REVIEW ARTICLE

T-Wave Alternans: Reviewing the Clinical Performance, Understanding Limitations, Characterizing Methodologies

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Accurate recognition of individuals at higher immediate risk of sudden cardiac death (SCD) is still an open question. The fortuitous nature of acute cardiovascular events just does not seem to fit the well-known model of ventricular tachycardia/fibrillation induction in a static arrhythmogenic substrate by a synchronous trigger. On the mechanism of SCD, a dynamical electrical instability would better explain the rarity of the simultaneous association of a correct trigger and an appropriate cardiac substrate. Several studies have been conducted trying to measure this cardiac electrical instability (or any valid surrogate) in an ECG beat stream. Among the current possible candidates we can number QT prolongation, QT dispersion, late potentials, T-wave alternans (TWA), and heart rate turbulence. This article reviews the particular role of TWA in the current cardiac risk stratification scenario. TWA findings are still heterogeneous, ranging from very good to nearly null prognostic performance depending on the clinical population observed and clinical protocol in use. To fill the current gaps in the TWA base of knowledge, practitioners, and researchers should better explore the technical features of the several technologies available for TWA evaluation and pay greater attention to the fact that TWA values are responsive to several factors other than medications. Information about the cellular and subcellular mechanisms of TWA is outside the scope of this article, but the reader is referred to some of the good papers available on this topic whenever this extra information could help the understanding of the concepts and facts covered herein.

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A majority of sudden cardiac death (SCD) cases are usually related to coronary artery disease, dilated nonischemic, and hypertrophic cardiomyopathies.¹ Equally important are those cases of SCD registered in otherwise healthy subjects. From 1994 to 2003, 453 SCDs were reported among individuals from 15 to 81 years old in the general population of the UK. In this set, 269 (59.3%) hearts were found to be macroscopically and microscopically normal.²

Accurate recognition of individuals at higher immediate risk of SCD is still an open question. Many factors (acquired or congenital; structural, functional, or genetic) are related to an increase in SCD risk, but they alone cannot pinpoint when someone will have this risk at its highest. Vigorous physical

activity (6 METS or higher) can potentially increase the risk of acute cardiovascular events, but the rarity of reported events associated with exercise is a clear indication that an additional specific cardiac substrate is needed, as stated in the joint communication of the American Heart Association and the American College of Sports Medicine.³

In other words, the apparent randomness of an acute cardiovascular event illustrates well that a static arrhythmogenic substrate and a trigger in correct synchrony to initiate ventricular tachycardia (VT) or fibrillation does not seem to be the typical pathophysiological mechanism of SCD. On the contrary, electrical instability would be dynamical, which would explain the low probability of the

correct trigger being associated with the appropriated cardiac substrate.⁴

Over the past decades, several studies have been conducted trying many ways to measure this cardiac electrical instability (or any valid surrogate) in an ECG beat stream. Briefly, they can be clustered in two main approaches: (1) how much the measured instability variable would be related to the proneness to future arrhythmias and (2) how fast the myocardium recomposes itself after a minor electrical arrhythmia (e.g., extrasystole) and its consequences on future prognosis. Building over usual clinical arrhythmia vocabulary, where primary and secondary preventions stand respectively for prevention before and after a cardiovascular event, we could call the former a *primary instability to arrhythmias* and the latter, *secondary instability to arrhythmias*. Among the clinical measures of primary instability we could number QT prolongation, QT dispersion, late potentials, T-wave alternans (TWA). Heart Rate Turbulence, on the other hand, would be a measure in the secondary instability group.

This review will highlight the role of TWA in the current cardiac risk stratification scenario. Due to the vast literature on the topic, this review will be divided in four parts. After TWA definition and a brief historical background, the second part will focus on TWA prognostic performance among different populations. Since TWA is essentially a technology-dependent exam, the third section will elaborate on a crucial aspect to TWA clinical performance: available technological approaches and their consequent analysis options and limitations. The fourth part will explore other factors that may modulate TWA values and influence TWA exams, like use of medication and physiological influences.

TWA Definition and Brief Historical Background

What is known as TWA is a regular amplitude oscillation in the ST-T part of the ECG tracing that occurs with half the frequency of the heart rate. In other words, the ST-T amplitude changes repeat themselves in an every-other-beat basis, so that one amplitude pattern can be created from even beats and another one can be related to odd beats. For didactic purposes, a chronological history of TWA research can be summarized in three distinct phases: the transition of research focus from macroscopic

to microscopic TWA; the impact of microvolt TWA in risk stratification protocols and health policies; and the establishment of a solid experimental background for current TWA clinical findings.

Visual (macroscopic) TWA has been reported since the electrocardiology beginnings,⁵ and it was always associated with poor prognosis. It was considered a rare finding until the invisible (microscopic) TWA was first published in the 1980s.⁶ Since then, several groups have been studying TWA, each in its own way. As a direct consequence of this, a review published in 2005 listed more than 10 different evaluation technologies,⁷ but their results were still restricted to particular segments of the cardiology research community. The next step—when TWA got the attention of the clinical cardiologist besides the researcher in cardiology—happened in the early 2000s, with published evidences that TWA could reduce the average number of implantable automatic defibrillators (ICD) needed to be used in order to actually save a life.⁸

By that time, intracardiac action potential duration alternans (APD-alternans) has also been extensively studied over the years. Back in 2002, there were already evidences that APD-alternans was the first link in an orderly progression of increasingly complex amplitude oscillation patterns toward ventricular fibrillation (VF) during ischemia,⁹ as well as that atrial APD-alternans were consistently recorded before the transition from atrial flutter to atrial fibrillation.¹⁰ Additionally, TWA and APD-alternans were already found to be associated with each other in experimental studies,¹¹ but so far there were no evidences that this relationship still held in clinical settings.

The third phase of TWA research has begun with the solution to this clinical puzzle. First, patients with cardiomyopathy and inducible VT or positive TWA test were shown to have greater repolarization heterogeneity (in both epicardium and endocardium) than those without inducible VT or negative TWA test.¹² Latter, TWA was consistently related to endocardial and epicardial APD-alternans in a way that a minimum number of sites with APD-alternans were always needed in order to TWA be successfully measured at the body surface, but APD-alternans alone were not always associated with TWA at the body surface.¹³

Nowadays, TWA presents itself as a valid clinical surrogate of APD-alternans, which is an important marker of cardiac electrical instability. The interested reader can refer to several publications on

the mechanisms of genesis of APD-alternans and its relationship with the cardiac arrhythmogenic substrate.^{4,14–17} This experimental foundation adds value on TWA stratification performances among different clinical populations, reviewed in the next section.

