A Novel Method for Determining the Phase of T-Wave Alternans: Diagnostic and Therapeutic Implications

Running title: Sayadi et al.; Method to Estimate the Phase of Repolarization Alternans

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Abstract

**Background** - T-wave alternans (TWA) has been implicated in the pathogenesis of ventricular arrhythmias and sudden cardiac death (SCD). However, in order to effectively estimate and suppress TWA, the phase of TWA must be accurately determined.

**Methods and Results** - We developed a method that computes the beat-by-beat integral of the T-wave morphology, over time points within the T-wave with positive alternans. Then, we estimated the signed derivative of the T-wave integral sequence which allows the classification of each beat to a binary phase index. In animal studies, we found that this method was able to accurately identify the T-wave phase in artificially induced alternans (p<0.0001). The coherence of the phase increased consistently after acute ischemia induction in all body-surface and intracardiac leads (p<0.0001). Also, we developed a phase resetting detection algorithm that enhances the diagnostic utility of TWA. We further established an algorithm that employs the phase of TWA in order to deliver appropriate polarity pacing pulses (all interventions compared to baseline, p<0.0001 for alternans voltage; p<0.0001 for Kscore), to suppress TWA. Finally, we demonstrated that using the phase of TWA we can suppress spontaneous TWA during acute ischemia; 77.6% for alternans voltage (p<0.0001) and 92.5% for Kscore (p<0.0001).

**Conclusions** - We developed a method to quantify the temporal variability of the TWA phase. This method is expected to enhance the utility of TWA in predicting ventricular arrhythmias and SCD and raises the possibility of using upstream therapies to abort a ventricular tachyarrhythmia prior to its onset.

**Key words:** alternans, arrhythmia (heart rhythm disorders), pacing, implantable cardioverter-defibrillator
Introduction

T-wave alternans (TWA) testing has been associated with an increased risk for ventricular tachyarrhythmic events (VTE), such as ventricular tachycardia/ventricular fibrillation (VT/VF) and sudden cardiac death (SCD)\textsuperscript{1}, during medium and long-term follow-up\textsuperscript{2,3}.

However, beyond medium- and long-term risk prediction, several lines of pre-clinical and clinical evidence suggest that the presence of heightened TWA may play a more proximal role in creating the substrate necessary for malignant ventricular arrhythmias\textsuperscript{4-6}. Analysis of intra-cardiac electrograms from ICD leads has demonstrated a sharp increase in TWA magnitude immediately prior to spontaneous ventricular arrhythmias\textsuperscript{7-9}, without a similar upsurge in TWA prior to induced ventricular arrhythmias or preceding inappropriate ICD shocks\textsuperscript{7}. Simultaneous measurement of TWA from body-surface and intra-cardiac electrograms by our group\textsuperscript{10} and others\textsuperscript{11} has shown a high degree of correlation, suggesting that these measurements are detecting the same electrical phenomenon. In aggregate, the clinical and experimental data suggest that the heart either passes through a state of heightened T-wave alternans on the way to VT/VF or heightened TWA occurs in close conjunction with developing VTE\textsuperscript{12,13}. In either scenario, these findings suggest that detecting significantly elevated levels of TWA may serve as an important short-term predictor of impending arrhythmias and also raise the possibility for a therapeutic role where TWA suppression therapies are delivered upstream to suppress the arrhythmogenic substrate and abort VT/VF prior to arrhythmia onset\textsuperscript{2,14}.

The clinical application of TWA, whether it serves a diagnostic role for medium- and long-term risk prediction or a therapeutic role for guiding short-term upstream anti-arrhythmic therapy, requires the ability to accurately estimate TWA. It has been reported that abnormal beats with altered morphology and/or timing present inherent difficulties in the estimation of
alternans\textsuperscript{15}, primarily by provoking changes in TWA phase\textsuperscript{16}. However, assessment of the alternans phase, a term which refers to the specific phase of an alternating sequence of long/short or large/small T-waves, has generally been underappreciated in clinical TWA testing. Current algorithms used to estimate TWA classify premature ventricular contractions (PVCs) and beats following transient prolongation or shortening of the cycle length as “bad” beats, but they do not estimate the phase of TWA either preceding or following such “bad” beats. However, a “bad” beat may cause phase resetting of TWA and therefore may either reduce the amplitude of alternans\textsuperscript{15} or potentiate the amplitude of alternans\textsuperscript{16}. In either case, PVCs may significantly hamper the TWA estimation by generating a false positive or a false negative result. Therefore, the development of methods to estimate the phase of TWA before and after a “bad” beat and consequently accounting for changes in phase, may significantly improve the performance of TWA as a diagnostic test for predicting medium- and long-term arrhythmia risk\textsuperscript{17,18}.

Similarly, the ability to estimate the phase of TWA is also likely to have a critical impact on designing potential therapeutic strategies to suppress TWA and mitigate short-term arrhythmia risk. However, as has been demonstrated\textsuperscript{2}, lack of a priori knowledge of the phase of TWA (long/short or large/small T-wave) may increase the amplitude of alternans and potentiate, rather than suppress, the arrhythmogenic substrate. Therefore, developing methods to estimate the phase of alternans in real-time is likely to play an important role in designing therapies to suppress TWA.

In this study, we aim to examine the hypothesis that algorithms to estimate the phase of TWA in intracardiac and body-surface signals can improve the diagnostic and therapeutic utility of TWA.
Methods

Animal Preparation

24 male Yorkshire swine (40-45 kg) were anesthetized and acutely instrumented in the Animal Electrophysiology Laboratory of the Massachusetts General Hospital. The protocol was approved by the Hospital’s Animal Care and Use Committee.

Anesthesia was induced with Telazol (4.4 mg/kg) IM and Xylazine (2.2 mg/kg) IM. Each subject was intubated and placed on a mechanical ventilator, and anesthesia was maintained with Isoflurane (1.5-5%).

