The Power of Exercise-Induced T-wave Alternans to Predict Ventricular Arrhythmias in Patients with Implanted Cardiac Defibrillator

Laura Burattini, PhD[1]*, Sumche Man, MD[2] and Cees A. Swenne, PhD, MD[2]
[1]Department of Information Engineering,
Polytechnic University of Marche, Ancona, Italy.
[2]Department of Cardiology, Leiden University Medical Center,
Leiden, The Netherlands.

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ABSTRACT

The power of exercise-induced T-wave alternans (TWA) to predict the occurrence of ventricular arrhythmias was evaluated in 67 patients with an implanted cardiac defibrillator (ICD). During the 4-year follow-up, electrocardiographic (ECG) tracings were recorded in a bicycle ergometer test with increasing workload ranging from zero (NoWL) to the patient's maximal capacity (MaxWL). After the follow-up, patients were classified as either ICD_Cases (n = 29), if developed ventricular tachycardia/fibrillation, or ICD_Controls (n = 38). TWA was quantified using our heart-rate adaptive match filter. Compared to NoWL, MaxWL was characterized by faster heart rates and higher TWA in both ICD_Cases (12–18 μ V vs. 20–39 μ V; $P < 0.05$) and ICD_Controls (9–15 μ V vs. 20–32 μ V; $P < 0.05$). Still, TWA was able to discriminate the two ICD groups during NoWL (sensitivity = 59–83%, specificity = 53–84%) but not MaxWL (sensitivity = 55–69%, specificity = 39–74%). Thus, this retrospective observational case-control study suggests that TWA's predictive power for the occurrence of ventricular arrhythmias could increase at low heart rates.

Keywords: T-wave alternans, ventricular arrhythmias, predictive power, implanted cardiac defibrillator

1. INTRODUCTION

T-wave alternans (TWA) is an electrophysiological phenomenon consisting in every-other-beat fluctuations of amplitude and/or shape of the electrocardiographic (ECG) T-wave at stable heart rate during sinus rhythm. Macroscopic TWA is quite rare and has been identified as a harbinger of malignant ventricular arrhythmias [1]. Instead,

*Corresponding author: Laura Burattini, Polytechnic University of Marche, Department of Information Engineering, via Brecce Bianche, 60131 Ancona, Italy. Phone: (071) 220-4461. Fax: (071) 220-4220. E mail: l.burattini@univpm.it. Other authors: S.Man@lumc.nl; C.A.Swenne@lumc.nl.

microvolt TWA is much more common, requires specifically designed algorithms for its automatic identification [2–9], and has been recognized as a promising noninvasive index for risk stratification [10–16].

Even though microvolt TWA has been also observed in resting conditions [17, 18], it is well known that its amplitude tends to increase at high heart rates [19, 20], which can be reached pharmacologically [21], by physical exercise [10, 20, 22–23] or, more invasively, by artificial pacing [21, 24, 25]. Beside increasing TWA amplitude (and, thus, the signal-to-noise ratio), fast heart rates reduce heart-rate variability [26]. Both conditions (high signal-to-noise ratio and low heart-rate variability) have been found to be fundamental for a reliable TWA identification by means of any automatic method [27], so that most risk-stratification studies have been performed under exercise conditions [10, 15, 16, 22–24, 28, 29]. In the risk-stratification studies involving ambulatory recordings during daily activities, TWA was often measured at maximum heart rates [11, 12, 30].

Exercise-induced TWA is more easily detectable than resting TWA, but the effect of exercise on TWA predictive power for the occurrence of ventricular arrhythmias remains unknown. Thus, the aim of the present study was to evaluate if exercise, besides inducing a higher amplitude of TWA, also enhances TWA's ability to discriminate patients at higher risk for malignant ventricular arrhythmias. To this aim, exercise ECG tracings of implanted cardioverter-defibrillator (ICD) patients were analyzed. More specifically, two populations of ICD patients were considered: developing (ICD_Cases) and not developing (ICD_Controls) ventricular tachycardia or ventricular fibrillation during the 4-year follow-up. TWA's ability to discriminate the two ICD groups was evaluated at the very beginning of the exercise test when the workload was null and heart-rate was close to resting, and at the end of the exercise test when the workload matched the patient's maximum capacity and the heart rate was high.

2. METHODS

2.1. Database of Exercise ECGs in Heart Failure Patients with ICDs

The collection (from August 2006 to September 2010) of routine clinical data from 266 patients with an ICD for primary prevention because of a depressed left ventricular ejection fraction (LVEF < 35%), constituting the Leiden University Medical Center (The Netherlands) database of exercise ECGs in heart failure patients with ICDs, was retrospectively selected for the present observational study on TWA. All patients underwent a 4-year follow-up starting from the date of ICD implantation. During the follow-up, patients received standard care, which included periodic visits to the outpatient clinic, amongst others, to assess validity by bicycle ergometry. The bicycle ergometer test consisted of an approximately 10-minute bicycle test during which the workload was incremented from zero (NoWL) to the patient's maximal capacity (MaxWL) by applying load-increments of 10% of the expected maximal capacity every minute. During the bicycle ergometer test, ECG recordings were obtained using a CASE 8000 stress test recorder (GE Healthcare, Freiburg, Germany; sampling frequency: 500 Hz; resolution: 4.88 μV/LSB) and the 3M Red Dot ECG Electrode Soft Cloth 2271 electrodes, specifically designed for conditions where skin moisture is an issue, like stress testing. The skin was first cleaned with alcohol and abraded, to reduce electrode

resistance. Electrode resistance was measured by the electrocardiograph, and considered acceptable if < 5 kOhm. Electrodes were applied in the Mason-Likar position.

