Assessment of spatial heterogeneity of ventricular repolarization after multi-channel blocker drugs in healthy subjects

Valentina D.A. Corino[1], Massimo W. Rivolta[2], Luca T. Mainardi[1], Roberto Sassi[2]

[1] Dipartimento di Elettronica, Informazione e Bioingegneria, Politecnico di Milano, via Golgi 39, 20133, Milan, Italy

[2] Dipartimento di Informatica, Università degli Studi di Milano, Via Celoria 18, 20133, Milan, Italy

Corresponding Author:

Valentina D.A. Corino

Dipartimento di Elettronica, Informazione e Bioingegneria, Politecnico di Milano

Via Golgi 39, 20133, Milano, Italy

Tel: +39 2 2399 3392; Fax: +39 2 2399 3360

E-mail: valentina.corino@polimi.it

Abstract:

Background and Objectives: In contrast to potassium channel blockers, drugs affecting multiple channels seem to reduce torsadogenic risks. However, their effect on spatial heterogeneity of ventricular repolarization (SHVR) is still matter of investigation. Aim of this work is to assess the effect of four drugs blocking the human ether-à-go-go-related gene (hERG) potassium channel, alone or in combination with other ionic channel blocks, on SHVR, as estimated by the V-index on short triplicate 10 s ECG.

Methods: The V-index is an estimate of the standard deviation of the repolarization times of the myocytes across the entire myocardium, obtained from multi-lead surface electrocardiograms. Twenty-two healthy subjects received a pure hERG potassium channel blocker (dofetilide) and 3 other drugs with additional varying degrees of sodium and calcium (L-type) channel block (quinidine, ranolazine, and verapamil), as well as placebo. A one-way repeated-measures Friedman test was performed to compare the V-index over time.

Results: Computer simulations and Bland-Altman analysis supported the reliability of the estimates of V-index on triplicate 10 s ECG. Ranolazine, verapamil and placebo did not affect the V-index. On the contrary, after quinidine and dofetilide administration, an increase of V-index from predose to its peak value was observed (ΔΔV-index values were 19 ms and 27 ms, respectively, $p<0.05$).

Conclusions: High torsadogenic drugs (dofetilide and quinidine) affected significantly the SHVR, as quantified by the V-index. The metric has therefore a potential in assessing drug arrhythmogenicity.

Keywords: calcium channel; dominant T-wave; human ether-à-go-go-related gene potassium channel; sodium channels; ventricular arrhythmias: ventricular repolarization;

1. Introduction

[*]It is well-known that certain drugs, either anti-arrhythmics or non-anti-arrhythmics, can also induce cardiovascular adverse effects and have been associated with drug-induced arrhythmias. Some might increase the spatial heterogeneity [1] of ventricular repolarization as well as prolong repolarization and cardiac refractoriness, e.g., class III antiarrhythmic drugs. The prominent theory today is that these pro-arrhythmic drugs can be identified by assessing whether they block relevant ion channels. Among the many, a relevant pro-arrhythmic block is the one that occurs to the human ether-à-go-go-related gene (hERG) potassium channel (an outward current) [2,3]. However, not all drugs blocking the hERG potassium channel are necessarily associated with a high pro-arrhythmic risk because they may also concurrently block calcium and/or sodium channels (inward currents), leading to a "compensatory" effect. Blocking inward currents, in fact, can prevent early afterdepolarizations, and consequently torsade de pointes [1,4,5], a life-threatening ventricular arrhythmia. Therefore, the interest has been focused on those drugs having a compensatory effect, thus seeming to be safer to use.

In this scenario, the US Food and Drugs Administration (FDA) funded a prospective randomized controlled clinical trial [6] to assess the effects on the electrocardiograms (ECG) of multiple marketed drugs that either block the hERG potassium channel alone or do so while also blocking calcium and sodium channels. In particular, the study included a pure hERG potassium channel blocker (dofetilide) and other three drugs with varying degrees of sodium and calcium channel block (quinidine, ranolazine, and verapamil). Channel patch clamps experiments have shown that the

[*] Abbreviations: ECG: electrocardiograms; FDA: US Food and Drugs Administration; hERG : human ether-à-go-go-related gene; NSTEMI: non-ST-elevation myocardial infarction; QTc: corrected QT interval; TMP: transmembrane potential

degree of hERG blockage is different for the four drugs [7]. They were administered (plus a placebo) to twenty-two healthy subjects and ECG was contemporarily recorded. This dataset was analyzed in previous studies, where QTc and J-Tpeak intervals were computed from the ECG recordings. In particular, QTc misclassified the risk associated to ranolazine [6], while J-Tpeak was less sensitive to this error [8], though its computation is more critical (mostly due to the lack of a definition for the location of the peak of the T-wave [9]). Recently, a new index assessing spatial heterogeneity of ventricular repolarization, the V-index, has been introduced [10]. The V-index is an estimate of the standard deviation of the repolarization times of the myocytes across the entire myocardium, obtained from multi-leads surface ECG. Being based on a biophysical model of the ECG [11], the physiological interpretation of the V-index is easier than with other metrics such as QT or Tpeak – Tend [10], when the assumptions of the model hold. Recent studies seem to support this indication: in patients with Chagas disease, an increased V-index was found to be significantly correlated with the risk of death in a survival analysis, after correction for other established risk factors [12]. Also, in patients with suspected non-ST-elevation myocardial infarction (NSTEMI), the V-index significantly improved the accuracy and sensitivity of the ECG for the diagnosis of NSTEMI and independently predicted mortality during follow-up [13]. Finally, when tested after administration of two drugs known to provide different alteration of the QT interval length, ranging from subtle (moxifloxacin) to evident (sotalol), the V-index increased with the drug's serum concentration and its maximal percent variation was larger than what displayed by QTc [14] (although with a higher variability).

Aim of this work is to assess the effect of hERG potassium channel block, alone or in combination with other ionic channel blocks, on spatial heterogeneity of ventricular repolarization, as estimated by the V-index, aiming to find an alternative and complementary index to QTc or J-Tpeak, that are solely based on the duration of the ventricular repolarization.

2. Material and methods

2.1. Data and Protocol

The design of the clinical study has been previously described [6]. Briefly, it was a randomized, double-blind, 5-period crossover clinical trial to assess the effect on the ECG of four hERG potassium channel blockers (either pure or displaying various degrees of concurrent calcium and sodium channel block). Twenty-two healthy young subjects (27 ± 6 years, range 18 – 35), without a family history of cardiovascular disease, were included in the study. In the morning of each period, subjects received either a single dose of 500 μg dofetilide (Tikosyn, Pfizer, New York, NY), or 400 mg quinidine sulfate (Watson Pharma, Corona, CA), or 1500 mg ranolazine (Ranexa, Gilead, Foster City, CA), or 120 mg verapamil hydrochloride (Heritage Pharmaceuticals, Edison, NJ) or placebo under fasting conditions. Dofetilide is the only strong pure hERG potassium channel blocker [15] (about 55% block at the mean maximum concentration in the population under analysis [7]). Quinidine is also a strong hERG potassium channel blocker, but has additional weak blocks of calcium (L-type) and late sodium currents (at the maximum serum concentration, about 71% block for hERG potassium channel, 8% for calcium current and 3% for late sodium current [7]). The other drugs block significantly additional channels: ranolazine blocks the late sodium current (26% block for hERG potassium channel and 21% for late sodium current) and verapamil the L-type calcium channels (7% block for hERG potassium channel and 17% for calcium current) [5,7].

