Quinidine-Induced Microvolt Electrocardiographic Alternans

Quinidine-Induced Microvolt Electrocardiographic Alternans

Ilaria Marcantoni, *Member, IEEE*, Erica Iammarino, *Student Member, IEEE*, and Laura Burattini, *Member, IEEE*

***Abstract*— Quinidine is a class Ia antiarrhythmic agent, but it seems also associated with an increased risk of ventricular arrhythmia and sudden death. This study aims to evaluate whether the possible risk of arrhythmia due to quinidine can be confirmed and tracked over time on electrocardiograms (ECGs) acquired on a healthy population before and within 24 hours after quinidine administration. The study population belongs to the "ECG Effects of Ranolazine, Dofetilide, Verapamil, and Quinidine in Healthy Subjects" database. The ECG analysis was performed through the enhanced correlation method (ECM) to quantify ECG alternans (ECGA), defined as beat-to-beat fluctuation of ECG wave morphology and recognized in the literature as an index of arrhythmia risk. ECM identified all forms of ECGA, thus not only T-wave alternans (TWA) as in its original formulation, but also P-wave alternans (PWA) and QRS-complex alternans (QRSA), quantified by amplitude and magnitude. The results showed that quinidine induced an increase in ECGA within 6 hours after administration, in accordance with its elimination half-life, and then returned to baseline (pre-dose) values. Depending on the ECGA form and quantification feature, the maximum increase was observed two to four times the baseline value. Furthermore, ECGA magnitude seemed to reveal transient changes better than amplitude, resulting in more post-dose time points, especially in the range 3.5-7 hours after quinidine administration, at which ECGA was statistically different than at the pre-dose time point. Thus, ECGA appears to disclose the higher risk of arrhythmia associated with quinidine.**

***Clinical Relevance*— The present study provides a contribution to guide therapy involving quinidine through the analysis of its proarrhythmic risk by electrocardiographic alternans.**

I. Introduction

The electrocardiogram (ECG) is a time-dependent voltage plot of cardiac electrical activity. It provides information on the functional characteristics of the heart and plays a key role in the diagnosis and treatment of heart electrophysiological disorders and arrhythmias [1]. Indeed, cardiac electrophysiological processes occurring at the cellular level are reflected in electrocardiographic waveforms and intervals [2]. The main ECG elements that determine a heartbeat are the P wave, the QRS complex and the T wave. Within each heartbeat, different intervals are defined, such as the RR, QT, or PR one. Features related to intervals and waves may reflect irregularities, as well as the effect of drugs that restore or impair normal heart rhythm. Among the medications that are able to intervene on the heart rhythm there is quinidine.

Quinidine is a class Ia antiarrhythmic agent used to treat heart rhythm disturbances. Quinidine acts on sodium and potassium channels. In particular, among its main mechanisms of action, it inhibits the fast inward sodium current and reduces potassium efflux during repolarization. Although quinidine acts as an antiarrhythmic agent, it also carries a proarrhythmic risk, such as QT prolongation, which can potentially result in sudden cardiac death. After administration, the drug reaches the highest plasma concentration in approximately 2 hours. The current recommended dose for quinidine is 900 mg/day to 1.5 g/day. Elimination of quinidine occurs through a combination of renal excretion and hepatic biotransformation. In healthy people, the elimination half-life of quinidine is between 5 and 12 hours, but clearance is reduced in the elderly, patients with cirrhosis, and those with congestive heart failure [3-5].

Historically, quinidine was the first drug introduced for the treatment of heart arrhythmias and demonstrated to be able to restore sinus rhythm after conversion from atrial fibrillation or atrial flutter, and to prevent recurrence of ventricular tachycardia or ventricular fibrillation. Quinidine shows its antiarrhythmic efficacy in several pathologies, such as Brugada syndrome, idiopathic ventricular fibrillation and early repolarization syndrome. Moreover, it is able to normalize QT interval in patients suffering congenital short QT syndrome. Nevertheless, much evidence shows that quinidine treatment is associated with an increased risk of ventricular arrhythmia and sudden death. This evidence has led to the withdrawal of this drug from use and its unavailability in many countries. In addition, since quinidine is a drug that induces prolongation of the QT interval, in some cases, this effect can evolve into Torsades de Pointes [6, 7].

One of the ECG features related to arrhythmias is T-wave alternans (TWA). TWA, one of the possible forms of ECG alternans (ECGA) manifestation (and the most studied one), is a beat-to-beat fluctuation in T-wave morphology occurring at stable heart rhythm and is linked to vulnerability to life-threatening ventricular tachyarrhythmias. TWA has frequently been reported in patients with long QT syndrome, sometimes accompanied by Torsade de Pointes [8]. Indeed, TWA has become a prognostic tool for arrhythmia risk stratification and guidance of antiarrhythmic therapy [9]. In a wider perspective, ECGA can manifest also on the other waves of the ECG, so P wave (P-wave alternans, PWA) and QRS complex (QRS-complex alternans, QRSA), reflecting a possible arrhythmic risk in any form [10].