According to the data collection protocol, during the follow-up, patients could undergo more than one exercise tests. Eventually, at the end of the follow-up, patients were classified as either "ICD_Cases" (76 patients) if, during the follow-up, they developed ventricular tachycardia or ventricular fibrillation (treated with antitachycardia pacing and/or shock therapy), or "ICD_Controls" (190 patients) otherwise. ICD_Cases exercise tests were excluded when a major cardiac event (infarction, VT ablation, coronary artery bypass graft) occurred between the exercise test and the moment of VT/VF, because ablation modified their arrhythmogenic substrate with respect to the state when the ICD was implanted. If more than one exercise test remained available for analysis, the one closest in time (either before or after) to the VT/VF episode was selected so that the substrate during the stress test would be as close as possible to the substrate during the actual arrhythmia. In ICD_Controls with more than one suitable exercise test, the earliest available one was selected. Eventually, only one ECG tracing per patient was made available for the database.

According to "Guideline for Good Clinical Practice" (European Medicines Agency, CPMP/ICH/135/95) and the data privacy law of the Netherlands, for being enrolled in the present study, which is retrospective, observational and on standard clinical data, no informed consent from patients (whose identity remained anonymous) was needed because no interventions had taken place.

2.2. Study Population Enrollment Criteria

Enrollment criteria were applied to screen the ICD patients in the Leiden University Medical Center database of exercise ECGs in heart failure to warrant a reliable TWA identification and a comparable clinical profile of the two ICD groups. More specifically, patients in the ICD_Cases (n = 76) and ICD_Controls (n = 190) groups were considered eligible for the present study if satisfying the following inclusion criteria:

- Criterion 1. The NoWL and the MaxWL phases of the exercise test were both characterized by stable heart rate. More specifically, in each phase, it had to exist at least a 64-beat ECG window characterized by NN standard deviation not exceeding 10% of mean NN.
- Criterion 2. ECG tracings from the NoWL and the MaxWL phases of the exercise test were both characterized by a small number of artifacts and ectopic beats in at least three of the six precordial leads (V1 to V6). More specifically, for each phase, it had to exist at least a 64-beat ECG window for which at least three leads were characterized by no more than 4 replaced beats because of artifacts and ectopic beats [27, 31].
- Criterion 3. Age, at the time of the exercise test, was between 45 and 75 years old.
- Criterion 4. LVEF, at the time of the exercise test, was < 35 %.
- Criterion 5. Body mass index (BMI), at the time of the exercise test, was between 20 and 34 Kg/m^2.

2.3. Clinical ECG Data

All ECG tracings were preprocessed for noise removal (0.5–35 Hz bandpass filter) and baseline subtraction by means of a third-order spline interpolation [3, 31]. Subsequently, sliding ECG windows including 64 consecutive beats were extracted every two seconds from the entire ECG recording in correspondence of the NoWL and the MaxWL phases of the exercise test, and preprocessed for artifacts and ectopic beats replacement [27, 31] before going through our heart-rate adaptive match filter procedure (see below) for TWA quantification. ECG windows characterized by unstable heart rate (NN standard deviation greater than 10% of mean) or by a number of replaced beats greater than 4 were rejected. On the contrary, ECG windows characterized by a stable heart rate and a low number (≤ 4) of artifacts and ectopic beats were considered suitable for TWA analysis. These ECG suitability requirements represent an external (i.e. not integrated into the TWA algorithm) test for TWA reliability. Multiple extraction of ECG windows inside the one-minute duration of the NoWL or MaxWL phases was used to minimize the number of rejected patients, since it had to exist at least one ECG window suitable for TWA analysis inside each exercise phase for a patient not to be rejected (criterion 2). When more than a suitable window was found within a specific exercise phase, TWA measurements from all suitable windows were averaged.

2.4. T-Wave Alternans Identification

TWA identification was performed in a completely automatic way by individuals who were blinded to outcomes. All the 64-beat ECG windows (characterized by 6 leads each) found to be suitable for TWA analysis went through our heart-rate adaptive match filter (AMF) procedure [5] for automatic TWA identification previously validated in simulated settings [2, 27, 31] and applied to clinical data [5, 17, 18, 32–34]. TWA was identified in each one of the six precordial leads independently.