TWA AND CARDIAC RISK STRATIFICATION

Coronary or Ischemic Heart Diseases

The second Multicenter Automatic Defibrillator Implantation Trial (MADIT-2) provided compelling evidence of mortality reduction by use of ICD. Its findings showed an overall reduction of 31% in the mortality risk among postinfarction patients with left ventricle ejection fraction (LVEF) $\leq 30\%$, with no distinction among patients with more advanced disease as measured by NYHA class and blood urea nitrogen (reduction of 28–35%, regardless of the baseline mortality risk among subgroups).¹⁸ In 2003, the American Centers for Medicare and Medicaid Services (CMS) decided to cover the costs of prophylactic ICD treatment for patients MADIT-2-like and QRS duration > 120 ms. In 2005, CMS expanded the coverage to all MADIT-2-like patients,¹⁹ but there are still concerns about the cost-effectiveness of this decision and the necessity of better risk stratification.²⁰

TWA evaluation in 177 MADIT-2-like patients yielded a better risk stratification performance than QRS duration. Patients with narrow QRS (QRS < 120 ms) were not free from sustained ventricular arrhythmias (SVA) during a 2-year follow-up (mortality rate of 14%, similar to MADIT-2). On the other hand, the group of patients with normal TWA registered just 2 deaths in the same follow-up period (actuarial mortality rate of 3.8%) and the abnormal TWA cluster had a hazard ratio(HR) of 4.8 ($P = 0.012$) for all-cause mortality, adjusted for QRS duration. In MADIT-2, it was needed 18 implanted ICDs to save 1 life, but just 7 ICDs were needed to save 1 life with a TWA screening strategy.⁸

A prospective cohort study comprising 768 ischemic patients with LVEF $\leq 35\%$ and no prior sustained VT (51% received ICDs) brought up strong evidences that ICD benefits differ according to TWA status, lowering all-cause mortality (HR = 0.45, 95% CI = [0.27, 0.76], $P = 0.003$) in TWA-nonnegative patients (TWA positive or indeterminate) but with no reduction in the mortality in the

TWA-negative cluster (HR = 0.85, 95% CI = [0.33, 2.20], $P = 0.73$). Moreover, it was also shown these findings were largely due to arrhythmic mortality reduction. Regarding to the efficacy of ICD therapy, 9 ICDs were needed to save 1 life in a 2-year span among TWA-nonnegative patients, and 76 ICDs were needed to save 1 life in the same period in the TWA-negative group.²¹

TWA and QRS Duration

In 2002, Rashba and colleagues evaluated 108 patients ($\geq 50\%$ stenosis in any of the three major vessels, LVEF $\leq 40\%$; 62 patients with QRS ≥ 120 ms) referred for EPS for standard clinical indications. TWA was found to be not useful in patients with prolonged QRS.²² However, in 2007, a large study analyzed 768 patients (LVEF $\leq 35\%$, $\geq 70\%$ stenosis in one or more coronary vessel or prior infarction/revascularization, no history of ventricular arrhythmias; 223 with QRS > 120 ms) and compared outcomes grouping by ICD status (with or without ICD). TWA was associated with all-cause mortality and arrhythmic events in the non-ICD group (HR = 2.27, $P = 0.01$ and HR = 3.32, $P = 0.001$, respectively) as well as with all-cause mortality and defibrillator shocks in the ICD group (HR = 2.42, $P = 0.04$). Prolonged QRS duration had no prognostic utility in predicting outcomes in the ICD group (HR = 1.33 for arrhythmic death, $P = 0.29$) as well as in the non-ICD group (HR = 0.71 for arrhythmic death, $P = 0.95$). Another major finding was the absence of interaction between TWA and QRS duration ($P = 0.19$ – 0.73 , depending on outcome and group of interest), which strongly suggested that TWA prognostic performance did not differ among different QRS duration groups in ischemic population.²³

TWA and Electrophysiologic Study (EPS)

Two recent large studies yielded strong, but not definitive, results about TWA and EPS comparative performances. The Alternans Before Cardioverter Defibrillator (ABCD) trial evaluated 566 patients with coronary disease, LVEF $\leq 40\%$ and nonsustained ventricular tachycardia (NSVT) aiming to test whether TWA-directed therapy was as effective as EPS-directed therapy in predicting ventricular tachyarrhythmic events. Preliminary findings have demonstrated that TWA and EPS have similar negative and positive predictive values only in the first year of follow-up, with TWA losing power in

the following year.²⁴ Cantillon and coworkers evaluated 286 patients of mixed etiologies (75% with ischemic disease), LVEF $\leq$ 35%, with NSVT and/or syncope. In common with the ABCD trial findings, TWA-negative patients presented a considerable 2-year event rate (19%). Contrary to ABCD findings, TWA performance was found superior to EPS: half of the patients presented discordant EPS and TWA results, with only TWA being an effective predictor or arrhythmia-free survival in a 2-year follow-up (multivariate analysis HR = 2.37, 95% CI = [1.49, 3.81], $P < 0.01$).²⁵ Careful comparison of inclusion/exclusion criteria, as well as clinical protocols, may explain these differences. Nevertheless, both studies suggested that TWA-negative patients may have a considerable event rate after 2 years of examination.

TWA and Appropriate ICD Therapy

Primary results from the Microvolt TWA Testing for Risk Stratification of post-MI patients (MASTER I) trial showed that TWA testing did not predict life-threatening ventricular tachyarrhythmic (LT-VT) events (as assessed by ICD shocks) in 654 patients MADIT-2-like (post-MI with LVEF $<$ 30%) and not in atrial fibrillation (HR = 1.26, 95% CI = [0.76, 2.09], $P = 0.37$). The analysis was conducted in 575 patients, minimum follow-up period was 2 years, and the occurrence of LT-VT events in negative and nonnegative (positive + indeterminate) TWA groups was respectively 10.3% and 13.3% ($P = 0.37$).²⁶ However, an appropriate ICD shock (A-ICD) should not be regarded as a surrogate for SCD. The first appears to occur more frequently than SCD in patients with nonischemic cardiomyopathy²⁷ as well as ischemic or dilated cardiomyopathy.²⁸ Indeed, the study of Koller and coworkers elegantly performed a competing risk analysis in which A-ICD is the event of interest and prior death is the competing risk event and their findings showed that 55% (16 of 29) of all deaths without prior A-ICD were cardiac and not associated with heart failure.²⁸

On this topic, Hohnloser et al. recently presented in Heart Rhythm Society's 2008 Scientific Sessions an analysis of several TWA clinical trials performed in primary prevention populations, grouped on low and high frequency of appropriate ICD therapy (low A-ICD, high A-ICD) clusters, with a total of 3682 patients in the low A-ICD group and 2234 patients in the high A-ICD group. The low A-ICD

group had a higher HR associated with nonnegative versus negative TWA (HR = 13.6, 95% CI = [8.5, 30.4], $P < 0.0001$) as well as a lower annual event rate (AER = 0.3% [0.1%, 0.5%]) among negative TWA patients when compared to the high A-ICD group (HR = 1.6, 95% CI = [1.2, 2.1], $P < 0.001$; AER = 5.4% [4.1%, 6.7%]). The excess A-ICD appears to distribute randomly between TWA negative and nonnegative patients in the evaluated clinical trials.²⁹

Brugada Syndrome

Findings from a multicenter retrospective investigation of outcomes in a population of type 1 Brugada patients with ICD ($n = 220$) stated well the problems of pinpointing the subjects who would benefit from ICD therapy in this population. No death was reported over a follow-up period of 38 ± 27 months (range 1–150 months) after ICD implantation. Moreover, only 8% of patients received an appropriate shock, against the 20% of patients with registered inappropriate shocks. The exact appropriate shock figures between symptomatic (resuscitated SCD or syncope) and asymptomatic patients were, respectively, 12% and 4% ($P = 0.05$). Finally, available data also indicated that EPS failed to predict arrhythmic events in this population.³⁰ What about TWA? On this topic, current information on macroscopic and microvolt TWA prognostic performances appear to be contradictory.

