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Quantification of ventricular repolarization heterogeneity during moxifloxacin or sotalol administration using $\mathcal{V}$-index

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Abstract

Drug-induced alterations of ventricular heterogeneity must be limited to avoid induction of lethal ventricular arrhythmias. In here, a new parameter called $\mathcal{V}$-index, able to measure the standard deviation of myocites' repolarization times, was evaluated after moxifloxacin and sotalol administration. The two drugs are known to provide different alteration of the QT interval length ranging from subtle (moxifloxacin) to evident (sotalol). In fact, while the former is employed as active-comparator in thorough QT studies, the latter might induce torsades de pointes. 24 h Holter ECGs of 39 (sotalol) and 68 (moxifloxacin) healthy subjects were retrospectively analyzed. The recordings were performed after infusion of the drugs and after the placebo (moxifloxacin) or at baseline (sotalol). The corrected QT interval (QT_c) was included as well in the study, for a direct comparison. In both populations, $\mathcal{V}$-index and QT_c increased along with the drugs' serum concentration and were statistically different from values in the placebo arm or at baseline ($p < 0.05$).

With sotalol, the maximum value of $\mathcal{V}$-index occurred, on average, after 5.64 h from the infusion, whereas for QT_c after about 4.27 h. The two metrics displayed evident changes ($\mathcal{V}$-index: 27.79 ms ± 4.89 ms versus 60.13 ms ± 18.52 ms; QT corrected: 387.07 ms ± 19.84 ms versus 437.76 ± 32.05 ms; $p < 0.05$). Regarding moxifloxacin, maximum values were reached, on average, 5.01 h after administration for $\mathcal{V}$-index (30.70 ms ± 8.32 ms versus 40.48 ms ± 7.61 ms; $p < 0.05$), and 4.37 h for QT_c (404.29 ms ± 29.05 ms versus 426.77 ± 36.67 ms; $p < 0.05$). They were statistically different from baseline values. With both drugs, the maximal percent variation after administration was higher for $\mathcal{V}$-index than

QT_c (moxifloxacin: 34.56% ± 24.60% versus 5.56% ± 2.98% ; sotalol: 114.77% ± 33.15% versus 12.13% ± 2.85% ; $p < 0.05$).

The study suggests that the standard deviation of the ventricular repolarization times, as quantified by the $\mathcal{V}$-index, might be an effective measure of spatial heterogeneity.

Keywords: ventricular repolarization heterogeneity quantification, drug-induced alterations, biophysical models

(Some figures may appear in colour only in the online journal)

1. Introduction

Ventricular arrhythmias, such as torsade de pointes or ventricular fibrillation, are among the most life threatening cardiac disorders. They stem from changes in the biochemical properties of the myocytes. In particular, those alterations which affect ventricular repolarization by increasing the spatial heterogeneity of ventricular repolarization (SHVR), and, thus, the likelihood of re-entry. Yan and Antzelevitch (1998) elucidated this by showing that an alteration of the normal balance of ionic currents, e.g. changed by means of administration of drugs as sotalol (an antiarrhythmic drug and β-blocker known to significantly increase the QT interval and which might potentially lead to torsade de pointes (Extramiana *et al* 1980)), could cause a disequilibrium in the transmural electrical gradient with a consequent higher spatial dispersion of ventricular repolarization and an increase in the QT interval on the ECG. Coherently, the QT interval was shown to be significatively associated with the risk of cardiac arrhythmias (Pueyo *et al* 2004).

Nowadays, the QT interval is the most employed parameter in repolarization studies and the one adopted by regulatory boards/agencies to assess undesired proarrhythmic effects on non-antiarrhythmic drugs. Before market introduction of any new drug, the US Food and Drug Administration (FDA) mandated a 'thorough QT study' (TQT) to screen for possible drug-induced alterations of ventricular heterogeneity. Other international regulatory agencies soon followed suit (ICH 2005) and since its implementation 9 years ago, the ICH E14 guideline proved very successful: no drug was recalled for repolarization-related issues. However, the high sensitivity is paired with low specificity (common harmless antibiotics fail to pass the test). It is thus questioned if this QT-based test might have prevented valuable molecules from being commercialized.

