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T-vector and T-loop morphology analysis of ventricular repolarization in ischemic heart disease

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To my family

CONTENTS

ABSTRACT

Background Sudden cardiac death (SCD) is responsible for about half of all cardiovascular deaths in the western world. Heterogeneous ventricular repolarization (VR) is a common denominator in the genesis of malignant ventricular arrhythmias responsible for SCD and the presence of coronary artery disease (CAD) is an aggravating factor. A non-invasive method reliably reflecting VR heterogeneity could therefore play a significant role in the preventive strategy against SCD. This thesis focuses on VR in CAD and acute ischemia.

Aims To study VR abnormalities in patients with CAD using 3-dimensional (3-D) vectorcardiography (VCG). To assess VR at rest and during acute ischemia in patients with/without major co-morbidities, including hypertension and left ventricular hypertrophy (LVH), applying recently developed VCG parameters. To assess VR alterations in relation to the amount of the ischemic myocardium. To explore the prognostic value of these parameters in terms of cardiovascular (CV) mortality and morbidity during long-term follow-up.

Studies I-II As a first step, VR measures at rest and during acute ischemia were analyzed in a subgroup of 56 CAD patients selected to create a relatively homogeneous group without obvious confounders affecting the VR response, e.g. previous myocardial infarction (MI) or LVH. They were identified from a cohort of 187 patients planned for an elective single-vessel percutaneous coronary intervention (PCI). In the next step, the electrophysiological consequences of myocardial hypertrophy were assessed in all 187 CAD patients, including 33 with LVH and 54 with a history of hypertension. VR was examined in terms of the maximum T-vector orientation in space by azimuth and elevation and the angular relationship with the main depolarization vector, the QRS-T angle. The planarity of the T loop (Tavplan), its shape and roundness (Teigenv) and the area under the 3-D T-wave (Tarea) were analyzed as well. At rest, the Tarea and Teigenv differed significantly between CAD patients and healthy controls. Acute ischemia most consistently reduced T-loop planarity and increased its roundness and area under the T-wave. Only occlusion of the left anterior descending artery (LAD) significantly changed the T-vector orientation. Patients with LVH had not only the most abnormal VR at rest but also a significantly more pronounced VR response during coronary occlusion. Patients with a history of hypertension (without LVH) had mean parameter values between the LVH patients and those with neither hypertension nor LVH.

Study III The relationship between the size and location of the myocardium at risk (MAR) and the VR response during ischemia was studied during elective PCI in another cohort of 35 CAD patients. Tc-99m-sestamibi was administered intravenously immediately after coronary occlusion. The perfusion defect severity and MAR were quantified by automated software. The VR measures during maximum ischemia was compared with baseline and the changes (Δ) were related to the MAR and the occluded artery. There were significant correlations between MAR size and ST-segment alterations (STC-VM, ΔST-VM), as previously shown, but also with ΔTavplan and ΔTeigenv, although they were most prominent during LAD occlusion, which induced the largest MAR size.

Study IV In a longitudinal cohort study, the 187 CAD patients (Study II) were followed for 8±1 years. There were 16 CV deaths, 19 new MIs and more then 70 additional revascularizations. CV death was independently predicted by a prolonged QRS duration and a widened QRS-T angle, along with left ventricular dysfunction or hypertrophy. MI was most consistently predicted by increased Tavplan. Repeat revascularization was predicted by the presence of diabetes and the absence of stent implantation.

Conclusion CAD patients displayed changes in VR compared with the healthy controls, even in the absence of major co-morbidities. Short-lasting LAD occlusion induced the most pronounced VR changes, which were associated with the largest amount of jeopardized myocardium compared with the other coronary arteries. Myocardial hypertrophy was associated not only with the most abnormal VR at baseline but also with the most exaggerated VR response during ischemia. These observations are consistent with epidemiological, experimental and autopsy data showing a predominance of LAD disease and/or myocardial hypertrophy in SCD victims. A widened QRS-T angle was independently associated with the CV deaths, which is consistent with previous studies, and an increased distortion of the T-loop (Tavplan) with subsequent MI, which is a novel finding. VCG-based VR analysis might prove to be a useful tool in assisting the identification of risk individuals and for following the effects of preventive therapies.

List of Original Papers

This thesis is based on the following studies, which will be referred to by their Roman numerals.

I

Rubulis A, Jensen J, Lundahl G, Tapanainen J, Wecke L, Bergfeldt L.

T vector and loop characteristics in coronary artery disease and during acute ischemia.

Heart Rhythm 2004;1:317-325

II

Rubulis A, Jensen J, Lundahl G, Tapanainen J, Bergfeldt L.

Ischemia-induced aggravation of repolarization abnormalities in left ventricular hypertrophy: a deleterious interaction.

J Appl Physiol 2006;101:102-110

III

Rubulis A, Jensen SM, Näslund U, Lundahl G, Jensen J, Bergfeldt L.

Ischemia-induced repolarization alterations in relation to the size and location of the ischemic myocardium – observations from a human model of short-lasting coronary occlusion.

In manuscript

IV

Rubulis A, Jensen J, Rydén L, Bergfeldt L.

Prediction of cardiovascular death and myocardial infarction by the QRS-T angle and T-vector loop morphology after angioplasty in stable angina pectoris - 8-year follow-up.

In manuscript

List of Abbreviations

APD	Action potential duration
Ca^{2+}	Calcium ion
CABG	Coronary artery bypass surgery
CAD	Coronary artery disease
CV	Cardiovascular
ECG	Electrocardiogram
EF	Ejection fraction
ERP	Effective refractory period
ICD	Implantable cardioverter defibrillator
IK_{atp}	Adenosine triphosphate (ATP) -sensitive potassium channel
K^{+}	Potassium ion
LAD	Left anterior descending artery
Lcx	Left circumflex artery
LV	Left ventricle
LVH	Left ventricular hypertrophy
MAP	Monophasic action potential
MAR	Myocardium at risk
MI	Myocardial infarction
MIDA	Myocardial Infarction Dynamic Analysis (Ortivus AB, Täby, Sweden)
Na^{+}	Sodium ion
PCI	Percutaneous coronary intervention
PPV	Positive predictive value
RCA	Right coronary artery
s	The intra-individual standard deviation
SCD	Sudden cardiac death
SPECT	Tc-99m-sestamibi single photon emission computed tomography
TWA	T-wave alternance
VCG	Vectorcardiography
VF	Ventricular fibrillation
VR	Ventricular repolarization
VT	Ventricular tachycardia
WHO	World Health Organisation
3-D	Three-dimensional

INTRODUCTION

General

Modern cardiology is the result of outstanding medical development over the past 60 years. At the beginning of 1950s, physicians were almost helpless when confronted by problems related to acute coronary thrombosis and malignant ventricular arrhythmias, including sudden cardiac death (SCD), disorders which are much better managed nowadays [1]. In spite of this, the death toll from SCD in the western world accounts for the majority of all cardiovascular (CV) deaths [2]. Abnormalities of ventricular repolarization (VR) and coronary artery disease (CAD) are often key players, having a deleterious effect when they coexist [2-4]. If detected in time, medical treatment (thrombolysis), percutaneous coronary interventions (PCI), or implantable cardioverter defibrillators (ICD) might greatly reduce the risk of SCD [5]. However, the prediction and thereby the prevention of future SCD events is still "terra incognita" and represents an unresolved problem.

Many SCD risk markers are available: clinical, such as previous myocardial infarction (MI), left ventricular function, left ventricular hypertrophy (LVH) and autonomous nervous system activity; and electrophysiological, such as QT interval dispersion, QTc interval, T-wave alternance (TWA), are good at the group level, whereas at the individual level they are relatively poor predictors [6-8] (Table 1). Moreover, they have been mainly

Table 1. Risk factors for sudden cardiac death. (Adapted from Zipes DP. Can J Cardiol 2005;21:37A-40A).

Male sex
Smoking
Diabetes
Obesity
Inactivity
Previous myocardial infarction or history of coronary artery disease
Low left ventricular ejection fraction and heart failure
Left ventricular hypertrophy
Previous sudden cardiac arrest or prior episode of ventricular tachycardia
Ventricular ectopy in chronic ischemic heart disease
Premature ventricular complexes during recovery from treadmill exercise
Atrial fibrillation
Abnormal electrophysiological parameters (QTc, QRS duration, QT dispersion, T wave altenance, decreased heart rate variability etc.)

tested in a high-risk population, which comprises only a small percentage of all potential SCD victims (Figure 1). In the lower-risk groups, SCD could be the first and last sign of CAD. For this reason, the search is on for reliable, non-invasive risk predictors of SCD. In this context, a well-established association between abnormalities in repolarization, malignant ventricular arrhythmia and SCD is promoting the 3-dimensional (3D) vectorcardiographic (VCG) analysis of the VR [2,4,9-10] in order to explore potential risk markers of this kind.

Based on previous work from this institution and Umeå University Hospital [11-14], the present thesis has validated recently introduced VCG based VR morphology descriptors. An evaluation of their behavior in patients with CAD at rest and during acute ischemia provoked by PCI, as well as analyses of prognostic value for CV risk estimation, was performed.

Sudden cardiac death

The definition of SCD has been evolving and has come in for a great deal of scrutiny and criticism. The previous definition of SCD established in the 1960s [15] was primarily time based and implied unexpected CV death occurring within 24 hours of the onset of the fatal event. A more recent definition defines SCD as unexpected death as the result of CV causes in a person with or without pre-existing heart disease, within 1 hour of the onset of change in clinical status [2]. The mechanism of death, whether arrhythmic or non-arrhythmic, cannot, however, always be inferred from the time course alone. Most SCD are unwitnessed and unmonitored and autopsies are rarely performed, so the categorization of death becomes somewhat arbitrary [16].

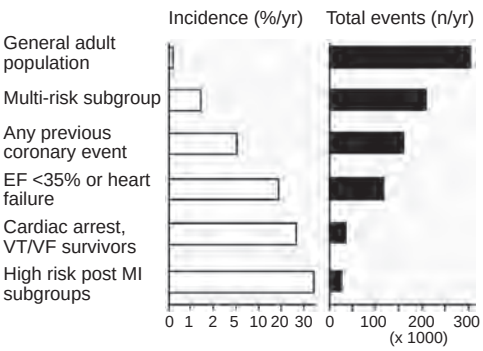


Figure 1. Epidemiology of sudden cardiac death. (Adapted from Myerburg RJ et al. Circulation 1998;97:1514-1521).

Epidemiology: So-called widow maker (because it often strikes unexpectedly, more frequently men and without previously known CAD) accounts for about 50% of all CV deaths and 5.6% of total annual mortality in the USA according to a relatively recent study [17]. Although age-adjusted mortality due to CAD has decreased, the incidence of SCD appears to be rising, in line with increasing global rates of CAD and heart failure. SCD is the first and last manifestation in 20% of coronary deaths. It spares no age, race, or gender, although its preponderance in older men is a reflection of the prevalence of CAD in the population. The underlying cause of SCD is CAD in 80%, cardiomyopathy in 15% and others in 5% [17]. Common risk factors are shown in Table 1.

Mechanism: Based on electrocardiographic (ECG) monitoring at the onset of SCD, it appears that VT or VF is the most common initial rhythm. Ambulatory ECG recordings obtained from a large series of patients who were wearing a Holter monitor at the time of cardiac arrest show that ventricular tachyarrhythmias, including monomorphic ventricular tachycardia (VT) that transformed to ventricular fibrillation (VF), were the primary event in 83% of patients, with bradyarrhythmias in 17% [18], regardless of the underlying heart disease.

The most common sequence of events leading to SCD appears to be the degeneration of VT into VF, followed by asystole or pulseless electrical activity (Figure 2).

In simple terms, SCD requires interac-

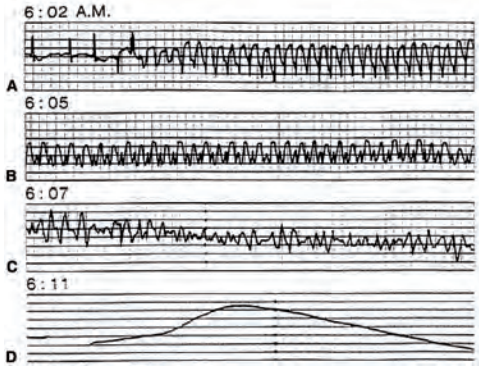


Figure 2. Typical sequence of events leading to sudden cardiac death. (From Akhtar M et al. Ann Intern Med 1991;114:499).

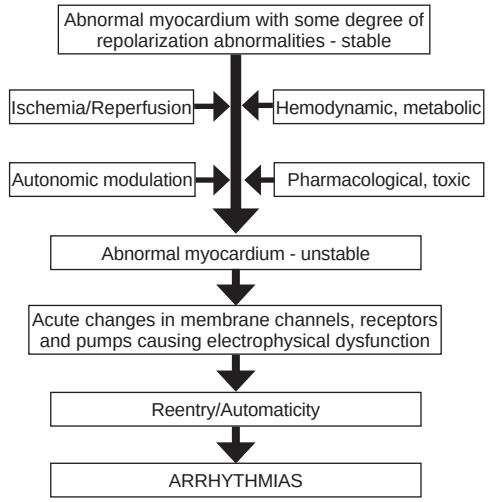


Figure 3. Pathophysiology of sudden cardiac death. (Modified from Myerburg RJ et al. PACE 1991;14:935-943).

tion between a structurally abnormal substrate and its modulation and a trigger induced by a transient perturbation (Figure 3). Pre-existing CAD and its consequences, such as acute myocardial ischemia, scarring from previous MI and heart failure, all usually accompanied by some degree of ***repolarization abnormalities,*** are manifest in a majority of SCD victims [3-4]. Other structural abnormalities include myocardial hypertrophy of different etiologies, cardiomyopathies, structural electrical abnormalities, ion channellopathies and valvular diseases [19].