Standard electrocardiographic electrodes were placed on the subject’s limbs and chest; the epidermis was excised at point of contact to maximize signal quality. An arterial line was used to monitor arterial blood pressure. Percutaneous intracardiac access was achieved in the jugular veins and femoral arteries and veins using standard Seldinger techniques. Decapolar catheters were placed under fluoroscopic guidance in the (i) right ventricle (RV), the distal lead being at the RV apex, (ii) coronary sinus (CS), the distal lead being at the distal CS, (iii) left ventricle (LV), the proximal lead being at the LV apex. An inferior vena cava catheter was inserted as a reference electrode for unipolar signals.

Regional myocardial ischemia was induced in 11 subjects via balloon occlusion of the proximal left circumflex coronary artery utilizing standard percutaneous cardiac catheterization techniques.

Equipment and Data Collection Methods

Intracardiac and body-surface (leads II and V4) electrocardiographic signals as well as arterial blood pressure were recorded through a Prucka Cardiolab (General Electric) electrophysiology system that provided 16 high fidelity analog output signals, as previously described. We
used a previously developed signal acquisition, analysis and display system, consisting of custom software written in LabView 8.5 (National Instruments, Austin, TX) and MATLAB 7.6 (Mathworks, Natick, MA) 10.

Details of the alternans estimation algorithm 10, 14 are provided in the Online Supplement. T-wave alternans estimates were obtained from two body-surface leads and 12 intracardiac unipolar leads (three in each of the RV, LV, and CS catheters).

We have employed a programmable stimulator that is capable of dynamically delivering pacing pulses on a beat-to-beat basis. Each pacing pulse is triggered upon detection of an ECG waveform (the R-wave) 14.

**T-Wave Alternans Phase Estimation**

The algorithm we developed to estimate the phase of TWA is presented in Figure 1A, and is described below. In order to compute a phase index (PI) that differentiates between the two phases of TWA (Figure 1B), we devised a method that is based on the integral of the T-wave. The method relies on computing the beat-by-beat integral of the T-wave morphology (Figure 1C) between the onset and offset T-wave points (as determined by the wavelet transform).

To maximize the effect of T-wave samples with significant alternans on the integral value and at the same time to account for the temporal characteristics of the TWA, we estimated the alternans voltage and K_{score} 8, 10 on a point-by-point basis throughout the T-wave, using a rolling 128-beat window that was shifted one beat at a time. Then, the integral was calculated at each time point within the T-wave with statistically significant TWA (that is points with K_{score}>3 and alternans voltage greater than a lead-dependant threshold 10) and normalized to the number of points used for the integral estimation. This improved integral provides a more accurate means of determining the phase of the alternating T-waves.
To overcome subtle changes in the beat-to-beat T-wave morphology that may affect the phase estimation, we introduce the signed derivative of the T-wave integral which allows the classification of each beat to a binary phase index that takes two different values: +1 or -1 corresponding to large/small integral values, respectively (Figure 1D).

Finally, to minimize the sensitivity of the algorithm to an arbitrary ECG reference channel, we have devised an artificial alternating reference phase index sequence (+1,-1,+1,-1,…) time series against which the phase time series of each lead is compared.

To quantitatively investigate the degree of synchronous alternation between two waveforms, the coherence of two signals (estimated phase and reference phase) has been estimated using the Welch's averaged periodogram method (for more information, please see the Online Supplement) 19.

**Statistical Methods**

Aggregate variables were expressed as mean±standard deviation. Box-plot representation including the median, 90-10% and 95-5% percentiles was used to demonstrate statistical properties of the estimated coherence in data sequences with/without repolarization alternans. For each channel, coherence values were compared before and after R-wave triggered pacing using the Wilcoxon signed-rank test. The phase of TWA was compared across catheters using the Kruskal-Wallis analysis of variance. Comparisons between alternans voltage and Kscore across leads before and after R-wave triggered pacing used 2-way ANOVA. The 95% confidence intervals for sensitivity, specificity, positive predictive value, negative predictive value and accuracy were calculated based on binomial distribution. A p value of <0.05 was used to determine statistical significance. Statistical analysis was performed using MATLAB (MathWorks Inc, Natick, MA) and STATA (StataCorp LP, College Station, TX).
Results

Evaluation of the Estimation of the TWA Phase in the Normal Heart

We evaluated the ability of the algorithm to accurately estimate the phase of TWA using R-wave triggered pacing during the absolute refractory period. Pacing pulses of (i) amplitude: ±1 mA and ±5 mA, (ii) width: 30 ms and (iii) coupling to the R-wave: 10 ms were delivered during the absolute refractory period on every other beat basis from two leads (RV12) of a decapolar catheter, at the apex of the RV, to modulate ventricular repolarization and induce TWA.

To investigate the ability of our algorithm to determine the phase of the T-wave, we estimated the PI at baseline and following R-wave triggered pacing. We then evaluated the coherence at 0.5 cycles/beat between the artificial alternating reference PI sequence and the PI series for each of the leads at the body-surface and CS, LV and RV catheters, before and after R-wave triggered pacing. For each Kscore and alternans voltage, TWA was considered positive if Kscore>3 and alternans voltage was greater than the lead-specific thresholds (body-surface: 0.55 μV; CS: 1.98 μV, LV: 4.18 μV, RV: 4.235 μV) 10. The box plot representation of the phase coherence distributions for sequences of negative and positive alternans during all 4 pacing protocols are shown in Figure 2A for body-surface and intracardiac leads. The summary statistics demonstrate that the coherence is significantly higher for positive alternans than negative alternans across all leads on the body-surface and intracardiac leads (p<0.0001 for all paired comparisons, n=36 for each of the body surface, CS, LV and RV leads; where n represents the overall sample size: 9 subjects for each of the ±1 mA and ±5 mA pacing interventions).

Then, to estimate the phase coherence thresholds for body-surface and intracardiac leads, we estimated the receiver operating characteristic (ROC) curve for coherence values ranging from 0 to 1 and computed the true and false detection rates for positive and negative alternans.
using the coherence values, as follows: using the TWA criteria described above (for $K_{\text{score}}$ and alternans voltage), the estimated coherence indicated a true positive or false negative outcome if it was greater or smaller than the corresponding threshold. Similarly, if alternans was negative, the coherence estimate was considered a false positive or true negative depending on whether its value was greater or smaller than the corresponding threshold.