In the setting of Brugada Syndrome, the evaluation of late potentials (LP), TWA (exercise-induced) and QT dispersion in 33 patients with manifested Brugada ECG phenotype found that only LP were predictive of life-threatening events.³¹ In 2004, another study with nine symptomatic Brugada patients also stated that exercise TWA was not an appropriate test for arrhythmia stratification in this population.³² The largest prospective study on non-invasive indices for risk stratification of Brugada patients so far ($n = 124$) also brought evidences that exercise TWA was present in 14 patients (11%) but was not a significant risk stratifier in the sample.³³

On the other hand, intravenous administration of pilsicainide (50 mg, class IC antiarrhythmic drug) in a Brugada patient-induced transient macroscopic TWA in leads V₂ and V₃, with posterior significant microvolt TWA in orthogonal leads Y and Z.³⁴ Another case-report of a Brugada patient brought evidences that both procainamide (450 mg, class IA antiarrhythmic agent) and pilsicainide (25 mg)

infusions accentuated ST-segment elevation and induced macroscopic TWA in leads V2 and V3, although no microvolt TWA testing was not performed at that time.³⁵ The most recent work on TWA and Brugada (n = 77 patients), published in 2008, stated that macroscopic TWA after pilsicainide infusion (1 mg/kg) was more related to occurrence of VF during the follow-up period (odds ratio (OR) = 22.21, P = 0.001) than LPs (OR = 12.46, P = 0.047), or even induction of VF during EPS (OR = 6.00, P = 0.080).³⁶

In conclusion, there are compelling evidences that current TWA testing by standard exercise protocols are not of prognostic value in Brugada patients. On the other hand, the infusion of class I antiarrhythmic drugs is used to unmask a typical Brugada ECG phenotype but it seems to induce macroscopic TWA, which in this setting appears to be well correlated to VF episodes in the follow-up period. These findings seem to be contradictory, as macroscopic and microvolt TWA should not be treated like distinct entities.

The same publication that stated the inefficacy of exercise TWA as a risk stratifier in Brugada patients also reported that Holter monitoring just before VF episodes showed a shift toward increased vagal activity, as assessed by heart rate variability.³³ The future use of Holter TWA, the assessment of TWA during spontaneous ST changes or standard drug challenge to unmask Brugada phenotype, as well as in vagal maneuvers in Brugada patients, may then set the final word on the TWA risk stratification value in this high-risk population.

Nonischemic Cardiomyopathy

In 2003, the Marburg Cardiomyopathy Study (MACAS) evaluated 263 patients with idiopathic dilated cardiomyopathy, sinus rhythm, LVEF $\leq$ 45%, NYHA class I–III, and determined that LVEF was the only significant predictor of major arrhythmic events during an 18-month follow-up.³⁷ In 2005, the TWA in Congestive Heart Failure trial (TWA in CHF) studied 549 patients (51% nonischemic) with LVEF $\leq$ 40%, NYHA class I–III, no prior SVA and reported that abnormal (positive or indeterminate) TWA tests were strongly associated with cardiac events (death or nonfatal SVA) during the 2-year follow-up period.³⁸ Discrepancies between the results of MACAS and TWA in CHF might be explained by the different study designs: the former treated separately positive and indeterminate TWA

tests, as well as could not guarantee a uniform use of beta-blockers.

The T-wave Alternans in Patients with Heart Failure (ALPHA) trial was designed to further elucidate the prognostic performance of TWA in this population. Published in 2007, it was comprised of 446 patients with LVEF $\leq$ 40% and NYHA class II–III, followed by 18–24 months. After 18 months, abnormal TWA tests were associated with a four-fold higher risk of cardiac death or life-threatening arrhythmias (HR = 4.01, 95% CI = [1.41, 11.41], P = 0.002) and TWA negative predictive value ranged from 97.3% to 98.6% among subgroups. In addition, no significant association was found between TWA and LVEF or QRS duration in this population.³⁹

Interestingly, quantitative TWA comparison among patients with ischemic or nonischemic cardiomyopathy revealed that nonischemic patients had larger TWA values and more TWA-positive ECG leads than ischemic ones. In both groups, patients with events during follow-up also had higher TWA values, but only nonischemic patients with events presented a higher percentage (38%) of positive TWA test already under resting conditions.⁴⁰

TWA and Peak VO₂

Peak VO₂ is considered as a marker of all-cause mortality in patients with congestive heart failure, independently of beta-blocker therapy, and is also an absolute key indication for heart transplantation. However, peak VO₂ is not suitable to risk stratification of SCD.⁴¹ The association of TWA and peak VO₂ in a sample of 70 patients with dilated cardiomyopathy was a significant predictor of either major cardiac events (death or documented VT or VF) or arrhythmic events. When considered alone, peak VO₂ failed to reach statistical significance for prediction of both endpoints and TWA only stratified for arrhythmic events.⁴²

Hypertrophic Cardiomyopathy

Patients with hypertrophic cardiomyopathy (HCM, n = 28) showed distinct TWA profiles from those with hypertensive left ventricular hypertrophy (HLVH, n = 29) and similar hypertrophy grade. TWA values were higher in HCM patients with severe histological changes (myocardial disarray and/or fibrosis) than in HCM patients with mild histological changes or HLVH subjects. Actually,

no significant TWA differences between the last two groups were reported.⁴³ In 2002, Kuroda and colleagues compared several parameters between TWA-positive and TWA-negative HCM patients ($n = 53$, of which 37 nonobstructive type and 12 with apical hypertrophy). From ECG, they evaluated resting SV1+RV5, QT dispersion, ST-T exercise-induced depression. Thicknesses of inter-ventricular septum and posterior wall, as well as end-systolic and end-diastolic diameters of left ventricle were evaluated from echocardiogram. The number of total ventricular ectopic (VE) beats, maximal sequence of VE and presence of NSVT (≥ 5 VE beats) were assessed from 24-hour Holter records. LP presence/absence, quantification of fibrosis, disarray and hypertrophy of myocytes, as well as family history of HCM or sudden death were also included. Interestingly, TWA-positive and TWA-negative patients showed similar ECG and echocardiographic parameters, similar LP patterns and family histories. Again, TWA-positive patients were only distinctive by a significantly higher degree of myocyte disarray (not fibrosis) and higher presence of VE and NSVT episodes.⁴⁴

The comparative analyses of pathological findings, mode of death and risk profile in HCM patients ($n = 75$, age ranging from 6 to 72 at time of death/transplantation) showed an association of disarray and ischemia as well as fibrosis and nonsustained ventricular tachycardia (NSVT).⁴⁵ In a similar study, disarray was found to be inversely related to age and apparently not related to sex or wall thickness, while fibrosis increased with age and was specifically influenced by sex. Hence, disarray would probably be a primary response to functional or structural abnormalities of the mutated protein, whereas fibrosis would be a secondary manifestation unrelated to the former.⁴⁶ Disarray was also associated with morphologically expressed adrenergic myocardial stress in hearts ($n = 340$) in conditions of sympathetic overtone,⁴⁷ and that would be a plausible justification to its link with TWA since TWA itself is elicited by stress situations or increased sympathetic activity in general (see "Factors that modulate TWA").

Pediatric and Congenital Heart Disease

There are sparse evidences of TWA in this population. In 2006, it was published a cohort study whose findings stated that TWA presence during clinical evaluation of pediatric patients with

mixed etiologies or adults individuals with congenital heart disease ($n = 304$) was associated with higher risk of cardiac arrest or ventricular arrhythmias (HR = 5.0–7.9 with $P \leq 0.009$, and specificity of 96–100% depending on the outcome examined). Even with the adaptation of typical TWA onset heart rate thresholds (see "TWA classification schemes") to a pediatric population, a TWA-negative result do not strongly exclude patients with events: 273 (96%) patients without events during follow-up were TWA-negative, but 74% (14 out of 19) of patients with events also showed TWA-negative tests.⁴⁸ Further studies with more homogeneous cohort would help to clarify aspects of TWA performance in this population.

