# The use of repolarization variability for arrhythmic risk monitoring
"The rocking of the boat"

**Peter Oosterhoff**

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# The use of repolarization variability for arrhythmic risk monitoring
"The rocking of the boat"

# Het gebruik van variabiliteit van repolarisatie voor het monitoren van aritmisch risico
"Het deinen van de boot"
(met een samenvatting in het Nederlands)

Proefschrift

ter verkrijging van de graad van doctor aan de Universiteit Utrecht op gezag van de rector magnificus, prof.dr. G.J. van der Zwaan, ingevolge het besluit van het college voor promoties in het openbaar te verdedigen op donderdag 19 mei 2011 des middags te 12.45 uur

door

**Peter Oosterhoff**

geboren op 5 januari 1971
te *Noordoostpolder*

**Promotor:**

Prof.dr. M.A. Vos

**Co-promotor:**

Dr. M.A.G. van der Heyden

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# Contents

*"You can help things move along by standing at the end of your boat, feet on opposing gunnels, and swaying in rhythm to the motion imparted by the sea. However slight you are, however large your lifeboat, you will be amazed at the difference this will make. I assure you, in no time you'll have your lifeboat rocking and rolling like Elvis Presley."*
*Life of Pi - Yann Martel*

# Chapter 1

# Introduction

P. Oosterhoff

# Introduction

Sudden cardiac death remains a major cause of cardiovascular death. Implantation of an implantable cardioverter-defibrillator (ICD) can be effective in terminating life threatening cardiac arrhythmias.[1,2] Since its introduction 30 years ago, it has become a widely used therapy for patients with high risk of sudden cardiac death.[3, 4] The two major studies used to derive current guidelines, report an absolute reduction in mortality of 5-7% over 2-5 years.[1,2] However, this reduction comes at a cost; studies have reported increased anxiety and depression in patients that received ICD therapy.[5] Furthermore, reports show that up to 47% of ICD shocks are delivered inappropriately for conducted supraventricular tachycardia.[6, 7] Current guidelines permit ICD implantation also for patients without a history of cardiac arrhythmias (primary prevention).[4] A recent analysis of survival data over an 8 year follow-up in the MADIT-II population found a number needed to treat of 8, which means most patients are subjected to the risk and discomfort of ICD implantation without receiving benefit.[8]

Continuous estimation of arrhythmic risk may be used to guide therapy in ICD patients to prevent shocks; either by conservative arrhythmia detection settings in patients with low risk, prevention of arrhythmias by physician intervention (e.g. prescribe antiarrhythmics, correction of hypokalemia), or preventive pacing algorithms initiated by the device (this was tested in chapter 3).

Variability in cardiac repolarization has been suggested as a marker of risk of cardiac arrhythmias or sudden cardiac death; Baseline beat-to-beat changes in QT interval from surface ECG, quantified as short-term variability (STV), have been related to spontaneous and drug induced ventricular arrhythmias in patients.[9-11] In a preclinical setting, STV has been associated with drug induced and spontaneous arrhythmias in anesthetized dogs with chronic atrioventricular block (CAVB).[12-14] The anesthetized CAVB dog shows a high susceptibility to drug induced Torsade de Pointes (TdP), which may be predicted by baseline STV. Alternatively, as seen in figure 1, after infusion of a proarrhythmic drug STV increases several minutes before an episode of TdP is induced; meanwhile a non-proarrhythymic dose leaves STV unchanged. Continuous monitoring of STV may be used to detect an increase in arrhythmic risk and take preventive measures.

In a cooperation between Medtronic Bakken Research Center (Maastricht, The Netherlands), and the department of Medical Physiology (Division heart & Lungs, UMC Utrecht, The Netherlands), we have explored feasibility of STV monitoring in an implantable ICD. In this thesis we present data on the feasibility of STV measurement in ICDs and prevention of TdP arrhythmia by cardiac pacing. Furthermore, we look

into the mechanisms of drug induced TdP and the link between arrhythmia and STV in the CAVB model.

## Thesis overview

In chapter 2, background of the CAVB dog model and a review of data on drug-induced arrhythmia in relation to STV is presented. Chapter 3 describes a proof of concept study into preventive effect of heart rate increase on TdP occurrence and on STV. Furthermore, technical requirements for measuring STV are defined and the STV measurement from chronic electrograms is explored. Chapter 4 deals with the fact that for elaborate testing of an STV guided algorithm for preventive pacing, longer experiments in awake dogs would be preferable. Therefore, in this chapter we determined the role of anesthesia for TdP induction in the CAVB dog. In chapter 5, as a first step to a clinical application of STV, we determined the long-term predictive value of baseline STV for sudden arrhythmic death in an ICD population. Chapter 6 addresses further improvement of sensitivity and specificity of STV. Therefore, the relation between STV and preload variation, a distinct feature of the CAVB dog, was investigated. Chapter 7 presents newly derived analysis methods for measurement of STV from monophasic action potentials and electrograms as developed during the course of the investigations described in this thesis. Finally, chapter 8 compares and discusses the findings presented in the experimental chapters, and provides views for further development of STV as an arrhythmia prediction application in ICD technology.

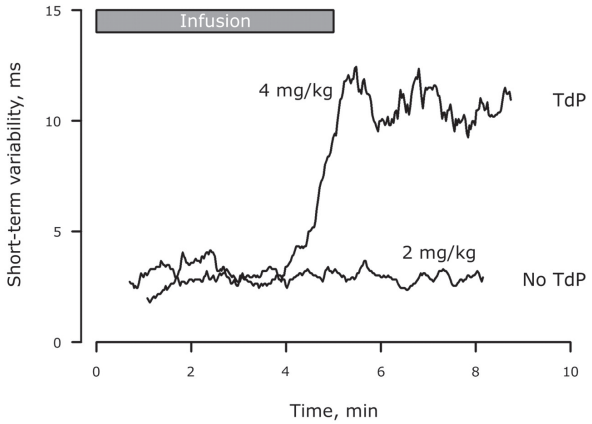


*Figure 1: Temporal development of short-term variability (STV) of left ventricular monophasic action potential duration after d-sotalol infusion (grey bar). In the experiment with TdP, there is instantaneous increase of STV 5 minutes after start of a high dose d-sotalol administration. This is 14 minutes before TdP occurred. At a lower dose, which did not trigger arrhythmia, STV remains unchanged. Figure from Thomsen et al.*[12]

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## Chapter 2

# Beat-to-Beat Variability of Repolarization A new parameter to determine arrhythmic risk of an individual or identify proarrhythmic drugs

Peter Oosterhoff *, Avram Oros and Marc A. Vos [$]

From the Department of Medical Physiology, Heart Lung Centre Utrecht, UMC Utrecht, Utrecht and from * Bakken Research Center, Medtronic, Maastricht, the Netherlands

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## Abstract

Hypertrophy and heart failure are associated with an enhanced propensity for cardiac arrhythmias and a high mortality rate. Altered repolarization might play a role in the occurrence of these ventricular arrhythmias. Beat-to-beat variability of repolarization duration (BVR) has been proposed as a parameter for detection of an unstable, and less controlled repolarization process that precedes the actual tachy-arrhythmia. To investigate the relevance of BVR in identifying individuals at risk for arrhythmic events, this parameter was studied in dogs with remodeled hearts and increased susceptibility to arrhythmias due to chronic complete atrio-ventricular block. Progression of electrical remodeling (prolongation of repolarization times), vulnerability to arrhythmias and sudden cardiac death were reflected in baseline values of BVR. Furthermore, BVR showed a strong predictive value in the screening for pro-arrhythmic effects of drugs. Thus, BVR can be used to identify 1) individuals at risk for ventricular tachycardias and 2) drugs with proarrhythmic properties.


## Introduction

Heart failure is a multi-factorial disease in which many different adaptations may be responsible for the very high mortality seen in this patient group. It has been estimated that about half of these patients die from an arrhythmia, accounting for > 500.000 deaths a year worldwide. The prevalence for sudden death is present in all categories of the New York Heart Association functional classification, indicating that a depressed cardiac contractility is only part of the arrhythmia story.[1] Many arrhythmogenic mechanisms have been identified to contribute to this predisposition, including reentrant and focal sources. One of the electrophysiological hallmarks of heart failure is the increase in ventricular repolarization times, as detected on the ECG (QT-time), through more local recorded signals with catheters (monophasic action potential or electrogram) and in tissue and isolated cardiomyocytes (trans-membrane action potential).[2-4] It has been assumed that this aspect of electrical remodeling is important to explain the enhanced susceptibility of arrhythmias in many patients. But how can we detect who is at risk? This information is not only relevant to guide treatment (ICD yes or no), but also to inform the physician and the patient about what situations and/or drugs should be avoided so that a further challenge on repolarization and possibly on life may be prevented. Especially, the proarrhythmic risk of drugs that block the rapid component of the delayed rectifier current ($I_{Kr}$) has been a topic of intense discussion in the scientific, pharmacologic and clinical societies.[5, 6] It is a future aim not only to define whether such a drug that affects repolarization is safe or unsafe, but also to identify patients at risk for certain

types of drugs. To quote Sir Richard Sykes (rector of Imperial College, London and former chairman of GlaxoSmithKline) "Future must lie in identifying sections of the population most likely to suffer from adverse effects from a drug, so they can be excluded".[7]

In this review, we will address this double challenge: 1) how to identify individuals at risk for repolarization dependent arrhythmias, and 2) how to establish the proarrhythmic risk of a repolarization-prolonging drug. For that it is necessary to elaborate on dog studies in which the severity of electrical remodeling is related to ventricular arrhythmias (1a) and to introduce a new parameter to quantify arrhythmic risk (2). Finally, drug induced arrhythmias will be discussed (3), before the concepts will be integrated.



## Ventricular Remodeling in the Chronic AV-block dog

Mechanisms of remodeling have been extensively studied by our group in dogs with chronic complete atrio-ventricular block. This model allows examination of electrical, mechanical and structural changes in the heart and their effect on susceptibility to arrhythmias as remodeling progresses.

