MIKKO MINKKINEN

# T-Wave Alternans as a Prognostic Marker in Patients Referred for Exercise Testing

## Quantitative Analysis and Combined Assessment with Exercise Capacity and Heart Rate Recovery

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ACADEMIC DISSERTATION
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To my family

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# 1 LIST OF ORIGINAL COMMUNICATIONS

This thesis is based on the following four original publications, which are referred to in the text by their Roman numerals **I–IV**. The original publications have been reprinted with the permission of the copyright holders.

**I** Minkkinen M, Kähönen M, Viik J, Nikus K, Lehtimäki T, Lehtinen R, Kööbi T, Turjanmaa V, Kaiser W, Verrier RL, and Nieminen T (2009): Enhanced Predictive Power of Quantitative TWA during Routine Exercise Testing in the Finnish Cardiovascular Study. J Cardiovasc Electrophysiol 20:408-415.

**II** Minkkinen M, Nieminen T, Verrier RL, Leino J, Lehtimäki T, Viik J, Lehtinen R, Nikus K, Kööbi T, Turjanmaa V, and Kähönen M (2009): Impaired Exercise Capacity Predicts Sudden Cardiac Death in a Low-Risk Population: Enhanced Specificity with Heightened T-Wave Alternans. Ann Med 41:380-389.

**III** Leino J, Minkkinen M, Nieminen T, Lehtimäki T, Viik J, Lehtinen R, Nikus K, Kööbi T, Turjanmaa V, Verrier RL, and Kähönen M (2009): Combined assessment of heart rate recovery and T-wave alternans during routine exercise testing improves prediction of total and cardiovascular mortality: the Finnish Cardiovascular Study. Heart Rhythm 6:1765-1771.

**IV** Minkkinen M, Nieminen T, Verrier RL, Leino J, Lehtimäki T, Viik J, Lehtinen R, Nikus K, Kööbi T, Turjanmaa V, and Kähönen M (2011): The prognostic capacity of clinically indicated exercise test is enhanced by combined analysis of exercise capacity, heart rate recovery and T-wave alternans. Submitted.

In addition, the study contains unpublished data.

Original publication **III** has also been used in the thesis of Johanna Leino.

# 2 ABBREVIATIONS

| | |
|---|---|
| $\mu V^2$ | Alternans power |
| AF | Atrial fibrillation |
| AECG | Ambulatory ECG |
| APD | Action potential duration |
| BMI | Body-mass index |
| CABG | Coronary artery bypass graft |
| $Ca_i$ | Calcium ion |
| CARISMA | Cardiac Arrhythmias and Risk Stratification After Acute Myocardial Infarction |
| CI | Confidence interval |
| CHD | Coronary heart disease |
| DI | Diastolic interval |
| ECG | Electrocardiogram |
| EPS | Electrophysiologic study |
| FINCAVAS | the Finnish Cardiovascular Study |
| HRT | Heart rate turbulence |
| HRV | Heart rate variability |
| HRR | Heart rate recovery |
| ICD | Implantable cardioverter-defibrillator |
| IQ | Interquartile range |
| K score | Alternans ratio (i.e., alternans power divided by the standard deviation of the noise frequency band) |
| LVEF | Left ventricular ejection fraction |
| METs | Metabolic equivalents |
| MI | Myocardial infarction |
| MMA | Modified Moving Average |
| NYHA | New York Heart Association functional classification |
| ROC | Receiver operating characteristic |

| | |
|---|---|
| REFINE | Noninvasive Risk Assessment Early After a Myocardial Infarction |
| RyR | Ryanodine receptors |
| SAECG | Signal-averaged ECG |
| SCD | Sudden cardiac death |
| SD | Standard deviation |
| SERCA | Sarcoplasmic-endoplasmic reticulum calcium adenosine triphosphatase |
| SR | Sarcoplasmic reticulum |
| TWA | T-wave alternans |
| $V_{alt}$ | Voltage of the alternans |
| $VO_2$ | Oxygen consumption |
| VF | Ventricular fibrillation |
| VPB | Ventricular premature beat |
| VT | Ventricular tachycardia |

# 3 ABSTRACT

T-wave alternans (TWA) is an electrocardiogram (ECG) phenomenon illustrating inhomogeneities in cardiac electrical repolarization. It can be measured from the surface ECG as microvolt-level beat-to-beat alternation in the shape, timing, or amplitude of the ST segment or T wave. TWA has been experimentally and clinically linked to ventricular tachyarrhythmias as well as to the related pathogenesis. Moreover, positive TWA testing has been shown to predict all-cause and cardiovascular mortality as well as sudden cardiac death (SCD) in diverse patient populations. The present study was designed to solve the methodological issues related to the prognostic power of TWA analysis, with quantitative TWA analysis in particular. Furthermore, the prognostic power of TWA in combination with exercise capacity and heart rate recovery (HRR), a marker of autonomic nervous system imbalance, were studied.

This study is part of the Finnish Cardiovascular Study (FINCAVAS), which enrolled 4,178 (2,537 men) consecutive patients attending an exercise stress test at Tampere University Hospital between October 2001 and the end of 2008 (Study **IV**). A sub-population of 2,212 (1,400 men) were recruited by the end of 2004 (Studies **I**, **II,** and **III**). A continuous digital ECG signal (500 Hz) was recorded during the entire exercise test from the pre-exercise to the post-exercise phase. The Modified Moving Average (MMA) analysis, which allows TWA analysis during a normal symptom-limited exercise test, was employed. Exercise capacity was assessed in the form of metabolic equivalents (METs) in a standard manner, and HRR was determined as the maximum heart rate minus the heart rate at 1 minute after the cessation of exercise. Hazard ratios for all-cause and cardiovascular mortality as well as SCD were estimated with Cox regression analysis.

During the median follow-up of 48 months (37–59 interquartile range [IQ]), there were 126 deaths, 62 cardiovascular deaths, and 33 SCDs in the sub-population (Studies **I**, **II,** and **III**). The overall follow-up time for the 3,609 patients investigated in Study **IV** was 57 months (35–78 IQ), during which 233 patients died—96 of these deaths were further categorized as cardiovascular deaths. Elevated TWA levels measured during the exercise phase were found to be independently associated with an increased risk of all-cause and cardiovascular mortality and SCD when grouped in increments of 10μV. All-cause and cardiovascular mortality, but not SCD, were also predicted when TWA was measured during the pre- or post-exercise phase (Study **I**).

When analyzed as a continuous variable, increased TWA voltage was a significant predictor of all-cause (Study **I**) and cardiovascular mortality (Studies **I** and **IV**).

Poor exercise capacity (METs <8) was a strong predictor of SCD (hazard ratio of 8.8, 95% confidence interval [CI] 2.0–38.9, p=0.004). The risk was further increased when combined with heightened TWA (≥ 65μV; hazard ratio 36.1, 95% CI 6.3–206.0, p<0.001 in comparison to patients with neither factor; Study **II**). The combination of poor HRR (≤ 18 beat/min) and elevated TWA (≥ 60μV) yielded a hazard ratio of 12.3 (95% CI 4.3–35.3, p<0.01) for cardiovascular mortality when analyzed in comparison to patients with neither factor, with a C-index of 0.713 (95% CI 0.648–0.777; Study **III**). When all three prognostic markers—namely exercise capacity in METs, HRR, and TWA—were combined, the prognostic capacity of exercise testing increased further. The linear model that contained all three study parameters predicted cardiovascular mortality significantly better than the model without METs (p<0.001), HRR (p=0.002), or TWA (p=0.01). The hazard ratio of cardiovascular mortality for the combination of the three parameters with the previously reported cut-off points of <8 for METs, ≤18 beats/min for HRR, and ≥60 μV for TWA was 5.7 (95% CI 1.8–18.2, p=0.003) when compared to all other patients included in the study. The corresponding Harrell C-index was 0.719 (95% CI 0.665–0.772; Study **IV**).

Measuring TWA from surface ECG is inherently challenging, and the future will show whether this non-invasive TWA assessment can be incorporated into clinical use or whether, for example, TWA analysis based on cardiac implantable electric devices will break through.

Finally, the present study produces new information concerning the predictive capacity and characteristics of TWA in patients referred for exercise testing. The evidence derived from our study, together with information uncovered by experimental and clinical studies, clearly shows that elevated levels of TWA are pathophysiologically linked with increased risk for cardiovascular mortality. The study also demonstrates that poor exercise capacity predicts SCD in a population of patients referred for exercise testing. Moreover, it shows that the combination of exercise capacity, HRR, and TWA enhances the prognostic capacity of exercise stress testing. These three parameters that can be measured during routine exercise testing offer an avenue for improving the risk stratification for cardiovascular mortality and SCD.

# 4 TIIVISTELMÄ (ABSTRACT IN FINNISH)

T-aallon vuorottelu (T-wave alternans, TWA) on sydänsähkökäyrästä (elektrokardiogrammi, EKG) mitattava ilmiö, joka kuvaa sydämen sähköisen palautumisvaiheen poikkeavuutta. Se voidaan mitata ihon pinnalta lyönnistä toiseen tapahtuvana mikrovolttitason vuorotteluna T-aallon ja/tai ST-segmentin muodossa, ajoituksessa tai amplitudissa. TWA on liitetty kokeellisissa ja kliinisissä tutkimuksissa kammioperäisten rytmihäiröiden patogeneesiin. Lisäksi positiivisen TWA-testin tuloksen on osoitettu ennustavan kokonais- ja sydänperäistä kuolleisuutta sekä sydänperäisiä äkkikuolemia useissa erityyppisissä populaatioissa. Tässä tutkimuksessa selvitimme TWA:n prognostiseen merkitykseen vaikuttavia metodologisia tekijöitä, erityisesti TWA:n kvantitatiivisen analyysin merkitystä. Lisäksi tutkimme TWA:n ja heikentyneen suorituskyvyn sekä autonomisen hermoston toimivuutta kuvaavan rasituksen jälkeisen sykkeen palautumisen (heart rate recovery, HRR) yhdistettyä ennustemerkitystä.

Tämä tutkimus on osa The Finnish Cardiovascular Study (FINCAVAS) -tutkimusta, johon rekrytoitiin 4178 potilasta (2537 miestä), jotka tulivat kliiniseen rasituskokeeseen Tampereen yliopistolliseen sairaalaan lokakuun 2001 ja vuoden 2008 lopun välisenä aikana (tutkimus **IV**). Alapopulaatio koostui vuoden 2004 loppuun mennessä rekrytoiduista 2212 potilaasta (1400 miestä; tutkimukset **I**, **II** ja **III**). Jatkuva digitaalinen EKG-signaali rekisteröitiin koko rasituskokeen ajan lepovaiheesta palautumisvaiheen loppuun asti. TWA:n analysointiin valittiin Modified Moving Average (MMA) -menetelmä, joka mahdollistaa TWA:n mittaamisen tavanomaisen kliinisen rasituskokeen aikana. Suorituskyky analysoitiin metabolisina ekvivalentteinä (METs) ja sykkeen palautuminen rasituksen aikaisen maksimisykkeen ja yksi minuutti rasitusvaiheen päättymisen jälkeen mitatun sykkeen erotuksena. Riski kuolleisuudelle, sydänperäiselle kuolemalle ja sydänperäiselle äkkikuolemalle määritettiin Coxin regressiomenetelmällä.

Alapopulaation mediaani seuranta-aika oli 48 kuukautta (kvartiiliväli 37–59), ja sinä aikana 126 potilasta kuoli; 62 oli sydänperäistä kuolemaa ja 33 sydänperäistä äkkikuolemaa. Kokonaisseuranta-aika tutkimuksessa **IV** (N=3609) oli 57 kuukautta (kvartiiliväli 35–78), minä aikana 233 potilasta kuoli ja 96 heistä koki sydänperäisen kuoleman. Kohonneet rasituksen aikaiset TWA-arvot liittyivät itsenäisesti kohonneeseen riskiin kuolla, kokea sydänperäinen

kuolema tai sydänperäinen äkkikuolema seuranta-aikana, kun TWA arvot analysoitiin 10 μV välein ryhmiteltyinä. Erikseen ennen ja jälkeen rasitusvaihetta mitatut kohonneet TWA-arvot ennustivat kuolleisuutta ja sydänperäistä kuolemaa mutta eivät sydänperäistä äkkikuolemaa (tutkimus **I)**. Kun kohonnut TWA analysoitiin jatkuvana muuttujana, se ennusti tilastollisesti merkitsevästi kokonais- (tutkimus **I**) ja sydänperäistä kuolleisuutta (tutkimus **I** ja **IV**).

Huono suorituskyky (METs <8) ennusti voimakkaasti sydänperäistä äkkikuolemaa (riskisuhde 8,8, 95% luottamusväli [LV] 2,0–38,9, p=0,004). Riski kasvoi edelleen, kun huono suorituskyky yhdistettiin kohonneen TWA:n (≥ 65μV) kanssa (riskisuhde 36,1, 95% LV 6,3–206,0, p<0,001 verrattuna potilaisiin, joilla molemmat muuttujat olivat normaalit; tutkimus **II**). Hitaan sykkeen palautumisen (≤ 18 lyöntiä/min) ja kohonneen TWA:n (≥ 60μV) kombinaation riskisuhde sydänperäiselle kuolemalle oli 12,3 (95% LV 4,3–35,3, p<0,01) verrattuna potilaisiin, joilla molemmat muuttujat olivat normaalit ja C-indeksi oli vastaavasti 0,713 (95% LV 0,648–0,777; tutkimus **III**). Kun kaikki kolme muuttujaa – suorituskyky, sykkeen palautuminen ja TWA – yhdistettiin, rasituskokeen ennusteellinen arvo parani. Lineaarinen malli, jossa oli kaikki kolme muuttujaa, ennusti tilastollisesti merkitsevästi paremmin sydänperäistä kuolemaa kuin sama malli ilman suorituskykyä (p<0,001), sykkeen palautumista (p=0,002) tai TWA:a (p=0,01). Kolmen muuttujan yhdistelmän riskisuhde sydänperäiselle kuolemalle oli 5,7 (95% LV 1,8–18,2, p=0,003), kun käytettiin aikaisemmin julkaistuja raja-arvoja (METs <8, sykkeen palautuminen ≤18 lyöntiä/min ja TWA ≥60 μV), verrattuna kaikkiin muihin tutkittuihin potilaisiin. Vastaava C-indeksi oli 0,719 (95% LV 0,665–0,772; tutkimus **IV**).

TWA:n määrittäminen ihon pinnalta mitattavasta EKG-signaalista on haastavaa. Tulevaisuus näyttää, pystytäänkö sitä hyödyntämään kliinisessä päätöksenteossa vai olisiko esimerkiksi sydämen sisälle kiinnitettävistä laitteista mitattavasta TWA:sta lääkäreiden työkaluksi.

Tutkimustulokset antavat uutta tietoa TWA:n ennustemerkityksestä kliiniseen rasituskokeen osallistuvilla potilailla. Tutkimuksemme ja aikaisempien kokeellisten sekä kliinisten tutkimusten tulokset osoittavat, että kohonneet TWA-tasot liittyvät patofysiologiseen ketjuun, joka johtaa kohonneeseen sydänperäisen kuoleman riskiin. Tässä tutkimuksessa myös osoitettiin, että heikentynyt suorituskyky ennustaa sydänperäistä äkkikuolemaa kliiniseen rasituskokeeseen osallistuvassa populaatiossa. Lisäksi osoitimme, että heikentyneen suorituskyvyn, matalan sykkeen palautumisen ja kohonneen TWA:n yhdistelmä parantaa kliinisen rasituskokeen kykyä ennustaa sydänperäistä kuolemaa. Koska nämä kaikki kolme ennustemuuttujaa pystytään määrittämään normaalin kliinisen rasituskokeen aikana, antaa niiden yhdistelmä mahdollisuuden parantaa sydänperäisen kuoleman ja sydänperäisen äkkikuoleman riskin arviointia.

# 5 INTRODUCTION

T-wave alternans (TWA) is an electrocardiogram (ECG) phenomenon describing cardiac repolarization instabilities. It was first reported in 1908 as visible beat-to-beat alternation in the shape, amplitude, or timing of the ST segment and the T wave (Herring 1909, Lewis 1910). It remained an interesting ECG curiosity until the 1980s, when Cohen and colleagues described a method for microvolt-level TWA analysis (i.e., not visible in surface ECG). They also reported that elevated levels of microvolt TWA are present in situations where the susceptibility to ventricular arrhythmias is increased (Adam et al. 1984, Smith et al. 1988).

Since the first reports, accumulating evidence from experimental and clinical studies has linked elevated TWA to an increased risk of cardiovascular mortality and sudden cardiac death (SCD). The association was first demonstrated in humans when TWA was measured invasively during atrial pacing (Rosenbaum et al. 1994). Thereafter, TWA as measured non-invasively during exercise has been the most studied means of TWA analysis (Gehi et al. 2005, Nieminen et al. 2007). More recently, the association between TWA and cardiovascular mortality has also been established in studies based on ambulatory ECG (AECG, i.e., Holter; Verrier et al. 2003, Sakaki et al. 2009). Moreover, it was also recently reported that elevated TWA levels precede ventricular fibrillation (VF) when TWA was analyzed from implantable cardioverter-defibrillator (ICD) -based ECGs (Swerdlow et al. 2011). Most of the clinical studies on TWA have been carried out with populations of patients at a high risk of life-threatening arrhythmias, such as patients with reduced left ventricular ejection fraction (LVEF) or patients with a prior myocardial infarction (MI; Gehi et al. 2005, Hohnloser et al. 2009). However, TWA has also been linked to increased risk in patients with a prior MI and preserved cardiac function (i.e., LVEF >40%; Ikeda et al. 2006) and, most notably, in patients referred for exercise stress testing (Nieminen et al. 2007).

There are two commercially available methods for TWA analysis from surface ECG. The spectral method is the most widely studied and better-validated method (Bloomfield et al. 2002a). However, it requires stationary data to allow TWA measurement, therefore requiring the use of a non-standard exercise test protocol with fixed heart rate and specialized electrodes. The other commercially available method, namely the Modified Moving Average (MMA) method,

was announced in the early 2000s (Nearing and Verrier 2002a). It makes TWA analysis possible during fluctuating heart rates, with no need for a special exercise protocol or electrodes. Furthermore, it also allows TWA measurement from AECG.

Exercise capacity is a powerful predictor of cardiovascular and all-cause mortality (Kodama et al. 2009). However, the data available concerning its association especially for SCD is lacking. Heart rate recovery (HRR), a factor related to the dysfunction of the autonomic nervous system, has also been shown to be a strong prognostic marker (Cole et al. 1999). Moreover, the combination of low exercise capacity and low HRR has been linked to a further increased risk for total and cardiovascular mortality (Mora et al. 2003, Mora et al. 2005). This complementary prognostic information may be caused by the different pathophysiological mechanisms behind these two parameters. Exercise capacity essentially measures mechanical cardiac function, whereas HRR is thought to be caused principally by a reactivation of the parasympathetic nervous system and, subsequently, by the withdrawal of sympathetic tone (Imai et al. 1994).

In the present study, TWA was assessed with the MMA method which enables TWA measurement during a standard clinical exercise stress test in which exercise capacity in terms of metabolic equivalents (METs) and HRR analysis is also possible. As a part of the Finnish Cardiovascular Study (FINCAVAS), we evaluated the prognostic capacity of TWA. FINCAVAS enrolled more than 4,000 patients undergoing a clinically indicated exercise stress test, making it the largest TWA study conducted to date. We concentrated particularly on methodological issues related to the prognostic power of TWA, such as its quantitative assessment. Furthermore, the predictive strength of exercise capacity and HRR were studied independently and in combination with TWA to further enhance the prognostic capability of exercise stress testing.

# 6 REVIEW OF THE LITERATURE

## 6.1 T-wave alternans

### 6.1.1 Definition

TWA is an ECG phenomenon described as beat-to-beat alternation in the shape, timing, or amplitude of the ST segment or T wave (Nearing et al. 1991, Rosenbaum et al. 1994).

Visible TWA was first linked to the heart's electrical instability over 100 years ago (Herring 1909, Lewis 1910). Subsequently, it has been linked to different pathophysiologic situations, and in many of these cases, visible TWA has preceded ventricular tachyarrhythmias; it is also occasionally seen in patients with long QT syndrome (Zareba et al. 1994). However, in other situations besides ion channelopathies, macrovolt TWA is a very uncommon ECG phenomenon.

In the 1980s, Adam, Cohen, and colleagues first described microvolt-level TWA (i.e., not detected with visual inspection of ECG) in animal studies as being present in situations where the susceptibility to ventricular arrhythmias is enhanced (Adam et al. 1984, Smith et al. 1988). Since then, microvolt-level TWA has been linked to an increased risk of ventricular arrhythmias and cardiovascular mortality in diverse patient populations.

### 6.1.2 Mechanisms of T-wave alternans

There is growing evidence that the action potential duration (APD; Fig. 1) at a level of a single cardiac myocyte plays a key role in the development of TWA (Weiss et al. 2006). The beat-to-beat alternation in the membrane repolarization of a single cell is thought to be caused either by voltage dynamics (the APD restitution hypothesis) or by cytosolic calcium cycling.

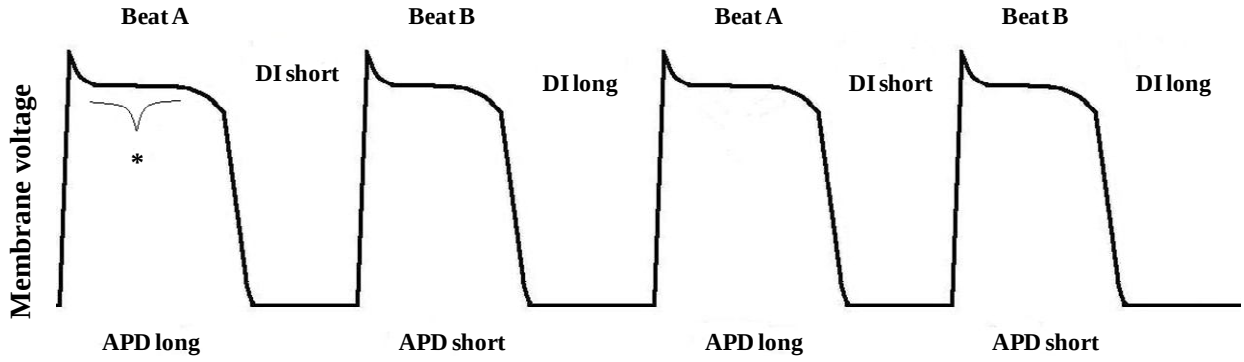



**Figure 1.** Action potential duration (APD) alternans of a single myocyte at a given cycle length. See text for details. * Showing the plateau phase caused by calcium ions entering and potassium ions exiting the cell. DI= diastolic interval. Redrawn from Naryan et al. (2006).

### 6.1.3 The action potential duration restitution slope hypothesis

The APD restitution slope hypothesis is a cell-level model that has been suggested to explain the development of APD alternans and, furthermore, the progression of TWA. Action potential restitution is the physiologic reduction of APD with increasing heart rate and, as a result, allows better diastolic filling at faster heart rates (Fig. 2). The APD restitution slope is described as a direct relationship between the APD of one beat and the diastolic interval (DI) of the preceding beat (Saitoh et al. 1989, Cutler and Rosenbaum 2009). A shortening in the DI will lead to a shorter APD, followed by a long DI which, in turn, will cause a long APD, thus resulting in an APD alternans (Weiss et al. 2006; Fig. 1).

Nolasco and Dahlen showed as early as in 1968 that when the APD restitution slope is less than 1 at a given cycle length (i.e., heart rate), the APD alternans is transient, indicating local electrical stability. In other words, when the APD restitution slope is more than 1, the APD alternans progressively increases and leads to a persistent alternation in APD, referring to a more unstable condition. Moreover, when the APD restitution slope is more than 1, small changes in DI caused by, for example, a premature beat can initiate the alternans (Karma 1993, Narayan 2006).

The APD restitution slope hypothesis is a useful mathematical model, and it is supported by computer simulation studies (Watanabe et al. 2001b, Fox et al. 2002, Qu 2004). However, the mechanisms of APD restitution and APD alternans at a cellular and molecular level in real cardiac tissue are multifractional. Hence, the hypothesis that the duration of action potential depends only on the length of the preceding DI is oversimplified (Weiss et al. 2006). Narayan

and others published an interesting case control study in 2007. They studied 53 subjects with reduced LVEF (≤40%) and 18 controls during an electrophysiologic study (EPS) and found that the maximum APD restitution slope did not differ between the two groups, whereas the TWA values were more likely to be abnormal in the study patients than in the controls ($p<0.01$). Furthermore, TWA, but not APD restitution slope >1, predicted ventricular arrhythmias in patients with reduced LVEF during follow-up (Narayan et al. 2007). However, as the authors discussed, the APD was measured from limited sites during the EPS and, therefore, APD alternans may have been under-detected.

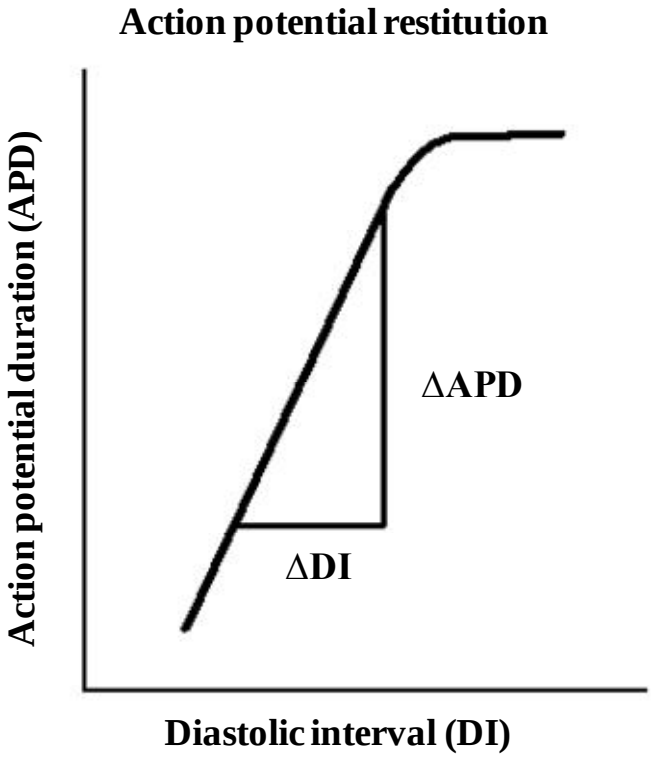



**Figure 2.** Action potential restitution is the relationship of action potential duration (APD) to preceding diastolic interval (DI). Please see the text for details. Redrawn from Naryan et al. (2006).

### 6.1.4 The calcium cycling hypothesis

The second major hypothesis that has been thought to explain the development of APD alternans and the progress of TWA is the calcium cycling hypothesis.

Calcium ions ($Ca_i$) are in a key position in the cascade of cardiac muscle contraction. In the beginning of the action potential, a small amount of $Ca_i$ enters the cell via the L-type calcium

channels due to the depolarization of the cell membrane as caused by the entry of sodium ions. $Ca_i$ entering through the L-type calcium channels then triggers the release of large amounts of $Ca_i$ from the stores of sarcoplasmic reticulum (SR), which extends the repolarization of a myocyte (Fig. 1). The release occurs via the ryanodine receptors (RyR) related to the $Ca_i$ channels. After this, most of the $Ca_i$ is pumped back into the SR by the sarcoplasmic-endoplasmic reticulum calcium adenosine triphosphatase (SERCA) pumps. A proportion of the $Ca_i$ is also removed to the extracellular space by the sodium-calcium exchanger (Weiss et al. 2006).

The APD and $Ca_i$ cycling are strongly coupled. Therefore, if APD alternans is caused by APD restitution, the intracellular $Ca_{i,}$ cycling will alternate as a result of the role of $Ca_i$ in the creation of action potential (Weiss et al. 2006). However, it has been recently shown that it is rather the $Ca_i$ cycling that initially alternates and actually causes the APD alternans.

In normal conditions, $Ca_i$ release from the SR in the myocyte equals the reuptake via SERCA pumps (Cutler and Rosenbaum 2009, Verrier et al. 2009). However, any condition that affects these processes can lead to $Ca_i$ cycling alternans and, further, to APD alternans. In a ventricular myocytes stimulation study by Diaz et al. (2004), the alternation of $Ca_i$ cycling was shown to depend on the alternation of the SR $Ca_i$ concentration. The $Ca_i$ release from the SR via RyR $Ca_i$ channels therefore varies with respect to the $Ca_i$ content in SR. On the contrary, it has been shown that the alternation in $Ca_i$ cycling does not necessarily require fluctuations in the SR's $Ca_i$ content. Hence, it seems likely that there is some other factor or factors that have the primary role in creating the $Ca_i$ cycling alternas, such as the alternation in the $Ca_i$ release from the SR caused by the RyR availability after the prior beat (Picht et al. 2006).

It has also been suggested recently that it is the alternans in action potential voltage, rather than in APD explained by reduced SR calcium uptake, that may lead to TWA and, further, to VF (Narayan et al. 2008, Bayer et al. 2010).

### 6.1.5 Mechanisms linking T-wave alternans to ventricular arrhythmias

APD alternans can occur in the same phase in every myocyte of the heart (i.e., concordant alternans). Hence, myocytes in all regions alternate in the same pattern (i.e, short-long-short). However, different regions of the heart can also alternate in opposite phases simultaneously. This is called discordant alternans (Weiss et al. 2006, Cutler and Rosenbaum 2009, Verrier et al. 2009).

Spatially discordant alternans has been experimentally demonstrated to be more arrhythmogenic than spatially concordant alternans (Pastore et al. 1999, Qu et al. 2000). Moreover, it has been shown that discordant alternans always precedes pacing-induced VF in experimental models (Pastore et al. 1999). It seems, however, that concordant alternans is necessary for the development of discordant alternans (Cutler and Rosenbaum 2009).

### 6.1.6 Concordant to discordant

Conduction velocity restitution has a key role in the transition from concordant to spatially discordant alternans. Like APD, conduction velocity is in a direct relationship with the DI of the preceding beat (Weiss et al. 2006).

When the DI gets shorter, as in the case of increasing heart rate, the sodium channels do not have enough time to recover completely, which leads to a decrease in conduction velocity and may convert concordant alternans to discordant. Heart rate affects both APD and conduction velocity restitution, and it has been suggested to be the mechanism underlying the TWA's dependency on heart rate (Cutler and Rosenbaum 2009). In a simulation study by Qu et al. (2000), the sustained discordant alternans did not appear without a deep conduction velocity restitution. However, it has been shown that a premature ventricular beat can elicit the transition from concordant to discordant also in the absence of deep conduction velocity restitution (Watanabe et al. 2001b). Other mechanisms like intercellular uncoupling in hearts with a macroscopic structural barrier (Pastore and Rosenbaum 2000) or spatial heterogeneities in calcium cycling have been suggested to be the harbingers in producing discordant alternans (Weiss et al. 2006).

### 6.1.7 Conduction block and re-entry

The experimental study by Pastore et al. (1999) demonstrated, for the first time, the link between TWA and the initiation of re-entry leading to VF. The study found that spatially discordant APD alternans produces spatial gradients of repolarization of such a magnitude that they can lead to a unidirectional block and, further, to re-entry and VF.

When the discordant alternans develops, the APD and, consequently, the refractory period in adjacent regions of the heart alternates in a short-long-short pattern simultaneously. Therefore, the dispersion of refractoriness strengthens and an ectopic beat can cause a unidirectional block

(Weiss et al. 2006). Moreover, when the tissue is heterogeneous enough, the unidirectional block can occur even without the premature ventricular beat (Cao et al. 1999, Pastore et al. 1999, Qu et al. 2000). In such a case, after a long APD, the DI can decrease to zero, resulting in a conduction block during the next wavefront with a short APD and, hence, a short refractory period. Moreover, a local conduction block allows impulses from neighboring areas of the cardiac tissue to re-enter the blocked regions. This mechanism has been shown to cause VF during rapid pacing in simulations (Qu et al. 2000) and in experiments (Cao et al. 1999, Pastore et al. 1999).

## 6.2 Methods for T-wave alternans analysis

There are currently two commercially available methods for a TWA analysis from body surface ECG. Several other methods have been studied as well, but they are beyond the scope of this thesis and are therefore not discussed here.

### 6.2.1 Spectral Method

The Spectral Method is the most widely used and studied method for TWA analysis from body surface ECG. It was generated in the 1980s by Smith and co-workers (1988) and commercialized by Cambridge Heart Inc., Bedford, MA, USA.

#### *6.2.1.1 T-wave alternans measurement*

The Spectral Method computes a spectrum created by 128 corresponding points of 128 consecutive T waves. The measurement of the T wave in each beat is obtained at exactly the same time point after the preceding QRS complex. The power spectrum from different points of the T wave is calculated and composited to detect any alternans in the T wave's morphology. The frequency of the alternans is given in units of cycles per beats, and, the spectrum at the exact frequency of 0.5 thus indicates the level of TWA.

The alternans at the frequency of 0.5 beats per cycles (i.e., TWA) and the alternans of the reference frequency (i.e., noise frequency band that is measured between 0.44 and 0.49 cycles per beat) are then squared and their difference is called the alternans power ($\mu V^2$). The voltage of the physiologic alternans ($V_{alt}$) in $\mu V$ is the square root of the $\mu V^2$. The $V_{alt}$ equals the root mean

square difference between the mean beat over the consecutive 128 beats and either the odd or even mean beats. The alternans ratio (K score) is defined as the ratio of the $\mu V^2$ divided by the standard deviation (SD) of the noise frequency band (Smith et al. 1988, Rosenbaum et al. 1994, Bloomfield et al. 2002a) .

*6.2.1.2 Noise handling with the spectral method*

The key questions in analyzing TWA from body surface ECG are: (a) Is there TWA, and if so, is it true TWA or caused by noise and artifacts? (b) If there is no TWA, is the ECG tracing of such a good quality that we can trust that it is a true negative finding (i.e., are there potential artifacts present that could cause a false negative finding)? It is therefore crucial to reduce and observe the noise caused by different kinds of factors, such as pedaling in bicycle exercise, respiration, ectopic beats, etc.

The first step in handling the noise with the spectral method is ensuring that the $V_{alt}$ measurement is based on 128 consecutive beats. Hence, it provides an accurate measurement of frequency on the beat-by-beat basis. The TWA occurs exactly at the frequency of 0.5, which allows its differentiation from artifacts that can possibly cause alternans close to that frequency. The K score is used to give a numerical value for the ratio between true physiologic alternans and noise and artifacts in a frequency of 0.44 to 0.49. However, it is possible that artifacts can also occur in an exact frequency of 0.5. The respiration frequency is normally between 0.2 and 0.33 cycles per beat during the exercise. It is possible, therefore, that the respiration frequency is 0.25 (i.e., one fourth of a heart rate) and can thus cause alternans in a frequency of 0.5. For this reason, the respiration frequency is measured during the exercise test, with an indicator informing the operator if the respiration occurs at a frequency of 0.25. The pedaling frequency is also considered as a possible artifactual factor in bicycle ergometer testing for the same reasons as in the case of respiration (Bloomfield et al. 2002a).

Secondly, specialized electrodes have been developed (Micro-V Alternans Sensors$^{TM}$, Cambridge Heart Inc., Bedford, MA, USA) to reduce noise. They detect and process ECG signals from multiple segments of an electrode as well as reduce the impedance together with careful skin preparation. It is also recommended that patients loosely rest their arms on handlebars when exercising either with an ergometer or a treadmill to reduce the muscle artifacts. A careful electrode placement is also considered. In addition, a disconnected lead can mask true alternans (Bloomfield et al. 2002a).

Ectopic premature beats or falsely detected beats can produce a false positive TWA, but they can also obscure alternans. A single premature ectopic beat can reset the alternans pattern from A-B-A-B to B-A-B-A, and if this occurs at exactly the midpoint of the 128 beat pattern, zero alternans will be shown despite the possible underlying true TWA. During TWA testing with the spectral method, the morphology of every beat is compared to a template beat and they are considered bad beats if the correlation coefficient is <0.9. The artifactual alternans caused by bad beats is usually non-sustained (Bloomfield et al. 2002a).

If the heart rate changes by more than 30 beats/min in a 128-beat window, an indicator will inform the operator about it. A rapid change in hear rate can cause an artifactual TWA or obscure true TWA. A rarer phenomenon that can lead to the detection of an artifactual TWA is R-R interval alternans (i.e., cycle length alternans). It is also measured during the spectral TWA testing, and the computer will alert the operator if the R-R interval alternans is more than 2 ms and if the R-R interval ratio is more than 3. Neither of these heart-rate-related factors normally create a sustained TWA (Bloomfield et al. 2002a).

Finally, the high noise level calculated from the noise frequency band can cause artifactual TWA. It has been suggested that it is usually of extremely short duration, especially in precordial leads. Moreover, a high noise level can also obscure true TWA. It is therefore important that there is a sequencet of artifact-free ECG available for every patient undergoing microvolt TWA testing with the spectral method to determine whether TWA is present or not (Bloomfield et al. 2002a).

#### *6.2.1.3 Interpretation of the results: the criteria*

The criteria for the interpretation of the TWA test results analyzed with the spectral method are well described (Rosenbaum et al. 1996, Bloomfield et al. 2002a), and they have been used in numerous clinical studies (Gehi et al. 2005, Hohnloser et al. 2009). However, the data available underlying the criteria is sparse.

The test is considered positive if sustained alternans is present at the onset heart rate of ≤110 beats/min or at the resting heart rate, even if the latter is more than 110 beats/min. Sustained alternans is determined when $V_{alt}$ is equal to or more than 1.9μV and the K score equal to or more than 3 for at least one minute in any orthogonal lead (the X, Y, Z, or the vector magnitude lead) or in any precordial lead, with $V_{alt}$ equal to or more than 1.9μV also in an adjacent precordial lead. Moreover, there has to be a period of artifact-free data available: ectopic or

premature beats are allowed in ≤10% of all the beats, the respiratory cycle cannot be exactly 0.25 cycles per beat, the variation in heart rate over the 128-beat period has to be under 30 beats/min, and, finally, the R-R interval (i.e., cycle length) variation must not be ≥2 ms (Bloomfield et al. 2002a).

The test is considered negative when it cannot be classified as positive and when the maximum negative heart rate is ≥105 beats/min (i.e., heart rate over 128 beats period is ≥105 beats/min, and noise level in the vector magnitude lead is ≤1.8 μV [or the sum of the noise level plus the $V_{alt}$ is ≤2.5 μV], with ≤10% ectopic beats and no lead malfunction). Furthermore, in some studies the test has been defined negative when the maximum heart rate during a maximal test has been ≥80 beats/min with a maximum negative heart rate of equal to or more than the maximum heart rate minus 5 beats/min. After all, the test is considered indeterminate if it cannot meet the criteria for being either positive or negative (Bloomfield et al. 2002a). However, prior studies have shown that indeterminate test results contain equal prognostic information in relation to positive tests (Kaufman et al. 2006), and they have been grouped together in the majority of the clinical studies since this discovery (Hohnloser et al. 2009).

Chan and co-workers published interesting data in 2007 from their population of patients with ischemic cardiomyopathy. Of their 768 consecutive patients, 159 (21%) had indeterminate TWA test results. Of these, 14 (9%) were due to an un-sustained TWA, 21 (13%) to excessive noise, 73 (46%) to ventricular ectopy, and 51 (32%) to an inability to reach the target heart rate. Moreover, the authors found that the indeterminate tests that were due to an inability to reach the target heart rate or frequent ventricular ectopy were associated with an increased risk for all-cause mortality, whereas non-sustained TWA was not. Chan et al. proceded to suggest that indeterminate TWA test results should be classified as positive or negative, depending on the underlying reason. As a result, only 3 percent of all tests would remain indeterminate because of excessive noise. Indeterminate results are reported to occur in 9%–47% of tests (Bloomfield et al. 2002a), and attempts have been made to to reduce the rate with test repeating (Chow et al. 2008). A great concordance has been described between the exercise-based TWA test results measured during a treadmill test and those obtained during a bicycle exercise test (Bloomfield et al. 2003). Moreover, short-term test repeatability has been verified in bicycle exercise tests with repeat tests performed within an average 15 minutes (concordant results in 18 out of 22 study patients, kappa 0.58; Bloomfield et al. 2002b) or within 4 hours (concordant results in 39 out of 42 study patients; Turitto et al. 2002) of the first test. Longer-term repeatability has been investigated by Wierzbowski et al. (2007). In their study with 22 patients receiving ICDs, they found that the reproducibility of the TWA test was 77% (kappa 0.602) when the second test was

carried out, on average, 12 months (mean value, range 7–16) after the first. However, their results may be biased, as they report the results of 30 repeated tests, suggesting that some patients were tested more than once.