The most common transient perturbation is an acute ischemia or reperfusion, which alters the regional conduction velocities and creates increased dispersion of conduction patterns and refractoriness, thereby facilitating re-entry. The autonomic nervous system also plays an important part as a modulator in SCD. Animal data show that, in the setting of acute MI, enhanced sympathetic activity promotes malignant ventricular tachyarrhythmias, while beta blockade or increased vagal tone provides a protective antifibrillatory effect [20-21]. Electrolyte disturbances can also provoke lethal arrhythmias. Antiarrhythmic drug therapy can facilitate reentry by stabilizing pre-existing circuits or by creating new areas of functional conduction block [22-23]. Hemodynamic factors, such as increasing ventricular volume, might change myocardial refractoriness and facilitate ventricular arrhythmias (mechano-electrical coupling) [24-25].

In some circumstances, SCD can be mimicked by non-cardiac causes, such as a rupture of an aortic aneurysm, intracerebral hemorrhage, massive pulmonary embolism and prosthetic valve dysfunction [16]. Clinical information may be helpful in identifying these as the mechanism of death, but a carefully performed autopsy is the most credible method.

Prevention: No specific anti-arrhythmic pharmacological therapies have been shown to reduce mortality. However, a reduction in mortality has been achieved by other agents: beta-blockers, acetylsalicylic acid, statins, angiotensin-converting enzyme inhibitors and spironolactone [26].

The role of ICD in the prevention of SCD in high-risk population is indisputable [27]. However, if the MADIT-II criteria and SCD-HeFT for ICD treatment are applied, only one in every five patients will receive the appropriate shock, while many will suffer from inappropriate treatment [28]. The investigation of new modalities for risk iden-

tification, risk stratification and for guiding prevention treatment for potentially fatal arrhythmias is therefore extremely prudent.

Coronary artery disease

This is a disease in which there is a narrowing or blockage of the coronary arteries most commonly caused by atherosclerosis. An insufficient supply of blood carrying oxygen and energy substrates for the demand driven by the actual work load translates into heart muscle ischemia [29]. Endothelial damage is followed by an inflammatory process involving macrophages, T-lymphocytes and chemokines [30]. Subsequently, it forms atherosclerotic plaque, which progresses alongside the accumulation of lipids and the infiltration of cells. While the process is slow, it might be totally silent or produce exercise induced angina. If the fibrous cap overlying the atherosclerotic plaque fissures or ruptures, it triggers the process of aggregation of platelets and the coagulation cascade within the coronary artery lumen, resulting in a sudden narrowing or total occlusion leading to an acute coronary event/MI [31-32].

Epidemiology: During the second half of the 20th century, CAD became the most common cause of morbidity and mortality in the western world [33]. CV mortality varies from 3 to 9 per 1,000 inhabitants in Europe (WHO mortality database 2002). In Sweden in 2002, CV disease caused 29,000 deaths (33% of total mortality). Although progress in the diagnosis, treatment and prevention of CV disease has resulted in a substantial age-adjusted mortality reduction, the total number of affected individuals is still increasing due to improved survival and increasing overall longevity in the population [34].

MI: Coronary atherosclerotic disease is the underlying substrate in nearly all patients with acute MI. The initiating event is a crack or fissure in the atherosclerotic plaque. If an occlusive thrombus forms, patients may develop an acute transmural MI unless the subtended myocardium is richly collateralized. If, the thrombus formed is not occlusive, but rather mural, the patient may develop unstable angina or myocardial ischemia that is not transmural.

The approach to the patient with acute MI radically changed since the 1980s. The ultimate goal is to save myocardium initiated by quickly reperfusion therapy, either fibrinolytic or PCI-based. The size and location of the infarct, is remarkably important for prognosis [35-36].

The benefit of thrombolytic therapy and primary angioplasty is independent of age, gender, and most of the baseline characteristics. The critical dogma is to restore myocardial perfusion as quickly as possible. The shortcomings of thrombolytic therapy is only the 50-60% plateau of TIMI III (normal flow) reperfusion for even the most effective thrombolytic therapy [37].

PCI: The first PCI was performed in Zurich in September 1977 by the Swiss radiologist Andreas Gruentzig [38]. The patient, 38-year-old Adolph Bachman, underwent successful angioplasty to a left coronary artery lesion and remains well to this day. Later this technique became convincingly superior to thrombolytic therapy for establishing good flow in a greater proportion of patients and was successfully applied in unstable angina pectoris and MI management [37,39].

In the management of chronic stable angina, two invasive techniques are available for myocardial revascularization: coronary artery bypass surgery (CABG) and catheter-attached devices. Nowadays, about half of all stable angina patients are managed by elective PCI, often with the help of an intracoronary stent technique [40]. Introduced in 1986, the objective for the stent was to take down dissection flaps and provide mechanical support preventing the recoil of the plaque and restenosis [41].

The widespread use of PCI techniques included a variety of angina pectoris patients with different co-morbidities: LVH, previous

MI, diabetes mellitus, the absence or presence of collaterals and so on.

A great many animal model studies have been performed to obtain an insight into the consequences of acute ischemia in the myocardium [42-43], but the translation of these models to human equivalents is cumbersome, while human studies are scarce. The opportunity to study the human heart in acute, short-lasting ischemia conditions makes PCI procedures attractive for clinical electrophysiological studies, where the effects of ischemia on the depolarization-repolarization processes can be observed [44-45].

An angiography technique has been used also to estimate the size of jeopardized myocardium, which is an important determinant of final infarct size and outcome in patients with acute MI [46-47]. However, the angiography reflects epicardial arteries, has limitations and has been shown to be inferior to nuclear imaging [48-49].

Myocardial scintigraphy: Prinzmetal was the first to study coronary arteries by injecting labelled micro-spheres in 1947 [52]. During the development of the myocardial scintigram technique, different radionuclides were used: cesium-131, potassium-42 and thallium-201. However, recently introduced technetium-99m is preferable, due to physical characteristics permitting high-quality images [51]. On the basis of phantom studies [52] and studies of a "normal" reference population [53], various quantitative methods have been developed to reduce the subjectivity of analysis and enhance diagnostic performance.

Ventricular repolarization

Einthoven recorded the first electrocardiogram (ECG) more than a century ago, but the repolarization process is still the subject of debate and is an issue that has not been fully resolved. In general terms, VR is the electrophysiological phenomenon associated with the recovery (resetting) of cardiac cells, following their depolarization or excitation (activation). The T wave of the ECG is the electrical manifestation of the repolarization process and reflects the influence of heart rate, autonomous nervous system activity, ventricular activation sequence and electrical disturbances [54].

Basic principles: The myocytes maintain a transmembrane potential difference of approximately –90 mV. This depends on the chemical gradient between the interior and exterior of the cell. The normal resting potential of a cardiac myocyte is approximately the potassium equilibrium potential of around -90 mV (inside negative). An equilibrium potential is a balance point as the net transmembrane flux (or current) of K+ is zero. It can be calculated using the Nernst equation in a simplified form by assuming typical human body temperature (37 C), reducing the constants and switching to Log base 10 [55]:

$$E_{eq,K}{}^{+}=61.54\log([K^{+}]_{o}/[K^{+}]_{i})$$

- $E_{eq,K}{}^{+}$ is the equilibrium potential for potassium, measured in volts
- $K^{+}]_{o}$ is the extracellular concentration of potassium, measured in $mol\cdot m^{-3}$ or $mmol\cdot l^{-1}$
- $[K^{+}]_{i}$ is likewise the intracellular concentration of potassium.

A sodium pump is responsible for sustaining a higher negative ion concentration inside the cell. Electrical stimuli (generated by pacemaking cells in the sinus or AV node, able to achieve spontaneous electrical impulse formation) transform the resting potential from –90 mV to approximately + 20/30 mV (depolarization).

The electrical activation front flows from the positively charged (depolarized) cells to the negatively charged (resting) cells. In the ventricles the depolarization wave starts in the septum, spreads into the apex of the left and right ventricles, finishing in their basal-inferior regions. It also spreads from the endocardium to the epicardium [56-57].

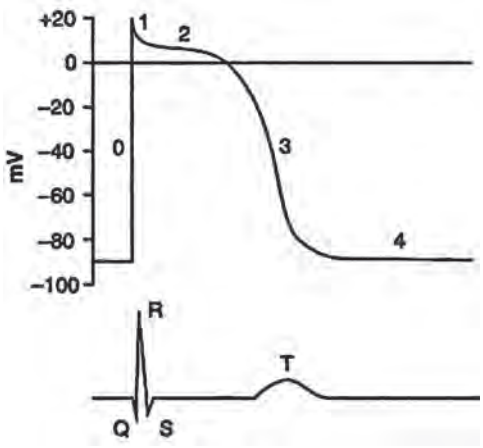


Figure 4. The myocardial action potential and corresponding surface electrocardiogram. *Phase 0* represents a rapid sodium ion inflow, resulting in the reversal of the transmembrane potential, lasting less than 1 ms and causing depolarization. *Phase 1*, brief repolarization because of transient outward potassium current, returns the membrane to the level of the plateau. This phase displays the greatest variability between various regions of the heart; it is, for example, more pronounced in the epicardium. *Phase 2* is the plateau phase of the action potential and lasts for up to several hundred milliseconds. An outward flow of potassium ions is counterbalanced by an inward flow of calcium and sodium ions. *Phase 3* marks the period of terminal repolarization when the membrane potential is restored to its resting level by the outflow of potassium ions. *Phase 4* describes the membrane potential during diastole. Working myocardial cells maintain a steady diastolic level of approximately –85/90 mV.

Repolarization of the heart: The restoration of a negative transmembrane potential to a steady state is the process of repolarization. Repolarization is arranged in such a way that differences between different parts of the heart are minimized to maintain electrical stability. It is achieved by adjusting the action potential duration (APD) so that sites activated at an early stage have longer action potentials and sites activated at a later stage have shorter action potentials. The present view is that the ventricular repolarization process begins in the epicardium and ends in the endocardium and the polarity of the repolarization waveform is therefore the same as that of depolarization. As a result, repolarization in the normal heart tends to be as homogeneous as possible, presumably to avoid the risk of re-entry VT [56].

Heterogeneity of repolarization: If all ventricular action potentials had the same magnitude and duration, the heterogeneity or dispersion of repolarization would be extremely small, but still present, because not all the parts of the myocardium are activated simultaneously [58]. In reality, the normal heart has some degree of heterogeneity on several levels: 1) Regional level, due to differences in the duration of the repolarization forces in some areas of the ventricles in the mammalian heart, between the base and apex or between the anterior or posterior side of the ventricles, as well as between the two ventricles [56,59]; 2) Transmural level, where endocardial, mid-myocardial and epicardial cell layers have different shapes and lengths of action potential, shown in the in vitro model [60]; 3) Temporal level/heterogeneity, or beat-to-beat changes [61-62]. As long as the ventricular myocardium is not ischemic, the recovery of excitability (end of refractoriness) directly follows repolarization. Both the APD and the effective refractory period (ERP), which protects the heart from too high a heart rate, shortens when the heart rate increases [63]. So, studying heterogeneity in refractoriness by invasive electrical stimulations in different parts of the ventricles may provide a clue to the dispersion in APD. In the case of arrhythmogenesis, the distance between the minimum and maximum values of APD is highly relevant. It can be large without pro-arrhythmic consequences, as long as they are gradual between distant sites, but heterogeneity between adjacent sites is highly arrhythmogenic (Figure 5) [4]. The increased heterogeneity of repolarization, with differences in the duration of activation

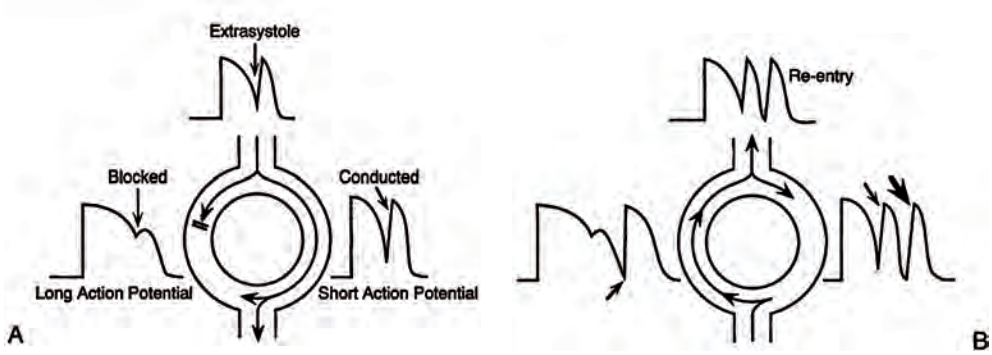


Figure 5. Mechanism of arrhythmia. The initiation and maintenance of re-entry in a ring model based on the dispersion of action potential duration and refractoriness. Action potentials from different sites within the circuit are depicted. **A:** An extra systole blocks after encountering tissue with long action potential durations and thereby refractoriness. It is able to conduct through the alternate pathway where action potential duration and refractory periods are shorter. **B:** The impulse conducts retrogradely through the previously blocked pathway, which has now repolarized and recovered excitability. It is then able to re-excite tissue at the site of origin of the original extra systole and potentially recirculate around the same pathway to cause re-entry. (From Wit AL et al. The ventricular arrhythmias of ischemia and infarction: the electrophysiological mechanisms. Mount Kisco, NY: Futura, 1992).

time, APD and critical localization, plays a major role in the mechanism of ventricular arrhythmias [4,64].

Factors accentuating heterogeneity of repolarization: Repolarization assessment is the focus of our attention in the work of this thesis. We are, however, aware that the depolarization process is also affected by ischemia with subsequent consequences for repolarization and this issue is approached in the discussion section.