We identify three stages in the development of a non-remodeled heart to a state of compensated hypertrophy: 1) sinus rhythm (SR), 2) acute AV-block (AAVB) and 3) chronic AV-block (CAVB). In SR, the atria and ventricles contract synchronously and the ventricular heart rate is determined by the sinus node (Figure 1, left panel). AAVB, the stage immediately after the ablation of the AV-node, is characterized by a slow idio-ventricular rhythm (IVR) (Figure 1, middle panel). Reduction of cardiac output is limited by neurohumoral activation.[8,9] The slow heart rate, altered activation and loss of atrio-ventricular synchrony all can trigger several remodeling mechanisms with which the heart tries to compensate in the long run. After several weeks this remodeling process reaches a stable situation (CAVB), in which compensated hemodynamics is now associated with biventricular hypertrophy, an increased cardiac performance and prolonged repolarization times (electrical remodeling).[10] At the cellular level current densities of components of the delayed rectifier ($I_{Kr}$ and $I_{Ks}$ ) are decreased, which is confirmed on the molecular level by a down-regulation of the ion channel subunit expression levels. This results in a reduction in repolarization strength, visible as QT prolongation and an increase in the duration of the left ventricular monophasic action potential (LV MAP) (Figure 1, right panel), which makes the animal susceptible to drug induced arrhythmias. A normal cell possesses redundancy in repolarizing currents, its repolarization reserve, which can be recruited to withstand internal and external factors that challenge the cell's control over the action potential duration.[11] Factors that decrease the repolarization strength

(i.e. electrical remodeling, bradycardia or pharmacological $I_{Kr}$ block) can reduce this reserve to a point where the repolarization process can no longer be controlled and becomes unstable. This results in ectopic beats and eventually triggered arrhythmias. In the CAVB model, administration of an $I_{Kr}$-blocker as the final hit can uncover this increased susceptibility to arrhythmias. Recorded electrophysiological parameters and arrhythmic response to a pharmacological challenge characterize the different stages of electrical remodeling in the AVB dog.

## Beat-to-beat variability of repolarization

Abnormal repolarization has been related to increased risk of cardiac arrhythmias and sudden cardiac death. Several parameters are used to quantify deviations in specific aspects of cardiac repolarization: either measuring T-wave morphology (Notched T-waves [12], microvolt T-wave alternans [13]) or measuring repolarization duration (QT interval [14], $T_{peak}$-$T_{end}$ [15] or QT variability index [16]).

Recently, our group proposed beat-to-beat variability of repolarization duration (BVR) as an additional parameter to quantify arrhythmic risk.[17] BVR is a measure of temporal dispersion which captures the variation in repolarization between subsequent beats and is evaluated at resting heart rates.

We quantify BVR using the duration of the left ventricular monophasic action potential (MAPD), but QT interval or transmembrane action potential duration of isolated cells can also be used. Monophasic action potentials (MAP) are

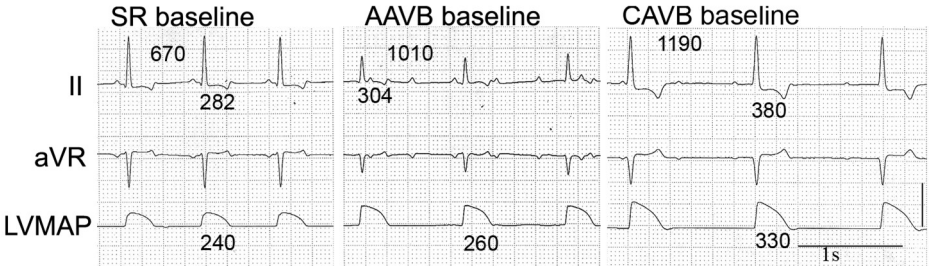


*Figure 1: Representative examples of ECG (lead II and aVR) and left ventricular monophasic acton potential (MAP) tracings at baseline recorded at the three stages of the dog model: Sinus rhythm (SR), acute AV-block (AAVB) and chronic AV-block (CAVB). Printed values (top to bottom) are RR, QT and LVMAP duration. ECG was calibrated to 1mV/cm. MAP signal to 20mV/cm. Printed at 25mm/s.*

recorded using catheters placed on the endocardium of the left ventricular wall. The morphology of the signals recorded from these catheters resemble local trans-membrane action potentials.[18] A Poincaré plot is created by plotting MAPD of each beat versus MAPD of the preceding beat. BVR can now be quantified as short-term variability (STV) of MAPD, which is calculated as the distance of the points in the plot to the line of identity, averaged over 30 consecutive beats: $STV = \sum_{1..30} |MAPD_n - MAPD_{n-1}| / (30 * \sqrt{2})$.[17] Figure 2a shows an example of a left ventricular MAP tracing (left panel) with a detailed view of the corresponding Poincaré plot (right panel).

## Drug-induced Torsade de Pointes

Torsade de pointes (TdP) is a ventricular polymorphic tachyarrhythmia characterized by a twisting shape of QRS complexes and T waves around the isoelectric line of the ECG (Figure 2b).[19] This arrhythmia can stop spontaneously or degenerate into ventricular fibrillation and sudden death. Although originally diagnosed in circumstances of AV-block and severe bradycardia, TdP can also be initiated by an adverse reaction to various pharmaceutical compounds with class-III effects. In the recent years, several cardio-vascular or non-cardiovascular drugs have been withdrawn from the market due to QT prolongation and TdP.[5, 6, 20] Drug induced TdP is a rare arrhythmia with, for some drugs, an incidence of less than 1 case in 10000 or 100000 exposures, creating difficulties for the detection of proarrhythmic properties of drugs.[5, 6, 21] Therefore, proarrhythmic animal models were developed, including the CAVB dog,[5] and several drugs that block $I_{Kr}$ (cardiovascular or non-cardiovascular) have been tested for cardiac safety assessment. Such models also offer the opportunity to study the mechanisms of proarrhythmia and TdP.

To study the potential of a drug to induce TdP, we prefer a serial experimental design in which several drugs can be administered i.v. in different experiments using the anesthetized CAVB animal as it own control. We often use dofetilide as a gold standard for induction of TdP arrhythmias. Dofetilide is an $I_{Kr}$ blocker used for the treatment of atrial fibrillation and ventricular tachycardia. But one of its known side-effects is TdP, with an incidence of 3.3 % in a selected patient population with congestive heart failure.[22] However, in our anaesthetized CAVB dog model a similar dose of dofetilide-induced TdP in 74% of the dogs.[23] confirming the high sensitivity of the model. Based on this arrhythmic response, we can split the CAVB dogs in two phenotypes: dofetilide susceptible and dofetilide resistant animals (26%).

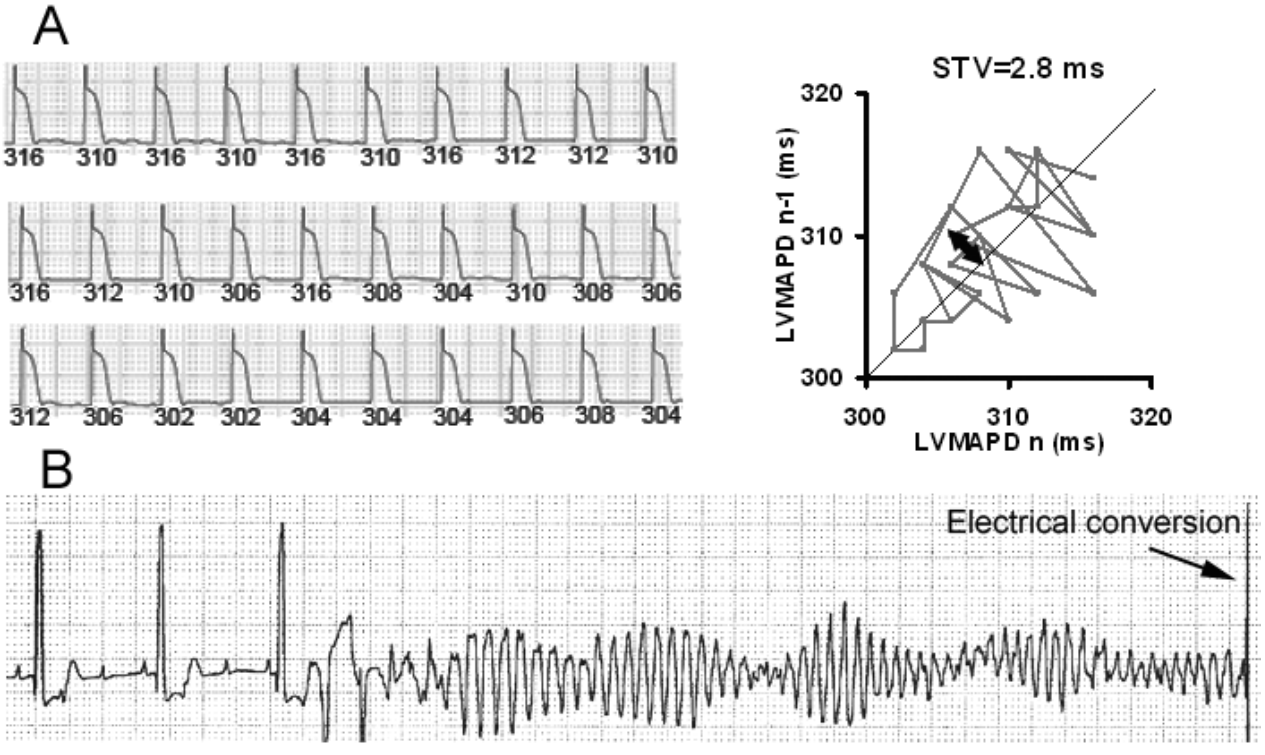


*Figure 2: A. In the left panel a tracing of thirty consecutive monophasic action potentials is shown with their respective MAP durations. Shown in the right panel is a Poincaré plot of MAP durations. Short-term variability (STV) is calculated as the average distance of the points of the plot to the line of identity (arrow).*
*B. ECG tracing (lead II) of a drug induced TdP episode, which needed cardioversion.*

## BVR as measure of severity of electrical remodeling

We investigated the use of BVR as a measure of severity of electrical remodeling in the AVB dog. As mentioned, several remodeling processes are initiated after the induction of AV-block. Among them electrical remodeling has been well described at several levels: in the intact heart (QT, MAPD), at the cellular (APD, ion currents) and at the molecular level (expression level of ion channel subunits). Over the years, several investigations using these dogs have been performed.[8-10, 17, 23-31] The response of the CAVB dog has been well preserved, but was not always quantitated on aforementioned cellular or molecular level. To compare severity of electrical remodeling with BVR in these investigations, the heart rate corrected QT interval ($QT_C$) will be used in this review (Table 1a).