Indeed, despite its popularity, the QT parameter suffers from any misplacement of fiduciary points on the ECG, a technical task known to be difficult and highly dependent on the implementation of the adopted detector (Kligfield *et al* 2014).

For the above reasons, the regulatory agencies encourage the research of novel parameters quantifying repolarization, which might move one step forward with respect to QT. To this aim, Sassi and Mainardi (2011) recently introduced the $\mathcal{V}$-index, a measure that provides an estimate of the standard deviation of the repolarization times of the myocytes across the entire myocardium from the surface ECG. Being the $\mathcal{V}$-index based on a biophysical model of the ECG (van Oosterom 2001), its physiological interpretation becomes easier than with other metrics such as QT or $T_{peak} - T_{end}$.

In this work, we performed two retrospective studies to evaluate changes in the $\mathcal{V}$-index after: (i) sotalol; and (ii) moxifloxacin administration. Preliminary results were presented in Rivolta *et al* (2012, 2014). Both drugs are known to increase the QT interval

(Bloomfield *et al* 2008, Extramiana *et al* 2010), even if very differently. Sotalol effects are very evident and can be easily detected on the ECG, while moxifloxacin is more subtle, and statistical tests are necessary to detect the changes it induces. With both drugs, changes are expected in the duration of the myocytes' action potentials. Furthermore, an inhomogeneous prolongation of the action potentials was found in wedge preparations in studies involving *in-vitro* animal models. Indeed, when sotalol was perfused, Akar *et al* (2002) found a significant longer prolongation for the action potential of the midcells. On the other hand, at different moxifloxacin concentrations, Chen *et al* (2005) noticed that the prolongation of the action potentials duration was more prominent in the endocardial than in the epicardial region. Thus, these two drugs are suitable tools as test-benches for the $\mathcal{V}$-index.

2. Method

2.1. The $\mathcal{V}$-index

Myocytes' transmembrane potentials (TMP) shapes and, particularly, durations differ when traversing the heart from apex to base and across the muscular tissue from the endo- to the epicardium. However, in first approximation, the slopes of the TMP during phase 3 do not differ significantly across myocytes. Let's divide the myocardium in M nodes and let's suppose that each node m shares the same TMP during repolarization, which we represent here with a common function $D(t - \rho_m(k))$, where $\rho_m(k)$ marks the repolarization time of the kth beat, as the point where the down-slope is maximal. $\rho_m(k)$ may be expressed as

$$\rho_m(k) = \bar{\rho}(k) + \Delta\rho_m(k), \tag{1}$$

where the repolarization delay $\Delta\rho_m(k)$ is the deviation from the average repolarization time $\bar{\rho}(k) = \frac{1}{M}\sum_{m=1}^{M} \rho_m(k)$ in the given heartbeat k. Sassi and Mainardi (2011) introduced a simple model to describe the distribution of these delays:

$$\Delta\rho_m(k) = \vartheta_m + \varphi_m(k), \tag{2}$$

where ϑ_m models the *spatial variability* of the repolarization times for a given subject at a given heart rate, and $\varphi_m(k)$ describes *temporal differences* in repolarization times which are observable among successive beats.