During each period, standard (Mason-Likar) 12-lead continuous ECGs were recorded at 500 Hz (H12+ Mortara Instruments) and then upsampled at 1 kHz. From the continuous recording, triplicate 10-second standard diagnostic ECGs were extracted at predose and at 15 predefined time points post-dose (0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 7, 8, 12, 14, and 24 h), during which the subjects were resting in a supine position for 10 minutes (for protocol details refer to [6]). The 10 s diagnostic ECGs

were the only signals made available by FDA for further analysis, thus our analysis did not consider the continuous recordings. A blood sample was drawn for pharmacokinetic analysis, after each ECG time point, and plasma drug concentration was measured using a validated liquid chromatography with tandem mass spectroscopy by Frontage Laboratories (Exton, Philadelphia, PA). The study was approved by the U.S. FDA Research Involving Human Subjects Committee and the local institutional review board. All subjects gave written informed consent.

Diagnostic ECG recordings were made available on physionet.org [16], together with three standard repolarization parameters: i) the QT interval; ii) the Tpeak-Tend duration; and iii) the interval between the J-point to the peak of the T-wave (J-Tpeak). The heart rate corrected QTc interval was obtained using the Fridericia formula, while the computation of the J-Tpeak corrected interval was based on the formula published in [6]. Tpeak-Tend did not need correction, as suggested in [17].

2.2. Background on V-index

The V-index is a metric that estimates the spatial heterogeneity of ventricular repolarization from multi-lead surface ECG recordings. It is an indirect assessment of the standard deviation of the ventricular cardiomyocytes' repolarization times ρ_m (e.g., the instants of the largest downslope of the transmural action potentials, or other common markers as APD_{90}) in a single beat. It is estimated by considering multiple beats under stable heart rate [10].

The V-index is derived by combining the electrophysiological model of the surface ECG proposed by van Oosterom [18], with a statistical model of the myocytes' repolarization times [10]. Van Oosterom showed that the shape of the T-wave on the surface ECG in one single beat can be modeled with a weighted sum of the myocytes' transmembrane potentials. Moreover, the repolarization phase of the action potential is equivalent across myocytes at a specific heart rate

[19], thus, in first approximation, the multi-lead surface ECG can be approximated using a weighted sum of a single function $T_d(t)$ (the so-called "dominant T-wave" [19]) and its derivatives , as follows

$$\boldsymbol{\Psi}(t) \approx \boldsymbol{w_1} T_d(t) + \boldsymbol{w_2} \frac{dT_d(t)}{dt} + \cdots + \boldsymbol{w_q} \frac{d^{(q-1)} T_d(t)}{dt^{(q-1)}} \quad (1)$$

where $\boldsymbol{\Psi}(t)$ is the [L x 1] ECG, with L being the number of leads, and the terms $\boldsymbol{w_1}$, $\boldsymbol{w_2}$ and $\boldsymbol{w_q}$ are [L x 1] vectors of lead factors (one element for each lead).

Sassi and Mainardi [10] proposed a model for the repolarization delay $\Delta\rho_m$ for each cell m as follows:

$$\Delta\rho(\mathrm{k}) = \rho_m(k) - \bar{\rho}(k) = \vartheta_m + \varphi_m(k) \quad (2)$$

where $\bar{\rho}(k)$ is the average repolarization time in the single beat k over the set of M cells; ϑ_m models the spatial variability of the repolarization times at a given HR; $\varphi_m(k)$ describes differences in repolarization times which are observable among successive beats (the temporal variability of the repolarization times). An estimate V_i of the standard deviation of ϑ_m is given by

$$V_i = \frac{\mathrm{std}[w_2(i)]}{\mathrm{std}[w_1(i)]} \approx \left(\sum_{m=1}^{M} \frac{\vartheta_m^2}{M} \right)^{1/2} = s_\vartheta \quad (3)$$

where the standard deviation (std) is computed across beats and for the *i*-th lead.

2.3. Preprocessing and V-index computation

Standard ECG preprocessing was performed in four steps, as in [14]. First, ECG signals were bandpass filtered (3rd order, Butterworth, 0.5 – 40Hz, zero-phase forward and reverse filtering) to reduce baseline wandering and high frequency noise.

Second, for each lead independently, the isoelectric line was approximately set to 0 mV by subtracting a horizontal line estimated as in [14] (briefly, obtained by averaging the ECG samples contained in the TP segments, roughly identified as the mode of the ECG's amplitude distribution).
Third, beats were detected by means of an ad-hoc implementation of the Pan-Tompkins detector on lead II and then, a QRS template was used to align the beats, using a cross-correlation-based algorithm, to obtain a common fiducial point.
Fourth, lead quality was assessed using the average cross-correlation between the QRS template and the aligned QRS complexes. Leads with an average cross-correlation higher than 0.9 were considered of enough quality and then further analyzed.

The V-index was computed on ECG signals having at least three high quality leads (out of 12) by means of an iterative numerical algorithm previously validated [10]. Briefly, the algorithm estimated alternatively the lead factors (i.e., $\mathbf{w_1}$ and $\mathbf{w_2}$) and $T_d(t)$ on each T-wave, using a discretized version of the Eulero-Lagrange equations. Then, the standard deviations (std) in eq. (3) were computed on each lead i, over the different beats, leading to a V-index value for each high-quality lead. We considered their average as an overall estimate of the V-index.
Since each diagnostic ECG recording lasted only 10 s, the number of beats available for the V-index computation was rather limited (please refer to section 2.4 for further considerations on this issue). To increase the robustness of the estimates in a specific time-point, we averaged the V-index values obtained from each of the three ECG replica for that time point. While the V-index was found not to depend on the heart rate [13], the three values were averaged only when the corresponding mean RR did not differ more than 50 ms (i.e., the heart rate was stable).

2.4. Validation of V-index on short recordings: synthetic signals

In previous literature, the V-index has been computed using as few as 41 cardiac beats [18]. To assess the reliability of the V-index as estimated on short recordings (i.e., the few beats available in 10 s), we first performed synthetic simulations using a re-implementation of the forward ECG model on which ECGSIM is based [20].

The model was composed by two 3D geometries (meshes) for heart (its surface was discretized into 257 nodes) and torso, plus the bio-electrical model. The myocytes associated with a given node m were lumped together and shared the same transmembrane potential (TMP). Virtual electrodes were placed on the torso mesh, by considering the 12 nodes closest to the position of the electrodes in the standard (Mason-Likar) ECG lead configuration. The electrical model linking the TMP and surface ECG was defined as follows:

$$\mathbf{\Psi}(\mathrm{t}) = A \begin{bmatrix} D(t-\rho_1) \\ D(t-\rho_2) \\ \dots \\ D(t-\rho_M) \end{bmatrix} \tag{4}$$

where $\mathbf{\Psi}(t)$ is the 12-lead surface ECG, t the time, A the 12x257 transfer matrix, $D(t-\rho_m)$ the TMP of node m, whose repolarization time ρ_m was approximately set as the time of its maximum negative slope [10].

In this context, $\boldsymbol{w_1}$ and $\boldsymbol{w_2}$ could be theoretically computed using

$$\begin{aligned} \boldsymbol{w_1} &= -A\Delta\boldsymbol{\rho} \\ \boldsymbol{w_2} &= \tfrac{1}{2}A\Delta\boldsymbol{\rho}^2 \end{aligned} \tag{5}$$

where $\Delta\boldsymbol{\rho}$ is a vector containing the repolarization delays $\Delta\rho_m$ (please refer to Eq.2) for each node m [10].

Ten-second twelve-lead ECGs were simulated by varying both s_θ, i.e., changing $\Delta\boldsymbol{\rho}$ (with $\sigma_\phi = 1$ ms) and the heart rate in the range 10 to 70 ms and 40 to 120 bpm, respectively. For each beat in a synthetic ECG, the theoretical value of $\boldsymbol{w_1}$ and $\boldsymbol{w_2}$ were computed using Eq.5, whereas their estimates $\widehat{\boldsymbol{w}}_\mathbf{1}$ and $\widehat{\boldsymbol{w}}_\mathbf{2}$ were determined using the numerical algorithm described in sec. 2.3. The theoretical and estimated V-index were then computed for the specific ECG by plugging $(\boldsymbol{w_1}, \boldsymbol{w_2})$ and $(\widehat{\boldsymbol{w}}_\mathbf{1}, \widehat{\boldsymbol{w}}_\mathbf{2})$ into Eq.3. Afterwards, other two ten-second twelve-lead ECG replicas were generated, and the V-index computed accordingly. The three V-index values (either theoretical or estimated) were then averaged to obtain the final V-index value (exactly as done on real data, please refer to Section 2.3). The process was repeated 30 times to reach statistical consistency. The difference between the estimated and theoretical values was assessed, and its mean and standard deviation were used to describe the error distribution.