The sudden bradycardia after the transition from SR to AAVB, before electrical remodeling is initiated, leaves $QT_C$ unchanged while uncorrected QT and LVMAPD are prolonged (normal frequency dependency). However, at chronic AVB, with electrical remodeling, $QT_C$ is severely prolonged (SR: 294±17, AAVB: 286±30, CAVB: 382±51 ms; Table 1a).

Baseline values of BVR respond to electrical remodeling in the different stages of the CAVB dog model in a similar way as $QT_C$ (Table 1b). Figure 3a shows representative examples of Poincaré plots for SR, AAVB and CAVB. Be aware of the difference in scale compared to figure 2a. At the transition from SR to AAVB, the value of BVR increases from 0.7±0.1 to 1.2±0.6 ms. Possible explanations might be the increased RR interval variability [23] or rate dependence of BVR. At chronic AVB, when electrical remodeling is complete, BVR stabilizes at an elevated level (2.6±0.9 ms). Figure 3b illustrates the relation between $QT_C$ and BVR. It confirms that severity of electrical remodeling (increase in $QT_C$) is reflected in an increase of BVR.

## Prognostic value of BVR at baseline

The severity of electrical remodeling also has consequences for the susceptibility to arrhythmias in the AVB dog. Where in SR or AAVB dofetilide in combination with anesthesia never induces TdP, there is a high TdP incidence in CAVB (74%). Moreover, some dogs (10%) die suddenly in the absence of proarrhythmic drugs or anesthesia.[25, 29]

Therefore, within the group of CAVB dogs we can discriminate three phenotypes: 1) animals that die from spontaneous arrhythmias (SCD), 2) animals that only show arrhythmias after a pharmacological challenge and 3) animals that are resistant to both spontaneous and drug induced arrhythmias. Most likely these differences in arrhythmic response can be explained by a different degree of electrical remodeling and baseline BVR values, expecting the highest values in dogs that die suddenly

*Table 1*: *Baseline values of $QT_C$ and beat-to-beat variability of repolarization (BVR) at different stages of the chronic AV-block dog model.*

*a. Baseline $QT_C$ in the 3 groups of dogs*

| Reference | SR | AAVB | CAVB |
|---|---|---|---|
| *17* | | | *413±42* |
| *31* | *310±10* | | *423±32* |
| *25* | | *282±29* | *378±52* |
| *24* | | | *460±67* |
| *26* | | | *361±54* |
| *23* | *288±18* | *293±38* | *376±46* |
| *30* | | *284±25* | *366±59* |
| **Pooled data** | *294±17 (n=16)* | *286±30 (n=31)* | *382±51*† (n=133)* |

** P<0.05 vs SR, † P<0.05 vs AAVB.*

*b. Baseline BVR in the 3 groups of dogs*

| Reference | SR | AAVB | CAVB |
|---|---|---|---|
| *17* | | | *3.3±1.2* |
| *31* | *0.8±0.1* | | *2.4±0.2* |
| *25* | | *1.3±0.3* | *2.7±0.9* |
| *24* | | | *2.0±0.8* |
| *26* | | | *2.3±0.7* |
| *23* | *0.7±0.1* | *0.7±0.1* | *2.3±0.6* |
| *30* | | *1.5±0.9* | *2.7±1.2* |
| **Pooled data** | *0.7±0.1* | *1.2±0.6** | *2.6±0.9*† |

** P<0.05 vs SR, † P<0.05 vs AAVB. SR: sinus rhythm, AAVB: acute AV-block , CAVB: chronic AV-block. All values in ms expressed as mean±sd.*

and the lowest values in drug resistant animals. This has been validated in a recent investigation.[23] Baseline BVR values were highest in the SCD animals (5.4±1.4 ms [25]), followed by CAVB dogs that show TdP arrhythmias only after dofetilide (2.5±0.4 ms [23]), while the lowest values of BVR are indeed seen in the dofetilide resistant group (1.7±0.4 ms [23], Figure 4, CAVB white bars). Thus, BVR captures the differences in repolarization reserve and susceptibility to spontaneous or drug induced arrhythmias.

Limited data are available using BVR in humans. Hinterseer et al. compared BVR derived from QT interval for patients with a history of drug induced arrhythmias (dLQTS, n=13) to a healthy control group (n=13).[32] Despite similar values of rate corrected QT interval, patients in the dLQTS group had a higher BVR compared to control (6.2±4.2 vs 4.2±2.1 ms, P<0.05). Even in a setting without any pharmacological challenge and normal repolarization duration, BVR identified the reduced repolarization reserve and higher propensity for drug induced arrhythmias in the dLQTS group.

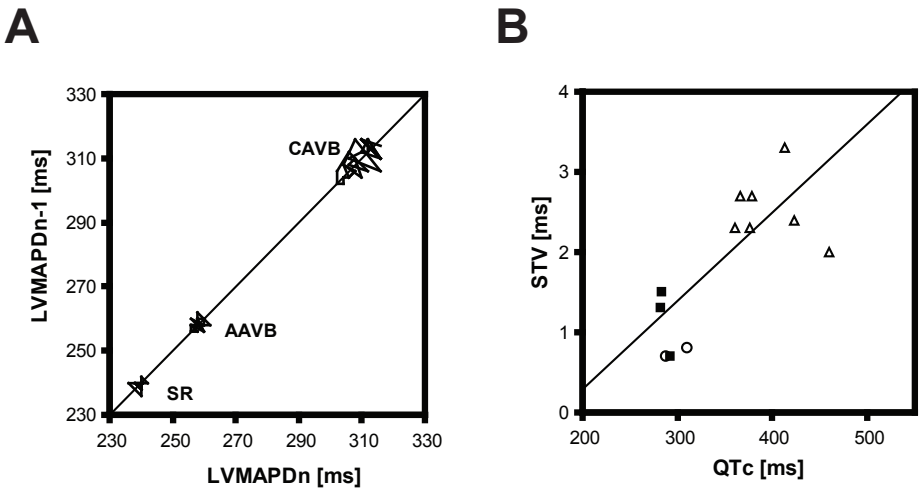


*Figure 3: A. Representative Poincaré plots of baseline left ventricular monophasic action potential durations (30 beats) at the three stages of the dog model.*
*B. Relation between baseline values of $QT_C$ and beat-to-beat variability of repolarization at sinus rhythm (SR, open circles), acute AV-block (AAVB, closed squares) and chronic AV-block (CAVB, open triangles) with regression line ($p<0.01$, $R^2=0.56$). Data from tables 1a and 1b.*

## BVR and drug-induced torsade de pointes

To further explore the relation between proarrhythmia and BVR, we assessed the effect of several proarrhythmic drugs on BVR. Repolarization parameters ($QT_C$ and BVR) were evaluated before the first drug induced ectopic beat and compared to baseline values. In the CAVB dog model, TdP can be induced by numerous $I_{Kr}$-blockers. After dofetilide, it can be seen that the drug prolonged the $QT_C$ interval and increased BVR (Table 2) leading to TdP in the majority of the animals. When this study population is divided according to their proarrhythmic outcome into dofetilide-susceptible and dofetilide-resistant animals, we found that BVR increased only in the group where TdP occurred (Figure 4), while $QT_C$ prolonged in both groups.[23] Furthermore, when these proarrhythmic doses of drugs are given in SR or AAVB dogs no arrhythmia was induced and no increase in BVR was observed, whereas QT duration was significantly increased in both situations.[23] This indicates that QT prolongation and TdP are not always causally linked. To evaluate alternative parameters, like BVR, in determining arrhythmic properties of medication, we set out a number of experiments: the dose dependent induction of TdP with d-sotalol, another class-III anti-arrhythmic, was one study methodology. A high dose (4 mg/kg) resulted in 75% TdP occurrence, whereas with a low dose (2 mg/kg) only 25% of the animals showed TdP (Table 2). The only parameter reflecting this dose dependency was BVR: with the high dose BVR increased, while with the low dose it did not change significantly, although the 25% inducibility still accounted for a tendency towards increasing values (3.5±1.5 to 5.5±1.6 ms, Table 2).[17]

A more black and white picture was seen with sertindole, an antipsychotic drug. At a clinically relevant dose (0.2mg/kg) there was no significant increase in BVR and no TdP, while at a high dose (1mg/kg) BVR increased and TdP was induced in 76% of the individuals.[26] A similar observation was seen for NS-7, a drug in development for anti-stroke therapy, but now by changing the infusion time. The fast infusion increased BVR and induced TdP in 50 % of the cases, while the slow infusion of NS-7 did not induce TdP nor did it increase BVR (from 2.1±0.2 to 2.5±1.0 ms, Table 2).[31] Thus, drug-induced TdP is associated with an increase in BVR.