#### *6.2.1.4 Interpretation of the results: the evidence*

In the first human atrial pacing studies with the spectral method, only the K score was used to determine whether alternans was present or not (Smith et al. 1988, Rosenbaum et al. 1994, Armoundas et al. 1998a, Armoundas et al. 1998b). In a pilot study by Smith et al. (1988), the alternans level was defined significant if the power of the alternans frequency exceeded the estimate of the noise mean (i.e., the K score ≥3) by three or more SDs. In their landmark paper in 1994, Rosenbaum et al. used the K score ≥2.5 as the threshold for the alternans. In addition, they also used the cumulative alternans voltage (i.e., square root of the alternas voltages summed over the 128-beat window) of ≥10μV in determining the presence of TWA. The cut-off point for the K score was simply judged to be the level of significance (Smith et al. 1988). After the review article by Rosenbaum et al., published in 1996, the K score ≥3 has been adopted without dispute.

The cut-off point for alternans voltage ($V_{alt}$ ≥1.9μV) was determined retrospectively in a pilot exercise-based TWA study by Estes et al. published in 1997. The threshold value provided was the most optimal predictor for vulnerability to ventricular arrhythmias in 27 patients undergoing EPS. Moreover, a cut-off point of $V_{alt}$ ≥1.0μV yielded the best results when the alternans was analyzed during rest. However, there is no information available about the methods used to optimize these cut-off points, and the results yielded with other cut-off points were not shown. Subsequently, the cut-off point of ≥1.9μV has been the standard for the alternans magnitude, and it has been used in numerous clinical studies with different patient populations and endpoints (Gehi et al. 2005, Hohnloser et al. 2009). Alternative cut-off points have been studied only in few clinical studies with atrial pacing at different heart rates (Narayan and Smith 2000, Tanno et al. 2004). For instance, it was found in an atrial pacing study with 60 patients that $V_{alt}$ ≥2.6μV at a heart rate of 120 beats/min provided optimal sensitivity (87.5%) and specificity (88.7%) for inducible ventricular tachycardia (VT) during EPS (Narayan and Smith 2000).

Orthogonal leads (X, Y, Z or vector magnitude) and standard precordial leads are used for analyzing TWA with the spectral method (Bloomfield et al. 2002a). As described earlier, if the alternans is present in any precordial lead, it also has to be present in the adjacent lead to be

determined significant. This has been explained with the higher noise levels in the precordial leads in comparison to the orthogonal leads. However, only one pacing study can be found where the prognostic information of different lead groups is evaluated. Kavesh et al. (1998) analyzed three different sets of leads during atrial pacing at the heart rates of 77, 100, and 120 beats/min. They concluded that when all the leads where analyzed in the fashion described earlier (i.e., any orthogonal or two precordial leads have to be abnormal), the TWA detection for inducible sustained VT (sensitivity 67% and specificity 72% at a heart rate of 100 beats/min) was little improved when compared to the vector magnitude lead alone (42% and 93%) or to the lead set containing all of the orthogonal leads and lead $V_4$ (59% and 72%).

As described in the previous chapter, the classification of TWA test results with the spectral method in regard to current guidelines requires a constant heart rate of between 105 and 110 beats/min for at least a few minutes. Therefore, it is important that the heart rate increases slowly during the exercise test and that once the heart rate of 95 to 100 beats/min is achieved, the workload should be kept constant. The exercise test protocol should be chosen with respect to the patient's physical fitness and the resting heart rate. The Modified Bruce protocol or Naughton Protocol is recommended for patients with limited exercise tolerance exercising on a treadmill and a ramp protocol for all patients exercising on a bicycle ergometer (Bloomfield et al. 2002a).

The hypothesis that TWA is predominantly a rate-dependent phenomenon is based on the observation that TWA increases independently of the autonomic condition, when it reaches a specific heart rate threshold (Cutler and Rosenbaum 2009). This was demonstrated clinically in a ventricular pacing study with 24 patients by Kaufman and co-workers (2000), in which they compared the effect of the elevation of heart rate to the effect of beta-adrenergic stimulation to the same heart rate in three different groups of patients (i.e., normal subjects, history of SCD, and patients with inducible VT). They concluded that it is increased heart rate rather than sympathetic tone that causes the TWA during exercise. However, Verrier and others challenged this conclusion in 2009 with the fact that in the group of patients with a history of SCD, beta-adrenergic stimulation with isoproterenol produced a 2.8-fold increase in TWA magnitude, suggesting that autonomic tone has a role in the genesis of TWA at least in patients with a history of cardiac arrest. Morever, it has also been shown that beta-blockage either with metoprolol (Klingenheben et al. 2001) or with esmolol (Rashba et al. 2002a) reduces the mean TWA magnitude as well as the number of positive tests.

The heart rate limits (i.e., 105–110) in spectral TWA testing during exercise are based in a few small studies. The effect of heart rate on TWA was first studied clinically in 1997 by

Hohnloser and co-workers. They compared the TWA test results during atrial pacing and exercise in 30 patients and found that TWA magnitude increased when the patient-specific heart rate threshold (mean 100 and SD 13 beats/min during exercise) was achieved and was significantly greater at maximum heart rate. However, paired T-tests (i.e., parametric test) were incorrectly used when comparing the TWA magnitudes in respect to the given SDs, which hampers the interpretation of the results.

In 1998, Kavesh and colleagues studied 45 patients during sinus rhythm and atrial pacing at the heart rates of 100 and 120 beats/min and found that the TWA magnitude increases with the increase in heart rate. Similar results were achieved in their study of the effects of procainamide on TWA (Kavesh et al. 1999). However, they also used parametric tests despite the fact that TWA magnitude values were not normally distributed. It was also shown that sensitivity for inducible VT increased when the pacing rate increased, while the specificity decreased. The authors concluded that the optimal target heart rate in TWA testing is between 100 and 120 beats/min (Kavesh et al. 1998).

In a series of two articles by Narayan and Smith (1999, 2000) where they studied the temporal distribution of TWA, it was also shown that the magnitude of TWA increases when the pacing length decreases (i.e., heart rate increases). However, the optimal combination of sensitivity and specificity was achieved in a pacing cycle length of 600 msec as opposed to 500 msec and 400 msec (Narayan and Smith 1999). It was also shown that TWA was more exaggerated during the deceleration of heart rate than during the acceleration of heart rate (Narayan and Smith 2000). Once again, the parametric methods were misused, which is evident in the non-normal distribution of the study variables.

A target heart rate of 115 beats/min showed the best predictive accuracy for malignant ventricular tachyarrhythmias in an exercise-based case-control study with 105 patients (Turitto et al. 2001). There is also evidence to the effect that the onset heart rate of TWA has prognostic value (Tanno et al. 2000, Kitamura et al. 2002). In a study by Kitamura and co-workers (2002), it was shown that an onset heart rate of under 100 beats/min has additional prognostic value in patients with dilated cardiomyopathy. Moreover, Tanno and others (2004) showed in an atrial pacing study that the incidence of VT, VF, or SCD is greater when TWA is present at lower heart rates.

There is only a limited amount of information available concerning the quantitative analysis of TWA with the spectral method. In 2005 Klingenheben and co-workers studied 204 patients with ischemic or non-ischemic cardiomyopathy. The alternas level (i.e. $V_{alt}$) was higher (10.8±10.0 [mean±SD] with the median value of 8.8 vs. 7.4±5.7 with the median value of 6.4,

p=0.05) in patients who suffered an arrhythmic event during a mean follow-up of 17 months. The number of the positive ECG leads ($V_{alt}$ ≥1.9μV) was also higher in patients with events. However, there was no survival analysis available.

## 6.2.2 The Modified Moving Average method

The MMA method for TWA analysis was developed by Nearing and Verrier (2002a) and commercialized by GE Healthcare Inc, Freiburg, Germany.

### *6.2.2.1 T-wave alternans measurement*

The goal for developing the MMA method was to make TWA analysis possible during routine clinical ECG monitoring (e.g., during rest, exercise, AECG, etc.; Nearing and Verrier 2002a) and, thus, without controlling heart rate or a need for specialized electrodes, but, at the same time, managing the noise.

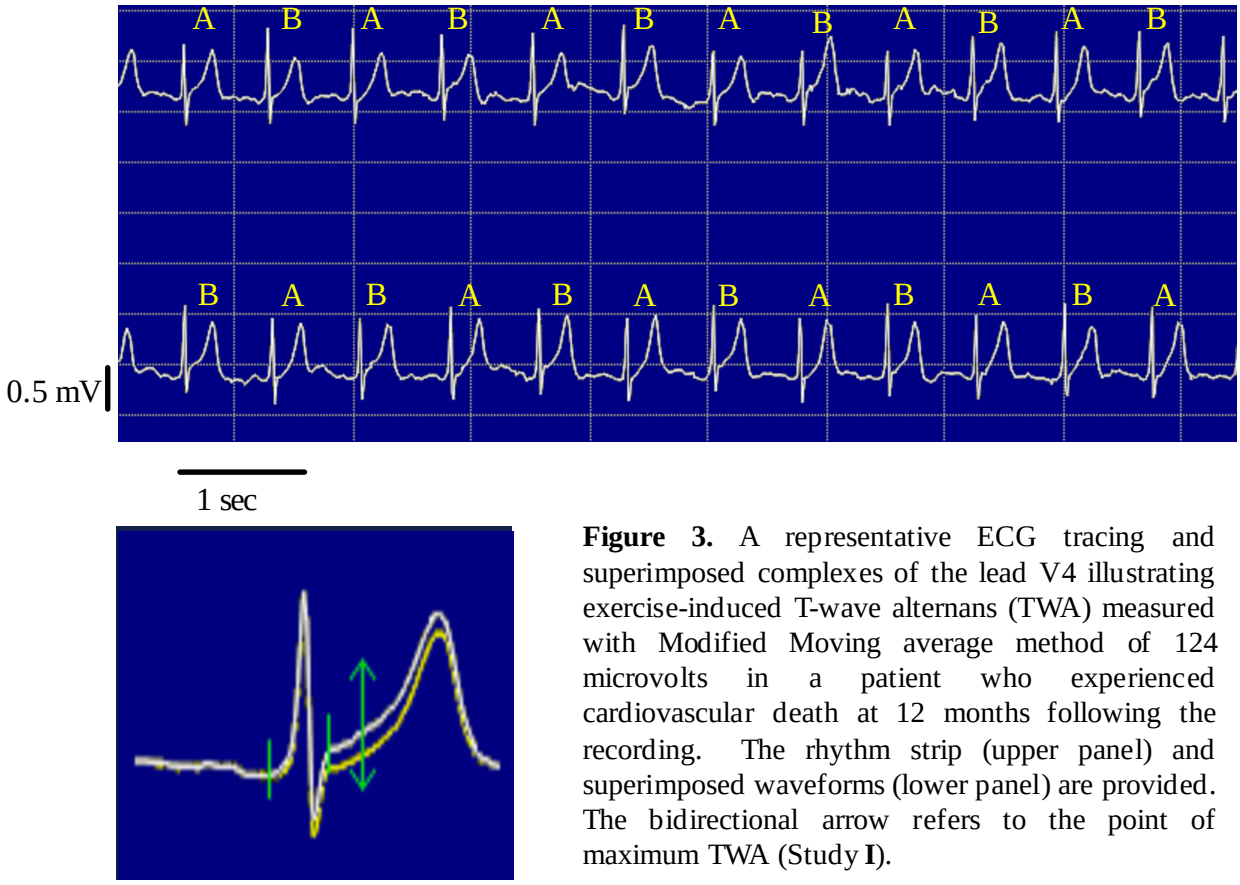



**Figure 3.** A representative ECG tracing and superimposed complexes of the lead V4 illustrating exercise-induced T-wave alternans (TWA) measured with Modified Moving average method of 124 microvolts in a patient who experienced cardiovascular death at 12 months following the recording. The rhythm strip (upper panel) and superimposed waveforms (lower panel) are provided. The bidirectional arrow refers to the point of maximum TWA (Study **I**).

The MMA algorithm separates the odd and even beats and then creates a median odd and even beat over the succeeding beats (Fig. 3). Therefore, if the coming ST-T segment is more positive than the present median beat, the following average beat will increase and vice versa. The median odd and even beats are updated continuously with an update factor of 1/8, 1/16, 1/32, or 1/64—the computed average beats thus illustrate the odd and even beats over the preceding 16, 32, 64, or 128 beats, respectively. However, because the MMA algorithm is modified rather than simple moving average analysis, it takes into account all the values from the beginning of the measurement period (i.e., from the onset of the exercise test or from the breakpoint caused by noise or other technical aspect). Nevertheless, the preceding 16, 32, 64, or 128 beats, as based on the specific update factor, are weighted and have thus more impact on the actual TWA value.

The actual TWA value is the maximum difference between the computed average odd and even beats over the ST segment and T wave, and it is given every 10 to 15 seconds.

#### *6.2.2.2 Noise with the Modified Moving Average method*

After the initial publication in 2002 by Nearing and Verrier (2002a), the management of noise with the MMA method has been further advanced by GE Healthcare (Kaiser W et al. 2004, Hostetler B et al. 2005).

Noise handling with the MMA method is essentially produced by a signal conditioning (Hostetler B et al. 2005). First, the baseline wander of the ECG is corrected by using a cubic spline (i.e., spline interpolation). A cubic spline is calculated over three succeeding beats from three different points of the isoelectric line of the QRS complexes. It removes baseline shift but has no negative effect on the TWA analysis. The signal is then filtered to reduce muscle artifacts with a 40-Hz low-pass filter. The last step before the separating of the odd and even beats is the detection and exclusion of noisy beats by analyzing the high and middle frequency content between the end of QRS complex and the end of the T wave. The exclusion of the beats is always made in pairs to maintain the odd and even balance (Kaiser W et al. 2004).

The calculation of the TWA value itself works as a nonlinear filter in handling high-frequency noise. The ST-T segment is divided in 20 ms pieces, and the minimal difference in each piece between the median odd and even beat is selected and stored. In the end, the maximum of the stored value is kept as the TWA value.

Lastly, the actual noise value is calculated as the intra-class variability. This is simply the average of the TWA value of the odd beats and the TWA value of the even beats, measuring the variability within the odd and the even beats. The noise value is then used in the calculation of the signal-to-noise ratio between the TWA value (inter-class variability) and noise value (intra-class variability; i.e., the TWA value divided by the noise value). The smaller the signal-to-noise ratio is, the less reliable is the TWA value. The noise ratio takes into account especially the artifacts caused by respiration, footfalls, or pedaling (Kaiser et al. 2004).

#### *6.2.2.3 Interpretation of the results*

The first clinical study using the MMA method in analyzing TWA by Verrier and co-workers (2003) with 44 post-MI patients in a case-control setting used 24-hour AECG monitoring in leads V1 and V5 with the update factor of 1/8. They discovered that a pre-specified cut-off point of >75$^{th}$ percentile at maximum heart rate (resulting in the cut-off points of 46.6 μV for V1 and 53.0 μV for V5) significantly predicted VF or arrhythmic death as an endpoint. Since the publication of this primary study, the predictivity of TWA as measured with the MMA method has been studied in several investigations (Cox et al. 2007, Exner et al. 2007, Nieminen et al. 2007, Stein et al. 2008, Maeda et al. 2009, Sakaki et al. 2009, Slawnych et al. 2009, Stein et al. 2010, Leino et al. 2011). However, there is no consensus concerning the criteria for the interpretation of the TWA test results measured with the MMA method in contrast to the spectral method as described earlier.

Because of the intrinsic flexibility of the MMA method that makes TWA analysis possible during fluctuating heart rates, most of the clinical studies with the MMA method have been carried out with 24-hour AECG monitoring in which TWA analysis with the spectral method is not possible. However, exercise-test-based data is also available (Exner et al. 2007, Nieminen et al. 2007, Slawnych et al. 2009, Leino et al. 2011).

In all of the prognostic clinical TWA studies with the MMA method, TWA values have been dichotomized with a few different cut-off values. However, it has also been speculated that the higher the TWA value, the higher the risk (Nearing and Verrier 2002a). The quantification of TWA would therefore contain additional risk stratification information, even though the data concerning the predictive power of quantitative TWA is limited. Nonetheless, TWA was found to predict cardiovascular mortality when analyzed as a continuous variable during the post-

exercise phase in coronary heart disease (CHD) patients (Slawnych et al. 2009). Moreover, a clear relationship was found between quintiles of the TWA voltage and mortality.

The cut-off point of ≥46 μV derived from the primary study by Verrier and others (2003) has also been shown to predict SCD (Stein et al. 2008). In case-control study with 138 post-MI patients with heart failure and/or diabetes with left ventricular dysfunction, Stein and colleagues (2008) achieved the best prediction for lead $V_3$ with the cut-off point of ≥46 μV, but for lead $V_1$, with the cut-off point of ≥42 μV. The cut-off point of ≥46 μV has also been shown to have prognostic power for all-cause and cardiovascular mortality as well as SCD in a population of patients referred for exercise testing. However, in a study with 1,037 participants by Nieminen and co-workers (2007), the cut-off point of ≥65 μV yielded the highest risk ratios in Cox regression analysis when the additional cut-off points of 46, 50, 60, and 70 μV were tested. In 2009 Slawnych, Nieminen, and others reported in their two-cohort post-exercise study with over one thousand CHD patients that the TWA cut-off points of 20 μV (high sensitivity) and 60 μV (high specificity) contain prognostic information. However, the cut-off points were identified by evaluating receiver operating characteristic (ROC) data that does not take into account the different follow-up times of each participant. A prior study by Exner and others (2007) found that the cut-off point of 5 μV contains prognostic information 10 to 14 weeks after MI, when TWA was analyzed during the post-exercise period. The low cut-off value may be due to the fact that the authors used the update factor of 1/16 and different noise handling criteria, as discussed later. However, in generating the optimal cut-off point, the ROC curves were again misused. Regardless of the fact that the cut-off point of ≥65 μV has been derived from an exercise-based TWA study, it has also been demonstrated to carry risk stratification information in 24-hour AECG-based studies (Maeda et al. 2009, Sakaki et al. 2009). Recently, Stein and co-workers (2010) studied 49 cases of SCD and 98 matched controls with AECG-based TWA and found that the cut-off point of >37 μV yielded the best separation of cases and controls for lead aVR and the cut-off point of >46 μV for lead V5. However, the results for lead V5 were not statistically significant. The cut-off point of 10.75 μV, which yielded the best prediction in the ROC analysis, has also been studied during pacing (Cox et al. 2007).

It is not known which ECG leads should be analyzed and which leads have the best prognostic power when TWA is analyzed with the MMA method. In the first exercise-based MMA-TWA study, all standard 12 ECG leads were used and the maximum value in any lead was selected for each patient (Nieminen et al. 2007). However, it has been shown in an experimental study with 61 dogs and 7 humans (Nearing et al. 1994) as well as a study with 95 patients undergoing coronary angioplasty (Martinez et al. 2006) that during ischemia TWA is

better detected in the precordial leads than the Frank orthogonal leads (Nearing et al. 1994) or limb leads (Martinez et al. 2006). However, in these studies TWA was not analyzed with the MMA method but with the complex modulation method (Nearing et al. 1994) and with an experimental method (Martinez et al. 2006). In a post-exercise TWA recording study with the MMA method, leads V1, V5, and Z have been used (Slawnych et al. 2009). In 24-hour AECG studies, the leads V1 and V5 have been mainly used, while V1 and V3 were used in one study (Stein et al. 2008). Interestingly, the risk was further increased when risk information for the leads V1 and V3 were combined with the optimal cut-off points in either lead, suggesting that the different leads should have different cut-off values. In a population-based case-control study by Stein and co-workers (2010), leads from two channels for ECG recordings were employed. The authors believed that they correspond to the leads V5 and aVR. However, they were unable to find anyone who could ensure the specific AECG hookup as the study enrollment had started as early as in 1989. TWA values above 37 μV for lead aVR were associated with SCD in conditional logistic regression analysis, but TWA values over 46 μV for lead V5 were not. In 2011, Leino and others evaluated the prognostic power of TWA separately in every precordial lead as well as in a selection of lead combinations with nearly 3,600 patients referred for exercise testing. Lead V5 was the only single lead that significantly predicted all-cause and cardiovascular mortality as well as SCD. In addition, lead V3 had prognostic significance for SCD. The hazard ratios for lead V5 were highly comparable to the results with the lead combinations (i.e., V1–V6; V2–V6; V3–V6; V4–V6; V5 and V6; V3–V5; V4 and V5), and the corresponding CIs (confidence interval) highly overlapped.

A certain issue that has to be validated in the future in TWA measurement with the MMA method, especially during the exercise, is the necessity of a heart rate limit. As one of the aims for developing the MMA method was to make TWA analysis possible during fluctuating heart rates, the need to limit the heart rate may not be necessary (Nearing and Verrier 2002a). In the study by Nieminen and co-workers (2007), the heart rate limit <125 beats/min was used over the entire exercise test from rest to recovery, based on the fact that the published experiences in TWA testing with the spectral method suggest that inaccuracies in TWA values may result when the heart rate exceeds this range (Bloomfield et al. 2002a). However, as discussed earlier, the data on the underlying reasons for this is sparse. In a post-exercise study in 2009, no heart rate limit was used, but the risk for cardiovascular death was, nevertheless, higher for those with elevated TWA values (Slawnych et al. 2009). The median heart rate when maximal TWA was observed was 76–93 beats/min regarding the specific study cohort.

In the context of exercise testing, TWA by the MMA method has been measured in the published literature from rest to recovery (i.e., over the entire test; Nieminen et al. 2007), during recovery only (Slawnych et al. 2009), or during the exercise phase only (Leino et al. 2011). It remains to be defined whether some part (i.e., rest, exercise, or recovery) of the test accumulates more risk information than others. In 2007, Exner and colleagues tested 322 post-MI patients with the spectral method during the exercise and with the MMA method during the post-exercise phase. The risk ratios for cardiovascular mortality or resuscitated cardiac arrest were highly comparable between the two phases, indicating that both contain potential risk stratification information. Because the spectral TWA analysis requires a specific exercise test protocol, the authors were unable to analyze TWA with both methods simultaneously during the exercise phase of the test.

The update factor of 1/8 has been used in most of the AECG studies (Verrier et al. 2003, Stein et al. 2008, Maeda et al. 2009, Sakaki et al. 2009, Stein et al. 2010) as well as the exercise-based TWA (Nieminen et al. 2007) and the post-exercise studies (Slawnych et al. 2009). However, the update factor of 1/32 has also been applied (Cox et al. 2007, Nieminen et al. 2007). Cox and others (2007) discovered that in 41 left ventricular dysfunction patients in whom TWA was measured with the MMA method during atrial or ventricular and atrial pacing, TWA did not significantly predict death or sustained ventricular arrhythmias. This may be due to the fact that the rapid update factor of 1/8 is more sensitive in detecting TWA than the less dynamic 1/32 but, on the other hand, also more vulnerable to noise and false positive tests. Similarly, the prediction of death was proved to be superior with the update factor of 1/8 in comparison to 1/32 in a population of patients undergoing a clinically indicated exercise test (Nieminen et al. 2007). However, the data concerning the results was not shown. In at least one study available the update factor of 1/16 has also been used (Exner et al. 2007).

The optimal noise limit for MMA-TWA analysis is not known. In a methodological study by Kaiser et al. (2004), the authors suggested that the TWA value is annotated with a question mark if the signal-to-noise ratio is less than three, the heart rate exceeds 125 beats/min, or too many noisy beats are excluded. They found that with these limits, the MMA algorithm has a sensitivity and specificity of circa 90% for the detection of true TWA when over 1,500 (1,426 exercise-based and 90 AECG-based) ECGs were analyzed. True TWA was confirmed by first analyzing the data with a highly sensitive threshold and then visually and manually confirming the TWA episodes. In 2002, Nearing and Verrier (2002a) used the SD of the TP segment in determining whether the beat was too noisy to be used with a predefined threshold value (typically 50 μV). Exner and others (2007) calculated the TWA value as a raw value minus the noise when the

signal-to-noise ratio exceeded >1.2. More recently, the noise threshold of >20μV has been applied, as measured during isoelectric segments (Maeda et al. 2009).

In their primary paper about the MMA method, Nearing and Verrier (2002a) visually reviewed the elevated TWA values for artifacts. However, the impact of over-reading on the prognostic significance of TWA is not known. In the primary exercise-based TWA study with the MMA method, the automatically derived TWA value was found to be a strong prognostic tool as discussed later (Nieminen et al. 2007). Moreover, the automatically derived TWA value has also been used during post-exercise TWA measurement (Slawnych et al. 2009). In the other clinical studies with the MMA-TWA method, the TWA values have been inspected visually.

### 6.2.3 Comparing the two methods

There are only a few studies to date where the spectral and the MMA method have been compared directly. In 2005, Hostetler and others found in their simulation study that TWA values derived from the MMA method are 3.23 times higher than the corresponding values derived with the spectral method. This may be due to the fact that the spectral method averages the amplitude over the 128 beats, whereas the MMA method uses the maximum TWA amplitude. Moreover, the MMA method was found to detect short run TWA that last only 20 to 30 beats in two actual stress ECG tracings, while the spectral method did not. However, in a stimulation study by Selvaraj and Chauhan in 2009, the MMA method was found to yield false measurements of TWA when noise was present, even when there was no true TWA present. When true TWA was present, the TWA values measured with the MMA method were consistently overestimated. On the other hand, the spectral method underestimated the simulated TWA. Similar results were found in AECG-based recordings from 18 normal subjects and 15 patients with congestive heart failure. It was also found that in stimulation, the signal-to-noise ratio of >1.2 eliminated the false TWA detection and thus improved the specificity. However, as the authors correctly stated in the limitations section, they did not compare the commercial application of the MMA method versus the spectral method in which the noise handling mechanisms have been extensively advanced after the publication of the algorithm (Nearing and Verrier 2002a). Therefore, the results have to be interpreted with caution.

TWA has been measured with both methods in two prognostic clinical studies (Cox et al. 2007, Exner et al. 2007). These are discussed in detail below.

## 6.3 Clinical studies on T-wave alternans

Clinical studies on TWA that have enrolled only patients with hypertrophic cardiomyopathy, Brugada syndrome, or long QT syndrome are beyond the topic of this dissertation. Hence, they are not included in this review of the literature. A consensus statement about the clinical utility of TWA as well as its physiological basis and the methods for analysis has been released in September 2011 (Verrier et al. 2011).

### 6.3.1 Pacing

In the first prognostic studies on TWA with follow-up and clinical endpoints, TWA was measured during atrial or atrio-ventricular pacing (Rosenbaum et al. 1994, Armoundas et al. 1998a, Armoundas et al. 1998b, Rashba et al. 2002b, Tanno et al. 2004, Narayan et al. 2005, Paz et al. 2006, Cantillon et al. 2007, Cox et al. 2007, Morin et al. 2007, Zacks et al. 2007, Sandhu et al. 2008). The studies with survival analysis considering TWA as a prognostic parameter are summarized in the Table 1. These studies have been conducted mainly with populations of patients referred for EPS. Therefore, the majority of the patients had a history of non-sustained VT or syncope without an explanation. Moreover, the latest studies have recruited only patients with reduced left LVEF, corresponding to the idea that TWA maybe helpful in guiding ICD therapy. The most common endpoint used in the studies has been a composite of SCD, sustained VT, VF, or appropriate ICD therapy (Table 1).

The association between TWA and the risk for ventricular arrhythmias in humans was first established in 1994, when Rosenbaum and others published the results of their prospective observational study with 66 patients. Of the 66 patients, 13 had an arrhythmic event during the median follow-up time of 20 months. The arrhythmia-free survival rate was 94 percent among patients without TWA, comparing with the 19 percent among patients with TWA ($p<0.001$). The univariate relative risk according to the Cox proportional hazard analysis was 9.0. No multivariable analysis was carried out. In 2007, Cantillon and co-workers followed 286 patients with left ventricular dysfunction prospectively and found that a non-negative TWA test predicts significantly arrhythmic events (hazard ratio 2.37, 95% CI 1.49–3.81, $p<0.01$) even when adjusted with other cardiac risk markers such as age, sex, QRS duration, LVEF, New York Heart Association functional classification (NYHA), etiology of cardiomyopathy (i.e., ischemic or non-ischemic), and subsequent ICD implantation. The 2-year arrhythmia free survival rates were 81% vs. 66% ($p<0.0001$ from the Kaplan-Meier survival analysis) for the TWA negative and

TWA non-negative groups, respectively. The authors concluded that the high event rate (19%) in the TWA negative group suggests that TWA may not be a sufficient marker for detecting low-risk individuals that may not benefit from ICD implantation. Interestingly, the EF ≤ 30% did not have prognostic value even in a univariate analysis. Morin and others (2007) discovered that positive TWA predicts arrhythmia-free survival (hazard ratio 1.64, p = 0.04) in patients with a narrow QRS complex (≤ 120ms), but not in patients with a wide QRS complex (hazard ratio 1.04, p = 0.91).

**Table 1.** Pacing-based T-wave alternans (TWA) studies with survival analysis.

| First author | Year | Test class. | Population type | N | Pros. | Primary End-point | Follow-up | HR | CI | p |
|---|---|---|---|---|---|---|---|---|---|---|
| Rosenbaum et al. | 1994 | N. vs P. only k score | Referred for EPS | 66 | Yes | SCD, VT or VF | 4.2 months | 9* | | <0.001* |
| Armoudas et al. | 1998 | N. vs P. only k score | Referred for EPS, no I or III drugs | 43 | No | SCD, VT or VF | 5.7 months | 2.77* | | <0.048* |
| Armoudas et al. | 1998 | N. vs P. only k score | Referred for EPS | 44 | No | SCD, VT or VF | 20 months | 10.50* | | <0.0008* |
| Rashba et al. | 2002 | N. vs P. | CHD, LVEF≤40%, referred for EPS | 178 | Yes | Death, ICD therapy, SVT or VF | 15 months | 1.1* | 0.6-1.9 | 0.8* |
| Tanno et al. | 2004 | N. vs P., >110beats/min | Referred for EPS | 248 | Yes | SCD, SVT, VF or ICD therapy | 45 months | | | 0.001 |
| Narayan et al. | 2005 | N. vs P. | CHD, LVEF≤45%, NSVT | 59 | Yes | Death, ICD therapy, SVT or VF | 4 months | | | 0.045* |
| Paz et al. | 2006 | N. vs non-N. | ICD | 25 | Yes | VT or VF | at least 6 months | | | 0.006* |
| Cantillon et al. | 2007 | N. vs non-N. | LVEF ≤35%, referred for EPS | 286 | Yes | Death, SVT, VF or ICD therapy | 38 months | 2.37 | 1.49-3.81 | <0.01 |
| Cox et al. | 2007 | N. vs P. | LVEF≤40%, referred for risk strafication | 41 | Yes | Death, ICD therapy, SVT or VF | 18 months | | | 0.016* |
| Cox** et al. | 2007 | Cut-off point of 10.75 μV | LVEF≤40%, referred for risk strafication | 41 | Yes | Death, ICD therapy, SVT or VF | 18 months | | | 0.061* |
| Morin et al. | 2007 | N. vs non-N. | CHD, LVEF≤40%, NSVT, Sinus rhythm | 386 | Yes | Death, SVT or VF | 40 months | 1.49 | | 0.048 |

* univariate analysis, ** TWA measured with the Modified Moving Averaged method. CHD=coronary heart disease; class.=classification; CI=confidence interval; EPS=electrophysiologic study; HR=hazard ratio; ICD= implantable cardioverter-defibrillator; LVEF=left ventricular ejection fraction; MI=myocardial infarction; N.=negative; NSVT=non-sustained ventricular tachycardia; P.=positive; Pros.=prospective; SCD=sudden cardiac death; SVT=sustained ventricular tachycardia; VF=ventricular fibrillation; VT= ventricular tachycardia

Rashba and others (2002b) compared the prognostic utility of atrial-pacing-induced and exercise-elicited TWA in a prospective study of 251 patients with CHD and LVEF≤ 40% who were referred for an electrophysiological study. Pacing-induced TWA did not predict arrhythmia-free survival in the univariate analysis (hazard ratio 1.1, 95% CI 0.6-1.9, p=0.8),

whereas exercise-induced TWA was a significant predictor of events in the univariate analysis (hazard ratio 2.2, 95% CI 1.1–4.7, p=0.03) and was also an independent predictor in multivariable analysis. The authors stated that TWA should be measured during the exercise rather than pacing. However, there was no direct comparison between the two methods, and even the CIs from the univariate analysis overlapped.

To date, there is only one follow-up study with a clinical end-point that has also measured TWA with the MMA method during pacing. Cox et al. found (2007) in their study with 41 patients with an LVEF≤ 40% that spectral TWA significantly separated patients with and without events in Kaplan-Meier analysis (p=0.016), whereas MMA-based TWA failed to reach significance (p=0.061). It seemed that the two methods may contain additional prognostic information due to the fact that when either of the tests was positive, there was slight improvement in statistical significance (p=0.014). However, because TWA was measured with software written by the authors rather than the commerlized versions of the two methods, it is possible that the noise handling, especially with the MMA algorithm, was not appropriate. Therefore, the results have to be interpreted with caution.

In 2008, Sandhu et al. reported that TWA measured during atrial pacing in patients referred for EPS correlated in 86% (50 out of 68) of patients with the intracardiac alternans (kappa value 0.60, 95% CI 0.31–0.81). The positive predictive values of TWA and intracardiac alternans for ICD-based ventricular arrhythmias were 17% and 14% at one year, respectively. However, no survival analysis was made.

### 6.3.2 Exercise testing

From the late 1990s onwards, TWA has been mainly measured during the exercise test, which allows TWA measurement non-invasively. Hohnloser et al. discovered in 1998 that exercise-based TWA predicted ventricular arrhythmias during a mean follow-up time of 15 months in a population of patients with ICD. Since then, exercise-based TWA has been shown to have great prognostic capacity in diverse patient populations. However, some contradictory results have also been published. Studies with multivariable prognostic analysis are summarized in Table 2.

As demonstrated in pacing-based TWA studies, Gold and others showed in 2000 that exercise-based TWA is also a strong prognostic marker for life-threatening ventricular arrhythmias in a population of patients undergoing diagnostic electrophysiological testing. In their study with 313 patients, the multivariable relative risk was 12.2 for a composite arrhythmic

end-point or all-cause mortality for patients with a positive TWA test ($p<0.0001$ for model chi-square). Moreover, the relative risk for those with a positive electrophysiological test was 3.0 ($p<0.0001$ for model chi-square). Therefore, elevated TWA is a strong risk marker in a population of patients with a history of sustained or non-sustained ventricular tachyarrhythmia or unexplained syncope. However, no CIs were given.

**Table 2.** Exercise-test-based T-wave alternans (TWA) studies with multivariable survival analysis. Please note that the average follow-up time is given in months.

| First author | Year | Method | Class. | Population type | N | Primary End-point | Follow -up | HR | CI | p |
|---|---|---|---|---|---|---|---|---|---|---|
| Groh et al. | 1999 | Spectral | N. vs P. | ICD at least 1 month | 44 | ICD therapy | 11 | | | NS |
| Ikeda et al. | 2000 | Spectral | N. vs P. | Post MI | 102 | SVT or VF | 13 | 6.5 | 0.7–62.2 | 0.11 |
| Gold et al. | 2000 | Spectral | N. vs P. | Referred for EPS | 313 | SCD, SVT, VF or ICD therapy | 10 | 12.2*** | | <0.0001 |
| Klingenheben et al. | 2000 | Spectral | N. vs P. | Congestive heart failure, LVEF≤45% | 107 | SCD, SVT or VF | 14 | ∞ | | 0.0036 |
| Adachi et al. | 2001 | Spectral | N. vs P. | Non-ishcemic dilated cardiomyop. | 82 | SCD, SVT or VF | 24 | | | NS |
| Tapanainen et al. | 2001 | Spectral | N. vs P. | Post AMI, <7days when enrolled | 379 | Death | 14 | | | NS |
| Sakabe et al. | 2001 | Spectral | N. vs P. | Dilated cardiomyop. | 34 | VT for ≥5s | 13 | | | 0.02 |
| Ikeda et al. | 2002 | Spectral | N. vs P. | TWA testing 2.7±5.4 months after MI | 834 | SCD or CA | 25 | 5.9 | 1.6–21.4 | 0.007 |
| Kitamura et al. | 2002 | Spectral | N. vs P:** | Non-ischemic dilated cardiomyop. | 83 | SCD, SVT or VF | 21 | | | <0.05 |
| Rashba et al. | 2002 | Spectral | N. vs P. | CHD, LVEF ≤40%, referred for EPS | 108 | Death or ICD therapy | 18 | | | <0.05 |
| Grimm et al. | 2003 | Spectral | N. vs P. | Idiopathic dilated cardiomyop., LVEF≤45% | 263 | SCD, SVT or VF | 52 | | | NS |
| Hohnloser et al. | 2003 | Spectral | N. vs P. | Dilated cardiomyop. | 137 | SCD, VF or unstable VT | 14 | | | 0.045 |
| Bloomfield et al. | 2004 | Spectral | N. vs non-N. | LVEF≤40% | 177 | Death | 20 | 4.7 | | 0.012 |
| Rashba et al. | 2004 | Spectral | N. vs P. | CHD, LVEF ≤40, indication for EPS | 144 | Death, SVT, VF or ICD therapy | 17 | | | <0.05 |
| Baravelli et al. | 2005 | Spectral | N. vs P. | NYHA II | 73 | SCD, SVT, VF or ICD therapy | 17 | | | 0.035 |

**Table 2.** (Continued.)

| First author | Year | Method | Class. | Population type | N | Primary End-point | Follow -up | HR | CI | p |
|---|---|---|---|---|---|---|---|---|---|---|
| Ikeda et al. | 2006 | Spectral | N. vs P. | Post MI, LVEF >40% | 1,041 | SCD, CA or VF | 32 | 15.8 | 4.2–59.1 | <0.0001 |
| Chow et al. | 2006 | Spectral | N. vs non-N. | Isch. cardiomyop, LVEF≤35% | 768 | Death | 18 | 2.24 | 1.34–3.75 | 0.002 |
| Bloomfield et al. | 2006 | Spectral | N. vs non-N. | LVEF ≤40% | 549 | Death, SVT, VF or ICD therapy | 20 | 6.3 | | <0.001 |
| Salerno-Uriarte et al. | 2007 | Spectral | N. vs non-N. | LVEF ≤40%, Non-isch. cardiomyop. | 446 | Cardiac death, VF, SVT or CA | 19 | 4.01 | 1.41–11.41 | 0.002 |
| Exner et al. | 2007 | Spectral | N. vs non-N. | LVEF ≤40% ≤48h or LVEF ≤50% >48h post MI | 322 | Cardiac death or CA | 47 | | | |
| | | | | Test 2-4 wks post MI | | | | 2.42 | 0.96–7.71 | 0.06 |
| | | | | Test 10-12 wks post MI | | | | 2.75 | 1.08–7.02 | 0.034 |
| | 2007 | MMA* | noise ratio | Test 2-4 wks post MI | | | | 2.09 | 0.95–4.60 | 0.067 |
| | | | ≥1.2; ≥5µV | Test 10-12 wks post MI | | | | 2.94 | 1.10–7.87 | 0.031 |
| Nieminen et al. | 2007 | MMA | ≥65µV | Referred for exercise testing | 1,037 | SCD | 44 | 7.4 | 2.8–19.4 | <0.001 |
| Chan et al. | 2008 | Spectral | N. vs non-N. | Isch. Cardiomyop., LVEF≤35%, NSVT | 768 | Death or ICD therapy | 18 | 2.37 | 1.47–3.84 | <0.001 |
| Gold et al. | 2008 | Spectral | N. vs non-N. | NYHA I, II or III and EF≤35% | 344 | SCD, SVT, VF or ICD therapy | 30 | 1.24 | 0.6–2.59 | 0.56 |
| Chow et al. | 2008 | Spectral | N. vs non-N. | Isch. cardiomyop., LVEF≤30% | 575 | SCD or ICD therapy | 25 | 1.16 | 0.68–1.99 | 0.58 |
| Huikuri et al. | 2009 | Spectral | N. vs P. | 6 wks post AMI, LVEF ≤40% | 212 | Fatal or near-fatal cardiac arrhyth. | 24 | | | NS |
| Slawnych et al. | 2009 | MMA* | Per 5µV | CHD patients from Exner et al and Nieminen et al | 1,003 | Cardiac death | 48 | 1.05 | 1.02–1.09 | 0.004 |
| Leino et al. | 2011 | MMA | Per 20µV | Referred for exercise testing | 3,598 | Cardiovascular death | 55 | 1.5 | 1.13–1.95 | 0.005 |

* post-exercise based analysis; ** onset heart rate for TWA analysis <100 beats/min; *** hazard ratio for primary end point + all-cause mortality (p-value for model chi-square). AMI=acute myocardial infarction; CA=cardiac arrest; Cardiomyop.=cardiomyopathy; CHD=coronary heart disease; Class.=classification; CI=confidence interval; EPS=elecrophysiologic study; h=hours; HR=hazard ratio; ICD= implantable cardioverter-defibrillator; Isch.=ischemic; LVEF=left ventricular ejection fraction; MI=myocardial infarction; MMA=Modified Moving Average; SCD=sudden cardiac death; SVT=sustained ventricular tachycardia; VF=ventricular fibrillation; VT=ventricular tachycardia, wks=weeks

### *6.3.2.1 Patients with dilated cardiomyopathy*

In patients with dilated or non-ischemic cardiomyopathy, the predictivity of TWA has been studied in several investigations (Adachi et al. 2001, Sakabe et al. 2001a, Sakabe et al. 2001b, Kitamura et al. 2002, Grimm et al. 2003, Hohnloser et al. 2003b, Baravelli et al. 2007), including

one with nearly 500 patients (Salerno-Uriarte et al. 2007). A positive or abnormal TWA test has also been shown to yield risk stratification information in this high-risk population (Table 2). In 2007, Salerno-Uriarte and colleagues studied 446 patients with non-ischemic cardiomyopathy (LVEF ≤40%) and found in their multicenter study that patients with abnormal TWA test results have a four-fold higher risk for cardiac death and life-threatening arrhythmias during a median follow-up time of 19 months, and they also confirmed the finding by Hohnloser and co-workers (2003b) that a positive TWA test was the only significant predictor of arrhythmia- free survival during a mean follow-up time of 14 months. However, the results of the Marburg Cardiomyopathy Study suggest that in a longer mean follow-up time of 52 months, TWA has no prognostic value for patients with dilated cardiomyopathy and a LVEF≤45% (Grimm et al. 2003). Nevertheless, an indeterminate test was found to predict arrhythmia-free survival during follow-up in univariate analysis, but it had no prognostic value in a multivariable analysis.