In Figure 2B we plot the sensitivity (Sn) and specificity (Sp) for body-surface and intracardiac leads as a function of coherence threshold. The corresponding ROC curves are shown in Figure 2C. The area under the curve for body-surface leads and intracardiac CS, LV and RV catheters was: 0.99, 0.95, 0.98 and 0.99, respectively. For each lead configuration, the optimal phase coherence threshold was determined as the point that has the minimum distance to the point $(\text{Sn}, 1-\text{Sp}) = (1,0)$, which is equal to minimizing $[(1-\text{Sn})^2 + (1-\text{Sp})^2]^{0.5}$ (Figure 2C). The estimated thresholds for body-surface leads and CS, LV and RV leads were: 0.419, 0.376, 0.406 and 0.647, respectively. These thresholds are shown by the horizontal lines in Figure 2A. The corresponding Sn and Sp pairs at the threshold were estimated to be: (0.95,0.95), (0.89,0.89), (0.93,0.91) and (0.96,0.98) for body-surface and CS, LV and RV leads, respectively.

**Evaluation of the Estimation of the TWA Phase in the Presence of Acute Ischemia**

To investigate the ability to estimate the phase of spontaneous TWA, we applied our algorithm on body-surface and intracardiac (CS, LV and RV leads) electrograms recorded before (baseline) and during acute ischemia. The number of beats per subject recorded before and during coronary artery occlusion was 848±334 and 954±532, respectively ($p=0.69$, signed-rank=23, n=10). The mean heart rate at baseline and during coronary artery occlusion was 106.7±9.2 bpm, and 109.2±20.1 bpm, respectively ($p=1$, signed-rank=0, n=10).

The alternans voltage was significantly higher ($p<0.0001$ for all paired comparisons,
n=80 where n represents the total number of comparisons: 40 for baseline and 40 during ischemia, 10 for each of the body surface, CS, LV, and RV leads) during coronary artery occlusion compared to baseline: 0.12±0.17 μV vs. 6.12±6.23 μV, 0.25±1.01 μV vs. 10.39±9.42 μV, 0.60±1.69 μV vs. 19.13±12.88 μV and 0.51±1.70 μV vs. 24.86±19.29 μV, for body-surface and CS, LV and RV leads, respectively. Similarly, the K_score was significantly higher (p<0.0001 for all paired comparisons, n=80 where n represents the total number of comparisons: 40 for baseline and 40 during ischemia, 10 for each of the body surface, CS, LV, and RV leads) during coronary artery occlusion compared to baseline: 0.87±1.61 vs. 189.72±366.96, 0.14±0.51 vs. 29.60±43.10, 0.27±0.76 vs. 68.08±126.66 and 0.15±0.48 vs. 90.44±176.76, for body-surface and CS, LV and RV leads, respectively.

In Figure 3 we summarize the compiled results (n=10) of the coherence distributions for electrograms recorded before and during acute ischemia. The coherence is significantly higher for ischemia-induced TWA than baseline across all leads on the body-surface and intracardiac leads (p<0.0001 for all paired comparisons, n=80 where n represents the total number of comparisons: 40 for baseline and 40 during ischemia, 10 for each of the body surface, CS, LV, and RV leads). No statistically significant difference was observed as a function of lead type, including between body-surface and any of the intracardiac catheter leads (baseline: Kruskal-Wallis test, p=0.62, n=10; post balloon occlusion: Kruskal-Wallis test, p=0.88, n=10; where n is the number of study subjects used in this comparison). These results suggest that an increase of the TWA magnitude during acute ischemia is associated with an increase of the corresponding coherence, while as expected during baseline when TWA was deemed negative, the associated coherence values were significantly lower.

To evaluate the performance of the algorithm to estimate the phase in the presence of
myocardial ischemia, we applied the previously ROC-derived phase coherence thresholds to the results reported in Figure 3 (Table 1). We observe that the accuracy of the estimation for body-surface, CS, LV and RV leads is 0.91, 0.94, 0.93 and 0.90, respectively. These results suggest that coherence thresholds estimated from artificially induced alternans can be applied to the acute ischemia model and yield highly accurate estimates of the TWA phase in both body surface and intracardiac leads.

**Detection of TWA Phase Resetting**

Having examined the ability of the proposed method to estimate the phase of TWA, we evaluated the use of the PI as a means to detect phase resetting (Figure 4). In order to detect potential TWA phase resetting, we estimated the cross-correlation of the estimated TWA phase with that of the reference phase index sequence, which at the point of phase resetting is expected to result to a zero-crossing and a change in the polarity of the correlation coefficient (please, see Online Supplement).

In Figures 4A-4D, we demonstrate the performance of the proposed phase resetting detection algorithm in two different study subjects in body surface electrograms recorded during myocardial infarction. The first subject shows a reduction of the alternans voltage and $K_{\text{score}}$ caused by phase resetting from a PVC at beat numbers 628 and 831 (lead V4). The second subject shows an artificial increase of the alternans voltage and $K_{\text{score}}$ caused by a PVC at beat number 350 (lead V4). Each of these two PVCs results in an abrupt decrease/increase in the estimated correlation of the phase of the signal with that of the reference channel, manifested by a zero-crossing of the correlation coefficient and changes in the sign of the correlation coefficient before and after the zero-crossing point.

We applied the phase-resetting detection algorithm to all 10 subjects during acute
ischemia and found a total of 67 incidents of phase resetting out of 224 QRS-complex morphology changes (i.e. PVCs), yielding a 30% probability of experiencing phase-resetting when a bad beat occurs. This signifies the relatively high incidence of artificial modulation of TWA estimation, which can now be determined by the proposed phase-resetting detection algorithm.

**Utility of the TWA Phase to Trigger Appropriate Electrical Therapy**

After evaluating the ability of the proposed method to estimate the phase of artificially induced and spontaneously occurring TWA, we evaluated its performance as a means to guide electrical therapy aimed to suppress TWA.