Ideally, at a fixed heart rate, TWA is characterized by a single frequency by definition equal to half heart rate. In our clinical 64-beat ECG windows with slowly (see enrollment criterion 1) increasing heart-rate, TWA was supposed to be characterized by a small frequency band centered in half mean (over the 64 beats) heart rate (f_{TWA}). On this basis, our AMF was conceived as a heart-rate (and, thus, f_{TWA}) adaptive narrow-band passing filter (ideally a match filter) with its passing band centered in f_{TWA}. In our implementation, the AMF is a 6th order bidirectional Butterworth band-pass filter characterized by a 0.12 Hz wide passing band centered in f_{TWA}, and consisted of a cascade of a low-pass filter (*LPF*; cut-off frequency $f_{LPF} = f_{TWA} + df_{TWA}$, with $df_{TWA} = 0.06$ Hz) and a high-pass filter (*HPF*; cut-off frequency $f_{HPF} = f_{TWA} - df_{TWA}$) [5, 31]. The squared module of the AMF is expressed by the following equation:

$$\left|H_{AMF}(f)\right|^2 = \left|H_{LPF}(f)\right|^2 \cdot \left|H_{HPF}(f)\right|^2 = \frac{1}{1+\left(\frac{f}{f_{LPF}}\right)^6} \cdot \frac{\left(\frac{f}{f_{HPF}}\right)^6}{1+\left(\frac{f}{f_{HPF}}\right)^6} \quad . \qquad (1)$$

Each time the AMF is fed with an ECG window which was found to be suitable for TWA analysis, it computes the ECG heart rate and the corresponding f_{TWA}, and filters out every ECG components, including those relative to noise but not those relative to TWA. Thus, the output of the AMF is an amplitude-modulated sinusoidal signal, i.e., the TWA signal, having the same length of the input ECG and characterized by a frequency which matches f_{TWA}. If really pertaining to TWA (and not, for example, to noise oscillations which match f_{TWA}), the TWA signal maxima and minima have to fall inside the JT intervals (Figure 1). Such requirement represents the internal (i.e., integrated in the TWA identification algorithm) test for a reliable TWA by the AMF. The local amplitude of the TWA signal corresponding with the i^{th} beat provides a quantification of the TWA amplitude ($TWAA_i$, in μ V) characterizing that beat (Figure 1). If the TWA signal has its maxima and minima outside the repolarization segment (as in the presence of QRS alternans, for example), all $TWAA_i$ values are set to zero. Eventually, in the case of an ECG tracing affected by no alternans of any kind, the TWA signal at the output of the AMF reduces to a zero constant signal. Consequently, all the $TWAA_i$ values are equal to zero.

The $TWAA_i$ values computed from each beat were averaged over the 64 beats to provide a characterization of an ECG window. (TWAA) Eventually, all the available TWAA values of the same exercise phase were averaged to obtain one TWA estimation for NoWL (TWAA_NoWL) and another TWA estimation for MaxWL (TWAA_MaxWL). For each patient, TWAA_NoWL and TWAA_MaxWL values relative to every lead were determined, together with the lead-system TWAA

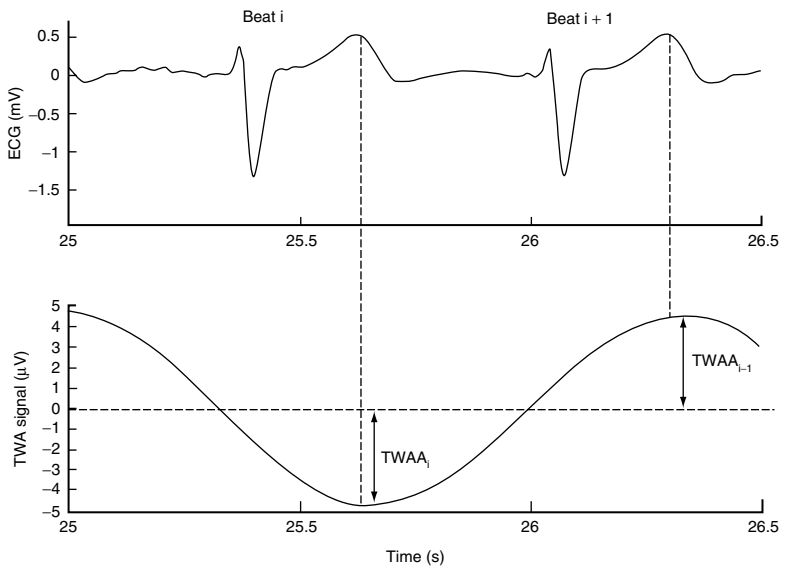

Figure 1. ECG signal (upper panel) and relative TWA signal (lower panel) at the output of the AMF from which the local TWA amplitude ($TWAA_i$) corresponding with the i^{th} beat is estimated.

measurements consisting of the mean and maximum TWAA values over the leads (mean V1–V6 and max V1–V6, respectively).