## BVR and safe drugs

To further validate the assumption that BVR reflects arrhythmic risk we assessed the effect of several non-proarrhythmic drugs on BVR; expecting that safe drugs would not increase the value of BVR. The anti-arrhythmic drug amiodarone is known to prolong repolarization duration, but is free of TdP in our experimental setting.[28] Although amiodarone prolonged the QT interval, it did not increase BVR (from 2.4±0.2 to 2.4±0.4 ms, Table 2).[17]

*Table 2: Drug-induced TdP and repolarization parameters ($QT_C$, BVR ) in chronic AV-block dogs.*

| | $QT_C$ | | BVR | | TdP | Reference |
|---|---|---|---|---|---|---|
| **Drug** | **control** | **drug(ms)** | **control** | **drug(ms)** | **(%)** | |
| **Dofetilide** | *376±46* | *467±66 ** | *2.3±0.6* | *4.2±1.5 ** | *74* | *23* |
| **d-Sotalol** 4 mg/kg | *415±47* | *484±52 ** | *3.0±0.7* | *8.6±3.8 ** | *75* | |
| **d-Sotalol** 2 mg/kg | *410±37* | *475±60 ** | *3.5±1.5* | *5.5±1.6* | *25* | *17* |
| **Sertindole** 1 mg/kg | *361±54* | *452±63 ** | *2.3±1.0* | *5.1±2.0 ** | *76* | |
| **Sertindole** 0.2mg/kg | *367±54* | *439±78 ** | *2.3±1.0* | *3.2±1.0* | *0* | *26* |
| **NS-7** 3 mg/kg in 5 min | *420±40* | *480±50* | *2.6±0.3* | *6.0±1.4 ** | *50* | |
| **NS-7** 3 mg/kg in 60 min | *425±20* | *460±30* | *2.1±0.2* | *2.5±1.0* | *0* | *31* |
| **Amiodarone** | *340±40* | *470±75 ** | *2.4±0.2* | *2.4±0.4* | *0* | *17, 28* |
| **Moxifloxacin** | *466±78* | *556±63 ** | *2.0±0.9* | *3.0±1.3* | *0* | |
| **Azithromycine** | *450±42* | *416±48* | *2.2±0.6* | *2.3±0.5* | *0* | *24* |

**, $p<0.05$ vs. control; values are expressed as mean ± sd.*

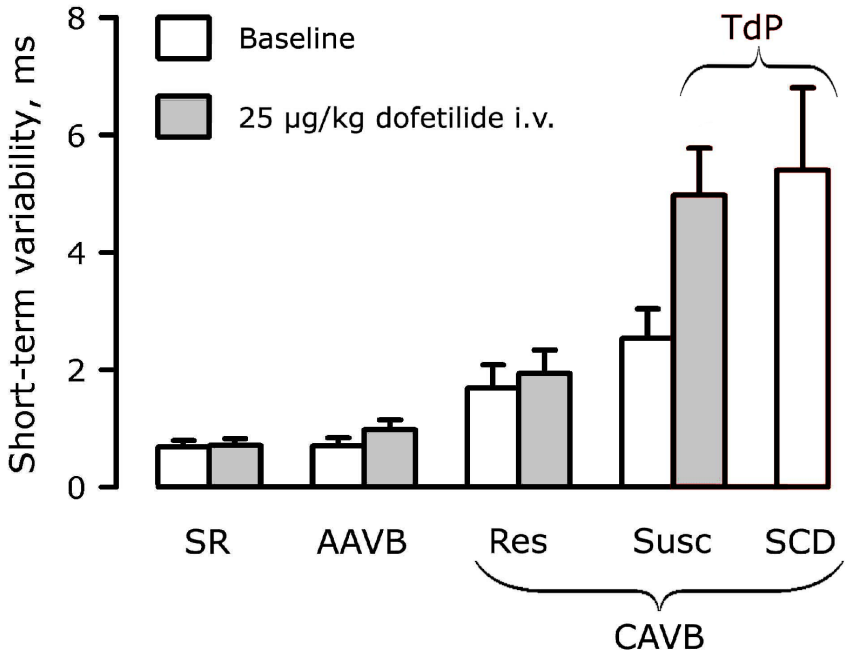


*Figure 4: Beat-to-beat variability of repolarization duration in anesthetized dogs at baseline (open bars) and after administration of dofetilide (grey bars) for sinus rhythm (SR), acute AV-block (AAVB) and three subgroups of chronic AV-block: dofetilide resistant (Res), dofetilide susceptible (Susc) and dogs that died suddenly (SCD). Data from 23 and 25.*

The antibiotic moxifloxacin, used as a gold standard for QT prolongation assessments in human volunteers (thorough phase-1 QT studies),[33] was administered serially in CAVB dogs. All dogs were found to be susceptible to dofetilide-induced TdP. This test revealed that an extensive prolongation of repolarization duration, similar to dofetilide, is not associated with induction of TdP.[24] Again, the absence of TdP with this drug was linked with an unchanged value of BVR. In the same susceptible group azithromycin, one of the most prescribed antibiotics today, was also tested. Again the absence of TdP at plasma concentrations relevant to the clinical practice, was associated with an unaltered BVR, supporting the idea that a stable BVR is characteristic for a safe drug (Table 2).[17, 24, 28]

## Integration

Abnormalities in cardiac repolarization have been linked to progression of heart failure and increased risk for sudden cardiac death. BVR has been proposed to quantify temporal variation as one aspect of altered repolarization. Figure 4 summarizes our findings; baseline values (open bars) reflect the severity of electrical remodeling, which determines the risk for spontaneous or drug induced arrhythmias. Individuals prone to drug induced arrhythmias present with higher BVR values than their drug resistant counter parts. This makes BVR a candidate parameter for identification of patients at risk.

Furthermore, BVR can detect the proarrhythmic potential of drugs (closed bars). Unsafe medication results in an increase of BVR, while safe drugs leave BVR unaffected (Table 2).

Further research is needed, including human studies, to evaluate the applications of BVR in risk stratification.

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## Chapter 3

# High rate pacing reduces variability of repolarization and prevents repolarization-dependent arrhythmias in dogs with chronic AV-block

Peter Oosterhoff [*†], Morten B. Thomsen [†‡] , Joep N. Maas [†], Nico J.M. Atteveld[†], Jet D.M. Beekman[†], Harold V.M. Van Rijen [†], Marcel A.G. van der Heyden[†], Marc A. Vos [†]

* Medtronic Bakken Research Center, Maastricht, The Netherlands. † Medical Physiology, Division Heart & Lungs, University Medical Center Utrecht, Yalelaan 50, 3584CM Utrecht, The Netherlands.

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## Abstract

*Introduction*: High rate pacing may have an inhibitory effect on the initiation of Torsade de Pointes arrhythmias (TdP). However, permanent pacing is only indicated in high risk patients. We performed a proof of concept study into automatic overdrive pacing for prevention of drug induced TdP, using short-term variability of repolarization (STV) as a feedback parameter of arrhythmic risk.

*Methods and results:* The minimal signal sampling frequency required for measuring STV was determined through computer simulation. Arrhythmogenic response to dofetilide (25 μg/kg/5 min) was tested at two different paced heart rates (60-65bpm versus 100-110bpm) in 7 dogs with chronic atrioventricular block, while recording right and left ventricular (LV) monophasic action potential (MAP) and LV electrogram (EGM).

Simulations showed a sampling frequency of 500 Hz is sufficient to capture relevant STV values. High rate pacing prevented dofetilide-induced TdP seen at the low rate (low: 6/7 versus high:1/7). At the low rate, STV from LV MAP duration increased before occurrence of spontaneous, ectopic activity and TdP (1.7±0.6 to 3.0±1.8 ms, $p<0.05$), but at the high rate STV did not change significantly (0.9±0.2 to 1.5±1.4 ms, NS). Regression analysis showed a close relation between STV calculated from LV MAP and from LV EGM. ($R^2=0.71$).

*Conclusions:* High rate pacing increases repolarization reserve in dogs with chronic atrioventricular block, preventing dofetilide-induced TdP. Changes in repolarization reserve are reflected in values of STV.


## Introduction

Torsade de Pointes arrhythmia (TdP) is a potentially life threatening complication in congenital and acquired long QT syndromes (LQTS).[1, 2] While these arrhythmias often self terminate, they can deteriorate into ventricular fibrillation, which requires immediate life saving intervention. Temporary high rate pacing is proven effective in prevention of drug induced TdP, in combination with administration of magnesium, normalization of potassium plasma levels, and withdrawal of the pro-arrhythmic drug.[2-4] Permanent pacing, on the other hand, is currently only indicated for high risk LQTS patients:[5] The increased heart rate may induce heart failure, making it only a viable option in patients with severe risk of arrhythmic death.[6] However, even in high risk LQTS patients, arrhythmias are infrequent and sometimes come in storms.[7] This indicates that the risk of arrhythmia is a transient phenomenon. Continuous assessment of arrhythmic risk could be used to limit cardiac pacing to periods of increased risk and make preventive overdrive pacing feasible for

patients with a more moderate risk profile. Beat-to-beat variability of repolarization has been proposed as a biomarker for arrhythmic risk in both animal models and patients.[8-10] This variability can be quantified as short-term variability (STV) of either QT intervals[9, 10] or LV monophasic action potential duration (LV MAPD).[11-13] In dogs with chronic atrioventricular block (CAVB) proarrhythmic remodeling leads to elevated STV values while drug-induced ectopic activity and subsequent episodes of TdP are often preceded by a further increase in STV.[11, 13, 14] Moreover, we have shown that STV of LV MAPD is reduced by interventions that prevent or suppress TdP episodes.[14] In the present study, we used the CAVB dog, temporarily paced from the high septum during experiments, to evaluate the effect of heart rate on STV and incidence of TdP before and after administration of $I_{Kr}$ block. Thus, the activation pattern was preserved throughout the study. We hypothesized that $I_{Kr}$ block only at the lower heart rate would decrease the already impaired repolarization reserve to critical values, resulting in a marked STV and TdP arrhythmias. We simulated the effect of various signal sampling frequencies to determine the technical requirements for STV monitoring. Additionally, as a proof of concept, we investigated whether STV can be determined using chronic cardiac electrograms (EGM) as are recorded by clinically available intracardiac defibrillators (ICD).