Specifically, we employed R-wave triggered pacing pulses during the absolute refractory period to induce and suppress TWA from two catheter leads in the RV (induction lead: RV12; suppression lead: RV34 or RV56; pulse amplitude: +/-7 mA; pulse width: 30ms; coupling to R-wave: 30ms). In Figure 5, baseline (intervention A) corresponds to alternans induced during R-wave triggered pacing from RV12 on an every other beat basis, while subsequent interventions refer to the effect of varying the R-wave triggered stimulus polarity delivered from RV34 or RV56 with respect to the phase of alternans (induced from RV12). In interventions (B)-(E) R-wave triggered pacing from RV12 continues on an every even beat basis; however, triggered stimuli are now delivered from RV34 or RV56 on every odd beat (B and C) or every even beat (D and E) with -7 mA (B and E) or +7 mA (C and D).

In order to establish a relationship between the outcome of the R-wave triggered pacing with the pacing pulse polarity and the phase of TWA, we estimated the alternans voltage and $K_{score}$ using the protocol described above and reported the product of: (pacing polarity)x(phase polarity). *Phase polarity* is defined as positive when the stimulus from RV34 or RV56 is
Triggered on beats with the opposite phase to the phase of alternans at baseline (out of phase pacing). In contrast, phase polarity is defined as negative when the RV34 or RV56 stimulus is triggered on beats with the same phase as the phase of alternans at baseline (in-phase pacing). The alternans voltage and Kscore for body surface, CS, LV and RV leads for each of the above interventions is shown in Figure 5 (n=8). We observe that in-phase pacing with negative pulse polarity, as well as out of phase pacing with positive pulse polarity, results in TWA increase, while in-phase pacing with positive pulse polarity or out of phase pacing with negative pulse polarity results in a reduction of TWA (comparison between baseline and each of the interventions used to suppress alternans for each lead type: p<0.0001 for alternans voltage; p<0.0001 for Kscore for the same paired interventions, n=8).

These results demonstrate that lack of a priori knowledge of the phase of TWA may result in an increase in the TWA amplitude (as shown in interventions C and E). Therefore, in order to effectively suppress TWA, one needs to know the phase of the estimated TWA. Based on these results, we propose that when the alternans phase and pacing pulse have opposite polarity, R-wave triggered pacing will decrease TWA.

**Utility of the TWA Phase to Suppress Spontaneous TWA During Acute Ischemia**

We have further evaluated the utility of the phase-dependent R-wave triggered pacing during the absolute refractory period to suppress spontaneous TWA in sinus rhythm, *in vivo.*

In Figure 6, we plot the Kscore of a triangular intra-cardiac bipolar lead configuration CS2CS8, LV4CS2 and LV10CS2 (an optimized intra-cardiac alternans detection lead system14), following coronary artery balloon occlusion. In intervention A, one observes significant spontaneously occurring TWA. Upon detection of significant TWA, we estimated the phase of TWA and applied in-phase R-wave triggered pacing with positive polarity from a catheter lead in
the right ventricle apex with the following parameters: amplitude: +4 mA; pulse width: 10 ms; pulse coupling: 10 ms, on every even beat (intervention B). We observe that R-wave triggered pacing results to a significant reduction of spontaneous TWA during acute ischemia (77.59% average reduction across the 3 leads of the alternans voltage compared to baseline, p<0.0001; 92.55% average reduction across the 3 leads of the K_score compared to baseline, p<0.0001). In intervention c, RV12 triggered pacing is discontinued, leading to increase of the alternans voltage and K_score (68.62% average increase across the 3 leads of the alternans voltage compared to TWA suppression, p<0.0001; 87.06% average increase across the 3 leads of the K_score, compared to TWA suppression, p<0.0001).

Incidentally, pacing on an every beat basis does not decrease the level of alternans (data not shown). This finding is further supported by our recent observation 14 that pacing during the absolute refractory period on every beat basis, induces a consistent, lead-dependent modulation on ventricular repolarization. These data provide an important proof of concept that the phase of TWA is a critical parameter of TWA that can be estimated in real-time and be used to suppress spontaneously occurring TWA.

**Discussion**

In light of extensive pre-clinical 12, 13, 20-22, 23 and clinical data 8, 9, 11, 24 demonstrating an association between heightened TWA and the onset of malignant arrhythmias including VT/VF, the accurate detection and potential modulation of TWA may hold promise as a method to predict and preempt lethal heart rhythm disturbances. We have previously shown that a premature beat may alter the phase of alternans with significant implications on the estimation of TWA and its predictive utility 15. The main aim of this study was to establish a robust method to quantify the temporal variability of TWA phase, and use it to enhance the medium- and long-
term SCD risk prediction of TWA as well as to guide short-term electrical therapy to suppress TWA.

This study is the first to provide a comprehensive and systematic approach to determine the phase of TWA in body-surface and intracardiac leads. Specifically, first we have shown that the method we have developed to estimate the phase of TWA is robust and sensitive to subtle changes of the T-wave morphology. Second, use of an artificial alternating reference phase index sequence allows the real time estimation of the temporal dynamics of the TWA phase. Third, during acute ischemia the phase-derived coherence can discriminate between negative and positive TWA levels, and provide highly accurate estimates of the TWA phase. Fourth, the phase-derived coherence can be used to determine phase-induced variability of TWA that results to significant improvement in the TWA estimation. Fifth, the phase of TWA and the polarity of a pacing pulse are sufficient to define a pacing strategy to suppress TWA in-vivo. Sixth, we have been able to demonstrate that the TWA phase can be used to suppress spontaneously occurring TWA following acute myocardial infarction, in-vivo.