#### *6.3.2.2 Post myocardial infarction patients*

A certain patient population in which TWA has been extensively studied are post-MI patients (Ikeda et al. 2000, Tapanainen et al. 2001, Ikeda et al. 2002, Hohnloser et al. 2003a, Ikeda et al. 2006, Exner et al. 2007, Huikuri et al. 2009). The data available suggest that TWA contains risk stratification information for this wide range of patients. However, the timing of the TWA testing after the MI seems essential.

Ikeda and others studied 102 post-MI patients in 2000. TWA was measured with the spectral method 7–30 days (with the mean value of 20) after acute MI. A positive TWA test was a significant predictor of spontaneous sustained ventricular arrhythmia in univariate analysis, but failed to reach significance in multivariable analysis. However, when combined with late potentials, TWA was a highly significant prognostic marker in multivariable analysis as well (hazard ratio 19.9, 95% CI 3.2–125.3, p=0.001). The results concerning the prognostic capacity of the late potentials alone in multivariate analysis were not given. The wide CI is presumably caused by the small amount of study participants. Moreover, there were only 15 patients with a primary endpoint; 8 of these tested positive for both TWA and late potentials. The total number of patients with both markers abnormal was 16, indicating that the combination of late potentials and TWA is a marker of elevated risk but that a negative finding does not ensure a good prognosis. Tapanainen and co-workers (2001) studied 379 patients after an acute MI and discovered that TWA was not predictive when measured 5–21 days after the MI. Nonetheless, an

incomplete TWA test (i.e., unable to exercise or reach the target heart rate >105 beats/min for one minute) was a significant risk marker for all-cause mortality during 14 months of follow-up (hazard ratio 9.28, 95% CI 1.99–43.30, p<0.01).

The finding that TWA testing early after an acute MI is not prognostic was confirmed in the Noninvasive Risk Assessment Early After a Myocardial Infarction (REFINE; Exner et al. 2007) and Cardiac Arrhythmias and Risk Stratification After Acute Myocardial Infarction (CARISMA; Huikuri et al. 2009) studies. In CARISMA, TWA was analyzed separately during exercise and pacing+exercise (i.e., TWA was considered positive when it was positive during pacing or exercise). Nevertheless, it did not have predictivity in either way. TWA testing was performed 2–4 weeks and 6 weeks after the acute MI in REFINE and CARISMA, respectively. However, when TWA testing was carried out 10–14 weeks after the AMI in REFINE, it was found to predict cardiac death or cardiac arrest over a 47-month follow-up period when analyzed during exercise with the spectral method (hazard ratio 2.75, 95% CI 1.08–7.02, p=0.034) and also when analyzed during post-exercise with the MMA method (hazard ratio 2.94, 95% CI 1.10–7.87, p=0.031) in patients with reduced left ventricular function.

Ikeda and colleagues (2002) studied 850 post-MI patients, 82% of whom had an LVEF ≥40% with a mean follow-up of 26 months. TWA was measured 2.7 months (mean value) after the MI, and a positive TWA test yielded a hazard ratio of 5.9 (95% CI 1.6–21.4, p=0.007) for SCD or arrest. In 2006 Ikeda and others also found that TWA testing was prognostic for post-MI patients with preserved cardiac function (LVEF ≥40%) when tested earlier after a MI. The mean time for TWA testing was 48 days with a SD of 66 days. However, the large SDs suggests that the time was not normally distributed and, moreover, that a large proportion of the tests were performed somewhat later rather than early after the MI. Nevertheless, a positive TWA test had the most significant hazard ratio in the multivariable analysis (hazard ratio 15.8, 95% CI 4.2–59.1, p<0.0001).

#### *6.3.2.3 Patients with reduced left ventricular function*

In the brief history of clinical TWA studies, the greatest interest has involved the question whether TWA might help in guiding ICD implantation. Therefore, numerous studies have been conducted with patient populations suffering from reduced left ventricular function. These include patients with dilated cardiomyopathies, as discussed earlier, as well as patients with ischemic cardiomyopathies or congestive heart failure.

In 2000, Klingenheben and others published the finding that a positive TWA test was the only significant predictor (p=0.0036) of arrhythmia-free survival in 107 patients with congestive heart failure and LVEF≤45% and, moreover, ejection fraction, non-sustained VT, baroreflex sensitivity, or signal-averaged ECG (SAECG) did not have independent predictive value. Bloomfield and co-workers (2004) studied 177 MADIT II (i.e., prior MI and LVEF≤30%) type patients to see whether TWA and QRS duration identify patients at a high or low risk among those who met the criteria for ICD prophylaxis. An abnormal TWA test yielded a hazard ratio of 4.7 (p=0.012) for all-cause mortality, while QRS duration did not significantly add to the prognostic ability of TWA. Moreover, the 2-year mortality rates were significantly greater in patients with abnormal TWA test results (17.8%) than in those with normal TWA (3.8%, p=0.02). The full cohort of this study contained 549 patients with LVEF≤40%, and in 2006 Bloomfield et al. published that an abnormal TWA test result was associated with increased risk for mortality and sustained ventricular arrhythmias (hazard ratio 6.3, p<0.001) also in this expanded database. The 2-year actuarial event rates were 15.0% and 2.5% for patients with abnormal TWA and normal TWA test results, respectively, indicating that TWA may also be helpful in identifying not only high-risk patients but also those at a low risk for an arrhythmic event.

In a study by Chow and others in 2006, 768 consecutive patients with ischemic cardiomyopathy and LVEF≤35% were followed for a mean value of 18 months. An abnormal TWA test was found to be an independent predictor of death from any cause (hazard ratio 2.24, 95% CI 1.34–3.75, p=0.002). In 2007, another study from the same cohort with a longer follow-up (mean value of 27 months) compared the mortality benefits separately for non-negative TWA patients (67%) and TWA negative patients (33%; Chow et al. 2007a). Multivariable analysis showed that ICDs were associated with significantly reduced all-cause mortality in the non-negative TWA group (hazard ratio 0.45, 95% CI 0.27–0.76, p=0.003) but not in the negative TWA group (hazard ratio 0.85, 95% CI 0.33–2.20, p=0.73). In a third publication with the same cohort, it was found that an abnormal TWA test predicts mortality in patients without ICD (hazard ratio 2.27, 95% CI 1.22–4.24, p=0.01), in addition to predicting mortality and ICD shocks in patients with ICD (hazard ratio 2.42, 95% CI 1.07–5.41, p=0.04; Chow et al. 2007b). Moreover, in the fourth publication on the same cohort, it was discovered that an abnormal TWA test result reliably predicts mortality beyond one year and thus throughout a 2–3-year follow up (Chan et al. 2008).

In 2008, the results from two large prospective multicenter clinical trials concerning the predictivity of TWA were published (Chow et al. 2008, Gold et al. 2008). The Microvolt T-wave

Alternans Testing for Risk Stratification of Post-myocardial Infarction Patients patients (MASTER) trial enrolled 575 patients with prior MI and LVEF≤30% who met the MADIT II criteria for ICD implantation at 50 centers in the United States (Chow et al. 2008). The primary end-point was SCD or appropriate ICD therapy. The annual event rates were 6.3% and 5.0% for TWA non-negative and negative patients, respectively, with the univariate hazard ratio of 1.26 (95% CI 0.76–2.09, p=0.37). There were only seven SCDs during the mean follow-up of 2.1 years, in comparison to 63 ICD shocks. In secondary multivariable analysis, abnormal TWA was predictive for all-cause mortality, yielding a hazard ratio of 2.16 (95% CI 1.13–3.78, p=0.02). The SCD in Heart Failure Trial (SCD-Heft) was a multicenter clinical trial in which 2,521 patients were randomized to receive ICD, placebo, or amiodarone (Bardy et al. 2005). The T-wave Alternans SCD-Heft Substudy evaluated 490 patients with LVEF≤35% and NYHA I, II, or III class symptoms who underwent TWA testing at the time of inclusion in the main trial (Gold et al. 2008). The primary analysis was defined prospectively to exclude the patients from the amiodarone arm, because amiodarone seems to reduce the prevalence of TWA (Groh et al. 1999). The primary end-point was SCD, sustained VT, VF, or ICD discharge. The event-free survival did not differ between the TWA positive and negative patients (hazard ratio 1.24 95% CI 0.60–2.59, p=0.56) or, similarly, between the TWA non-negative and negative patients (hazard ratio 1.28 95% CI 0.65–2.53, p=0.46). Nonetheless, it was discussed by Rosenbaum (2008) in the editorial that, actually, the event-free survival started to differ between the TWA positive and negative patients after approximately 20 months of follow-up. Moreover, that was the same point in time where the survival benefit from the ICD began to be evident in the primary publication.

The Alternans Before Cardioverter Debrillator (ABCD) trial was a multicenter non-inferiority study that compared an electrophysiological examination and TWA in guiding ICD insertion (Costantini et al. 2009). The ABCD conducted 566 patients with LVEF≤40% due an ischemic cause and a history of non-sustained VT. ICD implantation was mandated if either TWA or EPS was positive. In other cases (i.e., both TWA and EPS were negative or TWA was indeterminate and EPS negative), the decision regarding ICD implantation was left to the discretion of the investigators. The positive predictive value for the TWA-directed patients at one-year was 9.5% and for the EPS-directed patients 11.1% for SCD or ICD discharge. The pre-described non-inferiority limit was 10% and, therefore, the TWA strategy was comparable to the invasive risk stratification strategy. The sensitivity of the TWA-directed strategy was 74%, with a specificity of 44%, whereas the EPS-directed strategy yielded a sensitivity and specificity of 62% at 1 year.

In 2009, Hohnloser and others made an interesting finding in their meta-analysis that evaluated prospective clinical trials in primary prevention patients. They tested the hypothesis that TWA is predictive for ventricular arrhythmias in primary prevention patients without ICDs but that is not predictive for ICD therapy in such patients with ICD. They discovered that in a total of 3,682 patients from the studies where none or only a small fraction (≤15%) of the reported endpoints were ICD therapies (low ICD group), the hazard ratio for TWA non-negative versus negative patients was 13.6 (95% CI 8.5–30.4). In contrast, the hazard ratio of TWA non-negative versus TWA negative patients was only 1.6 (95% CI 1.2–2.1) in a total of 2,234 patients in studies where ICD therapy constituted more than 15% of the endpoints (high ICD group). Moreover, the annual event rates were 0.3% (95% CI 0.1%–0.5%) and 5.4% (95% CI 4.1%–6.7%) for the low and high ICD groups, respectively. This finding may be due to the fact that ICD therapy is not a surrogate for SCD (Connolly 2006). ICD seems to detect and treat arrhythmias that might be self-terminating without treatment and, moreover, ICDs are thought to be arrhythmogenic in themselves (Germano et al. 2006).

Chan and others published another interesting meta-analysis in 2010. They found nine prospective TWA studies with primary prevention patients suffering from left ventricular dysfunction. The main finding of the study with 3,939 patients was that the prognostic capacity of TWA for ventricular arrhythmic events varied widely based on whether the beta-blocking therapy was withheld 24 hours before the TWA testing or not. The overall pooled relative risk for abnormal TWA was 1.95 (95% CI 1.29–2.96; p=0.002) for arrhythmic events. Moreover, the pooled relative risk was 5.39 (95% CI 2.68–10.84, p<0.001) in the 4 studies in which beta-blocker therapy was not withheld prior to TWA testing and 1.40 (95% CI 1.06–1.84, p=0.02) in the five studies where the use of beta-blocker therapy was withheld prior to TWA measurement. This interesting finding may be caused by the fact that the administration of beta-blocking agents significantly reduces TWA levels (Klingenheben et al. 2001, Rashba et al. 2002a) and, in contrast, withholding the beta-adrenergic stimulation before the exercise testing might lead to a false positive finding. On the other hand, a positive TWA finding seems to be more arrhythmogenic when beta-blocking agents are not withheld prior the TWA testing. However, prospective studies are certainly needed to resolve this important issue.

#### *6.3.2.4 Patients referred for exercise testing*

The great majority of the clinical studies on TWA have been conducted in high-risk populations (Table 2). Nonetheless, Ikeda and others showed in 2006 that TWA also has prognostic power for post-MI patients with preserved cardiac function, as discussed earlier. Moreover, Nieminen and colleagues (2007) studied 1,037 patients referred for clinical exercise stress testing. In this lower-risk population (annual mortality rate of 1.6%), elevated TWA was found to be a strong prognostic marker for SCD (hazard ratio 7.4 95% CI 2.8–19.4, $p<0.001$) as well as for cardiovascular mortality (hazard ratio 6.0 95% CI 2.8–12.8, $p<0.001$) and total mortality (hazard ratio 3.3 95% CI 1.8–6.3, $p=0.001$) during a mean follow-up time of 44 months. Interestingly, the patients with atrial fibrillation (AF) or flutter were not excluded from the analyses. The authors stated that the MMA method does not hinder TWA assessment during the AF or flutter. With the spectral method, TWA analysis is possible only during sinus rhythm, and patients with AF or flutter have therefore been excluded from the clinical TWA studies using the spectral method.

Recently, Leino and others (2011) studied 3,598 patients referred for exercise testing. They evaluated the prognostic capacity of TWA measured with the MMA method during the exercise phase separately for different leads and lead combinations. The adjusted hazard ratio for each 20-μV increase in TWA, when analyzed from all precordial leads, was 1.49 (95% CI 1.13–1.95, $p=0.005$) for cardiovascular mortality and 1.25 (95% CI 1.02–1.52, $p=0.026$) for all-cause mortality. Lead V5 was the only single lead that significantly predicted all-cause and cardiovascular mortality as well as SCD. The hazard ratios of TWA analyzed for lead V5 and for the lead combinations (i.e., V2–V6, V3–V6, V4–V6, V5 and V6, V3–V5, and V4 and V5) were highly comparable to the results of TWA analyzed in all the precordial leads.

### 6.3.3 Ambulatory ECG-based T-wave alternans analysis

The development of the MMA method for detecting TWA has allowed TWA measurement during 24-hour AECG tracings. To date, a few clinical studies have evaluated the associating between AECG-based TWA and the risk for ventricular arrhythmias during follow-up (Verrier et al. 2003, Stein et al. 2008, Maeda et al. 2009, Sakaki et al. 2009, Stein et al. 2010; Table 3).

In 2003, Verrier and others published for the first time that AECG-based TWA contains risk stratification information. In their case control study with 15 cases with cardiac arrest due to documented VF or arrhythmic death and 29 controls, AECG recordings were carried out a mean

of 15 days after an acute MI. Elevated TWA (a pre-specified cut-off point of >75$^{th}$ percentile) was found to be a significant prognostic marker when measured during the maximum heart rate (hazard ratio 4.6 95% CI 1.1–18.7, p=0.04 in lead V1, and hazard ratio 55.3 95% CI 4.3–713.3, p=0.002 in lead V5) or at 8.00 a.m. (hazard ratio 4.9 95% CI 1.2–20.8, p=0.03 in lead V1, and hazard ratio 5.3 95% CI 1.2–23.9, p=0.03 in lead V5) but did not predict arrhythmic death or VF when measured at maximum ST-level deviation. The first prospective AECG-based TWA study was conducted by Sakaki and others in 2009. They studied 295 patients referred for AECG monitoring with an LVEF≤40%. Elevated TWA (≥65μV) predicted cardiac mortality highly significantly in multivariable analysis, yielding a hazard ratio of 17.1 (95% CI 6.3–46.6, p<0.0001).

**Table 3.** Ambulatory electrocardiogram (AECG)-based T-wave alternans studies with clinical end-points. Please note that TWA was measured with the Modified Moving Average method in all the studies.

| First author | Year | Type of study | Test classification | Population type | N | Primary end-point | Follow-up | HR | CI | p |
|---|---|---|---|---|---|---|---|---|---|---|
| Verrier et al. | 2003 | Case-control | ≥46.6μV in V1 at max heart rate | Post AMI | 44 | Arrhythmic death or VF | 21 months | 4.6 | 1.1–18.7 | p=0.04 |
| Stein et al. | 2008 | Case-control | ≥46μV in V1 or V3 | LVEF≤40%, post AMI | 138 | SCD | Not known | 7.1* | 2.7–18.3 | p<0.001 |
| Sakaki et al. | 2009 | Prospective follow-up | ≥65μV in V1 or V5 | LVEF≤40%, referred for AECG | 295 | Cardiac death or ICD therapy | 13 months | 17.1 | 6.3–46.6 | p<0.0001 |
| Maeda et al. | 2009 | Case-control | ≥65μV in V1 or V5 | Referred for AECG | 63 | History of MI and SVT | 72 months | 4.9 | 1.2–19.6 | p<0.05 |
| Stein et al. | 2010 | Case-control | ≥37μV in channel 2 (aVR) | ≥65 years | 147 | SCD | 14 years | 4.8 | 1.5–15.8 | p=0.009 |

* univariate analysis. AMI=acute myocardial infarction; CI=confidence interval; HR=hazard ratio; ICD=intracardiac defibrillator; LVEF=left ventricular ejection fraction; MI=myocardial infarction; SCD=sudden cardiac death; SVT=sustained ventricular tachycardia; VF=ventricular fibrillation

## 6.3.4 T-wave alternans precedes ventricular arrhythmias in humans

In 2006, Shusterman and colleagues studied 42 patients with sustained VT in AECG. TWA was measured with MMA and intrabeat average analysis as well as with the spectral method. TWA was found to increase before the arrhythmia and reached the peak value 10 minutes before the onset of VT (23.6±11.7μV, p=0.007 as compared to the mean value 60 to 120 minutes before the VT). However, no follow-up data was available.

Recently, a few studies have evaluated TWA from ICD electrograms, and the results have shown that TWA levels increase before VT or VF (Swerdlow et al. 2008, Kim et al. 2009, Swerdlow et al. 2011).

Because the stored ICD electrograms contain only a 10–20-beat rhythm strip before the onset of VT or VF, TWA measurement is not possible with either the spectral or the MMA method. In 2008, Swerdlow and co-workers published a simple average method for TWA analysis from intracardiac electrograms and found in their retrospective study with 10 patients suffering from ischemic or dilated cardiomyopathy that TWA values before the VT or VF (83±67μV) were significantly ($p<0.0001$) higher than in control electrograms during atrial pacing (12±18μV) or sinus rhythm (15±12μV). The control data was available for six out of ten patients. Kim and colleagues (2009) studied 74 patients with ICD implantation and showed that TWA magnitudes were significantly higher before spontaneous VT than immediately after inappropriate ICD therapy.

The first prospective study on ICD-based TWA was published in 2011, when Swerdlow and others conducted a multicenter investigation with 63 ICD patients. During a follow-up time of 6 months, there were 166 episodes of VT or VF in 28 patients. TWA was greater before VT or VF (62.9±3.1μV) than during baseline rhythm in 62 patients (12.8±1.8 μV, $p<0.0001$), during rapid pacing in 52 patients (14.5±2.0 μV, $p<0.0001$), before supraventricular tachycardia in 9 patients (27.5±6.1μV, $p<0.0001$), or during 16 time-matched ambulatory control patients (12.3±3.5μV, $p<0.0001$). Moreover, the area under the ROC curve was 0.818 as unadjusted and 0.916 when adjusted with multiple VT or VF episodes within a patient, showing that TWA is effective in discriminating patients with preonset VT or VF and controls.

## 6.4 Other non-invasine risk markers for sudden cardiac death

Cardiovascular diseases are a major public health challenge and the most common cause of death in developed countries (Myerburg et al. 1993, Myerburg et al. 1997). In 2009 there were almost 50,000 deaths in Finland; approximately 40% of those were classified as cardiovascular deaths and, furthermore, two thirds of them were cardiac deaths (http://www.stat.fi/til/ksyyt/2009/01/ksyyt_2009_01_2011-02-22_tau_001_fi.html, referred 31 May 2011). Moreover, 50% of cardiac deaths have been estimated to be sudden (Myerburg et al. 1993, Zipes and Wellens 1998, Huikuri et al.2001), with values of more than 60% reported in some studies (Zheng et al. 2001), yielding circa 6,000 to 8,000 SCDs in Finland every year. The incidence is higher in high-risk groups, such as patients with prior MI or low LVEF. However, the greatest number, approximately 300,000 a year in the Unites States, occurs in the general population of patients with no known risk factors (Myerburg et al. 1998). Moreover, it has been

suggested that approximately 80% of all SCDs are principally caused by fatal ventricular tachyarrhythmias originating from a myocardial scar or acute plaque destabilization (i.e., CHD), and that 10%–15% are due to dilated and hypertrophic cardiomyopathies and 5% due to uncommon causes such as genetic ion-channel abnormalities (Huikuri et al. 2001). The identification of patients at risk for SCD and thus the possibility to reduce the number of these events has a great potential not only to reduce the cardiovascular mortality and morbidity but also the effect on national economies (Goldberger et al. 2011).

**Table 4.** Selection of noninvasine risk markers for sudden cardiac death

| Resting ECG | Exercise | AECG | Others |
|---|---|---|---|
| QRS duration | METs | Ventricular ectopy | LVEF |
| PR interval | HRR | NSVT | NYHA class |
| QT dispersion | ST-level | HRT | Baroreceptor sensitivity |
| SAECG | Recovery ventricular ectopy | HRV | Positive family anamnesis |
| QT interval | RPP | Deceleration capacity | Genetic testing |
| Short term HRV | ST/HR index | | |
| ST level | ST/HR hysteresis | | |
| Q waves | ST slope | | |
| T wave changes | ST integral | | |
| QT variability | Blood pressure decrease | | |

HR=heart rate; HRR=heart rate recovery; HRT=heart rate turbulence; HRV=heart rate variability; LVEF=left ventrivular ejection fraction; METs=metabolic equivalents; NYHA= New York Heart Association functional classification; NSVT=non-sustained ventricular tachycardia; RPP=rate pressure product; SAECG=signal averaged electrocardiogram

During the last few decades, a vast number of studies have been conducted in the field of noninvasive SCD risk stratification. There are a few promising ECG, AECG, and exercise test risk stratification techniques, including TWA, and numerous others have been studied (Table 4). However, only LVEF is widely accepted in clinical use, because its usability in guiding therapy has been demonstrated. The focus of this dissertation is on TWA and, therefore, the next few section discuss only briefly the current evidence regarding LVEF, a selection of resting ECG and AECG-based techniques, as well as exercise-test-derived risk stratifiers.

### 6.4.1 Left ventricular ejection fraction

LVEF is used as a measure of systolic pump function of the left ventricle. It is measured most commonly with 2-dimensional echocardiography but can be also determined with, for example, ventriculography during angiography, with radioisotopetechniques, or with cardiac magnetic

resonance imaging. The association between low LVEF and outcome was first observed in the 1980s in patients with a prior MI (Sanz et al. 1982, Bigger et al. 1984). In the late 1990s and early 2000s, the results from multicenter randomized clinical trials showed that in patients with reduced LVEF (≤30% or ≤35%), the prophylactic ICD reduces mortality as compared to conventional medical therapy (Moss et al. 1996, Moss et al. 2002, Kadish et al. 2004, Bardy et al. 2005). Therefore, the merits of LVEF as a prognostic tool are well recognized. However, results showing no benefit from ICD implantation in selected patients with reduced LVEF—such as those with low heart rate variability (HRV) or a resting heart rate of more than 80 beats/min (Hohnloser et al. 2004), or patients with positive SAECG test results (Bigger 1997)—have also been reported. In 2009, an interesting meta-analysis showed no benefit from ICD implantation for women with reduced LVEF (hazard ratio for total mortality of 1.01, 95% CI 0.76–1.33, p=0.95; Ghanbari et al. 2009). Hence, studies concentrating specifically on ICD implantation in women are needed, because thousands of ICDs are being implanted every year in women with low LVEF without evidence. Moreover, the majority of SCDs occur in a population of patients with more preserved LVEF (>40%), indicating that the sensitivity of LVEF for SCD is only moderate. Therefore, other risk stratification techniques are needed.

## 6.4.2 Resting-ECG-based techniques

### *6.4.2.1 QRS duration*

The duration of ventricular activation can be easily measured from the standard 12-lead ECG. A prolongation in QRS duration is caused by either intra- or interventricular conduction delay. QRS duration is thought to be a marker of more advanced myocardial disease, but it may also have an independent effect on mortality, as dyssynchronous ventricular activation may lead to a reduced cardiac function (Park et al. 1985). Prolonged QRS duration (≥120ms) has been shown to predict mortality as well as SCD in population-based studies (Baldasseroni et al. 2002, Iuliano et al. 2002) as well as in substudies of ICD trials (Zimetbaum et al. 2004, Bardy et al. 2005). More specifically, left ventricular bundle branch block and non-specific interventricular conduction delay, but not right bundle branch block, have been linked to increased risk (Zimetbaum et al. 2004). However, results showing no associating between QRS duration and outcome also exist (Hofmann et al. 1988, Greenberg et al. 2004, Kadish et al. 2004, Huikuri et al. 2009).

#### *6.4.2.2 QT interval and dispersion*

A prolonged QT interval as a measure of ventricular APD in patients with no genetically confirmed long QT syndrome has yielded mixed results in studies evaluating its risk for mortality or SCD (Goldberger et al. 2008). Moreover, inter-observer and intra-observer variability reduce the reproducibility of QT interval assessment. QT dispersion is another cardiac repolarization marker evaluated from the surface ECG as a maximum difference between the QT intervals of different ECG leads (Malik and Batchvarov 2000). It has been linked to increased mortality and arrhythmia risk (Pinsky et al. 1997, Malik and Batchvarov 2000, Huikuri et al. 2009), but a multitude of negative studies are also available (Brendorp et al. 2001, Sakabe et al. 2001a). QT dispersion is a measure of abnormalities during myocardial repolarization. It is highly dependent on the quality of the ECG tracing and the operator, and the reproducibility of the test is poor (Malik 2000). Moreover, the pathophysiological mechanisms behind it are only loosely understood, and QT dispersion as an independent ECG phenomenon has been questioned (Malik 2000).

#### *6.4.2.3 Signal Averaged ECG*

SAECG refers to time-domain based ECG techniques to reduce noise and thus to allow the measurement of late potentials from surface ECG. SAECG recording requires a few minutes of high-quality ECG measuring (i.e., free of noise and artifacts) to average multiple QRS complexes. Late potentials indicate the low amplitude signals that occur after the end of the QRS complex, and they are thought to refer to delayed or prolonged activation of some regions of the ventricles and, moreover, are thought to be substrates for re-entry (El-Sherif et al. 1990). Three time-domain measures describing late potentials are generally defined (i.e., QRS duration, low-amplitude signal duration, and root mean square voltage of the terminal 40 ms of the QRS complex; Goldberger et al. 2008).

SAECG has been associated with an increased risk for mortality and arrhythmic events (Gold et al. 2000, Gomes et al. 2001). The CABG (coronary artery bypass graft) Patch trial randomized patients undergoing CABG surgery with positive SAECG to receive or not to receive ICD (Bigger 1997, Bigger et al. 1999). The rate of arrhythmic events was reduced in the ICD group. However, the rate of all-cause mortality did not significantly differ between the two groups. In CARISMA, a QRS width of ≥120 ms, but not the signal duration or root mean square voltage, was associated with increased risk for fatal or near fatal cardiac arrhythmia (Huikuri et al. 2009).

Hence, further studies are suggested before the possible clinical use of SAECG (Goldberger et al. 2008).

## 6.4.3 Ambulatory ECG-based techniques

### *6.4.3.1 Ventricular ectopy and non-sustained ventricular tachycardia*

Non-sustained VT (i.e., VT≤30s) and the presence of VPBs (≥10 per hour) during 24-hour AECG recording have been shown to predict mortality and SCD especially in a population of patients with reduced LVEF (Julian et al. 1997) as well as a general population of post-MI patients (Maggioni et al. 1993). In 1989, the results of the Cardiac Arrhythmia Suppression Trial showed that reducing ventricular arrhythmias with class IC antiarrhythmic drugs actually increases the death rate (Anonymous 1989), indicating that risk markers are not necessarily optimal therapeutic targets (Goldberger et al. 2008).

It has been discussed that patients with LVEF between 35% and 40% should undergo AECG recording for risk stratification with NSVT and VPBs and, moreover, if the testing is positive, further risk stratification with EPS (Buxton et al. 1999, Goldberger et al. 2008). However, it seems that patients with preserved LVEF (>40%) do not benefit from risk stratification with AECG (Goldberger et al. 2008). Moreover, it seems that NSVT and the presence of VPBs do not have prognostic value in multivariable analysis when measured early (i.e., up to 6 weeks) after acute MI (Huikuri et al. 2009).

### *6.4.3.2 Heart rate variability*

HRV is a measure of autonomic nervous system modulation by the sinus node (Kleiger et al. 1987). It can be addressed from normal surface ECG as short-term HRV, but it has been studied more extensively during the AECG as long-term HRV (Goldberger et al. 2008). An increase in sympathetic tone or dismissal of parasympathetic tone often precedes ventricular arrhythmias. Therefore, low HRV has been thought to be a predictor of SCD. The prognostic capacity of HRV has been evaluated in numerous studies, and it has been shown to have independent predictive capacity (Huikuri et al. 1998, La Rovere et al. 1998, La Rovere et al. 2001). However, no difference in survival was observed when patients with low LVEF and low HRV where randomized to receive or not to receive ICD (Hohnloser et al. 2004). In CARISMA, low HRV

was predictive of the composite primary arrhythmic endpoint when measured at 6 weeks after acute MI, but not at 1 week (Huikuri et al. 2009). In REFINE, HRV measurement at both 2 to 4 weeks and 10 to 14 weeks after acute MI failed to reach significant prognostic power (Exner et al. 2007). It seems that HRV is a better predictor of non-arrhythmic deaths than of SCD (Goldberger et al. 2008).

#### *6.4.3.3 Heart rate turbulence and deceleration capacity*

Heart rate turbulence (HRT) is a promising risk stratification tool. It measures short-term fluctuation of autonomic tone after VPBs. It was launched by Schmidt and co-workers at 1999 (Schmidt et al. 1999) and has been shown to predict mortality especially in post-MI populations (Schmidt et al. 1999, Ghuran et al. 2002, Bonnemeier et al. 2003, Exner et al. 2007). HRT can be obtained from a relatively small numbers of VPBs (> 5) and does not require blood pressure measurement or intervention (Bauer et al. 2008).

Another interesting novel risk marker describing autonomic failure is deceleration capacity. It is derived from a signal-processing algorithm that analyses the acceleration and deceleration of heart rate separately from 24-hour AECG (Bauer et al. 2006). It has been shown to be a powerful predictor after MI alone (Bauer et al. 2006) and together with HRT (Bauer et al. 2009).

### 6.4.4 Exercise-testing-based techniques

#### *6.4.4.1 ST level deviation*

Horizontal or down sloping ST level depression (≥1.0mm) during exercise testing is a well-known diagnostic tool for patients with mid-level pretest probability for CHD (Gibbons et al. 1997). Moreover, it has been shown to have prognostic power for future cardiac events in patients with CHD (Weiner et al. 1987, Detrano et al. 1989) as well as those with no previous diagnosis of CHD (Bruce et al. 1983, Laukkanen et al. 2001). In 2009, Laukkanen and co-workers studied a population-based sample of 1,769 asymptomatic men. The hazard ratio for SCD was 2.1 (95% CI 1.2–3.9) for those with asymptomatic ST level depression during exercise and 3.2 (95% CI 1.7–6.0) for those with asymptomatic ST segment depression during the recovery period over a median follow up of 18 years.

#### *6.4.4.2 Exercise capacity*

Exercise capacity provides a measure of cardiovascular, pulmonary, or neural function, in addition to reflecting a response to the action of exercising muscles. Reduced exercise capacity may therefore be caused by dysfunction in any of these components. An increase in heart rate or arterial pressure may be inadequate, or cardiac output may fail to fulfil the needs of a higher rate of metabolism. Moreover, the pulmonary capacity may be decreased or the neural response to the exercise may be inadequate, as the control of the autonomic nervous system by the brain is imbalanced (Balady et al. 2010).

Exercise capacity has been linked to an increased risk for cardiovascular and total mortality for decades (Kodama et al. 2009). Myers and co-workers showed in 2002 that exercise capacity predicts mortality in a clinically relevant population of 6,213 individuals (i.e., among men referred for exercise testing). They found that low exercise capacity as expressed in METs was the best predictor of death from any cause in normal subjects as well as in those with cardiovascular disease, as compared to a history of congestive heart failure, history of MI, pack-years of smoking, left ventricular hypertrophy in ECG, pulmonary disease, or exercise-induced ST segment depression. In the total population, every 1-MET increase was associated with a 12% improvement in survival and predicted mortality more accurately that the percentage of age-predicted value in the Cox proportional hazard model as well as in the ROC analysis ($p<0.01$). However, the ROC analysis did not take into account the different follow-up times of the studied subjects.

In 2008 Kokkinos et al. reported that exercise capacity predicts mortality similarly among black and white men. The adjusted hazard ratio for mortality for every 1-MET increase was 0.87 (95% CI 0.86–0.88, $p<0.001$) in the total cohort of 15,660 patients with a mean follow-up time of 7.5 years. Recently, another study by Kokkinos and co-workers (2010) showed that exercise capacity in METs is an independent predictor for all-cause mortality in older men as well (i.e., >65 years old).

Gulati and others (2003) studied 5,721 asymptomatic women at the age of 35 or older who were able to walk on a treadmill. Every 1-MET increase resulted in a 17% ($p<0.001$) improvement in survival when adjusted with the Framingham risk score. Interestingly, adjustements with the Framingham risk score strengthened the association between exercise capacity and death, when exercise capacity was grouped in three groups (METs <5, METs 5 to 8, and METs >8). Moreover, in the another study on the same population with the validation cohort of 4,471 women referred for diagnostic exercise testing, the percentage of the age-predicted

value of the exercise capacity in METs was discovered to be associated with an increased risk for all-cause and cardiovascular mortality (Gulati et al. 2005).

In 2010, Laukkanen and co-workers showed that low exercise capacity also predicts SCD in a population-based study of 42 to 60 years old men. In their cohort of 2,368 men, a 1-MET increase yielded a hazard ratio of 0.78 (95% CI 0.71–0.84, $p<0.001$) in multivariable Cox regression analysis with a Harrel's C-index of 0.767 for total model discrimination. However, the exercise capacity in METs produced only a modest improvement (i.e., from 0.760 to 0.767).

#### *6.4.4.3 Heart rate recovery*

Heart rate is regulated from the sinoatrial node by pacemaker currents establishing "the voltage clock" (i.e., intrinsic regulation), as well as by the autonomic nervous system and circulating hormones, in addition to reflex regulation via cardiorespiratory and baroreseptors inputs (i.e., extrinsic regulation; Verrier and Tan 2009). The intrinsic heart rate of healthy individuals is ~100 beats/min, when autonomic input has been completely blocked (Katona et al. 1982). The main mechanisms to accelerate the heart rate (e.g., during exercise) are steepening the slope of spontaneous diastolic depolarization and hypopolarizing the resting potential as a result of a release of noradrenalin and adrenaline by the sympathetic nervous system. In slowing heart rate, the reverse occurs via the vagus nerve (i.e., the parasympathetic nervous system) as a decrease in the slope of diastolic depolarization and through hyperpolarization of the resting potential (Verrier and Tan 2009).

HRR is a marker of the autonomic nervous system's response after the end of exercise. The reduction in heart rate during the first minutes after the exercise has been principally thought to be caused by the reactivation of the vagal activation, but also by withdrawal of the sympathetic tone (Imai et al. 1994). However, Savin and co-workers showed as early as in 1982 that even when both the sympathetic and the parasympathetic systems are blocked with propranolol and atropine, respectively, heart rate decelerates exponentially after the exercise in healthy men. Hence, significant independent factors, such as alternations in venous return leading to stretch of the atrial receptors of pacemaker tissue, may play an important role in the physiology of HRR.

Cole and co-workers (1999) followed for six years 2,428 patients who were referred to exercise myocardial perfusion imaging and were candidates for first-time coronary angiography. Low HRR ($\leq$12 beats/min) was found to be a significant predictor of all-cause mortality when adjusted with standard cardiac risk factors, including exercise capacity in METs (hazard ratio

2.0, 95% CI 1.5–2.7, p<0.001). Moreover, 56% of the patients who died had an abnormal HRR value, indicating a modest sensitivity for mortality. Since the landmark publication by Cole and colleagues (1999), abnormal HRR has been found to be risk marker for mortality in a cardiovascularly healthy cohort (Cole et al. 2000) as well as when the Duke treadmill exercise score (Nishime et al. 2000), LVEF (Watanabe et al. 2001a), or the severity of CHD (Vivekananthan et al. 2003) has been accounted for.

In 2005 Jouven and others described, for the first time, the association between HRR and SCD. They studied 5,713 asymptomatic men with a 23-year follow-up period. An HRR of less than 25 beats/min (the lowest quintile) was found to be a significant predictor of SCD when compared to the highest quintile (relative risk 2.20, 95% 1.02–4.74) and also remained significant in multivariable analysis.

In the studies on HRR, a multitude of different cut-off values have been used depending on the exercise protocol (i.e., a cool-down period or abrupt cessation of the exercise) and the time point of HRR measurement (i.e., 1 or 2 minutes after the end of exercise). In a validation study in 2001, the cut-off point of ≤22 beats/min, when measured 2 minutes after the cessation of exercise with the cool-down period, yielded the best prediction for death (Shetler et al. 2001) when compared to the cut-off points of ≤12 beats/min or ≤18 beats/min at 1 minute or ≤42 beats/min at 2 minutes that have been used in other publications (Cole et al. 1999, Cole et al. 2000, Nishime et al. 2000).

The data available concerning the optimal cut-off point for an exercise test with an abrupt end is limited. In 2001, Watanabe and others (2001a) followed 5,438 patients referred for exercise echocardiography for 3 years. The patients were positioned to a lateral left decubitus position after the exercise without a cool-down period. The HRR cut-off point of 18≤ beats/min at 1 minute yielded the highest log-rank chi square statistic and was chosen for the survival analysis. An abnormal HRR had predictive capacity for death from any cause (adjusted hazard ratio 2.09, 95% CI 1.49–2.82, p<0.001).

### 6.4.5 Combination of the exercise test variables

One method to improve the prognostic power of an exercise stress test and, furthermore, to enhance its overall predictivity for cardiovascular mortality and SCD is to combine several risk markers. There is ample information available concerning the prognostic capacity of different combinations of exercise test variables. Some of these are discussed briefly here.

A treadmill exercise risk score that takes into account exercise time, ST deviation and angina was developed by Mark and co-workers in 1987. They established with their cohort of 2,842 patients who underwent both a treadmill exercise test with the Bruce protocol and cardiac catheterization that the treadmill exercise score added independent prognostic information to the 5-year survival rate in patients with three-vessel disease. Since then, the prognostic capacity of the treadmill exercise score has also been validated in different patients populations, such as in outpatients with suspected CHD (Mark et al. 1991) and symptomatic patients with non-specific ST-T changes in their resting ECG (Kwok et al. 1999). However, in patients referred for exercise testing, the prognostic capacity of treadmill exercise score seems to be caused principally by functional capacity (i.e., exercise time; Nishime et al. 2000).

Mora and others reported in 2003 that the combination of low exercise capacity and low HRR strongly predicts cardiovascular and total mortality in an asymptomatic cohort of 2,994 women with 20-years follow-up. The adjusted hazard ratio for cardiovascular mortality in patients with both low exercise capacity in METs and low HRR was 3.52 (95% CI 1.57–7.86) when compared to patients with neither factor. Moreover, 103 cardiovascular deaths occurred in patients with both parameters abnormal, whereas only 43 occurred in other patients included in their study. In 2005, Mora and co-workers showed that the combination of low exercise capacity and low HRR also strongly predicts cardiovascular and total mortality in an asymptomatic cohort of more than 6,000 individuals. Recently, Kokkinos and others (2011) established that the combination of low exercise capacity (≤6 METs) and impaired HRR (≤14 beats/min) is associated with an approximately seven-fold risk for all-cause mortality in male veterans when compared to patients with neither factor.