Under a few (common) hypotheses, usually enforced in forward and inverse electrocardiographical solvers (Sassi *et al* 2013), the link between $\Delta\rho_m(k)$ and the T-wave $\Psi(t)$ on the ECG (being $\Psi(t)$ a $L \times 1$ vector containing the T-wave values for each lead) can be derived by analytically simplifying a biophysical model (van Oosterom 2001). Specifically,

$$\Psi(t) \approx -A\Delta\rho T_d(t) + \frac{1}{2}A\Delta\rho^2 \dot{T}_d(t) = w_1 T_d(t) + w_2 \dot{T}_d(t), \tag{3}$$

where the function $T_d(t)$ is the first derivative of $D(t)$ (which, with a sign reversal, is often termed 'dominant T-wave' (van Oosterom 2001)) and $\Delta\rho = [\Delta\rho_1(k), \Delta\rho_2(k), \ldots, \Delta\rho_M(k)]^T$ is a vector of repolarization delays. A is a patient-dependent $[L \times M]$ transfer matrix accounting for the contribution of each node to the L-leads electrocardiographic recording in $\Psi(t)$. The terms w_1 and w_2 are $[L \times 1]$ vector of scalars ('lead factors'), one for each lead.

An estimate of the SHVR, quantified as the sample standard deviation of the repolarization times across the myocardium, can be derived from the lead factors through the $\mathcal{V}$-index, defined as:

$$\mathcal{V}_i = \frac{\text{std}\,[w_2(i)\,]}{\text{std}\,[w_1(i)\,]} \quad s = \left(\frac{1}{M}\sum_{m=1}^{M} z_m^2\right)^{\frac{1}{2}}, \tag{4}$$

where the standard deviations (std) are computed on the lead factors of lead i across a certain number of successive beats (not across different leads).

SHVR, as measured by the $\mathcal{V}$-index, has a straightforward physiological interpretation and does not suffer from an imperfect location of ECG fiducial points. Moreover it was proved to be consistent by extensive numerical simulations (Sassi and Mainardi 2011, Sassi and Mainardi 2012) and promising preliminary clinical validations (Sassi *et al* 2014).

2.2. Dataset (sotalol)

The study population was the same employed in Extramiana *et al* (2010). It is composed by 39 healthy subjects in which 12-leads 24 h digital Holter recordings were collected in three consecutive days (sampling frequency: 180 Hz; LSB: 2.50 μV). During the first day no drugs were administrated, and we used the data collected in this day as reference baseline values. A 160 mg dose of sotalol was injected the second day and a double dose was given the third one. In the third day, only 22 subjects were involved in the study.

Sotalol was administered at 8 : 00 a.m., while plasma concentrations were measured at 16 predefined subsequent instants, which in the following will be referred to as 'time-points'. For protocol details, please refer to Sarapa *et al* (2004).

Heart rate corrected QT (QT_B) intervals (Bazett formula) were provided with the dataset, and then used for comparison with the $\mathcal{V}$-index.

2.3. Dataset (moxifloxacin)

The E-HOL-12-0140-008 dataset from the Telemetric and Holter ECG Warehouse (THEW) was retrospectively analyzed. It contains 24 h digital Holter recordings (12 standard leads, sampling frequency: 1 kHz, LSB: 3.75 μV) collected from 68 healthy subjects, enrolled in a TQT study. For each participant, two registrations were performed when either a placebo or moxifloxacin (a 400 mg dose) were administrated. Drug's serum concentration was assessed at 11 predefined instants ('time-points') during the entire day.

QT intervals were determined using the algorithm described in section 2.4 and then corrected using the Fridericia formula (QT_F) for a direct comparison with the Bloomfield's results (Bloomfield *et al* 2008). In practice, they were obtained as an average of QT values for beats with similar preceding RR values, then corrected with the average of these RR interval lengths.

2.4. Preprocessing and fiducial point detection

ECG recordings were preprocessed using a bandpass Butterworth filter (3rd-order, pass-band: 0.5–40 Hz) to reduce powerline interference, baseline wandering and high frequency noise. After filtering, the baseline of all signals was adjusted: for each channel, the mode of the ECG's samples distribution (computed using a bin size of 75 μV) was identified. Then, ECG

samples belonging to the modal bin were linearly fitted, and the obtained regression line subtracted from the signal.