## Methods

*Simulation model:* To explore the effect of signal sampling frequency on recorded STV a simulation model was written in Matlab (The Mathworks Inc, MA, USA). Values of STV were simulated for signal sampling frequencies from 10 kHz down to 250Hz. For each sampling frequency 10,000 values of STV were generated, each based on 31 simulated activation and repolarization moments. Repolarization duration was defined as the time from activation moment to repolarization moment and can represent either MAPD or activation-recovery interval (ARI) from EGM. Repolarization duration was modeled as being normally distributed; standard deviation (SD) was changed to generate a range of target STV values. Signal sampling was simulated by rounding activation moments and repolarization moment to a multiple of the sampling time. STV was calculated from the resulting approximate repolarization durations.

*Animal handling:* Animal handling was in accordance with Dutch law on animal experiments and the European Directive for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (86/609/EU). All experiments were approved by the Animal experiment committee of the University of Utrecht.

All experiments were performed under general anesthesia. Premedication consisted of an intramuscular injection with vetranquil, methadone and atropine (10 mg, 10 mg and 0.5 mg respectively). Complete anesthesia was induced by thiopental (15 mg/kg i.v.) and maintained by isoflurane (1.5% in $O_2$ and $N_2O$, 1:2).
Nine purpose bread mongrel dogs (20-25 kg, Marshall, New York) were used in this study. Two animals were excluded for technical reasons: one for pacing failure as a result of electrode dislocation, and one for intrinsic rhythm above 70 bpm prohibiting the study at low heart rate (see below).

*A V nodal ablation and instrumentation:* In an initial procedure, under general anesthesia, complete atrioventricular block was achieved by radiofrequency ablation.[15] The chest was opened through the 4$^{th}$ or 5$^{th}$ intercostal space, and a stimulation electrode (Medtronic 10627) was placed in the high ventricular septum, near the bundle of His and connected to a subcutaneously placed pacemaker (Diamond III, Vitatron, The Netherlands), as described previously.[16] Lead location was accepted when the QRS axis was similar to sinus rhythm, and the QRS width was below 90ms. During subsequent experiments the animals were paced from this site to provide a reproducible, constant and physiological relevant ventricular activation pattern, without the asynchronous activation associated with right ventricular pacing.[16]
A custom-made recording electrode (BRC, Maastricht) was inserted into the left ventricular wall just above the apex, from epicardium to sub-endocardium. The tip of the electrode consisted of a 10 mm screw which was electrically isolated except for the most distal part. The electrode was connected to a subcutaneous short range (<10 cm) telemetry device (Vitatron, The Netherlands) for recording of sub-endocardial electrograms (EGM) during experiments. The animals were left at a spontaneous idioventricular rhythm for 4 weeks, to allow cardiac remodeling to complete.[17]

*Arrhythmia induction in dogs with remodeled hearts:* In anesthetized CAVB dogs, after 4 weeks of remodeling, monophasic action potential catheters (MAP) were temporarily placed on the endocardial surface of the left and right ventricular apex. The left MAP catheter was positioned as close as possible to the EGM electrode. ECG and MAP signals were digitized with a 500 Hz sampling frequency and stored for off-line analysis. Unipolar, left sub-endocardial electrograms were digitized at 800Hz. Animals were paced from the high septum in VVI mode throughout the experiment. Baseline ECG, MAP and EGM signals were recorded for 10 min at the lowest captured ventricular rate between 60 and 70 bpm (low heart rate) and at a

40% shorter paced cycle length (100 to 115bpm; high heart rate). One dog showing intrinsic rhythm at a pacing rate of 70bpm was excluded from the study.
Repolarization reserve was probed by monitoring for spontaneous TdP episodes in response to pharmacological $I_{Kr}$ block during fixed rate pacing: The $I_{Kr}$-blocker dofetilide (25 μg/kg i.v.) was administered over 5 minutes during constant high septal pacing at the low heart rate, and ECG MAP and EGM signals were recorded. Two weeks later, 6 weeks after AV-block, the experiment was repeated using the high heart rate during drug administration.



*Analysis:* QT interval was determined manually from lead II. Heart rate corrected QT ($QT_C$) was calculated using both the Bazett and the Fridericia formula. MAPD was determined semi-automatically at 90% repolarization using a user defined template to determine relevant fiducial points (AutoECG, EMKA, France).
ARI was derived from the unipolar EGM according to the modified Wyatt method:[18, 19] The time of minimum of the first derivative during the QRS complex was used as the moment of activation. For negative or biphasic T-waves the moment of repolarization was determined as the time of maximum derivative during the T-wave (Figure 1a), whereas for positive T-waves the time of minimum derivative was used (Figure 1b).
Beat-to-beat variability of repolarization was quantified as short-term variability (STV) over 31 beats using the formula: $STV = \sum_{1..30} |D_n - D_{n-1}| / (30 * \sqrt{2})$, where D represents LV MAPD or ARI, as described earlier.[11] This formula represents the average distance to the line of identity for 30 points in a Poincaré plot (See inset Figure 2b).
To prevent influence from spontaneous, extrasystolic activity on electrophysiological parameters, all electrophysiological parameters after dofetilide administration must be determined before the time of first drug-induced extrasystolic activity. To be able to compare values at identical dofetilide challenge within each animal, the time to the first extrasystolic beat was determined in the high-rate and the low-rate experiments; the earliest of these 2 time points was used for analysis.
TdP was defined based on ECG tracings as a polymorphic ventricular arrhythmia lasting at least 5 beats, with complexes twisting around the iso-electric line. The ECG was monitored for TdP starting 10 minutes before, until 10 minutes after start of dofetilide. This interval includes maximum plasma levels and maximum QT prolongation.[20] Animals were considered inducible when 3 or more TdP episodes occurred. Both the number of inducible animals and the individual number of TdP arrhythmias were quantified.

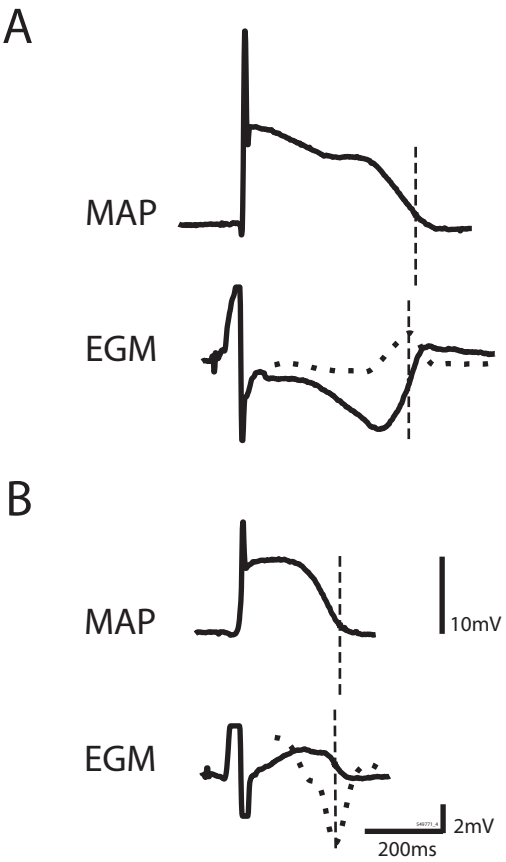
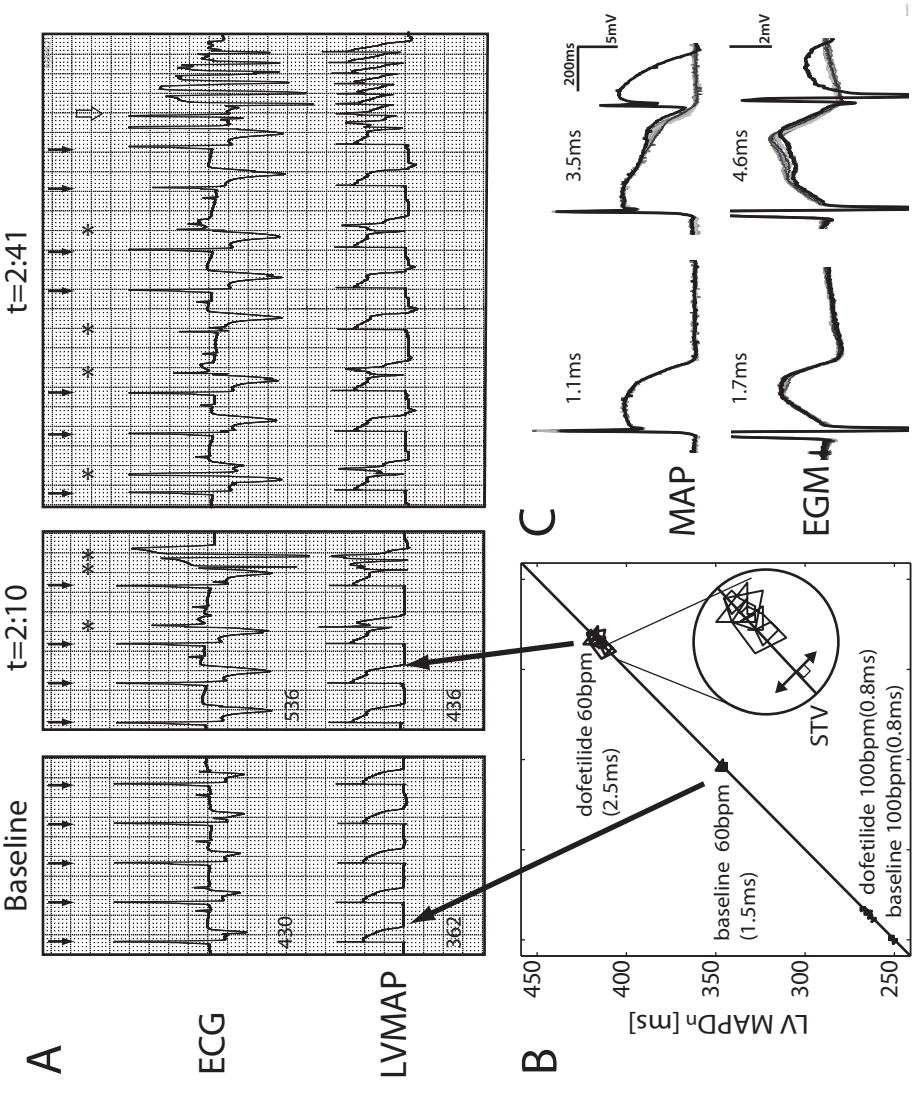