Since the experiment aimed to understand the impact of using a limited number of beats on the V-index computation, we compared the error distribution with one computed using 300 beats (hereafter called reference). To obtain the reference values, the procedure above was repeated (using thirty different simulations for each s_θ), but longer synthetic signals were generated (300 beats).

2.5. Validation of V-index on short recordings: ECG data from a TQT study

We further verified the feasibility of the computation of the V-index on short recordings by analyzing a portion of the ECGs collected during a Thorough-QT- Study (TQT), when the subjects are supposed to be mostly at rest. The data in the E-HOL-12-0140-008 dataset from the Telemetric and Holter ECG Warehouse (THEW) were used. The set contained 24 h digital Holter recordings (12 standard leads, sampling frequency: 1 kHz, LSB: 3.75 μV), enrolled in a TQT study (only the placebo and moxifloxacin arms were made available). For this study we analyzed the placebo arm. In particular, we considered

the ECG signals collected in the 20-min before the administration of the placebo and we further analyzed only the first 300 stable beats, to be coherent with the simulations performed in section 2.4 (the criteria employed to consider a beat as "stable" were previously described in [14]). On these 300 beats, the mean HR and the V-index were computed and considered as reference value. Then, the V-index was also computed on each of three sets, each containing a number of beats (out of the 300) accounting to 10-seconds of ECG (given the HR). Similarly to our study protocol, the three V-index values were averaged together.

Finally, the V-index obtained from the 300 beats was compared to the one derived from the three 10-seconds sets, using a Bland-Altman plot. Out the 68 subjects contained in the dataset, only 26 displayed 300 stable beats in the segment under analysis and were thus considered for the study.

2.6. Statistical analysis

A one-way repeated-measures Friedman test was performed to compare the parameters over time. When the Friedman test was statistically significant ($p<0.05$), a paired Wilcoxon test with Holm's correction was applied. A paired t-test or Wilcoxon test was also used to compare drugs and placebo parameters at the same time point. One-way ANOVA was used to test whether the reference error was different than the other errors, at a specific s_ϑ.

p values smaller than 0.05 were considered statistically significant in all cases. All analyses and statistical tests were performed using MATLAB R2017b (The MathWorks).

3. Results

3.1 Validation of the V-index on simulated signals

Figure 1 shows the results obtained from the analysis of the simulated data: the average difference (Δ) between the "true" V-index value and its estimates is shown, obtained considering different numbers of beats and different values of s_ϑ. First, for large values of s_ϑ, the numerical method led to significant Δ values. This is likely due to limiting the approximation in eq. (1) to the second order (i.e., using two lead factors), which breaks down with high spatial heterogeneity of ventricular repolarization [20]. On the contrary, for $s_\vartheta \leq 60$ ms, the average error was below 5 ms. Second, the standard deviation of Δ decreased at higher heart rate (equivalent to a higher number of beats).

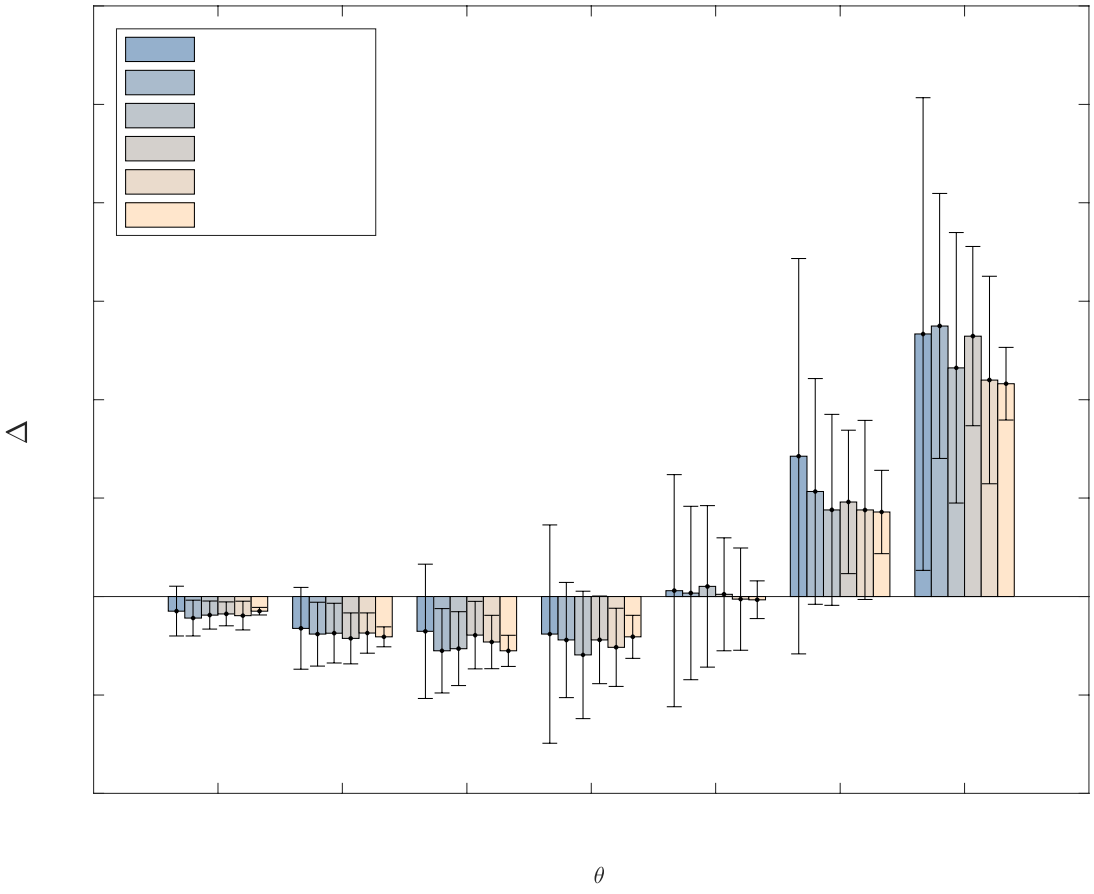

Figure 1: Average and standard deviation of the error between the "true" V-index and its estimated value. Each bar corresponds to a different number of beats used for the estimation, whereas each group of bars is for a specific s_ϑ value.

Third, when comparing the estimates performed on short (3 times 10 s) and long synthetic ECG segments (300 beats) at a given value of s_ϑ, there was no significant difference (across heart rate

values) in the average error with respect to reference (ANOVA test ns), while the variance was larger. These results suggest that the computation of the V-index on short recordings, using three 10 s replicas, might be feasible.

3.2 Validation of the V-index on ECG coming from a TQT study

Figure 2(a) shows the Bland-Altman plot comparing the V-index obtained from the longer (300 beats) reference ECG recordings with the one derived from the triplicate 10-second ECGs (both extracted from a TQT study). It can be observed that there is no bias between the two estimates ($p<0.05$). Only two subjects had a difference slightly outside the 95% confidence interval. Figure 2(b) shows the values of average V-index versus the reference V-index. It can be noted they are highly correlated: the Pearson's correlation coefficient between the two sets of V-index values was 0.83, with a residual error of -1.6 ± 5.4 ms. Also, figure 2(c) shows the difference between the two estimates as a function of heart rate. The difference was not significantly different than zero in any bin ($p > 0.05$).