### 6.4.6 Combination of T-wave alternans with other prognostic markers

There are a several studies available where the prognostic power of TWA has been investigated in combination with other non-invasive parameters. However, only few have concentrated especially on the combined risk stratification.

In 2000, Ikeda and co-workers concluded that the combined assessment of TWA and late potentials yielded the highest positive predictive value in 102 post-MI patients as compared to the parameters alone, or to LVEF. The positive predictive value for arrhythmic events in patients with positive TWA and late potentials test results was 50% (i.e., half of the patients died during the follow-up of 13 ± 6 months). However, the different follow-up times of study participants

was not taken into account in the analyses of positive predictive value. In the univariate Cox regression analysis, TWA alone yielded a hazard ratio of 16.8 (95% CI 2.2–127.8, p=0.006) for arrhythmic events, whereas the combination of TWA and late potentials produced a hazard ratio of 8.6 (95% CI 3.1–23.9, p<0.0001).

Baravelli and others (2007) prospectively studied 70 patients with idiopathic dilated cardiomyopathy who underwent symptom-limited cardiopulmonary exercise testing with oxygen consumption ($VO_2$) recording as well as TWA alternans testing with the spectral method. They found that only the combination of peak $VO_2$ uptake and TWA, but not either of the parameters alone or LVEF, was associated with the composite primary endpoint of cardiac mortality or ventricular arrhythmias (hazard ratio 0.28, 95% CI 0.12–0.95, p=0.03) in a mean follow-up of 19.2 months.

In the REFINE study, the combination of impaired autonomic function (i.e., HRT), exercise-based TWA, and LVEF < 50% beyond 8 weeks after acute MI was associated with increased risk for cardiac death or resuscitated cardiac arrest (hazard ratio 5.08, 95% CI 2.17–11.89, p<0.001; Exner et al. 2007). Similar results were achieved with the combinations of exercise-based TWA, baroreflex sensitivity, and LVEF; recovery based TWA, HRT, and LVEF; as well as recovery based TWA, baroreflex sensitivity, and LVEF.

In respect to these three studies, TWA has been investigated in several other studies combination with different parameters, such as baroreflex sensitivity (Hohnloser et al. 1998), LVEF (Hohnloser et al. 1998, Adachi et al. 2001), SAECG (Gold et al. 2000), VPBs, and HRV (Stein et al. 2010), showing that TWA may have complementary prognostic capacity with these parameters. However, three of these four studies (Hohnloser et al. 1998, Adachi et al. 2001, Gold et al. 2000) were initially designed to evaluate the prognostic power of TWA alone or in comparison with the other risk markers, and the fourth was a case-control study (Stein et al. 2010). Therefore, the possible supplementary prognostic significance of these parameters has to be tested in future prospective studies.

# 7 AIMS OF THE STUDY

The aims for the present study were:

1. To test the hypothesis that the quantification of TWA magnitude enhances its prognostic capacity for SCD as well as for cardiovascular and all-cause mortality (**I**);

2. To test the prognostic capacity of TWA separately during the pre-exercise, standard exercise, and post-exercise phases (**I**); and to analyze the predictive capacity of TWA measured in the limb leads (**data addition**);

3. To evaluate whether low exercise capacity predicts SCD alone and in combination with elevated TWA (**II**);

4. To analyze HRR in combination with TWA to enhance risk assessment in a population undergoing a clinically indicated exercise testing (**III**);

5. To test the hypothesis that the prognostic capacity of a clinically indicated exercise test is further enhanced by combined analysis of exercise capacity, HRR, and TWA (**IV**).

# 8 MATERIALS

## 8.1 Patients

Consecutive patients referred for a clinically indicated exercise stress test at Tampere University Hospital and who were willing to participate in FINCAVAS (Nieminen et al. 2006) were recruited between October 2001 and the end of 2008.

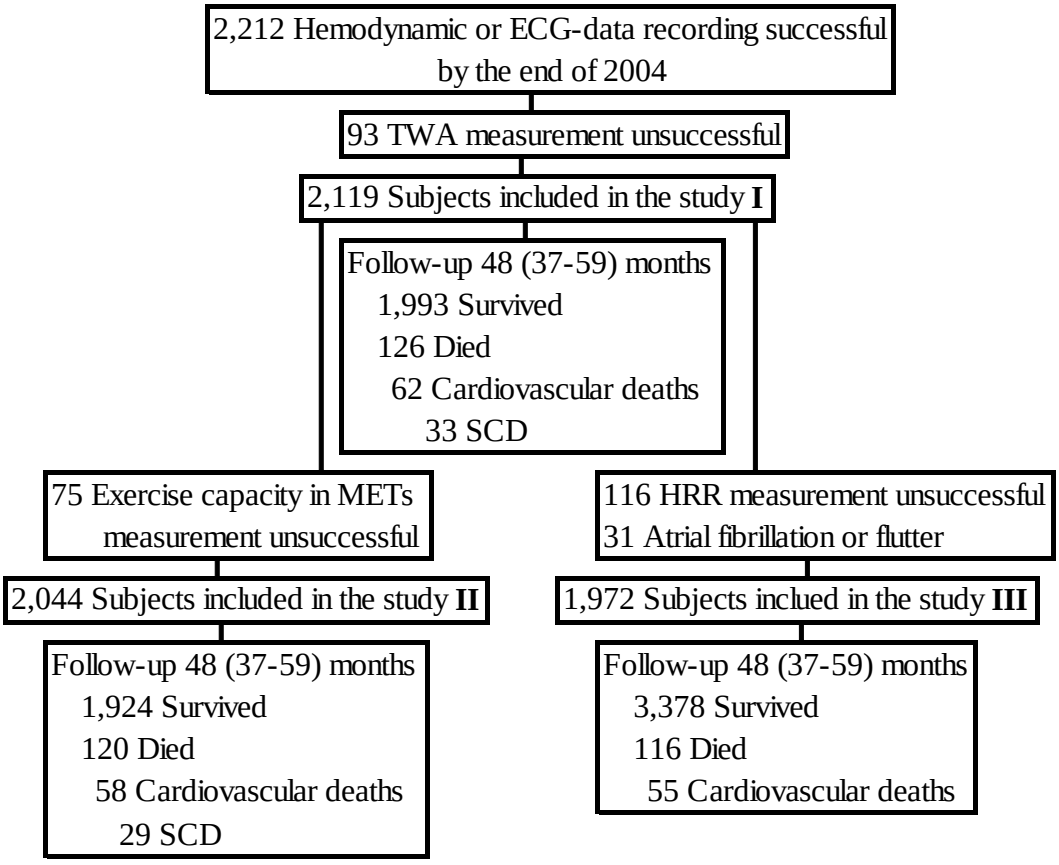



**Figure 4.** Flow chart of Studies **I**, **II**, and **III**. The median value of follow-up time is given with the interquartile range in parentheses. ECG = electrocardiogram; FINCAVAS = the Finnish Cardiovascular Study; HRR = heart rate recovery; MET = metabolic equivalent; SCD = sudden cardiac death; TWA = T-wave alternans

A total of 2,212 (1,400 men) patients had hemodynamic data and continuous digital ECGs technically successful measured and were recruited until the end of 2004 (Studies **I**, **II** and **III**, Fig. 4). Of these, 2,119 patients (1,342 men) also had TWA successfully assessed and were studied in Study **I**. Furthermore, 2,044 patients (1,305 men) underwent exercise capacity recording in METs and TWA assessment during the exercise phase of the test successfully and were enrolled in Study **II**.

For the purposes of Study **III,** patients (N=1,972 [1,254 men]) with successfully measured HRR and TWA were recruited. Moreover, patients with AF or flutter (N=31) were excluded, as these patients have been excluded from the previous HRR studies (Elhendy et al. 2003, Vivekananthan et al. 2003)**.**

Between October 2001 and the end of 2008, a study population of 4,178 patients (2,537 men) was enrolled (Fig. 5). Patients with AF, flutter, or implantable cardiac devices were excluded from the analyses. Thereafter, a total of 3,609 patients (2,157 men) underwent technically successful exercise tests (i.e., the storing of the hemodynamic data and the continuous digital ECG as well as the TWA assessment during exercise, the exercise capacity recording in METs, and the HRR measurement were successful; Study **IV**).

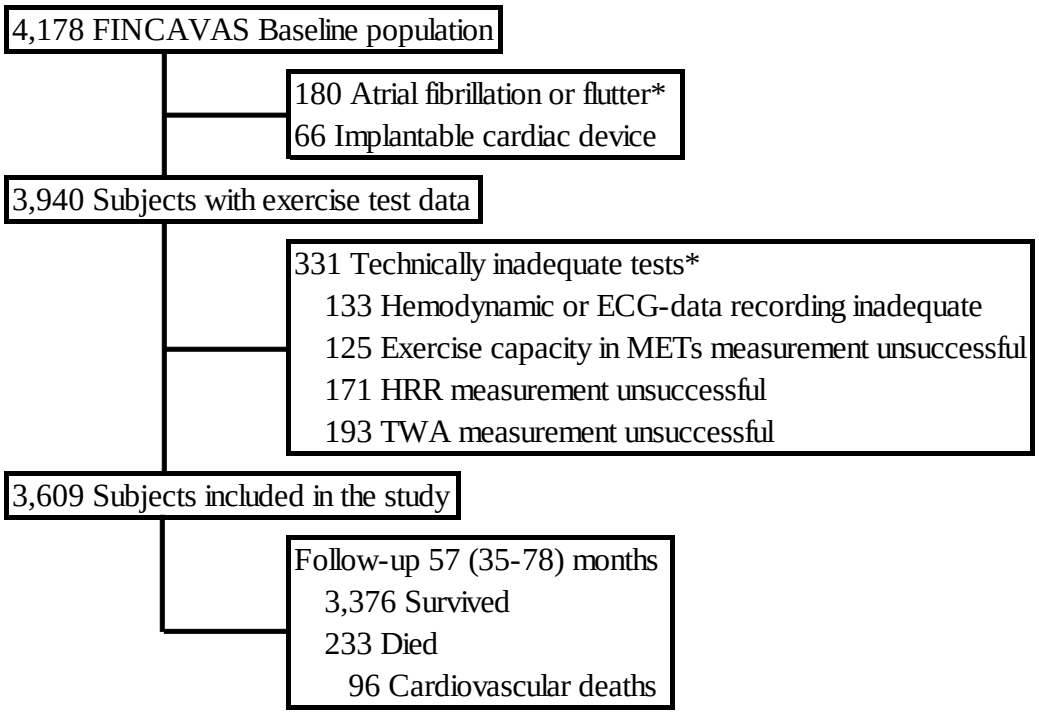



**Figure 5.** Flow chart of Study **IV**. * Some patients had more than one criterion. The median value of follow-up time is given with the interquartile range in parentheses. ECG = electrocardiogram; FINCAVAS = the Finnish Cardiovascular Study; HRR = heart rate recovery; MET = metabolic equivalent; TWA = T-wave alternans

The main indication for the exercise test was a diagnosis of CHD (47%). The other indications were an evaluation of work capacity (26%), palpitation or a sensation of arrhythmia (25%), and an assessment of the adequacy of CHD treatment (13%), in addition to obtaining an exercise test profile prior to an invasive procedure (9%) or after an MI (8%); some patients had more than one indication (Study **IV**).

## 8.2 Ethical aspects

The study protocol was approved by the Ethics Committee of Tampere University Hospital, Pirkanmaa Hospital District, Finland, and all patients gave informed consent prior to the interview and measurements, as stipulated in the Declaration of Helsinki.

# 9 METHODS

## 9.1 Study flow

After an informed consent was signed, the medical history of each patient was collected with a computer-based questionnaire form. Thereafter, the exercise test was performed.

## 9.2 Exercise test protocol

Prior to the exercise stress test, subjects lay down in the supine position for 10 minutes, and the resting ECG was digitally recorded. Exercise testing was performed using a bicycle ergometer with electrical brakes. The lead system used was the Mason-Likar modification of the standard 12-lead system (Mason and Likar 1966). The initial workload varied from 20 W to 30 W, and the load was increased stepwise by 10–30 W every minute. Continuous ECGs were digitally recorded at 500 hertz with the CardioSoft exercise system (Version 4.14, GE Healthcare, Freiburg, Germany).

Heart rate was continuously registered with ECG during the tests, while systolic and diastolic arterial pressures were measured with a brachial cuff every two minutes. Exercise capacity in METs was estimated on the standardized basis of maximum workload and weight of the patient, with 1 MET equivalent to a 3.5 mL oxygen uptake per kilogram per min. HRR was determined as the maximum heart rate during exercise minus the heart rate after the first minute following the cessation of exercise.

## 9.3 Measurement of T-wave alternans

TWA was analyzed automatically with the released version of GE Healthcare MMA software (Studies **I**, **II** and **III**). Moreover, all the TWA values over 46 μV were over-read by a physician blinded to clinical outcomes in Study **IV**.

MMA analysis (Nearing and Verrier 2002a) calculates and compares separate average morphologies of odd and even beats and is discussed in detail earlier in the *Review of the literature* section. Continuous updating for every incoming beat by a weighting factor of 1/8 of the difference between the ongoing average and the new incoming beat produces continuous moving averages of odd and even beats. The following steps were taken to ensure quality control of TWA values. Throughout the analysis, beat-labeling was performed to exclude the suspect and preceding beat based on noise and prematurity according to several criteria. These included: beats with >20 μV of noise, which was measured during the isoelectric segments; regions with >25% of noisy beats; and ventricular premature beats (VPBs).

TWA magnitude was calculated continuously during the entire exercise test for all precordial leads (V1 to V6; Studies **I**, **II**, **III,** and **IV)**. In addition, the TWA magnitude from limb leads (I, II, III, aVF, aVL, and aVR) was calculated (**data addition**, studied in the population of patients of Study **I**). The maximum TWA value was derived separately during the pre-exercise (Study **I**), exercise (Studies **I**, **II**, **III** and **IV**), and post-exercise (Studies **I** and **III**) phases. TWA results for the limb leads were excluded in Studies **I**, **II**, **III,** and **IV,** as these leads are subject to significant motion artifact, as confirmed by visual inspection of the templates of superimposed ECGs in the GE Healthcare system. Precordial leads have also been shown to be optimal for TWA measurement (Nearing et al. 1994; Martinez et al. 2006).

TWA values at heart rates >125 beats/min were not included in the analyses, based on the published experience with the spectral method (Bloomfield et al. 2002a), indicating that inaccuracies in TWA measurement can result at heart rates exceeding this range (Studies **I**, **II,** and **III**). However, in Study **IV** no heart rate limit was used.

## 9.4 Left ventricular ejection fraction

Measurement of LVEF is not routine for patients referred for a clinical exercise test. However, LVEF was determined for 1,117 study patients with echocardiography or isotope techniques within 6 months of exercise testing (Study **II**).

## 9.5 Follow-up and end-points

Death certificates listing causes of death using the tenth revision of the International Classification of Diseases (ICD-10) were received from the Causes of Death Register, maintained by Statistics Finland; this source has been shown to be reliable (Pajunen et al. 2005). Diagnosis numbers and certificate texts were used to classify deaths as all-cause, cardiovascular, or SCD, i.e., cardiovascular death within 24 hours after onset of symptoms. The investigators who analyzed the TWA test results were blinded to events.

All-cause and cardiovascular mortality as well as SCD were studied as end-points in Studies **I,** and **II**. In Study **III**, all-cause and cardiovascular mortality were used, whereas in Study **IV,** cardiovascular mortality was chosen as a primary end-point. In addition, all-cause mortality was employed in the secondary analyses in Study **IV**.

## 9.6 Statistical analysis

Differences between patient and exercise characteristics according to the survival status were compared using the Student T-test, Mann-Whitney U test, or Chi Square test, as appropriate.

The risks to experience the selected end-points during follow-up were analyzed with the Cox proportional hazards model. The proportionality assumption for all covariates was checked by using correlations on the survival rankings with the Schoenfeld residuals. All of the covariates fulfilled the proportionality assumption. The use of β-blockers was defined as “no” if patient did not use β-blockers or had not used β-blockers for three or more days before the test.

Statistical analyses were performed with the SPSS releases 14.0 (Study **III**), 15.0 (Studies **I** and **II**), and 17.0 (Study **IV**) for Windows (SPSS Inc, Chicago, Illinois), STATA (version 10.1, StataCorp LP, College Station, Texas; Studies **III** and **IV**), in addition to R (version 2.10.1, The R Foundation for Statistical Computing c/o, Vienna, Austria; Study **IV**). All statistical tests were two-tailed and used an alpha level of 0.05.

### 9.6.1 Study **I** and **data addition**

The Hazard ratios were analyzed for the pre-exercise, exercise, and post-exercise phases, separately; for TWA results grouped in 10μV increments with the cut-off points of 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, and 120μV; and also for TWA as a continuous variable using

the following covariates: sex, age, body-mass index (BMI), daily smoking (yes/no), use of β-blockers (yes/no), MET, as well as prior diagnoses of CHD (yes/no), MI (yes/no), and diabetes (yes/no).

### 9.6.2 Study **II**

Analyses of exercise capacity in METs were performed with the cut-off point of <8, which has been used in studies on women (Mora et al. 2003, Mora et al. 2005) but, to our knowledge, not in studies on men. In subgroup analyses in women, the cut-off point of < 5 was also used (Gulati et al. 2003).

For analyses of TWA, the cut-off point of 65μV for precordial leads was adopted, because it had the best prognostic power in the previous FINCAVAS study (Nieminen et al. 2007). Low exercise capacity and TWA were combined into one categorical variable with three different groups of patients: MET ≥8 and TWA <65μV, MET <8 or TWA ≥65μV, and MET <8 and TWA ≥65μV. Thereafter, the risk for all-cause and cardiovascular death as well as for SCD was estimated using the following covariates: sex, age, BMI, daily smoking (yes/no), use of β-blockers (yes/no), as well as prior diagnoses of CHD (yes/no), MI (yes/no), and diabetes (yes/no).

### 9.6.3 Study **III**

The HRR cut-off point of ≤18 beats/min was employed as it has been suggested for exercise tests with an abrupt end (Watanabe et al. 2001a). The exercise-based TWA cut-off point of 60μV, which yielded excellent Cox regression results in our previous study (Study **I**), was employed. Recovery-based TWA values were analyzed according to the cut-off points of 20 μV and 60μV (Slawnych et al. 2009).

The risks for total and cardiovascular mortality were analyzed for HRR, TWA, and their combinations, as well as for ST segment deviation after adjustment by the covariates of sex, age, BMI, smoking (yes/no), use of β-blockers (yes/no), the reached maximum heart rate, and prior diagnoses of CHD (yes/no), history of MI (yes/no), diabetes (yes/no), and hypercholesterolemia (yes/no). Harrell's C-indices were also calculated. The calculations for the combinations variables were based on three categories—no parameter positive, either parameter positive, and both parameters positive.

### 9.6.4 Study **IV**

The prognostic significance of exercise capacity, HRR, and TWA was tested with linear Cox proportional hazard model using sex and age as covariates, because the linear models did not differ significantly from the non-linear models using splines.

A linear model with exercise capacity in METs, HRR, and TWA as continuous variables was created and compared separately to the three models that consisted two of the three variables—i.e., exercise capacity and HRR, exercise capacity and TWA, or HRR and TWA—using Chi-square test of likehood ratios. Moreover, the hazard ratios for cardiovascular mortality were analyzed for the combination of exercise capacity in METs, HRR, and TWA, with the cut-off points of 6, 8, 10, and 12 for MET; 12, 15, 18, and 21 beats/min for HRR; and 40, 50, and 60 μV for TWA. Patients with abnormal results for all the variables were compared to all other patients included in the study. In addition, adjusted survival curves from the Cox regression analyses were created for the combination of the three variables with the previously used cut-off points of <8 for MET (Study **II**; Mora et al. 2003, Mora et al. 2005), ≤18 beats/min for HRR (Study **III**; Watanabe et al. 2001a), and ≥60 μV for TWA (Studies **I** and **III**).

The unadjusted absolute event rates were calculated for cardiovascular mortality separately for patients with all three parameters normal and for all other patients included in the study, with previously employed cut-off points as well as with the loosest (i.e., METs<12, HRR ≤21 beats/min, and TWA ≥40 μV) and strictest (i.e., METs<6, HRR ≤12 beats/min, and TWA ≥60 μV) cut-off points.

The Harrell's C-indices were calculated with previously determined cut-off points. The calculations for the combinations of parameters were based on four categories: no parameter positive, one of the parameters positive, two of the parameters positive, and all parameters positive.

# 10 RESULTS

## 10.1 Patients characteristics

The patient selection is described in Figures 4 and 5. Patient characteristics are given in Tables 5 (Study **II**) and 6 (Study **IV**). Exercise test variables are summarized in Table 7 (Study **III**). Patient characteristics and exercise test variables were similar in Studies **I**, **II** and **III**.

**Table 5**. Patient characteristics (N=2,044; Study **II**)

| | Men | | Women | |
|---|---|---|---|---|
| | (N=1,305) | | (N=739) | |
| | Mean | SD | Mean | SD |
| Age (years) | 57 | 13 | 57 | 13 |
| BMI (kg/m$^2$) | 27.5 | 4.2 | 27.4 | 5.0 |
| Weight (kg) | 85 | 14 | 72 | 13 |
| Height (cm) | 176 | 7 | 162 | 6 |
| | % | | % | |
| Beta blockers | 63 | | 51 | |
| Smoking | 32 | | 15 | |
| CHD | 44 | | 29 | |
| Prior MI | 25 | | 14 | |
| Hypercholesterolemia | 53 | | 45 | |
| Hypertension | 40 | | 40 | |
| Diabetes | 13 | | 12 | |

β-blockers = beta-adrenergic blocking agents; BMI = body-mass index; CHD = coronary heart disease; MI = myocardial infarction; SAP = systolic arterial pressure; SD = standard deviation

## 10.2 Left ventricular ejection fraction

LVEF, reported for 1,117 patients, was 66±14% (mean ± SD). Of these, 103 patients (9.2%) presented with ejection fraction <50%, 39 patients (1.9%) with ejection fraction <40%, and 10 patients (0.9%) with ejection fraction <30% (Study **II**).

**Table 6**. Patient characteristics of the study population according to survival status for cardiovascular deaths (N=3,609; Study **IV**)

| | Survivors (N=3,513) | | Non-survivors (N=96) | | |
|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | p |
| Age (years) | 55.3 | 12.9 | 63.2 | 12.4 | <0.001 |
| BMI (kg/m$^2$) | 27.5 | 4.6 | 28.1 | 5.0 | 0.19 |
| Height (cm) | 171.3 | 9.4 | 171.5 | 9.7 | 0.79 |
| Weight (kg) | 80.7 | 15.7 | 82.9 | 18.1 | 0.24 |
| Heart rate at supine rest (beats/min) | 63.8 | 11.7 | 65.0 | 14.1 | 0.31 |
| SAP at supine rest (mmHg) | 136.8 | 18.9 | 134.8 | 23.3 | 0.41 |
| METs | 7.8 | 2.9 | 5.1 | 2.4 | <0.001 |
| | Median | IQ | Median | IQ | |
| TWA (μV) | 23.0 | 16-31 | 25.5 | 19-37 | 0.002 |
| HRR (beats/min) | 25.0 | 18-31 | 16.0 | 9-22 | <0.001 |
| Reached maximum heart rate (beats/min) | 155.0 | 133-172 | 124.0 | 106-144 | <0.001 |
| Age-predicted maximum heart rate (%) | 87.6 | 76-96 | 71.5 | 61-81 | <0.001 |
| | % | | % | | |
| Women | 41 | | 17 | | <0.001 |
| Beta blockers | 51 | | 80 | | <0.001 |
| Smoking | 23 | | 32 | | 0.03 |
| CHD | 28 | | 46 | | <0.001 |
| Prior MI | 18 | | 38 | | <0.001 |
| Diabetes | 11 | | 14 | | 0.44 |

β-blockers = beta-adrenergic blocking agents; BMI = body-mass index; CHD = coronary heart disease; HRR = heart rate recovery; IQ = interquartile range; MET = metabolic equivalent; MI = myocardial infarction; SAP = systolic arterial pressure; SD = standard deviation; TWA = T-wave alternans

## 10.3 T-wave alternans

### 10.3.1 Dichotomous variable

#### *10.3.1.1 The cut-off point of 20μV*

The TWA cut-off point of 20μV did not reach the level of significance for all-cause or cardiovascular mortality when analyzed during the post-exercise phase (Study **III**).

**Table 7**. Exercise test variables of the study population according to survival for all-cause deaths (N=1,972; Study **III**).

| | Survivors (N=1,856) | | Deaths (N=116) | | |
|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | p |
| Duration of test (minutes) | 7.5 | 2.1 | 6.3 | 2.0 | <0.001 |
| Maximum SAP during the exercise (mmHg) | 194.2 | 28.5 | 175.8 | 30.7 | <0.001 |
| Maximum DAP during the exercise (mmHg) | 92.2 | 11.8 | 85.1 | 13.2 | <0.001 |
| SAP at supine rest (mmHg) | 136 | 18.5 | 133.7 | 24.6 | 0.33 |
| DAP at supine rest (mmHg) | 79.6 | 9.6 | 75.7 | 11.4 | <0.001 |
| MET | 7.3 | 2.9 | 5.3 | 2.4 | <0.001 |
| | Median | IQ | Median | IQ | |
| Precordial TWA at rest before exercise (μV) | 14 | 9-20 | 17 | 11-28 | <0.001 |
| Precordial TWA during exercise (μV) | 25 | 19-34 | 29 | 23-39 | <0.001 |
| Precordial TWA during recovery (μV) | 19 | 14-28 | 23 | 17-34 | <0.001 |
| Heart rate at supine rest (beats/min) | 62 | 55-70 | 61 | 55-71 | 0.74 |
| Reached maximum heart rate (beats/min) | 150 | 129-167 | 127 | 106-150 | <0.01 |
| Age-predicted maximum heart rate (%) | 80 | 68-90 | 72 | 60-84 | <0.001 |
| HRR at 1 min post-exercise (beats/min) | 25 | 18-32 | 17 | 10-23 | <0.001 |
| ST segment deviation during exercise (mV) | −0.059 | −0.12-−0.015 | −0.077 | −0.14-−0.031 | 0.011 |

DAP = diastolic arterial pressure; HRR = heart rate recovery; IQ = interquartile range; MET = metabolic equivalent; SAP = systolic arterial pressure; SD = standard deviation TWA = T-wave alternans

### *10.3.1.2 The cut-off point of 60μV*

The TWA cut-off point of 60μV significantly predicted all-cause (adjusted hazard ratio 2.5 95% CI 1.4–4.5, p<0.01) and cardiovascular mortality (adjusted hazard ratio 5.8 95% CI 3.1–11.1, p<0.01) during exercise as well as during recovery (adjusted hazard ratio 2.4 95% CI 1.3–4.4, p<0.01 for all-cause mortality and 3.5 95% CI 1.6–7.9, p<0.01 for cardiovascular mortality). C-indices are given in Table 8 (Study **III**).

**Table 8**. Harrell's C- indices for cardiovascular mortality

| | Study | C-index | 95 % Lower | CI Upper |
|---|---|---|---|---|
| HRR ≤ 18 beats/min | **III** | 0.650 | 0.583 | 0.718 |
| Exercise-based TWA ≥ 60μV | **III** | 0.594 | 0.535 | 0.653 |
| Recovery-based TWA ≥ 60μV | **III** | 0.550 | 0.535 | 0.653 |
| HRR≤18 beats/min and exercise-based TWA ≥ 60μV | **III** | 0.713 | 0.648 | 0.777 |
| ST-segment deviation (0.1mV) in exercise test | **III** | 0.580 | 0.511 | 0.649 |
| | | | | |
| METs ≤ 8 | **IV** | 0.648 | 0.605 | 0.691 |
| HRR ≤ 18 beats/min | **IV** | 0.659 | 0.607 | 0.711 |
| Exercise-based TWA ≥ 60μV | **IV** | 0.524 | 0.498 | 0.550 |
| METs < 8, HRR ≤ 18 beats/min, and TWA ≥ 60μV | **IV** | 0.719 | 0.665 | 0.772 |

CI = confidence intervals; HRR = heart rate recovery; METs = metabolic equivalent; TWA = T-wave alternans

#### *10.3.1.3 The cut-off point of 65μV*

The TWA cut-off point of 65μV developed in the initial FINCAVAS study (Nieminen et al. 2007) resulted in an adjusted hazard ratio of 2.4 (95% CI 1.2–4.5, p=0.009) during exercise for total mortality, 4.6 (95% CI 2.2–9.9, p<0.001) for cardiovascular mortality, and 4.4 (95% CI 1.5–12.7, p=0.007) for SCD (Study **I**).

#### *10.3.1.4 The quantification of T-wave alternans*

Quartile distribution in peak precordial TWA amplitude during the pre-exercise and exercise phase is graphed for survivors (controls) and victims of all-cause and cardiovascular mortality and SCD (Fig. 6). Increasing TWA values resulted in a progressive increase in the percentile level, which was markedly accelerated when the 40-μV range was exceeded.

The adjusted hazard ratios for elevated TWA grouped in 10μV increments for all-cause mortality, cardiovascular death, and SCD during routine exercise are shown in Figure 7. The adjusted hazard ratios for total and cardiovascular mortality were significant when TWA values reached 50μV. The highest adjusted hazard ratios for total and cardiovascular death were obtained at the cut-off point of 90μV (Fig. 7) and were 3.1 (95% CI 1.1–8.5, p=0.03) and 6.4 (95% CI 2.0–21.2, p=0.002), respectively. SCD was strongly predicted by TWA levels from 60μV upwards, and this TWA value yielded the highest adjusted hazard ratio, 4.6 (95% CI 1.7–12.3, p=0.002; Study **I**).

When TWA in the limb leads was analyzed during the exercise phase in 10μV increments, none of the cut-off points yielded the level of significance for all-cause mortality, cardiovascular mortality, or SCD. During the pre- and post-exercise phases, the results for the limb leads were highly comparable to the results from the precordial leads (**data addition**).

### 10.3.2 Continuous variable

As a continuous variable, increasing TWA voltage significantly predicted all-cause and cardiovascular mortality during the pre-exercise phase (adjusted hazard ratio 1.08 per 5μV; 95% CI 1.04–1.13, p<0.001 for all-cause and 1.08 per 5μV; 95% CI 1.02–1.14, p=0.008 for cardiovascular mortality). During exercise, the adjusted hazard ratio was 1.04 per 5μV (95% CI 1.00–1.07, p=0.05) for all-cause and 1.07 per 5μV (95% CI 1.03–1.11, p=0.001) for cardiovascular mortality. During the post-exercise phase, the adjusted hazard ratio was 1.04 per

5µV (95% CI 1.01–1.07, p=0.01) for cardiovascular death. TWA as a continuous variable did not reach significance for SCD prediction during any of the phases of the routine exercise test (Study **I**).

Increasing TWA voltage in limb leads, when analyzed as a continuous variable, did not significantly predict all-cause, cardiovascular, or sudden cardiac death during the exercise phase. The adjusted hazard ratio was 1.00 per 5µV (95% CI 0.95–1.05, p=0.95) for all-cause mortality, 1.02 per 5µV (95% CI 0.96–1.08, p=0.47) for cardiovascular mortality, and 1.02 per 5µV (95% CI 0.93–1.11, p=0.71) for SCD. During the pre- and post-exercise phases, the results from the limb leads were highly comparable to the results from the precordial leads (**data addition**).

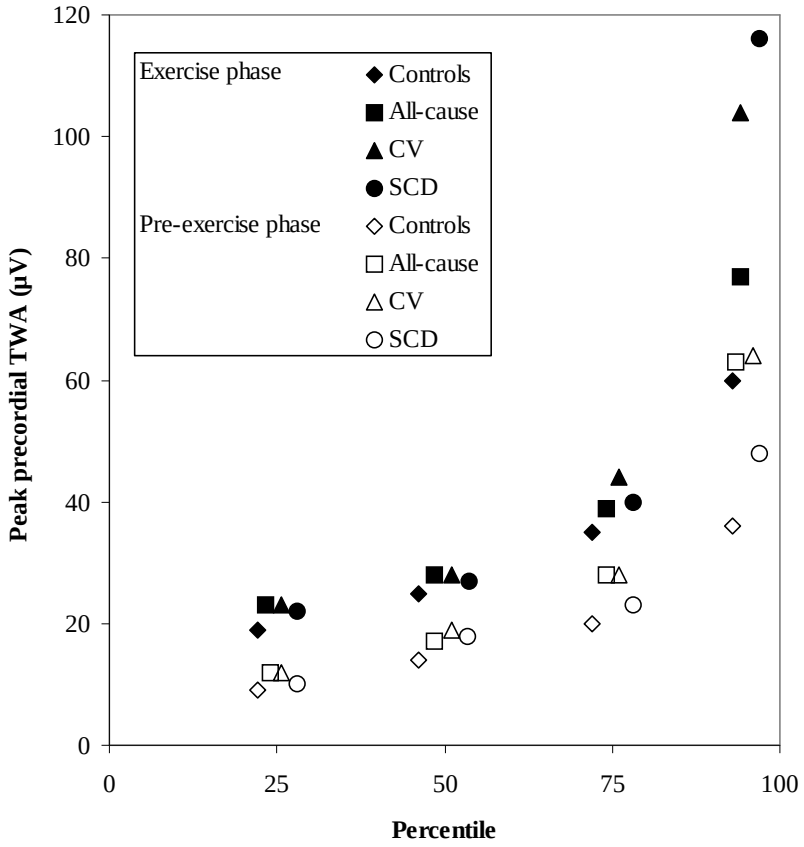



**Figure 6.** Percentiles (25%, 50%, 75%, and 95%) of peak T-wave alternans (TWA) magnitude during the pre-exercise and exercise phases as registered for survivors (controls) and patients with all-cause, cardiovascular (CV), or sudden cardiac death (SCD). The values during the post-exercise phase have been omitted for clarity; the data points lie between the values for the other two phases (Study **I**).

In the expanded database, TWA was a significant predictor of cardiovascular mortality also when adjusted with HRR and MET (Table 9). However, in the secondary analyses, TWA was not a significant independent predictor for all-cause mortality (adjusted hazard ratio 1.11; 0.98–1.26, p=0.105; for 1 SD increase; Study **IV**).

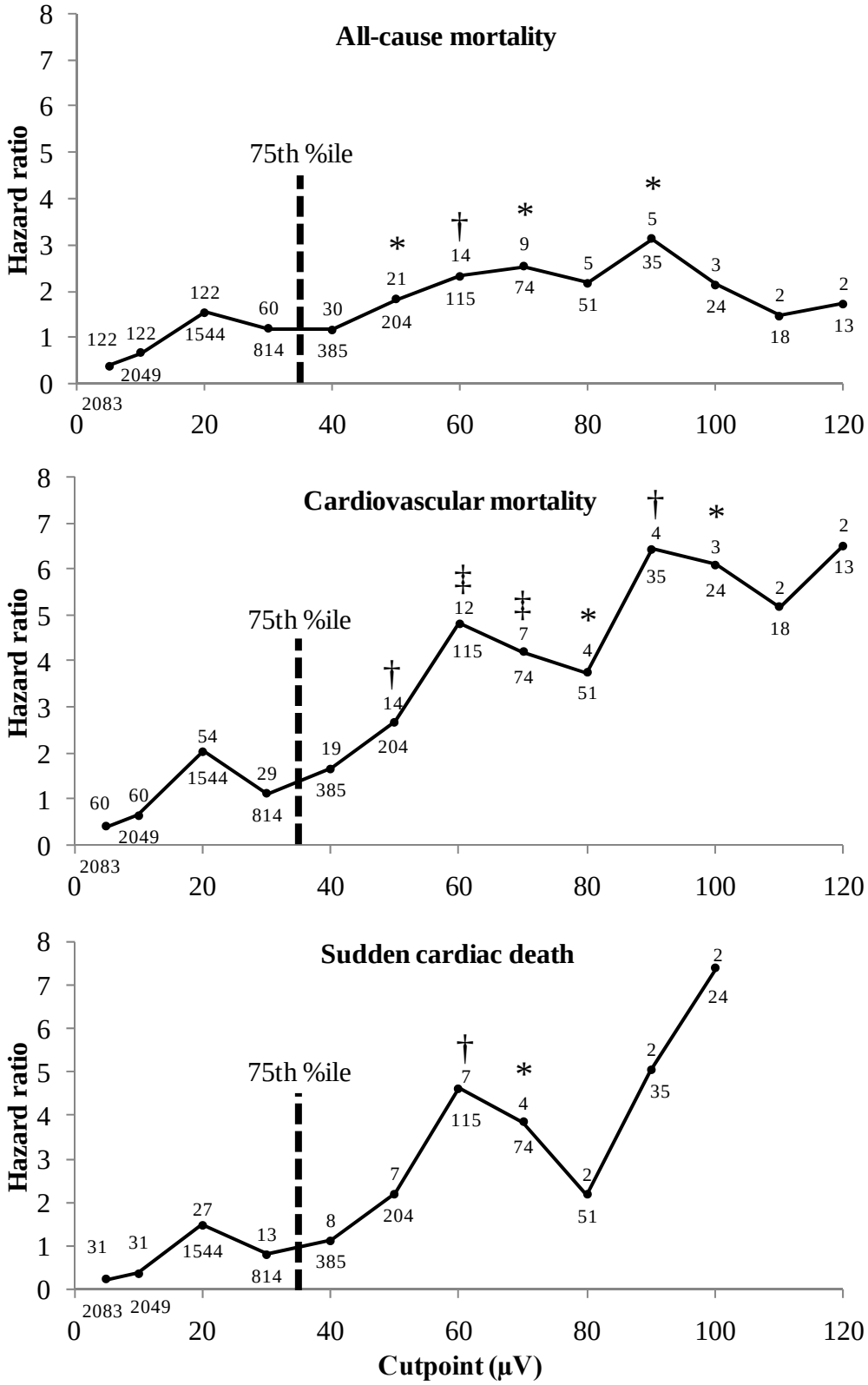



**Figure 7.** Adjusted hazard ratios of maximum T-wave alternans (TWA) during a routine exercise protocol at different cut-off points. The 75$^{th}$ %ile cut-off point for TWA in controls (35 µV) is indicated by the vertical dotted line. The numbers above the line indicate the number of events and the numbers below the line indicate the number of patients for each TWA cut-off point. The line for sudden cardiac death (SCD) is shorter than others, because there were no SCD cases for the highest TWA cut-off points. * $p<0.05$, † $p<0.01$, ‡ $p<0.001$ (Study **I**)

**Table 9**. Adjusted hazard ratios and confidence intervals for cardiovascular mortality from the linear Cox regression model (Study **IV**)

| | HR for 1 SD increase | 95 % CI Lower | CI Upper | p |
|---|---|---|---|---|
| MET | 0.41 | 0.31 | 0.54 | <0.001 |
| HRR | 0.69 | 0.55 | 0.88 | 0.002 |
| TWA | 1.29 | 1.07 | 1.54 | 0.007 |
| Age (y) | 1.21 | 0.94 | 1.57 | 0.15 |
| Sex (f/m) | 3.97 | 2.31 | 6.82 | <0.001 |

CI= confidence interval; HR=hazard ratio; HRR=heart rate recovery; MET=metabolic equivalent; SD=standard deviation; TWA=T-wave alternans

## 10.4 Exercise capacity

In our population of consecutive patients (N=2,044) referred for clinical exercise testing, 58.5% had reduced exercise capacity (MET<8). The mean value (± SD) for exercise capacity in METs was 7.4±3.0 for men (N=1,305) and 6.7±2.8 for women (N=739; Study **II**). Moreover, in our extended database (N=3,609), 48.9% (N=1,766) patients had a reduced exercise capacity (MET <8), with a mean value of 7.8 (±2.9) for survivors and 5.1 (±2.4) for patients experiencing cardiovascular death during follow-up (Table 6; Study **IV**).

The adjusted hazard ratio risk for those with poor exercise capacity (MET <8) was 8.8 (95% CI 2.0–38.9, p=0.004) for SCD, 5.2 (2.0-13.6, p=0.001) for cardiovascular mortality, and 3.3 (95% CI 1.9–5.6, p<0.001) for all-cause mortality.

As a continuous variable, increasing METs significantly improved survival in terms of SCD as well as cardiovascular and all-cause mortality (adjusted hazard ratios 0.67 per 1 MET increase, 95% CI 0.57–0.83, p<0.001 for SCD; 0.69 per 1 MET increase, 95% CI 0.60–0.80, p<0.001 for cardiovascular mortality; and 0.77 per 1 MET increase, 95% CI 0.70–0.84, p<0.001 for all-cause mortality; Study **II**). Exercise capacity in METs also had a strong association with cardiovascular mortality (adjusted hazard ratio 0.41 per 1 SD increase, 95% CI 0.31–0.54, p<0.001) as well as death from any cause (adjusted hazard ratio 0.56 per 1 SD increase, 95% CI 0.47–0.66, p<0.001) in our extended database. C-indices are given in Table 8 (Study **IV**).

