

In our results based on standard 12-lead ECGs, the TpTe interval was not associated with drug-induced changes when using univariate analysis whereas QT and QT apex intervals were significantly prolonged by the drug. The hypothetical explanation for this inconsistency is the validity of the TpTe interval measured from surface ECG as a surrogate marker of transmural dispersion of repolarization.

The new repolarization measurement and the statistical model we have developed demonstrate that specific I_{Kr}-inhibition can be better identified and better assessed than using QT interval prolongation. The main limitation of the study resides in the lack of an independent dataset for the validation of our model.

V. Conclusion

We evidenced the presence of electrocardiographic markers of I_{kr}-related abnormalities in surface ECGs other than a prolongation of the QT interval. Our new parameters provide increased sensitivity and specificity for the detection of moxifloxacin-induced repolarization delay. Once these results are validated on an independent dataset, these new parameters may play a role in the future of drug safety evaluation. This study was supported by ECG signals from the US-FDA ECG Warehouse and the role of this database in future development of better ECG markers of drug cardiotoxicity is crucial.

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