Our results may have important clinical applications for diagnostic and therapeutic procedures that are based on TWA estimation. From a diagnostic point of view, microvolt TWA testing has emerged as an important predictor of SCD during medium- and long-term follow-up. However, although the negative predictive value of TWA testing has generally been good, the positive predictive value has been much less robust. Our data suggest that methods to improve the positive and negative predictive value of TWA testing may significantly improve its predictive utility. It has previously been shown that the presence of PVCs or other “bad” beats (with altered morphology and/or timing) may have a profound detrimental effect on TWA testing by either underestimating or overestimating the level of TWA. Our study extends these
prior findings by demonstrating in a swine model of acute coronary ischemia that PVCs frequently lead to modulation of TWA. The presence of PVCs is very common during clinical TWA testing and the presence of frequent PVCs is the most common reason for indeterminate test results. Additionally, the likelihood of indeterminacy increases with progressively more impaired left ventricular systolic function, suggesting that patients at highest risk of SCD may be particularly susceptible to false-positive or false-negative TWA results due to PVC induced phase resetting. The algorithm developed and validated in this study may provide a robust method for detecting scenarios where phase reversals are likely to lead to false-positive or false-negative TWA estimates and thus may significantly improve the sensitivity, positive and negative predictive value of TWA testing.

Furthermore, studies in humans in which TWA was measured during ventricular pacing have demonstrated that the presence of alternans phase reversal after PVCs carries important prognostic information and predicts a significantly lower arrhythmia-free survival when compared to patients without post-PVC phase reversal. However, extension of these findings has been limited by the lack of a validated method to measure the alternans phase in an efficient manner. Our data suggest that in addition to improving positive and negative predictive value, incorporating information about phase reversal into clinical TWA testing algorithms may independently improve the utility of non-invasive TWA testing for medium- and long-term SCD risk prediction.

From a therapeutic perspective, several lines of pre-clinical and clinical evidence suggest that the presence of heightened TWA may play a causative or permissive role in creating an arrhythmogenic substrate and setting the stage for impending ventricular arrhythmias. It has been suggested that appropriately timed pacing therapy, potentially delivered from an
implantable device (e.g. ICD), may be capable of suppressing the amplitude of TWA and potentially pre-empting the onset of VT/VF. In order for such upstream therapies to be viable, several conditions must be met. First, TWA must be detectable from an implantable device in real-time with a high degree of sensitivity and fidelity. In this regard, we have developed and tested a novel intra-cardiac lead configuration which is capable of highly sensitive spatial and temporal alternans detection. Second, once a state of heightened TWA is detected, device-delivered therapy must be administered with the goal of rapidly suppressing the alternans magnitude and quelling the arrhythmogenic substrate. We have recently demonstrated that R-wave triggered pacing pulses delivered during the absolute refractory period are capable of suppressing TWA. However, the outcome of R-wave triggered pacing therapy is critically dependent on the alternans phase and if pacing therapy does not consider the TWA phase, the amplitude of TWA may be potentiated rather than suppressed. Therefore, being able to measure the TWA phase in real-time and then deliver customized therapy is a critical step in being able to reproducibly suppress TWA. In this study, we have demonstrated the importance of phase and pacing pulse polarity in determining the response to pacing therapy. Specifically, in-phase pacing with positive polarity pulses or out of phase pacing with negative polarity pulses provides a consistent suppression effect on TWA. It is likely that TWA suppression helps reduce repolarization gradients and thus reduce arrhythmia susceptibility. The important relationships established in this paper between phase polarity and response to R-wave triggered therapy are likely to become a crucial step in designing future studies that specifically assess the effect of alternans suppression algorithms on arrhythmia inducibility with the aim of validating the concept of upstream therapy. However, it should be noted that acute ischemia induced alternans and SCD may have a different physiology than chronic ischemic related alternans and SCD.
In conclusion, our findings support the idea that the temporal variability of the phase of TWA can be effectively estimated by analysis of subtle changes of the T-wave amplitude that occur on a beat-to-beat basis. The algorithm presented in this study aims to enhance both the diagnostic and therapeutic clinical utility of TWA estimation.

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**Table 1** Performance evaluation of phase estimation during acute ischemia. For each of the body-surface, CS, LV and RV leads the ROC-derived thresholds, determined in artificially induced TWA in control swine, have been applied to the coherence estimation results reported in Figure 3. The 95% confidence intervals are shown in parentheses.

<table>
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<tr>
<th>Lead</th>
<th>Surface</th>
<th>CS</th>
<th>LV</th>
<th>RV</th>
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<tbody>
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<td>0.93 (0.92-0.94)</td>
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<td>0.90 (0.89-0.90)</td>
<td>0.93 (0.92-0.94)</td>
<td>0.93 (0.93-0.94)</td>
<td>0.83 (0.83-0.84)</td>
</tr>
<tr>
<td>Accuracy</td>
<td>0.91 (0.90-0.92)</td>
<td>0.94 (0.94-0.94)</td>
<td>0.93 (0.92-0.94)</td>
<td>0.90 (0.90-0.90)</td>
</tr>
</tbody>
</table>

(Sn: Sensitivity; Sp: Specificity; PV+: Positive predictive value; PV-: Negative predictive value).

**Figure Legends:**

**Figure 1** Algorithm for estimating the phase of repolarization alternans (A). Example of an intracardiac left ventricular lead (LV1U) electrogram (B) used to estimate the integral of the T-wave (C) and the binary phase index (D).

**Figure 2** Statistical coherence analysis of 4 pacing protocols (amplitude: ±1/±5 mA, pulse width: 30 ms, coupling: 10 ms) in the normal heart (n=9) for negative and positive alternans sequences. (A) Box-plot representation of the estimated coherence value at 0.5 cycle/beat for negative
alternans (white boxes) and positive alternans (gray boxes) during baseline and following R-wave triggered pacing for body-surface and CS, LV and RV leads. For each set of body-surface leads and intracardiac leads, the median (horizontal solid line), 90-10% percentiles (box) and 95-5% percentiles (error bars) are shown. The dashed lines indicate the lead-dependent coherence threshold determined using the corresponding ROC curve. (n=36 for each of the body surface, CS, LV and RV leads; where n represents the overall sample size: 9 subjects for each of the ±1 mA and ±5 mA pacing interventions) (B) Sensitivity (Sn) and specificity (Sp) for body-surface and intracardiac leads as a function of their respected coherence threshold. (C) ROC curves for body-surface leads and intracardiac leads determined by changing the coherence threshold from 0 to 1 in steps of 0.001. For each lead, statistically significant comparisons (negative alternans vs. positive alternans) are marked by an asterisk.