In the subgroup analyses in women (N=739), increasing METs as a continuous variable significantly improved the survival in terms of cardiovascular and all-cause mortality (adjusted hazard ratios 0.52 per 1 MET increase, 95% CI 0.32–0.83, p=0.006 for cardiovascular mortality; and 0.77 per 1 MET increase, 95% CI 0.62–0.95, p=0.016 for all-cause mortality). The cut-off

point of METs <8 did not reach significance in women, but the cut-off point of METs <5 predicted statistically significantly cardiovascular mortality (adjusted hazard ratio 15.0, 95% CI 2.0–111.8, p=0.008) in women. In men, the results were highly comparable to the results of the all participants (Study **II**).

## 10.5 Heart rate recovery

HRR was abnormal (≤18 beats/min) in 29.5% (N=590) of the population (N=1,972; Study **III**) and in 28.8% (N=1,040) in our extended database (N=3,609; Study **IV**). The mean and median values of HRR at 1 minute post-exercise according to survival status are given in Tables 6 and 7 (Studies **III** and **IV**).

Abnormal HRR (≤18 beats/min) yielded an adjusted hazard ratio of 2.5 (95% CI 1.6–3.7, p<0.01) for all-cause mortality and 2.3 (95% CI 1.3–4.2, p=0.01) for cardiovascular mortality. The corresponding C-indices are given in Table 8 (Studies **III** and **IV**).

When analyzed as a continuous variable, HRR was a strong predictor of cardiovascular mortality (adjusted hazard ratio 0.69 per 1 SD increase, 95% CI 0.55–0.88, p=0.002) as well as all-cause mortality (adjusted hazard ratio 0.64 per 1 SD increase, 95% CI 0.55–0.74, p<0.001; Study **IV**).

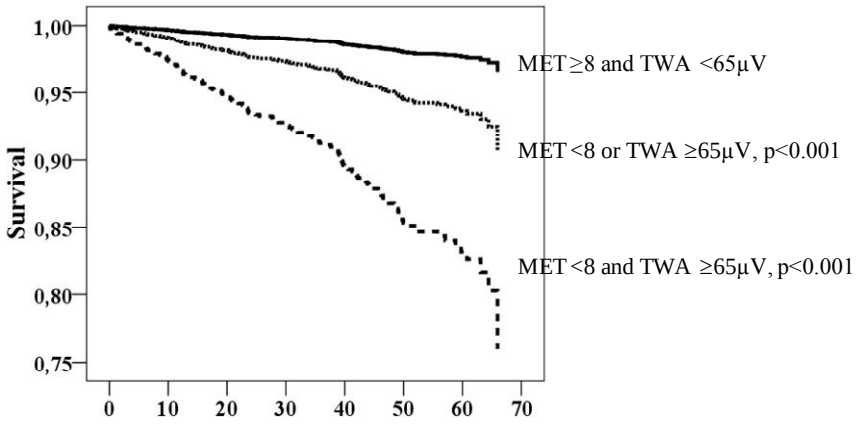
## 10.7 Combination of the variables

### 10.7.1 Exercise capacity and T-wave alternans

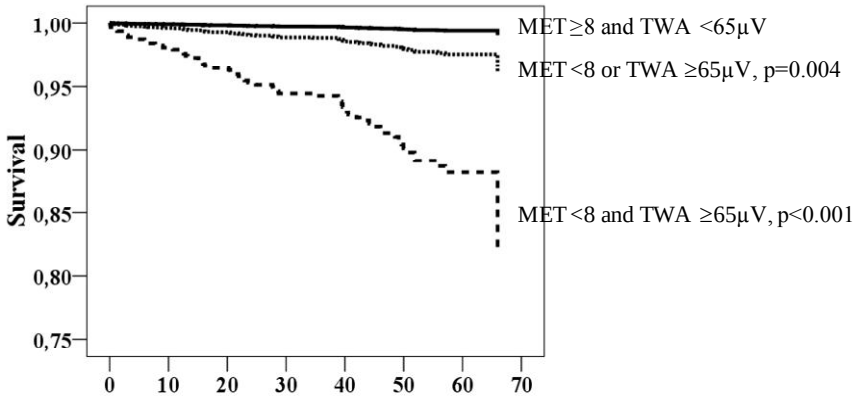
The three groups of patients with METs ≥8 and TWA <65μV, METs <8 or TWA ≥65μV, and METs <8 and TWA ≥65μV comprised 811, 1177, and 56 patients, respectively. The survival curves depict events across 4 years of follow-up for the combined analysis of reduced METs <8 and elevated TWA (≥65μV; Fig. 8).

The adjusted hazard ratios for patients with both reduced exercise capacity (METs <8) and heightened TWA (≥65μV) was 36.1 (95 % CI 6.3–206.0, p<0.001) for SCD, 21.1 (95 % CI 6.7–66.2, p<0.001) for cardiovascular mortality, and 7.8 (95 % CI 3.5–17.4, p<0.001) for all-cause mortality in comparison to patients with neither factor (Study **II**).

**a. All-cause mortality**




**b. Cardiovascular mortality**




**c. Sudden cardiac death**

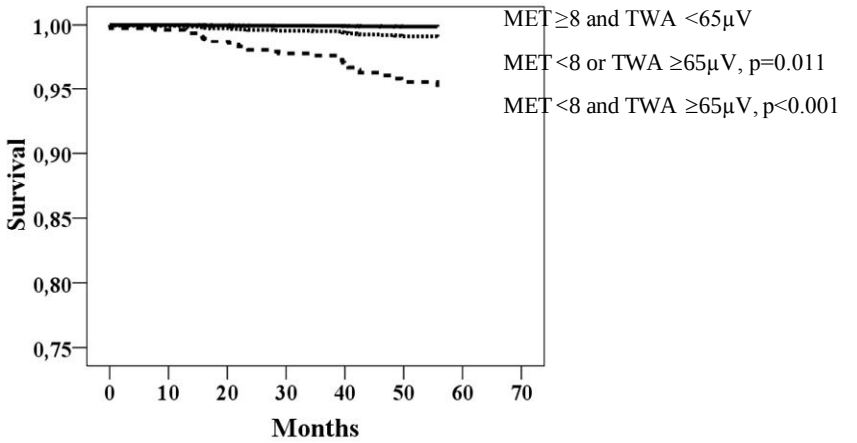



**Figure 8.** Adjusted survival curves by Cox regression for subjects with metabolic equivalents (METs) ≥ 8 and T-wave alternans (TWA) < 65 µV (the upper curve in each panel), METs < 8 or TWA ≥ 65µV (the middle curve), and METs < 8 and TWA ≥ 65µV (the lower curve) for a) all-cause mortality, b) cardiovascular mortality, and c) sudden cardiac death. Please note that the scale for the y-axis is from 0.75 to 1.00 (Study **II**).

The combination of low exercise capacity (METs <8 or METs <5) and elevated TWA (≥65μV) did not reach significance in women. In men, the results were highly comparable to the results of all participants (Study **II**).

### 10.7.2 Heart rate recovery and T-wave alternans

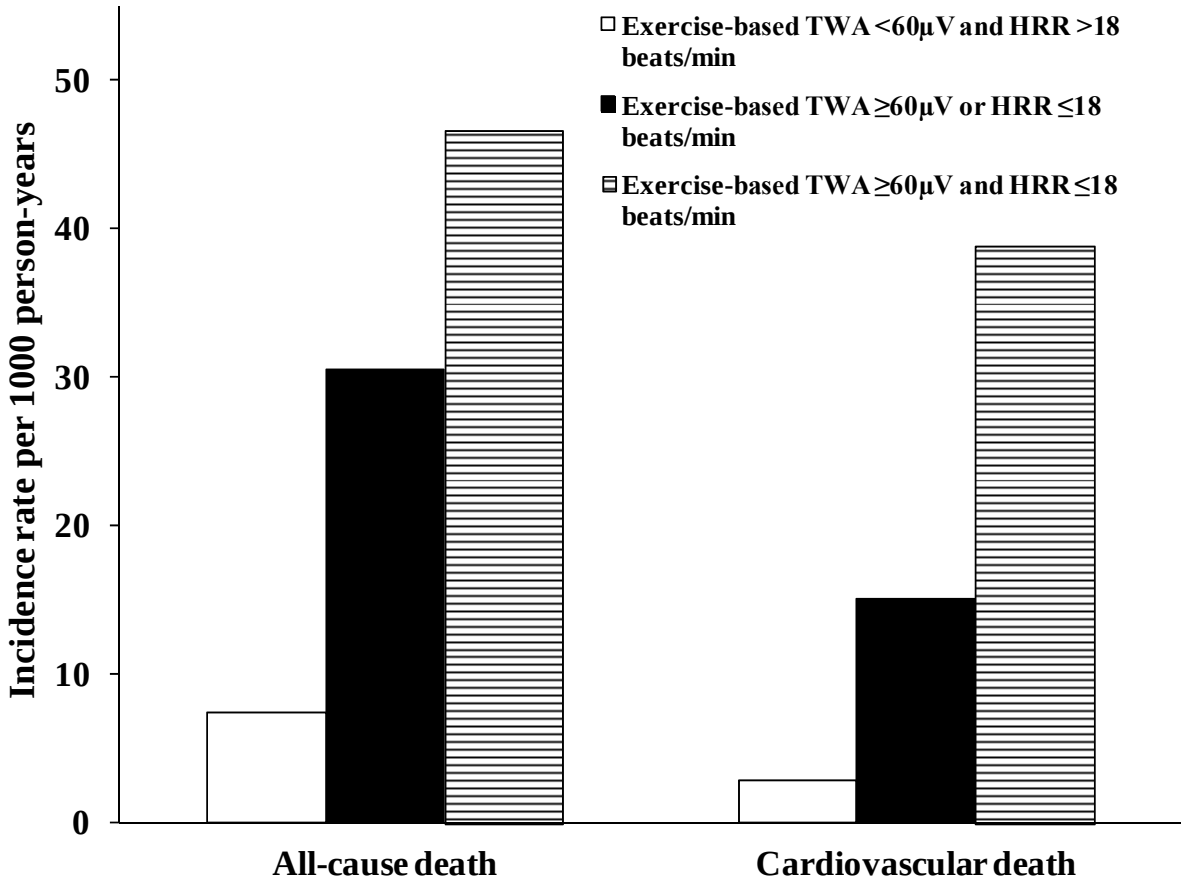


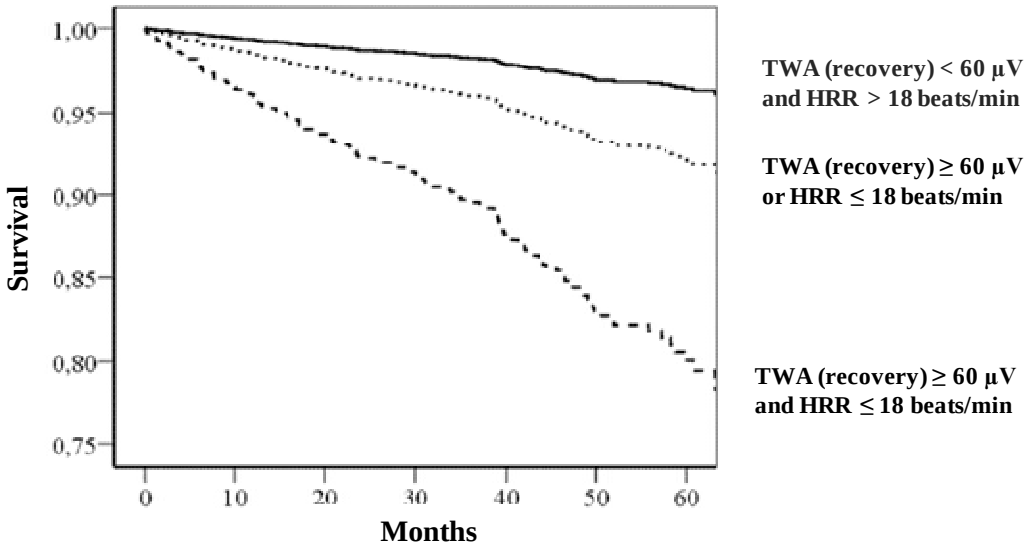

**Figure 9.** Incidence rate of all-cause and cardiovascular mortality per 1,000 person-years among patients according to exercise-based T-wave alternans (TWA) and heart rate recovery (HRR; Study **III**).

The combined Cox proportional hazard analysis of depressed HRR and heightened exercise- or recovery-based TWA more than doubled the prognostic capacity for total and cardiovascular mortality after adjustment for standard risk factors and exceeded exercise-induced ST segment deviation (Table 10). The incidence rates of all-cause and cardiovascular deaths in subgroups are shown in Figure 9.

Adding exercise-based TWA ≥60μV to reduced HRR yielded the highest C-index for all-cause and cardiovascular mortality, although the confidence intervals overlapped with HRR

alone (Table 8). Survival curves depict events across 4 years of follow-up for the combined analysis of reduced HRR and elevated TWA during recovery (Fig. 10; Study **III**).

**a. All-cause mortality**




**b. Cardiovascular mortality**

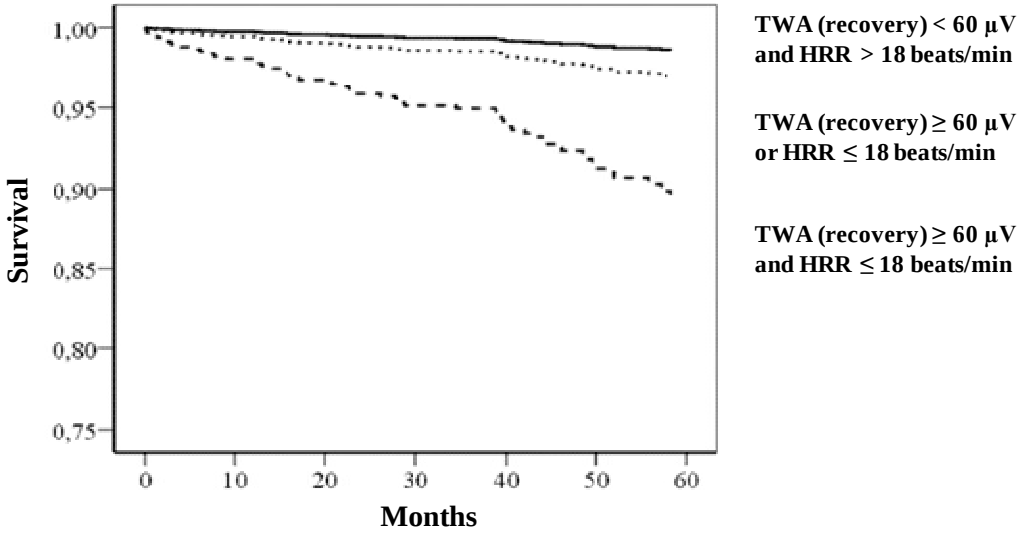



**Figure 10.** Adjusted survival curves by Cox regression for subjects with recovery-based T-wave alternans (TWA) < 60 µV and heart rate recovery (HRR) > 18 beats/min (the upper curve in both panels), TWA ≥ 60 µV or HRR ≤ 18 beats/min during recovery (the middle curve), and TWA ≥ 60 µV and HRR ≤ 18 beats/min during recovery (the lower curve) for (a) all-cause mortality and (b) cardiovascular mortality. Note that the scale for the y-axis is from 0.75 to 1.00 (Study **III**).

**Table 10**. Results of Cox multivariable regression analysis (N=1,972; Study **III**).

| | All-cause mortality | | | | Cardiovascular mortality | | | |
|---|---|---|---|---|---|---|---|---|
| | HR | 95 % CI Lower | 95 % CI Upper | p | HR | 95 % CI Lower | 95 % CI Upper | p |
| HRR≤18 beats/min and exercise-based TWA≥60μV | 5.0 | 2.1 | 12.1 | <0.01 | 12.3 | 4.3 | 35.3 | <0.01 |
| HRR≤18 beats/min or exercise-based TWA≥60μV | 2.8 | 1.8 | 4.3 | <0.01 | 3.4 | 1.8 | 6.6 | <0.01 |
| HRR and recovery-based TWA≥60μV | 6.1 | 2.8 | 13.2 | <0.01 | 8.0 | 2.9 | 22.0 | <0.01 |
| HRR or recovery-based TWA≥60μV | 2.3 | 1.5 | 3.5 | <0.01 | 2.2 | 1.2 | 4.2 | 0.01 |
| ST segment deviation (0.1mV) during exercise | 1.3 | 0.9 | 1.9 | 0.18 | 1.8 | 1.0 | 3.0 | 0.04 |

Results after adjustment by sex, age, body-mass index, smoking (yes/no), use of β-blockers (yes/no), the reached maximum heart rate, as well as prior diagnoses of coronary heart disease (yes/no), myocardial infarction (yes/no), diabetes (yes/no), and hypercholesterolemia (yes/no). CI = confidence interval; HR = hazard ratio; HRR = heart rate recovery; TWA = T-wave alternans.

### 10.7.3 Exercise capacity, heart rate recovery, and T-wave alternans

The linear model that contained all three study parameters predicted cardiovascular mortality significantly better than the model without METs (p<0.001), without HRR (p=0.002), or without TWA (p=0.01; Table 9). Therefore, the prediction of cardiovascular mortality was enhanced when each of the parameters was added to the model.

The combination of the three parameters by the Cox regression analysis identified 7 (METs <6, HRR ≤12 beats/min, and TWA ≥60 μV) to 171 (METs <12, HRR ≤21 beats/min, and TWA ≥40 μV) patients whose risk for cardiovascular death was significantly elevated (Fig. 11). The highest adjusted hazard ratio (16.5, 95% CI, 4.0–67.7, p<0.001) for cardiovascular death was achieved with the cut-off points of 60 μV for TWA, 12 beats/min for HRR, and 6 units for METs. The risk thus rose eight-fold when TWA level was increased from ≥40 to ≥60 μV (Fig. 11), with a C statistic of 0.719 (95% CI, 0.665–0.772). The adjusted hazard ratio for cardiovascular mortality for the combination of the three parameters with the previously reported cut-off points of <8 METs, ≤18 beats/min for HRR, and ≥60 μV TWA was 5.7 (95% CI, 1.8–18.2, p=0.003). The absolute event rates for 1,000 person years are given in Figure 12.

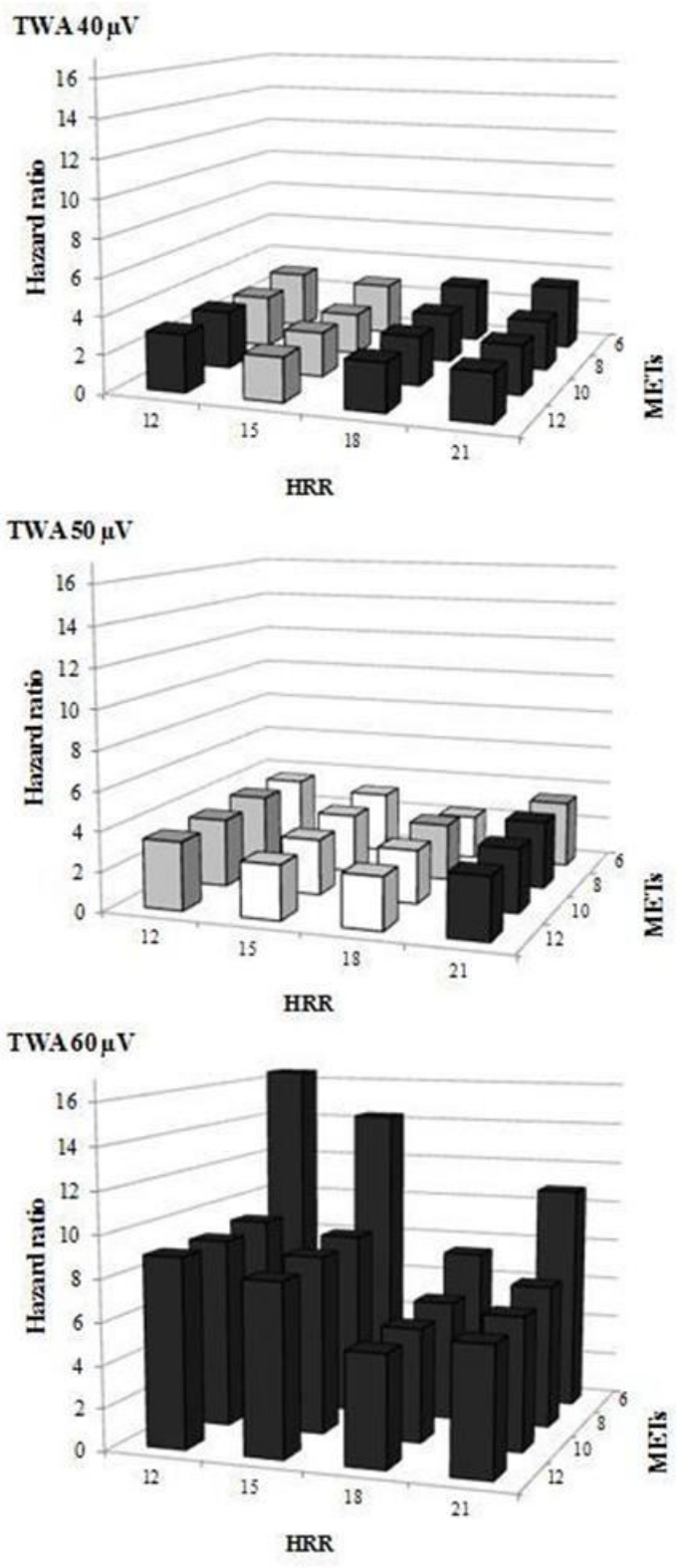



**Figure 11.** Y-axis represents the adjusted hazard ratio for cardiovascular mortality from Cox regression analysis of poor exercise capacity in metabolic equivalents (METs), low heart rate recovery (HRR) after exercise, and high T-wave alternans (TWA) levels at different cut-off points. The color of the bar indicates the level of significance: dark grey ($p<0.01$), light grey ($0.01\leq p<0.05$), and white ($p\geq 0.05$; Study **IV**).

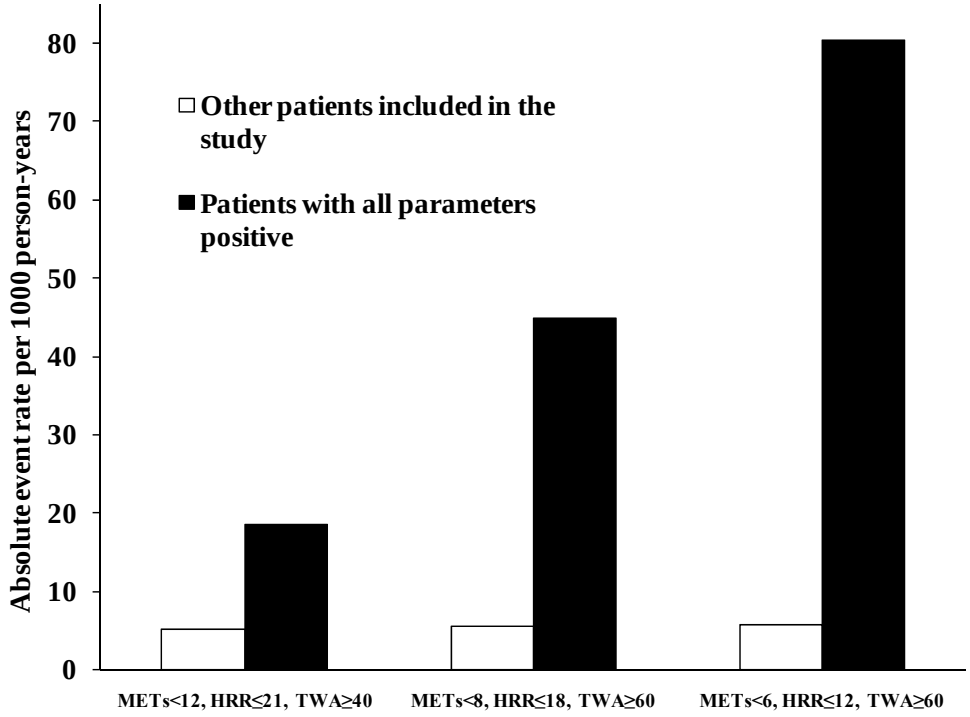



**Figure 12.** The absolute event rates for cardiovascular mortality per 1,000 person years among patients according to exercise capacity in metabolic equivalents (METs), heart rate recovery (HRR), and T-wave alternans (TWA; Study **IV**).

# 11 DISCUSSION

## 11.1 General aspects

Randomized controlled trials and the systematic reviews relying on them are considered as the golden standard in medical research; they are graded at the top of the hierarchy of evidence lists (Glasziou et al. 2004). However, observational cohort studies also form an important source of evidence in medical research (von Elm et al. 2007). Moreover, they can answer the questions concerning the etiology, diagnosis, prognosis, or adverse effects of treatment that are not possible or not necessary to evaluate with randomized controlled trials. Occasionally, even case reports produce scientifically important information of uncommon adverse effects or treatment of very rare diseases. Therefore, different types of research are needed to respond to different types of research problems.

In 2009, the American Heart Association published a scientific statement concerning the evaluation of novel markers of cardiovascular risk (Hlatky et al. 2009). They suggested that the evaluation process of a novel risk marker should consist of six phases analogous to the three to four phases used in the development of a new drug. Hence, different types of study settings are needed on different phases.

Firstly, the novel risk marker has to be demonstrated to separate individuals according to the outcome. For instance, are TWA levels higher in patients suffering cardiovascular events during follow-up than in others? Several other questions also have to be answered, the second of which is: does the novel risk marker have a predictive capacity for hard future events (i.e., cardiovascular death or ventricular arrhythmias)? Thirdly, does the novel risk marker add predictive information on established risk markers? Fourthly, what is the clinical utility of the novel risk marker? For instance, does the TWA testing change the individual risk enough to move patients in the high-risk group, and, furthermore, to change the recommended therapy? Lastly, does the use of the novel risk marker improve survival of clinical outcomes tested in randomized clinical trials, and, moreover, is the use of the risk marker cost-effective enough to justify the additional costs of testing and treatment?

Hlatky and co-workers (2009) also suggested the adoption of different approaches when reporting on novel risk markers. In addition to hazard ratios and absolute event rates, the discrimination in terms of the C-index with its confidence limits should also be reported. The C-index is a variation of the ROC curve that takes into account the different follow-up times of study participants (Cook 2007). For example, the value of 0.65 in a model that contains only exercise capacity in METs means that there is a 65% probability that a case has lower exercise capacity than a non-case. The use of C-index as a sole measure of risk prediction utility of a novel marker has been criticized, because especially in low risk-populations, the rank order used by the C-index does not take into account the distribution of different risk classes (Cook 2007). For example, a population-based cohort may have a small proportion of patients at high risk and more patients at a low or even very low risk. The rank for 2 patients at low risk (i.e., risk for event 1.2% versus 1.4%) may be the same as for 2 patients who are at a moderate versus high risk (i.e., risk of death 7% versus 18%). Hence, the use of different approaches when accounting the utility of novel risk markers is essential and, furthermore, a close cooperation between clinicians and biostatisticians has been recently suggested to enhance the statistical approaches used in risk stratification studies (Goldberger et al. 2011).

One principal approach in non-invasive risk stratification is combined analysis of different risk factors in multivariable models and their utilization in creating composite risk algorithms or scores. It has been suggested that the prediction for cardiovascular events can be improved by using several variables in combinations (Redwood et al. 1997). Moreover, multivariable risk models are frequently used in observational studies to estimate the effect of a single factor with uncontrolled confounding factors or known predictive factors (Harrell et al. 1996). It is essentially important to validate the prediction capability of the combination of parameters in independent cohorts (Hlatky et al. 2009). It has also been suggested that in the studies for multifactorial prediction, the dichotomy limits of a single parameter should be derived in respect to other studied parameters, and that the cut-off points derived from studies including single parameters should not be used as such (Redwood et al. 1997).

## 11.2 Study population

This study is a part of the FINCAVAS (Nieminen et al. 2006) which enrolled patients attending an exercise stress test at Tampere University Hospital who were willing to participate in the study. Between October 2001 and December 2008, over 4,000 patients have been recruited.

Because of the selection criteria, there is great variety in the patient population. This is one of the strengths of FINCAVAS, as the population demonstrates a genuine clinical group of patients with diverse characteristics as seen in the real life of physicians all over the world. On the other hand, the multiformity of the population is challenging because the different subgroups (i.e., women, patients with CHD, post-MI patients, etc.) have the most likely different risk profiles and the study parameters may also have different prognostic capabilities from one patient group to the other. This was evident in the present study when it was discovered that the combination of exercise capacity and TWA was not predictive in subgroup analysis in women (Study **II**). This finding may be related to the smaller number of events in that specific subgroup. Although the FINCAVAS population is large, the small amount of events has limited the possibility for subgroup analyses. The study enrolment has already concluded, but the longer follow-up time in the future may also make the subgroup analyses possible.

The main indication for a clinical exercise test in Finland is a suspicion of CHD, as was also the case in our population (47%, Study **IV**). Moreover, in Finland the exercise tests are performed in various healthcare settings, such as in primary health centers, private clinics, local and central hospitals, as well as university hospitals. It is obvious that the patients referred for exercise testing in these facilities differ from one population to the other. The FINCAVAS population was recruited at a university clinic, which may have lead to an over-representation of some group of patients, such as those tested prior to an invasive procedure (9%) or after MI (8%).

It is also noteworthy that the only follow-up data available in FINCAVAS are the death certificates. There have not been any follow-up visits and, therefore, we do not have information on subsequent changes in parameters affecting mortality risk (e.g., smoking, lifestyles, and medication) during the follow-up. This has to be kept in mind when interpreting the results. Moreover, the ejection fraction data is available for approximately 55% of patients in Studies **I**, **II,** and **III,** as well as for 29% of patients in Study **IV**. The coronary angiography data was available for 602 patients (Study **IV**).

## 11.3 Endpoints

The endpoints used in this study were all-cause and cardiovascular mortality as well as SCD. Because the only follow-up data used in FINCAVAS are the death certificates, the use of other endpoints (e.g., ventricular arrhythmias, MI, pacemaker implantation, hospitalization) was not

possible. Establishing definitively that an event is cardiovascular death or especially SCD is inherently challenging (Huikuri et al. 2001). In the present study, the classification for different death categories was made by two independent experts based on the certificate texts that have previously been shown to be a reliable source (Pajunen et al. 2005). The certificate texts were based on autopsy information in approximately 40% of all cases, and in 60% of the patients who suffered an SCD (Study **II**). It is possible that an independent event committee having more information than death certificates would have lead to a more accurate determination of the causes of deaths with regard to specific death classes. However, that was not planned in the study design (Nieminen et al. 2006), and, moreover, all-cause death was also used as an endpoint based on its precise nature.

In this study, the definition of SCD was death within 24 hours after the onset of symptoms. It has been reported that the 24-hour limit leads to an overestimation of the true SCD incidence, whereas the other commonly used definition, the 1-hour limit, underestimates the actual event rate (Adabag et al. 2010). In FINCAVAS, 1.9% of all patients suffered an SCD during follow-up (Studies **I**, and **II**). The proportion of all deaths that were sudden was 34% and of cardiovascular deaths 47% in Study **IV** and up to 56% in Studies **I**, **II**, and **III**. The majority of deaths that were classified as SCD in Study **II** were caused by acute coronary events, which have been shown to be triggers of ventricular tachyarrhythmias leading to SCD (Huikuri et al. 2001). There were no signs of pulmonary embolism or pulmonary edema in the autopsy information on patients with SCD (Study **II**).

## 11.4 T-wave alternans

### 11.4.1 Methodological issues

There is growing evidence available to support the finding that TWA, when measured with the MMA method, is associated with an increased risk of mortality. However, the criteria for a positive TWA test have not been validated. The other commercially available method, namely the spectral method, for TWA analysis has been well validated. However, as reviewed in the *Review of the literature* section, the evidence behind the criteria is sparse and has not been critically viewed after their publication (Bloomfield et al. 2002a).

#### *11.4.1.1 Over-reading*

TWA analysis with the MMA method allows the investigator to inspect visually the superimposed QRS-aligned complexes that show the alternation. In the present study, the automatically derived TWA values were used in Studies **I**, **II,** and **III,** and they were visually inspected and corrected when equal to or over 46μV in Study **IV**. TWA had prognostic value in all the sub-studies, but the effect of the over-reading itself remains to be determined. Moreover, the MMA-based TWA has been demonstrated to be prone to noise and, therefore, to overestimate the actual TWA levels (Selvaraj and Chauhan 2009). Hence, all TWA values used in research or in future in a clinical decision-making should be over-read.

#### *11.4.1.2 Patients with atrial fibrillation and flutter*

Patients with AF have typically been excluded from clinical TWA studies. TWA measurement with the spectral method requires stationary data that is difficult to achieve in patients with irregular R-R intervals such as in patients with AF. However, it has been speculated that AF does not hinder TWA measurement with the MMA method, but there is no data available concerning TWA in patients with AF and, especially, its prognostic capacity in that specific subgroup. Interestingly, Narayan et al. (2011) showed recently that atrial APD alternans precedes AF in an atrial pacing study with 33 subjects (12 with persistent AF, 13 with paroxysmal AF, and 8 controls with no known AF). As the authors concluded, atrial APD alternans may expose a dynamic substrate for AF. However, no information about the ventricular APD alternans or TWA was available.

In the present investigation, the patients with AF or flutter were included in Studies **I** and **II** and excluded from Studies **III** and **IV**, because patients with AF or flutter have been excluded from the previous HRR studies. TWA had prognostic power in all these substudies. No comparison between the patients with AF and flutter was made. However, in our population of patients (N=3,747), there was a significant difference in the median TWA value between the patients in sinus rhythm (23, 16–31 IQ, N=3,667) and in AF or flutter (N=164; 43, 35–57.75 IQ, $p<0.001$ from the Mann Whitney U-test). It is known that patients with AF or flutter have a worse prognosis (Levitt and Coplan 2009) than patients in sinus rhythm. Hence, as it seems that AF has an effect on TWA values by itself, it is possible that the inclusion of patients with AF in TWA studies may bias the results, including ours (Studies **I** and **II**). Therefore, before there is

evidence about the association between TWA and AF, patients with this type of arrhythmia should also be excluded from the TWA studies using the MMA method.

#### *11.4.1.3 Phases*

In the prior FINCAVAS study, TWA was measured with the MMA method over the entire exercise test from rest to recovery (Nieminen et al. 2007). In the present dissertation, TWA measured during the exercise phase of the clinical exercise test seemed to have superior prognostic capacity especially for SCD when compared to the pre- or post-exercise phases in Study **I** and to the post-exercise phase in Study **III**. However, no direct statistical comparison was made, and the corresponding CIs overlapped. Nevertheless, TWA measured either during the pre- or during the post-exercise stage did not significantly predict SCD (Study **I**). That maybe due to the fact that in order to expose latent electrical ventricular instability, a provocative challenge such as exercise is needed. Estes and co-workers (1997) studied 27 patients referred for EPS—in their population, six patients were TWA positive at rest, only one of whom became negative during exercise, whereas six of the patients who were TWA negative at rest became positive during exercise. TWA measured either during exercise ($p<0.01$) or during rest and exercise ($p<0.003$) was predictive for inducible arrhythmias during EPS, but TWA analyzed at rest was not ($p<0.08$). Nonetheless, the TWA measured at rest tended to be a highly specific marker (88%) for arrhythmias but lacking sensitivity (44%).

In our study, the pre-exercise-measured TWA strongly predicts all-cause and cardiovascular mortality (Study **I**). There are at least two possible explanations behind this somewhat surprising finding. In previous studies, it has been shown that mental stress can induce TWA (Kop et al. 2004, Lampert et al. 2005) and, moreover, that anger-induced TWA predicts arrhythmic events in patients with ICDs (Lampert et al. 2009). In our exercise protocol, the pre-exercise phase of the test started when the patients sat on the bicycle and are thus physically at rest. However, some degree of mental stress and, furthermore, sympathetic nerve activity were evident especially at elevated heart rates (from 63±12 to 71±14 beats/min, $p<0.001$ in paired T-test) but also in blood pressures (from 136±19 to 139±22 mmHg, $p<0.001$) over the pre-exercise phase. Although there was a raise in the heart rates in the supine position, the actual heart rates were relatively low, indicating that the sympathetic activation, and not only the heart rate itself, also provokes TWA.

Secondly, it is possible that the inclusion of the patients with AF biased the results at the pre-execise phase. As discussed above, the TWA levels in patients with AF were higher than in patients with sinus rhythm during the exercise phase. Moreover, it is probable, albeit speculative, that similar results or an even bigger difference would be found during the pre-exercise phase.

TWA measured during the post-exercise period was predictive for all-cause and cardiovascular death in our population of patients referred for exercise testing (Studies **I** and **III**). However, it also lacked significance considering the predictivity for SCD (Study **I**). In a previous study of 681 FINCAVAS patients with CHD together with 322 post-MI patients from the REFINE cohort, post-exercise-based TWA was also predictive of SCD in secondary analysis (Slawnych et al. 2009). Moreover, the noise values of the FINCAVAS patients during exercise (median value of 8μV) were significantly ($p<0.001$) higher than during post-exercise (5μV). In the ROC analysis, the area under the curve tended to be higher during post-exercise-based TWA than during exercise-measured TWA when cardiovascular mortality was an endpoint. However, the ROC analysis was misused, based on the fact that it does not take in account the different follow-up times of each participant. Because the noise values during exercise are higher than during the post-exercise phase mainly because of the motion artifacts, it is possible that the post-exercise TWA measurement with the MMA method has some benefits. On the other hand, it has to be noted that no over-reading was performed in the studies discussed above, including ours (Studies **I** and **III**). Hence, future studies are needed to evaluate and compare the prognostic significance of exercise-based and post-exercise-based TWA.

#### *11.4.1.4 Leads*

In the MMA-based TWA study by Nieminen et al. (2007), all 12 leads were used for TWA measurement. In the present study, limb leads were excluded from the analyses in Studies **I**, **II**, **III,** and, **IV**, because the precordial leads have been shown to be optimum for TWA measurement (Nearing et al. 1994, Martinez et al. 2006). Furthermore, limb leads are prone to significant motion artifact, as confirmed by the visual inspection of the templates of superimposed ECGs in the GE Healthcare system. This finding was confirmed in the current study (**data addition**) when the limb leads were also analyzed in the population of patients examined in Study **I**. During exercise, the limb leads did not have any prognostic power (**data addition**). Hence, the limb leads should be excluded from TWA analyses during exercise.

Whether or not the measurement of limb leads add to the prognostic power of precordial TWA during pre-or post-exercise phase needs to be determined.

In Studies **I**, **II**, **III,** and **IV** the maximum TWA value in any of the precordial leads was selected and used for risk stratification. Thus, the same cut-off point was used in all leads. Stein and co-workers (2008) studied 46 non-survivors and 92 matched controls with AECG-based TWA and found that different cut-off points, namely 43 μV and 47 μV in leads V1 and V3, respectively, maximized the survival difference. Interestingly, the risk was further increased when risk information from leads V1 and V3 were combined, suggesting that the different leads should have a different cut-off value. It may be also possible that optimizing the cut-off points separately in every precordial leads could also improve the prognostic power of TWA when measured during exercise testing.

Recently, Leino and others (2011) published an interesting study with nearly 3,600 FINCAVAS patients. They evaluated the prognostic power of TWA separately in every precordial lead as well as in a selection of lead combinations. They concluded that TWA measured in anterolateral lead V5 is the strongest predictor for cardiovascular mortality and SCD. It was the only lead that significantly predicted all-cause and cardiovascular mortality as well as SCD. However, the results for the lead combinations (i.e., V1–V6; V2–V6; V3–V6; V4–V6; V5 and V6; V3–V5; V4 and V5) were highly comparable to the results with V5, and the corresponding CIs highly overlapped. On the other hand, if TWA is measured from only one lead, a great amount of information is lost by excluding the others. Moreover, it seemed that the exclusion of lead V1 improves the prediction of TWA, although the corresponding CIs were again highly overlapping. Therefore, more information is needed concerning the optimal lead selection for TWA assessment. Nonetheless, the limb leads should be excluded from TWA measurement during exercise.

#### *11.4.1.5 Cut-off points*

In our study, the previously used TWA cut-off point of 65μV (Nieminen et al. 2007) was a highly significant risk marker also in this expanded database of over 2,000 participants (Studies **I** and **II**). It was originally developed in the first FINCAVAS study with ≈1,000 patients, yielding the highest hazard ratio in the Cox regression analysis (Nieminen et al. 2007). TWA was measured over the entire exercise phase, and a maximum value from the standard 12 lead set was derived. Despite the fact that the TWA values used in the present study were from the precordial

leads only and analyzed separately during the three phases of the stress test, the cut-off point of 65μV clearly has capacity to separate patients according to the risk status.