Apparently Healthy Individuals and General Population

Adults

The prevalence of TWA at rest and during exercise in apparently healthy subjects (none on permanent medication) was evaluated in 48 individuals (age 21–53 years, 29 men). Functional and structural heart diseases were excluded by clinical history and evaluation, resting and exercise ECG, Doppler echocardiography. TWA transients were observed in 5 (10.4%) individuals. Sustained TWA were registered in 2 (4.2%) subjects, but only 1 (2.1%) met all positivity criteria. And none of the 48 individuals developed arrhythmia morbidity during the follow-up period of 12–40 months.⁴⁹ A larger study (110 healthy subjects, 20–75 years, 76 men) was published in the same year with 5 (5%) subjects showing a positive TWA test, 98 (89%) individuals TWA-negative, and 7 (6%) with indeterminate TWA tests. Again, no arrhythmia morbidity or mortality was registered during a follow-up of 32 ± 15 months.⁵⁰

Under 18

TWA was evaluated in 100 normal volunteers (no heart disease history, normal clinical examination and 12-lead rest ECG; 8–17 years). Excessive noise prevented adequate exercise data in 16 volunteers and adequate resting data in 24 volunteers, but all other 76 volunteers were TWA-negative at rest. Nine volunteers (11% of the valid tests) had sustained alternans, all with onset heart rates higher than usual adult criteria: they had a range from

120 to 158 bpm, whereas the usual onset heart rate threshold is less or equal than 110 bpm.⁵¹

General Population

A substudy of the Finnish Cardiovascular Study (FINCAVAS) reported the TWA evaluation in a general population cohort of 1037 patients (61.4% men, 58 ± 13 years), all referred to exercise stress test. Clinical indications for exercise test comprised diagnosis of coronary heart disease (46%), vulnerability to exercise-induced arrhythmia (18%), evaluation of work capacity (19%), adequacy of coronary heart disease treatment (24%), patient profiling before an invasive operation (13%), and evaluation after a myocardial infarction (10%). Exercise-induced TWA with either a TWA magnitude cutpoint of $47 \mu\text{V}$ or $65 \mu\text{V}$ was a strong predictor of SCD (RR = 2.9, $P = 0.02$ and RR = 7.4, $P < 0.001$, respectively) as well as cardiovascular death (RR = 2.6, $P = 0.01$ and RR = 6.0, $P < 0.001$), yielding excellent negative predictive values, both around 98%.⁵²

Athletes

Amateur soccer players with or without mitral valve prolapse and age-matched sedentary subjects (three groups of 20 individuals) did not show any TWA-positive tests in standard exercise stress protocols.⁵³ TWA and EPS were also performed in professional competitive athletes, of several sports, either healthy ($n = 48$) or with important arrhythmias but no arrhythmogenic pathology ($n = 52$). No healthy athlete had a TWA-positive test or any event during an average follow-up of 36 months. On the other hand, 7 of 52 (13.5%) arrhythmic athletes showed a TWA-positive test, of which 5 also had an EPS-positive test for VT and 1 was positive for severe supraventricular tachycardia (the other one refused EPS). In the arrhythmic athletes' cluster, all 42 negative TWA tests were also followed by negative EPS but one, specifically on treatment with amiodarone. However, this TWA-negative/EPS-positive subject did not show any events during a 25.3-month follow-up.⁵⁴

A recent study on athletes with ventricular arrhythmias ($n = 85$, 61 male) stressed the good correlation between TWA and EPS results in this population. Similar figures were reported for TWA-positive tests (15 of 85, 18%), with less frequent negative TWA tests (57 of 85, 68%) and more

indeterminate tests (13 of 85, 14%). All TWA-positive athletes had EPS-positive results and all TWA-negative athletes were EPS-negative. No correlation was found between indeterminate TWA and EPS results. Regarding to the occurrence of events during an average follow-up of 30 months, TWA-negative athletes had no events, and 5 TWA-positive subjects reported events as well as 2 TWA-indeterminate subjects.⁵⁵

Type 2 Diabetes

Patients with type 2 diabetes have significant higher prevalence of VF, which appears to be independent of coronary artery disease or congestive heart failure.⁵⁶ Also, diabetes mellitus has been associated with increased risk of cardiac conduction abnormalities.⁵⁷ Although sparse, current evidences suggest that abnormal TWA (positive + indeterminate) tests are common in diabetic subjects without manifest cardiovascular disease (being reported at rates of approximately 25%) and are strongly related to glycemic control: abnormal TWA values were jointly reported with higher hemoglobin A1c.^{58,59}

Following Acute Myocardial Infarction

On prediction of serious arrhythmic events in the setting of preserved cardiac function after acute myocardial infarction (MI), a Japanese prospective study ($n = 1041$; 79% men) found that TWA showed a prognostic performance similar to studies on post-MI populations with depressed LVEF. TWA testing was performed at least 14 days after acute MI, with 169 (17%) positive tests, 747 (74%) negative and 87 (9%) indeterminate tests, overall sensitivity and negative predictive value of, respectively, 81% and 99.6% and a multivariate analysis HR = 19.7, 95% CI = (5.5, 70.4), $P < 0.0001$ for arrhythmic events.⁶⁰

The Risk Estimation Following Infarction, Non-invasive Evaluation (REFINE) cohort study evaluated the prognostic performance of autonomic tonus and/or cardiac electrical substrate assessment in the identification of patients at higher cardiac risk early after MI. Briefly, no single parameter (TWA, heart rate turbulence, baroreflex sensitivity) evaluated at 2 to 4 weeks (acute phase) after MI successfully predicted outcomes. The best diagnostic accuracy in the nonacute phase (10 to 14 weeks) after MI was achieved combining abnormal TWA and HRT plus LVEF < 50%. This composite indicator

correctly identified two-thirds of all patients who suffered a cardiac arrest, with sensitivity of 55%, specificity of 86%, and negative predictive value of 96%. Importantly, TWA measured during exercise stress or Holter recording presented similar performances, even though it was evaluated with different technologies at each time. In a multivariate analysis adjusted for age, gender, previous MI, and diabetes, the composite indicator with Holter-TWA resulted in HR = 6.22, 95% CI = (2.88, 13.47), $P < 0.001$ and the composition with exercise-TWA yielded HR = 5.08, 95% CI = (2.17, 11.89), $P < 0.001$.⁶¹

Distinct from REFINE findings, another study on TWA assessment in the period from 7 to 30 days after MI ($n = 119$) yielded 17 (14%) indeterminate tests, 50 (42%) positive tests, and 52 (44%) negative tests. In a follow-up period from 3 to 23 months, TWA had the best prognostic value among indicators (TWA, LPs, ejection fraction): 14 of the 15 patients with arrhythmic events were TWA-positive, with the best sensitivity and negative predictive value among all analyzed parameters (93% and 98%, respectively; relative hazard of 16.8, $P = 0.006$).⁶²

TWA Temporal Variations after MI

To collect evidences about the best timing of TWA evaluation after MI, Oliveira and colleagues assessed TWA twice in 51 post-MI patients: with less than one month and at 6 months after acute MI. TWA tests were discordant in 15 (29.4%) patients, of which 7 (13.7%) changed from TWA-negative to TWA-positive and only 1 (1.9%) changed from positive to negative. All other changes were due to indeterminate tests.⁶³ Temporal variations on TWA testing after MI may well follow ventricular remodeling after MI. On this topic, a pooled analysis from the placebo arms of five multicenter trials (total of 3431 subjects) showed that the arrhythmic death risk in the first 6 months was two to fourfold that in the subsequent 6 months. In fact, further analysis even suggests that two-thirds of all arrhythmic deaths happen in the first 45 days after MI.⁶⁴ Provided that these numbers apply to the clinical population in a hospital's catchment area, they alone may explain discordant TWA testing results during patient evolution. However, temporal TWA variations are also subject to reproducibility issues. TWA testing reproducibility, along with the clinical relevance of the appropriate choice of technology used

in TWA evaluation, is further discussed in the next section topic.