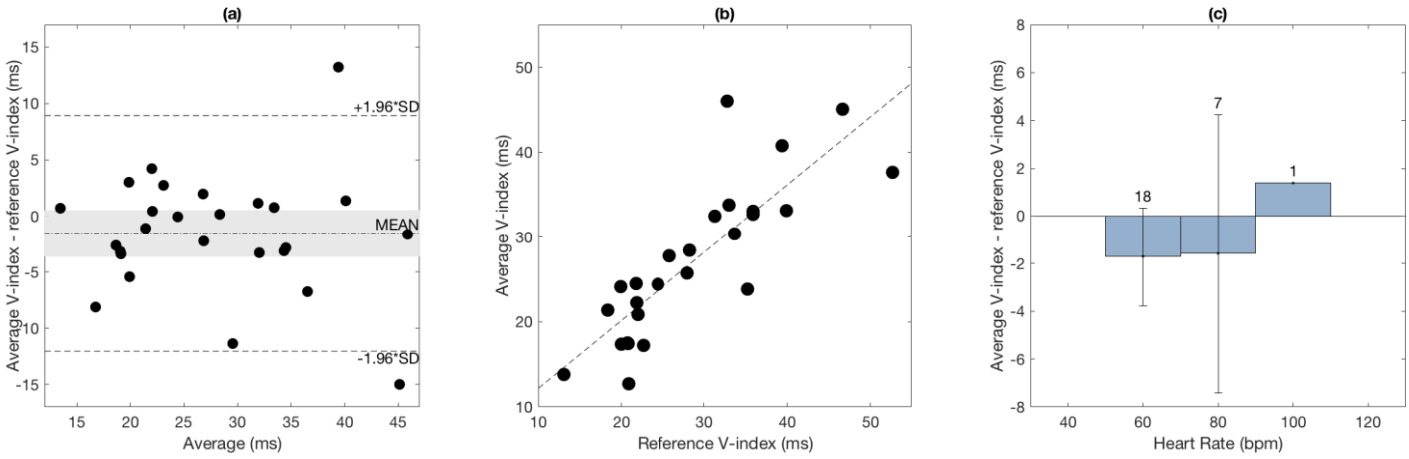

Figure 2: (a) Bland-Altman plot comparing the V-index derived from triplicate 10-second ECGs (average V-index) with the one obtained from reference ECG recordings (reference V-index, 300 beats long), both extracted from a TQT study. Each dot represents a subject and the grey band is the 95% standard error of the mean. (b) Values of average V-index versus reference V-index. Each dot represents a subject and the dashed line is the regression line. (c) Difference between the two estimates, as a function of heart rate, using 20-bpm wide bins. The error bar represents the 95%

standard error of the mean. The label above each bar indicates the number of subjects with that heart rate.

3.3 Spatial heterogeneity of ventricular repolarization estimated using the V-index after drug administration

The heart rate in the three 10 s ECG collected at each time point was generally stable, with variations between different ECGs in the mean RR of 32.8 ± 18.8 ms. The percentage of segments excluded due to an unstable heart rate was therefore very limited (4.9% of the total).

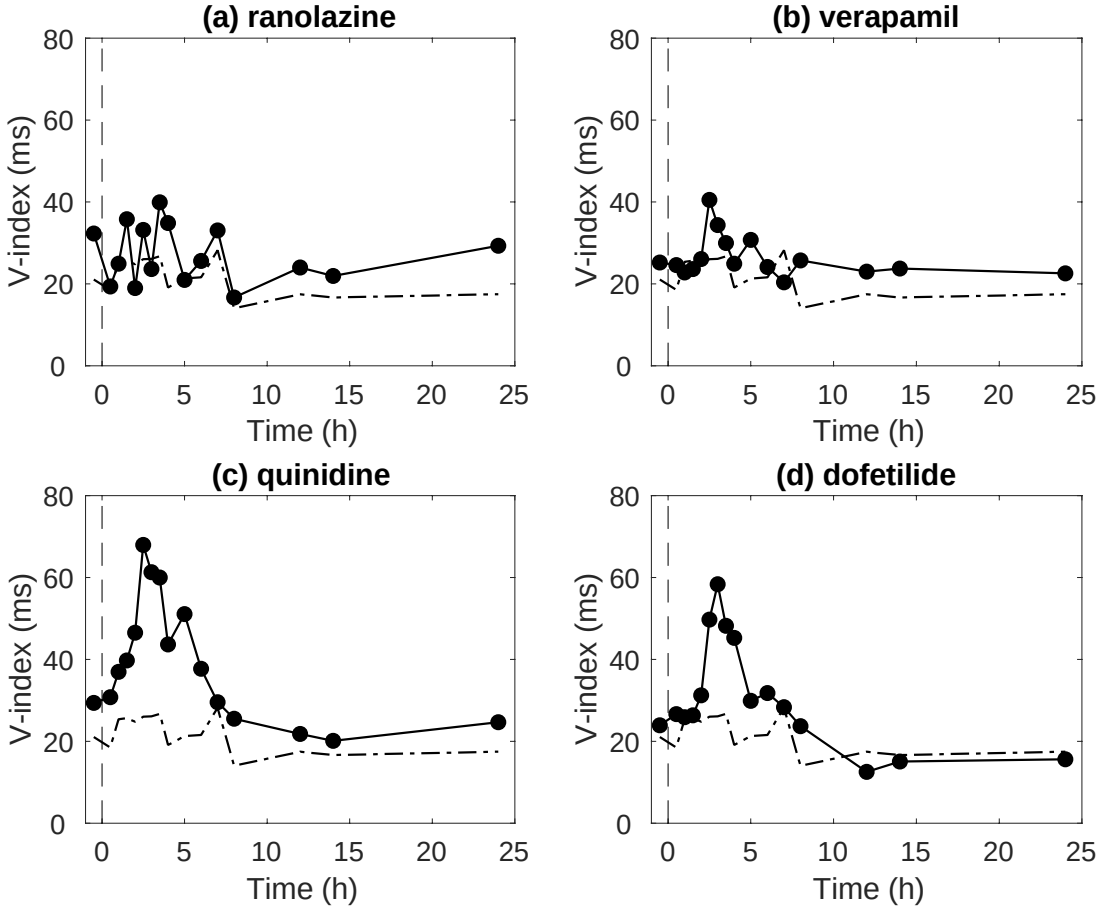

Figure 3: Evolution of V-index values after administration of the different drugs included in the protocol and placebo, for subject 1009: (a) ranolazine, (b) verapamil, (c) quinidine, (d) dofetilide. In each panel, the dash-dot line is the trend obtained after placebo. The dashed vertical line marks the time of drug administration.

Figure 3 shows the trend of the V-index during the administration of the different drugs and the placebo, in one of the subjects. It can be observed that the effects induced on SHVR by both ranolazine and verapamil are undistinguishable from placebo. On the contrary, after quinidine and dofetilide administration, an increase of V-index from predose to its peak value is observed, varying from 29 to 68 ms and from 24 to 58 ms, respectively. The V-index peak value appeared 2.5 and 3 hours after administration of quinidine and dofetilide, respectively.

These results are confirmed on the whole population, as shown in Figure 4. In fact, also in the aggregated results, the administration of ranolazine and verapamil did not lead to a significant increase of V-index, as instead occurred after both quinidine and dofetilide. Moreover, from the average concentration of each drug (also shown in picture), it can be observed that the V-index reached its peak at the same time of peak serum concentration for quinidine and dofetilide. In particular, comparing the V-index value obtained at each of the time points with the corresponding predose value, a significant ($p<0.05$) increase can be observed already after 1 h of quinidine, and about after 1.5 h of dofetilide administration.