Beat locations were provided for the moxifloxacin dataset and detected for the sotalol data using a multilead detector based on a modified version of OSEA (EP Limited, MA, USA, 2003). Then, the *T*-waves were segmented; in particular, the end was determined using the Surawicz method (Lepeschkin and Surawicz 1952). This procedure does not need to be accurate because the $\mathcal{V}$-index is robust to displacement of fiducial points (Sassi and Mainardi 2012).

The quality of the leads was determined as the average crosscorrelation between a mean QRS complex and each ones. A lead was considered good when such average was higher than 0.9.

2.5. Data analysis and parameter computation

Three consecutive windows, 10 min each, were analyzed at every time-point (in both studies). The values of QT or $\mathcal{V}$-index obtained in the three windows were then averaged.

The computation of both QT and $\mathcal{V}$-index requires heart rate to be approximately 'stationary'. Therefore, beats were selected using a criterion similar to the 'binning' procedure proposed by Badilini *et al* (1999). A beat was considered 'stable', and included into the computation, if the two preceding RR values were within ± 25 and ± 50 ms, respectively, with respect to a constant RR value. Considering the beats in a given ECG segment, RR was selected such to maximize the number of beats (typically it corresponded to the median value, but not necessarily).

The $\mathcal{V}$-index was estimated using the algorithm described in Sassi and Mainardi (2011). Briefly, a numerical iterative procedure estimated alternatively the lead factors (i.e. w_1 and w_2) and T_d, on the J-T interval of each beat independently from the others. A value of $\mathcal{V}$-index was obtained for each leads. We employed their average as overall estimate of the $\mathcal{V}$-index. The $\mathcal{V}$-index was determined only when, at least 3 good-quality leads were available (see section 2.4) and 64 stable beats were available in the ECG window.

QT_F intervals in the moxifloxacin dataset were determined on the same stable beats selected for the $\mathcal{V}$-index computation.

2.6. Statistical analysis

A statistical comparison was performed among the values of both $\mathcal{V}$-index and QT at each time-point (paired single-tail Wilcoxon test; $p < 0.05$). Multiple comparisons were considered applying the Bonferroni's correction.

3. Results

The plasma concentrations of both drugs are shown in figure 1. The maximum values were measured after around 3 h from administration in both cases.

3.1. Sotalol administration

The time evolution of $\mathcal{V}$-index and QT_B is shown in figure 2.

After each sotalol's dose administration, both $\mathcal{V}$-index and QT_B were statistically larger than the time-matched values at baseline (day 1 versus day 2 and day 2 versus day 3), for the

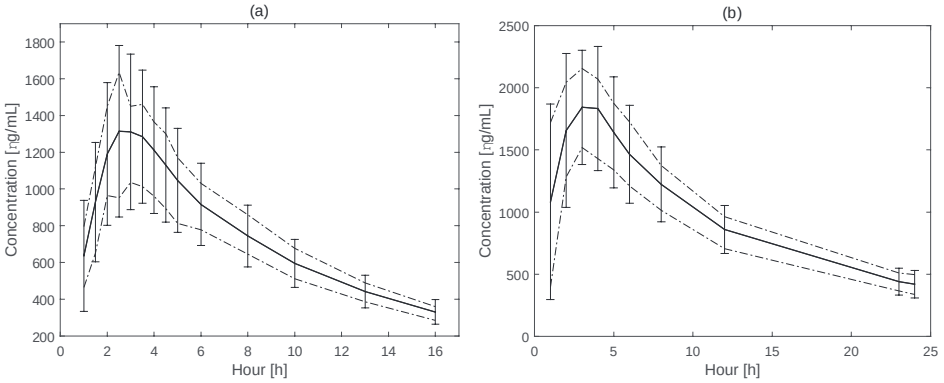

Figure 1. Mean and standard deviation of the serum concentration of sotalol (*a*) and moxifloxacin (*b*) over time. Dashed lines are the 25th and 75th percentiles, respectively.