2.5. Statistics

As a consequence of screening criterion 2, some patients could have some missing TWA measurements in up to three leads. Missing data were replaced with the mean value of the corresponding population. More specifically, for each ICD group, no more than 10% of replacements (maximum 2 and 3 replacements among the ICD_Cases and ICD_Controls, respectively) were allowed in the TWAA distribution relative to a specific lead and specific phase of the exercise test.

Comparison between clinical and/or TWA parameters relative to the two ICD groups or measured during the NoWL or MaxWL phases of the exercise test were performed using parametric and non-parametric tests for normal and non-normal distributions, respectively. Normality of a parameter distribution was tested using the Lilliefors test. Comparisons between continuous and normally distributed parameters were performed using the t-test for equal means (unpaired Student's t-test for comparing the ICD_Cases against ICD_control, and the paired t-test for comparing parameters belonging to the same ICD group but measured during the NoWL vs. MaxWL phases of the exercise test), whereas comparisons between continuous and not-normally distributed parameters were performed using the Wilcoxon rank-sum test for equal medians. Eventually, differences in the binary parameters distributions between the two groups were evaluated using the chi-square test. The statistical significance level was set at 5%.

To evaluate the TWA predictive power for the occurrence of ventricular arrhythmias, the receiver operating characteristic (ROC) and its area under the curve (AUC) were used. Sensitivity (Se) and specificity (Sp) values were also computed after the definition of a threshold value identified as the point where the minimum distance between Se and Sp digital curves occurred (ideally, for continuous curves, at the intersection of the ROC curve and the Sp = 1-Se line).

3. RESULTS

Patients screening based on the criteria in the order listed in Methods was as follows. Of the 76 ICD_Cases, 47 (62%) patients were rejected for ECG-related reasons. More specifically, 36 patients were rejected because not characterized by stable heart rate (criterion 1 not satisfied) while the remaining 11 showed too many artifacts or ectopic beats (criterion 2 not satisfied). No patients were rejected for clinical ECG-unrelated reasons (i.e., criteria 3 to 5 always satisfied) among the ICD_Cases. On the other hand, among the 190 ICD_Controls, 94 patients were rejected because not showing stable heart rate, and 27 were rejected because showing too many artifacts and ectopic beats. Thus, globally, 121 (64%) patients were rejected for ECG-related reasons. In addition, another 31 (16%) ICD_Controls patients were rejected because of age (10 patients), LVEF (17 patients) and BMI (4 patients). Overall, 47 (62%) ICD_Cases and 152 (80%) ICD_Controls patients were rejected, and the final study population counted 29 ICD_Cases and 38 ICD_Controls.

Clinical parameters characterizing the two groups of ICD patients analyzed in this study are exhibited in Table 1. No statistically significant differences were observed between the ICD_Cases and ICD_Controls in terms of gender, age, body mass index,

heart rate, therapies and medications. The ICD patients distributions over the NYHA functional classes were also comparable. LVEF was smaller for the ICD_Cases than for ICD_Controls (28.0±10.0% vs. 32.3±7.2%).

During the NoWL phase of the exercise test, the two ICD groups were characterized by comparable heart rate (ICD_Cases: 81±10 bpm; ICD_Controls: 79±10 bpm; Table 1); however, compared to the ICD_Controls, the ICD_Cases showed significantly higher TWAA in all leads and lead systems (Table 2). The values of the AUC, Se and Sp quantifying the ability of TWAA to discriminate the two populations are exhibited in Table 3. Lead V6, although not characterized by the highest levels of TWAA, was the

Table 1. Clinical parameters (mean ± standard deviation or number of occurrences) for the ICD_Cases and ICD_Controls, and P-values for comparing the two groups

	ICD_Cases (29)	ICD_Controls (38)	P-value
General:			
Age (years)	59.1±8.5	61.7±7.6	0.2013
Gender (male)	22 (75.9%)	33 (86.8%)	0.20–0.30
BMI (Kg/m^2)	26.3±3.5	26.3±3.4	0.9931
LVEF (%)	28.0±10.0	32.3±7.2	0.0484*
CRT-D	10 (34.5%)	16 (42.1%)	0.50–0.70
NYHA functional class:			
I–II	22 (75.9%)	30 (79.0%)	0.70–0.80
III–IV	7 (24.1%)	8 (21.1%)	0.70–0.80
Medications:			
Beta-blocker	28 (96.6%)	36 (94.7%)	0.70–0.80
Amiodarone	8 (27.6%)	4 (10.5%)	0.05–0.10
Calcium antagonists	0 (0.0%)	2 (5.3%)	0.20–0.30
Flecainide	1 (3.4%)	0 (0.0%)	0.20–0.30
Digoxin	2 (6.9%)	4 (10.4%)	0.50–0.70
ACE inhibitor / AT antagonist	27 (93.1%)	37 (97.4%)	0.30–0.50
Diuretics for CHF	22 (75.8%)	30 (79.0%)	0.70–0.80
Statins	23 (79.3%)	32 (84.2%)	0.50–0.70
Heart rates:			
HR at rest (bpm)	71±11	70±12	0.7876
HR NoWL (bpm)	81±10	79±10	0.5854
HR MaxWL(bpm)	122±13	124±14‡	0.5009

*Statistical significance ($P < 0.05$).