Figure 3 Statistical coherence analysis before (white boxes) and during (gray boxes) circumflex coronary artery balloon occlusion for body-surface and RV, CS, LV and RV leads. For each group of body-surface and intracardiac leads, the median (horizontal solid line), 90-10% percentiles (box) and 95-5% percentiles (error bars) are shown. Coherence increased during acute ischemia in all leads (p<0.0001, n=80 where n represents the total number of comparisons: 40 for baseline and 40 during ischemia, 10 subjects for each of the body surface, CS, LV, and RV leads). No statistically significant difference was observed between any lead type (baseline: Kruskal-Wallis test, p=0.62, n=10; post balloon occlusion: Kruskal-Wallis test, p=0.88, n=10; where n is the number of study subjects used in this comparison). For each lead, statistically significant comparisons (baseline vs. ischemia) are marked by an asterisk.
**Figure 4** Use of phase estimation to detect phase resetting. Effect of phase resetting on the alternans voltage (A and C) and $K_{\text{score}}$ (B and D) estimation on a body-surface lead in two different study subjects (subject 1: panel A and B, subject 2: panel C and D). Artificial decrease (A) or increase (C) of the alternans voltage with concomitant $K_{\text{score}}$ artificial decrease (B) or increase (D), are in each case associated with the presence of phase resetting caused by a premature ventricular contraction. Detection and removal of phase resetting (in black) results to the corrected alternans voltage and $K_{\text{score}}$ estimation (in gray). Dashed vertical lines indicate the index of the PVC.

**Figure 5** Algorithm to suppress T-wave alternans using the phase of TWA and the pacing pulse polarity. The distribution of alternans voltage (left column) and $K_{\text{score}}$ (right column) is shown for alternans baseline (through R-wave triggered pacing on alternate beats from lead RV12) and following phase perturbation (through R-wave triggered pacing from RV34 or RV56 leads) for body surface, CS, LV and RV leads ($n=8$). The (pacing polarity)$\times$(phase polarity) for the interventions following the alternans baseline is shown on horizontal axis. For each intervention, the median (horizontal solid line), 90-10% percentiles (box) and 95-5% percentiles (error bars) are shown. Both alternans voltage and $K_{\text{score}}$ increased for interventions with positive (pacing polarity)$\times$(phase polarity). In contrast, both alternans voltage and $K_{\text{score}}$ decreased for interventions with negative (pacing polarity)$\times$(phase polarity).

**Figure 6** Utility of the T-wave alternans (TWA) phase to suppress spontaneously occurring alternans in sinus rhythm during acute myocardial ischemia. Alternans voltage and $K_{\text{score}}$ are plotted for the intra-cardiac lead configuration CS2CS8, CS2LV4 and CS2LV10. In-phase
suppression pacing intervention is delivered from the right ventricle apex (RV12; amplitude: +4 mA, width: 10 msec, coupling to R-wave: 10 msec). **A**: spontaneously occurring alternans is visible at baseline, **B**: R-wave triggered pacing is delivered from RV12 on every even beat with a positive polarity pulse, which results to significant reduction of TWA (77.59% reduction of the alternans voltage compared to baseline, p<0.0001; 92.55% reduction of the K_{score} compared to baseline, p<0.0001), **C**: R-wave triggered pacing is discontinued and both alternans voltage and K_{score} increase to the baseline level during sinus rhythm. Transitions **A to C** occur correspondingly at times marked by solid vertical black lines, while the colored horizontal lines during each intervention indicate the mean value of the alternans voltage and K_{score} during that intervention.
A. Input Signal

- T-wave boundary detection
- Power spectrum estimation
  - $K_{\text{score}}$ and alternans voltage estimation
  - Integration of points with $K_{\text{score}} > 3$ and voltage $> \text{threshold}$
  - Slope determination
  - Sign thresholding

B. ECG (mV) vs. Time (sec)

C. T-wave Integral (mV) vs. Time (sec)

D. Phase Index vs. Time (sec)
Alternans Voltage (ȝV)

Pacing polarity: – + + –
Phase polarity: + + – –

Surface

CS

LV

RV

Pacing polarity: – + + –
Phase polarity: + + – –
A Novel Method for Determining the Phase of T-Wave Alternans: Diagnostic and Therapeutic Implications
Omid Sayadi, Faisal M. Merchant, Dheeraj Puppala, Theofanie Mela, Jagmeet P. Singh, E. Kevin Heist, Chris Owen and Antonis A. Armoundas

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Supplemental Material

Body Surface and Intracardiac Electrograms

T-wave alternans (TWA) estimates were obtained from the two body surface leads, 12 intracardiac unipolar leads (three in each of the right-ventricular, left-ventricular, and coronary sinus catheters) seen in Figure OS1.

Figure OS2 shows representative examples of body surface and intracardiac electrograms obtained prior to R-wave triggered pacing. These electrograms demonstrate that all catheter leads provide artifact-free and high-fidelity signals with high signal to noise ratio, appropriate for the TWA analysis.

Delineation of the T-wave Boundaries Using the Wavelet Transform

The first step in the phase analysis involves the robust estimation of the T-wave boundaries. Considering the variability in the morphology as well as the timing of the T-wave among different leads, the T-wave boundaries were independently determined on a beat-by-beat basis for each of the body-surface and intracardiac leads. Therefore, we developed a software-based T-wave delineation algorithm. This method is an updated version of the previously developed technique for surface ECG delineation based on the wavelet transform (WT)\(^1\).

The wavelet transform provides a multi-resolution decomposition of the signal as a combination of a set of basis functions, obtained by means of dilation and translation of a single prototype function, namely the mother wavelet\(^2\). It is possible to identify the significant points of the ECG signal using the information of local maxima, minima and zero crossings of the WT coefficients at different scales.