In this context, the aim of the present study is to assess whether the possible risk of arrhythmia due to quinidine

I. Marcantoni is with the Department of Information Engineering, Università Politecnica delle Marche, Ancona, Italy (corresponding author, phone: 071 2204465; e-mail: i.marcantoni@staff.univpm.it).

E. Iammarino and L. Burattini are with the Department of Information Engineering, Università Politecnica delle Marche, Ancona, Italy (e-mail: e.iammarino@pm.univpm.it; l.burattini@univpm.it).

intake, which has sometimes been detected, can be confirmed and tracked over time on ECG by ECGA. The analyzed population is healthy, and the ECG was acquired both before and after quinidine, thus assessing ECGA in resting condition unaffected by the drug and its trend in the 24 hours after administration of the drug.

II. Materials and Methods

The study population belongs to the "ECG Effects of Ranolazine, Dofetilide, Verapamil, and Quinidine in Healthy Subjects" (ECGRDVQ) database, created by Johannesen et al. to evaluate the effects induced by four QT-prolonging drugs on electrophysiological parameters [11]. The database is freely available on PhysioNet, a repository of medical research data, managed by the MIT Laboratory for Computational Physiology [12]. The population included healthy volunteers who were enrolled in an acquisition protocol involving the administration of quinidine.

The method used for the identification and quantification of ECGA possibly induced by the drug administration represents an updated and improved version of the correlation method (CM). The original CM [13] was implemented to quantify only TWA (as were all others present in the literature except for the enhanced adaptive matched filter method [10]), while the enhanced CM (ECM) was proposed to provide an overall assessment of ECGA. The details about CM implementation can be found in [13].

A. Database Description

The ECGRDVQ database resulted from a prospective randomized double-blind controlled clinical trial, in which 22 healthy volunteers participated. The enrollment criteria were: good general health determined by a clinician; no history of heart disease or unexplained syncope; no family history of long QT syndrome; 18-35 years of age; 50 kg of weight at least; 18-27 kg/m^2 of body mass index; no more than 10 ectopic heartbeats during a continuous 3-hours ECG at screening. Eventually, the enrolled study population had an age of 26.9 ± 5.5 years and a body mass index of 23.1 ± 2.6 kg/m^2 [11]. The clinical trial involved five 24-hour periods, each involving the administration of four known QT prolonging drugs (specifically, ranolazine, dofetilide, verapamil, and quinidine) and placebo. In the morning of each period, participants received, under fasting conditions, a single dose of 500 µg dofetilide, 400 mg quinidine sulfate, 1500 mg ranolazine, 120 mg verapamil hydrochloride or placebo. These periods were interspersed with a washout lasting 7 days. The present study focused on the quinidine administration period.

During the treatment, continuous 12-lead ECG was recorded (amplitude resolution: 2.5 µV; sampling frequency: 500 Hz; recorder: Surveyor, Mortara Instrument, Milwaukee, WI; electrode configuration: Mason–Likar). Then, three standard (10 s-long) ECGs were taken from the continuous tracing at sixteen predefined pre- and post-dose time-points (0.5 hours before the dose administration, and at 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 7, 8, 12, 14, 24 hours after the dose administration), during which participants were resting for 10 minutes in a supine position. These ECGs were selected on the basis of stable heart rate and good signal quality as assessed by Antares software (AMPS, New York, NY), resulting in 48 planned ECGs per participant and per treatment period. The resulting ECGs were up-sampled from 500 to 1000 Hz. For one participant (the second in the numbering of the database, which is maintained in this work), ECGs related to the period of quinidine administration were not available, so 21 participants were included in our study population and a total of 1008 raw digital ECGs were analyzed. Being a database coming from PhysioNet, all data were de-identified and do not need further independent ethics committee approval to be used for research purposes [14].

B. Preprocessing of Electrocardiograms

ECG recordings were analyzed in MATLAB R2024b. Before being processed for ECGA identification and feature-engineering-based quantification, a pre-processing step was performed on the raw ECGs. Each lead of the 10-s ECGs (henceforth referred to as the 'ECG tracing' and 'ECG recording', respectively) was filtered through a 6th-order bidirectional band-pass Butterworth filter with a low-frequency cut-off frequency of 0.3 Hz and a high-frequency cut-off frequency of 45 Hz. R-peak detection was followed by the application of the Pan-Tompkins algorithm, whose performance was optimized by adjusting the R-peak position on the local maximum searched in a 0.04-s window. Then, the baseline oscillation was linearly subtracted from the ECG tracing, where the baseline was previously reconstructed as a 3rd-order spline interpolation of fiducial points located 0.08 s before the R peak. The mean RR interval (mRR, ms) and heart rate variability (HRV, %) were calculated for each ECG recording, where HRV was calculated as standard deviation of RR intervals and expressed as a percentage of mRR.

C. Definition and Alignment of Electrocardiogram Sections

ECG main sections (P wave, QRS complex, T wave) landmarks were estimated by experimental formulas, often dependent from mRR (expressed in seconds).