The most common cause of increased repolarization heterogeneity is acute or chronic ischemia. During ischemia/infarction, APD changes dynamically. Initially, it is prolonged and it then shortens. Shortening is more pronounced in epicardial areas than in endocardial areas during transmural ischemia.

APD is generally prolonged in the hypertrophied myocardium, but not homogeneously in different areas, resulting in the dispersion of repolarization [65]. It is known that changes in blood pressure and fast-developing hypertension are arrhythmogenic factors [66].

Sympathetic stimulation can produce an increase in the dispersion of refractoriness and in QT intervals. Inhomogeneity in innervation, may be a plausible explanation of this [54]. The effect of systemic catecholamines is less pro-arrhythmogenic, as a more homogeneous effect is obtained after norepinephrine infusion [54].

Genetic anomalies may also increase the dispersion of repolarization by different means. The congenital long QT syndrome is subdivided into seven genotypes distinguished by mutations in at least six different ion channel genes. It is characterized by the appearance of long QT intervals in the ECG, a polymorphic VT (Torsades de Pointes) and an increased risk of SCD [67]. Acquired long QT syndrome is similar to the congenital form, but it is caused by exposure to drugs that prolong the duration of APD or QT prolongation, secondary to cardiomyopathy, bradycardia or electrolyte imbalances [67-68]. The short QT syndrome is a recently discovered inherited syndrome characterized by a QTc < =300 ms and a high incidence of malignant ventricular arrhythmia. At least two genes are reported to be responsible [69-70].

The Brugada syndrome is another condition characterized by an accentuated ST-segment elevation or J wave appearing in the right precordial leads, often followed by a negative T wave and a high incidence of rapid polymorphic ventricular arrhythmias [71]. It is thought that the arrhythmogenic substrate is secondary to the amplification of heterogeneities intrinsic to the early phases of the APD of cells residing in different layers of the right ventricular wall of the heart [67].

Recording and measuring repolarization and its heterogeneity: There are two invasive ways directly to measure repolarization. In open-heart surgery or invasive electrophysiological studies, monophasic action potentials (MAP) or ERP can be recorded [72-73]. The limitation of the invasive technique is that invasive procedures represent an inherent risk for patients and are mainly used as part of open-heart surgery and invasive electrophysiological studies. In the case of MAP and similarly in the case of the ERP technique, only a limited number of sites are available, permitting only very local repolarization measures. Moreover, only relatively selected groups of people can be studied, i.e. not allowing widespread risk assessment in the general population. These techniques can be combined with mapping tools such as the CARTO system to enhance spatial assessment [74].

Non-invasive recording of repolarization by ECG or VCG might enable the measurement of repolarization in a more global manner, permitting less expensive, risk-free routine assessments in large population cohorts.

The QT interval from the surface ECG estimates the time from the earliest activation to the latest repolarization of the ventricular myocardium. The prolongation of the QT interval was associated with increased global heterogeneity of repolarization and pro-arrhythmia risk. However, in serial experiments using various doses of the same drugs, pro-arrhythmic outcome was not dependent on the degree of QT interval prolongation [75]. In spite of this, the QT interval prolongation is still the most commonly used non-invasive indicator of pro-arrhythmia in routine risk assessment**.**

QT dispersion is the difference between the longest and shortest QT interval in the 12-lead ECG. The QT dispersion does not reflect local differences in repolarization moment [76]. However, a large QT dispersion on the body surface ECG is related to an increased propensity for arrhythmias. Dispersion in APD has been related to dispersion in QT intervals [77-78]. Nevertheless, QT dispersion has several methodological limitations, including difficulties with correct T-wave end identification, and has been shown to be a risk predictor only at the group level [61] (see Discussion).

T-wave morphology descriptors were developed for spatial, temporal and wavefront direction characteristics of VR from scalar 12-lead ECGs [79]. These descriptors, reconstructed from 12-lead ECGs, are said to be more reproducible than the conventional QT interval-related descriptors and to assess different ECG characteristics from conventional parameters [79]. They are relatively recently introduced repolarization descriptors.

T-wave alternance measures the alternating amplitude of T waves from beat to beat on the ECG at the microvolt level. It therefore represents a temporal measure of repolarization heterogeneity and has been linked to ventricular arrhythmias and SCD [80]. Measurements are usually made during exercise stress testing. However, problems exist when it comes to the interpretation of microvolt TWA tracings and patients' ability to perform this test [81]. Pacing-induced T-wave alternance needs to be explored further.

Recently introduced ***T-loop morphology*** descriptors by means of VCG to study and validate VR [82] were applied at our institution to follow interventions [11] and will be discussed in the VCG and methods sections.

Concept of vectorcardiography

Electrical bases for VCG, Figure 6: Differences in the cardiac potentials of a single cardiac cell or a small group of cells do not produce enough current to be detected on the body surface. On the other hand, the sum of all the cardiac electrical potentials during each cardiac cycle produces an electrical field. This field can be represented by a single vector that originates at the centre of the Einthoven triangle (which in VCG is assumed to be in the centre of the heart mass throughout the cardiac cycle), with an arrowhead that points at the positive pole. The length of the vector represents the magnitude and the direction depicts the spatial orientation of the electrical forces. When all the instantaneous single vectors are plotted consecutively, a continuous vector loop is formed in 3-D space, the so-called spatial VCG (Figure 6 A). It can be projected and recorded along three mutually perpendicular axes (X, Y, and Z). Using simultaneous analysis of the configuration of the loops in all three planes, it is possible to calculate the direction and magnitude of the spatial vector from a Pythagorean formula (Figure 6 B). The VCG integrates two surface leads of three (named X, Y, and Z) in an orthogonal system and depicts a separate loop for each of the ECG components: the P loop reflecting atrial depolarization, the QRS loop representing ventricular depolarization and the T loop representing VR (Figure 6 C). The advantage of the VCG over the 12-lead ECG is that it provides information not only about the magnitude and direction (i.e. positive or negative) of the signals but also about the spatial orientation. Furthermore, the ECG can be recalculated from the VCG with a good approximation [83]. Because of the aim of the present thesis, we concentrated on studies of T-vector and T-loop morphology.

Directional characteristics of repolarization, the T vector: Each vector can be represented in 3-D space in X, Y, Z (rectangular) coordinates or in polar (spherical) coordinates. Polar coordinates are analogous to geographical lines of latitude and longitude and to the altitude with respect to the center of the globe (in the case of VCG, the center of the heart). However, in VCG, the terms azimuth and elevation are used rather than longitude and latitude, respectively. The orientation of the T-loop vector can be defined by azimuth and elevation. Polar coordinates therefore contain the same information as X, Y, and Z coordinates while requiring less space and they might therefore be preferable for describing the orientation of the T vector [83].

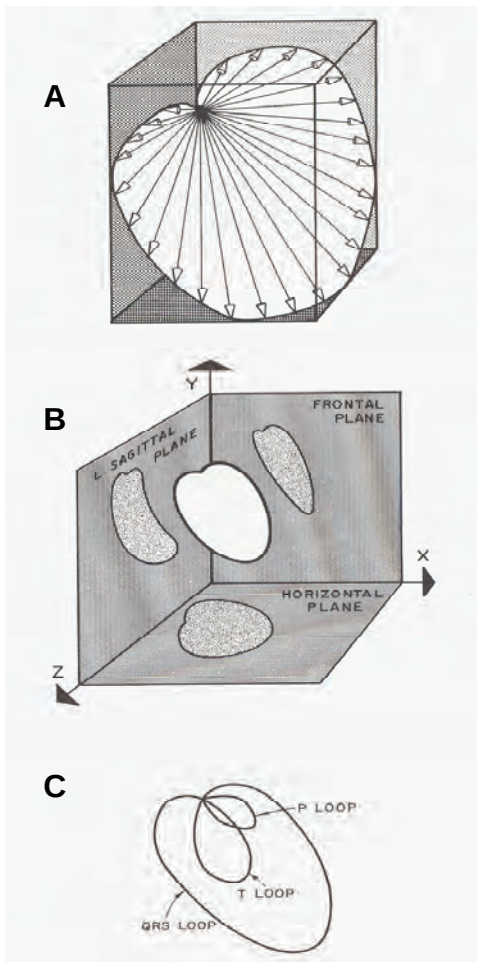


Figure 6. (Adapted from Wartak J. Simplified vectorcardiography. 1970, J. B. Lippincott Company, Philadelphia, Toronto)**.**

The maximum T vector, or the major axis of the T loop, would represent the direction of the main electrical forces in space during repolarization. It is measured from the center of polar coordinates to the most remote point of the T loop.

Repolarization morphology, the T loop: The T-loop configuration and symmetry in 3-D can be analyzed to characterize its morphology. The shape of the T loop can be expressed by the parameters Tavplan, Teigenv and Tarea [11], discussed later in the methods section. The association between increased repolarization heterogeneity (reflected by QT dispersion) and changes in the shape of the T loop has already been shown previously, but the data relating to this are still scarce [11,82-84]. It should be noted, however, that it is the periphery of the loop that is characterized; we know nothing about its interior.

Depolarization/repolarization interrelationship, the QRS-T angle: This is the angle subtended by the maximum QRS vector (calculated in a similar fashion to the maximum T vector) and the maximum T vector [83]. Theoretically, the QRS-T angle represents the spatial deviation between the depolarization and repolarization waves and reflects the heterogeneity of APD appearing in the human heart [85]. It has previously been shown that it reflects the degree of LVH and it was simultaneously suggested that the QRS-T angle might reflect the heterogeneity of VR beyond LVH [86].

Rationale for repolarization heterogeneity assessment by VCG: Because of a well-established link between VR abnormalities and malignant ventricular arrhythmias including SCD, our interest focused on the studies of heterogeneity of VR. The VCG has several advantages over conventional ECG in describing repolarization: 1) the ability to provide information about the spatial orientation of repolarization (not only positive or negative) [83]; 2) the opportunity to construct a more anatomically reliable T-vector loop in contrast to ECG; 3) T-loop analysis is less sensitive to operator variability in the detection of the T-wave end, unlike QT interval and QT dispersion analysis, where it is crucial [87-88]. Moreover, the association between the increased heterogeneity of repolarization and changes in T-loop morphology has already been demonstrated [11,82,84].

AIMS OF THE STUDY

The overall objective for this thesis was to study the influence of acute ischemia on VR by means of VCG, more specifically by analyzing alterations in the T-vector and T-loop morphology. Coronary occlusion during elective PCI on patients with stable CAD was chosen as the appropriate human model.

In detail:

1. To examine the diagnostic value and the reproducibility of recently developed T-vector and T-loop morphology descriptors

2. To study the behavior of the new parameters at rest and during acute ischemia

3. To assess differences in VR changes between CAD patients with and without signs of LVH at rest and during acute ischemia

4. To correlate VR changes during acute ischemia and the amount of ischemic myocardium, as well as its localization

5. To investigate the prognostic value of the new VR descriptors in CAD patients in relation to new CV event/death and repeat revascularization

MATERIAL AND METHODS

Patients

Studies I-II, IV: One hundred and ninety-two consecutive CAD patients undergoing elective PCI and initially enroled for a VCG study of the ST-segment response during PCI were included [12,13]. This study was approved by the Ethics Committee at Karolinska Institutet and all the participants gave their informed consent to participate. The inclusion criteria were angina pectoris, a positive exercise test, or both, and ≥ 1 significant angiographic stenosis (≥ 60% vessel diameter reduction). The exclusion criteria were MI within 48h, ongoing ischemia or angina pectoris at the start of the procedure, atrial fibrillation, bundle branch block, pacemaker rhythm and previous revascularization procedure (PCI or CABG). Five patients had to be excluded for technical reasons. In order to assess VR in patients without major co-morbidities, such as previous MI, LVH and PCI-related MI, and without visible collaterals to the target vessel, we selected 56 patients from 187 for the specific purpose of **Study I**. The purpose was to assess the effect of fully developed (no flow) ischemia in a relatively healthy human myocardium.

A group of 10 apparently healthy volunteers (5 men) with a mean age of 41±7 years were examined to determine the reproducibility of the new VCG parameters at rest and they served as controls for the baseline values. They were selected from staff and personnel on the basis of no chronic or acute disease and a normal 12-lead ECG.

In Study II, all 187 patients were allocated to three subgroups on the basis that they would represent a spectrum of increasing degrees of LVH. One group comprised 33 patients with LVH according to ECG criteria [89]; in 20 (87%) of the 23 patients with available echocardiography records, the presence of myocardial hypertrophy was supported. A history of hypertension was present in 18 and 5 more patients had blood pressure measurements suggesting at least intermittent hypertension. Ventricular remodeling after MI was the probable cause of LVH in six, whereas one patient each had aortic stenosis and obesity. Two patients had no identifiable cause except their CAD. Fifty-four were allocated to the "hypertensive" subgroup, on the basis of a history of arterial hypertension, and 100 to the "normotensive" subgroup, because they had neither a history of hypertension nor ECG signs of LVH.

In Study IV, all 187 patients were followed for 8 ± 1 years.

Study III: Forty-five patients with stable CAD were enroled in a study with Tc-99m-sestamibi-single photon emission computed tomography (SPECT) estimated myocardium at risk (MAR) and its relationship to the VCG parameters, ST-VM and STC-VM [14]. All these patients were selected for elective PCI and gave their informed consent to participate in the study. The Ethics Committee at University Hospital, Umeå, Sweden, approved this study. The exclusion criteria were chronic atrial fibrillation, bundle

branch block, MI, or a pacemaker rhythm. Ten patients had to be excluded because of ECG signs of previous MI (7), atrial fibrillation during PCI (1) and for technical reasons (2).