In Study **I,** the cut-off point of 90μV yielded the highest hazard ratios for all-cause and cardiovascular mortality, whereas the cut-off point of 60μV was the strongest significant predictor for SCD. It therefore seems that the cut-off point of 60μV–65μV had a strong association especially with SCD when TWA was measured during the exercise. In the study by Slawnych and Nieminen and others (2009), the ROC analysis of the REFINE patients showed that the sensitive cut-off point of 20μV and the more specific cut-off point of 60μV were optimal for predicting cardiovascular mortality when TWA was measured during the post-exercise period. These cut-off points were also studied in the current Study **III,** and the TWA cut-off point of 60μV significantly predicted cardiovascular mortality during exercise as well as during the recovery phase. The cut-off point of 20μV did not have an independent association with mortality. However, in all these studies, the automatically derived TWA value was used and no visual correction was therefore made. It is probable that the optimal TWA cut-off point when derived during exercise, but also during post-exercise, is lower than the cut-off point discussed above, when TWA is visually inspected and corrected. Nevertheless, the cut-off point of 65μV was also a highly significant predictor in an AECG-based prospective TWA study where the TWA values were visually inspected and corrected if data was not available due to noise and artifacts (Sakaki et al. 2009).

In summary, future studies are needed to evaluate the optimal cut-off point when TWA is analyzed with the MMA method and the TWA values are visually over-read. Meanwhile, the cut-off point of 60–65μV may be used in clinical TWA studies, but not without a degree of uncertainty.

#### *11.4.1.6 Quantification*

In clinical medicine, it is typically necessary to have a decision threshold, i.e., a cut-off value for a test, especially in the field of diagnostics but also in prognostics. For instance, a clinician needs to know at which TWA levels the risk for SCD is so great that more examinations are required. However, when continuous information is dichotomized, information is always lost. In this study, we discovered that the quantification of TWA enhances it prognostic capacity, as we showed that the risk for all-cause and cardiovascular mortality as well as SCD increases with the magnitude of TWA (Study **I**). This was especially well-defined when TWA values were

measured during the exercise. TWA was also associated with increased risk when analyzed as a continuous variable, demonstrating that there is some sort of linear relationship with TWA magnitude and increased mortality (Study **I**). The same was also found in Study **IV** where the TWA values over 46μV were visually inspected and corrected.

It has also been demonstrated in the experimental studies (Smith et al. 1988, Nearing et al. 1991, Nearing et al. 1994, Nearing and Verrier 2002b) and on AECG- and ICD-based studies on humans (Shusterman et al. 2006, Swerdlow et al. 2011) that TWA magnitude increases before the onset of VF. Studies applying the spectral method have also shown that TWA magnitudes derived during pacing (Tanno et al. 2004) or exercise (Klingenheben et al. 2005) are higher in patients who experience arrhythmic events during follow-up. Therefore, together with the findings of our study, the evidence supports that TWA could be considered more as a continuous risk index in the future.

#### *11.4.1.7 Heart rate limit*

TWA has been thought to be a rate-dependent phenomenon (i.e., increasing hear rate provokes and elevates TWA levels; Cutler and Rosenbaum 2009). This is based on the possible underlying pathophysiological mechanisms such as APD and conduction velocity restitution as well as $Ca_i$ cycling leading to the development of TWA, as reviewed earlier. It has been shown that TWA, when measured with the spectral method, can occur in normal children aged 8 to 17 years at heart rates exceeding 120 beats/min (Cheung et al. 2001). Gibelli and others (2008) studied eight healthy trained subjects with the MMA method and found out that there was no TWA present at rest but that TWA (19 to 27 μV) was provoked in all the participants during exercise at a heart rate of less than 125 beats/min. Furthermore, Tanno et al. (2004) reported that patients with elevated TWA ($V_{alt}$ >1.9 μV) at a heart rate of 90 beats/min suffered more frequently from VT, VF, or SCD than those with elevated TWA levels at 100, 110, or 120 beats/min when TWA was measured with the spectral method during pacing.

In this study, the heart rate limit of <125 beats/min was used in the TWA measurement in Studies **I**, **II**, and **III**. However, no heart rate limit was used in Study **IV**, where the TWA values over 46 μV were over-read by physician. In all the substudies, elevated levels of TWA were independently associated with increased risk. As discussed earlier, there are many confounding factors that have an effect on the results of the current study (**I**, **II**, **III**, and **IV**). It is therefore not possible to make any conclusions about the need or non-need of a heart rate limit in MMA-based

TWA assessment. Moreover, it is evident that TWA is dependent on heart rate, but the effect of the heart rate on the prognostic utility of TWA needs to be studied thoroughly in the future, especially when TWA is measured with the MMA method that enables TWA assessment during fluctuating heart rates.

### 11.4.2. T-wave alternans as a risk marker

The TWA predicts all-cause and cardiovascular mortality as well as SCD in our population of patients referred for exercise testing (Studies **I**, **II**, **III** and **IV**). Moreover, it has independent prognostic capacity for cardiovascular mortality in more than 3,600 patients (Table 9). However, when the prognosis for cardiovascular mortality was assessed with a C-index in the expanded database, TWA failed to reach significance (Table 8, Study **IV**). This may reflect the fact that the C-index may underestimate the predictivity especially in a low risk population (Cook 2007).

## 11.5 Exercise capacity

The present study demonstrated that reduced exercise capacity in terms of METs predicts SCD in a general population of patients referred for exercise testing. Having METs less than eight conveys a roughly nine-fold risk of SCD (Study **II**). This finding was recently confirmed in a population-based follow-up study with 42 to 60 years old men (Laukkanen et al. 2010).

In our expanded database with over 3,600 FINCAVAS patients, exercise capacity was found to be a highly significant marker for cardiovascular mortality (Table 9) also when analyzed as a continuous variable with the C-index of 0.648 (Table 8; Study **IV**). The current evidence, based on the results of the present study and the available literature, clearly shows that exercise capacity has to be taken into account when defining a patient's risk for cardiovascular events, including SCD. The pathophysiologic basis of the predictivity of exercise capacity in METs is discussed below.

## 11.6 Heart rate recovery

The present study confirms the findings of a previous publication (Watanabe et al. 2001a) in that reduced HRR (≤ 18 beats/min) significantly predicts all-cause and cardiovascular mortality in an

exercise test with an abrupt end (Study **III**). It also discriminates well patients at risk for cardiovascular mortality as defined with C-indices (Table 8). Furthermore, when HRR was measured as a continuous variable, the risk for cardiovascular mortality was significantly increased (Study **IV**; Table 9).

Cole et al. (1999) studied 2,428 patients with no history of cardiac failure or revascularization and found that reduced HRR was the strongest predictor of death in multivariable analysis including exercise capacity. However, in our population of patients referred for exercise testing, the exercise capacity in METs was found to have stronger prognostic power than HRR (Table 9; Study **IV**).

## 11.7 Combination of exercise test variables

### 11.7.1 Exercise capacity and T-wave alternans

The literature provides clues supporting the rationale for combined analysis of exercise capacity with TWA. Tapanainen and co-workers (2001) demonstrated with post-MI patients that the inability to exercise or reach the target heart rate of >105 beats/min for one minute, as required for spectral TWA testing, was in itself predictive of all-cause mortality (hazard ratio 9.28, 95% CI 1.99–43.30, $p<0.01$). More recently, Baravelli and others (2007) prospectively studied 70 patients with idiopathic dilated cardiomyopathy who underwent symptom-limited cardiopulmonary exercise testing with $VO_2$ recording as well as TWA alternans testing with the spectral method and found that only the combination of peak $VO_2$ uptake and TWA significantly predicted cardiac mortality and ventricular arrhythmias (hazard ratio 0.28, 95% CI 0.12–0.95, $p=0.03$) but not either of the parameters alone.

In the present study, we discovered that the prognostic capacity for cardiovascular mortality is enhanced with the combination of reduced exercise capacity and elevated TWA (Study **II**). When low exercise capacity (METs < 8) was combined with elevated TWA ($\geq$ 65 μV), the risk of SCD increased 36-fold when compared to patients with neither factor. As seen in Figure 8, the survival for all-cause and cardiovascular mortality as well as SCD was markedly reduced in patients with either and, especially, both of the parameters abnormal.

### 11.7.2 Heart rate recovery and T-wave alternans

The potential to improve the prediction of cardiovascular and all-cause mortality by combining TWA with another AECG-based autonomic marker, namely HRT, was first announced in 2007 in the REFINE study (Exner et al. 2007). Our study demonstrated that the presence of high levels of TWA during exercise or recovery significantly adds the prognostic strength of reduced HRR for all-cause and cardiovascular mortality in a population of patients referred for exercise testing (Study **III**).

When analyzed together, TWA and HRR provide high hazard ratios for all-cause death and cardiovascular mortality after adjustment for standard risk factors when analyzed in comparison to patients with neither factor (Table 10). The combination of reduced HRR with heightened TWA was superior to exercise-induced ST-segment deviation using Cox proportional hazards models (Table 10) and Harrell's C-indices (Table 8).

### 11.7.3 Exercise capacity, heart rate recovery and T-wave alternans

This study demonstrates that the combination of poor exercise capacity, low HRR after exercise, and high levels of TWA during exercise improves the prognostic strength of a clinically indicated exercise test. Adding METs, HRR at 1 minute after the exercise, or TWA to the linear Cox regression model significantly improved the predictive capacity for cardiovascular mortality (Study **IV**; Table 9).

Patients with poor exercise capacity, low HRR, and high TWA were at a 5 to 16-fold higher risk for cardiovascular death when the TWA cut-off point of 60 μV was reached (Fig. 11). The figure indicates that the risk is elevated in a large variety of combinations of different cut-off points as well as with the previously used cut-off points. However, no specific approach was adopted to find optimal cut-off values, as is sometimes suggested (Redwood et al. 1997), since we preferred using pre-established cut-off points. Moreover, it would have been essentially important to validate the results in another cohort if the cut-off points had been optimized in the present study. The absolute events rates were clearly higher in patients with all three parameters abnormal than in other patients included in the study (Fig. 12). However, the absolute numbers of patients who had all three parameters abnormal concerning the different cut-off values was fairly low (i.e., 7–171 patients). It is possible that some other cut-off points or even different weighting factors for each parameter could lead to more clinically useful figures. On the other

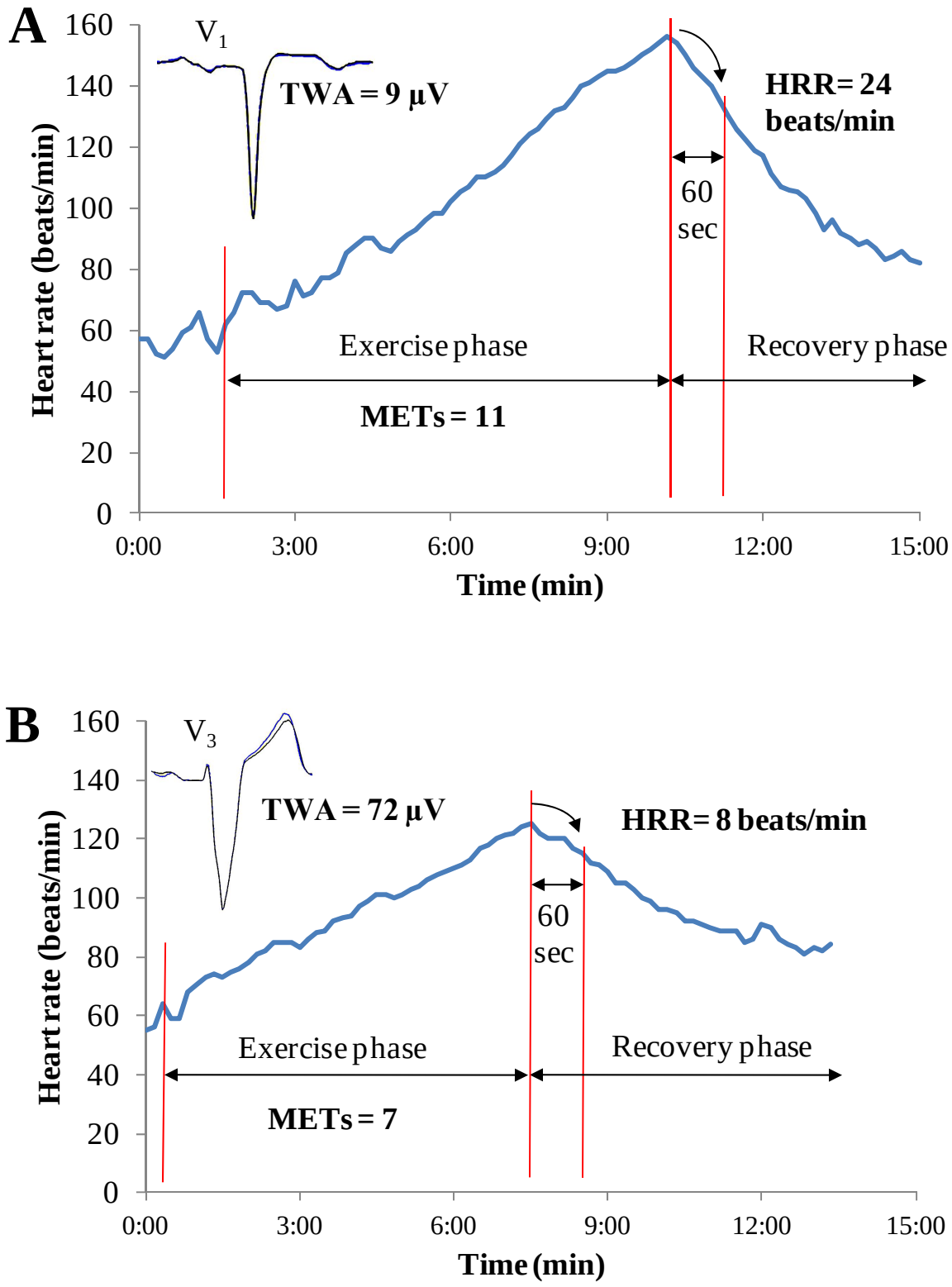



**Figure 13**. Schema about the relationship between exercise capacity in metabolic equivalents (METs), heart rate recovery (HRR), and T-wave alternans (TWA). Panel A shows the course of the exercise test of a 61–year-old man who survived the follow-up. Panel B shows the course of the exercise test of a 69–year-old man who died a cardiovascular death at 27 months during the follow-up (Study **IV**).

hand, two of the seven patients whose exercise capacity in METs was <6, HRR ≤ 12 beats/min, and TWA ≥ 60μV experienced cardiovascular death during follow-up. If those deaths could be prevented with extensive treatment and preventive strategies, the combination of exercise capacity, HRR, and TWA would be a potentially useful risk stratification tool.

The C statistic of 0.719 (95 % CI 0.665–0.772) for cardiovascular death improved with a combined analysis of these three factors when compared to the results for each of them separately, and to the combined analysis of TWA and HRR in Study **III** (Table 8). However, no statistical comparison was made and the corresponding CIs overlapped. The C statistic of 0.719 also compares well with the area under the ROC curve of other noninvasive markers, such as deceleration capacity, which has been found to be 0.740–0.80 for all-cause mortality (Bauer et al. 2006) and HRT, which has been found to be 0.66 for cardiac death or resuscitated cardiac arrest (Exner et al. 2007).

The mechanistic basis for the improvement in the prediction resulting from a combined analysis of the mentioned variables may be due to the fact that these parameters together provide a more complete picture of the contributions of abnormal mechanical function, depressed autonomic activity, and cardiac electrical instability to cardiovascular risk. Moreover, these three parameters, namely exercise capacity in METs, HRR and, TWA, give a multifaceted view about the severity of the potential heart disease. The schema about the relationship between the three study variables is seen in the Figure 13. Exercise capacity indicates cardiovascular, pulmonary, and neural function, in addition to reflecting muscular strength. Reduced exercise capacity may be caused by dysfunction in any of these components. Increases in heart rate, arterial blood pressure, or cardiac output may fail to meet metabolic requirements. Pulmonary capacity may be decreased or the neural response to exercise may be inadequate when the central nervous system's control of the autonomic nervous system is imbalanced (Balady et al. 2010). HRR especially is thought to reflect the dynamic interplay between sympathetic and parasympathetic nerve activity as influenced by baroreceptor gain (La Rovere et al. 2002). Low HRR reflects impaired vagus nerve activation and depressed capacity to withdraw sympathetic nerve tone (La Rovere et al. 2001), both of which are conditions that predispose to arrhythmias (Kolman et al. 1975). TWA indicates the presence of abnormal repolarization and electrophysiologic inhomogeneities that underlie vulnerability to VF (Narayan 2006).

## 11.8 Study strengths and limitations

The leading strength of the present study is its size. Even in a worldwide perspective, FINCAVAS is one of the largest, if not the largest, exercise test databases with continuous ECG signal. Moreover, the FINCAVAS population represents a real clinical group of patients referred for exercise testing in a university hospital. The results demonstrate the risk of a wide spectrum of patients and could thus be used as a source of hypotheses tested in more specific future patient populations.

In the present study, TWA was analyzed with the MMA method, which enables TWA measurement during a routine symptom-limited exercise test that is performed millions of times annually worldwide with no special protocols or electrodes. The number of patients with an unsuccessful TWA measurement in the present study (≈5% in Study **IV**; Fig. 5) was highly comparable to the rate of technically inadequate tests (≈3-6%) and markedly lower than the proportion of indeterminate TWA tests (≈9-47%) in series where TWA has been assessed with the spectral method (Bloomfield et al. 2002a, Kaufman et al. 2006, Chan et al. 2007). Therefore, TWA measured with the MMA method carries great potential in terms of risk stratification as it can be integrated in the standard daily practise of physicians with only moderate extra cost and time. In particular, the MMA method allows the simultaneous combined assessment of TWA with other cardiac risk markers derived from the routine exercise test.

On the other hand, one limitation of FINCAVAS is the moderate number of patient exclusion due to technical reasons. In Study **IV,** the drop–out rate was ≈7% of those recruited (Fig. 5). One possible reason for this is the fact that the data collection was made alongside usual clinical practice. This may have biased the results in either way. However, the proportional numbers of events for those who were excluded due to technical reasons were highly comparable to those investigated in Study **IV** (data not shown). Moreover, the diversity of the FINCAVAS patient population makes it impossible to extrapolate the results to any specific group of patients, such as those with CHD, reduced LVEF, or post-MI patients.

The number of endpoints, especially in the cardiovascular death and SCD categories, is fairly low, and because no power calculations were made at the time of study design or subsequently, some of the analyses may have been underpowered. Hence, the true risk has possibly been underestimated. Furhermore, it has been recommended that the C-index should be calculated separately for the model containing established risk markers and for the model including the studied parameter (Hlatky et al. 2009). It may have provided a better picture of the overall predictive capacity of the risk markers if the C-index had been calculated for the whole model.

However, because the main idea behind the FINCAVAS study was to produce new information about the prognostic variables and, therefore, to work more as a factory for new hypotheses than to produce information that could be readily incorporated into clinical practice, the C-indices were calculated separately for each study variable to allow direct comparison.

Lastly, we do not have information on the changes in the parameters affecting the risk during the follow-up, such as smoking, revascularizations, and medications. The hazard ratios were analyzed with the Cox regression model that assumes that the study variables are constant over time. It is plausible to speculate, however, that the net effect of patients changing their lifestyles for the better or worse would be close to zero in respect to the study variables in such a large population as FINCAVAS.

# 12 SUMMARY AND CONCLUSIONS

In conclusion, the evidence derived from our study, together with information derived from experimental and clinical studies, clearly shows that elevated levels of TWA are pathophysiologically linked with increased risk for cardiovascular mortality and that high level of TWA precedes VF.

The study also demonstrated that poor exercise capacity predicts SCD in a population of patients referred for exercise testing in a university hospital. Moreover, it showed that the combination of exercise capacity, HRR, and TWA enhances the prognostic capacity of the exercise stress test. These three parameters that can be measured during routine exercise testing have the potential to improve the risk stratification for cardiovascular mortality and SCD.

Finally, the study provides some answers, albeit raising a few new questions, about the methodological issues related to TWA analysis, especially with regard to the MMA method. Measuring TWA from surface ECG is inherently challenging, and the future will show whether this noninvasive TWA assessment could be incorporated into clinical use or whether, for example, TWA analysis based on cardiac implantable electric devices will eventually break through.

The principal findings and conclusions are:

1. TWA is an independent predictor for SCD as well as for cardiovascular and all-cause mortality in a population of patients referred for exercise testing. Its prognostic ability is enhanced with quantitative analysis (Study **I**).

2. TWA analyzed during a standard exercise phase seems to be superior to TWA analyzed during the pre- or post-exercise phases (Study **I**). TWA measured in the limb leads during the exercise phase has no predictivity (**data addition**).

3. Exercise capacity in terms of METs powerfully predicts the risk for SCD as well as cardiovascular and all-cause mortality. The risk prediction is further increased by a combined analysis with TWA (Study **II**).

4. The combination of HRR and TWA predicts all-cause and cardiovascular mortality (Study **III**).

5. The prognostic capacity of the clinical exercise stress test is significantly enhanced by the combined analysis of exercise capacity, HRR, and TWA (Study **IV**).

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# 15 ORIGINAL COMMUNICATIONS

# Enhanced Predictive Power of Quantitative TWA During Routine Exercise Testing in the Finnish Cardiovascular Study

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**Quantitative TWA and Prognostics.** ***Introduction:*** **We examined whether quantification of T-wave alternans (TWA) enhances this parameter's capacity to evaluate the risk for total and cardiovascular mortality and sudden cardiac death (SCD).**

***Methods and Results:*** **The Finnish Cardiovascular Study (FINCAVAS) enrolled consecutive patients (n = 2,119; 1,342 men and 777 women) with a clinically indicated exercise test with bicycle ergometer. TWA (time domain-modified moving average method) was analyzed from precordial leads, and the results were grouped in increments of 10 $\mu$V. Hazard ratios (HR) for total and cardiovascular mortality and SCD were estimated for preexercise, routine exercise, and postexercise stages. Cox regression analysis was performed. During follow-up of 47.1 ± 12.9 months (mean ± standard deviation [SD]), 126 patients died: 62 were cardiovascular deaths, and 33 of these deaths were sudden. During preexercise, TWA ≥ 20 $\mu$V predicted the risk for total and cardiovascular mortality (maximum HR >4.4 at 60 $\mu$V, P < 0.02 for both). During exercise, HRs of total and cardiovascular mortality were significant when TWA measured ≥50 $\mu$V, with 90 $\mu$V TWA yielding maximum HRs for total and cardiovascular death of 3.1 (P = 0.03) and 6.4 (P = 0.002), respectively. During postexercise, TWA ≥60 $\mu$V indicated risk for total and cardiovascular mortality, with maximum HR of 3.4 at 70 $\mu$V (P = 0.01) for cardiovascular mortality. SCD was strongly predicted by TWA levels ≥60 $\mu$V during exercise, with maximum HR of 4.6 at 60 $\mu$V (P = 0.002), but was not predicted during pre- or postexercise.**

***Conclusion:*** **Quantification of TWA enhances its capacity for determination of the risk for total and cardiovascular mortality and SCD in low-risk populations. Its prognostic power is superior during exercise compared to preexercise or postexercise.** *(J Cardiovasc Electrophysiol, Vol. 20, pp. 408-415, April 2009)*

*ventricular tachycardia, sudden cardiac death, electrocardiography, T-wave alternans*

## Introduction

T-wave alternans (TWA) testing has been employed clinically for more than a decade.[1-4] The most widespread contemporary approach utilizes spectral analysis, in which the presence or absence of TWA is based on a single cutpoint of >1.9 microvolts ($\mu$V) achieved at a heart rate of 105 beats per minute (bpm) during exercise. If TWA does not meet this criterion, the test is deemed "negative." Tests in which the target heart rate is not achieved or excess premature beats or unsustained TWA are present were previously classified as "indeterminate" and are now classified as "abnormal." The current practice does not involve providing the actual TWA values, and thus, the test is essentially qualitative in nature.

Recently, Klingenheben and coworkers[5] explored the possibility that quantitative assessment of TWA magnitude might yield prognostic and pathophysiologic information that would complement this qualitative approach to spectral TWA testing. They found in patients with ischemic or nonischemic cardiomyopathy that the magnitude of TWA was associated with an incidence of tachyarrhythmic complications, which they postulated reflected the extent of myocardial damage. The authors indicated that more extensive studies are

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Mr. Willi Kaiser is an employee of GE Healthcare Information Technologies, Freiburg, Germany. Dr. Tuomo Nieminen reports receiving honoraria from GE Healthcare for travel to Heart Rhythm Society Symposium 2007. Dr. Richard L. Verrier is principal investigator and/or collaborator on grants from the American Heart Association, National Institutes of Health, and Medtronic, Inc., on T-wave alternans. He is co-inventor of patents for T-wave alternans measurement including the modified moving average method, which have been licensed to GE Healthcare. He reports receiving honoraria for lectures related to this topic.

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warranted to determine whether quantitative TWA assessment should be routinely performed to enhance the predictive power of this parameter. The rationale for quantifying TWA is also supported by extensive data from experimental studies indicating that higher TWA levels are associated with an increased likelihood of ventricular tachycardia (VT) and fibrillation (VF).[6-8] In a clinical study, Shusterman and coworkers[9] demonstrated that TWA magnitude is increased in ambulatory ECG records prior to onset of ventricular arrhythmias in patients enrolled in the Electrophysiologic Study Versus Electrocardiographic Monitoring (ESVEM) trial. The value of assessing TWA magnitude is also supported by Stein and coworkers,[10] who found in ambulatory ECG recordings in high-risk post-myocardial infarction (MI) patients with left ventricular dysfunction enrolled in the Eplerenone Post-Acute Myocardial Infarction Heart Failure Efficacy and Survival Study (EPHESUS) study that modified moving average (MMA)-based TWA measurement powerfully predicts sudden cardiac death (SCD).

The main goal of the present study was to test the hypothesis that quantification of TWA magnitude enhances the predictive power of this parameter in a sizeable, full-cohort study of consecutive patients referred for routine exercise testing. We employed the time domain-based, FDA-cleared MMA method for TWA analysis[11] because of its intrinsic flexibility and demonstrated capacity to measure TWA accurately under dynamic conditions including changing heart rates, myocardial ischemia, exercise, and behavioral stress.[9,12-16] The present study builds on our previous investigation of the Finnish Cardiovascular Study (FINCAVAS), in which we found that TWA is a risk marker in patients referred for a routine clinical exercise stress test.[14] The patient population of over 2,000 is nearly double the previous database, rendering the present study as the largest TWA investigation conducted. As these patients typically had a normal ejection fraction, the results are relevant to a sizeable group of individuals whose elevated risk for major cardiovascular events is not disclosed by other contemporary tests.

## Methods

The study cohort, study flow, exercise test protocol, measurement of TWA, and follow-up were performed as described earlier in detail.[17]

### *Study Cohort*

All consecutive patients referred for a routine exercise test at Tampere University Hospital and willing to participate in the study were recruited between October 2001 and the end of 2004. A total of 2,119 patients (1,342 men and 777 women) with technically successful exercise tests (95.8% of all the tests) were enrolled. A test was technically adequate if storing the hemodynamic data and continuous digital ECG signal as well as the TWA assessment was successful. Patients with atrial fibrillation were not excluded, as this condition does not hinder TWA assessment by the method applied.

The main indications for the exercise test were diagnosis of coronary heart disease (CHD; frequency 45%), palpitation or other sense of arrhythmia (21%), and evaluation of work capacity (18%) and adequacy of the CHD treatment (16%), as well as obtaining an exercise test profile prior to surgery (13%) or after an MI (8%); some patients had more than one indication. The study protocol was approved by the Ethics Committee of the Tampere University Hospital, District of Pirkanmaa, Finland, and all patients gave informed consent prior to the interview and measurements as stipulated in the Declaration of Helsinki.

### *Study Flow*

After an informed consent was signed, the medical history of each patient was collected through a computer-based questionnaire form. Thereafter, the exercise test was performed.

### *Exercise Test Protocol*

Prior to the exercise stress test, the subjects lay down in the supine position for 10 minutes, and the resting ECG was digitally recorded. The exercise test was performed using a bicycle ergometer with electrical brakes. The lead system used was the Mason–Likar modification of the standard 12-lead system.[18] The initial workload varied from 20–30 W, and the load was increased stepwise by 10–30 W every minute. Continuous ECGs were digitally recorded at 500 Hz with CardioSoft exercise ECG system (version 4.14; GE Healthcare, Freiburg, Germany) and analyzed fully automatically by the released version of the GE Healthcare MMA software.

Heart rate was continuously registered with ECG during the test while systolic (SAP) and diastolic arterial pressures (DAP) were measured with a brachial cuff every 2 minutes.

### *Measurement of TWA*

The algorithm employed in the identification and quantification of TWA is time domain MMA analysis.[11] In brief, the MMA algorithm separates odd from even beats. The average morphologies of both the odd and the even beats are calculated separately and continuously updated by a weighting factor of 1/8 of the difference between the ongoing average and the new incoming beat. The update is calculated for every incoming beat and results in continual moving averages of the odd and even beats. This approach is intrinsically robust and makes MMA suitable for TWA analysis during periods of activity or fluctuating heart rates.[19] In addition, algorithms have been incorporated to decrease the influence of noise and artifacts, such as those caused by pedaling and respiration.[20] Quality control of automatically derived TWA values was achieved throughout the analysis by beat labeling and exclusion of the suspect and preceding beat based on noise and prematurity according to several criteria, namely beats with $>20\ \mu$V of noise, measured during the isoelectric segments, regions with $>25\%$ of noisy beats, and ventricular premature beats.

The TWA values were calculated continuously during the entire exercise test from the precordial leads ($V_1$–$V_6$). Maximum TWA values at heart rates $<125$ bpm were derived. TWA results were grouped in increments of 10 $\mu$V for analysis of their capacity to stratify risk for sudden, cardiovascular, and total mortality. TWA results in limb leads were excluded as these leads are subject to significant motion artifact, as confirmed by a visual inspection of templates of superimposed ECGs in the GE Healthcare system. Precordial leads have also been shown to be optimum for TWA measurement in an experimental study in dogs and humans.[7,21] TWA values at heart rates $>125$ bpm were not included, based on the published experience indicating that inaccuracies in

TWA measurement can result at heart rates exceeding this range.[22]

### *Follow-Up*

Death certificates were received from the Causes of Death Register, maintained by Statistics Finland, in April 2007; this source has been shown to be reliable.[23] The certificates contained causes of death using the tenth revision of the International Classification of Diseases (ICD-10). The diagnosis numbers and certificate texts were used to classify the deaths as all-cause, cardiovascular, and SCD (defined as a cardiac death within 24 hours after the onset of symptoms).

### *Statistical Analysis*

The hazard ratios (HR) of TWA for all-cause and cardiovascular death as well as for SCD were estimated with a Cox proportional hazards model using the following covariates:[17] sex, age, body mass index (BMI), daily smoking (yes/no), use of beta-blockers (yes/no), metabolic equivalent (MET) as well as prior diagnoses of CHD (yes/no), MI (yes/no), and diabetes (yes/no). The use of beta-blockers was defined as "no" if a patient did not use beta-blockers or had not used beta-blockers before the test for 3 or more days. HRs were analyzed for the preexercise, exercise, and postexercise phases, separately, for TWA results grouped in 10-$\mu$V increments with cutpoints of 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, and 120 $\mu$V and also for TWA as a continuous variable. The statistical analyses were performed with the SPSS release 15.0 for Windows (SPSS, Inc., Chicago, IL, USA). All statistical tests were two-tailed and used an alpha level of 0.05.

## Results

Tables 1 and 2 summarize patient characteristics. There were 126 deaths (5.9% of the population) over the succeeding 47.1 $\pm$ 12.9 months (mean $\pm$ SD). Of those, 62 (2.9%) were categorized as cardiovascular deaths, and 33 (1.6%) further as SCD. Thus, the cardiovascular mortality of the present patients was 0.74% per year. Only 25 (1.1%) of the patients had an implantable cardioverter defibrillator (ICD), and only 32 patients (1.4%) were tested during atrial fibrillation or flutter.

The TWA cutpoint of 65 $\mu$V developed in our initial study[14] remained highly significant in this expanded database, resulting in an HR of 2.4 (95% confidence interval [CI] = 1.2–4.5, P = 0.009) during exercise for total mortality, 4.6 (95% CI = 2.2–9.9, P < 0.001) for cardiovascular mortality, and 4.4 (95% CI = 1.5–12.7, P = 0.007) for SCD. In this extended database, during exercise, the sensitivity, specificity, and positive and negative predictivity were 16.1, 95.7, 10.2, and 97.4, respectively, for cardiovascular mortality using a cutpoint of 65 $\mu$V.

**TABLE 1**

Patient Characteristics for Men and Women

| | Men | | Women | |
|---|---|---|---|---|
| | **Mean** | **SD** | **Mean** | **SD** |
| Age (years) | 57 | 13 | 58 | 13 |
| BMI (kg/m$^2$) | 27.6 | 4.2 | 27.4 | 4.9 |
| Weight (kg) | 86 | 14 | 72 | 13 |
| Height (cm) | 176 | 7 | 162 | 6 |
| HR at supine rest (bpm) | 62 | 12 | 65 | 12 |
| SAP at supine rest (mmHg) | 135 | 18 | 138 | 20 |
| MET | 7.3 | 3.0 | 6.8 | 2.8 |
| Reached HR of expected maximum (%) | 78 | 14 | 80 | 14 |

BMI = body mass index; HR = heart rate; SAP = systolic arterial pressure; MET = metabolic equivalent of the task.

**TABLE 2**

Unadjusted Percentage of Women, Frequency of Beta-Blocker Use, as Well as Prevalence of Cardiovascular Diseases, Symptoms, and Risk Factors for All Participants (n = 2,119)

| | % |
|---|---|
| Women | 37 |
| Beta-blockers | 59 |
| Smoking | 26 |
| CHD | 39 |
| Prior MI | 22 |
| Hypercholesterolemia | 50 |
| Hypertension | 40 |
| Diabetes | 12 |

CHD = coronary heart disease; MI = myocardial infarction.

### *TWA and Mortality*

The adjusted HRs for all-cause mortality, cardiovascular death, and SCD using the Cox regression analysis during the preexercise, routine exercise, and postexercise phases are shown in Figures 1, 2, and 3, respectively. An illustrative ECG with TWA from a patient who died from cardiovascular causes is provided in Figure 4.

During the preexercise phase (Fig. 1), TWA levels from 20 $\mu$V significantly predicted total and cardiovascular mortality. The 60-$\mu$V cutpoint yielded the highest HRs for all-cause mortality and cardiovascular death, 4.6 (95% CI = 2.0–10.7, P < 0.001) and 4.4 (95% CI = 1.3–14.8, P = 0.016), respectively. SCD was not predicted by TWA during this phase.

During exercise, HRs for total and cardiovascular mortality were significant when TWA values reached 50 $\mu$V. The highest HRs for total and cardiovascular death were obtained at the cutpoint of 90 $\mu$V (Fig. 2) and were 3.1 (95% CI = 1.1–8.5, P = 0.03) and 6.4 (95% CI = 2.0–21.2, P = 0.002), respectively. SCD was strongly predicted by TWA levels from 60 $\mu$V, and this TWA value yielded the highest HR, 4.6 (95% CI = 1.7–12.3, P = 0.002).

During the postexercise phase (Fig. 3), TWA levels from 60 $\mu$V significantly predicted total and cardiovascular mortality, but TWA test results during this period did not reach significance for SCD prediction. The highest statistically significant HR in this phase for all-cause mortality was 4.7 (95% CI = 1.1–20.0, P = 0.03) at the cutpoint of 100 $\mu$V. For cardiovascular death, the highest HR during this phase was 3.4 (95% CI = 1.3–8.7, P = 0.01) at the cutpoint of 70 $\mu$V.

As a continuous variable, increasing TWA voltage significantly predicted all-cause and cardiovascular mortality during preexercise (HR = 1.08 per 5 $\mu$V, 95% CI = 1.04–1.13, P < 0.001 for all-cause mortality; and HR = 1.08 per 5 $\mu$V; 95% CI = 1.02–1.14, P = 0.008 for cardiovascular mortality). During exercise, the HR was 1.04 per 5 $\mu$V (95% CI = 1.00–1.07, P = 0.05) for all-cause mortality, and 1.07 per

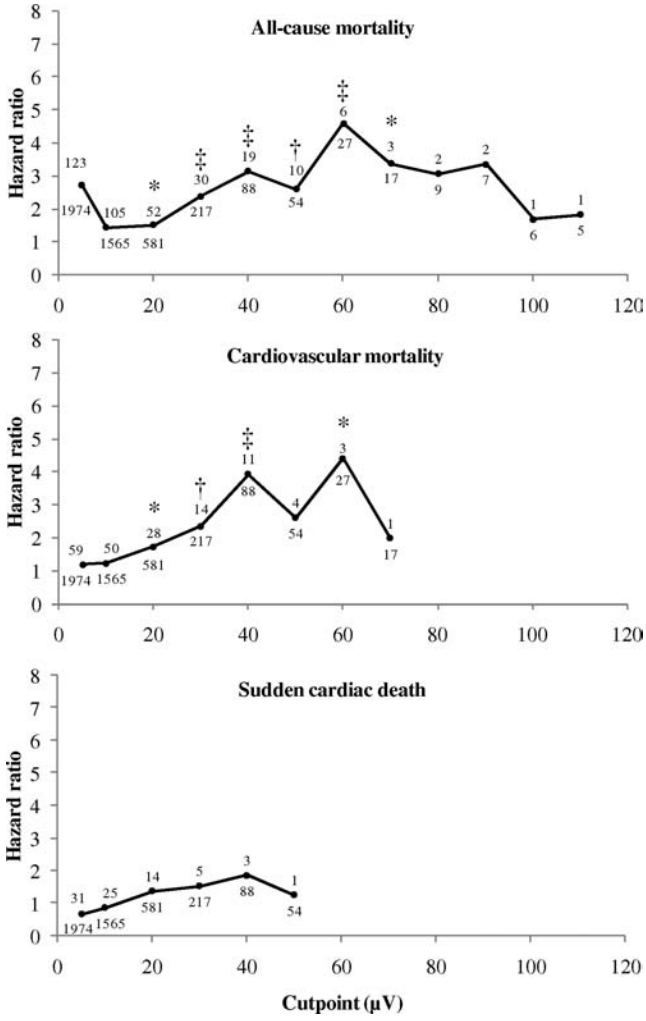


**Figure 1.** *Hazard ratios of maximum T-wave alternans (TWA) in the precordial leads during the preexercise phase at different cutpoints. The data were analyzed for all-cause mortality (top panel), cardiovascular death (middle panel), and sudden cardiac death (bottom panel) (*P < 0.05, †P < 0.01, ‡P < 0.001). The numbers above the line indicate the number of events, and the numbers below the line indicate the number of patients for each TWA cutpoint. The lines end if there were no cases in the respective mortality group for higher TWA cutpoints.*

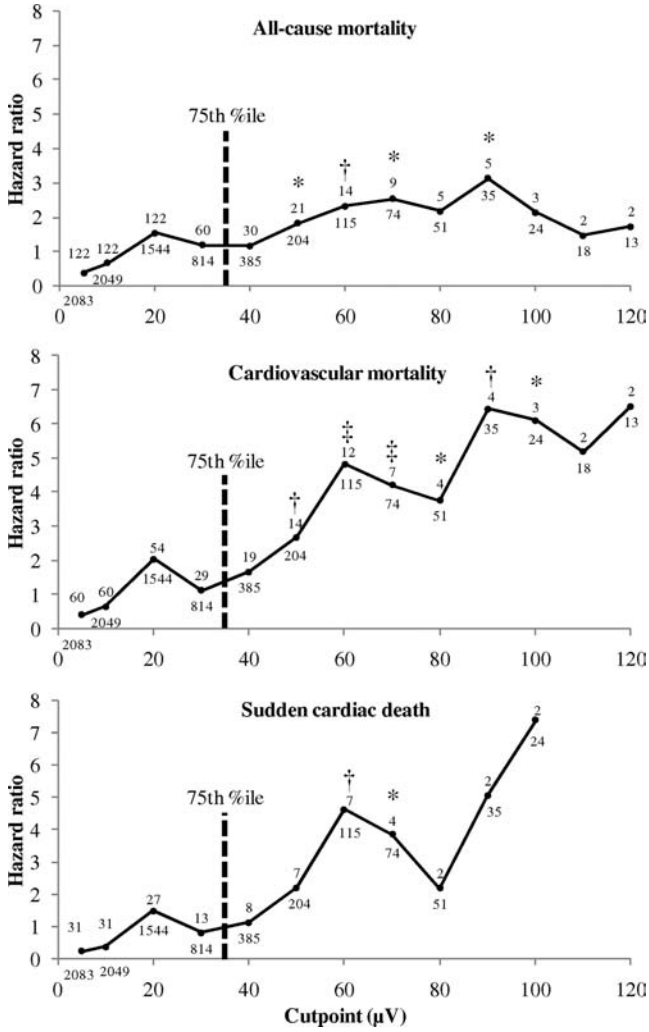


**Figure 2.** *Hazard ratios of maximum T-wave alternans (TWA) in the precordial leads during routine exercise protocol at different cutpoints. The data were analyzed for all-cause mortality (top panel), cardiovascular death (middle panel), and sudden cardiac death (SCD, bottom panel) (*P < 0.05, †P < 0.01, ‡P < 0.001). The 75th percentile cutpoint for TWA in controls (35 μV) is indicated by the vertical dotted line. The numbers above the line indicate the number of events, and the numbers below the line indicate the number of patients for each TWA cutpoint. The line for SCD is shorter than others, because there were no SCD cases for the highest TWA cutpoints.*

5 $\mu$V (95% CI = 1.03–1.11, P = 0.001) for cardiovascular mortality. During the postexercise phase, the HR was 1.04 per 5 $\mu$V (95% CI = 1.01–1.07, P = 0.01) for cardiovascular death. TWA as a continuous variable did not reach significance for SCD prediction during any of the phases of the routine exercise test.