- Referring to the P wave: for mRR shorter than 0.6 s, between 0.6 and 1.1 s, and longer than 1.1 s, P-wave onset (Pon) was located 0.18 s, 0.20 s, and 0.25 s before Qon, respectively, while the P-wave ending (Poff) was found $0.2 \cdot \sqrt{\text{mRR}}$ after the Pon.
- Referring to the QRS complex: the QRS onset (Qon) and QRS ending (J) were located 0.05 s before and after the R peak, respectively.
- Referring to the T wave: for mRR shorter than 0.6 s, between 0.6 and 1.1 s, and longer than 1.1 s, T-wave onset (Ton) was located 0.06 s, 0.10 s, and 0.15 s after R peak, respectively, while the T-wave ending (Toff) was found $0.35 \cdot \sqrt{\text{mRR}}$, $0.4 \cdot \sqrt{\text{mRR}}$, and $0.45 \cdot \sqrt{\text{mRR}}$ after the Ton for mRR shorter than 0.6 s, between 0.6 and 1.1 s, and longer than 1.1 s, respectively.

For each ECG tracing, the ECG sections were subjected to an alignment step. Specifically, after calculating the template ECG section as the median of all the ECG sections included in the ECG tracing, each ECG section was aligned with the template. The alignment procedure performed was based on correlation, calculated through Pearson's coefficient. Indeed, the alignment with the template corresponded with the maximum correlation found by moving the original landmarks, calculated through the experimental formulas described above, within a -0.03 s − +0.03 s window, keeping

the ECG-section length constant. In turn, the alignment of each ECG section with the same template results in the alignment of all ECG sections with each other.

D. Electrocardiographic Alternans Analysis by the Enhanced Correlation Method

ECM is a time-domain ECGA detection method based on a similarity index of each ECG wave with respect to a reference one. For each ECG tracing, the analysis procedure was applied in parallel for each ECG section (*i.e.*, for P-wave, QRS-complex and T-wave sections). Specifically, the ECM was fed with the set of P-wave/QRS-complex/T-wave sections coming out of the alignment phase. From each section, the median section was computed, and it was used to compute an alternans correlation index (ACI) as in (1):

$$ACI_j = \frac{\sum_{i=1}^{N_s} S_j(i) \cdot S_{mdn}(i)}{\sum_{i=1}^{N_s} {S_{mdn}}^2(i)}, \qquad (1)$$

where $j = 1 : N_T$ and N_T was the number of heartbeats in the ECG tracing, N_s was the number of samples in the section, S_j was the considered ECG section and S_{mdn} the median section.

ACI represented the maximum value of cross-correlation function of the single section against the template, over the maximum value of the auto-correlation function of the template. If ECGA was present and the sections did not change polarity, ACI fluctuated around 1, and PWA/QRSA/TWA was actually detected and quantified only if ACI fluctuated for at least five consecutive heartbeats. Originally, TWA detection by CM was arbitrarily based on the presence of at least seven consecutive alternating heartbeats, usually considering 128-beat ECG tracings; here, given the short duration (10 s) of ECG tracing, we modified this threshold by setting it to five, as done in a previous work analyzing the same study population to detect the dofetilide-induced TWA by CM [15].

Lastly, PWA/QRSA/TWA intra-heartbeat amplitude was quantified as A_{ECM}, where $A_{ECM} = A_{CM}$ (subscript refers to the method), and was computed according to (2):

$$A_{CM}(j) = 2 \cdot \left| ACI_j - 1 \right| \frac{\sum_{i=1}^{N_s} {S_{mdn}}^2(i)}{\sum_{i=1}^{N_s} |S_{mdn}(i)|}, \qquad (2)$$

A_{ECM} was quantified only for sections involved in alternating episodes; otherwise, it was set to zero. Thus, the value of A_{ECM} (different or equal to zero in case of presence or absence of ECGA, respectively) was assigned to each heartbeat and each form of alternans (PWA/QRSA/TWA). Summarizing, the ECM provided in output the PWA, QRSA, and TWA local amplitudes (A_{ECM}), so a quantification of alternans per heartbeat and per ECG tracing, expressed in μV.

E. Statistics

Within each ECG tracing, two features were computed from the output of the ECM (A_{ECM}): PWA/QRSA/TWA amplitude and magnitude. The amplitude was the mean local amplitude computed over all the heartbeats contained in the ECG tracing (μV). The magnitude was the multiplication of zero-excluded amplitude by duration (μV·beats, abbreviated as μV·b), where the zero-excluded amplitude was the mean local amplitude computed over all the heartbeats contained in the ECG tracing, excluding the heartbeats with zero-amplitude alternans (μV) while the duration was the number of heartbeats that were affected by a local amplitude alternans greater than 0 μV (beats, abbreviated as b). If the amplitudes were all equal to 0 μV a missing value was assigned to the magnitude. Thus, ECGA episodes were characterized not only in their manifestation strength (through the amplitude), but also their time length (through the duration).