Vectorcardiography

Continuous VCG recording was started at least 5 minutes before the PCI procedure and ended ~5 minutes after the last balloon inflation. In the control group (**Study I**), each volunteer had a one-minute VCG recording in the supine position twice with a one-week interval. A MIDA 1000 system (Myocardial Infarction Dynamic Analysis, Ortivus Medical AB, Täby, Sweden) was used in all the studies. In **Study III,** a more recent VCG recording system, "Coronet" (Ortivus Medical, AB, Danderyd, Sweden), was also used. VCG signals were collected continuously from 8 electrodes positioned according to the Frank orthogonal lead system (*X, Y,* and *Z)*. The sampling frequency for each electrode is 500 Hz. The method has been described in detail elsewhere [12]. At the start of the recording, the dominant QRS pattern is automatically determined from the first 10 complexes and it is then used as the template to exclude subsequent artifacts or ectopic ventricular complexes. Signals are averaged every 10 s (in **Study III** every 15 s) and the averaged complex is compared with the template. The changes are presented as trend curves over time.

Conventional ECG parameters such as the heart rate (in bpm), QRS and QT intervals were measured automatically in each averaged complex. The QT interval was corrected for heart rate (QTc) by Bazett's formula $QTc=QT/\sqrt{RR}$ (in seconds). The following conventional VCG parameters, which describe the *early* repolarization phase, were used as references: ST vector magnitude (ST-VM), which constitutes the deviation of the ST segment from the isoelectric level (defined as the average value of 10 samples in the period 12 to 30 ms before QRS onset), measured 60 ms after the J point, and ST change vector magnitude STC-VM), which constitutes the spatial difference vector between the initial and the current ST vector [12,13,90]. For each 10-second period (in **Study III** each 15-second period), an averaged 3-D T-vector loop and the direction of the maximum QRS and T vectors were determined. The T-vector and T-loop morphology was analyzed off-line using customized software developed earlier [11].

T vector: We expressed the spatial characteristics of the T vector (the maximum T vector) by the T-vector elevation (*Tel*) and the T-vector azimuth (*Taz*) and its relationship to the main depolarization vector as the *QRS-T angle*. The orientation of the maximum T vector in the horizontal plane is reflected by Taz on a scale of 0º to 180º (left-front-right) and 0º to –180º (left-back-right), whereas Tel represents the angle in the vertical plane on a scale of 0º (caudal direction) to 180º (cranial direction) (Figure 7 **A-B**). The QRS-T angle reflects the simplified spatial (angular) deviation between maximum QRS vector and maximum T vector on a scale of 0º to 180º.

The T-vector loop (from here: T loop): was characterized by three parameters: *Tarea*, *Tavplan* and *Teigenv,* as defined previously [11]. Tarea is the 3D area between the curve and the baseline from the QRS end (J point) to T end in X, Y and Z Frank leads, calculated as $\sqrt{(Tx^2 + Ty^2 + Tz^2)}$. Tavplan expresses the bulginess of the loop or, in other words, its planarity expression defined as the mean distance from the preferential plane for the sample values in the T loop. Considering the sample values of the T loop as mass points, the matrix of inertia can be defined [11] (Figure 8 **A**). Teigenv expresses the form and the symmetry of the T loop and was calculated as the quotient between the two highest eigenvalues (~diameters) of the matrix of inertia [91]. Teigenv = $(d1/d2)^2$, where d1>d2. For the special case of a circle, the Teigenv = 1 (Figure 8 **B**). In other words, the T loop can be viewed as a more or less elliptical ring

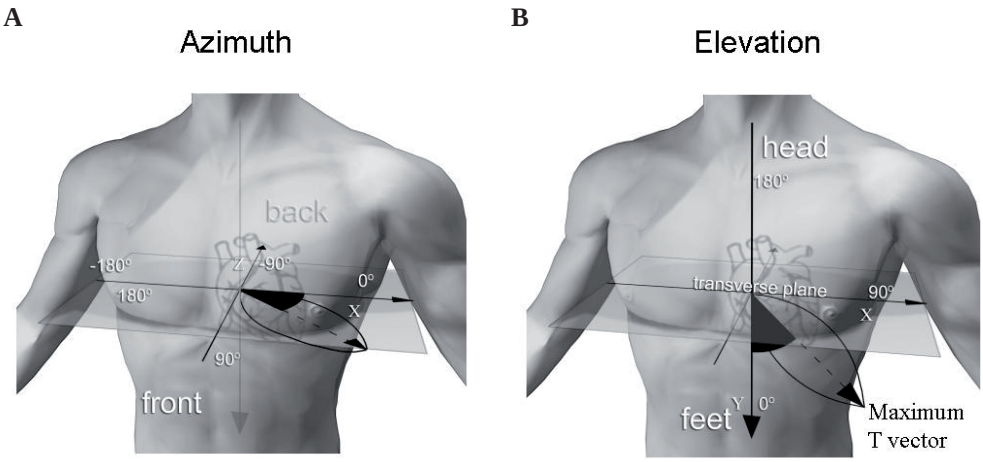
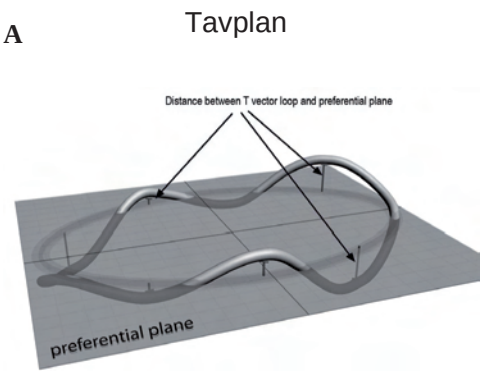


Figure 7. (*Reprinted with permission from L. Wecke: Cardiac Memory Studies in Two Human Models. Doctoral thesis 2006, Karolinska Institutet, Stockholm, Sweden).*



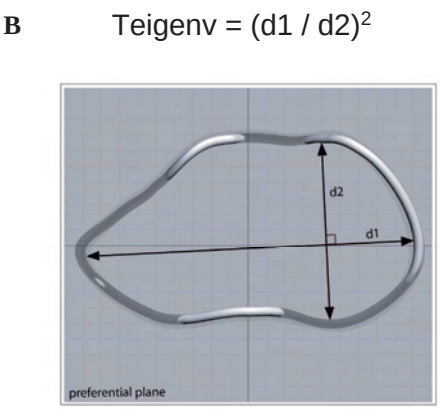


Figure 8. (*Reprinted with permission from L. Wecke: Cardiac Memory Studies in Two Human Models. Doctoral thesis 2006, Karolinska Institutet, Stockholm, Sweden).*

capable of rotation, where the longest axis around which it rotates most easily is called d1. The axis perpendicular to d1 allowing the easiest rotation is called d2. The third axis perpendicular to the first two is normally close to zero and is therefore disregarded.

Tpeak-Tend parameter: In addition, we included another parameter, which measures part of the repolarization process, namely from the time-point at the maximum amplitude of T wave in the averaged 3-D QRST complex to the T end, defined according to the same principles as for ECG. According to *in vitro* studies, this measure has been associated with the transmural gradient of repolarization (Figure 9). The amplification of the Tpeak-Tend interval predisposes for ventricular arrhythmias [92-93]. According to *in vivo* experiments in both a porcine and a dog model, this measure reflects a more global gradient of VR [94]. The Tpeak and Tend were defined on the MIDA 1000 system at a signal amplification of 0.5 mV/10mm with a time resolution of 2 ms.

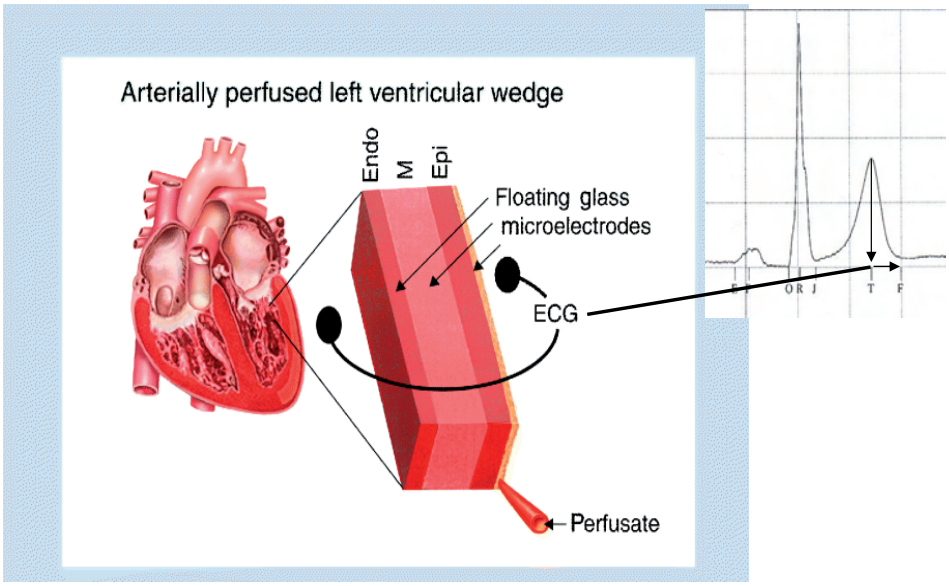


Figure 9. Possible mechanism of the transmural gradient of repolarization, where the top of the T wave coincides with the end of repolarization in the epicardium, whereas the T-wave end coincides with the end of repolarization in M cells (mid-myocardial cells) (adapted from Yan, Shimizu & Antzelevitch, Circulation 1998;98:1928-36).

PCI and single-photon emission computed tomography (Study III)

The PCI procedure was performed according to routine. Fifteen seconds after the start of *the first balloon inflation,* 900 MBq (1-2 ml) Tc-99m-sestamibi (Cardiolite, Du Pont Scandinavia, Stockholm, Sweden) was injected intravenously and SPECT imaging was started approximately 1 h later. The image acquisition technique, including the construction of a volume weighted bull's eye plot applying the *CEqual* algorithm (General Electric Medical System, Milwaukee, USA), has been described previously (Figure 10) [14,95]. Briefly, each short-axis tomogram (slice) was compared with a slice from a reference group. This consisted of patients with < 5% likelihood of CAD based on a sequential Bayesian analysis of age, gender, symptom classification and the results of an exercise ECG. The percentage of abnormal pixels was calculated. MAR was expressed as a percentage of the estimated size of the

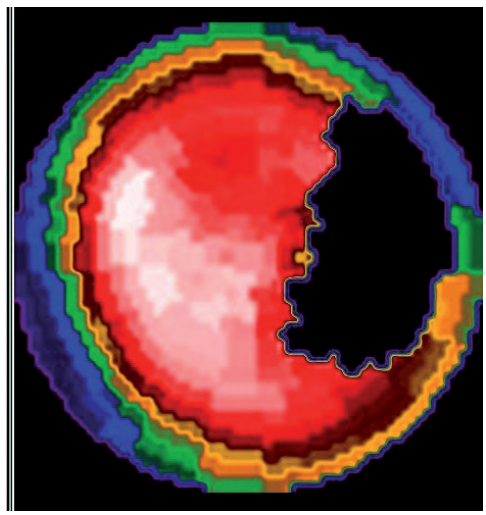


Figure 10. A "volume-weighted 2-D bull's eye plot (polar map)" demonstrates a perfusion defect in the lateral part of the left ventricular in a patient during left circumflex artery occlusion. The polar map is constructed by mapping sequential maximum-count circumferential profiles, extending from the apex to the base, into successive rings on the polar map. The apex is mapped into the center of the polar map and the base is mapped into the periphery of the map (From Jensen SM et al. Scand Cardiovasc J 2002;36:11-18 91)

left ventricle. The limit of abnormality varied between 1.75 standard deviations below the mean normal profile for inferior defects and 3.75 standard deviations below the mean normal profile for lateral defects. The sensitivity of this method is 89% and the normalcy rate (true negative rate in low likelihood patients) is 81% [96]. The *severity* of the perfusion defect was calculated to reflect both its degree and extent. It was defined according to Berman et al. by the sum of the product of all profile points with an uptake below the normal limits multiplied by their respective number of standard deviations below the normal mean count [97].

Measurement protocol

For each patient, 4 measurements were performed: 1) the baseline was defined at the beginning of the recording, before the contrast injection. For each parameter, the mean ± SD was calculated during a 3-minute recording selected as having the lowest noise level from ≤ 18 averaged cycles (in **Study III** from ≤ 12 cycles, because of 15-s sampling period); 2) at the end of the 1st balloon inflation, a single value (one 10-s/15-s averaged complex) was obtained; 3) at the time of the maximum STC-VM (the reference for maximum ischemia) [47], all parameters were measured as a single value; 4) 2 min after the last balloon deflation, the mean ± SD was again calculated from 3-minute averages. The QRS, QT and QTc intervals were measured only at baseline and at the end of the 1st inflation, because we had previously demonstrated their lack of sensitivity to ischemia [11]. In **Study III,** we performed only the first and second measurements, because of the different aim of the study.

In the control group, two separate 1-minute baseline VCG recordings were performed with a one-week interval. The mean value of every parameter calculated from each of the two recordings was used for comparison with patients in **Study I**.

Analyses in Study III

This study was an extension of a previous study of SPECT-estimated myocardium at risk (MAR) and its relationship to the VCG parameters ST-VM and STC-VM study, which reflect part of the early VR [14]. In the new and extended analysis, we wanted to relate the new 3-D VR parameters, which describe the entire VR, to MAR and to the target vessel (for the purpose of simplified localization). The data analysis was therefore performed with regard to both the size of the MAR and its location. For the first purpose, the patients were divided into two subgroups according to the median MAR value: MAR ≤ 11% (n=18) and > 11% (n=17). The data were then analyzed with regard to the location of the threatened myocardium and the patients were divided according to the target vessel: 1) LAD (n=14); 2) the right coronary artery (RCA, n=7); 3) the left circumflex artery (Lcx, n=14).