Left Ventricular Dysfunction and Heart Failure or Diabetes Mellitus

The Eplerenone Postacute Myocardial Infarction Heart Failure Efficacy and Survival Study (EPHESUS) has yielded an interesting substudy about the TWA analysis of the recorded 24-hour ambulatory ECGs of alive controls ($n = 92$) and patients who died of cardiovascular causes during follow-up ($n = 46$, 18 from SCD). Sample profile included patients with LVEF $\leq 40\%$, predominantly male ($n = 90$, 65%), with either clinical signs of heart failure (67%) or diabetes (33%), of whom 24-hour AECGs were recorded 2–10 days post-MI. Maximum TWA in either modified V_1 or V_3 leads was associated with SCD. In V_1 , the relative risk (RR) of SCD was 5.2 (1.8, 14.6) ($P = 0.002$) with 45% (5 of 11) of the patients whose TWA was above 47 μV dying suddenly. As for V_3 , RR was 5.5 (2.2, 13.8) ($P < 0.001$) with the death of 42% (8 of 19) of the patients above the same cutpoint.⁶⁵

TWA Analytical Methodologies

TWA is essentially a technology-dependent exam since its typical amplitude oscillations (in magnitude order of few 1/50 mm at standard gain of 10 mm/mV) are beyond the visual resolution of the practitioner. As mentioned in the historical background, there are several distinct methodologies of TWA assessment. So, the choice of methodology will have a direct impact on TWA measured values and clinical limitations, even if the different TWA algorithms have similar clinical performances.^{61,66} This section aims to summarize the basic concepts, distinguish features and clinical limitations of the most relevant TWA analysis methodologies developed. Relevance criteria included the only two commercially available TWA analysis algorithms (spectral method and modified moving average) and the most similar research-only available methodologies (complex demodulation and intra-beat average).

How is TWA measured? Recalling from TWA definition, frequency is the key concept. TWA, whenever present, always happens at half the heart rate or, in other words, at a frequency of 0.5 cycles per beat (cpb). Whenever we think of automatic TWA detection this is the only prior information available, as no one knows in advance whether it

is present or not, or which part of the ST-T complex will alternate, or even how big alternation will be registered. It is much like tuning a radio station in a car stereo: one knows the interested radio frequency, but cannot foretell whether there will be any relevant information at all or just noise. From this distinctive TWA feature—fixed frequency—it seemed rather logical (or natural) that the earliest forms of TWA analysis were based on tracking this frequency of 0.5 cpb. This approach is currently available in the spectral method (SM) and the complex demodulation (CD).

Spectral Method

The SM measures T-wave fluctuations by computing point-to-point differences among 128 equally spaced sites in the ST-T from a stream of

128 aligned consecutive beats (ectopic and noisy beats already discarded).⁶⁷ In other words, there are 128 tachograms similar to those used in heart rate variability analysis. Latter, 128 frequency spectra are computed (hence the methodology name—SM) and averaged. The TWA value is then evaluated at the frequency of 0.5 cpb (Fig. 1). In 1994, the adaptation of this technique to human patients was first published.⁶⁸ Since then, it is the most used TWA analysis approach, with the widest range of applications.

Complex Demodulation

The CD approach was presented later than SM,⁶⁹ as an alternative algorithm. Basically, this method evaluates only the energy in the close vicinity of the alternans frequency of 0.5 cpb, instead of

Figure 1. The four main steps of the SM methodology. (A) Selection of 128 equally spaced points in the ST-T complex of a 128-beat ECG stream. (B) Graphical descriptions of the amplitude variation along the 128 beats from ST-T point 1 to ST-T point 128 (128 tachograms). (C) Each tachogram has its spectrum computed by the Fourier Transform, in a total of 128 spectra. (D) All 128 spectra are averaged to create a composite spectrum. From this composite spectrum, the alternans power is computed as the power at 0.5 cpb minus the average noise power measured. The correspondent TWA amplitude is the square root of the alternans power.

calculating fluctuations over a wide range of frequencies like SM does.

As the TWA research field matured a new family of algorithms emerged, all based on the comparison of beat patterns. This approach is currently available in the modified moving average (MMA) and the intrabeat average.

Modified Moving Average

The MMA recursively creates two-beat templates from any stream of valid beats (one associated only

with even beats and another, with odd beats). For each beat template the algorithm is as follows: The amplitude differences between the current (even or odd-beat) template and the next valid (even or odd) beat are measured over several equally spaced sites in the ST-T. Each of these differences are divided in X equal parts (where X may be 8, 16, 32, or 64) and the contribution of the current valid beat to the next template instance is bounded to 1/X (called update factor or bounding fraction) of the differences between template and beat (Fig. 2). Finally, TWA values are available every 15 seconds, as the

Figure 2. Illustration of the main steps of the MMA algorithm. Reprinted from Figure 1, reference 92.

Figure 3. Schematic diagram showing the most used three-category classification schemes in TWA testing with the SM technology. Most two-category schemes are based on grouping together positive and indeterminate tests in an “abnormal” cluster, though some may group only those indeterminate tests with specific indeterminacy causes. See text for information. Abbreviations used: MaxHR = maximum heart rate achieved in the test; MaxNegHR: maximum heart rate in the test with clearly no TWA. It is calculated backward, from the maximum heart rate to the basal heart rate.

difference between even-beat and odd-beat recursive (continuously updated) templates.⁷⁰

Intrabeat Average

Intrabeat Average (IBA) concepts and features are much the same as those presented in the MMA approach. Its hallmark is the splitting of the ST-T complex in three time intervals (T-onset to T-peak—that includes ST segment; T-peak to T-end; T-onset to T-end) and the computation of distinct TWA values for each one of them.^{71–73}

Adaptability to Clinical Settings and Protocols

The selection of a TWA analysis algorithm for clinical (routine and research) purposes cannot rely solely on sensitivity, specificity, or predictive values found in the scientific literature on the subject. That is because none of the most used methods had its application reported in all the relevant clinical settings. SM—the most prolific method—has been reported in the majority of studies on TWA in all clinical populations involved in TWA screening so

Table 1. Theoretical Applicability and Published Clinical Studies on TWA Clustered by TWA Evaluation Methodology, Study Protocol for TWA Assessment and Clinical Population

TWA Evaluation Methodology					
Pacing	Theoretical Applicability?*	SM Yes	CD Yes	MMA Yes	IBA Yes
Published clinical studies	Atrial	<i>CARISMA substudy</i> ⁷⁴ LVEF ≤ 40%, WMA ^{12,13} LVEF ≤ 40%, EPS ^{66(#)} LVEF ≤ 35%, EPS ²⁵	EPS ^{68,75,80} CAD, EPS ^{76,101} Brugada ³⁴	VT or SVT ¹⁰⁴ ICM, VT ¹⁰³	LVEF ≤ 40%, EPS ^{66(#)}
	Ventricular Atrial + Ventricular	EPS ⁷⁵ <i>CARISMA substudy</i> ⁷⁴ ICD electrogram ^{94(#)}	LVEF ≤ 40%, EPS ^{66*}	LVEF ≤ 40%, EPS ^{66*}	ICD testing ⁷³
Stress ECG	Theoretical Applicability?*	Yes	Yes	Yes	Yes
Published clinical studies	Not specified	<i>REFINE</i> ⁶¹ <i>SCS</i> ⁹⁸	<i>MASTER I</i> ²⁶ Brugada ³³	<i>MACAS</i> ³⁷	<i>ABCD</i> ²⁴
	Ergometer	<i>CARISMA substudy</i> ⁷⁴ MADIT-2-like ⁸ Type 2 DM ^{58,59} Healthy subjects ^{49,50} NIHD ^{42–44,95,100}	<i>TWA in CHF</i> ^{38,89} Post-MI ^{60,62} EPS ⁷⁹ CHF ^{40,77}	<i>ALPHA</i> ³⁹ Athletes ^{53–55} Brugada ^{31,32} CAD, EPS ⁷⁶	<i>FINCAVAS Substudy</i> ⁵²
	Treadmill	<i>TWA in CHF</i> ^{38,89} EPS ⁷⁹	Pediatric ^{48,51} <i>ALPHA</i> ³⁹ CHF ⁷⁷	Post-MI ⁶⁰	
	Pharmacological	IHD, LVEF ≤ 35% ^{21,23,90} Dobutamine ⁸¹	Athletes ⁵⁴ Atropine ⁸⁰		
Ambulatory ECG	Theoretical Applicability?*	N.P.	Yes	Yes	Yes
Published clinical studies			<i>ST-T database Data analysis</i> ⁸⁴	<i>ATRAMI 92 (§)</i> <i>REFINE trial</i> ⁶¹ <i>EPHESUS</i> ⁶⁵ Mental stress ⁹³ Guidelines ⁸⁵	<i>ESVEM</i> ⁷² Mental stress ⁹⁶ Prior TV onset ⁷¹