Among the features extracted from the three ECG recordings referring to the same pre- and post-dose time-point the average was computed for each ECG tracing. If a missing value was assigned to the magnitude of at least one ECG tracing, the average over the three ECG tracings was also assigned a missing value.

Then, among the 12 ECG tracings available per pre- and post-dose time-point, only the maximum value of each feature was retained (given the dependency of ECGA from the considered lead [16], this made it possible to select the ECG lead that was best able to capture the electrophysiological phenomenon), attaining the quantification of the two features per participant and per pre- and post-dose time-point.

PWA/QRSA/TWA amplitude and magnitude trends, sampled at each pre- and post-dose time-point, were accomplished per participant (with a total of six trends per participant). Each trend was characterized in terms of:

- baseline (B), as the value of the trend at the -0.5 hours time-point (μV or μV·beats);
- Peak (P), as the maximum value reached after the drug administration (μV or μV·beats);
- Time of the peak (TP), as the time point corresponding to P (hours);
- difference (D), as the difference between P and B (P-B, μV or μV·beats);
- gain (G), as the ratio of P over B (P/B, adu)

In turn, distributions of B, P, TP, D, and G over the population were described as 50^{th} [25^{th},75^{th}] percentiles.

At each pre- and post-dose time-point, the 25^{th}, 50^{th}, and 75^{th} percentiles of the population-related distributions for both the amplitude and magnitude were computed, after excluding the possible missing values for the magnitude. Thus, PWA/QRSA/TWA amplitude and magnitude trends, sampled at each pre- and post-dose time-points, were finalized for the entire population (with a total of six trends).

Evaluation of statistical difference of pre- vs post-dose PWA/QRSA/TWA amplitude and magnitude was performed at each post-dose time-point through the non-parametric Wilcoxon Rank-Sum Test for independent samples, setting the statistical significance p at 0.05. Analogously, possible differences between the female and male subpopulations were also evaluated.

III. Results

Results are reported in Fig. 1 and Tables I-V. Fig. 1 shows the trends in amplitude (first row) and magnitude (second row)

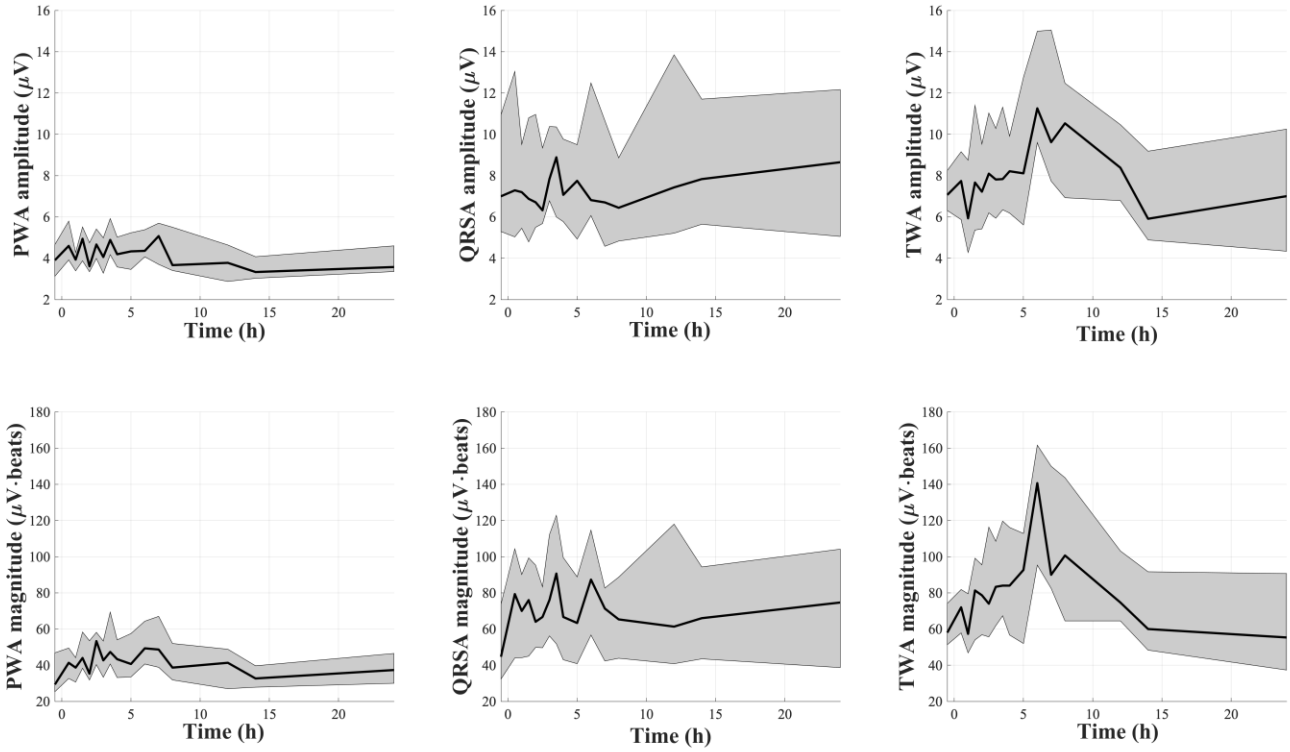

Figure 1. ECGA amplitude and magnitude trends. PWA, QRSA, and TWA amplitudes and magnitudes on the first and second row, respectively.

relative to PWA (first column), QRSA (second column) and TWA (third column), sampled at each time point pre- and post-dose, referring to the entire population. The thick black line represents the 50th percentile (median), while the gray area indicates the interquartile range, of which the lower contour is the 25th percentile and the upper contour is the 75th percentile.