Follow-up and analyses in Study IV

During elective PCI performed at the beginning of the study, 15 patients developed elevation of the cardiac enzymes above reference level, of whom only one had an enzyme level above 5 times the normal. Only the latter case might be regarded as having had a prognostically significant per-procedural myocardial injury [98-99].]

All 187 patients were followed for 8 ± 1 years, making a total of 1,328 patient-years. The end-points were defined as: 1) CV death; 2) non-CV death; 3) MI; and 4) repeat revascularization including PCI or CABG. Copies of death certificates were obtained from the Center of Epidemiology at the Swedish National Board of Health and Welfare. The occurrence of subsequent MI, PCI, or CABG was obtained from hospital records.

In order to describe the natural course of this patient cohort and to analyze the prognostic value of different variables and measures in relation to outcome, the events were organ-

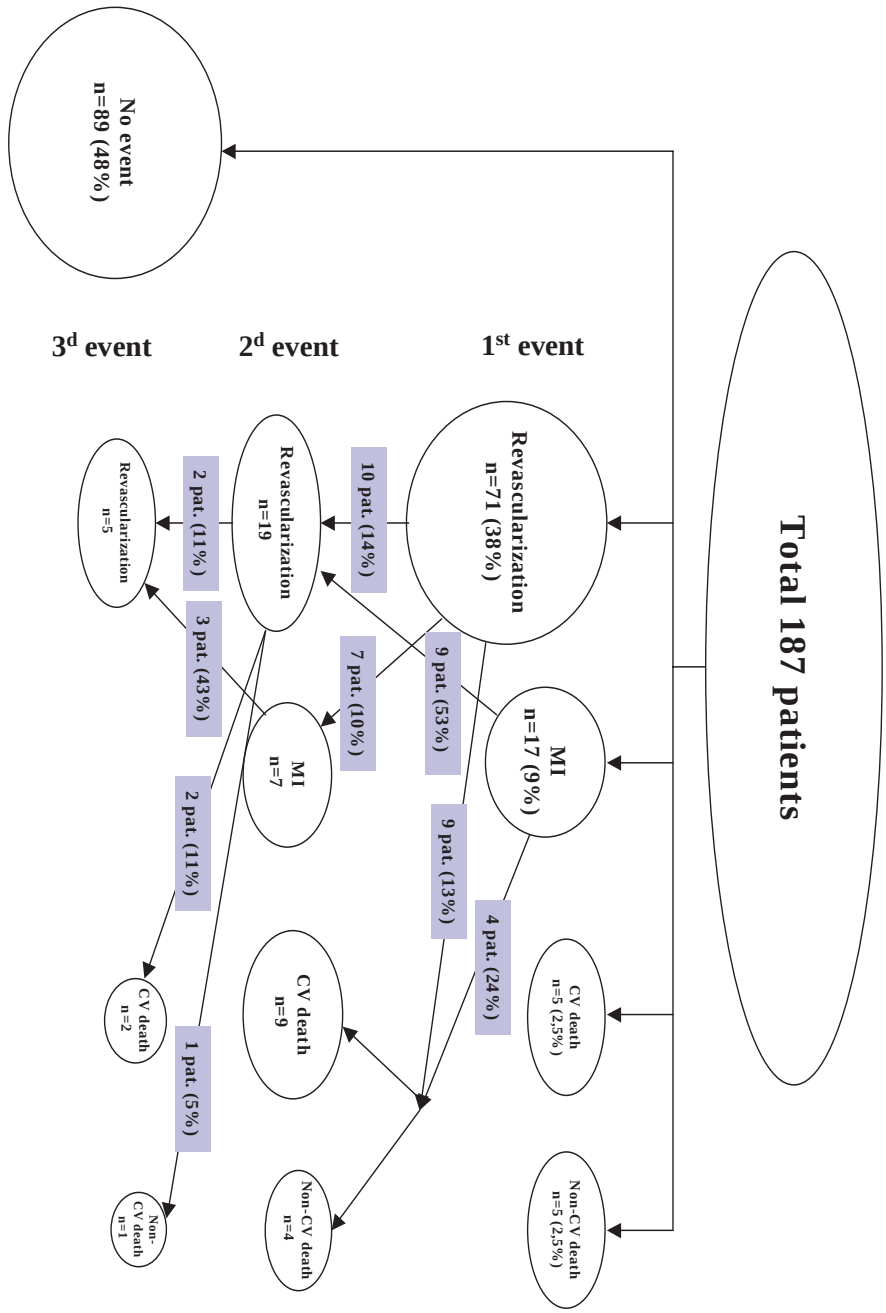


Figure 11. Adapted from **Study IV**.

ized as first, second or third (Figure 11). The data were then analysed in 2 steps: firstly, the patient group with a first event, such as CV death, MI or repeat revascularization, was compared with the patient group without any CV event. Secondly, patients with a CV death or MI as the major event were compared with those without such events.

Statistical analysis

The mean and standard deviation or median and 25th and 75th percentiles were used for descriptive statistics. Categorical variables were compared by chi-square analysis. The significance of the skewness was tested by Shapiro-Wilk's W test. Extremely skewed data (Teigenv) were logarithmically transformed before analysis. Between-group analysis was performed using an unpaired *t* test and by analysis of variance and co-variance (for normally distributed data) and by the Mann-Whitney U test and Kruskal-Wallis statistics (for skewed data). Analyses of statistical non-homogeneity between repeated measurements were conducted using ANOVA (repeated-measures analysis of variance) and skewed data were tested with the Friedman two-way analysis of variance by ranks. Analysis of co-variance (ANCOVA) was used to test parameter dependence on acute ischemia during balloon occlusion, MAR size and location of ischemia. The Bonferroni correction of p-values was applied for multiple comparisons where appropriate. A backward stepwise multiple linear regression analysis, with inclusion at the F 4.00 level and exclusion at the F 3.00 level, was used to evaluate the influence of conventional variables on every VCG parameter, which served as dependent variables in **Study III**. Simple linear regression analysis was used and correlation matrices were constructed for the correlation assessment; alternatively, the correlation was tested using the Spearman R method. Univariate and multivariate logistic regression analysis was applied to assess the predictive values in relation to outcome during follow-up. All parameters with a p-value of < 0.05 were then tested together in a multivariate logistic regression analysis, where non-significant parameters were excluded in a step-by-step fashion. Adjustments for age, gender, LV hypertrophy and QRS duration were performed separately.

Receiver Operating Characteristic (ROC) curves were constructed to obtain optimal cut-off values for ejection fraction (EF), QRS duration, QRS-T angle and Tavplan (Microsoft Excel, Windows 2000; **Study IV**). The area under the curve was calculated and compared with the area under the identity line, which is 0.5, meaning no predictive value [100]. Kaplan-Meier analysis was used to construct graphs describing the cumulative proportion of survivals divided according to the cut-off levels. The survival analysis was then performed using the Gehan test.

The intra-individual time-dependent variability over one week in the control group was expressed as the intra-individual standard deviation (s) calculated from the paired observations, as previously described ($s=\sqrt{(d1^2/2 + d2^2/2 + \ldots.+ d10^2/2)/10}$, where d is the difference between the two compared measurements for each individual) [101] (**Study I**).

The positive predictive value (PPV) of several parameters was calculated to assess the diagnostic capacity to distinguish CAD patients from controls at baseline, where PPV (%) = (true positives/all positives))* 100; here, true positives are patients with parameters exceeding 1 or 2 s (both were tried) from the average of the two observations in controls (**Study I**). Additionally, the PPV of LV dysfunction, QRS duration, QRS-T angle and Tavplan was calculated for the second-step analysis (**Study IV**), where true positives were CV death or MI patients with pathological values according to the cut-off level.

A *p*-value of < 0.05 was considered statistically significant. All the analyses were performed using Statistica for Windows software, release 6.0 (StatSoft, Tulsa, Oklahoma, USA).

Ethical considerations

This thesis is based on a *de novo* analysis of VR based on VCG recordings made in two series of patients with stable angina undergoing elective PCI at two university

clinics in Stockholm and Umeå respectively. These patients were studied according to protocols approved by the Ethics Committees at Karolinska Institutet and Umeå University Hospital. In **Study IV**, an extended follow-up analysis (up to 8 years) was made. This extension was beyond the short-term period for which approval had initially been sought. Data were obtained from hospital records and, in a few patients, by direct telephone inquiry, to which all the approached patients gave their consent. Part of this extended follow-up was a documentation of the causes of death. A formal application to the Swedish National Board of Health and Welfare was made in April 2004 in order to obtain this information. Approval was granted without requests for new or additional consent from the Ethics Committee at Karolinska Institutet.

RESULTS

Baseline measurements (Studies I-II)

In healthy controls, the T loop had an elongated shape and its maximum vector pointed downwards, anteriorly, and to the left, similar to previous observations [102].

At baseline, ST-VM, Tarea and Teigenv were significantly lower when the CAD group was compared with the control group of healthy individuals (**Study I**). There were no significant differences in heart rate, QRS, QT or QTc intervals and T-vector parameters (QRS-T angle, Taz and Tel).

In **Study II,** the presence of LVH was associated with increased ST-VM, a wider QRS-T angle, a more anterior and cranial T-vector direction and a more symmetrical rounded T loop (lower Teigenv). In addition, the QRS-T angle was significantly increased, even in the group of hypertensive patients without ECG signs of LVH. There was a general pattern in which the mean values of most parameters in the hypertensive group, such as Tel, Taz, Teigenv and ST-VM, were between those of the normotensive and LVH subgroups, probably reflecting a continuous spectrum of myocardial hypertrophy. In multiple regression analysis, the width of the QRS-T angle was increased in the presence of LVH, hypertension and previous MI (with remodeling-induced myocardial hypertrophy) respectively ($p<0.001$; $p<0.01$; $p<0.05$; $R^2 = 0.15$).

The time-dependent variability, reflecting the reproducibility of the new VCG parameters, was estimated in the healthy controls and presented in **Study I**. These data also provided an estimate of the normal range for these parameters. The coefficient of variation [CV= (s/mean)x 100; %] and intra-individual standard deviation reflecting the time-dependent variability was relatively low for ST-VM, Tarea and Tel (7% and 8 μV, 9% and 6 μVs and 4% and 3° respectively). The corresponding values for Ttop-end (calculated subsequently) were 7% and 8 ms. In contrast, the time-dependent variation was more pronounced for the QRS-T angle (49% and 21°) and Teigenv (47% and 47).

The PPV for VR abnormalities in the cohort of CAD patients without major comorbidities with 1 and 2 s, respectively, were 86% and 79% for Tarea, 80% and 20% for Teigenv. The PPV in total population of 187 CAD patients with 1 and 2 s, respectively were 73% and 55% for widened QRS-T angle, 97% and 27% for decreased Teigenv. Similarly, the PPV in relation to both LVH and hypertension were 56% (1s) and 41% (2s) for widened QRS-T angle.

Acute ischemia (Studies I-III, Figure 12)

During acute, short-term occlusion of the coronary arteries (**Studies I-II**: ~ 1 min; **Study III**: ~ 2 min), the most pronounced response was observed in ST-VM and STC-VM (**Studies I-III**). In the population of CAD

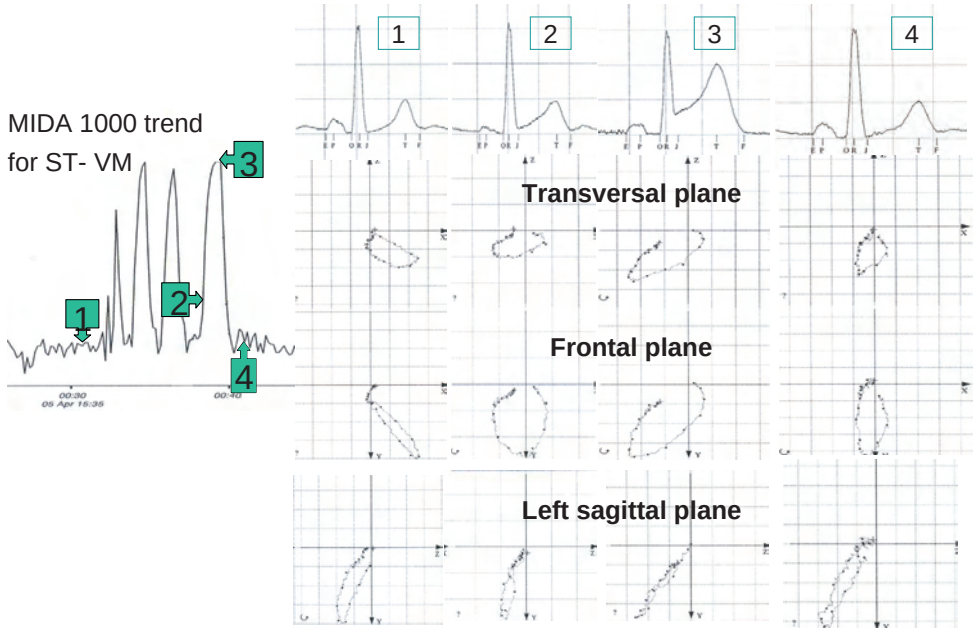


Figure 12. An example of the T-loop transformation during balloon occlusion of the right coronary artery during elective PCI (~ 10 minutes). The ST-VM trend shows 4 peaks, one for each occlusion (left panel). Sequence of events (the 4 panels to the right): 1) baseline state before the balloon inflation; 2) about 15 seconds from the beginning of the 4th inflation; 3) maximum ST-VM change, approximately 60 seconds from the start of the occlusion; 4) 20 seconds after the deflation of the balloon.

patients without major co-morbidities, all the T-loop parameters responded to ischemia in such a way that the T loop became more bulgy (distorted) and less elliptical and the Tarea increased (**Study I**). However, in the entire CAD cohort including patients with previous MI and LVH, the QRS-T angle also widened and the T vector moved more cranially during acute ischemia **(Study II**).