Papers related to randomized trials (either trials originally involving TWA, specific substudies or data reanalyses) or international databases are italicized. * = Evaluated according to the methodological principles framework published by Martinez and Olmos.⁷ # = noncommercial algorithm implementation; § = TWA analysis of trial data (either partial or total); N.P. = not possible with the current commercial implementation of this methodology; LVEF = left ventricle ejection fraction; WMA = wall motion abnormalities; EPS = referred for electrophysiological study; CAD = coronary artery disease; ICM = ischemic cardiomyopathy; VT = ventricular tachycardia; SVT = supra-ventricular tachycardia; ICD = implanted Cardio-defibrillator; NIHD = nonischemic heart disease; IHD = ischemic heart disease; CHF = congestive heart failure.

far (Table 1). However, SM has major protocol restrictions: it requires a smooth heart rate acceleration, whether by pacing,^{74,75} bicycle exercising^{76–78} treadmill,⁷⁹ or pharmacological stress.^{80,81} This definitely rules out this approach whenever TWA analysis of ambulatory ECG is concerned. And there is

still some controversy on which protocol should be preferred.

Atrial pacing is traditionally used in SM-TWA since it was first published in clinical studies in humans.⁶⁸ Later, bicycle exercising was shown to be superior to atrial pacing for prediction of death,

SVA, and appropriate implantable cardioverter defibrillator therapy.⁷⁶ In 2005, as a substudy of the CARISMA trial, Raatikainen and colleagues have reported that simultaneous ventricular and atrial pacing has significantly less indeterminate test results ($n = 7$) than bicycle exercise ($n = 34$) and atrial pacing ($n = 21$), with a higher incidence of sustained TWA (24% during exercise test and 50% during V+A pacing).⁷⁴

In general, exercise protocols (bicycle or treadmill stress) are considered more physiological than pacing TWA tests. The reliability of bicycle-based SM-TWA testing was found acceptable in several works. Results of determinate (positive or negative) tests were concordant in 82% (18 of 22 patients) of sequential SM-TWA tests within an average 15-minute period⁷⁷ and concordant in 93% (39 of 42) when tests were performed within a 4-hour time gap.⁷⁸ On the other hand, treadmill test results were considered equivalent to those obtained in bicycle SM-TWA tests, with the advantage of a lessening in the rate of indeterminate tests (9 of 58 on treadmill, 14 of 58 on pedaling).⁷⁹ Exercise protocols vary according to the patient clinical conditions: on treadmill either Naughton Protocol or Modified Bruce Protocol should be used, whereas in bicycle ergometer test, ramp protocols are optimal.⁸² Dobutamine and exercise stress tests yielded concordant results in 27 out of 29 patients with recent myocardium infarction.⁸¹ Atropine and atrial pacing had similar results in 22 of 27 subjects referred to EPS.⁸⁰

Another frequency-domain method, the CD algorithm runs with shorter streams of beats than those required by the SM commercial approach (i.e., less than 128 beats) and with less strict heart frequency stability conditions. CD use was reported in clinical TWA studies with acute coronary artery occlusion and reperfusion during angioplasty⁸³ as well as with annotated records from an international Holter database (the European ST-T Database) and computer simulations.⁸⁴ Nevertheless, the major contribution of CD algorithm to TWA base of knowledge is that this methodology can be easily extended to allow dynamic tracking of T-wave heterogeneity beyond TWA. Thanks to that it was possible to show that increasingly complex T-wave oscillations (tripling or 3:1; quadrupling or 4:1) were effectively involved in TWA transition to induced VF during ischemia.⁹

Time-domain methods (both MMA and IBA) are theoretically the most flexible TWA analysis ap-

proaches, even though their application was not reported in all possible clinical settings yet. These algorithms do not have technical restrictions of use in pacing, exercise, sinus rhythm during clinical procedures, and/or ambulatory ECG. Actually, these approaches are undisputed in Holter ECG TWA analysis, with methodological guidelines already published.⁸⁵ The differences of adaptability among methods are summarized in Table 1.

Quantitative TWA Assessment and Noise Influences

How do TWA algorithms measure TWA amplitude and background noise, and how can these measurement differences weaken the comparisons among test results obtained with different algorithms? SM, CD, and MMA algorithms measure both local (point-wise) and global (over the entire interval) TWA values over the ST-T complex. What differs among them is how they generate local TWA values, how they assemble them into global TWA estimates and how they isolate TWA from noise (Table 2). CD local TWA value is just the magnitude verified in the 0.5 cpb frequency at any specific point in the ST-T.⁷ In MMA approach, local TWA values are just the point-to-point differences between average beat templates.⁷⁰

SM-based calculation of TWA values is more complicated. Local TWA values are the square root of the power at frequency of 0.5 cpb—the alternans frequency—minus the power of the background noise (measured over the range 0.44–0.49 cpb) in every single point in the ST-T complex. Here it should be noted that local TWA amplitudes computed from a spectrum are approximately half the correspondent amplitude differences between the averaged clusters of even and odd beats.⁸² IBA algorithm does not measure local TWA values in the sense of a point-to-point difference among beat templates. This is because IBA methodology first calculates the mean amplitude in every segment of interest (T-onset to T-peak—that includes the ST segment; T-peak to T-end; T-onset to T-end) before computation of TWA values.⁷¹

The overall (global) TWA value is actually what each method yields in its test reports, and there are differences among TWA analysis algorithms on this topic too (Table 2). Global TWA amplitude, in the MMA algorithm, is the maximum of all local values over the entire ST-T complex.^{7,70} IBA methodology returns three global values, each one the averaged

Table 2. Summary of the Most Relevant Technical Differences between SM and MMA Analysis Algorithms

Features	TWA Analysis Algorithm	
	Spectral Method (SM)	Modified Moving Average (MMA)
Noise measurement	Measured over the range 0.44–0.49 cpb	Measured on the average templates outside the ST-T complex
Influence of background noise in test results	Local TWA estimations are adjusted for noise	Disregarded during local TWA estimations.
Decision rule for presence of TWA	Measured TWA is threefold or higher than noise SD	Measured noise is lower than a high-noise fixed (but adjustable) threshold; Less than 25% of noisy beats were excluded in template computation
Final TWA value reported in test results	Average (RMS value) of all local TWA estimations over ST-T	Maximum local TWA estimation over ST-T
Estimation of TWA waveform	Partial (absolute values)	QRS-aligned templates for over-reading of TWA output in a way similar to ST-segment evaluation

These technicalities may result in very different quantitative TWA values between the methodologies, see text for more information.

difference of amplitude over each segment of interest.^{71–73} SM global TWA amplitude is the square root of the alternans power (the average of the local values, calculated from the composite spectrum resultant from the 128 original spectra) or, in other words, the root mean square (RMS) value of local values (i.e., similar to the average of all local values) over the entire ST-T complex.^{68,82} Finally, CD algorithm generates global TWA amplitudes like the SM approach.⁷