Table I reports the mRR and HRV in relation to gender and age. Tables II-IV summarize for each participant results related to the characterization of PWA (Table II), QRSA (Table III), and TWA (Table IV) amplitude and magnitude trends, in terms of B, P, TP, D, and G. Their distributions over the whole population, over the female sub-population, and over the male sub-population are described as 50th [25th,75th] percentiles in Table V. Tables II-V confirmed the inter-subject variability noticeable from the grey area extent in Fig. 1.

PWA amplitude and magnitude increased in more than 70% and 85% of subjects, respectively. QRSA amplitude and magnitude both increased in more than 85% of subjects. TWA amplitude and magnitude increased in more than 85% and 90% of subjects, respectively.

Lastly, the statistical analysis that tested the differences between each ECGA feature at all post-dose versus pre-dose time points revealed that: (1) PWA amplitude was statistically higher 3.5 hours post-dose, and PWA magnitude was statistically higher 0.5, 1.5, 2.5 and 3.5-7 hours post-dose; (2) QRSA magnitude was statistically higher 3, 3.5, 6, and 24 hours post-dose; (3) TWA amplitude was statistically higher 3.5 hours post-dose, and TWA magnitude was statistically higher 0.5, 2.5, and 3.5-7 hours post-dose.

IV. Discussion

We considered the ECGRDVQ database to be appropriate for studying ECGA in relation to quinidine intake because the electrophysiological phenomenon could be observed both before administration and at different time intervals in the later 24 hours, following the time course. Moreover, all of the subjects involved were healthy; thus, it was possible to assess

TABLE I. General Characteristics of the Study Population

Subject	*1*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*	*15*	*16*	*17*	*18*	*19*	*20*	*21*	*22*
Age (years)	25	30	32	23	22	27	33	29	21	31	21	32	35	19	25	21	30	26	20	35	35
Gender [a]	F	F	F	F	F	F	F	F	F	F	M	M	M	M	M	M	M	M	M	M	M
mRR (ms)	765	939	819	971	860	747	775	818	951	799	938	885	918	1071	972	973	943	1068	884	1017	1055
HRV (%)	2	5	2	4	5	3	2	2	5	3	2	3	1	3	3	3	4	2	4	2	2

a. F - female, M - male

TABLE II. PWA-RELATED AMPLITUDE AND MAGNITUDE FEATURES

Subject	*1*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*	*15*	*16*	*17*	*18*	*19*	*20*	*21*	*22*
Amplitude																					
B (μV)	3	6	4	4	6	5	4	5	4	3	4	4	2	4	4	3	3	3	8	4	4
P (μV)	7	6	5	11	6	6	8	9	18	7	7	7	6	6	4	10	7	12	14	9	5
TP (h)	3.5	0.5	4	2	4	5	8	4	3.5	3.5	14	7	6	1.5	6	1.5	7	0.5	0.5	12	8
D (μV)	4	0	1	7	0	1	4	4	14	4	3	3	4	2	0	7	4	9	6	5	1
G	2	1	1	3	1	1	2	2	5	2	2	2	3	2	1	3	2	4	2	2	1
Magnitude																					
B (μV·b)	26	49	46	39	54	53	35	49	20	29	29	39	11	26	27	12	23	23	61	29	33
P (μV·b)	89	49	53	76	77	83	119	103	190	79	71	83	66	49	43	118	82	105	178	83	51
TP (h)	3.5	-0.5	4	3	4	5	8	4	3.5	3.5	14	7	6	7	6	1.5	8	0.5	0.5	12	8
D (μV·b)	63	0	7	37	23	30	84	54	170	50	42	44	55	23	16	106	59	82	117	54	18
G	3	1	1	2	1	2	3	2	10	3	2	2	6	2	2	10	4	5	3	3	2