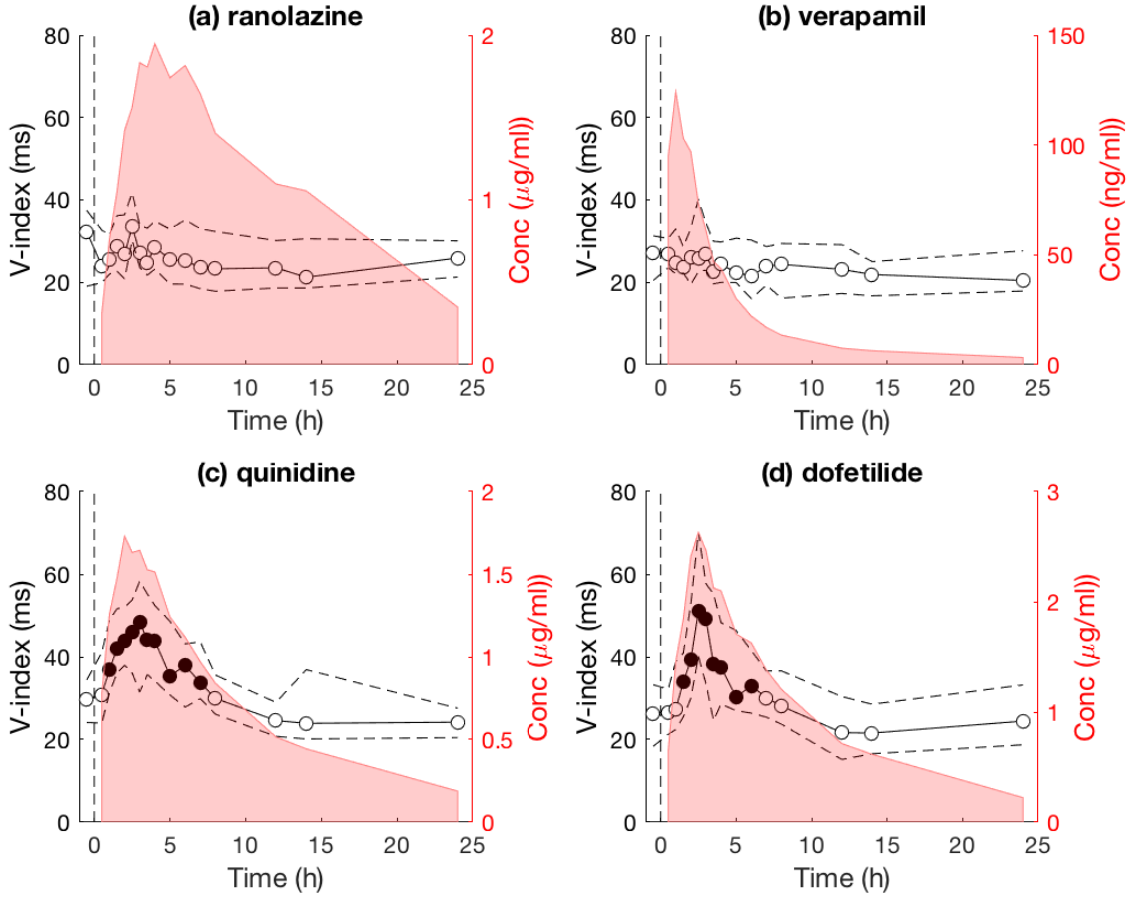

Figure 4: Evolution of the median (solid line) and 25th and 75 percentiles (dashed lines) values of V-index, computed across subjects at each time point, after administration of the different drugs included in the protocol: (a) ranolazine, (b) verapamil, (c) quinidine, (d) dofetilide. The dashed vertical line marks the time of drug administration. In each subplot, the average plasma concentration (Conc) is superimposed (red area). Black dots tag values significantly different from predose ($p<0.05$).

In order to consider the inter- and intra-subject variability, as well as the circadian rhythm, in the evaluation of the effects of the drug on V-index over time, the ΔΔ operator was applied [9], i.e., a time matched difference between the drug and placebo arms (each preliminary corrected by subtracting the respective predose values) was computed for all subjects independently. Figure 5 shows the trend over time of the ΔΔV-index (median and IQR), for each of the drugs. The V-index significantly increased ($p<0.05$) only after quinidine and dofetilide administration.

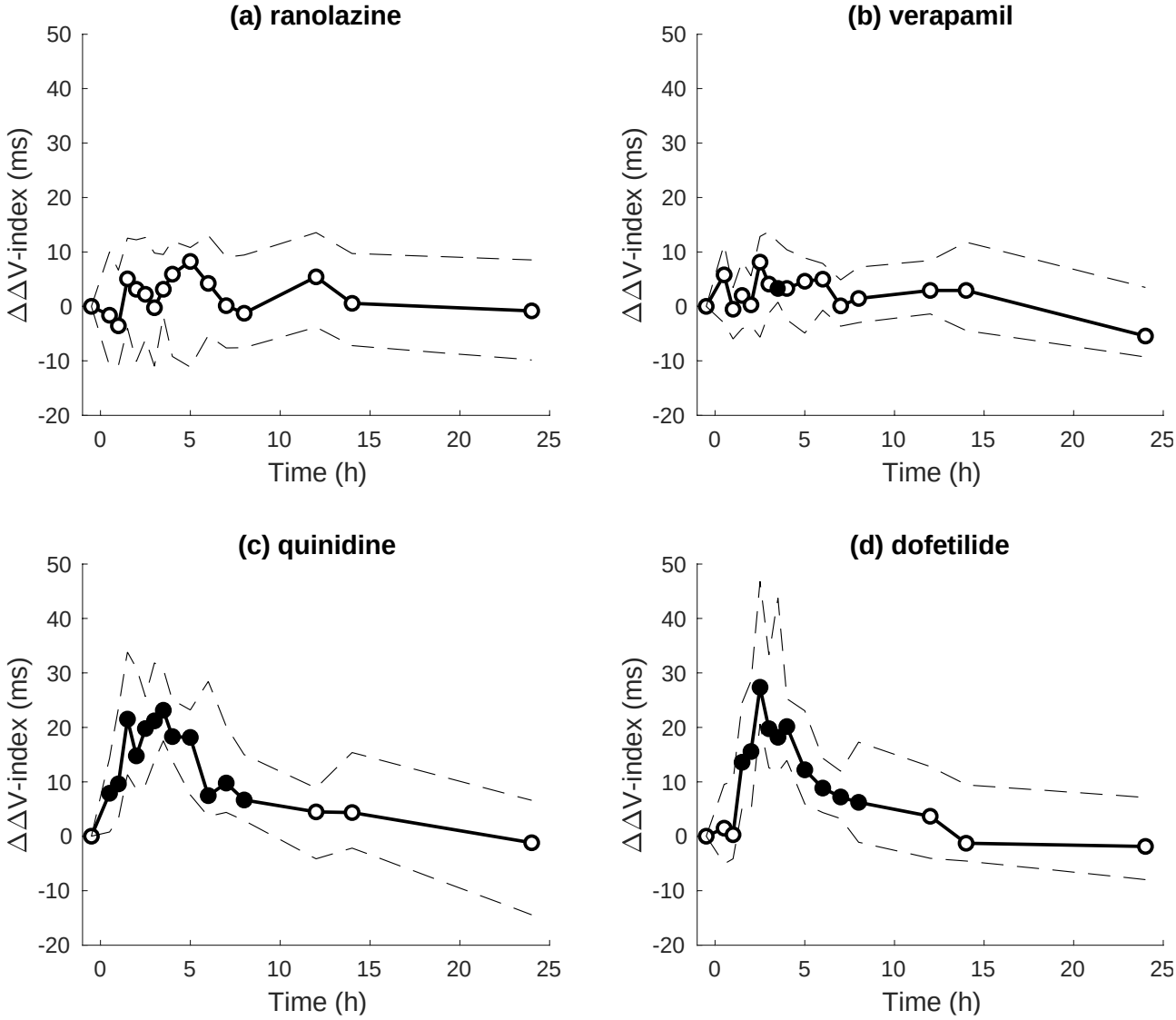

Figure 5: Evolution of the median (bold solid line) and 25th and 75 percentiles (solid lines) values of the ΔΔV-index (see text for details), computed across subjects at each time point, after administration of the different drugs included in the protocol: (a) ranolazine, (b) verapamil, (c) quinidine, (d) dofetilide. Black dots tag values significantly ($p < 0.05$) different from zero.