Briefly, for each beat, the dyadic wavelet transform of the signal was estimated using a quadratic spline wavelet for the first five scales, wherein most of the energy of the signal lies. We obtained QRS complex annotations (QRS onset, Q, S and QRS offset) by the zero crossing detection of the wavelet coefficients between a positive maximum-negative minimum pair at the first scale, which corresponds to the highest frequency component of the ECG beat. Afterwards, a search window relative to the R position and depending on the RR interval was defined. Within this window, the maximum modulus of WT in scale \(2^4\) was computed, based on which we
assigned one out of six possible morphologies to the T-wave as positive, negative, positive-negative biphasic, negative-positive biphasic, ascending and descending

It is noteworthy that the presented WT algorithm can be applied directly over the raw ECG signal without any pre-processing. In fact, the frequency domain filtering is implicitly performed when computing the WT, eliminating the need to pre-filtering the digitized signal and allowing the direct application over the raw data.

**T-wave Alternans Estimation Algorithm**

We employed a previously described alternans analysis algorithm for the estimation of intracardiac TWA.

Preliminary R-wave time points were obtained by applying a software-based QRS detection algorithm to surface electrogram lead V4. Then, QRS detections were refined and abnormal beats, for example, premature ventricular complexes (PVCs) and aberrantly conducted beats were identified using a template-matching QRS algorithm. Briefly, for each new beat, an 80-ms window centered at the peak of the QRS complex was formed from the preliminary beat detection; an isoelectric PR segment was automatically subtracted as a zero amplitude reference point (by estimating the mean voltage in a 10-ms window preceding the start of each QRS complex). A median QRS template was generated from all “normal” QRS complexes across the previous 127 beats, and the beat was aligned to the QRS template using cross-correlation. Cross-correlation was repeated twice for each new QRS complex to ensure proper QRS alignment. A beat was considered “abnormal” if its correlation coefficient was less than a threshold value of 0.95 or if the preceding R-to-R (R-R) interval was at least 10% shorter than the mean R-R interval of the previous 7 beats. Once all abnormal beats were identified in a 128-beat sequence, each abnormal beat was substituted with a median odd or even template beat on a lead-by-lead basis (derived from the odd or even “normal” beats respectively in the 128-beat sequence), depending on whether the abnormal beat was an even or odd beat.

Then, delineation of the T-wave boundaries (T onset, T offset) was performed using the wavelet transform, as described above. Spectral alternans analysis was performed on a beat-by-beat basis for each 128-beat data sequence using a 512-point power spectrum to improve the frequency-domain resolution. To account for the spatial variability of TWA, spectral analysis was independently performed for each lead. Repolarization alternans indices were estimated as follows:
alternans voltage (µV) = √alternans peak - µnoise

where, the alternans peak is the peak in the aggregate power spectrum corresponding to 0.5 cycles/beat and the mean (µ\textsubscript{noise}) and the standard deviation (σ\textsubscript{noise}) of spectral noise are estimated from a predefined aggregate power spectrum noise window (0.40-0.46 cycles/beat). The alternans voltage is a direct measure of the presence of alternans while the alternans K\textsubscript{score} is a measure of the statistical significance of the alternans voltage. For each lead, alternans was estimated on a beat-by-beat basis using a rolling 128-beat window that was shifted one beat at a time.

**Signal Processing Methods**

To quantitatively investigate the degree of synchronous alternation between two waveforms, the coherence of two signals (x[n] and y[n]) has been estimated using the Welch's averaged periodogram method \textsuperscript{5}. The magnitude squared coherence is defined as the ratio of the power spectral densities of signals x[n] and y[n] to the cross power spectral density of x[n] and y[n]. The cross-spectral density indicates the part of y[n] that is linearly related to x[n] as a function of frequency, or alternatively it can be interpreted like the cross-correlation function except it provides the expected results as a function of frequency. The coherence estimate is a function of frequency with values between 0 and 1 that indicates how well y[n] corresponds to x[n] at each frequency (0/1 indicating the lowest/highest coherence); in the case of T-wave alternans, we focus on the coherence values at 0.5 cycles/beat.

In order to detect TWA phase resetting, we estimated the cross-correlation of the estimated phase of TWA with the reference phase-index sequence which at the point of phase resetting, results in a zero-crossing of the correlation coefficient and a change in the polarity of the correlation coefficient in the vicinity of the zero crossings.

**Triggered Pacing Induced TWA in the Normal Heart**

R-wave triggered pacing during the absolute refractory period was used to induce alternans in the normal heart.
Pacing pulses were delivered on every other beat basis from two leads (RV12) of a decapolar catheter at the apex of the RV. We used pacing pulses of (i) amplitude: ±1 mA and ±5 mA, (ii) width: 30 ms and (iii) coupling to the R-wave: 10 ms. Figure OS3 demonstrates the $K_{score}$ for a single subject before and after R-wave triggered pacing (initiated at beat #177), for body-surface, CS, LV and RV leads. The increase in $K_{score}$ suggests the ability to modulate ventricular repolarization and induce TWA.

**Detection of TWA Phase Resetting**

Having examined the ability of the proposed method to estimate the phase of TWA, we evaluated the use of the PI as a means to detect phase resetting (Figure OS4). In order to detect potential TWA phase resetting, we estimated the cross-correlation of the estimated TWA phase with that of the reference phase-index sequence, which at the point of phase resetting is expected to result in a zero-crossing and a change in the polarity of the correlation coefficient.

We generated an N point time series of an ideal alternating phase-index. Two phase resetting points were introduced into this sequence by reverting the PI at two different pre-specified points, $i=70$ and $j=130$. An M-point ($M=36$) running window was used to estimate the cross correlation with the reference alternating time series. In computer simulated data (Figures OS4, panels A and B), we demonstrate that in the presence of phase resetting, the cross correlation of the phase index changes from 1 to -1 (or vice versa), crossing the zero point at the phase resetting point. The transient decrease/increase starts $M/2$ points before the phase resetting point and lasts for $M/2$ points after the phase resetting point. The change in the polarity of the estimated cross correlation in the M-point window is used to determine the phase resetting location at the zero crossing point.