*Figure 1. Determining the moment of repolarization from electrograms (EGM). In each panel a monophasic action potential (MAP, top) and an EGM (bottom) is depicted with a vertical line at the moment of repolarization. For the MAP 90% repolarization is used, while for the activation-recovery interval EGM repolarization is determined by the signal derivative (dashed line), using the modified Wyatt methodology.[18] A: Example with a negative EGM T-wave morphology. B: Example with a positive EGM T-wave morphology.*

*Figure 2 (right page). Effect of dofetilide on ECG (lead II), left ventricular monophasic action potential (LV MAP) and electrogram (EGM) at the two paced rates. A: At a paced rhythm of 60 bpm ECG and LV MAP at baseline (left panel), at first ectopic activity after dofetilide (middle panel) and at first Torsade de Pointes arrhythmia (right panel). When the experiment was repeated at 100 bpm no ectopic activity was seen over this time frame (not shown). Paced beats annotated with a black arrow. Ectopic beats are marked with a star. Start of Torsade de Pointes is marked with an open arrow. Note that in VVI mode spontaneous beats will postpone scheduled paces. Printed values are QT and LV MAP duration. ECG calibrated to 1 mV/cm. B: In the same animal, Poincaré plot of LV MAP duration from 30 beats recorded at baseline and just prior to first extrasystolic beat after dofetilide at 60 bpm, and at the same time points at 100 bpm. Arrows identify the last beat included in STV calculation. The zoomed detail illustrates short-term variability (STV) as the average distance to the line of identity of the points in the Poincaré plot. Printed numbers are STV of LV MAP duration. C: In a different animal, overlay of LV MAP and EGM of 10 beats (grey to black) at baseline (left) and at first dofetilide-induced ectopic beat (right), in an inducible chronic AV-block dog paced at 65 bpm. Plots are synchronized on moment of depolarization: signal maximum for MAP and minimum slope for EGM. The numbers represent the short-term variability of MAP duration and activation recovery interval, at baseline and at the last normal beat.*


A
Baseline
t=2:10
t=2:41
ECG
LVMAP
430
362
536
436
B
LV MAPDn [ms]
LV MAPDn-1 [ms]
dofetilide 60bpm
(2.5ms)
baseline 60bpm
(1.5ms)
dofetilide 100bpm(0.8ms)
baseline 100bpm(0.8ms)
STV
250
300
350
400
450
C
MAP
EGM
1.1ms
3.5ms
1.7ms
4.6ms
200ms
5mV
2mV
Baseline
Dofetilide

*Statistical analysis:* Electrophysiological parameters were tested using a repeated measures 2-way ANOVA with post-hoc Bonferroni paired t-test, when appropriate. A log-transformation was applied to STV values to correct for a skewed distribution. Arrhythmia incidence was compared using an exact McNemar test for paired proportions. Number of TdPs was tested with a paired Wilcoxon test. Statistical significance was acknowledged at $p<0.05$. Presented data are mean±standard deviation (SD).

# Results

## Signal sampling frequency limits STV measurement

The effect of signal sampling frequency was investigated by computer simulation. The relation between STV values before and after sampling is presented in figure 3a. For frequencies above 2 kHz the line coincides with the line of identity, meaning the sampling process will not have a significant effect on the recorded STV value. At lower sampling frequencies the graphs diverge at lower values of STV. Here the beat-to-beat changes in sampled repolarization duration are dominated by quantization errors rather than variation in repolarization, limiting measurement of small STV values. The minimum detectable STV values vary from 1 ms at 250 Hz to 0.01 ms at 10 kHz.

Figure 3b shows there is a clear linear relation between average STV and the SD of STV. This results in a skewed distribution, which can be corrected for by logarithmic transformation. The group averages from several publications of baseline values before (sinus rhythm, acute AV-block) and after remodeling (CAVB) are shown in the same graph.[8] Both the simulated and the experimental data show a positive relation between SD and average value. The SD of the experimental data both includes inter- and intra-individual variation, whereas simulated values are generated at fixed target STV values. Thus, 35% and 45% of the experimentally recorded SD at AAVB and CAVB respectively, can be explained by STV methodology alone (Figure 3b). The variation in recorded STV values will also be influenced by signal sampling (Figure 3b): SD greatly increases for lower sampling frequencies at small STV values. This can be overcome by incorporating more beats in the STV estimation, but at the expense of time resolution. For a clinical application the optimal time window will depend on the time available between a change in arrhythmic risk and the latest opportunity for preventive intervention.

Based on the simulation results, we chose a modified implantable pacemaker with a sampling frequency of 800 Hz to record intracardiac electrograms. The acquisition circuitry is identical to currently available pacemaker devices (Vitatron C and

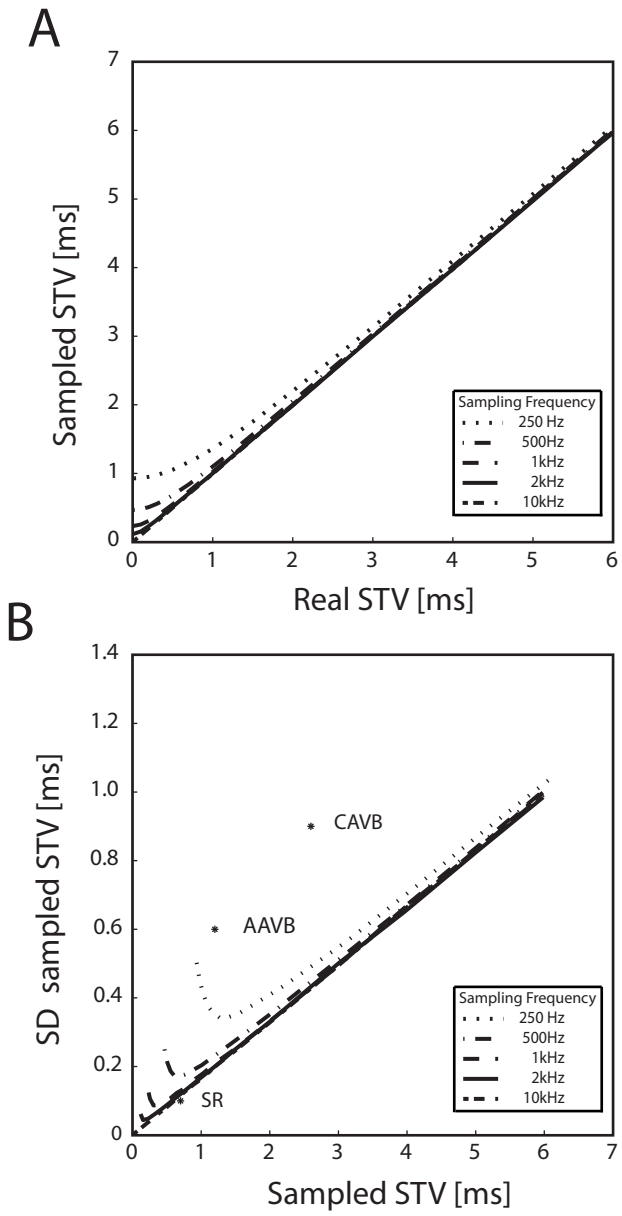



*Figure 3. Simulation of the effect of signal sampling frequency on short-term variability (STV). A: Relation between simulated sampled STV versus target STV values for several sampling frequencies. B: Standard deviation (SD) of sampled STV versus average sampled STV for several sampling frequencies. The stars represent reported baseline values for sinus rhythm (SR), acute AV-block (AAVB) and chronic AV-block (CAVB).*[8]

T-series). For in-vivo MAP registration, we chose a 500 Hz sampling frequency, limiting the minimal detectable STV to 0.5 ms.

## Reverse-use dependence of dofetilide

Six animals were tested at paced heart rates of 60 and 100bpm (low and high rate respectively), while one animal showed spontaneous idioventricular rhythm at 60bpm and required pacing rates of 65 and 110bpm. Baseline characteristics of electrophysiological parameters (QT, $QT_C$, ARI, RV and LV MAPD) collected at identical heart rates in experiments at 4 and 6 weeks showed no differences (data not shown). Baseline values of QT were higher at the low heart rate compared to the high heart rate; whereas heart-rate corrected QT intervals were comparable, as expected (Table 1). Dofetilide significantly prolonged both QT and $QT_C$ at the low rate ($p<0.05$), with a trend towards prolongation at the high rate ($p=0.12$ for both QT and $QT_C$). A similar pattern of more pronounced increases at the low heart rate was seen with LV and RV MAPD and LV ARI (Table 1).

## Dofetilide is only proarrhythmic at low heart rate

No TdP arrhythmias were seen during baseline recording at either pacing rate. At the low heart rate, administration of dofetilide led to ectopic activity intervening with the paced rhythm in 6 out of 7 animals (114±41 s after start infusion; Figure 2a middle panel). In the same 6 animals this was followed by multiple spontaneous TdP episodes (inducibility 86%, $p<0.05$ versus baseline; Figure 2a right panel, Figure 4a).