ACE: angiotensin converting enzyme; AT: angiotensin; BMI: body mass index; CHF: congestive heart failure; CRT_D: cardiac resynchronization therapy with defibrillator; HR: heart rate; LVEF: left ventricular ejection fraction; MaxWL: maximum workload; NoWL: no workload; NYHA: New Your heart association

lead that showed the highest ability to discriminate ICD_Cases from ICD_Controls, with an AUC of 81%, a Se of 83%, and a Sp of 84% (Table 3). With the only exception of V6, lead-system TWA measurements demonstrated advantages over single-lead

Table 2. TWAA median values (μV) with relative [2.5th; 25th; 75th; 97.5th] percentiles in the two groups of ICD patients measured during the NoWL and MaxWL phases of the exercise test, along with P-values for the comparison between the two ICD groups and for the comparison between NoWL and MaxWL measurements within a group

		ICD_Cases (29)	ICD_Controls (38)	P-value (cases vs. controls)
Exercise test phase	Lead			
NoWL				
	V1	12 [6; 8; 12; 31]	9 [3; 7; 11; 18]	0.0251*
	V2	16 [6; 10; 20; 69]	10 [4; 7; 13; 31]	0.0010*
	V3	13 [5; 11; 19; 66]	9 [4; 7; 16; 37]	0.0082*
	V4	14 [6; 11; 18; 53]	10 [5; 8; 15; 46]	0.0158*
	V5	18 [7; 12; 18; 56]	12 [3; 7; 15; 51]	0.0035*
	V6	17 [5; 13; 17; 44]	11 [4; 7; 11; 34]	$<10^{-4}$*
	Mean V1-V6	15 [8; 12; 17; 48]	10 [5; 9; 14; 33]	$<10^{-3}$*
	Max V1-V6	18 [13; 18; 26; 72]	15 [7; 11; 20; 51]	$<10^{-3}$*
MaxWL				
	V1	20 [9; 17; 21; 43]	20 [5; 10; 20; 57]	0.7223
	V2	26 [6; 17; 34; 89]	23 [5; 14; 27; 66]	0.2460
	V3	27 [8; 13; 42; 163]	23 [5; 17; 31; 82]	0.3686
	V4	27 [7; 16; 31; 122]	27 [9; 16; 30; 115]	0.5810
	V5	34 [12; 22; 42; 86]	25 [8; 17; 29; 65]	0.0071*
	V6	35 [5; 18 37; 104]	24 [8; 22; 26; 65]	0.1398
	Mean V1–V6	26 [13; 22; 36; 86]	24 [10; 18; 30; 56]	0.1094
	Max V1–V6	39 [21; 35; 58; 163]	32 [15; 26; 49; 115]	0.0215*
P-value (NoWL vs. MaxWL)				
	V1	$<10^{-5}$*	$<10^{-5}$*	–
	V2	0.0083*	$<10^{-6}$*	–
	V3	0.0104*	$<10^{-5}$*	–
	V4	$<10^{-3}$*	$<10^{-7}$*	–
	V5	$<10^{-5}$*	$<10^{-5}$*	–
	V6	$<10^{-3}$*	$<10^{-9}$*	–
	Mean V1–V6	$<10^{-5}$*	$<10^{-8}$*	–
	Max V1–V6	$<10^{-6}$*	$<10^{-8}$*	–

*Statistical significance ($P < 0.05$).

analysis since they allowed discrimination between ICD groups with better values of AUC (77% and 76% for mean V1–V6 and max V1–V6, respectively), Se (72% and 79% for mean V1–V6 and max V1–V6, respectively), and Sp (71% and 68% for mean V1–V6 and max V1–V6, respectively).

During the MaxWL phase of the exercise test, the two ICD groups were also characterized by similar heart rates (ICD_Cases: 122 ± 13 bpm; ICD_Controls: 124 ± 14 bpm; Table 1), which were significantly higher than those of NoWL ($P < 10^{-18}$ for the ICD_Cases and $P < 10^{-24}$ for the ICD_Controls). In both groups, such increment in the heart rate was accompanied by a significant increment of TWAA (Table 2). However, when comparing ICD_Cases vs. ICD_Controls, significant differences were only occasionally detected (in lead V5 and max V1–V6; Table 2). Consequently, TWA's ability to discriminate the two groups was quite poor during MaxWL (V5: AUC = 69%, Se = 66% and Sp = 71%; max V1–V6: AUC = 67%, Se = 69% and Sp = 61%). A comparison between the ROC curves during the NoWL (solid line) and the MaxWL (dotted line) phases of the exercise test is displayed in Figure 2.