TABLE III. QRSA-RELATED AMPLITUDE AND MAGNITUDE FEATURES

Subject	*1*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*	*15*	*16*	*17*	*18*	*19*	*20*	*21*	*22*
Amplitude																					
B (μV)	9	11	4	7	5	7	5	13	4	4	20	7	6	10	5	11	6	21	9	7	11
P (μV)	9	11	14	36	16	12	19	31	17	10	40	14	10	11	10	28	12	34	25	22	20
TP (h)	-0.5	-0.5	4	2	14	6	1	6	3.5	3.5	4	6	3	0.5	24	1.5	6	12	1.5	6	5
D (μV)	0	0	10	29	11	5	14	18	13	6	20	7	4	1	5	17	6	13	16	15	9
G	1	1	4	5	3	2	4	2	4	3	2	2	2	1	2	3	2	2	3	3	2
Magnitude																					
B (μV·b)	95	92	43	61	44	70	49	125	27	42	13	24	45	73	11	77	34	22	49	45	83
P (μV·b)	112	97	110	323	101	129	230	395	170	122	412	163	117	91	91	311	119	284	292	105	174
TP (h)	4	14	3.5	2	5	2	6	6	3.5	3.5	4	6	3	2	3.5	1.5	6	12	1.5	12	5
D (μV·b)	17	5	67	262	57	59	181	270	143	80	399	139	72	18	80	234	85	262	243	60	91
G	1	1	3	5	2	2	5	3	6	3	32	7	3	1	8	4	4	13	6	2	2

whether quinidine can induce or modulate ECGA, excluding comorbidities possibly acting as confounding factors.

Differently from the already mentioned enhanced adaptive matched filter method, the ECM implementation approach made analysis reliable even if ECGs were short in duration; this meant that possible transient ECGA episodes could be detected (reducing false negative rate). Moreover, the preprocess prevented other types of variability or artifacts were erroneously attributed to the ECGA (reducing false positives). HRV is one of the principal variability-related confounding factors, but we did not apply an ECG-tracing exclusion criterion based on it, because the database creators included ECGs only if signal quality was good and heart rate was stable. Indeed, intra-subject HRV turned out to be 5% of mRR at most from our verification (Table I) and no correlation was found between PWA/QRSA/TWA features (Tables II-IV) and HRV (Table I) across the population. Similarly, we could not notice a correlation between RR and ECGA across subjects. This can be due to the fact that they were all in resting condition with a relatively low inter-subject RR variability (less than 11%).

Results showed that ECGA was not always null at baseline (*i.e.*, at pre-dose time point), even if participants were all healthy subjects (Tables II-IV). This observation supports the assumption that ECGA is a continuous and not an on-off phenomenon, thus always present but to a small extent in healthy subjects and gradually becoming more pronounced with increasing cardiovascular risk [17]. In addition, the TWA magnitude at baseline was greater in females than in males, and the same seemed to be true for the PWA magnitude (although less obvious). We also observed that PWA-amplitude peak tended to occur before in females than in males, and that both QRSA-amplitude gain and TWA-magnitude peak tended to be greater in females than in males.

TABLE IV. TWA-RELATED AMPLITUDE AND MAGNITUDE FEATURES

Subject	*1*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*	*15*	*16*	*17*	*18*	*19*	*20*	*21*	*22*
Amplitude																					
B (μV)	15	8	7	7	8	7	7	7	10	6	4	7	5	7	15	7	7	5	12	4	7
P (μV)	17	12	11	38	13	20	18	28	41	17	15	13	10	15	15	24	20	23	15	34	28
TP (h)	4	14	4	2	6	24	6	8	3.5	3.5	7	8	24	6	-0.5	1.5	8	2	6	12	6
D (μV)	2	4	4	31	5	13	11	21	31	11	11	6	5	8	0	17	13	18	3	30	21
G	1	2	2	5	2	3	3	4	4	3	4	2	2	2	1	3	3	5	1	9	4
Magnitude																					
B (μV·b)	158	70	74	56	77	65	71	55	75	58	29	61	43	51	105	51	57	27	97	29	53
P (μV·b)	207	115	129	345	157	267	263	333	424	193	141	143	109	141	129	269	227	144	173	311	248
TP (h)	4	3.5	4	2	6	5	6	8	3.5	3.5	7	8	24	6	3.5	1.5	8	2.5	6	12	6
D (μV·b)	49	45	55	289	80	202	192	278	349	135	112	82	66	90	24	218	170	117	76	282	195
G	1	2	2	6	2	4	4	6	6	3	5	2	3	3	1	5	4	5	2	11	5

These observations were statistically supported only for the TWA magnitude at baseline, and generalization was not possible in any case considering the small population size. Nevertheless, they were in agreement with the literature, which shows sex-related differences in response to risk factors and treatments [18].

In this study, we observed that ECGA increased after quinidine administration with respect to its baseline values. This finding is in line with the drug association to an increased risk of ventricular arrhythmia and sudden death reported by the literature [6, 7]. Specifically, the increment observed in ECGA, most likely attributable to quinidine intake, was found within 6 hours after administration (with a certain degree of subjectivity), in accordance with elimination half-life of the drug, and then returned towards baseline values.