Table I shows ΔΔ values for the V-index and the three other parameters made available with the dataset (QTc, J-Tpeak corrected, Tpeak-Tend), computed at peak plasma concentration for each subject. First, the variation displayed by the V-index was statistically significant ($p<0.01$) only for quinidine and dofetilide, the two most high torsadogenic drugs, and not for the multichannel blocker ranolazine or verapamil ($p>0.05$). The corrected J-Tpeak behaved similarly, while QTc and Tpeak-Tend differed from placebo for all drugs. The results are in line with what reported for QTc,

J-Tpeak corrected and Tpeak-Tend by [6,7], with the exception that QTc [6,7] and Tpeak-Tend [6] did not significantly changed after verapamil, likely due to the different statistical test employed (parametric there vs non-parametric in here). The changes were marginal also in our results, however.

Table I: Median ΔΔ values at drug's peak plasma concentration. The values within brackets are the 25th percentile and 75th percentile. The asterisk (*) marks those values which are significantly different from zero (p<0.05).

	Ranolazine	Verapamil	Quinidine	Dofetilide
ΔΔV-index (ms)	1 (-10 – 11)	3 (-4 - 5)	19 (11 - 26) *	27 (14 - 47) *
ΔΔQTc (ms)	6 (2-15) *	8 (1-11) *	80 (66-86) *	69 (59-89) *
ΔΔJ-Tpeak corrected (ms)	0 (-5 - 10)	0 (-7 – 9)	36 (15 – 49) *	43 (17 – 48) *
ΔΔTpeak-Tend (ms)	7 (3 – 11) *	4 (2 - 6) *	36 (23 – 61) *	28 (20 – 41) *

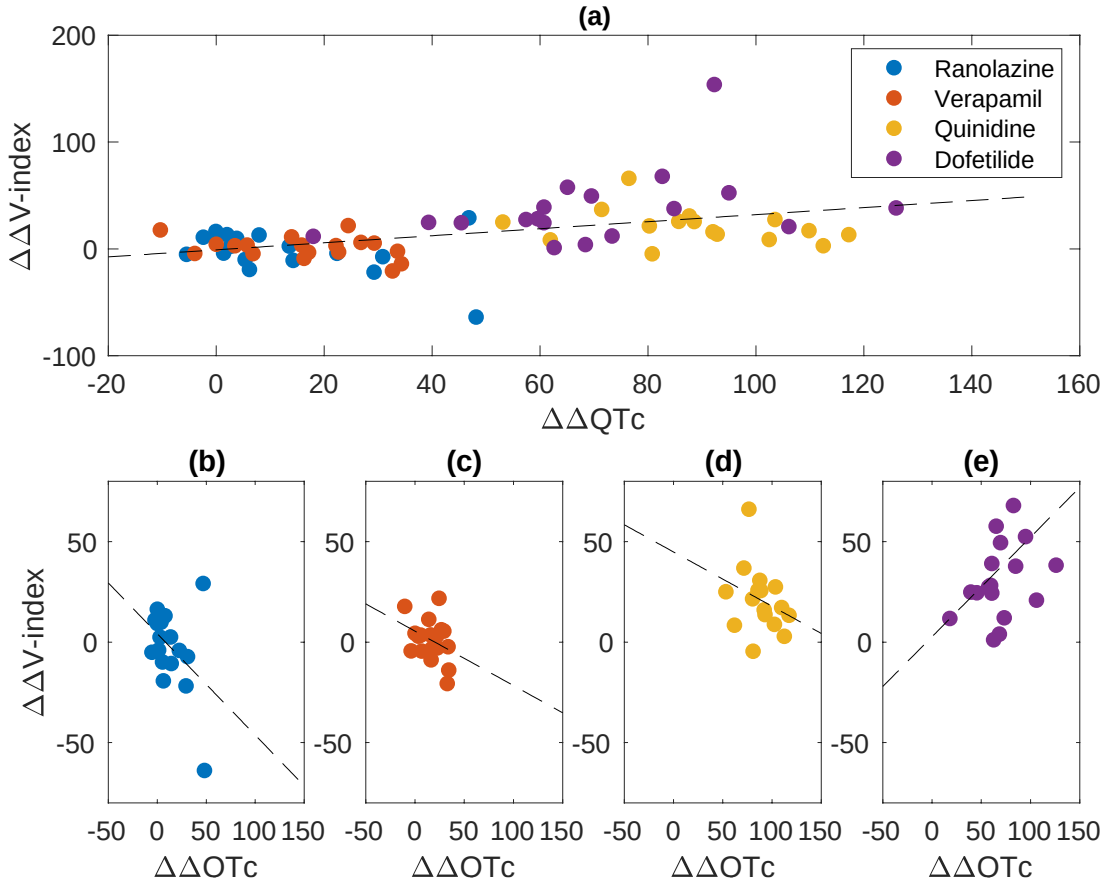

Figure 6: (a) ΔΔV-index as a function of ΔΔQTc (a single dot corresponds to one patient): a low significant correlation can be observed (p < 0.05). No significant correlation (p > 0.05) can be found for ranolazine (b), verapamil (c), quinidine (d) and dofetilide (e).

Second, Vicente et al. reported [7] the following average 5-day predose values (in ms, mean ± SD): 395.9 ± 17.1 for QTc, 225.6 ± 19.8 for J-Tpeak corrected and 73.1 ± 6.4 for Tpeak-Tend. The corresponding average predose value for the V-index was 29.5 ± 11.4 ms. As a consequence, using the data of Table I, the ratios between the ΔΔ value at drug's peak plasma concentration with respect to the predose value were: 64.4% (V-index), 20.2% (QTc), 15.9% (J-Tpeak corr.) and 49.3% (Tpeak-Tend) for quinidine and 91.5% (V-index), 17.4% (QTc), 19.0% (J-Tpeak corr.) and 38.4% (Tpeak-Tend) for dofetilide.

Third, Figure 6a reports the ΔΔV-index as a function of ΔΔQTc for each patient and drug; values were obtained at maximum serum concentration of the drug, as the results in Table I. A low correlation can be observed when all the drugs are considered together (0.46, $p<0.05$). While this correlation might be dictated by the relation that both QTc and V-index have with SHVR, it could as well merely be a sort of "batch effect" of the different drugs on QTc and V-index measurements. In fact, considering the ΔΔV-index as a function of ΔΔQTc on a drug-by-drug basis (thus removing the "batch effect"; Figures 6b-e), this correlation disappears.

Finally, the Spearman correlation between the V-index and each drug's serum concentration showed a high and significant correlation for quinidine and dofetilide (0.64 ± 0.23 and 0.66 ± 0.26, respectively, meaning that a higher concentration is reflected on higher V-index values), while the correlation with ranolazine and verapamil was poor or weak (0.15 ± 0.33 and 0.31 ± 0.33, respectively). Moreover, following the methodological suggestion of [21], we assessed the Spearman correlation between the V-index and RR value at predose, and a negligible correlation was found, independently of the drug. The lack of correlation confirmed that there was no need of correcting the V-index values for the heart rate, as in fact we did in our analysis. Furthermore, we

assessed the Spearman correlation between the V-index and the QTc at predose, and no correlation was found.

4. Discussion

In this study, for the first time, we computed the V-index on very short recordings (10 seconds), both on simulated and real data. In particular, our results showed that the estimate of spatial heterogeneity of ventricular repolarization assessed with the V-index, using a lower number of beats (as those included in three 10 s diagnostic ECGs) than what done in previous studies, was reasonably comparable to what obtained using longer recordings (no significant differences in the mean values, even if at the expenses of a larger variance of the estimates). Therefore, it was meaningful to compute the V-index on the data obtained from the prospective randomized controlled clinical trial funded by the FDA and described in section 2.1, for which only short 10 s recordings were available.