According to the subgroup analysis, the most pronounced reaction was seen in the LAD group (**Studies I, III**), in the presence of LVH (**Study II**) and in the group with the larger ischemic zone (MAR>11%, **Study III**).

Target vessel: The VR response during ischemia was dependent on the vessel that was occluded. As a result, the occlusion of the LAD induced the most pronounced changes in ST-VM and STC-VM (LAD > Lcx and RCA) and also the most consistent changes in the T vector and T loop. The T vector moved to the right and upwards (towards the ischemic area). The T loop became more bulgy (distorted) and rounded and the Tarea increased. The occlusion of the Lcx was followed by similar T-loop alterations, while RCA occlusion resulted in increased Tarea.

Myocardial hypertrophy: The extent of the VR response during coronary occlusion was also dependent on the presence of ventricular hypertrophy. The most prominent VR changes were thus seen in the LVH group (**Study II**), followed by the hypertensive patients, who presumably had some degree of hypertrophy, and lastly by the CAD patients with neither LVH nor hypertension. This exaggeration of the VR response during ischemia was also statistically significant in the LVH group when correction was made for the significantly more abnormal VR already at

baseline. As discussed below (Discussion), the increased vulnerability to ischemia observed in the presence of LVH is presumably not solely dependent on the amount of myocardium, which is threatened by coronary occlusion. During ischemia in the hearts of hypertensive patients, most parameter mean values remained between the values observed in LVH and those in normotensive patients, although a significant difference was only observed in Tarea, which increased significantly more in hypertensive patients compared with normotensive patients (ANCOVA, $p<0.05$).

Myocardium at risk: The importance of the amount of ischemic myocardium for the acute VR changes, during balloon occlusion of one of the coronary arteries, was the focus for **Study III**. In the subgroup with MAR >11% (= median value), the most significant and prominent changes were seen in ST-VM, STC-VM, Tavplan and Teigenv (**Study III**). In logistic regression analysis, STC-VM and ST-VM correlated best with MAR size, in line with the previous observations [14]. Additionally, the change in T-loop planarity (Δ Tavplan) and symmetry/morphology (Δ Teigenv) between baseline and maximum ischemia was significantly associated with MAR ($r=0.46$, $p<0.005$; and $r=0.42$, $p<0.02$ respectively), even though these associations were relatively weak according to the r^2 values **(Table 2)**.

Myocardium at risk and its location (Study III): During maximum ischemia, the following parameters increased significantly in both subgroups (MAR ≤11% and MAR > 11%): ST-VM, STC-VM, Tavplan and Tarea, similarly to the results in **Study I** in another series of CAD patients. However, ST-VM, STC-VM and Tavplan changed significantly more in the group with MAR > 11%. In this subgroup, Teigenv also reacted significantly (decreased, $p < 0.01$). In contrast, only in the group with MAR ≤ 11% were there significant changes in QRS duration (on average 6%, 98±9 vs. 104±11, $p < 0.05$) and QTc (on average 5%, 432±19 vs. 454±39, $p < 0.01$), similarly to the LAD subgroup in **Study I**. As in **Study I,** there were no significant changes in any group in heart rate, Tpeak-end, T-vector angles or the QRS-T angle.

In all groups, acute ischemia induced the most significant changes in ST-VM and STC-VM, but these changes were more pronounced in the LAD and Lcx groups compared with **Study I**, where the most pronounced changes were seen in the LAD and RCA subgroups. All T-loop parameters reacted to maximum ischemia but mainly in the LAD and Lcx groups **(Table 3).**

According to the simple linear regression analysis in subgroups, STC-VM and ΔTavplan correlated almost equally well with the perfusion defect severity in the LAD group. In the RCA group, only STC-VM correlated significantly with MAR size. In the Lcx group, STC-VM, ΔST-VM and ST-VM correlated significantly with MAR size **(Table 2)**.

According to the ANCOVA, only the QRS duration at maximum ischemia was significantly dependent on both MAR size ($p<0.04$) and target vessel. The longest duration at maximum ischemia appeared during the occlusion of the Lcx (111 ms), followed by the RCA (103 ms) and LAD (98 ms), $p<0.055$. Otherwise, there was no significant independent association between any repolarization parameter and both MAR size and the location of the ischemic myocardium.

Risk assessment of CV morbidity and mortality (Study IV)

During the follow-up of 8±1 years, 16 patients had a CV death, 24 an MI (5 later died) and 89 patients remained free of CV events (Figure 11). This study cohort should be regarded as a “low-risk population sample”, because of a very low risk of CV death (~1%/year) and MI (1.6%/year).

The overall pattern was the most abnormal values for most parameters in patients with subsequent CV death, less abnormal

Table 2. Simple linear regression analysis of MAR and repolarization parameters.

	All n=35		**LAD, n=14**		**RCA n=7**		**Lcx n=14**	
Parameters	CEqual, % of LV	Severity	CEqual, % of LV	Severity	CEqual, % of LV	Severity	CEqual, % of LV	Severity
Maximum ischemia at first inflation								
ST-VM, µV	0.57 (0.0004)	0.60 (0.0002)	ns	ns	ns	ns	0.66 (0.01)	0.68 (0.01)
STC-VM, µV	0.70 (0.000002)	0.73 (0.000001)	0.67 (0.01)	0.74 (0.003)	0.85 (0.02)	0.80 (0.04)	0.74 (0.01)	0.77 (0.01)
Tavplan, µV	ns	0.48 (0.004)	ns	0.66 (0.02)	ns	ns	ns	ns
Absolute difference between baseline and ischemia								
ST-VM, µV	0.64 (0.00004)	0.65 (0.00003)	ns	0.57 (0.04)	ns	ns	0.70 (0.01)	0.69 (0.01)
Tavplan, µV	0.38 (0.03)	0.55 (0.0007)	0.52 (0.057)	0.74 (0.003)	ns	ns	ns	ns

Data presented as simple linear regression coefficient r (p-value). LAD = left anterior descending artery; LV = left ventricle; Lcx = left circumflex artery; MAR = myocardium at risk; RCA = right coronary artery.

values in the MI subgroup and least abnormal values in patients with neither.

Both uni- and multivariate analyses revealed an association between CV death as the first event and a widened QRS-T angle (OR=19.5, p<0.01; and OR=34, p<0.025 respectively), whereas the QRS duration was predictive only in the univariate analysis (OR=10, p<0.01). A distorted T loop (increased Tavplan) predicted subsequent MI in both the uni- and multivariate analysis (OR=5, p<0.003; and OR=11, p<0.003 respectively), while a more rounded T loop (low Teigenv) predicted MI as a first event only in the univariate analysis (OR=11, p<0.02). Repeat revascularization was not predicted by any parameter.

In the second step analysis, CV death was associated with both a widened QRS-T angle and QRS duration in the uni- and multivariate analyses (for QRS-T angle OR=6, p<0.0013; and OR=6, p<0.03: for QRS duration OR=8, p<0.001; and OR=8, p<0.02 respectively). Subsequent MI was predicted only by an increase in Tavplan (OR=4, p<0.006; and OR=4, p<0.02 respectively).

Combinations between conventional predictors, such as LVH and the new VCG parameters (QRS-T angle and Tavplan), did not improve the predictive power for CV death or subsequent MI. The correlation between different repolarization parameters was statistically significant but biologically relatively weak, as exemplified by the QRS-T angle with Tavplan (r=0.40; p<0.000).

After ROC curve analysis, the cut-off values were chosen for QRS duration at 111 ms, for Tavplan at 0.71 µV and for the QRS-T angle at 101°. PPV was calculated for CV death: QRS duration (26%), QRS-T angle (24%) and Tavplan (12%). Similarly, the PPV for MI was for QRS duration (9%), QRS-T angle (12%) and Tavplan (19%).

The predictive power of the Tpeak-end parameter was also tested in relation to CV death or subsequent MI and did not display any predictive capabilities in this cohort of patients.

Table 3. Comparison between the target vessels at baseline and during ischemia.

		LAD n=14	RCA n=7	Lcx n=14	All n=35
B	Heart rate, bpm	57 ± 9	57 ± 14	53 ± 10	56 ± 11
I		59 ± 13	60 ± 13	57 ± 13	59 ± 13
B	QT interval, ms	443 ± 27	456 ± 35	462 ± 35	453 ± 32
I		449 ± 35	458 ± 65	457 ± 45	454 ± 45
B	QTc interval, ms	429 ± 19	438 ± 23	432 ± 18	432 ± 19
I		442 ± 36	455 ± 62	439 ± 31	443 ± 40
B	Tp-e interval, ms	102 ± 8	99 ± 11	113 ± 14†‖	106 ± 12
I		109 ± 17	113 ± 45	110 ± 20	110 ± 25
B	ST-VM, µV	72 ± 38	80 ± 28	87 ± 39	79 ± 36
I		183 ± 116**	144 ± 82*	193 ± 156*	179 ± 126***
I	STC-VM, µV	163 ± 92***	104 ± 55*	201 ± 160**	166 ± 122***
B	Tazimuth, degree	28 ± 52	54 ± 55	67 ± 32†	49 ± 48
I		32 ± 72	69 ± 60	64 ± 42	52 ± 59
B	Televation, degree	52 ± 16	71 ± 14‡	70 ± 14††	63 ± 17
I		62 ± 24	70 ± 28	60 ± 23	63 ± 24
B	QRS-T angle, degree	38 ± 23	70 ± 53	69 ± 37	57 ± 38
I		51 ± 33	73 ± 49	66 ± 33	61 ± 36
B	Tavplan, µV	0.55 ± 0.18	0.58 ± 0.13	0.58 ± 0.13	0.57 ± 0.15
I		1.22 ± 1.06**	0.62 ± 0.18	1.43 ± 0.91**‖	1.19 ± 0.92***
B	Teigenv, unitless	29 [11;71]	16 [5;68]	13 [5;18]	16 [7;47]
I		4 [3;12]**	12 [5;23]	8 [4;27]	9 [3;19]*
B	Tarea, µVs	60 ± 26	52 ± 16	64 ± 23	60 ± 23
I		84 ± 41**	69 ± 26	83 ± 32*	80 ± 35***
	MAR estimate				
	cequel% of LV	18 ± 11	7 ± 9	14 ± 17	14 ± 14
	severity	366 ± 262	111 ± 144	279 ± 307	280 ± 274

Abbreviations as in Table 2. Data presented as mean ± SD or median [25th; 75th percentile]. B= baseline, I= maximum ischemia, referenced by STC-VM.

Comparison between baseline and maximum ischemia in a respective parameter: *= p<0,05; **=p<0,01; ***=p<0.001

Comparison: between LAD and Lcx: †=p<0.05; ††=p<0.01, between LAD and RCA:‡=p<0.05 and between RCA and Lcx: ‖=p<0.05.

GENERAL DISCUSSION

VR abnormalities are mechanistically linked to the occurrence of malignant ventricular arrhythmias and SCD in different disease states, including MI (acute ischemia) [2,10,17,19,103]. VR is also very sensitive to structural and functional changes in the ventricular myocardium. Assessments of VR duration and heterogeneity have therefore been of clinical interest for a very long time, because they could provide crucial information about myocardial vulnerability and the propensity for ventricular arrhythmias (Figure 13).

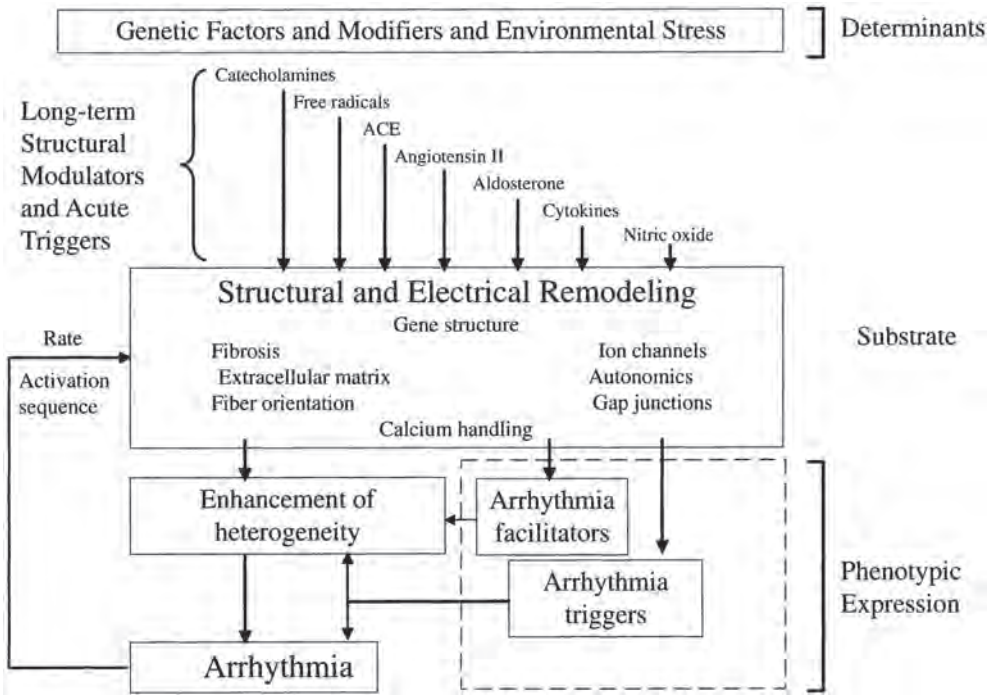


Figure 13. Determinants of cardiac arrhythmias. Genetic factors and modifiers and environmental stresses provide the background for the structural and electrical remodeling of the myocardium by different factors, which presumably trigger arrhythmias and/or facilitate their occurrence mainly via the enhancement of heterogeneity of repolarization (From members of the Sicilian Gambit. Eur Heart J 2001;22:2148-2163).