Table 3. Area under the curve (AUC), sensitivity (Se), specificity (Sp) and TWAA thresholds for discriminating ICD_Cases from ICD_Controls during the NoWL and MaxWL phases of the exercise test

		AUC	Se	Sp	TWAA threshold (μV)
Exercise test Phase	Lead				
NoWL					
	V1	0.66	0.59	0.53	10
	V2	0.74	0.69	0.68	12
	V3	0.69	0.72	0.66	11
	V4	0.67	0.62	0.66	13
	V5	0.71	0.66	0.58	14
	V6	0.81	0.83	0.84	11
	Mean V1–V6	0.77	0.72	0.71	13
	Max V1–V6	0.76	0.79	0.68	17
MaxWL					
	V1	0.53	0.55	0.39	19
	V2	0.58	0.62	0.53	23
	V3	0.56	0.55	0.55	25
	V4	0.54	0.55	0.47	26
	V5	0.69	0.66	0.71	25
	V6	0.60	0.62	0.74	24
	Mean V1–V6	0.62	0.59	0.58	26
	Max V1–V6	0.67	0.69	0.61	35

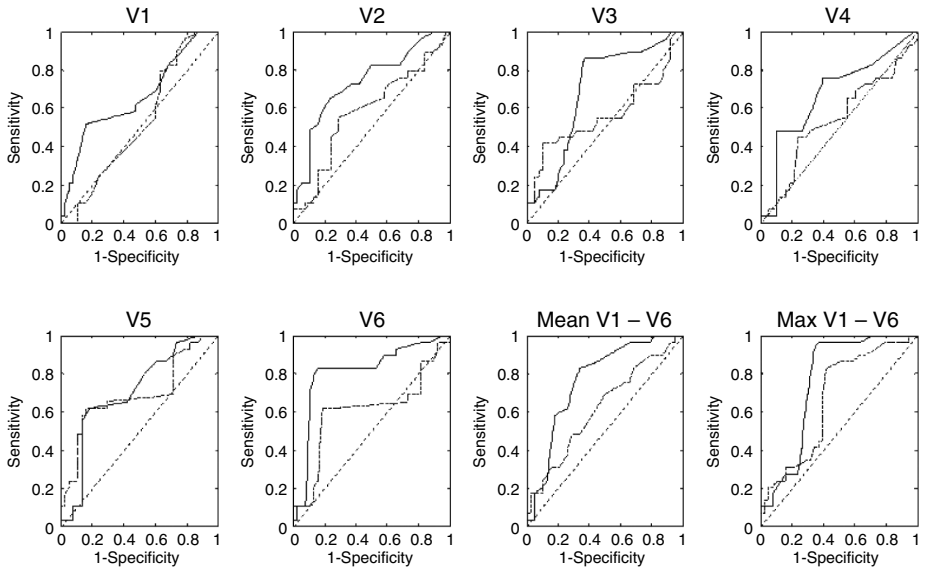

Figure 2. Receiver operating characteristics (ROC) curves for each lead or lead system during the NoWL (solid line) and the MaxWL (dotted line) phases of the exercise test.

4. DISCUSSION

The present study is the first to investigate if faster heart rates, reached through exercise, beside increasing TWA amplitude, also enhance the TWA's predictive power of ventricular arrhythmias. To this aim, the Leiden University Medical Center database of exercise ECGs in 266 heart failure patients with ICDs, grouped in 76 ICD_Cases (i.e. patients who developed VT/VF during follow-up) and 190 ICD_Controls (i.e. patients who did not develop ventricular arrhythmias during follow-up), was employed. Of these 266 ICD patients, 199 (75%), corresponding to 47 ICD_Cases and 152 ICD_Controls, did not satisfied the study screening criteria and were excluded. Such high rejection rate was due to both ECG related and unrelated reasons. Being TWA a phenomenon defined at stable and sinus rhythm, ECG tracings have to be characterized by a stable heart rate and a low number of artifacts and ectopic beats [2, 3, 27] for a reliable TWA identification by any method, including our AMF. In addition, with the aim to compare TWA's predictive power during the NoWL phase vs. the MaxWL phase of the exercise test, this study required that such conditions had to be satisfied in both phases, making the enrollment criteria even more restrictive. The aforementioned ECG features required for TWA identification are hardly found in routine clinical exercise ECG recordings that are mainly for the measurement of the maximum workload and not specifically for TWA testing. Neither technology nor measurement protocol of the routine clinical exercise ECG is TWA-specific, and the levels of noise and heart-rate variability were often not acceptable. As a result, rejections due to ECG-related reasons

were 168 (63%, including 47 ICD_Cases and 121 ICD_Controls). The need of having two ICD groups characterized by the same clinical profile caused the rejections due to ECG-unrelated reasons, which globally counted 31 ICD patients (12%, including 0 ICD_Cases and 31 ICD_Controls). The reported rejection rates for ECG-related vs. ECG-unrelated reasons, however, depend on the temporal order according to which the enrollment criteria were applied, as presented in Methods. By applying first criteria 3 to 5 and then criteria 1 to 2, the rejection rates due to ECG-related and ECG-unrelated reasons would likely decrease (for possible previous rejections due to clinical parameters) and increase, respectively. The final number of rejected patients, instead, is independent of the criteria application order. Eventually, 29 (38%) ICD_Cases and 38 (20%) ICD_Controls were found to be qualified for the present study.