In our analysis, we found a significant increase of the V-index after administration of either dofetilide or quinidine. On the contrary, no significant change of the V-index was associated with administration of verapamil or ranolazine. Thus, the V-index was found to increase only with drugs associated to torsade risk. Dofetilide, being a pure hERG potassium channel blocker, has a direct relationship with torsade risk. Quinidine is not only a strong hERG potassium channel blocker, but it also stops calcium and sodium currents at high concentrations [15], and it has been shown to provoke torsade at low concentrations [22]. We observed no difference between quinidine and dofetilide in terms of ΔΔV-index values at peak plasma concentration. Thus, the V-index increase seems to be not dependent on the single or multiple channel block, but on a predominant potassium channel block (and not on balanced ion channel block as described in [23]). As such, it is difficult to say if quinidine administration at the given dose produced a lower dispersion of the ventricular repolarization than dofetilide, just because it blocks multiple channels. Indeed, according to Vicente

et al. [15], quinidine blocks about 71% of the potassium channel while dofetilide about 55%. It might be the case that the concurrent blocks of both calcium and late sodium currents reduced the spatial heterogeneity of ventricular repolarization, making quinidine and dofetilide's effects comparable, but this needs to be verified further. Verapamil and ranolazine, blocking L-type calcium channel and the late sodium current, respectively, are associated with a low risk of torsade [24], likely because they balance inward and outward current blocks. Previous studies on the same dataset tried to characterize the effects of these drugs. Johannesen et al. [6] hinted that by separating the QT interval duration into early repolarization (J-Tpeak) and late repolarization (Tpeak-Tend), it was possible to differentiate between a pure hERG potassium channel blocker drug with high torsade risk (blocking only the potassium channels, as dofetilide) from drugs that also stop inward currents (calcium or late sodium) during repolarization. Dofetilide was found to prolong both J-Tpeak and Tpeak-Tend. Both ranolazine and quinidine prolonged QTc and Tpeak-Tend, whereas only quinidine, that is associated with risk of torsade, prolonged also J-Tpeak. Thus, ranolazine that is associated with a low risk of torsade, was still associated with a prolonged QTc.

The fact that ranolazine significantly prolonged QTc of about 6 ms at peak plasma concentration, while V-index did not change, might be due to a lack of sensitivity. However, this is not likely to be the case. In fact, in [14] a significant V-index increase was observed after administration of moxifloxacin, an active comparator used in TQT studies with a QTc prolongation of about 10 ms. Therefore, it is possible that ranolazine slightly prolonged ventricular repolarization without significantly altering its spatial heterogeneity.

In fact, according to the mathematical theory on which the V-index was derived, at first order, the T-wave (and its position in time) is dictated (mainly) by the average time of repolarization of the myocytes and by the heterogeneity of repolarization (dispersion around the average repolarization

time). When assessing repolarization using the QT interval, these factors are captured together. The V-index is instead only dependent on the second (repolarization heterogeneity). Being both the QT and the V-index dependent on prolongation at myocytes' transmembrane potentials, there are situations in which they both increase. The advantage of using the V-index is however that we focalize on changes in repolarization heterogeneity only (if all repolarization times are simply translated forward in time, the V-index is not affected).

A dedicated session was organized at the International Society for Computerized Electrocardiology (ISCE) congress in 2017 ("JTpeak initiative for the ISCE 2017 meeting"), aimed at analyzing the J-Tpeak interval on the same data we analyzed in this work. In particular, different algorithms designed to measure the heart-rate corrected J-Tpeak interval from standard 12-lead ECGs were compared. Such investigation was promoted by the Comprehensive in vitro Proarrhythmia Assay (CiPA) initiative, supported by FDA, HESI, CSRC, SPS, EMA, Health Canada, Japan NIHS, and PMDA. CiPA aims to shift the emphasis away from QT prolongation and focuses on predicting torsadogenic hazard at an early stage of drug development [25]. While some studies [9,26] hinted that the corrected J-Tpeak interval prolongation might be technology-independent and well differentiate among drugs, others showed differences between methods [27,28]. However, the clear point was that early or late repolarization (as measured by prolonged J-Tpeak and Tpeak-Tend, but also, by significant changes in T-wave morphology) not only alter the entire duration of ventricular repolarization, but also its spatial heterogeneity, which, in the present work, we assessed by the V-index.

T-wave morphology was also quantified in this dataset [7], showing that both a pure hERG potassium channel blocker drug, dofetilide, and multichannel blocking drugs, quinidine and ranolazine, changed T-wave characteristics. Thus, changes in T-wave morphology were associated

to hERG block, being it pure or associated to other ion channel blocks (still overestimating the risk connected to ranolazine). In another study [8] on different data, the ability of eight ECG morphology biomarkers was assessed to detect late sodium current block in the presence of QTc prolongation. It was showed that J-Tpeak was the biomarker most predictive of the presence of inward current block, making this index useful to confirm its presence. However, the computation of J-Tpeak can be critical (for example due to differences in the definition of the peak of the T-wave, in the presence of notched and flat T-waves). In this respect, V-index has the advantage of being only marginally affected by misdetection of T-waves fiduciary points: only the time window containing the T-wave has to be identified, with no need to find the precise T-wave fiduciary points. Also, while the V-index and J-Tpeak corrected both significantly changed after quinidine and dofetilide administration, the relative change with respect to predose of the V-index was larger (but also the coefficient of variation of the V-index estimator is larger [14]). Summarizing the results obtained on the FDA funded clinical trial, QTc misclassified the risk associated to ranolazine [6], while J-Tpeak was less sensitive to this error [8]. However, J-Tpeak computation shares similar issues to the QTc because based on fiducial points. The V-index correctly classified the risk associated with these drugs, and its computation was not affected by the proper location of the ECG fiducial points, making it a good candidate marker for detecting the risk of torsade.

To conclude, rather than estimating the duration of the repolarization, the V-index, differently than QTc, is an attempt to perform a direct estimate of the standard deviation of the repolarization times of the ventricular myocytes. In this regard, V-index assessment made sense in the chosen assay, and our results further corroborate the evidence that both dofetilide and quinidine prolong the entire duration of the ventricular repolarization, as well as increase its spatial heterogeneity. In addition, the work was also in line with the CiPA's vision of shifting the emphasis away from QT prolongation, towards a more effective evaluation of torsadogenic risk for drug development.

5. Conclusions

The V-index, assessing the spatial heterogeneity of ventricular repolarization, has been computed for the first time on triplicate 10 s segments, with simulated and real data results supporting its use on short recordings. After drug administration, the V-index was found to increase only with drugs associated to torsade risk, encouraging its use in detecting the risk of torsade of antiarrhythmic drugs.

Acknowledgement

Some of the data used for this research were provided by the Telemetric and Holter ECG Warehouse of the University of Rochester (THEW), NY.

References

[1] C.T. January, J.M. Riddle, Early afterdepolarizations: mechanism of induction and block. A role for L-type Ca2+ current., Circ. Res. 64 (1989) 977–990. doi:10.1161/01.RES.64.5.977.

[2] N. Stockbridge, J. Morganroth, R.R. Shah, C. Garnett, Dealing with Global Safety Issues, Drug Saf. 36 (2013) 167–182. doi:10.1007/s40264-013-0016-z.

[3] M.C. Trudeau, J.W. Warmke, B. Ganetzky, G.A. Robertson, HERG, a human inward rectifier in the voltage-gated potassium channel family, Science. 269 (1995) 92–95. doi:10.1126/science.7604285.

[4] L. Wu, S. Rajamani, J.C. Shryock, H. Li, J. Ruskin, C. Antzelevitch, L. Belardinelli, Augmentation of late sodium current unmasks the proarrhythmic effects of amiodarone, Cardiovasc. Res. 77 (2008) 481–488. doi:10.1093/cvr/cvm069.