**Phase-Dependent Coherence Estimation During R-wave Triggered Pacing**

In Figure OS5, we show the phase coherence variation as a result of applying R-wave triggered pacing pulses to induce and suppress TWA from two catheter leads in the RV (induction lead: RV12; suppression lead: RV34; pulse amplitude: -7mA; pulse width: 30ms; coupling to R-wave: 30ms).

Intervention A shows that the level of coherence in the presence of atrial pacing (100 bpm), is low. Intervention B demonstrates a significant rise in the coherence (due to an increase in the alternans voltage and $K_{score}$), resulting from R-wave triggered pacing from RV12 on an every even beat basis. In intervention C, triggered pacing continues from RV12 on even beats, however, triggered stimuli are now delivered from RV34 on every odd beat, and the
coherence substantially decreases (due to a decrease in the alternans voltage and $K_{\text{score}}$) because the distinction between odd and even beats is essentially lost by delivering R-wave triggered pacing pulses during each beat. In intervention D, R-wave triggered pacing continues from RV12 on every even (-7 mA) and from RV34 on every odd beat; however, the polarity of the pacing stimulus delivered on each odd beat from RV34 is reversed (+7 mA). Reversing the polarity of RV34 results in a significant increase in the coherence (due to an increase in the alternans voltage and $K_{\text{score}}$) because the opposite polarity pacing pulses accentuate the distinction between odd and even beats. In the following intervention (E), R-wave triggered pacing from RV12 continues on every even beat (-7 mA) but triggered pacing is now delivered from RV34 also on every even beat but with an opposite polarity pulse (+7 mA). Finally, in intervention G, the coherence again decreases (due to a decrease in the alternans voltage and $K_{\text{score}}$) during triggered pacing from RV12 on every even beat because the distinction between odd and even beats is eliminated. Thus, lack of a priori knowledge of the phase of TWA may result in an increase in the TWA amplitude (as shown in interventions B, D and F). Therefore, in order to effectively suppress TWA, one needs to know the phase of the estimated TWA.

**ECG Morphology Changes During TWA Suppression in the Presence of Acute Ischemia**

In Figure 6 of the manuscript we present the utility of phase-dependent R-wave triggered pacing during the absolute refractory period to suppress spontaneous TWA.

In Figure OS6, we present the change of ECG morphology from baseline in sinus rhythm (when significant TWA is present), to TWA suppression by pacing during the absolute refractory period (one may see the pacing pulse and appreciate that the discrimination of even/odd beats is lost) and back to baseline (when significant TWA is manifested again). In Figure OS6, the median odd/even beats (in a 128-beat sequence) of the triangular intra-cardiac bipolar lead configuration CS2CS8, CS2LV4 and CS2LV10 are shown during spontaneous TWA (intervention A), in-phase R-wave triggered pacing with positive pulse polarity (intervention B) and baseline TWA (intervention C). While we observe visible TWA in both baselines (interventions A and C), intervention B demonstrates a significant reduction in the alternans level, to the extent that TWA is no longer visible.
Online Supplement Figure Captions

**Figure OS1** Typical catheter position in the right ventricle (RV), left ventricle (LV), coronary sinus (CS) and right atrium (RA).

**Figure OS2** Representative electrograms from surface lead V4 and unipolar intracardiac leads from Coronary Sinus (CS), Left Ventricular (LV) and Right Ventricular (RV) catheters with reference to an electrode in the inferior vena cava. The amplitudes of signals have been normalized.

**Figure OS3** Repolarization alternans induction following R-wave triggered pacing on every other beat during absolute refractory period in the normal heart. $K_{\text{score}}$ is plotted for a body-surface lead (ECGII), one CS lead (CS1U), one LV lead (LV9U) and one RV lead (RV3U) from a single study subject. There is a significant increase in $K_{\text{score}}$ after the onset of R-wave triggered pacing during the absolute refractory period (initiated at beat no 177). The TWA increase in RV lead is more profound due to its proximity to the pacing site.

**Figure OS4** Use of phase estimation to detect phase resetting. (A) Computer simulated data indicate two examples of phase resetting, at points $i=70$ and $j=130$. (B) Detection of the phase resetting points using the cross-correlation of the simulated data shown in (A) with the reference phase using an M-point ($M=36$) running window.

**Figure OS5** Phase-dependant average coherence in body-surface leads, and intracardiac CS, LV and RV leads resulting from RV12 and RV34 ventricular triggered pacing (amplitude: -7 mA; pulse width: 30 msec; coupling to R-wave: 30 msec) in the presence of atrial pacing at 100 bpm. The following pacing interventions were performed from the pacing leads: A: baseline, atrial pacing only, B: RV12 every even beat, C: RV12 every even & RV34 every odd beat, D: RV12 every even beat (-7 mA) & RV34 every odd beat but with the opposite polarity current pulse (7 mA), E: RV12 every even (-7 mA) & RV34 every even beat but with the opposite polarity current pulse (7 mA), F: RV12 every even beat & RV34 every even beat, G: RV12 every beat.

**Figure OS6** ECG morphology changes during spontaneous TWA suppression in the presence of acute ischemia. Intervention A: visible TWA in sinus rhythm following coronary artery balloon occlusion; Intervention B: in-phase triggered pacing with positive pulse polarity delivered from
RV12 on every even beat decreases alternans level; Intervention C: R-wave triggered pacing is discontinued and TWA becomes visible during sinus rhythm. Panels show the median odd(red)/even(blue) beats in a 128-beat sequence of the triangular intra-cardiac bipolar lead configuration CS2CS8, CS2LV4 and CS2LV10 during each intervention.
References


Online Supplement Figure 1
Online Supplement Figure 2
Online Supplement Figure 3

![Graph showing ECGII, CS1U, LV9U, and RV3U data over beat numbers.]
A

Phase Index

B

Cross Correlation

Online Supplement Figure 4
Online Supplement Figure 5

Coherence at 0.5 cycle/beat

RV
LV
CS
Surface

A B C D E F G