Invasive catheter-based methods have been used for VR assessment by measuring local refractoriness and MAP duration and its relationship to local activation times. Recently, so-called mapping systems (e.g. the CARTO system) have been applied for similar purposes [74]. For practical, ethical and economic reasons, invasive methods have limited applicability and are therefore basically research tools. For more general use, non-invasive methods need to be applied and the conventional ECG has also been used for this purpose for decades. Commonly used assessments of VR by conventional 12-lead ECG provide time (the QT interval and its dispersion), as well as positive or negative voltage characteristics, which provide prognostic information [104-105]. The conventional ECG is, however, a 2-D projection of the 3-D VCG, which we have preferred for reasons discussed in the next section. This thesis focused on studying VR abnormalities in patients with CAD, who constitute the largest group at risk of malignant ventricular arrhythmia and SCD, by means of 3-D VCG parameters developed previously at our institution [11]. The overall purpose was to learn more about ischemia-induced VR abnormalities in order to better understand their nature, their relationship to the threat of SCD and thereby find approaches to prevent premature SCD.

Methodological advantages, reproducibility and sensitivity of 3-D VCG parameters

Measurement of the QT interval (reflecting VR duration) has played and still plays a major role in the diagnosis of the long QT syndrome with an associated risk of ventricular arrhythmia and SCD. The long QT syndromes are the "prototypes" for VR-related disorders. In the wake of the CAST study published in 1989, the interest in non-invasive assessment of repolarization heterogeneity increased [106] and QT dispersion (QTd) was introduced as a measure of VR heterogeneity and was applied in a number of studies [104-105,107]. QT dispersion is the difference between the longest and shortest QT intervals in the 12-lead ECG. The disadvantages of this measurement were subsequently pointed out: in a global sense, VR is finished at one time point (simple electric field theory), whereas QTd is or at least was thought to reflect regional differences in time. It is, however, a fact, that the QT interval and its variation in different leads reflect the T-loop morphology and its maximum axis [84]. Furthermore, the T-wave end is often difficult to define (considerable inter-observer variability) and the potentially pro-arrhythmic, and therefore important, U wave is usually excluded from the analysis of VR duration [6-7]. QTd also has notable time-dependent variation, which is 6 to 10 times higher than that of the QT interval [8]. Due to the limitations discussed above, the application of QTd measurements in clinical research has greatly diminished and has even been banned from use by some people [7,76].

By applying VCG in the assessment of VR, some of the problems discussed above could be overcome. This issue was first addressed by Badilini et al. in 1995 [82], when they compared QTd and T-loop morphology in a CAD population versus controls at rest. Firstly, the T-vector loop morphology describes the entire VR and is not dependent on operator-defined T-wave end. Secondly, it shows global VR deviations and does not contradict "simple electric field theory", which states that VR of the heart ends at one time point and is not dispersed between different parts of the myocardium. Thirdly, the reproducibility of some T-vector loop parameters is high and similar to that of the QT interval. Furthermore, recent diagnostic and prognostic studies have applied different T-vector and T-loop morphology parameters reconstructed from the surface ECG with some success [108-110]. Because such reconstructions do not represent 3-D from a

strictly anatomical perspective and involve some approximation error, VCG data would presumably correlate more accurately with the "true" anatomy.

Since there is a well-established link between VR alterations and the risk of malignant ventricular arrhythmias including SCD, it would be logical to attempt to obtain a more reliable and comprehensive picture of VR for improved risk assessment and for monitoring interventions. Bearing all the above-mentioned issues in mind, we wanted to use the VCG-based method in this thesis for studies of CAD and acute short-lasting ischemia, in parallel with studies of pacing-induced ventricular remodeling, which were the basis of another, recent thesis at our institution [46].

The time-dependent variation expressed as the coefficient of variation of the new parameters ranges from 4 to 49%, with the lowest value of 4% for Tel comparable with that of the QT interval, which is also around 4%. Teigenv and the QRS-T angle have the poorest reproducibility (32-49%), which is similar to that of QTd [8,111]. A parameter with relatively modest reproducibility is cumbersome for the following interventions at individual level and requires a larger sample size in order to detect systematic deviations. In spite of this, the reproducibility of Teigenv and QRS-T angle is modest to poor, although their ability to reflect biological deviations in the myocardium compensate to some extent for this limitation, i.e. if appropriate reference limits are used, their discriminative ability to distinguish health and disease is useful. For example, a wide QRS-T angle reflects myocardial hypertrophy (and left bundle branch block) and has predictive capabilities in relation to CV mortality, whereas Teigenv is sensitive to the presence of LVH and CAD and is also sensitive to ischemia in some areas.

The sensitivity of the VCG parameters to detect CAD in patients without major comorbidities was demonstrated in **Study I**. The Tarea appeared to be the best and Teigenv the next best VCG parameter for distinguishing CAD patients from healthy controls.

Notably, the QRS-T angle, which was not sensitive to CAD alone, appeared to be the best parameter for detecting LVH and also hypertension, or probably hypertension-induced remodeling, in CAD patients. One explanation might be that CAD and LVH induce biologically different alterations in VR, where CAD influences the morphology/shape of the repolarization and LVH its orientation in space. Another explanation could be limited statistical power in **Study I** compared with that in **Study II**, where the QRS-T angle appeared as a significant parameter, differentiating patients with/without LVH and hypertension.

Ischemia-induced effects on ventricular depolarization and repolarization – the PCI model

During acute ischemia, the myocytes partially depolarize and their transmembrane resting potential is reduced (less negative). As a consequence, the initial rate of rise of the action potential (phase 0) and its amplitude decrease, because the sodium influx is voltage dependent (the more negative the inside of the cell, the more rapidly sodium will enter). Reduced sodium channel availability, intracellular Na^+ and Ca^{2+} overload, post-repolarization refractoriness (a dissociation between APD and refractoriness [112]) and cellular uncoupling lead to conduction disturbances [112-113]. Acute ischemia depresses the excitability and conduction velocity more rapidly in the epicardium than in the endocardium, leading to increased dispersion or transmural heterogeneity [114]. Acute ischemia thus significantly affects ventricular activation, which precedes the recovery phase or VR. When assessing the VR, the topic of this thesis, depolarization and its alterations are one of several factors that influence measures of VR (see also Limitations).

Local injury currents develop between ischemic and non-ischemic cells, as well as voltage gradients between the layers of the ventricular wall, which manifest as ST-segment deviation in the involved area. During acute ischemia, changes mainly involve the ST-T waveforms and, to a far smaller extent, the QRS complex, because electrical depolarization is less susceptible to ischemia than electrical recovery. The current of injury occurs when the subendocardial cells complete their activation process and this affects the amplitude but not the duration of the QRS complex [115]. Moreover, acute ischemia opens IK_{atp} channels and causes the acidosis and hypoxia of myocardial cells. As a result, the extracellular potassium concentration increases in the ischemic zone (peaked T waves) and causes a larger dispersion in the repolarization across the border zone. The K^+ concentration modulates the cardiac automaticity, excitability and refractoriness. Subsequently, the ischemic zone is electrically more negative than its surrounding myocardial area during the recovery phase. As a result, the orientation of the repolarization forces changes so that it points towards the location of injury [83]. Later, when myocardial necrosis develops, the electrical forces of repolarization point away from this site [83]. Generally, our knowledge of the electrophysiological effects on VR related to acute ischemia is based on animal experiments. In order to study the effects of acute ischemia on VR in humans, the model of balloon occlusion during elective PCI appeared to be most appropriate. It is known that PCI induces regional ischemia in 70-90% of patients during balloon inflation [116-117]. The duration of inflation has changed over time, corresponding to the acquired knowledge and advances in the PCI technique [11,14]. Nevertheless, it is the best-known human model of acute ischemia in use nowadays [45].

Ischemia significantly affects VR, as reflected by changes in all T-loop and T-vector parameters. The early VR parameters (ST-VM and STC-VM = “old” or “conventional” VCG parameters) are most sensitive to ischemia, followed by the T-loop parameter Tarea. Generally speaking, the new VCG parameters are not better then the conventional ones for detecting acute ischemia. However, Teigenv and Tavplan appeared to be sensitive to acute ischemia in Lcx, unlike ST-VM. These parameters therefore emerged as potential tools for detecting and monitoring acute ischemia in the myocardium supplied by this artery, which is a well-known challenge for conventional 12-lead ECGs. However, the observations from **Study III** were not as convincing in this respect as those from **Study I**, although there were similar changes in Tavplan and Teigenv during Lcx occlusion. Further study of this issue is required.

Even though acute ischemia created changes in all parameters, the amount of these changes was related to vessel and MAR size.

The most prominent VR changes were seen during balloon inflation in the LAD. Moreover, the occlusion of this artery created the largest MAR size, which probably reflects anatomically a more widespread area of LV supplied by the LAD [11,14,118]. LAD occlusion was thus associated with the most rounded and distorted (bulgy) T loop, suggesting the most pronounced VR heterogeneity, while the T vector pointed more anteriorly and cranially compared with baseline conditions (towards the ischemic area). The increased propensity for malignant arrhythmias and SCD associated with LAD occlusion might be linked to these observations of the VR response during short-lasting ischemia [11,119]. Moreover, autopsy observations reveal a predominance of LAD changes in SCD victims [120-121].

Tentatively and expressed in more general terms, these data taken together suggest that acute ischemia caused by LAD occlusion results in relatively larger MAR size and more prominent VR heterogeneity, which results in a higher propensity for arrhythmogenesis and SCD.

The occlusion of the RCA and Lcx resulted in the contradictory changes in VR. In **Study I,** RCA occlusion induced more pronounced VR changes compared with Lcx occlusion (according to ST-VM, STC-VM, Tarea, and Teigenv), whereas the opposite was found in **Study III** (according to ST-VM, STC-VM, and Tarea). However, the Tavplan and Teigenv changes were concordant in both studies. These discrepancies could be explained along two lines. Firstly, there was a difference in inflation time (approximately 30 sec for the first occlusion and 60 sec for the occlusion causing maximum ischemia in **Study I** vs.128 sec in **Study III** for the first occlusion), which might affect the extent of response between different parameters. Secondly, the patient groups could perhaps differ. For example, in **Study III,** there was a somewhat higher predominance of males compared with **Study I**.

LVH and VR

While planning and executing **Study I,** we excluded patients with major ”confounders”, because, in our first study in this PCI model, we had a study population with a very mixed clinical background [11]. Patients with hypertension were, however, not excluded. Later, the publication of Dilaveris et al. raised the question of whether hypertension itself could be another confounding factor for VR abnormalities [122]. We therefore performed a post-hoc analysis of the hypertensive patients, which revealed that they reacted more profoundly to acute ischemia compared with their normotensive counterparts in this cohort of CAD patients according to Tarea and STC-VM. Moreover, the T loop was significantly rounder in shape (lower Teigenv) in hypertensive patients at baseline and after the procedure. This led us to study the ischemia-induced effects on VR in hypertension and LVH in **Study II**.

Myocardial hypertrophy is a condition in which the salient feature of all models is a prolongation of APD and refractoriness. Importantly, this prolongation is not uniform; in the epicardium, the APDs are more prolonged than in the endocardium, altering the transmural gradient seen in normal hearts [123-126]. Prolongation of the APD is associated with a diminished outward repolarizing current secondary to down-regulated K^+ channel expression in hypertrophied and failing hearts [127-128]. Non-uniform prolongation of the APD may be pro-arrhythmic by increasing the dispersion of repolarization or refractoriness and changing the electrical gradient [123-125]. Indeed, increased dispersion of repolarization and refractoriness within the left ventricle of hypertrophied hearts has been associated with increased inducibility of VT and VF [123,126]. Moreover, there is a hypothesis that polymorphic VT occurring in the setting of LVH might share the same or similar mechanistic elements as those observed in patients with QT interval prolongation, whereby it has been speculated that LVH might be another form of acquired long-QT syndrome or at least a latent form [129]. Additionally, myocardial hypertrophy is also characterized by a “reduced repolarization reserve”, which means reduced compensatory mechanisms for counteracting perturbations of repolarization [130]. The dispersion of repolarization between ischemic and non-ischemic areas is an important pro-arrhythmic mechanism during acute ischemia, as has been demonstrated previously [131]. If acute ischemia is added to myocardial hypertrophy, it is reasonable to anticipate a more profound VR response. **Study II** revealed that LVH patients displayed the most abnormal repolarization both at baseline (as expected) and during coronary occlusion and were therefore most sensitive to ischemia, followed by the hypertensive patients. These results are in line with animal data showing a decreased tolerance to induced ischemia and reperfusion in myocardial hypertrophy [42-43,132-133]. Moreover, epidemiological observations point to nearly 50% of deaths occurring in patients exhib-

iting LVH being sudden and unexpected. It is believed that most are likely due to the onset of polymorphic VT rapidly evolving into VF [129,134-137]. Our finding is also in line with the early clinical observations of a so-called “stone heart” after cardiac surgery in patients with cardiac hypertrophy, i.e. the ability to recover after ischemia [138]. The results of **Study II** thus provide a human link in this chain of evidence.

Several mechanistic factors involved in reduced tolerance to ischemia have been identified in myocardial hypertrophy: 1) impaired coronary vasodilator reserve; 2) decreased capillary density and increased diffusion distance; 3) decreased high-energy phosphate content; 4) impaired fatty acid oxidation leading to increased dependence on glucose metabolism; and 5) diminished myocardial glucose delivery into the cell [42-43,132-133]. The net result is an increased ischemic zone on top of heterogeneous repolarization.