*Table 1: Electrophysiological parameters at baseline and after dofetilide, at high and low heart rate.*

| | Low heart rate | | | High heart rate | | |
|---|---|---|---|---|---|---|
| | **Baseline** | **Dofetilide** | | **Baseline** | **Dofetilide** | |
| **Paced rate, bpm** | *61 (60-65)* | | | *101 (100-110)* | | |
| **QT, ms** | *444±25* | *533±67** | *+20%* | *365±18 †* | *420±67 †* | *+15%* |
| **$QT_{C,\ Bazett}$, ms** | *445±25* | *535±70** | *+20%* | *476±27* | *547±85* | *+15%* |
| **$QT_{C,\ Fridericia}$, ms** | *445±24* | *534±69** | *+20%* | *435±23* | *501±78* | *+15%* |
| **LV MAPD, ms** | *320±28* | *427±97** | *+34%* | *251±16 †* | *296±59 †* | *+18%* |
| **RV MAPD, ms** | *283±16* | *365±82** | *+29%* | *228±24* | *269±30 †* | *+19%* |
| **ARI, ms** | *305±47* | *405±78** | *+33%* | *230±8* | *271±46 †* | *+18%* |

*HR, Heart rate (range). LV (RV) MAPD, left ventricular (right ventricular) monophasic action potential duration. ARI, activation-recovery interval. * $p<0.05$ versus baseline, † $p<0.05$ versus low rate. Two-way, repeated measures ANOVA. with post-hoc Bonferroni T-test*

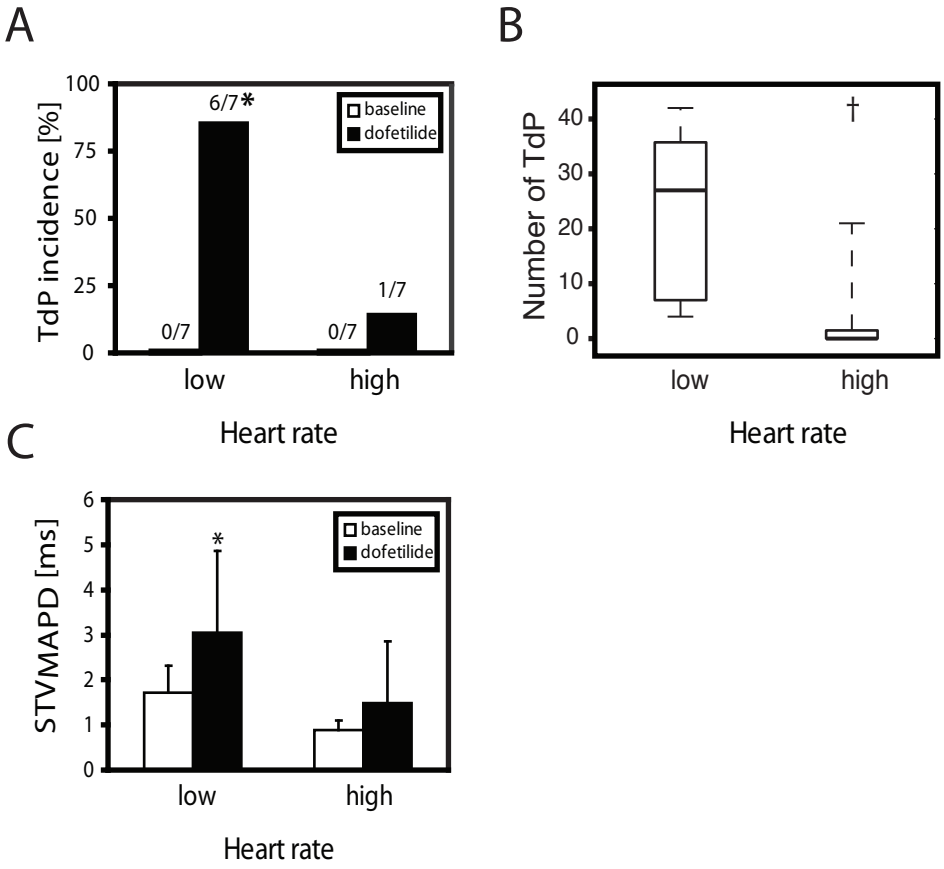



*Figure 4. Incidence of Torsade de Pointes (TdP) and development of short-term variability after administration of dofetilide at 2 different paced heart rates. A: An increase in TdP incidence after dofetilide is seen only at the low pacing frequency. B: Box plot of number of TdP episodes at high and low rate after dofetilide within the 10 minute monitoring period. Horizontal line represents the median, box indicates 25$^{th}$ to 75$^{th}$ interquartile range; whiskers show total range of values. C: Short-term variability of left ventricular monophasic action potential duration ($STV_{MAPD}$) shows a statistically significant increase with dofetilide only at the low pacing rate. *: p<0.05 versus baseline, †: p<0.05 versus low rate.*

In contrast, at the high rate drug induced ectopic activity was seen in only 2 of 7 animals (at 140 and 510 s), while only one animal presented with reproducible TdP episodes (14%, p>0.99 versus baseline; Figure 4a). The increased arrhythmic response to dofetilide at the low rate was not only reflected in TdP incidence (6/7 versus 1/7, p=0.06), but also in the number of TdPs during the first 10 minutes after dofetilide (23±15 versus 3±8, at low and high rates, respectively; p<0.05; Figure 4b). At the low heart rate, the average time from start of dofetilide infusion to the first TdP was 170±36 s, which corresponds to 57±12% of the total dofetilide dose. The one animal that was inducible in both experiments showed its first TdP episode at the same time point, irrespective of pacing rate (135 and 137 s).

## Variability of repolarization reflects repolarization reserve

Analysis of STV of LV MAPD at baseline, collected at identical heart rates in both experiments, showed no differences between the 4 week and 6 week experiments (data not shown). A small but significant effect of heart rate on STV of LV MAPD was detected at baseline (1.7±0.6 at low versus 0.9±0.2 ms at high rate, p<0.05.),
When compared to baseline values, dofetilide induced a significant increase in STV of LV MAPD at the low heart rate (1.7±0.6 to 3.0±1.8 ms, p<0.05; Figure 2, Figure 4c). In a Poincaré plot of LV MAPD this is visible as a widening of the plot perpendicular to the line of identity (Figure 2b). At the high rate, however, STV of LV MAPD during dofetilide remained low (0.9±0.2 to 1.5±1.4ms, NS.; Figure 4c). Thus, in accordance with earlier findings, STV increases after proarrhythmic challenges, before the onset of spontaneous extrasystoles and TdP.

## Variability of repolarization can be determined from EGMs

ARIs before and after dofetilide from the left sub-endocardial EGM were compared to LV MAPD values. Positive T-waves were seen in 3 of 14 LV EGM recordings (1 high and 2 low rate experiments). Linear regression between individual ARI and MAPD values (Figure 5a) shows a close relation, with ARI slightly shorter than MAPD (MAPD=1.01*ARI+19 ms, $R^2$=0.83, p<0.001).
STV of ARI shows similar response to dofetilide as STV of LV MAPD (2.0±0.7 to 3.1±1.7 ms at low rate, 1.1±0.5 to 1.8±1.2 ms at high rate; Figure 2c). However these changes were not significant at either rate, suggesting higher intra-individual variation in STV of ARI. Figure 5b compares STV of ARI and MAPD obtained in individual experiments. Regression analysis reveals a strong similarity between both methods for quantifying repolarization variability ($R^2$= 0.71, p<0.001), especially at the clinically relevant higher values (>2.5 ms) seen before drug-induced TdP.

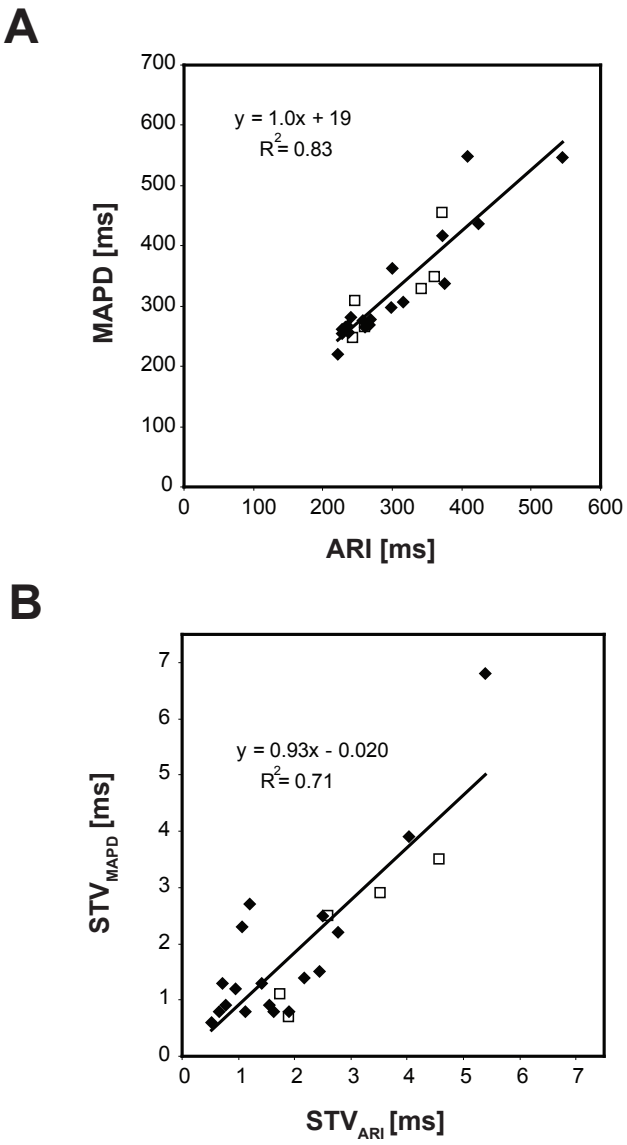



*Figure 5. Relation between LV monophasic action potential duration (MAPD) and activation-recovery interval (ARI). A: Scatter plot of LV MAPD and ARI recorded at baseline and after dofetilide, with the regression line. Positive T-waves on the electrogram are represented by open markers, negative or biphasic T-waves by closed markers. B: Same plot for short-term variability (STV) calculated from LV MAPD and ARI.*

# Discussion

Simulations showed that a sampling frequency of 500 Hz will suffice to capture relevant STV values in this model. We have shown that a transient increase in heart rate is able to almost completely prevent dofetilide-induced TdP arrhythmias. The improved repolarization reserve at the high pacing rate was reflected in lower baseline STV of LV MAPD and absence of a significant increase after dofetilide. Using activation-recovery intervals from chronic sub-endocardial EGM, we were able to derive similar STV values from ARI as were calculated from LV MAPD.