SM methodology has derived a new statistic to be used as a decision rule for the presence/absence of TWA, called *alternans ratio* or *K-score*. It is computed as the ratio of the alternans power to the SD of the measured background noise and should be above a specific threshold to define the presence of TWA.⁸² MMA evaluates noise from the TP segments, apart from TWA calculations, and compares it to fixed (but adjustable) threshold. However, its key feature—in the commercial version—is the availability of the superimposed QRS-aligned even- and odd-beats templates for computer-aided visualization of the TWA pattern to allow successful manual TWA revision by the practitioner.⁶⁵

Lately, a resurgent issue on TWA analysis (in editorials,^{86,87} original papers,⁴⁰ or commentaries⁸⁸) is whether TWA magnitude information may add to the prognostic performance of current TWA classification schemes. The point to be made here is that any discussion about this topic should take into account the TWA analysis methodology used in the measurement. Application of the two commercially available algorithms SM and MMA (al-

though both were not in their commercial version) on the same patient set demonstrated that MMA-TWA values are amplified compared to paired SM-TWA values.⁶⁶ That is so because local SM-based estimates are not only approximately half the difference of their MMA-based counterparts but they are also averaged to generate the final TWA amplitude value, whereas MMA-based test reports yield the maximum among local estimates. In other words, MMA returns the maximum alternating difference among even and odd averaged beats and SM returns the average of half these differences. For example, provided a stream of beats with T waves of maximum alternating amplitude of 30 μ V and cumulative alternans voltage of 40 μ V over 150 ms of T wave, MMA-based screening would return an TWA value of 30 μ V, while an SM-based test report would show nearly 4.6 μ V (approximately 6.5 times lower).

Evaluation of Sustained and Short TWA Bursts

The following (generally accepted) definition of “sustained TWA” is quoted from consensual SM-based interpretation criteria, discarded the SM-specific part: “Sustained alternans is defined as alternans that is consistently present at heart rates above a patient-specific onset heart rate (except for gaps believed to be caused by obscuring factors such as ectopies, noise or heart rate dips) with at least 1 minute of magnitude above the threshold of significant TWA amplitude.”⁸²

However, recent evidence shows that short bursts of TWA may also be prognostically significant.⁸⁹ This new clinical fact can be approached by readjusting the technical compromise between resolution and noise robustness. Briefly, shorter analysis windows show better tracking of abrupt TWA changes, transient TWA episodes, or dynamic TWA values than long ones. On the other hand, longer analysis windows achieve better noise reduction in the detection of TWA presence and amplitude estimations. SM methodology usually uses a 128-beat window, which presents a very good noise reduction but does not allow for a correct tracking of transient TWA, with dynamic TWA changes coming to knowledge in different frequencies other than 0.5 cpb, and therefore considered as noise.⁷ Moreover, many biological sources can generate spurious signals (artifacts) that contaminate the energy readings at the alternans frequency of 0.5 cpb: RR interval alternans, rapidly changing heart rate during the 128-beat stream, pedaling frequency (on an ergometer), and/or respiratory frequency. Respiratory frequency actually lies in the range of 0.2 to 0.33 cpb, but whenever it falls exactly on 0.25 cpb its multiples (harmonics) can be misleading.⁸²

In other words, the SM algorithm, in its current commercial implementation, experience problems in recognizing short TWA bursts or TWA episodes of varying amplitude. CD algorithm, on the other hand, uses a shorter analysis window—with an effective length of 30 beats approximately. More than any other feature, this window length is responsible for its good compromise between TWA tracking and denoising.⁷

Time-domain methodologies are better suited than frequency-domain algorithms with regards to the analysis of short MTWA episodes, for nonlinear averaging methodologies are intrinsically more immune to artifacts like ectopic beats and respiratory movements.⁷ This comparison was verified in practice for CD and MMA approaches.⁷⁰ Several factors may help to explain this distinction. First, MMA returns TWA values after processing ECG records of just 15 seconds of length. In addition, the proper selection of one available bounding fraction (1/8, 1/16, 1/32, or 1/64) also makes the algorithm more sensitive to changes in ST-T complex morphology. For MMA, the larger the update fraction (noting that 1/8 is larger than 1/32), the faster the template is updated, and shorter TWA episodes can be traced. As a rule of thumb, SM with a 128-beat window shows similar TWA resolution to MMA with a 1/32

update factor, but still different TWA values since the former returns the average figures and the latter the maximum alternans.

TWA Classification Schemes

SM currently presents validated positive and negative test criteria (*A-rules*, *B-rules*, *C-rules* in Fig. 3), but there are divergences among classification schemes: *A-rules* is the consecrated set, but it is also the one that generates the higher number of indeterminate tests—up to 40% of total cases—so other schemes (*B-rules* and *C-rules*) were devised.⁸² In common, all aforementioned classification schemes are based on the SM assumption of sustained TWA, with the significant TWA magnitude threshold being equal or larger than 1.9 μV . Moreover, it is also required that *alternans ratio* or *K-score* to be equal to 3 or higher.⁸²

However, since 2000 there is mounting evidence favoring two-category classification schemes (normal; abnormal) against the three-category classification schemes (positive; negative; indeterminate) in SM-based TWA studies. Kaufman and colleagues (during the TWA in CHF trial) originally used a standard three-category scheme and observed that indeterminate TWA tests (191 tests from a total of 549 ischemic and nonischemic patients, LVEF $\leq$ 0.40) were as good predictors of death or SVA as positive tests, provided that the cause of indeterminacy is due to “patient factors” (high level of ventricular ectopy, failure to achieve an adequate heart rate, or unsustained TWA at frequencies below 110 bpm).⁸⁹ Later, Chan and colleagues evaluated 768 ischemic patients with LVEF $\leq$ 35%, with 159 indeterminate tests, and observed improvements on the overall prognostic utility of TWA tests when classification was carried out in clusters of abnormal (positive tests + indeterminate tests with frequent ventricular ectopy or insufficient maximum heart rate) and normal (negative tests + indeterminate tests due to unsustained TWA or noise).⁹⁰

The MMA approach is more robust to heart rate fluctuations than SM methodology, so that some of the SM-based contraindications for TWA testing (patients in atrial fibrillation or the need for smooth heart rate acceleration) do not apply. Neither does the definition of sustained alternans play a role in the classification of MMA-TWA testing. Currently, its classification scheme is based on achieving or not a threshold of significant TWA magnitude before the heart rate of 125 bpm. The most used

thresholds are in the range of 47 μ V (in 24 hour-Holter^{65,85} and exercise TWA⁵²) and 65 μ V (in exercise TWA⁵²), even though the REFINE study optimally chose 5 μ V to the categorization of 30 minute-Holter TWA measurements.⁶¹ Cox and coworkers applied both SM and MMA algorithms on the same patient set and observed that MMA-TWA and SM-TWA testing had similar clinical performances. The slightly higher MMA sensitivity (91% to 82%) resulted on a little higher negative predictive value at costs of few more false-positives.⁶⁶ Finally, there are sparse publications on IBA⁷¹ and CD⁶⁹ classification schemes and significant TWA thresholds.