Because there are more factors than increased muscle mass or volume that might lead to altered VR and an exaggerated response to acute ischemia, it was thought to be prudent to assess the relationship between the VR changes and the amount of threatened myocardium; this led to **Study III**.

Relationship between MAR size, occluded vessels and the extent of VR changes (Study III)

From **Study II,** a relationship between the myocardial mass and the number of VR changes was anticipated. Other factors discussed below could also influence the VR response and it was therefore also logical to assess any relationship to the target vessel. As it turned out, VR parameters reflecting the earliest phase (STC-VM and ST-VM) were most sensitive to ischemia and correlated most significantly with MAR size, as shown previously [14]. Moreover, STC-VM correlated with MAR size independently of the occluded vessel. Two T-loop parameters, Tavplan and Teigenv, also correlated with MAR size; however, this association was weaker.

LAD occlusion created the largest MAR size, which correlated equally well with the STC-VM and ΔTavplan, followed by ST-VM and Teigenv. In contrast, there was no significant correlation between MAR size and Tavplan and Teigenv during RCA and Lcx occlusion. An explanation of this lack of correlation could perhaps be found along the following lines. Firstly, our VR parameters probably reflect complex interplay between apico-basal, transmural and right-to-left ventricular VR heterogeneities [139-140], which are not affected to the same extent during the occlusion of different vessels (locations) at a given MAR size. For example, the Tpeak-end interval, which was recently shown to be a measure of global VR heterogeneity [94,141], did not differ between the subgroups during ischemia. Tarea appeared to be another example of a possible lack of vessel-dependent difference; it showed almost no difference in absolute changes during acute ischemia between the LAD, RCA and Lcx subgroups (24, 17 and 18 μVs respectively). Secondly, VR and arrhythmogenesis are determined by factors other than the size of the ischemic myocardium and heart rate (which was low and did not differ in this study), such as the autonomous nervous activity. The sympathetic innervation varies in different parts of the heart. Among patients with CAD, such differences might be augmented and might interplay with local electrophysiological properties of the myocardium and the amount of ischemic myocardium to affect the extent of the VR changes [142-143]. Thirdly, a low or even a lack of correlation between the VR parameters and MAR size in the RCA group could be attributed to limited statistical power.

To summarize; there is a good correlation between MAR size and the early VR parameters STC-VM and ST-VM. The cor-

relation between MAR size and the T-loop morphology parameters Tavplan and Teigenv is mainly due to its association with ischemia in the LAD. Only the QRS duration was independently associated with vessel and MAR size but not with any of the T-loop parameters.

Risk prediction potential of the new 3-D VCG parameters

Up to this point, our group has shown that VR analysis by VCG is a useful research tool for following interventions such as acute, short-lasting ischemia and pacing-induced ventricular remodeling. Could this methodology be used for prognostic purposes? This issue was explored in **Study IV**. CV mortality and morbidity was assessed in a cohort of CAD patients followed up for an average of 8 years. The main findings were that VCG-derived morphology descriptors of VR had independent predictive values with regard to subsequent adverse CV events. A wide QRS-T angle correlated significantly with CV death (OR=5.8, CI [1.2-27.9]) and was comparable to the established risk factors such as LV dysfunction (EF≤45%, OR=8.2, CI [1.8-38.0]) and increased QRS duration (OR=7.6, CI [1.6-35.8]).

It was previously suggested that a narrow QRS-T angle represents depolarization, followed by a normal (similarly directed) repolarization process, and that the opposite, a widened QRS-T angle, represents the true VR heterogeneity [72,144]. A widened QRS-T angle predicts cardiac death in a general population, in postmenopausal women and in a post-MI population [10,145-146]. A widened QRS-T angle could be partly associated with myocardial hypertrophy, as was shown in **Study II** and by Ishizawa et al. [86]. However, Ishizawa proposed that a widened QRS-T angle is not only associated with hypertrophy of the myocardium but also reflects repolarization abnormalities beyond simple hypertrophy. Following that line, we performed a meticulous statistical analysis, adjusting for LVH, and acquired similar results.

The occurrence of MI during follow-up was most consistently associated with a distorted or bulgy T loop, which is a novel finding. Loss (or reduction) of the normal T-loop planarity has previously been observed during acute ischemia in humans and in a porcine model (Odenstedt et al. personal communication) [11, **Study I**] and is presumably associated with increased VR heterogeneity. Several patients had hypertension with or without ECG signs of LVH and others had had a previous MI (presumably with some degree of myocardial hypertrophy as part of a remodeling process). There is a well-known relationship between the degree of repolarization abnormalities and the degree of myocardial hypertrophy. Furthermore, there is an important relationship between myocardial hypertrophy and reduced tolerance to coronary occlusion [42,147]. We therefore interpret the association between increased Tavplan and subsequent MI as reflecting increased vulnerability to ischemia [**Study II**].

Importantly, these observations were made in a patient cohort which, according to present knowledge, is defined as representing a low-risk population (see Limitations).

Methodological considerations and limitations

In this project, we have studied the VR response to acute, short-lasting ischemia, but ischemia is added to several interacting factors, as delineated in Figures 3 and 13. The substrate is therefore different (LVH, previous MI, diabetes and so on), the autonomous nervous system activity varies (however, 93% of the patients had beta-blockers), the hemodynamic consequences depend on previous myocardial injuries and so forth. The metabolic consequences (such as lactate

production) are completely unaccounted for. The pharmacological background varies, with the exception of beta-blockade (**Studies I-II, IV**). However, these differences reflect the CAD population and the clinical reality in which people die suddenly.

While planning this project, we were aware that the sudden and short-lasting coronary occlusion during PCI is not a perfect model of the "naturally" appearing ischemia induced by a coronary thrombus. Nevertheless, it is probably the best experimental situation available in humans and its validity and reliability have been demonstrated previously [44].

In **Study I**, we attempted to avoid "confounding" factors such as peri-procedural MI, LVH, multivessel PCI and the presence of collaterals. However, collateral flow between coronary arteries has a gradual and sometimes obscure appearance and cannot always be completely ruled out from angiographic images [148]. Moreover, ECG-detected LVH has a high specificity (>95%) but a relatively low sensitivity [149]. Furthermore, because of recent observations by Dilaveris et al., suggesting that hypertension might be another "confounder", the repolarization response to ischemia was compared in patients with and without a history of hypertension [122]. In hypertensive patients, we found a more rounded T loop (decreased Teigenv) and these patients responded to ischemia more profoundly in the LAD group compared with those without a history of hypertension. Hypertension therefore appeared as another "confounder" and this finding led us to initiate **Study II**.

The data obtained at maximum STC-VM and at the end of the PCI (**Study I-II**) could have been influenced by the different degrees of preconditioning, the role of which is not fully understood. The end of the first inflation therefore served as the reference for comparisons with the data obtained at maximum STC-VM and for comparison with the previous studies.

In **Study II,** taking account of the problems with ECG-based diagnosis of LVH, we checked the available echocardiographic data. Although not complete, they arrived at a very similar result and basically confirmed the presence of LVH. Moreover, the hypertensive and normotensive subgroups also presumably included patients with some degree of myocardial hypertrophy, e.g. as a consequence of post-MI remodeling. In fact, our results very much agree with the notion that there was a more or less continuous spectrum of increasing degrees of myocardial hypertrophy, when going from the normotensive patients (55% with previous MI) via hypertensive patients to the LVH subgroup, rather than sharp borders between them. This would reduce rather than augment the between-group differences and support the validity of our results. Furthermore, at the time of recruitment, the criterion for a diagnosis of hypertension was repeated measurements of blood pressure >160/95 mmHg, which is too conservative a criterion according to a recent classification [150]. However, because both blood pressure and left ventricular mass are continuous variables, the entire patient group probably reflects continuity in these aspects and the main results of our study should be valid. In fact, this overlap should also act to reduce rather than augment any differences between the subgroups.

There was a lack of correlation between the ST-segment changes and changes in T-vector or T-loop parameters (except for Tarea, where the Spearman correlation coefficient was $r=0.80$, $p<0.00001$, **Study II**). The lack of correlation was possibly due to the fact that the other repolarization parameters were poor measures of acute ischemia or that any correlation was confounded by the alterations already observed at baseline in the hypertensive and LVH groups. In overall terms, there was a significant inverse relationship between the baseline values of the T-vector and T-loop parameters and the changes at maximum ischemia. Turned the other way around, an abnormal baseline repolarization possibly had a limited range within which to

become even more abnormal during coronary occlusion. Within-group comparison revealed that, in the normotensive subgroup with the fewest baseline alterations, significant changes were observed in both the T-vector (all 3) and T-loop parameters during ischemia. In contrast, with increasingly more pronounced baseline abnormalities, there were fewer significant changes; 2 and 1 T-loop parameter/s respectively changed significantly in the hypertensive and LVH subgroups. The exception was Taz (see "Acute ischemia"). STC-VM and ST-VM were, however, sensitive to ischemia, regardless of the subgroup.

Unequal numbers of patients in all the studies might affect the statistical power and may also contribute to an underestimation of subgroup differences. A significantly different pharmacological background could also influence the results in **Studies II** and **IV**.

Heart rate, which might at least partly reflect autonomous nervous activity, is an important determinant for at least the early repolarization pattern (STC-VM) [151]. However, we mainly observed VR alterations during a low and stable heart rate (~60 bpm), which did not differ between subgroups (**Studies I-III**). Furthermore, there appears to be a difference in the activation of the autonomous nervous system when the coronary occlusion is caused by balloon inflation as compared to a spontaneously forming thrombus [152].

In **Study III,** the presence of hypertension could potentially have influenced our results. Because the distribution of the hypertensive patients was almost equal between the groups, we believe that myocardial hypertrophy is unlikely to have affected our results and patients with MI were excluded. Moreover, the possibility that unequal wall thickness within different coronary artery areas or that the influence of the LV diameter in different subgroups might influence VR cannot be completely ruled out. However, recently published data did not reveal any significant correlation between the ST-T changes and ECG voltage in the subgroup of patients with modest LVH, unequal myocardial wall thickness and different LV diameters [153].

Study IV was exploratory in nature. The generalization of the study results should therefore be avoided, pending the results of studies of larger patient cohorts and of patients with a moderate and high risk of SCD. However, when our group was compared with the report from the Swedish coronary angiography and angioplasty registry, no significant discrepancies regarding the population's structure, angiographic and angioplasty data were observed. Furthermore, the results relating to the conventional risk factors (low EF, QRS duration) suggest that our study group is fairly representative of CAD patients studied by other authors.

Elective PCI has only a symptomatic effect and does not influence the prognosis. However, one PCI-related MI could be viewed as prognostically meaningful according to present knowledge (CK-MB elevation > than 5 times above normal values) [98-99,154]. In order to exclude any effect of PCI-related MI on our results, uni- and multivariate analysis was performed, also adjusting for this group of patients; basically the same results were obtained.

Implications

Since it became apparent that alterations in VR are linked to CV mortality and morbidity including SCD, a reasonably good non-invasive method to assess such changes in VR has been searched for. Our results point to the potential value of applying VCG for VR analysis. The potential utility of different T-vector and T-loop parameters was assessed by comparing their reproducibility, ability to distinguish CAD patients without major co-morbidities, capability to separate CAD patients with and without hypertension and LVH and the response to coronary occlusion. Risk assessment with regard to the subsequent need for revascularization and

the occurrence of MI and CV death was performed by VCG parameter analysis.

For diagnostic and epidemiological purposes, the Tarea and Teigenv appeared to be the best T-loop parameters for distinguishing CAD patients from the controls. Moreover, this method can be used for monitoring and assessing the effect of both short- and long-term therapeutic or modifying interventions, as has been shown for pacing-induced ventricular remodelling [155-156]. More specifically, the QRS-T angle might become an important tool for epidemiological studies and risk stratification, because it appears to be a sensitive marker of altered repolarization in LVH and hypertension, it correlates with the severity of LVH [86], which itself is a prognostic factor for the future CV event [134-136], and it has shown prognostic value in CAD and post-MI cohorts and in a general population [**Study IV**,145-146]. The Tavplan also might become useful for risk stratification for subsequent MI in the CAD population.

Finally, VCG recording and analysis might be easily incorporated into daily clinical routine by adapting contemporary recording devices with the T-vector and loop analysis algorithm.

CONCLUSIONS

- The baseline T-loop morphology of CAD patients without major co-morbidities differed significantly compared with controls. The reproducibility of the T-vector and T-loop parameters varied from very good, comparable to that of the QT interval (Tarea, Tel, Tpeak-end), to modest (QRS-T angle, Teigenv).

- At rest, the T-vector loop is elongated and points to the left and downwards. Brief coronary occlusion had a significant impact on VR, reflected by changes in the T-vector angle (pointing towards the ischemic zone) and the T-loop morphology (becoming rounded and more distorted). The most prominent repolarization changes occurred during LAD occlusion.

- A history of hypertension and/or the presence of LVH were associated with more abnormal repolarization at baseline and also a markedly aggravated response to acute ischemia induced by coronary occlusion. These results are in line with the animal experiments, showing reduced tolerance to ischemia in myocardial hypertrophy, and to available epidemiological data, demonstrating an increased risk of non-fatal and fatal CV events in the presence of LVH.

- Acute ischemia due to LAD occlusion was associated with a threat to a larger part of the left ventricular myocardium compared with RCA and Lcx occlusion and was significantly correlated with more pronounced VR changes, presumably reflecting an increased propensity for arrhythmogenesis. This finding is in line with experimental, anatomical and epidemiological data, as well as with autopsy data in SCD victims.

- In a relatively low-risk CAD cohort, LV dysfunction and increased divergence between depolarization and repolarization waves (increased QRS-T angle) were the best markers of increased risk of CV death during follow-up, which is in line with existing evidence. One novel finding was an association between a distorted repolarization loop and the risk of future MI.

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