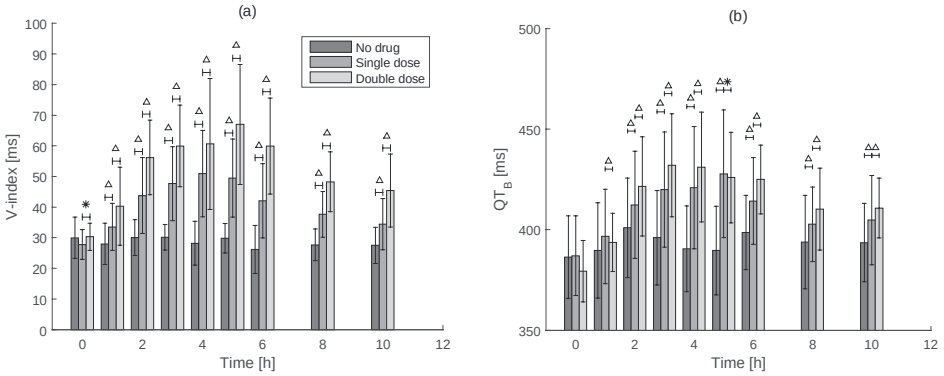

Figure 2. Mean ± standard deviation of $\mathcal{V}$-index (*a*) and QT_B (*b*) values during day 1 (no drug), day 2 (single dose of sotalol) and day 3 (double dose of sotalol). *: time-instants at which statistically significant differences were obtained (paired single-tail Wilcoxon test, $p < 0.05$); ▵: significance was retained after Bonferroni's correction. The standard deviation was estimated as 1.4826 × MAD, where MAD is the median absolute deviation, to reduce the possible impact of outliers. For clarity, only a selected number of time-points was included in the figure.

majority of the time-points ($p < 0.05$, after Bonferroni's correction for repeated comparisons). An estimate of $\mathcal{V}$-index was not available for each subject at every time-point, due to poor signal quality or to a small number of stable beats (the average percent of subjects for which a $\mathcal{V}$-index value was available in a time-point was 75.3%).

The maximum value of $\mathcal{V}$-index occurred on average 5.64 h administration, on day 2, and after 2.71 h, on day 3. QT_B peaked about 4.27 h from administration, at day 2, and after 2.05 h, at day 3. Both indexes' maximum values were statistically different (paired single-tail Wilcoxon test $p < 0.05$) from baseline, at day 2 ($\mathcal{V}$-index baseline: 27.79 ms ± 4.89 ms versus peak: 60.13 ms ± 18.52 ms; QT_B baseline: 387.07 ms ± 19.84 versus peak: 437.76 ms ± 32.05 ms) and at day 3 ($\mathcal{V}$-index baseline: 30.32 ms ± 4.46 ms versus peak: 79.79 ms ± 27.60 ms; QT_B baseline: 379.36 ms ± 15.26 ms versus peak: 447.97 ms ± 20.39 ms).