Quartile distribution in peak precordial TWA amplitude during exercise is graphed for survivors (controls) and victims of all-cause and cardiovascular mortality and SCD (Fig. 5). Increasing TWA values resulted in a progressive increase in the percentile level, which was markedly accelerated when the 40-$\mu$V range was exceeded.

## Discussion

The present investigation confirms and extends the findings of our initial study of the low-risk FINCAVAS cohort. Previously, we reported in ~1,000 FINCAVAS patients that TWA using the MMA method is a strong predictor of all-cause and cardiovascular mortality as well as SCD.[14] This study expands those results with a larger number of patients and provides evidence that quantification significantly enhances the prognostic capacity of this parameter. The study supports the concept that provocative testing with exercise is superior to the preexercise or postexercise state, although it appears that the anxiety associated with the anticipation of the test may divulge latent electrical instability.

### *Previous Studies Quantifying TWA*

Until recently, the main evidence in favor of TWA quantification derived from experimental studies employing diverse interventions including rapid pacing, sympathetic nerve stimulation, behavioral stress, and myocardial ischemia.[6-8,24] Increased TWA levels were associated with a greater likelihood of the onset of VT and VF. Clinical clues of the relevance of

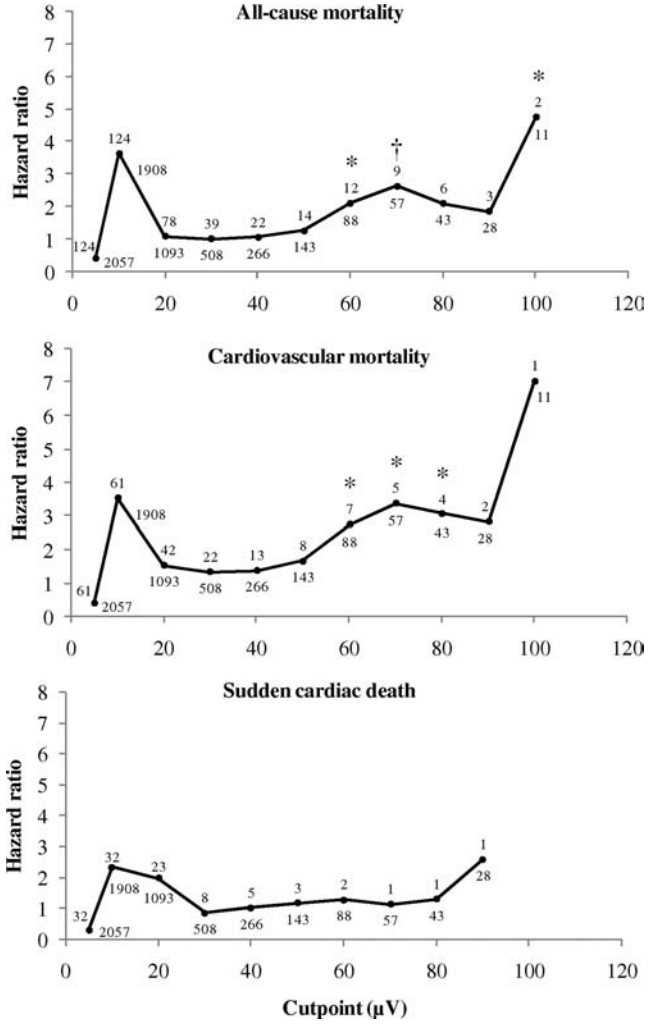


**Figure 3.** *Hazard ratios of maximum T-wave alternans (TWA) in the precordial leads during the postexercise phase at different cutpoints. The data were analyzed for all-cause mortality (top panel), cardiovascular death (middle panel), and sudden cardiac death (SCD, bottom panel) (*P < 0.05, †P < 0.01). The numbers above the line indicate the number of events, and the numbers below the line indicate the number of patients for each TWA cutpoint. The lines end if there were no cases in the respective mortality group for higher TWA cutpoints.*

TWA magnitude have emanated from studies by Tanno and coworkers,[25] who demonstrated that patients with high TWA levels during atrial pacing at low heart rates experienced an increased incidence of VT during follow-up. Recurring clinical reports[9,26–28] indicate that sizeable levels of TWA herald the onset of VF. Klingenheben and colleagues[5] provided the limited existing clinical literature on TWA magnitude with the spectral method, demonstrating that when patients with dilated or ischemic cardiomyopathy exercised to 105 bpm, higher TWA levels were found in those who experienced ventricular tachyarrhythmias.

Although most studies using spectral TWA analysis have yielded favorable results in assessing the risk of cardiovascular and arrhythmic mortality,[1] the negative outcome of the recent sizeable Microvolt T-Wave AlternanS Testing for Risk Stratification of Post MI Patients (MASTER) I trial in Multicenter Automatic Defibrillator Implantation Trial (MADIT)-II-type ICD-treated patients has raised questions about the value of TWA testing in estimating the risk for ventricular tachyarrhythmic events.[29] Two major limitations of that study that could account for this lack of predictivity have been identified. These include the use of ICD treatment of VT/VF as a surrogate endpoint for SCD, as shocks have been shown to overestimate arrhythmic mortality by a factor of two.[30] Second, the devices themselves are known to be proarrhythmic.[31] In their meta-analysis of studies comprising nearly 6,000 patients, Hohnloser and coworkers[32] determined that the HRs for predicting SCD were 13.6 (95% CI = 8.5–30.4) versus 1.6 (95% CI = 1.2–2.1) comparing studies in which few patients had implanted ICDs and with a low percentage of ICD treatments to studies in which a majority of the reported endpoint ventricular tachyarrhythmic events were ICD therapies. In a side-by-side comparison of the spectral and MMA methods, Exner *et al.*[16] demonstrated that these methods exhibited significant, comparable results in post-MI patients with moderately depressed ejection fraction but without ICDs enrolled in the Risk Estimation Following Infarction, Noninvasive Evaluation (REFINE) study. In the present study, only 25 (1.1%) patients had received an ICD, and thus this issue did not likely alter the predictivity of MMA-based TWA analysis.

### *Current Investigation*

The main goal of the study was to evaluate TWA's predictivity during routine exercise testing using standard protocol and electrodes in the normal flow of patient evaluation. Accordingly, heart rate was allowed to increase progressively during the stress test, and the maximum TWA level that was obtained at a heart rate <125 bpm was employed for risk stratification. This approach differs from the requirements of the spectral method, which necessitates the use of specialized electrodes and a nonstandard exercise protocol designed to fix heart rate between 105 and 110 bpm to allow sufficient stationarity of the R-R interval to generate reliable power spectra. The TWA values derived by the MMA method are larger by a factor of approximately 4–6 than the values reported by the spectral method. This difference is attributable to the fact that the time domain MMA method determines the maximum difference in the T-wave amplitude between successive beats (Fig. 4), in a window of approximately 16 beats, while the spectral method derives an average value from its spectra, which are generated across the entire T-wave and over 128 beats.

Our results demonstrate the importance of TWA magnitude by the finding that higher TWA levels are associated with greater HRs for total and cardiovascular mortality and SCD (Figs. 1, 2, and 3). HRs for these events rise sharply when the 75th percentile level is exceeded (Fig. 5). It is noteworthy that at this percentile level, TWA in the range of 40 $\mu$V was associated with comparable odds ratios for cardiac arrest or arrhythmic death in ambulatory ECG recordings of post-MI patients in the Autonomic Tone and Reflexes after Myocardial Infarction (ATRAMI) study.[13] Collectively, these observations are consistent with the view that TWA magnitude reflects a continuum of cardiac electrical instability. This inference is further supported by experimental[8] and clinical studies[9] that demonstrate that life-threatening ventricular arrhythmias emerge from a crescendo in TWA magnitude. In Figure 2, the drop in HRs for SCD at 80 $\mu$V and the lack of significance for TWA at 80–120 $\mu$V are

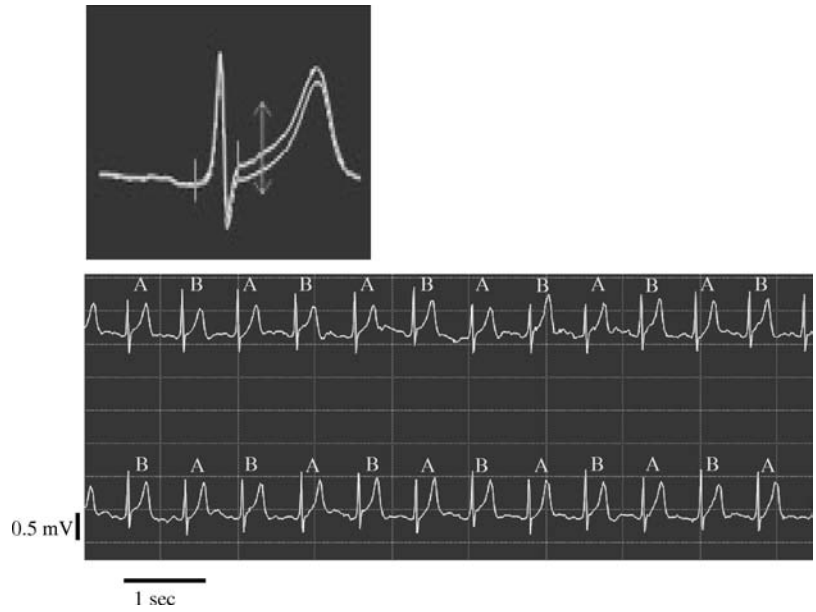


**Figure 4.** *A representative ECG tracing and superimposed complexes of the lead V4 illustrating exercise-induced TWA of 124 $\mu$V in a patient who experienced cardiovascular death at 12 months following the recording. The superimposed waveforms (upper panel) and rhythm strip (lower panel) are provided. The bidirectional arrow refers to the point of maximum TWA.*

probably due to the smaller number of events in this TWA range.

The preexercise phase of our clinical protocol starts when the patient sits on the bicycle. Although the patient is physically at rest, increased sympathetic nerve activity and behavioral stress are evident in the elevated heart rates (from 63 $\pm$ 12 to 71 $\pm$ 14 bpm, $P < 0.001$ in paired *t*-test) and systolic blood pressure (from 136 $\pm$ 19 to 139 $\pm$ 22 mmHg, $P < 0.001$) over the supine position. This is also clearly reflected as an increase in the rate–pressure product, an index of cardiac metabolism (from 8,600 $\pm$ 2,030 to 9,860 $\pm$ 2,460, $P < 0.001$). This finding is in accordance with previous observations that acute mental stress elevates TWA in patients prone to ventricular arrhythmia.[12,15] Importantly, TWA was measured at relatively low heart rates, indicating that during psychological stress, TWA provides risk information that is not a function of heart rate alone. As a result, for patients who cannot complete a routine exercise test, the preexercise phase data may be analyzed for risk stratification for total and cardiovascular mortality.

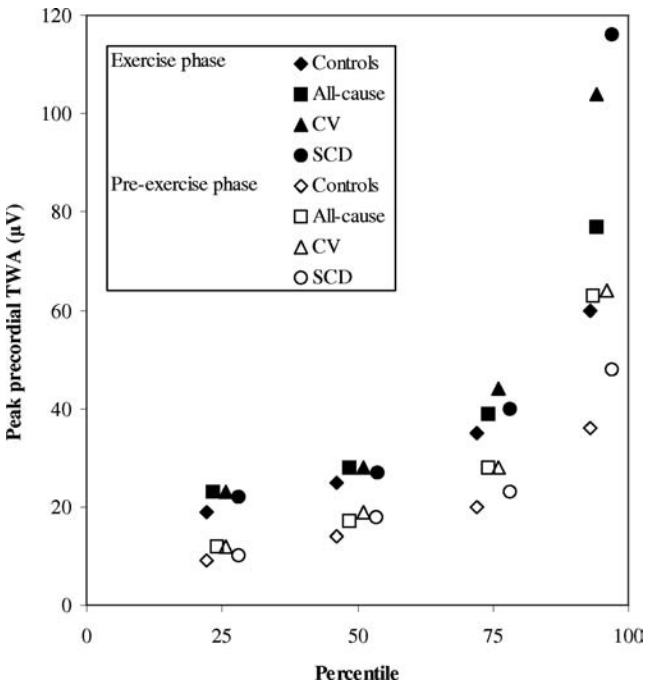


**Figure 5.** *Percentiles (25%, 50%, 75%, and 95%) of peak precordial T-wave alternans (TWA) magnitudes during the preexercise and exercise phases registered for survivors (controls) and patients with all-cause, cardiovascular (CV), or sudden cardiac death (SCD). The values during the postexercise phase have been omitted for clarity; the data points lie between the values for the other two phases.*

However, it should be noted that during neither the pre- nor the postexercise phases did TWA predict SCD. A possible explanation is that in order to expose the latent electrical instability, a provocative challenge such as intense physical activity is required.[33] This concept is consistent with the experience with the spectral method, described by Estes and coworkers,[34] who found that a number of patients who were TWA-negative at rest became positive during exercise.

We are uncertain regarding why, when analyzed as a continuous variable, TWA did not predict SCD, while it did predict all-cause and cardiovascular mortality. During the exercise phase, there was a sizeable increase in SCD HR in parallel with TWA magnitude (Fig. 2), which achieved statistical significance at the 60-$\mu$V and 70-$\mu$V levels. A likely possibility is that the overall number of these events was not as large as the number of total and cardiovascular deaths. This consideration is inherent to low-risk populations.

### *Limitations*

The definition of SCD is never clear-cut. We used death within 24 hours after the onset of symptoms as a definition for SCD. It is possible that some of these deaths were not due to ventricular tachyarrhythmia, which is a study limitation. However, TWA was a stronger predictor of cardiovascular mortality and SCD than of total mortality. This observation indicates that the occurrence of TWA during exercise reflects abnormal cardiac electrical, or mechanical function predisposing to cardiac death. Another limitation is that we do not have information on changes in parameters affecting mortality risk (e.g., smoking, lifestyles, and medication) during

the follow-up. Third, corrections for multiple testing procedures have not been made. The Cox regression itself does not possess multiple testing corrections.

## Conclusion

Our investigation reports the ranges of significant TWA values that may be employed in screening low-risk populations for the risk of total and cardiovascular mortality and SCD. As the measurement may be performed during routine exercise testing in the typical flow of clinical care, a significant opportunity is provided to identify individuals whose risk is elevated but who are otherwise not identified by contemporary tests. The largest number of events, approximately 300,000 in the United States, occurs in this broad group, although the incidence is low.[35]

Quantification represents a significant advance in the field of TWA testing, since knowing the extent of disease helps to ascertain the urgency of intervention and gauge the efficacy of therapy.[5,36] Whether spectral or time domain-based MMA analysis of TWA is employed, quantification provides an additional advantage over binary assessment as it reflects a continuum of cardiac electrical instability. The fact that antiarrhythmic drug therapy may reduce TWA magnitude without affecting its prognostic utility[37,38] suggests that this parameter can be used to guide medical therapy. Beta-blockade administration is a significant example, as it has been shown to reduce TWA by nearly 40%[37] as well as to reduce the incidence of SCD.[39] Conversely, there are reports of sizeable levels of TWA in association with drug-induced proarrhythmia.[27]

Thus, while TWA testing has been focussed largely on guiding ICD implantation for primary prevention of SCD, quantitative TWA may pave the way for a greater role in screening low-risk populations and in gauging the effectiveness of antiarrhythmic therapy and potential for proarrhythmia.

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**ORIGINAL ARTICLE**

# Impaired exercise capacity predicts sudden cardiac death in a low-risk population: Enhanced specificity with heightened T-wave alternans

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**Abstract**

*Aims.* Because sudden cardiac death (SCD) is due to cardiac electrical instability, we postulated that prediction of this mode of death by exercise capacity will be enhanced by combined assessment with T-wave alternans (TWA), an index of repolarization abnormality.

*Material and methods.* The Finnish Cardiovascular Study enrolled consecutive patients ($n = 2{,}044$) with a routine clinically indicated exercise test. Exercise capacity was measured in metabolic equivalents (METs) and TWA by time-domain modified moving average method.

*Results.* During $47.2 \pm 12.8$-month follow-up (mean $\pm$ SD) 120 patients died; 58 were cardiovascular deaths, and 29 were SCD. In multivariate analysis after adjustment for sex, age, smoking, use of β-blockers, as well as other common coronary risk factors, the relative risk of patients whose exercise capacity was depressed (MET $<8$) was 8.8 (95% CI 2.0–38.9, $P = 0.004$) for SCD. The combination of low exercise capacity (MET $<8$) and elevated TWA ($\geq 65$ μV) yielded relative risks for SCD of 36.1 (6.3–206.0, $P < 0.001$), for cardiovascular mortality of 21.1 (6.7–66.2, $P < 0.001$), and for all-cause mortality of 7.8 (3.5–17.4, $P < 0.001$) over patients with neither factor.

*Conclusions.* Reduced exercise capacity, particularly in combination with heightened TWA, indicating enhanced cardiac electrical instability, powerfully predicts risk for SCD in patients referred for exercise testing.

**Key words:** *Arrhythmia, electrocardiography, exercise, mortality, tachyarrhythmia*

## Introduction

Exercise capacity predicts all-cause and cardiac mortality in men more powerfully than do other cardiac risk factors (1). This prognostic utility is similar among women (2,3) and across racial groups (4). Surprisingly, its potential to estimate risk specifically for sudden cardiac death (SCD) has not been investigated. This is an important gap in our knowledge, as reduced exercise capacity could reflect the extent of heart disease, including myocardial scarring and poor myocardial perfusion that create a heterogeneous myocardial substrate with increased susceptibility to lethal re-entrant arrhythmias. Combined testing with T-wave alternans (TWA), a marker of cardiac electrical instability, during routine symptom-

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**Key messages**

- Reduced exercise capacity powerfully predicts risk for sudden cardiac death in a general population of patients referred for a clinical exercise test.
- T-wave alternans, an indicator of ventricular electrical instability, adds significantly to the prognostic strength of reduced exercise capacity.

**Abbreviations**

| | |
|---|---|
| β-blockers | beta-adrenergic blocking agents |
| CI | confidence interval |
| FINCAVAS | Finnish Cardiovascular Study |
| MET | metabolic equivalent |
| μV | microvolt |
| NPV | negative predictive value |
| PPV | positive predictive value |
| SCD | sudden cardiac death |
| TWA | T-wave alternans |
| W | watt |

limited exercise tests could demonstrate an association with SCD as well as increase the strength of prediction.

The literature provides clues supporting the rationale for combined analysis of exercise capacity with TWA. Tapanainen and co-workers (5) demonstrated in postmyocardial infarction patients that inability to achieve a target heart rate of 105–110 beats/min, as required for spectral TWA testing, was itself predictive of cardiovascular mortality. More recently, it has been advised that spectral TWA tests previously considered 'indeterminate' based on not achieving a target heart rate should be classified as 'abnormal' or 'non-negative' (6). However, target heart rate is not a reliable measure of exercise capacity, as patients' inability to increase heart rate may be influenced by many factors including sinus node responsiveness or medications, particularly beta-adrenergic blocking agents (β-blockers), as well as by mechanical function of the heart. Metabolic equivalents (METs) have been shown to be a more reliable measure of exercise capacity (7).

Based on extensive evidence that SCD is due to cardiac electrical instability, we postulated that prediction of this mode of death by exercise capacity will be enhanced by combined assessment with TWA, an index of repolarization abnormality. We applied the time-domain modified moving average method for TWA analysis (8), which, because of its intrinsic flexibility, permits TWA analysis during routine symptom-limited exercise protocols in which exercise capacity can be measured. The method has undergone extensive validation and performs at a resolution of 1 microvolt, equivalently to the spectral method (9,10). We tested its utility to improve SCD risk stratification in a general population of patients referred for a clinical exercise test.

## Material and methods

### *Study cohort*

All consecutive patients coming for an exercise test at Tampere University Hospital and willing to participate were enrolled in the Finnish Cardiovascular Study (FINCAVAS). Between October 2001 and the end of 2004, a study population of 2,212 patients (1,400 men and 812 women) was recruited. Results of analysis of TWA alone in about half of the patients (1,037) have been reported (11). A total of 2,044 patients (1,305 men and 739 women) had technically successful exercise tests (92.4% of all tests) and were studied in the current investigation (Table I). A test was technically adequate if storing hemodynamic data and continuous digital electrocardiogram as well as TWA assessment and exercise capacity recording in METs were successful.

The main indications for exercise testing were suspicion of coronary heart disease (46%), palpitation or sense of arrhythmia (21%), evaluation of work capacity (18%) and adequacy of coronary heart disease treatment (16%), as well as obtaining an exercise test profile prior to an invasive operation (14%) or after myocardial infarction (8%); some patients had more than one indication. The Ethics Committee of Tampere University Hospital District of Pirkanmaa, Finland, approved the study protocol, and all patients gave informed consent prior to the interview and measurements, as stipulated in the Declaration of Helsinki.

### *Study flow*

After an informed consent was signed, the medical history of each patient was collected with a computer-based questionnaire form. Thereafter, the exercise test was performed.

### *Exercise test protocol*

Prior to the exercise stress test, subjects lay down in the supine position for 10 minutes, and the resting electrocardiogram was digitally recorded. Exercise testing was performed using a bicycle ergometer with electrical brakes. The lead system used was the Mason-Likar modification of the standard 12-lead

Table I. Patient characteristics and unadjusted percentage of beta-adrenergic blocking agent (β-blocker) users as well as prevalence of cardiovascular diseases, symptoms, and risk factors for all participants ($n=2,044$) divided by gender and separately for men with sudden cardiac death (SCD) ($n=28$). The differences between men with and without SCD were tested by using *t* test for independent samples for continuous variables and chi-square test for categorical variables.

| | Men ($n=1305$) | | Women ($n=739$) | | Men with SCD ($n=28$) | | |
|---|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | Mean | SD | *P* |
| Age (years) | 57 | 13 | 57 | 13 | 61 | 13 | 0.069 |
| BMI (kg/m$^2$) | 27.5 | 4.2 | 27.4 | 5.0 | 27.1 | 4.3 | 0.593 |
| Weight (kg) | 85 | 14 | 72 | 13 | 83 | 16 | 0.457 |
| Height (cm) | 176 | 7 | 162 | 6 | 175 | 8 | 0.516 |
| Heart rate at supine rest (bpm) | 62 | 11 | 65 | 11 | 60 | 9 | 0.177 |
| SAP at supine rest (mmHg) | 135 | 18 | 138 | 20 | 132 | 28 | 0.552 |
| Reached heart rate of expected maximum (%) | 78 | 14 | 80 | 14 | 69 | 13 | 0.001 |
| | % | | % | | % | | |
| β-blockers | 63 | | 51 | | 89 | | 0.003 |
| Smoking | 32 | | 15 | | 25 | | 0.540 |
| CHD | 44 | | 29 | | 75 | | 0.002 |
| Prior MI | 25 | | 14 | | 54 | | 0.001 |
| Hypercholesterolemia | 53 | | 45 | | 64 | | 0.254 |
| Hypertension | 40 | | 40 | | 29 | | 0.246 |
| Diabetes | 13 | | 12 | | 21 | | 0.154 |

SCD = sudden cardiac death; BMI = body mass index; SAP = systolic arterial pressure; β-blockers = beta-adrenergic blocking agents; CHD = coronary heart disease; MI = myocardial infarction.



system (12). The initial work-load varied from 20 watts (W) to 30 W, and the load was increased stepwise by 10–30 W every minute. Continuous electrocardiograms were digitally recorded at 500 hertz with CardioSoft exercise system (Version 4.14, GE Healthcare, Freiburg, Germany).

Heart rate was continuously registered with electrocardiograms during the tests, while systolic and diastolic arterial pressures were measured with a brachial cuff every 2 minutes. Exercise capacity in METs was estimated on the standardized basis of maximum work-load and weight of the patient, with 1 MET equivalent to 3.5 mL oxygen uptake/kilogram/min.

### *Measurement of TWA*

TWA was analyzed fully automatically by investigators blinded to clinical outcomes with the released version of GE Healthcare Modified Moving Average software. Modified moving average analysis (8) calculates and compares separate average morphologies of odd and even beats. Continuous updating for every incoming beat by a weighting factor of 1/8 of the difference between the on-going average and the new incoming beat produces continuous moving averages of odd and even beats. This approach is intrinsically robust and is suitable for TWA analysis during periods of activity or fluctuating heart rates (13). Algorithms have also been incorporated to decrease the influence of noise and artifacts, such as those caused by pedaling and respiration (14). The following steps were taken to ensure quality control of TWA values. Throughout the analysis, beat-labeling was performed to exclude the suspect and preceding beat based on noise and prematurity according to several criteria. These included: beats with $>20$ microvolts of noise, which was measured during the isoelectric segments; regions with $>25\%$ of noisy beats; and ventricular premature beats.

Standard precordial leads (V1 to V6) were recorded continuously during the entire exercise test. The highest TWA value in any lead during the exercise-phase at heart rates $<125$ beats/min was derived. This heart rate limit was set, as inaccuracies in TWA measurement can result at heart rates exceeding this range (15). TWA results in limb leads were excluded as these leads are subject to significant motion artifact, as confirmed by visual inspection of templates of superimposed electrocardiograms in the GE Healthcare system. Precordial leads have also been shown to be optimum for TWA measurement (16,17).

The TWA values obtained by the modified moving average method are 4- to 6-fold higher than the values reported by the spectral method. This difference is due primarily to the fact that the time-domain modified moving average method

calculates the maximum difference in amplitudes of successive T-waves, while the spectral method computes an average value from its spectra, which are determined across the entire T-wave and across 128 beats.

### *Ejection fraction*

Measurement of left ventricular ejection fraction is not routine for patients referred for a clinical exercise test. However, ejection fraction was determined for 1,117 (55%) of study patients with echocardiography or isotope techniques within 6 months of exercise testing.

### *Follow-up*

Death certificates listing causes of death using the tenth revision of the International Classification of Diseases (ICD-10) were received from the Causes of Death Register, maintained by Statistics Finland, in April 2007; this source has been shown to be reliable (18). Diagnosis numbers and certificate texts were used to classify deaths as all-cause, cardiovascular, or SCD, i.e., cardiovascular death within 24 hours after onset of symptoms. Autopsy rate was 40% for all deaths and 60% for patients with SCD.

### *Statistical analysis*

Predictivity for SCD and for cardiovascular and all-cause mortality by METs with and without elevated TWA was analyzed using Cox proportional hazards models. Analyses of exercise capacity in METs were performed with the cut-point of $<8$, which has been used in studies in women (19,20) but to our knowledge not in studies with men. In subgroup analyses in women, the cut-point of $<5$ (2) was also used. The Pearson correlations between maximum heart rate and exercise capacity in METs and between TWA magnitude and maximum heart rate were calculated.

For analyses of TWA, the cut-point of 65 microvolts ($\mu$V) in precordial leads was used, because it had the best prognostic power in our previous study (11). Low exercise capacity and TWA were combined in one categorical variable with three different groups of patients: MET $\geq 8$ and TWA $<65$ $\mu$V; MET $<8$ or TWA $\geq 65$ $\mu$V; and MET $<8$ and TWA $\geq 65$ $\mu$V. Thereafter, risk for all-cause and cardiovascular death as well as for SCD was estimated with Cox regression analysis using the following covariates (21): sex, age, body mass index, daily smoking (yes/no), use of β-blockers (yes/no), as well as prior diagnoses of coronary heart disease (yes/no), myocardial infarction (yes/no), and diabetes (yes/no) (Table II). Use of β-blockers was defined as 'no' if the patient did not use β-blockers or if the pause in β-blocker use before the test was 3 days or more. Sensitivity and specificity as well as positive and negative predictive values were calculated for exercise capacity alone and in combination with TWA compared to patients with neither factor (Table III).

Statistical analyses were performed with the SPSS release 15.0 for Windows (SPSS Inc., Chicago, Illinois). All statistical tests were two-tailed and used an alpha level of 0.05.

## Results

Among the 2,044 enrolled patients, 120 deaths (5.9%) occurred over the succeeding $47.2 \pm 12.8$ months (mean $\pm$ SD). Of those, 58 (2.8%) were categorized as cardiovascular deaths and 29 (1.4%) further as SCD. Thus, the cardiovascular mortality of the present patients was 0.71%/year. Prevalence of all-cause death, cardiovascular death, and SCD in women was 3.8% ($n=28$), 1.1% ($n=8$), and 0.1% ($n=1$), respectively; in men, the prevalence was 7.0% ($n=92$), 3.8% ($n=50$), and 2.1% ($n=28$), respectively. Left ventricular ejection fraction, reported for 1,117 patients, was $66 \pm 14$% (mean $\pm$ SD). Of these, 103 patients (9.2%) presented with ejection fraction $<50$%, 39 patients (1.9%) with ejection fraction $<40$%, and 10 patients (0.9%) with ejection fraction $<30$%. Among the 29 SCD cases, ejection fraction was reported for 15 (52%) and was $60.5 \pm 15.7$ (mean $\pm$ SD). Only 24 patients (1.2%) had an implantable cardioverter defibrillator. Male patients with SCD reached lower percent of expected heart rate, more frequently had coronary heart disease and prior myocardial infarction, and were more often on β-blocker treatment than those who did not experience SCD in the follow-up (Table I). The mean value ($\pm$SD) for peak TWA levels measured during exercise in precordial leads was $30 \pm 21$ $\mu$V. The Pearson correlation was 0.537 ($P<0.001$) between maximum heart rate and exercise capacity in METs and $-0.099$ ($P<0.001$) between maximum heart rate and TWA magnitude.

### *Exercise capacity and mortality*

In our population of consecutive patients referred for clinical exercise testing, 58.5% had reduced exercise capacity. The mean value ($\pm$SD) for exercise capacity in METs for men (1,305) was $7.4 \pm 3.0$ and for women (739) was $6.7 \pm 2.8$. For patients with reduced exercise capacity (MET $<8$, $n=1,195$), the

Table II. Adjusted relative risks for all-cause mortality, cardiovascular mortality, and sudden cardiac death according to exercise capacity in metabolic equivalents (MET) and T-wave alternans (TWA), adjusted by covariates used in the Cox regression models ($n=2,044$).

| | All-cause mortality | | | | Cardiovascular mortality | | | | Sudden cardiac death | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 95% CI | | | | 95% CI | | | | 95% CI | | |
| | RR | Lower | Upper | *P* | RR | Lower | Upper | *P* | RR | Lower | Upper | *P* |
| MET <8 and TWA ≥65* | 7.8 | 3.5 | 17.4 | <0.001 | 21.1 | 6.7 | 66.2 | <0.001 | 36.1 | 6.3 | 206.0 | <0.001 |
| MET <8 or TWA ≥65* | 2.8 | 1.6 | 4.8 | <0.001 | 4.1 | 1.6 | 10.8 | 0.004 | 7.0 | 1.6 | 31.1 | 0.011 |
| Age (y) | 1.0 | 1.0 | 1.1 | <0.001 | 1.0 | 1.0 | 1.1 | 0.014 | 1.0 | 1.0 | 1.0 | 0.773 |
| Sex (f/m) | 1.9 | 1.2 | 3.0 | 0.004 | 3.3 | 1.6 | 7.2 | 0.002 | 14.8 | 2.0 | 110.8 | 0.009 |
| BMI (kg/m$^2$) | 0.9 | 0.9 | 1.0 | <0.001 | 0.9 | 0.9 | 1.0 | 0.008 | 0.9 | 0.8 | 1.0 | 0.072 |
| Smoking (no/yes) | 1.3 | 0.9 | 2.0 | 0.180 | 0.9 | 0.5 | 1.6 | 0.650 | 0.6 | 0.2 | 1.4 | 0.249 |
| Prior MI (no/yes) | 1.8 | 1.0 | 3.1 | 0.036 | 2.1 | 1.0 | 4.1 | 0.044 | 1.9 | 0.7 | 4.9 | 0.193 |
| Diabetes (no/yes) | 1.3 | 0.8 | 2.1 | 0.297 | 1.7 | 0.9 | 3.2 | 0.107 | 1.7 | 0.7 | 4.2 | 0.282 |
| β-blockers (no/yes) | 1.8 | 1.1 | 2.9 | 0.014 | 2.9 | 1.3 | 6.5 | 0.012 | 3.4 | 1.0 | 11.8 | 0.055 |
| CHD (no/yes) | 0.5 | 0.3 | 0.9 | 0.026 | 0.9 | 0.4 | 1.9 | 0.702 | 1.2 | 0.4 | 3.5 | 0.761 |

RR = relative risk; CI = confidence interval; μV = microvolt; BMI = body mass index; MI = myocardial infarction; β-blockers = beta-adrenergic blocking agents; CHD = coronary heart disease.
*Compared to patients with neither factor

unadjusted prevalence of all-cause death was 8.6%, cardiovascular death 4.4%, and SCD 2.3%. For those with preserved exercise capacity (MET ≥8, $n=849$), the prevalence was 2.0%, 0.6%, and 0.2%, respectively.

The adjusted relative risk for SCD for those with poor exercise capacity (MET <8) was 8.8 (95% confidence interval (CI) 2.0–38.9, $P=0.004$), was 5.2 (2.0–13.6, $P=0.001$) for cardiovascular mortality, and was 3.3 (1.9–5.6, $P<0.001$) for all-cause mortality. Age, sex, body mass index, use of β-blockers, and prior diagnosis of myocardial infarction and coronary heart disease were significant covariates for death from any cause. Age, sex, body mass index, and use of β-blockers were significant covariates for cardiovascular mortality, while only sex was a significant covariate for SCD. MET was a highly sensitive predictor (93.1%) of SCD, with high negative predictive value (NPV) (99.8%) (Table III). Similar predictivity was determined for cardiovascular and total mortality (Table III).

As a continuous variable, increasing MET significantly improved survival in terms of all-cause and cardiovascular mortality and SCD (relative risk 0.77 per 1 MET increase, 95% CI 0.70–0.84, $P<$ 0.001 for all-cause mortality; relative risk 0.69 per 1 MET increase, 95% CI 0.60–0.80, $P<0.001$ for cardiovascular mortality; and relative risk 0.67 per 1 MET increase, 95% CI 0.57–0.83, $P<0.001$ for SCD).

In the subgroup analyses in women ($n=739$) increasing MET as a continuous variable significantly improved the survival in terms of all-cause and cardiovascular mortality (relative risk 0.77 per 1 MET increase, 95% CI 0.62–0.95, $P=0.016$ for all-cause mortality; and relative risk 0.52 per 1 MET increase, 95% CI 0.32–0.83, $P=0.006$ for cardiovascular mortality). The cut-point of MET <8 did not reach significance in women, but the cut-point of MET <5 predicted statistically significantly cardiovascular mortality (relative risk 15.0, 95% CI 2.0–111.8, $P=0.008$) in women.

In men the results were highly comparable to results of the all participants.

### *Exercise capacity, TWA, and mortality*

The predictive power of TWA (≥65 μV) alone with covariates used in this study remained highly significant in this expanded database, resulting in a relative risk of 2.2 (1.1–4.2, $P=0.018$) for total mortality, 4.0 (1.9–8.5, $P<0.001$) for cardiovascular mortality, and 3.9 (1.4–11.5, $P=0.011$) for SCD.

SCD risk was further categorized according to TWA test results. Three groups of patients,

Table III. Sensitivity (Sn), specificity (Sp), as well as positive (PPV) and negative (NPV) predictive values for sudden cardiac death and for cardiovascular and all-cause mortality ($n = 2{,}044$).

| | Sn | Sp | PPV | NPV |
|---|---|---|---|---|
| MET <8:* | | | | |
| Sudden cardiac death | 93.1 | 42.0 | 2.3 | 99.8 |
| Cardiovascular death | 91.4 | 42.5 | 4.4 | 99.4 |
| All-cause death | 85.8 | 43.2 | 8.6 | 98.0 |
| MET <8 and TWA ≥65 μV:* | | | | |
| Sudden cardiac death | 66.7 | 94.0 | 7.1 | 99.8 |
| Cardiovascular death | 61.5 | 94.4 | 14.3 | 99.4 |
| All-cause death | 37.0 | 94.5 | 17.9 | 97.9 |

MET = metabolic equivalents; TWA = T-wave alternans
*Compared to patients with neither factor

MET ≥8 and TWA <65 μV, MET <8 or TWA ≥ 65 μV, and MET <8 and TWA ≥65 μV, contained 811, 1177, and 56 patients, respectively. The combination of poor exercise capacity and elevated TWA identified patients with the highest prevalence of SCD and of cardiovascular and total mortality (Figure 1). Survival curves depict events across 4 years of follow-up for the combined analysis of reduced MET <8 and elevated TWA (≥65 μV) (Figure 2). The adjusted relative risk for SCD for patients with both reduced exercise capacity (MET <8) and heightened TWA (≥65 μV) was 36.1 (6.3–206.0, $P < 0.001$), for cardiovascular mortality was 21.1 (6.7–66.2, $P < 0.001$), and for all-cause mortality was 7.8 (3.5–17.4, $P < 0.001$), over patients with neither factor (Table II). For SCD, the only significant covariate was sex. Combined analysis of SCD risk with METs and TWA ≥65 μV yielded high specificity (94.0%) while retaining high NPV (99.8%) (Table III). A representative example of exercise-induced TWA is provided (Figure 3).

The combination of low exercise capacity (MET <8) and elevated TWA (≥65 μV) did not reach significance in women. In men the results were highly comparable to the results of all participants.

## Discussion

Our study is the first to demonstrate that reduced exercise capacity is a risk factor for SCD. It also provides evidence that TWA, an indicator of ventricular electrical instability, adds significantly to the prognostic strength of reduced exercise capacity. This full-cohort examination of risk stratification with TWA enrolled more than 2,000 patients. In half of the patients, left ventricular ejection fraction was measured, and in 90% of these, it was found to be normal. It is probable although not documented that the remaining half of the cohort, in whom ejection fraction was not measured, had even better cardiovascular health, because ejection fraction determination was not indicated. Thus, the present results are relevant to a large group of individuals whose elevated risk for SCD and major cardiovascular events is not disclosed by other contemporary tests.

### *Previous studies*

Exercise capacity is a superior predictor of all-cause and cardiovascular mortality (1–4). The usefulness of exercise-induced TWA in predicting arrhythmic events and death has been investigated (11,22–25). We reported in ~1,000 FINCAVAS patients that TWA assessed during routine symptom-limited exercise testing is a strong risk marker for SCD and cardiovascular and total mortality in a general population of patients referred for a clinical exercise test (11). By contrast, most studies of TWA have been performed in populations with high risk of life-threatening arrhythmias (22,23,25) or lower-risk patients with prior myocardial infarction (24) and employed spectral analysis of TWA during a target heart rate exercise protocol. These TWA test results are indeterminate in 20%–40% of cases (6) due to patient factors, in the majority to inability to achieve the target heart rate of 105–110 beats/min. Classification of these indeterminate tests as 'abnormal' conferred prediction to avoid repetition of capacity although exercise capacity itself was not measured. Thus, the present study, in which exercise capacity was measured, is the first to provide direct evidence of its predictive value for SCD, particularly when combined with TWA.

### *Current investigation*

Our study provides new evidence that in a general population of patients referred for a clinical exercise test, reduced exercise capacity increases risk for SCD as well as for cardiovascular death and total mortality. When heightened TWA, a validated marker of arrhythmia risk, is also present, risk of SCD is further elevated over that of patients with neither factor (Table II). Poor exercise capacity alone was found to be a highly sensitive (93.1%) marker of SCD risk, with specificity of 42.0%, as it detected 27 of 29 cases of SCD (Table III). Combined analysis with elevated TWA greatly improved the specificity of the test, to 94.0%.