Overall, the extent of ECGA increase varied depending on form and feature. Specifically, the maximum increase in PWA and QRSA amplitudes was two times the baseline value and in TWA amplitude three times the baseline value, while the maximum increase in PWA, QRSA, and TWA magnitude was two, three, and four times the baseline value, respectively. Of the two ECGA features considered (*i.e.*, amplitude and magnitude), magnitude seemed to better reveal transient changes, resulting in a greater number of post-dose time points at which ECGA was statistically different than at the pre-dose time point, taken as the baseline. To date, there is a known association between the presence of ECGA and cardiovascular risk (as a predisposition of the subject to develop severe or even malignant arrhythmias), although there is no direct and simple entity-based correlation. Further studies evaluating the

TABLE V. AMPLITUDE AND MAGNITUDE FEATURE DISTRIBUTIONS OVER THE WHOLE POPULATION, OVER THE FEMALE SUB-POPULATION, AND OVER THE MALE SUB-POPULATION, EXPRESSED AS 50^{TH} [25^{TH},75^{TH}] PERCENTILES

		WHOLE POPULATION			***FEMALE SUB-POPULATION***			***MALE SUB-POPULATION***		
		PWA	***QRSA***	***TWA***	***PWA***	***QRSA***	***TWA***	***PWA***	***QRSA***	***TWA***
Amplitude	***B (μV)***	4 [3;4]	7 [5;11]	7 [7;8]	4 [4;5]	6 [4;9]	7 [7;8]	4 [3;4]	9 [6;11]	7 [5;7]
	P (μV)	7 [6;9]	16 [11;26]	17 [15;25]	7 [6;9]	15 [11;19]	18 [13;28]	7 [6;10]	20 [11;27]	15 [15;24]
	TP (h)	4 [2;7]	4 [2;6]	6 [4;8]	4 [4;4]	4 [1;6]	5 [4;8]	6 [2;8]	5 [2;6]	6 [3;8]
	D (μV)	4 [1;5]	10 [5;15]	11 [5;19]	4 [1;4]	11 [5;14]	11 [4;21]	4 [2;6]	9 [5;16]	11 [5;18]
	G	2 [1;2]	2 [2;3]	3 [2;4]	2 [1;2]	3 [2;4]	3 [2;4]	2 [2;3]	2 [2;3]	3 [2;4]
Magnitude	***B (μV·b)***	29 [25;47]	45 [32;74]	58 [51;74]	43 [29;49]	55 [43;92]	71 [58;75]	27 [23;32]	45 [23;67]	51 [33;60]
	P (μV·b)	82 [63;104]	129 [109;286]	193 [141;268]	81 [76;103]	126 [110;230]	235 [157;333]	82 [55;100]	163 [108;290]	144 [141;243]
	TP (h)	4 [3;7]	4 [3;6]	6 [4;7]	4 [4;4]	4 [4;6]	4 [4;6]	7 [3;8]	4 [2;6]	6 [4;8]
	D (μV·b)	50 [23;68]	85 [60;236]	117 [74;206]	44 [23;63]	74 [57;181]	164 [55;278]	54 [28;76]	91 [74;241]	112 [78;189]
	G	2 [2;3]	3 [2;6]	4 [2;5]	2 [1;3]	3 [2;5]	4 [2;6]	3 [2;5]	4 [2;8]	4 [2;5]

features considered here to reveal the change in ECGA, *i.e.*, the gain and difference assuming individual baseline value as reference, may evaluate whether they play a specific role in risk stratification. In the present study, it was not possible to conduct this kind of analysis, even preliminary, because the subjects' actual risk was not objectively assessed by a clinician and provided as annotation, nor is the subjects' follow-up known, as far as we know.

The results obtained in this pilot study suggest that, from a clinical point of view, ECGA, particularly when episodes are quantitatively assessed through the analysis of both manifestation strength and time length, may serve as a reliable parameter for indirectly evaluating the pharmacodynamic cardiovascular effects of quinidine. Thus, in a wider perspective, ECGA quantification holds potential as a non-invasive biomarker for assessing drug-induced alterations in cardiovascular function, thereby contributing to therapeutic decision-making and effective clinical management.

Recognizing the limitations of the study, we have to highlight that the sampling of ECG tracing acquisition was not performed regularly by the database creators. This implies that behaviors not revealed by this study may have occurred in the time intervals in which the ECG is not monitored, and likely the longer the interval the greater this probability. The limited population size may have adversely affected the statistical power of the study, potentially compromising the generalizability of the results. However, this study, even in its pilot version, may represent an important contribution to the evaluation of the application of ECGA as a risk index in various application fields such as personalized therapeutic guidance, increasingly affirming and strengthening its role in clinical settings. Future studies, possibly conducted in a larger population, including consideration of the placebo and considering better temporal resolution of ECG acquisition, may confirm the results obtained in this investigation.

V. Conclusion

The present study indicated that ECGA in all its possible forms appears to disclose the higher risk of arrhythmia associated to quinidine, particularly in the following 6 hours after administration.

References

[1] P. A. Iaizzo, *Handbook of cardiac anatomy, physiology, and devices: Second edition*. in Handbook of Cardiac Anatomy, Physiology, and Devices: Second Edition. 2005, p. 659. doi: 10.1007/978-1-60327-372-5.

[2] K. Gima and Y. Rudy, "Ionic Current Basis of Electrocardiographic Waveforms," *Circulation Research*, vol. 90, no. 8, pp. 889–896, May 2002, doi: 10.1161/01.RES.0000016960.61087.86.