Recently, TWA classification schemes in general are being put into question. Klingenhoben and colleagues brought into light the idea that magnitude information can actually be clinically relevant in TWA testing—with the extent of myocardial damage being possibly related to TWA magnitude rather than its presence.⁴⁰ Another strong point against classification schemes is the statistically proven loss of information due to the conversion of a continuous variable (TWA magnitude) into a categorical one (the classification scheme). Dichotomizing is effectively equivalent to losing a third up to a half of the study data.⁹¹

FACTORS THAT MODULATE TWA

Physiological and Pathophysiological Changes

Initial findings from Holter studies stated that TWA magnitude is responsive to circadian fluctuations and physiological changes.^{72,85,92,93} Multivariate analyses of 24-hour ambulatory ECGs recorded from patients of the Autonomic Tone and Reflexes After Myocardial Infarction (ATRAMI) trial database have pointed out that TWA levels above the 75th percentile at 8:00 AM or at maximum heart rate (about 47 μ V) were associated with higher odds of cardiac arrest (documented VF) or arrhythmic death during the follow-up period (OR ranged from 4.2 to 7.9, depending on the lead and time period).⁹² Later, Stein and coworkers noted that on average the highest levels of TWA followed the circadian timing of increased sudden death risk in heart failure patients. Moreover, TWA levels above 47 μ V was found to be associated with increased SCD risk.⁶⁵

On pathological processes affecting TWA magnitude, Shusterman and coworkers demonstrated

an upsurge in TWA magnitude preceding spontaneous onset of ventricular tachyarrhythmias (VTA). Holter records (n = 59) with spontaneous VTA were selected from the database of the Electrophysiologic Study Versus Electrocardiographic Monitoring (ESVEM) clinical trial. Their results show that TWA magnitude continuously increased from baseline up to a peak of 25% 10 minutes before the event.⁷² Investigators from the Triggers Of Ventricular Arrhythmias (TOVA) study also verified the existence of significant TWA magnitude before arrhythmia onset using CDI electrograms.⁹⁴ Postinfarction temporal TWA changes in the first 6 months after the event were also registered, probably following ventricular remodeling after acute myocardial infarction.⁶³ Eventually, TWA at pathological levels were strongly associated to cardiac sympathetic denervation and accelerated sympathetic nervous activity in patients with idiopathic dilated cardiomyopathy evaluated by Iodine-123 (I-123) metaiodobenzylguanidine (MIBG) imaging and echocardiogram.⁹⁵

Other studies have investigated the effects of acute mental stress (anger recall and mental arithmetic) on TWA. Kop and coworkers concluded that it enlarges TWA amplitude among ICD patients with documented coronary artery disease (n = 23) at lower heart rates than with current exercise protocols used in TWA evaluation.⁹³ Lampert and coworkers not only registered augmented TWA due to mental stress among ICD patients (n = 33) but also found that TWA changes correlated well with changes in heart rate, systolic blood pressure, and catecholamines,⁹⁶ in line with previous evidence that mental stress alters both cycle length and termination of VT in ICD patients without ischemia.⁹⁷

Finally, some external influences can also affect TWA. Spinal cord stimulation (SCS) is currently used in patients with intractable angina and it is thought to have an antiarrhythmic effect on the arrhythmogenic substrate. The study of TWA patterns to assess changes in arrhythmic substrate indicated that patients originally with high-amplitude TWA-positive tests when the stimulator was off experienced a lowering on TWA values (but still TWA-positive tests) after 2 hours of SCS. After 24 hours of SCS, all patients became TWA-negative. In this sample, all patients were already on full therapy with beta-blockers and no changes in basal heart rate or atrioventricular conduction were observed among consecutive TWA tests. These findings suggest a time-dependent remodeling

effect on the arrhythmogenic substrate (assessed by TWA) independent of sympathetic withdrawal.⁹⁸

Another external influence, ICD shocks during standard defibrillation testing ($n = 65$ patients) acutely increased TWA magnitude mediated in part by sympathetic stimulation.⁷³ In addition, studies are needed to demonstrate whether this TWA augmentation can be associated with the most common mechanism of sudden death in ICD patients:⁹⁹ the postshock electromechanical dissociation following a treated VT/VF. On this topic, one key aspect to be considered is that impaired calcium cycling—that is related to ventricular mechanical dysfunction—is strongly associated with APD-alternans.^{4,14,15,17}

Oral Drug Therapy

Beta-Blockers

Echocardiography and MIBG imaging were performed at baseline and after a 3-month beta-blocker oral therapy in a group of patients with nonischemic heart disease of mixed etiologies. Several beta-blockers were used (metoprolol, carvedilol, bisoprolol, atenolol), sometimes simultaneously. In the end, oral beta-blocker therapy was held responsible by the improvements verified in the left ventricular systolic function, the lessening of the abnormalities in the cardiac sympathetic nervous system and the concomitant improvements in TWA levels.¹⁰⁰ On the contrary, a 2007 study provided evidence that oral beta-blocker therapy appears to have no effect on TWA results and predictive power among patients ($n = 387$) with coronary artery disease, LVEF $< 40\%$ and NSVT referred for EPS.¹⁰¹

Intravenous Drug Therapy

Beta-Blockers

Current evidence states that TWA patterns appear to be responsive to intravenous therapy with beta-blockers. Administration of metoprolol (0.1 mg/kg) or d,l,sotalol (1.0 mg/kg) reduced TWA voltage in one-third, approximately.¹⁰² In addition, intravenous esmolol reduced TWA voltage in such a way that the number of positive TWA tests fell 50%.¹⁰³ Komiya and coworkers published a study where patients with diagnosed VT ($n = 15$) or supraventricular tachycardia (SVT, $n = 20$) were referred to TWA testing with temporary right atrial pacing at 90 and 110 bpm. All individuals in the VT group had an underlying cardiac disease and im-

paired left ventricular function, while subjects with SVT only manifested hypertension. Baseline (no medication) TWA values increased in both groups during heart rate pacing, with larger TWA values in the VT group. Propranolol infusion (2 mg/kg) reduced basal heart rate as well as TWA magnitude during pacing in both groups, with larger reductions in the VT group.¹⁰⁴

Isoproterenol and Atropine

In the same study by Komiya and colleagues, beta agonist infusion (isoproterenol, 0.1 μ g/kg per minute) increased basal heart rate in both groups and TWA values during pacing only in the SVT group.¹⁰⁴ Atropine is an antagonist of the muscarinic acetylcholine receptors, showing blocking effects on the parasympathetic autonomic system. TWA testing with atropine infusion (starting bolus of 0.5 mg + 0.25 mg steps, until maximum cumulative dose of 3.0 mg) yielded similar results to TWA testing with right atrial pacing in 22 of 27 patients referred to electrophysiological study. Atropine at the prescribed doses failed to achieve the minimum heart rate (105 bpm) needed for successful TWA evaluation in the other five patients.⁸⁰

Rotigaptide

Rotigaptide is an antiarrhythmic peptide that affects primarily the gap junction conductance, with not detectable effects on action potential duration. Hence, this drug stays outside of the current Vaughan-Williams classification scheme, which groups antiarrhythmic drugs based on their effects on ion channels (Na^+ , K^+ , Ca^{2+}) and beta-receptors. This new drug is still under development, but initial results from experimental studies have shown it may suppress the most arrhythmogenic form of APD-alternans.¹⁰⁵

FINAL REMARKS

Current available literature on TWA provided compelling evidences of its good prognostic performance (notably, its negative predictive value) in specific clinical populations, such as ischemic heart disease, after acute MI, nonischemic cardiomyopathy. TWA also equals or outperforms EPS prognostic accuracy in other populations (e.g., athletes with arrhythmias). On the other hand, the clinical value of TWA assessment in populations like Brugada patients, pediatric, or congenital diseases

is subject to further investigation, specially with clinical protocols apart from those routinely used nowadays.

The path ahead to derive new protocols and applications, to further explore TWA functionalities, or to fill the current gaps in the TWA base of knowledge runs toward one direction: practitioners and researchers should better explore the technical features of the several technologies available for TWA evaluation—each with its own strengths and weaknesses—and be aware of the fact that TWA values are responsive to several factors besides medications.

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