Although the ECG tracings of the enrolled patients underwent preprocessing for noise filtering and baseline subtraction, and were found to be suitable for TWA analysis, a small heart-rate variability and a low level of noise could still affect them. Consequently, automatic TWA identification was performed using our AMF method, which, compared to several other techniques, proved to be particularly robust to the presence of such interferences [27]. AMF robustness to noise is due to the fact that, each time the AMF is fed with a suitable ECG, it computes the ECG heart rate and, thus the corresponding TWA frequency, and filters out all ECG components including those related to noise, but not those related to TWA. Still, the presence of noise characterized by a frequency band overlapping f_{TWA} could jeopardize TWA estimation. Indeed, the noise component at f_{TWA} will survive preprocessing, corrupt the TWA signal determination and, consequently, ruin the TWAA estimation. Muscular noise and motion noise are two very common types of noise affecting real ECGs and characterized by a wide frequency band possibly overlapping f_{TWA}. However, their presence often causes artifacts in the ECG tracing whose identification in the pre-processing stage should lead to the tracing rejection [2, 3, 27].

In our study, pedaling frequency could also be an issue. Indeed, if f_{TWA} is an integer multiple of the pedaling frequency, the noise will have (according to the Fourier's analysis) a frequency component matching TWA frequency. For this reason, exercise tests specifically designed for TWA analysis should keep pedaling frequency under control. In the present retrospective study, we used exercise data in which pedaling frequency was not controlled since, as mentioned before, these data consisted of routine clinical exercise ECG recordings not specifically for TWA testing. However, in the presence of this rather unlikely but still possible situation of a pedaling noise component matching the TWA frequency, TWA would not be identified by the AMF unless the noise component (i.e. sinusoid) at the TWA frequency also matches TWA phase. In fact, to warrant a reliable TWA identification, the maxima and the minima of the sinusoidal signal at the output of the AMF must occur in correspondence of the JT segment for TWA to be identified. Only in the case of a noise component of the same frequency and phase of TWA, the noise would be falsely recognized as TWA by the AMF. Thus, for what concerns the presence of noise, while it is true that muscular, motion or pedaling noise could affect TWA measurements, the occasional occurrence of such cases should result in a limited statistical relevance. AMF robustness to subtle

heart-rate variability is due to the fact that, in real clinical cases, TWA is assumed to be characterized not only by a single frequency at half heart rate, as it would ideally be at fixed heart rate, but also by a small frequency band centered in f_{TWA} (i.e., mean half heart rate). These features of the AMF method make it a reliable technique to detect TWA in both rest and exercise conditions. To limit the noise propagation and the number of computations, the AMF was applied only to the six precordial leads, which were found to be particularly suitable for TWA identification in these [33] and other [28–30, 35, 36] groups of patients.

TWA may occur as a regionally specific phenomenon [35, 37] and can be lead-dependent. For this reason, a TWA value was provided for each one of the 6 precordial leads, as previously found to be particularly suitable for TWA identification in ICD patients [33]. In addition, lead-system (mean V1–V6 and max V1–V6) TWA values were also provided, and proved to have a greater ability to discriminate the two groups of ICD patients than most of the single lead measurements. However, mean V1–V6 and/or max V1–V6 TWAA values provide no insights into the spatial localization of TWA in the heart. By definition, max V1–V6 TWA may be more significantly affected by noise than mean V1–V6 TWA; therefore, the latter is supposed to provide a more robust TWA estimation. On the other hand, max V1–V6 TWA is more likely to be able to detect spatially specific TWA episodes which, on the surface ECG, will appear only in a few leads and would remain hidden by the averaging procedure.

During the NoWL phase of the exercise test, heart rates were similar in the two ICD groups (about 80 bpm). Nevertheless, TWA was quite low but significantly higher in the ICD_Cases (12–18 μV) than in the ICD_Controls (9–15 μV). This finding was observed in all leads and lead system measurements, even though V6 appeared to be the most powerful for discrimination of the two ICD groups, and therefore, for TWA's prediction of ventricular arrhythmias (Se = 83%, Sp = 84%). During the MaxWL phase of the exercise test, heart rates remained similar in the two ICD groups (around 123 bpm) and, as expected, was significantly higher than during NoWL. Accordingly, TWAA increased significantly in both the ICD_Cases (20-39 μV) and ICD_Controls (20–32 μV). Such finding, by confirming the well known TWA dependency on heart rate [20], also validates the AMF's ability to provide reliable TWA measurements in both resting and exercise conditions. Even though significantly higher values of TWA were observed in all leads and lead-system measurements, when comparing ICD_Cases vs. ICD_Controls, statistical significance was found only for V5 and max V1–V6 measurements (with values of Se for V5 being 66% and max V1–V6 being 69%; and values for Sp for V5 being 71% and max V1–V6 being 61%), which made TWA's ability to discriminate the two ICD groups practically negligible during MaxWL. Thus, in our ICD patients, AMF-derived TWA ability to discriminate patients at risk for malignant ventricular arrhythmias is higher at lower heart rates where TWAA is also smaller, than during exercise where both heart rate and TWA are higher.