[5] T. Aiba, W. Shimizu, M. Inagaki, T. Noda, S. Miyoshi, W.-G. Ding, D.P. Zankov, F. Toyoda, H. Matsuura, M. Horie, K. Sunagawa, Cellular and ionic mechanism for drug-induced long QT syndrome and effectiveness of verapamil, J. Am. Coll. Cardiol. 45 (2005) 300–307. doi:10.1016/j.jacc.2004.09.069.

[6] L. Johannesen, J. Vicente, J.W. Mason, C. Sanabria, K. Waite-Labott, M. Hong, P. Guo, J. Lin, J.S. Sørensen, L. Galeotti, J. Florian, M. Ugander, N. Stockbridge, D.G. Strauss, Differentiating drug-induced multichannel block on the electrocardiogram: Randomized study of dofetilide, quinidine,

ranolazine, and verapamil, Clin. Pharmacol. Ther. (2014). doi:10.1038/clpt.2014.155.
[7] J. Vicente, L. Johannesen, J.W. Mason, W.J. Crumb, E. Pueyo, N. Stockbridge, D.G. Strauss, Comprehensive T wave morphology assessment in a randomized clinical study of dofetilide, quinidine, ranolazine, and verapamil, J. Am. Heart Assoc. (2015). doi:10.1161/JAHA.114.001615.
[8] J. Vicente, L. Johannesen, M. Hosseini, J.W. Mason, P.T. Sager, E. Pueyo, D.G. Strauss, Electrocardiographic biomarkers for detection of drug-induced late sodium current block, PLoS ONE. (2016). doi:10.1371/journal.pone.0163619.
[9] J.P. Couderc, S. Ma, A. Page, C. Besaw, J. Xia, W.B. Chiu, J. de Bie, J. Vicente, M. Vaglio, F. Badilini, S. Babaeizadeh, C. hao S. Chien, M. Baumert, An evaluation of multiple algorithms for the measurement of the heart rate corrected JTpeak interval, J. Electrocardiol. (2017). doi:10.1016/j.jelectrocard.2017.08.025.
[10] R. Sassi, L.T. Mainardi, An estimate of the dispersion of repolarization times based on a biophysical model of the ECG, IEEE Trans. Biomed. Eng. (2011). doi:10.1109/TBME.2011.2166263.
[11] A. van Oosterom, Genesis of the T wave as based on an equivalent surface source model, J. Electrocardiol. 34 Suppl (2001) 217–227.
[12] R. Sassi, M.W. Rivolta, L.T. Mainardi, R.C. Reis, M.O.C. Rocha, A.L.P. Ribeiro, F. Lombardi, Spatial repolarization heterogeneity and survival in Chagas disease, Methods Inf. Med. 53 (2014) 464–468. doi:10.3414/ME14-01-0002.
[13] R. Abächerli, R. Twerenbold, J. Boeddinghaus, T. Nestelberger, P. Mächler, R. Sassi, M.W. Rivolta, E.K. Roonizi, L.T. Mainardi, N. Kozhuharov, M. Rubini Giménez, K. Wildi, K. Grimm, Z. Sabti, P. Hillinger, C. Puelacher, I. Strebel, J. Cupa, P. Badertscher, I. Roux, R. Schmid, R. Leber, S. Osswald, C. Mueller, T. Reichlin, Diagnostic and prognostic values of the V-index, a novel ECG marker quantifying spatial heterogeneity of ventricular repolarization, in patients with symptoms suggestive of non-ST-elevation myocardial infarction, Int. J. Cardiol. (2017). doi:10.1016/j.ijcard.2017.01.151.
[14] M.W. Rivolta, L.T. Mainardi, R. Sassi, Quantification of ventricular repolarization heterogeneity during moxifloxacin or sotalol administration using {V}-index, Physiol. Meas. (2015). doi:10.1088/0967-3334/36/4/803.
[15] J. Kramer, C.A. Obejero-Paz, G. Myatt, Y.A. Kuryshev, A. Bruening-Wright, J.S. Verducci, A.M. Brown, MICE models: Superior to the HERG model in predicting torsade de pointes, Sci. Rep. (2013). doi:10.1038/srep02100.
[16] A.L. Goldberger, L.A. Amaral, L. Glass, J.M. Hausdorff, P.C. Ivanov, R.G. Mark, J.E. Mietus, G.B. Moody, C.K. Peng, H.E. Stanley, PhysioBank, PhysioToolkit, and PhysioNet: components of a new research resource for complex physiologic signals, Circulation. 101 (2000) E215-220.
[17] K. Hnatkova, L. Johannesen, J. Vicente, M. Malik, Heart rate dependency of JT interval sections, 2017. doi:10.1016/j.jelectrocard.2017.08.005.
[18] R. Sassi, L.T. Mainardi, P. Laguna, J.F. Rodriguez, Validation of the ??-index through finite element 2D simulations, in: Comput. Cardiol., 2013.
[19] A. van Oosterom, T.F. Oostendorp, ECGSIM: an interactive tool for studying the genesis of QRST waveforms, Heart. 90 (2004) 165–168. doi:10.1136/hrt.2003.014662.
[20] E.K. Roonizi, M.W. Rivolta, L.T. Mainardi, R. Sassi, A comparison of three methodologies for the computation of V-index, in: Comput. Cardiol., 2015. doi:10.1109/CIC.2015.7410980.
[21] M. Malik, C. Garnett, K. Hnatkova, J. Vicente, L. Johannesen, N. Stockbridge, Implications of Individual QT/RR Profiles—Part 2: Zero QTc/RR Correlations Do Not Prove QTc Correction Accuracy in Studies of QTc Changes, Drug Saf. (2019). doi:10.1007/s40264-018-0735-2.
[22] D.M. Roden, R.L. Woosley, R.K. Primm, Incidence and clinical features of the quinidine-associated long QT syndrome: implications for patient care, Am. Heart J. 111 (1986) 1088–1093.
[23] J. Vicente, R. Zusterzeel, L. Johannesen, J. Mason, P. Sager, V. Patel, M.K. Matta, Z. Li, J. Liu,

C. Garnett, N. Stockbridge, I. Zineh, D.G. Strauss, Mechanistic Model-Informed Proarrhythmic Risk Assessment of Drugs: Review of the “CiPA” Initiative and Design of a Prospective Clinical Validation Study, Clin. Pharmacol. Ther. (2018). doi:10.1002/cpt.896.
[24] W.S. Redfern, L. Carlsson, A.S. Davis, W.G. Lynch, I. MacKenzie, S. Palethorpe, P.K.S. Siegl, I. Strang, A.T. Sullivan, R. Wallis, A.J. Camm, T.G. Hammond, Relationships between preclinical cardiac electrophysiology, clinical QT interval prolongation and torsade de pointes for a broad range of drugs: Evidence for a provisional safety margin in drug development, 2003. doi:10.1016/S0008-6363(02)00846-5.
[25] T. Colatsky, B. Fermini, G. Gintant, J.B. Pierson, P. Sager, Y. Sekino, D.G. Strauss, N. Stockbridge, The Comprehensive in Vitro Proarrhythmia Assay (CiPA) initiative — Update on progress, J. Pharmacol. Toxicol. Methods. (2016). doi:10.1016/j.vascn.2016.06.002.
[26] F. Badilini, G. Libretti, M. Vaglio, Automated JTpeak analysis by BRAVO, J. Electrocardiol. (2017). doi:10.1016/j.jelectrocard.2017.07.010.
[27] W.B. Chiu, J. de Bie, D.W. Mortara, The J to T-peak interval as a biomarker in drug safety studies: A method of accuracy assessment applied to two algorithms, J. Electrocardiol. (2017). doi:10.1016/j.jelectrocard.2017.07.011.
[28] S.C. Chien, R.E. Gregg, The algorithmic performance of J-Tpeak for drug safety clinical trial, J. Electrocardiol. (2017). doi:10.1016/j.jelectrocard.2017.08.018.