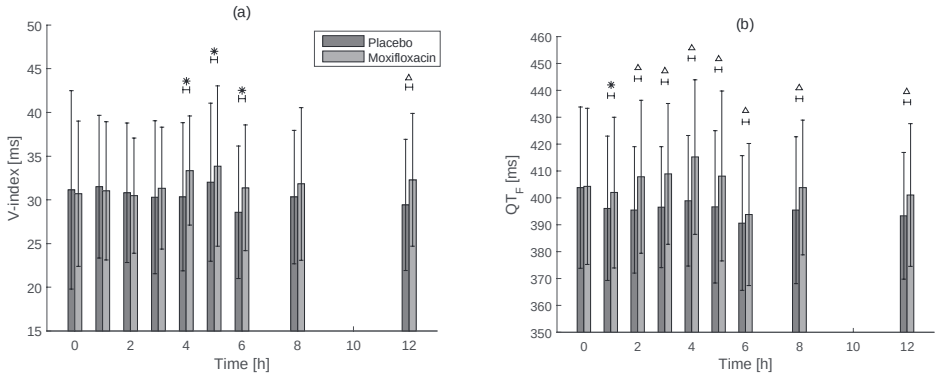

Figure 3. Mean ± standard deviation of $\mathcal{V}$-index (*a*) and QT_F (*b*) values over time, after placebo and moxifloxacin administration. *: time-instants at which statistical significant differences were obtained (paired single-tail Wilcoxon test, $p < 0.05$); ▵: significance was retained after Bonferroni's correction. To reduce the possible impact of outliers, the standard deviation was estimated as 1.4826 × MAD, where MAD is the median absolute deviation. For clarity, only a selected number of time-points was included in the figure. In panel (*b*), the difference in QT_F after moxifloxacin administration was significantly larger than 10 ms, confirming that the statistical sensitivity of our setup was coherent with what expected in TQT studies (Bloomfield *et al* 2008, Kligfield *et al* 2014).

However, the relative percent variation of $\mathcal{V}$-index at peak was statistically higher than that of QT_B in both day 2 and day 3 (day 2: $\mathcal{V}$-index% peak: 114.77% ± 33.15% versus QT_B% peak: 12.13% ± 2.85% ; day 3: $\mathcal{V}$-index% peak: 188.75% ± 53.58% versus QT_B% peak: 18.47% ± 2.85% ; paired single-tail Wilcoxon test $p < 0.05$).

3.2. Moxifloxacin administration

The time evolution of $\mathcal{V}$-index and QT_F is shown in figure 3. On average, in each time-point, $\mathcal{V}$-index values were available for 94.28% of the subjects. As shown, at several time-points, $\mathcal{V}$-index and QT_F values were significantly different from the corresponding values in the placebo arm.

Maximum values were reached, on average, after 5.01 h for $\mathcal{V}$-index and after 4.37 h for QT_F. Peak values were statistically larger than those during baseline (moxifloxacin arm, $\mathcal{V}$-index baseline: 30.70 ± 8.32 ms versus peak: 40.48 ± 7.61 ms and QT baseline: 404.29 ± 29.05 ms versus peak: 426.77 ± 36.67 ms; paired single-tail Wilcoxon test $p < 0.05$). The relative percent variation of $\mathcal{V}$-index at peak was statistically higher than that of QT_F ($\mathcal{V}$-index: 34.56% ± 24.60% versus QT_F: 5.56% ± 2.98% ; paired single-tail Wilcoxon test $p < 0.05$).

4. Conclusion

In this study, $\mathcal{V}$-index values, at different sotalol's and moxifloxacin's serum concentrations, were estimated and compared with the time evolution of the corresponding QT corrected values. We found that both indexes increased with drug serum concentration, but the changes

were larger, in percentage, in the $\mathcal{V}$-index than in QT_c. Also, the maximum $\mathcal{V}$-index value occurred, on average, later than then maximum QT_c value, for both drugs.

Our results evidence a direct link between SHVR, the QT interval and $\mathcal{V}$-index. In respect to QT measurements, $\mathcal{V}$-index has the advantages of being (i) a direct estimator of SHRV and (ii) only marginally affected by misdetection of *T*-waves fiduciary points. However, algorithm for $\mathcal{V}$-index computation are fairly more complex than those available for QT interval computation and they require longer observation windows to obtain reliable estimates of the lead factors w_1 and w_2. Both indexes are affected by HR changes and need appropriate selection of stationary HR sequences. However, while in here the QT values were also directly corrected for the heart rate, the same did not happen for $\mathcal{V}$-index. This possible improvement will be investigated in future studies.

In conclusion, the results confirm the capability of $\mathcal{V}$-index to assess changes in SHVR and evidence the applicability of this index for assessing drug-induced pro-arrhythmic effects.

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The E-HOL-12-0140-008 data, used for this research, were provided by the Telemetric and Holter ECG Warehouse of the University of Rochester (THEW), NY.

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