Several studies have shown that exercise-induced TWA is capable of stratifying risk for cardiovascular death and lethal arrhythmias [10, 15, 16, 22–24, 28, 29]. However, other studies have not confirmed the usefulness of TWA in risk stratification [38, 39], but rather conclude that the TWA related benefits seem to vary with studied population, so that further investigations are needed before TWA's incremental prognostic role can

be properly defined [40]. In a recent document providing a consensus guideline for TWA, authors conclude that even though it is reasonable to consider TWA evaluation whenever there is suspicion of vulnerability to lethal cardiac arrhythmias, there is as yet no definite evidence from interventional trials that it can guide to therapy [41]. Our results simply suggest that TWA at rest, though having lower amplitude, could discriminate better than TWA during exercise, when its amplitude is larger. Such finding, which specifically relates to our AMF method, is not in contrast with the aforementioned studies on exercise TWA performed using other techniques. Indeed, it does not imply that TWA has no predicting power when measured during exercise, but rather, invite new evaluations, eventually performed using other methods, on the TWA predictive power at rest. Other works have also suggested that TWA is more specific when measured at low heart rates, but it is also less sensitive, while at frequencies higher than 110-120 bpm, it has a good sensitivity but a poor specificity [42, 43]. In our study, we found that both sensitivity and specificity are better during the NoWL phase of the exercise test when heart rate is lower, than during the MaxWL when heart rate is higher. Such a difference from the previous studies is probably due to the different axis along which the study is performed. Indeed, our axis is the workload, not the HR, even though the two quantities are clearly correlated. Still, low HR to us means 81±10 bpm and 79±10 bpm for ICD_Cases and ICD_Controls, respectively, and not exactly 90 bpm. Analogously, high HR to us means 122±13 bpm and 124±14 bpm, and not exactly 120 bpm. In other words, our low and high heart-rate values are characterized by a significant variability due to their patients' dependency. Thus, if Tanno *et al*. [42] and Kitamura *et al*. [43] studied TWA as a function of frequency, our study is an analysis of TWA in resting vs. exercise conditions, respectively, approximated and defined as the NoWL and the MaxWL phases of the exercise test. Eventually, the possibility of TWA's predictive power of ventricular arrhythmias being higher at rest than under exercise also parallels the observation that several characteristics (such as QRS width and QRS-T spatial angle) have been identified in ordinary 10 s resting ECGs to also have predictive value [44–46]. Nevertheless, given the small but still representative number of the enrolled patients, which represents the major limitation of the present study, future analysis on ECG recordings specifically performed for TWA testing (to reduce the number of rejected patients) are desirable. More specifically, special TWA electrodes (e.g., the Micro-V Alternans Sensors proposed by the Cambridge Heart, usually used for TWA studies performed by the standardized spectral method) and measurement protocols in which heart rate is kept constant during the NoWL and MaxWL phases of the exercise test, together with TWA quantification performed by other methods, should be considered. Optimization of ECG recordings for TWA testing, however, was beyond the scope of the present work, in which ECG signal quality has been the major cause of exclusions.

5. CONCLUSION

In our 66 ICD patients, increments in the heart rates, reached through exercise, are accompanied not only by an increase in the TWA amplitude but also by a decrease in the TWA's ability to discriminate ICD_Cases from ICD_Controls. The present study, performed using our AMF-method, suggests that TWA's predictive power for ventricular

arrhythmias could be better at low heart rates (when TWA amplitude is smaller) than at high heart rates (when TWA amplitude is higher). However, given the relatively small number of patients involved in the study and the data quality, future studies possibly performed on better data and using other methods, are needed to confirm our findings.

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CONFLICT OF INTEREST

All authors have no financial or personal relationships with other people or organizations that could inappropriately influence (bias) this work.

NOMENCLATURE

ACE	Angiotensin Converting Enzyme
AMF	Adaptive Match Filter
AT	Angiotensin
AUC	Area Under the Curve
BMI	Body Mass Index (Kg/m^2)
CHF	Congestive Heart Failure
CRT_D	Cardiac Resynchronization Therapy with Defibrillator
ECG	Electrocardiogram
HR	Heart Rate (bpm)
ICD	Implanted Cardiac Defibrillator
LVEF	Left Ventricular Ejection Fraction, %
MaxWL	Maximum Work Load
NoWL	No Work Load
NYHA	New Your Heart Association
ROC	Receiver Operating Characteristic
Se	Sensitivity
Sp	Specificity
TWA	T-Wave Alternans
TWAA	T-Wave Alternans amplitude, μV
VF	Ventricular Tachicardia
VT	Ventricular Fibrillation

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