[3] H. K. Jeong, N. Yoon, Y. R. Kim, K. H. Lee, and H. W. Park, "Artemisinin-Quinidine Combination for Suppressing Ventricular Tachyarrhythmia in an Ex Vivo Model of Brugada Syndrome," *J Korean Med Sci*, vol. 40, no. 1, Jan. 2025, [Online]. Available: https://doi.org/10.3346/jkms.2025.40.e2

[4] Jain A, *Quinidine*. StatPearls [Internet], 2025. [Online]. Available: https://www.ncbi.nlm.nih.gov/books/NBK542193

[5] H. R. Ochs, D. J. Greenblatt, and E. Woo, "Clinical Pharmacokinetics of Quinidine," *Clinical Pharmacokinetics*, vol. 5, no. 2, pp. 150–168, Mar. 1980, doi: 10.2165/00003088-198005020-00003.

[6] B. Bozic, T. V. Uzelac, A. Kezic, and M. Bajcetic, "The role of quinidine in the pharmacological therapy of ventricular arrhythmias 'quinidine,'" *Mini-Reviews in Medicinal Chemistry*, vol. 18, no. 6, pp. 468–475, 2018, doi: 10.2174/1389557517666170707110450.

[7] S. Chigullapali, R. Jain, and R. Patil, "Drug-induced Macro-T-Wave Alternans with QTc Prolongation," *Indian Journal of Cardiovascular Disease in Women - WINCARS*, vol. 7, no. 3, p. 159, 2022, doi: 10.25259/mm_ijcdw_470.

[8] N. Takasugi *et al.*, "Prevalence of Microvolt T-Wave Alternans in Patients With Long QT Syndrome and Its Association With Torsade de Pointes," *Circulation: Arrhythmia and Electrophysiology*, vol. 9, no. 2, p. e003206, Feb. 2016, doi: 10.1161/CIRCEP.115.003206.

[9] G. Kanaporis and L. A. Blatter, "The Mechanisms of Calcium Cycling and Action Potential Dynamics in Cardiac Alternans," *Circulation Research*, vol. 116, no. 5, pp. 846–856, Feb. 2015, doi: 10.1161/CIRCRESAHA.116.305404.

[10] I. Marcantoni, A. Sbrollini, M. Morettini, C. A. Swenne, and L. Burattini, "Enhanced adaptive matched filter for automated identification and measurement of electrocardiographic alternans," *Biomedical Signal Processing and Control*, vol. 68, 2021, doi: 10.1016/j.bspc.2021.102619.

[11] L. Johannesen *et al.*, "Differentiating Drug-Induced Multichannel Block on the Electrocardiogram: Randomized Study of Dofetilide, Quinidine, Ranolazine, and Verapamil," *Clinical Pharmacology & Therapeutics*, vol. 96, no. 5, pp. 549–558, Nov. 2014, doi: 10.1038/clpt.2014.155.

[12] A. L. Goldberger *et al.*, "PhysioBank, PhysioToolkit, and PhysioNet," *Circulation*, vol. 101, no. 23, pp. e215–e220, Jun. 2000, doi: 10.1161/01.CIR.101.23.e215.

[13] L. Burattini, W. Zareba, and A. J. Moss, "Correlation method for detection of transient T-wave alternans in digital holter ECG recordings," *Annals of Noninvasive Electrocardiology*, vol. 4, no. 4, pp. 416–424, 1999, doi: 10.1111/j.1542-474X.1999.tb00232.x.

[14] M. Morettini, C. Peroni, A. Sbrollini, I. Marcantoni, and L. Burattini, "Classification of drug-induced hERG potassium-channel block from electrocardiographic T-wave features using artificial neural networks," *Annals of Noninvasive Electrocardiology*, vol. 24, no. 6, p. e12679, Nov. 2019, doi: 10.1111/anec.12679.

[15] I. Marcantoni *et al.*, "Dofetilide-Induced Microvolt T-Wave Alternans," presented at the Proceedings of the Annual International Conference of the IEEE Engineering in Medicine and Biology Society, EMBS, 2019, pp. 95–98. doi: 10.1109/EMBC.2019.8857486.

[16] L. Burattini, S. Man, R. Burattini, and C. A. Swenne, "Comparison of standard versus orthogonal ECG leads for T-wave alternans identification," *Annals of Noninvasive Electrocardiology*, vol. 17, no. 2, pp. 130–140, 2012, doi: 10.1111/j.1542-474X.2012.00490.x.

[17] L. Burattini, W. Zareba, and R. Burattini, "Assessment of Physiological Amplitude, Duration, and Magnitude of ECG T-Wave Alternans," *Annals of Noninvasive Electrocardiology*, vol. 14, no. 4, pp. 366–374, Oct. 2009, doi: 10.1111/j.1542-474X.2009.00326.x.

[18] L. Cho, *et al.*, "Summary of Updated Recommendations for Primary Prevention of Cardiovascular Disease in Women: JACC State-of-the-Art Review," *Journal of the American College of Cardiology*, vol. 75, no.20, pp. 2602–2618, May 2020, doi: 10.1016/j.jacc.2020.03.060.