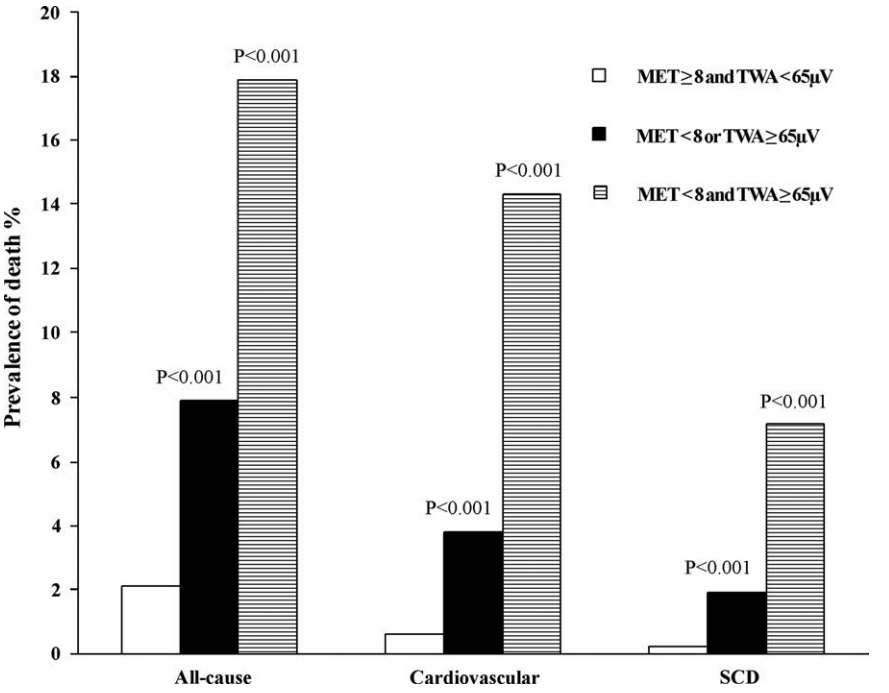


Figure 1. Prevalence of all-cause and cardiovascular mortality as well as sudden cardiac death (SCD) among patients according to metabolic equivalents (MET) and T-wave alternans (TWA). The *P*-values are from chi-square test. The subjects with metabolic equivalents (MET) $\geq$8 and T-wave alternans (TWA) $<$65 microvolts ($\mu$V) were compared to the subjects with either MET $<$8 or TWA $\geq$65 $\mu$V and to the subjects with MET $<$8 and TWA $\geq$65 $\mu$V.

The NPV for SCD for patients with both low exercise capacity and high exercise-induced TWA was 99.8% over patients with neither factor (Table III). This finding is similar to our previous results using only exercise-induced TWA as predictor (98.6%) (11) and to results of TWA testing with the spectral method, for which NPV averages 97.2% (95% CI 96.5–97.9) (22). Reduced exercise capacity alone does not provide high positive predictive value (PPV) (2.3%) for SCD in our low-risk population (Table III). However, when low exercise capacity is combined with TWA $\geq$65 $\mu$V, PPV for SCD rose to 7.1% (Table III), which is highly comparable to the 8.0% result achieved with only TWA as a predictor in our previous study (11) and to the 6.0% level (95% CI 4.5–7.4) provided by TWA testing with the spectral method for cardiac arrhythmic events in low-risk patients (22).

With the exception of sex differences, the relative risks linked to traditional cardiovascular risk markers (Table II) were lower than those for reduced exercise capacity, with or without TWA. Thus, the combination of depressed exercise capacity and heightened TWA provides a marked prognostic index independent of traditional risk factors.

In the subgroup analyses in women, low exercise capacity (MET $<$5) predicted cardiovascular mortality, and increasing MET as continuous variable improved survival for all-cause and cardiovascular mortality. However, the low exercise capacity (MET $<$8) alone or in combination with elevated TWA ($\geq$65 $\mu$V) did not reach significance as predictor of all-cause or cardiovascular mortality in women. This may be due to the smaller number of events in this subgroup. Thus, further studies are needed to evaluate the prognostic power of combined analysis of low exercise capacity and elevated TWA in women.

SCD in a general population without congestive heart failure most commonly results from ventricular fibrillation triggered by an ischemic event (26). TWA reflects the presence of abnormal repolarization and electrophysiologic inhomogeneities that underlie vulnerability to ventricular fibrillation during myocardial ischemia (27). Exercise testing serves to expose latent electrical instability, as indicated by elevated levels of TWA. When analyzed together, exercise capacity and TWA provide supplementary information that strengthens the predictive value of either parameter alone, to 36-fold over risk in the absence of both factors (Table II). The fact that the end-points measure largely different characteristics is likely to underlie the additive effect. Exercise capacity essentially provides a measure of cardiac

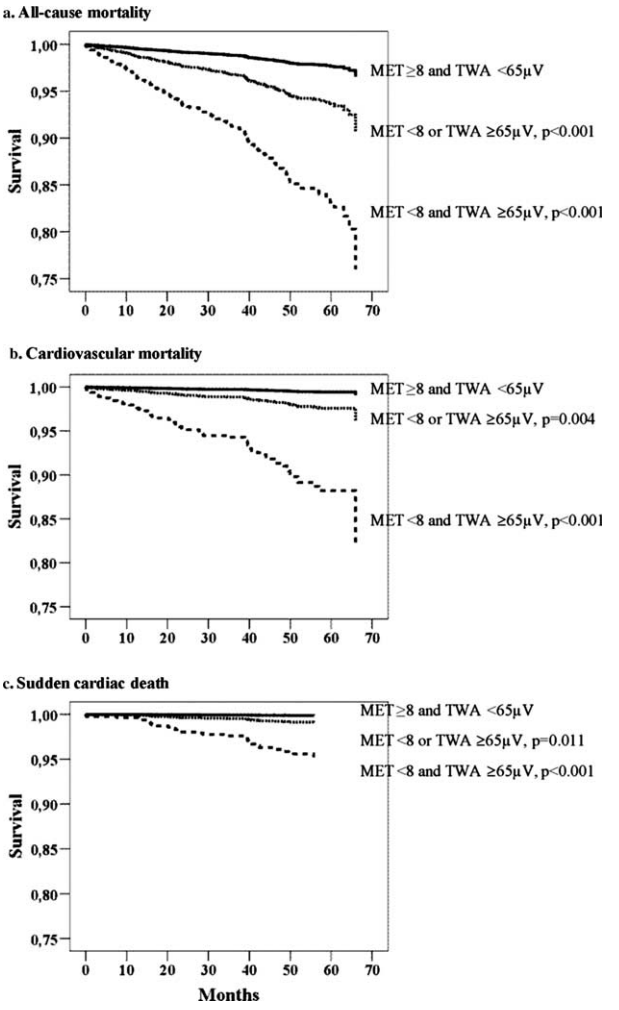


Figure 2. Adjusted survival curves by Cox regression anlysis for subjects with metabolic equivalents (MET) $\geq 8$ and T-wave alternans (TWA) $< 65$ microvolts ($\mu$V) (the upper curve in each panel), MET $< 8$ or TWA $\geq 65$ $\mu$V (the middle curve), and MET $< 8$ and TWA $\geq 65$ $\mu$V (the lower curve) for a) all-cause mortality, b) cardiovascular mortality, and c) sudden cardiac death. Please note that the scale for the y-axis is from 0.75 to 1.00.

mechanical function, whereas TWA is an indicator of cardiac electrical instability.

There are some limitations in our study. Establishing definitively that an event is SCD is inherently challenging. Our main criterion was death within 24 hours following onset of symptoms. The majority of deaths that were classified as SCD in this study were caused by acute coronary events, which have been shown to be the triggers for ventricular tachyarrhythmias leading to SCD (26,28,29). There were no signs of pulmonary embolism or pulmonary edema in autopsy information in patients with SCD. The presence of elevated exercise-induced TWA in patients with reduced exercise capacity was a stronger predictor of SCD than of either cardiovascular mortality or total deaths (Table II). The combination of heightened TWA with reduced exercise capacity also improved prediction of all-cause mortality, which is a definite end-point. A second limitation is the low PPV for SCD of exercise capacity alone, which is typical of low-risk groups, and which is improved by combined assessment with TWA. A third limitation is that we do not have information on changes in parameters affecting mortality risk (e.g. smoking, life-style, and medications) during follow-up. As with any observational study, it is not possible to draw causal inferences, and differences in variables that were not adjusted for or residual confounding may exist. Although the data reported in our study are from bicycle ergometer tests, it is likely that the results can be also generalized to populations undergoing a clinically indicated treadmill exercise test.

A broad implication of the present finding is that a mainstay measurement, namely exercise capacity, especially when combined with TWA assessment, is capable of identifying individuals whose risk for SCD is elevated but whose ejection fraction is normal. As exercise capacity was reduced in 58.5% of our population of consecutive patients referred for clinical exercise testing, TWA measurement can provide useful confirmatory information regarding their cardiac status. Because both parameters can be acquired automatically during the course of routine, symptom-limited exercise testing, without a specialized protocol or non-standard electrodes, this test has the potential for screening broad, diverse populations. The population tested was at relatively low risk of events, the group in which the greatest incidence of SCD occurs but in which identification of SCD risk has been elusive (26). Because combined measurement of mechanical function by exercise capacity and of cardiac electrical instability by TWA provides important insights into a potential basis for patients' risk for arrhythmia, it could prove helpful in identifying therapeutic targets for SCD reduction.

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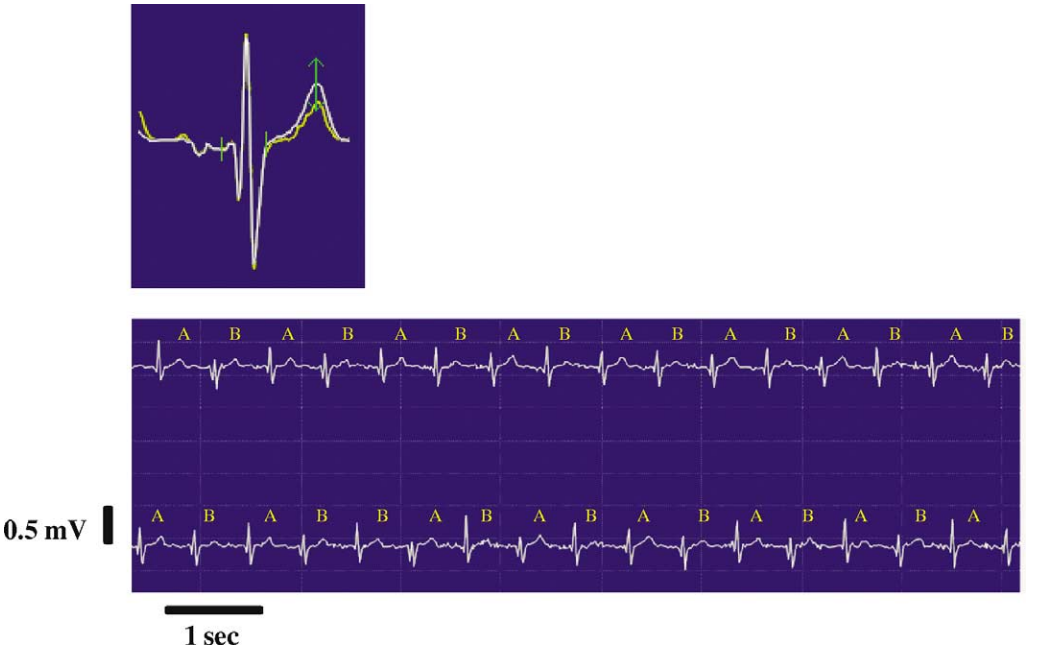


Figure 3. A representative electrocardiogram tracing and superimposed complexes of the lead V5 illustrating exercise-induced T-wave alternans (TWA) of 71 microvolts in a patient who experienced sudden cardiac death caused by an acute myocardial infarction at 3 months following the recording. The superimposed waveforms (upper panel) and rhythm strip (lower panel) are provided. The bidirectional arrow refers to the point of maximum TWA.

***Declaration of interest:*** Dr Richard L. Verrier is co-inventor of patents for T-wave alternans measurement, including by the modified moving average method, which were assigned to Georgetown University and Beth Israel Deaconess Medical Center and licensed to GE Healthcare. The other authors do not have any conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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# Combined assessment of heart rate recovery and T-wave alternans during routine exercise testing improves prediction of total and cardiovascular mortality: The Finnish Cardiovascular Study

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**BACKGROUND** Identification of individuals who are at risk for cardiovascular death remains a pressing public health challenge. Derangements in autonomic function acting upon an electrically unstable substrate are thought to be critical elements in triggering cardiovascular events.

**OBJECTIVE** The purpose of this study was to analyze heart rate recovery (HRR) in combination with T-wave alternans (TWA) to improve risk assessment.

**METHODS** The Finnish Cardiovascular Study (FINCAVAS) enrolled consecutive patients (N = 1,972 [1,254 men and 718 women], age 57 ± 13 years [mean ± SD]) with a clinically indicated exercise test using bicycle ergometer. TWA was analyzed continuously with the time-domain modified moving average method. Maximum TWA at heart rates <125 bpm was derived.

**RESULTS** During 48 ± 13 months of follow-up (mean ± SD), 116 patients died; 55 deaths were cardiovascular. In multivariable Cox analysis after adjustment for common coronary risk factors, high exercise-based TWA (≥60 μV) and low HRR (≤18 bpm) yielded relative risks for all-cause mortality of 5.0 (95% confidence 2.1–12.1, $P < .01$) and for cardiovascular mortality of 12.3 (95% confidence interval 4.3–35.3, $P < .01$). High recovery-based TWA (≥60 μV) and low HRR (≤18 bpm) yielded relative risks for all-cause death of 6.1 (95% confidence interval 2.8–13.2, $P < .01$) and for cardiovascular mortality of 8.0 (95% confidence interval 2.9–22.0, $P < .01$). Prediction by HRR and TWA, both singly and in combination, exceeded that of standard cardiovascular risk factors.

**CONCLUSION** Reduced HRR and heightened TWA powerfully predict risk for cardiovascular and all-cause death in a low-risk population. This novel approach could aid in screening of general populations during routine exercise protocols as well as improve insights into pathophysiology.

**KEYWORDS** Exercise test; Heart rate recovery; Mortality; Prognosis; T-wave alternans

**ABBREVIATIONS** **EF** = ejection fraction; **FINCAVAS** = Finnish Cardiovascular Study; **HRR** = heart rate recovery; **SCD** = sudden cardiac death; **TWA** = T-wave alternans

(Heart Rhythm 2009;6:1765–1771) 

## Introduction

An abnormal autonomic nervous system response in terms of heart rate recovery (HRR) during or after clinical exercise testing predicts all-cause and cardiovascular mortality in a variety of relatively low-risk cohorts,[1–7] including ours.[8] The reduction in heart rate during the first 30 to 60 seconds after exercise appears to be caused principally by reactivation of the parasympathetic nervous system but subsequently by withdrawal of sympathetic tone.[9]

T-wave alternans (TWA) is an ECG phenomenon indicating an electrically unstable myocardial substrate.[10] This beat-to-beat alternation in the shape, amplitude, or timing of the ST segment and the T wave has been found to predict sudden cardiac death (SCD) and cardiovascular and total mortality independent of standard factors in relatively low-

Dr. Verrier is co-inventor of the modified moving average method for T-wave alternans analysis, with patent assigned to Beth Israel Deaconess Medical Center and licensed by GE Healthcare. Financial support was received from the Medical Research Fund of Tampere University Hospital, Tampere Tuberculosis Foundation, and Finnish Cultural Foundation. **Address reprint requests and correspondence:** Dr. Mika Kähönen, Department of Clinical Physiology, Tampere University Hospital, FI-33520, Tampere, Finland. E-mail address: mika.kahonen@uta.fi. (Received March 15, 2009; accepted August 12, 2009.)

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risk populations,[11,12] including ours[13,14] as well as in higher-risk groups.[15–19] We applied the time-domain modified moving average method,[20] which permits TWA measurement during routine symptom-limited exercise.[13,14]

HRR and TWA reflect different pathophysiologic mechanisms. The aims of this study were to determine whether the combined analysis of HRR and TWA during routine exercise testing enhances their predictive power for cardiovascular and all-cause mortality over independent assessment of either variable and to compare their predictive strength to that of other standard risk factors.

## Methods

### Study cohort

All consecutive patients who were referred for an exercise stress test at Tampere University Hospital between October 2001 and the end of 2004 and were willing to participate in The Finnish Cardiovascular Study (FINCAVAS)[21] were recruited. A total of 1,972 patients (1,254 men and 718 women) with technically successful exercise tests were enrolled in the study. A test was considered technically adequate if storing the hemodynamic data and continuous digital ECG signal was successful. Patients with atrial fibrillation (N = 31) were excluded because atrial fibrillation is an exclusion criterion in HRR studies.[2,3] The main indications for the exercise test were suspicion of coronary heart disease (frequency 45%); testing vulnerability to arrhythmia during exercise (22%); evaluation of work capacity (18%) and the adequacy of treatment of coronary heart disease (16%); and obtaining an exercise test profile prior to an invasive procedure (13%) or after a myocardial infarction (8%). Some patients had more than one indication. The study protocol was approved by the Ethics Committee of the Tampere University Hospital District, Finland, and all patients gave informed consent prior to the interview and measurements as stipulated in the Declaration of Helsinki.

### Study flow

After written informed consent was obtained, the medical history of each patient was collected via a computer-based questionnaire form. The exercise test then was performed.

### Exercise test protocol

The subject lay down in the supine position for 10 minutes, and the resting ECG was digitally recorded. The upright routine exercise test then was performed using a bicycle ergometer with electrical brakes. The lead system consisted of the Mason-Likar modification of the standard 12-lead system. The initial workload varied from 20 to 30 W, and the load was increased stepwise by 10 to 30 W every minute. Continuous ECGs were digitally recorded at 500 Hz using the CardioSoft exercise ECG system (version 4.14, GE Healthcare, Freiburg, Germany). During the test, heart rate and ST segment deviation were continuously registered on the ECG, while systolic arterial pressure and diastolic arterial pressure were measured with a brachial cuff every 2 minutes.

### Measurement of HRR

HRR was determined as the difference between maximum heart rate during exercise minus heart rate during the first minute following cessation of exercise. We used the HRR cutpoint of ≤18 bpm, which has been suggested for exercise tests with an abrupt end.[22] Differences in recovery protocols have not negated the predictive strength of HRR.[22]

### Measurement of TWA

Assessing the relationship between TWA and mortality is one of the original goals of FINCAVAS.[21] We used the time-domain, Food and Drug Administration–cleared modified moving average method because of its intrinsic flexibility and demonstrated capacity to measure TWA accurately under dynamic conditions, including changing heart rates, myocardial ischemia, exercise, activity, and behavioral stress.[11,13,14,16,19,23] In brief, the modified moving average algorithm reports TWA as the maximum difference in T-wave morphology between successive beats. It separates odd from even beats, calculates average morphologies of both the odd and even beat streams separately, and continuously updates the result by a weighting factor of 1/8 of the difference between the ongoing average and the new incoming beat. The method performs at a resolution of 1 $\mu$V and has undergone extensive validation.[20]

TWA values were calculated automatically and continuously by the released version of GE Healthcare's modified moving average algorithm during rest, exercise, and recovery using all standard precordial leads ($V_1$–$V_6$). Maximum TWA values at heart rates <125 bpm were derived. TWA values at higher heart rates were excluded because inaccuracies in TWA measurement can result at heart rates exceeding this range. Precordial leads have been shown to be optimum for TWA measurement.[24,25] The exercise-based TWA cutpoint of 60 $\mu$V, which yielded excellent Cox regression results in our previous study,[14] was used. Recovery-based TWA values were analyzed according to cutpoints 20 $\mu$V and 60 $\mu$V.[14,26] TWA cutpoint of 20 $\mu$V was chosen because it has shown the highest sensitivities compared with other cutpoints.[26]

### Left ventricular ejection fraction

Measurement of left ventricular ejection fraction (EF) is not routine for patients referred for a clinical exercise test. However, EF was determined for 1,200 (55%) of the study patients using echocardiography or isotope techniques within 6 months of the exercise test. More than one fifth (N = 408 [21%]) of the patients were examined with coronary angiography.

### Follow-up

Death certificates were received from the Causes of Death Register, maintained by Statistics Finland, in May 2007, a source that has been shown to be reliable.[27] The certificates included causes of death using the tenth revision of the International Classification of Diseases (ICD-10). The diagnosis numbers and certificate texts were used to classify the

**Table 1** Patient characteristics of the study population

| | Survivors (N = 1,856) | | Deaths (N = 116) | | |
|---|---|---|---|---|---|
| Parameter | Mean | SD | Mean | SD | *P* value |
| Age (years) | 56.5 | 13.1 | 65.1 | 11.3 | <.01 |
| Weight (kg) | 80.5 | 15.2 | 79.0 | 15.8 | .27 |
| Height (cm) | 171.0 | 9.3 | 171.6 | 9.3 | .49 |
| Body mass index | 27.5 | 4.5 | 26.7 | 4.1 | .07 |
| | N | % | N | % | *P* value |
| Sex: female | 776 | 37.8 | 30 | 23.6 | <.01 |
| Smoking: yes | 534 | 26.0 | 41 | 32.3 | .12 |
| Nitrates | 695 | 33.9 | 66 | 52.0 | <.01 |
| Beta-blockers | 1,174 | 57.3 | 99 | 78.0 | <.01 |
| Hypercholesterolemia | 1,024 | 49.9 | 64 | 50.4 | .91 |
| Diabetes | 238 | 11.6 | 23 | 18.1 | .03 |
| Coronary heart disease | 784 | 38.2 | 64 | 50.4 | .01 |
| Left ventricular hypertrophy | 91 | 4.4 | 7 | 5.5 | .57 |
| History of myocardial infarction | 425 | 20.7 | 44 | 34.6 | <.01 |

deaths as all cause or cardiovascular. The investigators who analyzed TWA test results were blinded to events.

## Statistical analysis

The t-test for independent samples was used to compare continuous parameters of patient characteristics (Table 1) and exercise test variables (Table 2) for survivors and nonsurvivors. The Chi-square test was applied for dichotomous variables. *P* values were derived with the t-test and the Chi-square test for independent samples. Relative risks for total and cardiovascular mortality were analyzed for HRR, TWA, and their combinations as well as for ST-segment deviation by Cox regression analysis after adjustment by standard covariates (Table 3). The proportionality assumption for all covariates was checked by using correlations of the survival rankings with the Schoenfeld residuals. All of the covariates fulfilled the proportionality assumption. Harrell's C indices also were calculated (Table 4). The calculations for combination variables were based on three categories: no parameter positive, either parameter positive, and both parameters positive. Harrell's C index is a generalization of the area under the receiver operator characteristic (ROC) curve for survival data with censored cases. Values above 0.5 show better than random prediction, and a value of one represents perfect concordance between predicted and observed numbers.

Statistics were analyzed using SPSS release 14.0 for Windows (SPSS, Inc., Chicago, IL, USA) and Stata 10.1 for Windows (StataCorp LP, College Station, TX, USA). All statistical tests were two-tailed and used an alpha level <.05.

## Results

### Baseline characteristics

During the follow-up period of 48 ± 13 months (mean ± SD) in our study population of 1,972 consecutive patients referred for clinical exercise testing, there were 116 deaths (5.9% of the population), including 55 (2.8% of the popu-

**Table 2** Exercise test variables of the study population

| | Survivors (N = 1,856) | | Deaths (N = 116) | | |
|---|---|---|---|---|---|
| Parameter | Mean | SD | Mean | SD | *P* value |
| Duration of test (minutes) | 7.5 | 2.1 | 6.2 | 2.0 | <.01 |
| Age-adjusted expected maximum HR (bpm) | 176.8 | 6.7 | 172.5 | 5.6 | <.01 |
| Reached maximum HR (bpm) | 146.6 | 26.4 | 127.0 | 29.6 | <.01 |
| Maximum SAP during the exercise (mmHg) | 193.6 | 28.5 | 175.6 | 32.0 | <.01 |
| Maximum DAP during the exercise (mmHg) | 92.3 | 11.9 | 85.1 | 13.4 | <.01 |
| HR at rest (bpm) | 63.1 | 11.6 | 64.5 | 14.5 | .19 |
| SAP at rest (mmHg) | 136.1 | 18.6 | 135.2 | 25.8 | .62 |
| DAP at rest (mmHg) | 79.7 | 9.6 | 75.7 | 11.4 | <.01 |
| Maximum TWA at rest before exercise ($\mu$V) | 19.4 | 11.5 | 25.5 | 17.2 | <.01 |
| Maximum TWA during exercise ($\mu$V) | 35.8 | 21.8 | 39.9 | 23.3 | .04 |
| Maximum TWA during recovery ($\mu$V) | 26.7 | 23.4 | 31.3 | 19.5 | .03 |
| Maximum left ventricular ejection fraction | 65.9 | 13.8 | 60.2 | 15.6 | <.01 |
| HRR at 1 minute postexercise (bpm) | 24.7 | 11.5 | 18.2 | 13.8 | <.01 |
| ST-segment deviation during exercise (mV) | 0.08 | 0.10 | 0.11 | 0.14 | .01 |

DAP = diastolic arterial pressure; HR = heart rate; HRR = heart rate recovery; SAP = systolic arterial pressure; TWA = T-wave alternans.

**Table 3** Results of Cox multivariable regression analysis (N = 1,972) of relative risks for all-cause mortality and cardiovascular mortality

| | All-cause mortality | | | | Cardiovascular mortality | | | |
|---|---|---|---|---|---|---|---|---|
| | | 95% CI | | | | 95% CI | | |
| | RR | Lower | Upper | *P* value | RR | Lower | Upper | *P* value |
| HRR ≤18 bpm | 2.5 | 1.6 | 3.7 | <.01 | 2.3 | 1.3 | 4.2 | .01 |
| Exercise-based TWA ≥60 μV | 2.5 | 1.4 | 4.5 | <.01 | 5.8 | 3.1 | 11.1 | <.01 |
| Recovery-based TWA ≥20 μV | 1.1 | 0.7 | 1.6 | .73 | 1.5 | 0.8 | 2.5 | .18 |
| Recovery-based TWA ≥60 μV | 2.4 | 1.3 | 4.4 | <.01 | 3.5 | 1.6 | 7.9 | <.01 |
| HRR ≤18 bpm and exercise-based TWA ≥60 μV | 5.0 | 2.1 | 12.1 | <.01 | 12.3 | 4.3 | 35.3 | <.01 |
| HRR ≤18 bpm or exercise-based TWA ≥60 μV | 2.8 | 1.8 | 4.3 | <.01 | 3.4 | 1.8 | 6.6 | <.01 |
| HRR and recovery-based TWA ≥20 μV | 3.0 | 1.6 | 5.5 | <.01 | 5.2 | 1.8 | 14.4 | <.01 |
| HRR or recovery-based TWA ≥20 μV | 2.0 | 1.2 | 3.5 | .01 | 3.5 | 1.3 | 9.1 | .01 |
| HRR and recovery-based TWA ≥60 μV | 6.1 | 2.8 | 13.2 | <.01 | 8.0 | 2.9 | 22.0 | <.01 |
| HRR or recovery-based TWA ≥60 μV | 2.3 | 1.5 | 3.5 | <.01 | 2.2 | 1.2 | 4.2 | .01 |
| ST-segment deviation (0.1 mV) during exercise | 1.3 | 0.9 | 1.9 | .18 | 1.8 | 1.0 | 3.0 | .04 |

Results after adjustment for sex, age, body mass index, smoking (yes/no), use of beta-blockers (yes/no), reached maximum heart rate, and prior diagnoses of coronary heart disease (yes/no), history of myocardial infarction (yes/no), diabetes (yes/no), and hypercholesterolemia (yes/no).

CI = confidence interval; HRR = heart rate recovery; RR = relative risk; TWA = T-wave alternans.

lation; 47.4% of all deaths) that were classified as cardiovascular deaths. Thus, the cardiovascular mortality of the present patients was 0.7% per year. Patient characteristics and exercise test variables for survivors (N = 1,856) and nonsurvivors (N = 116) are given in Tables 1 and 2, respectively.

## Mortality, HRR, and TWA

HRR was abnormal in 29.5% (N = 590) of the population. Exercise-based TWA ≥60 μV was found in 5.2% (N = 107). During recovery, 51.3% (N = 1,063 patients) had TWA ≥20 μV, including 3.9% (N = 81 patients) with TWA ≥60 μV. Thus, the present approach classified the majority of the patients as low risk. Combined Cox proportional hazard analysis of depressed HRR and heightened exercise- or recovery-based TWA more than doubled the prognostic capacity for total and cardiovascular mortality after adjustment for standard risk factors and exceeded exercise-induced ST-segment deviation (Table 3). In addition to standard covariates, maximum left ventricular EF, blood pressures at rest, maximum blood pressures during exercise, and resting heart rate were added to the multivariate analysis with the combination of HRR and TWA. None of these factors exceeded the predictive power of the combination of HRR and TWA. Incidence rates of all-cause and cardiovascular deaths in subgroups are shown in Figure 1.

Harrell's C indices were calculated for all single and combination parameters as well as for ST-segment deviation (Table 4). For the single parameters, HRR provided the highest C index for both total and cardiovascular mortality. Adding exercise-based TWA ≥60 μV to reduced HRR yielded highest C index for all-cause and cardiovascular mortality, although confidence intervals overlapped with HRR alone.

Survival curves depict events across 4 years of follow-up for the combined analysis of reduced HRR and elevated TWA during exercise (Figure 2) and recovery (Figure 3).

## Discussion

Our study is the first to demonstrate that the presence of high levels of TWA during exercise or recovery adds significantly to the prognostic power of poor HRR for all-cause and cardiovascular mortality. Because both markers are automated and widely used parameters that can be moni-

**Table 4** Harrell's C indices for cardiovascular and all-cause mortality

| | All-cause death | 95% CI | | Cardiovascular death | 95% CI | |
|---|---|---|---|---|---|---|
| | | Lower | Upper | | Lower | Upper |
| HRR ≤18 bpm | 0.655 | 0.609 | 0.702 | 0.650 | 0.583 | 0.718 |
| Exercise-based TWA ≥60 μV | 0.539 | 0.507 | 0.570 | 0.594 | 0.535 | 0.653 |
| Recovery-based TWA ≥20 μV | 0.555 | 0.508 | 0.601 | 0.606 | 0.544 | 0.668 |
| Recovery-based TWA ≥60 μV | 0.526 | 0.499 | 0.553 | 0.550 | 0.535 | 0.653 |
| HRR ≤18 bpm and/or exercise-based TWA ≥60 μV | 0.677 | 0.631 | 0.723 | 0.713 | 0.648 | 0.777 |
| HRR ≤18 bpm and/or recovery-based TWA ≥20 μV | 0.655 | 0.608 | 0.702 | 0.691 | 0.633 | 0.749 |
| HRR ≤18 bpm and/or recovery-based TWA ≥60 μV | 0.661 | 0.614 | 0.709 | 0.671 | 0.602 | 0.740 |
| ST-segment deviation (0.1 mV) in exercise test | 0.558 | 0.510 | 0.606 | 0.580 | 0.511 | 0.649 |

Calculations for combination variables were based on three categories (0, 1, or 2 parameters positive).

CI = confidence interval; HRR = heart rate recovery; TWA = T-wave alternans.

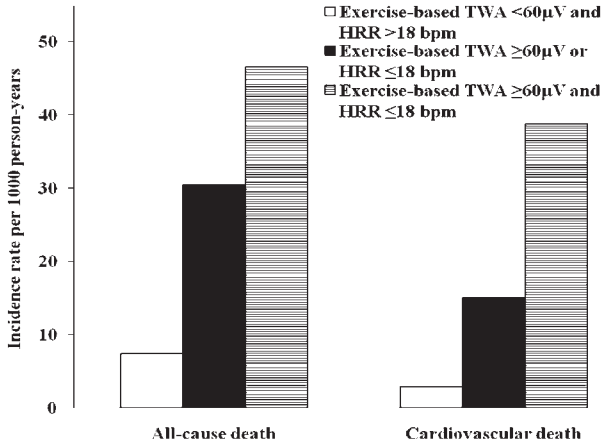


**Figure 1** Incidence rate of all-cause and cardiovascular mortality per 1000 person-years among patients according to exercise-based T-wave alternans (TWA) and heart rate recovery (HRR).

tored in conjunction with routine exercise testing, their combination may serve as a new risk stratification tool for screening low-risk patient populations.

## Previous studies

The significant influence of autonomic nervous system activity on cardiovascular and total mortality has been amply

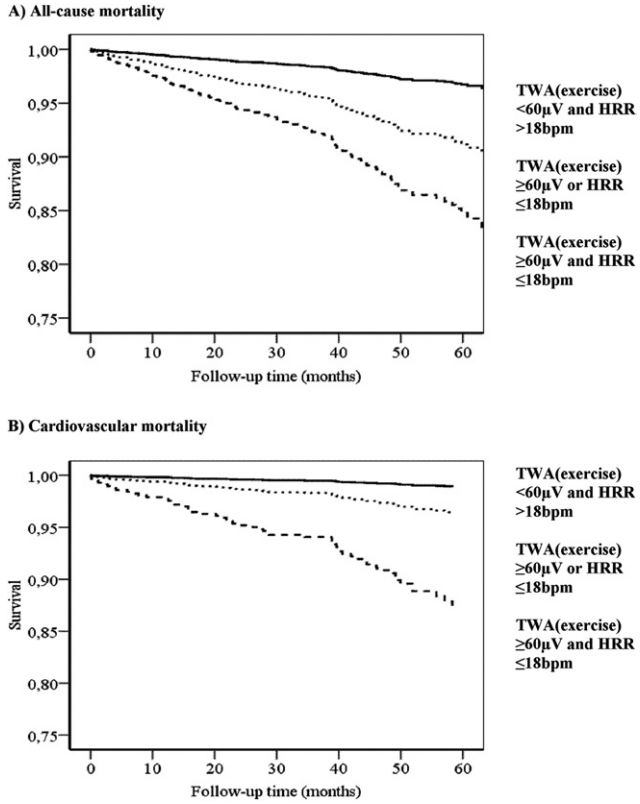


**Figure 2** Adjusted survival curves by Cox regression for subjects with exercise-based T-wave alternans (TWA) <60 $\mu$V and heart rate recovery (HRR) >18 bpm (*top curve* in both panels), TWA ≥60 $\mu$V or HRR ≤18 bpm (*middle curve* in both panels), and TWA ≥60 $\mu$V and HRR ≤18 bpm (*bottom curve* in both panels) for all-cause mortality (**A**) and cardiovascular mortality (**B**). Note that the scale for the y-axis is from 0.75 to 1.00.

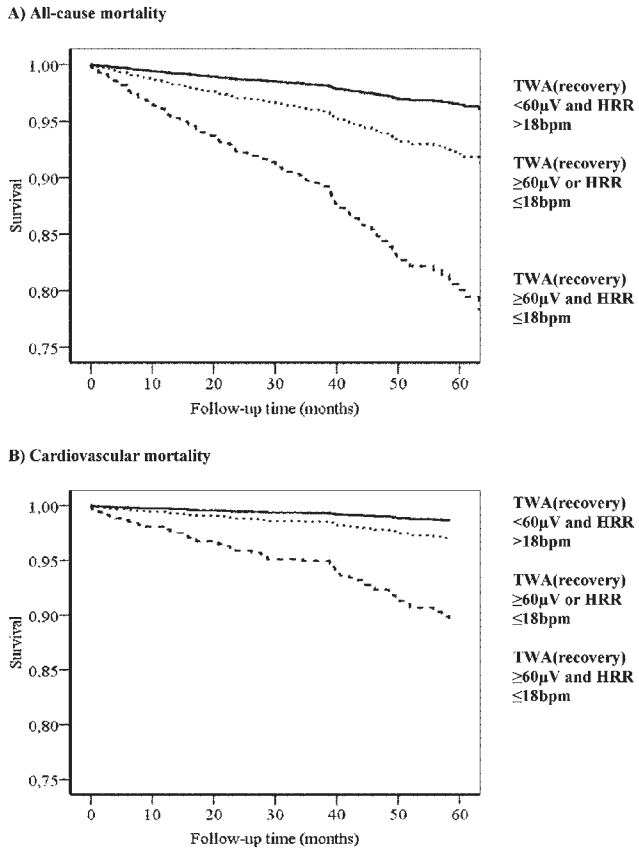


**Figure 3** Adjusted survival curves by Cox regression for subjects with recovery-based T-wave alternans (TWA) <60 $\mu$V and heart rate recovery (HRR) >18 bpm (*top curve* in both panels), TWA ≥60 $\mu$V or HRR ≤18 bpm during recovery (*middle curve* in both panels), and TWA ≥60 $\mu$V and HRR ≤18 bpm during recovery (*bottom curve* in both panels) for all-cause mortality (**A**) and cardiovascular mortality (**B**). Note that the scale for the y-axis is from 0.75 to 1.00.

demonstrated, most recently by baroreceptor sensitivity (BRS)[28] and noninvasive assessment with heart rate variability,[28,29] heart rate turbulence,[30] and HRR.[1–8] The latter is a strong predictor of cardiovascular mortality in asymptomatic patients[5,7,8] and in broad populations[4] as well as of SCD.[1] Importantly, impaired HRR is not attributable to ischemic burden[3] or lipid abnormalities.[6] Treadmill exercise scores strongly predict mortality among intermediate- to high-risk patients if HRR is abnormal.[6]

The accuracy and utility of exercise-based TWA in predicting arrhythmic events and death have been investigated.[12–15] Most TWA studies have been performed in high-risk populations, such as patients with heart failure,[15,17–19] cardiomyopathies,[15,18] or history of myocardial infarction.[11,12,15–19] We previously reported in approximately 2,000 FINCAVAS patients that TWA analyzed with the modified moving average method is a strong predictor of all-cause and cardiovascular mortality as well as of SCD in this low-risk population.[14] Especially high specificity when compared with other cardiovascular parameters has characterized the prognostic value of elevated TWA,[13] suggesting suitability to confirm suspected risk.

The potential to improve prediction of cardiovascular and total mortality by combining TWA with the ambulatory ECG-based autonomic marker of heart rate turbulence was recently confirmed in a high-risk population of postmyocardial infarction patients with left ventricular dysfunction.[16] The present study, which enrolled a 6.9-fold larger, lower-risk population of almost 2,000 patients, demonstrated further improvements in odds ratio.

### Current investigation

The present study confirms and extends the findings of our previous investigations of TWA[13,14] and HRR[8] in the low-risk FINCAVAS patient population. When analyzed together, TWA and HRR provide high relative risk ratios for all-cause death and for cardiovascular mortality after adjustment for standard risk factors (Table 3), indicating a marked independent prognostic capacity and exceeding the predictive value of either parameter alone or ST-segment deviation. The combinations of reduced HRR with heightened TWA were superior to exercise-induced ST-segment deviation in our low-risk population using Cox proportional hazards models (Table 3) and Harrell's C indices (Table 4). The incidence rate of all-cause as well as cardiovascular deaths was clearly higher among patients with reduced HRR and heightened TWA compared to patients with normal values (Figure 1).

The mechanistic basis for the improvement in prediction resulting from combined analysis of HRR and TWA is unclear. A plausible explanation is that a more complete picture of underlying pathophysiologic factors is rendered by information regarding both autonomic function and cardiac electrical instability. As HRR is thought to reflect the dynamic interplay between sympathetic and parasympathetic nerve activity as influenced by changes in baroreceptor gain,[1] a reduced HRR may indicate autonomic imbalance as a basis for cardiovascular events. Moreover, HRR may reflect aerobic capacity and physical fitness, which have been linked to prognosis.[31] The independent association between increased risk for all-cause and cardiovascular mortality and TWA[13,14] is consistent with the finding that TWA indicates increased heterogeneity of repolarization.[10,32] Although the incidence of SCD was not evaluated in the current investigation, because both TWA[13,14] and reduced HRR[1] have been independently associated with SCD in low-risk populations, it is possible that a number of the cardiovascular deaths were arrhythmic in origin. Atherosclerotic heart disease, typical of 29 (48%) of patients who died of cardiovascular causes, predisposes to ventricular fibrillation and SCD.[33] Accordingly, reduced HRR could indicate impaired vagus nerve activation and lessened capacity to withdraw sympathetic nerve tone, both influences known to be arrhythmogenic.[27,34] Thus, the presence of both abnormal HRR and elevated TWA, reflecting derangements in autonomic function as well as in cardiac electrical instability, would be expected to be associated with the highest risk for cardiovascular events, as demonstrated in the present study.

## Study limitations

We do not have information on changes in parameters affecting mortality risk (e.g., smoking, lifestyles, medication) during follow-up. In addition, data on EF were not available for 45% of patients. It is likely that patients in whom no need was found for EF determinations had even better cardiovascular health than did those with EF measurement. EF is an arrhythmia risk stratifier only when EF levels are below normal.[35]

## Conclusion

A broad implication of the study finding is that routine exercise testing discloses increased risk for cardiovascular as well as all-cause death among patients with both depressed HRR and abnormal TWA who are not identified by standard risk factors. In addition to improving predictivity, the combined assessment of HRR and TWA may be helpful in gaining insight into the pathophysiologic mechanisms on an individual patient basis that could help to guide therapy. In particular, patients with markedly depressed HRR could be directed toward an exercise training regimen that improves vagus nerve tone, BRS, and long-term prognosis.[31] TWA results reflective of an unstable cardiac substrate could signal the need for antiarrhythmic therapy. Finally, particularly as the measurements can be performed noninvasively during routine exercise testing in the typical flow of clinical care, these parameters can readily be incorporated, either singly or in combination, into routine risk assessment paradigms.

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