## Effect of heart rate on proarrhythmia

At the low heart rate, a significant number of animals showed reproducible TdP after dofetilide. In this study, TdP incidence was 86%, which is comparable to results from previous experiments performed at spontaneous idioventricular rhythm.[13] At the high rate, TdP incidence was reduced to only 14% (1/7), demonstrating a strong protective effect of elevated heart rate. Induction of TdP is further dependent on the administered dose of $I_{Kr}$ block,[11] hence it is central to note that dofetilide infusion was discontinued at the onset of the first TdP. Consequently, in experiments addressing the low heart rate, in 5 animals the dofetilide infusion was prematurely stopped at an average dose of 59% due to TdP. In contrast, at the high rate, these 5 animals were able to withstand the full dose of dofetilide, which illustrates the magnitude of increase in repolarization reserve.

Temporary overdrive pacing has been used clinically to prevent polymorphic tachycardia in congenital and acquired LQTS, but controversies remain about the mechanism by which it induces its protective effect.[2-4, 6, 21] TdP is often preceded by a long-short sequence: a long coupled beat, mostly resulting from a ventricular extrasystole, is followed by another short coupled extrasystolic beat that initiates the TdP arrhythmia.[4, 22] The same pattern of RR interval changes is seen preceding other ventricular tachycardia as well.[23] To explain the anti-arrhythmic effect of overdrive pacing it was hypothesized that preventing these sudden rate changes by cardiac pacing would prevent initiation of tachycardia. Special rate smoothing algorithms were developed for use in ICDs that did show promising results in congenital LQTS patients.[21] In large prospective studies in general ICD populations, these algorithms were effective in preventing sudden rate changes; however the expected reduction in cardiac arrhythmias was not established or only observed in a small subset of patients.[24-26] A more direct effect of high-rate pacing is shortening of QT interval and reduction in dispersion of repolarization, which one can imagine could be anti-arrhythmic in both congenital and acquired LQTS.[27] Reports of clinical applications

of rapid pacing in LQTS are limited to case reports and small retrospective studies, possibly because of the expected detrimental effect of a prolonged increase in heart rate.[6] Several publications report patients requiring pacing rates well above 70 bpm, sometimes up to 140 bpm, suggesting a mechanism that goes beyond stabilization of RR intervals.[3, 4, 28, 29] In the current serial study, we compared two paced rhythms, while all pause-promoting features of the pacemakers were turned off.[3] The difference in arrhythmogenic response to dofetilide is striking and can only be attributed to the difference in heart rate. All other parameters were kept constant, including activation pattern and degree of cardiac remodeling. Furthermore, as a result of the ventricular pacing, heart-rate variability was absent in all experiments. Rapid pacing proves to be an effective therapy for prevention of ventricular tachycardia, but continuous feedback on arrhythmic risk is required to determine when this protective effect outweighs the drawbacks of the elevated heart rate.



## STV at different heart rates

In our studies in the CAVB dog, elevated STV values are closely linked to a reduced repolarization reserve and pro-arrhythmia.[11, 13, 30] Even in the absence of drugs, a small but significant decrease of STV of LV MAPD at the high rate provides feedback that repolarization reserve has improved. At the low rate, dofetilide decreases repolarization reserve to critical values, reflected in a substantial increase in STV prior to the development of extrasystolic activity that often precedes TdP (Figure 4). In contrast, at the high rate, dofetilide does not significantly change STV of LV MAPD, suggesting that the improved repolarization reserve is able to protect the heart from arrhythmia.

A second, indirect, way to quantify repolarization reserve is by using the relative increase in repolarization parameters induced by a challenge:[31] The lower the initial reserve, the larger the increase. The repolarization times summarized in table 1, suggest that dofetilide delays repolarization around 15-19% at the high pacing rate, compared to 20-34% at the low rate. The magnitude of this reverse-use dependence is a function of the residual repolarization reserve.

Prolonged repolarization by itself is a known risk marker for TdP.[1] However, in our hands in the CAVB dog, STV of LV MAPD has still higher predictive value than QT prolongation or MAPD alone.[11-13] In a recent publication by Hinterseer et al,[9] STV from QT interval outperformed $QT_C$ interval in identifying patients susceptible to drug-induced arrhythmia. Furthermore, in our hands the increase in STV can precede first ectopic activity by minutes, allowing more time for preventive interventions.[11] STV in the low-rate experiments was similar to previously reported values in CAVB dogs with spontaneous idioventricular rhythm, both at baseline and after dofetilide

administration. This confirms results of previous publications that STV of LV MAPD is not dependent on beat-to-beat changes in RR interval or variation in activation pattern, often seen in this model at non-paced rhythms.[13, 14]

## STV determined from electrograms

Clinical use of MAPs is limited by the fact that MAP catheters can only be used in an acute setting under anesthesia. Chronic EGMs, on the other hand, might provide similar electrophysiological information, while being constant in signal quality and position for years. Comparing ARIs from electrograms with MAPD collected at the same time from approximately the same location, we found a close match between MAPD and ARI, both in absolute value as well as in STV (Figure 2c, Figure 5).

A limitation of the use of EGM in this study is the change in signal morphology seen after dofetilide, which will directly influence the quality of the ARI measurement, and thereby STV of ARI. In some cases intermittent interference by P-waves hampered ARI measurement. However, frequent overlap of P-waves with the moment of ventricular repolarization is typical for the AV-block dog and not very common in the clinical settings.

Recent publications have highlighted the diagnostic value of device recorded EGM: Swerdlow et al. detected high amplitude T-wave alternans from ICD stored EGM recorded just before the start of ventricular tachycardia.[32] Furthermore, Tereshchenko et al. recently proposed baseline QT variability index derived from device based right ventricular EGM as a long-term predictor of appropriate ICD therapy.[33] Continuous monitoring of measures of repolarization variability in implantable devices has the potential to greatly improve management of arrhythmic risk.

## Clinical implications

Implantable devices are becoming increasingly important for patient management, as the amount of collected diagnostic information increases. Additionally, with the introduction of home monitoring systems physicians have daily access to device data.[34] We have shown that recording STV is within the technical capabilities of modern devices. Furthermore, a temporary increase in pacing rate after detection of a transient increase in arrhythmic risk might help prevent extrasystolic beats and ventricular tachycardia, with only minimal detrimental remodeling.

### Study limitations

The current study is limited by the fact that bradycardia is part of this arrhythmogenic model. Therefore, it is not possible to predict whether this protective effect of heart rate increase can be extended to conditions of normal sinus rhythm. However, acquired LQTS patients often need faster than normal pacing rates for effective tachycardia prevention.[3, 29]

Secondly, due to the high arrhythmia incidence at the low rate and the strong preventive effect at the high rate, we were not able to separate the effects of heart rate and arrhythmic risk on STV. On the other hand, studies in animals[35] and patients[9, 10] have confirmed the diagnostic value of STV at more physiological heart rates.



## Conclusions

An increase in heart rate, without changing variability in RR interval or activation pattern, can increase repolarization reserve and prevent drug-induced TdP arrhythmia in anesthetized CAVB dogs. This anti-arrhythmic effect is associated with decreased values of STV. STV from chronic electrograms, although not as robust as from MAP recordings, may be a valuable tool for monitoring arrhythmic risk in implantable devices.

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## Chapter 4

# Anesthesia and arrhythmogenesis in the chronic AV-block dog model

A. Dunnink *, S. Sharif *, P. Oosterhoff *[$], S. Winckels, MD, D. Montagne, H. Beekman, R. van der Nagel, M.A.G. van der Heyden, M.A. Vos

Medical Physiology, Utrecht, and [$]Medtronic Bakken Research Center, Maastricht, the Netherlands.

* these authors contributed equally

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## Abstract

Background: Drug induced Torsade de Pointes (TdP) arrhythmias can readily be induced in anesthetized dogs with remodeled hearts (chronic AV-block dog: CAVB). Similar studies in conscious CAVB dogs reveal lower TdP incidences. Regulations forced us to reconsider our anesthetic regimen, which consisted of pentobarbital followed by halothane (P+H). We investigated the relevance of anesthesia for this enhanced susceptibility (part 1) and compared 3 anesthetic regimens (part 2).
Methods: Part 1. 10 CAVB dogs paced from the high septum at 1000 ms were challenged with dofetilide (25 µg/kg/5') twice: once under anesthesia and once awake. Anesthesia consisted of P+H (n=5) and thiopental maintained by isoflurane (T+I). Part 2. In CAVB dogs (n=6) with spontaneous idioventricular rhythm (IVR), the electrophysiological and arrhythmogenic consequences of different anesthetic regimens (P+H, T+I, and P+I) were serially compared.
Results: Part 1: In paced dogs, dofetilide-induced TdP was higher under anesthetized than in conscious circumstances, with the more severe outcome seen after T+I as compared to P+H or control (2x): 5/5, 2/5, 0/5, 0/5, $p<0.05$. Part 2: Electrophysiologically, T accelerated IVR, increased $QT_C$, and transiently induced polymorphic VTs in 2/6 dogs. This was not seen after P. At 120 min (end of the preparation), $QT_C$ increase was highest after T+I, intermediate with P+I and the smallest after P+H. Dofetilide in combination with T+I induced the most severe arrhythmogenic outcome.
Conclusion: Thiopental anesthesia causes arrhythmias sec whereas anesthesia in general predisposes for drug induced TdP in the CAVB dog. In combination with dofetilide, T+I has a more proarrhythmic outcome than P+I or P+H.


## Introduction

Drug induced Torsade de Pointes arrhythmias (TdP) are a feared adverse effect and because of the very low clinical incidence a real challenge to be early identified by safety pharmacology experts.[1,2] One of the animal models in use to test for pro-arrhythmic properties of drugs is the dog with chronic, complete atrio-ventricular block (CAVB).[3-5] Due to AV-nodal ablation, there is a necessity for an alternative pacemaker to generate the ventricular rate (idioventricular rhythm, IVR). The location of the pacemaker is unpredictable but the discharge of IVR is much slower as compared to sinus rhythm. To overcome the acute drop in cardiac output, the dogs develop ventricular remodeling,[5] that is present at the mechanical, electrical and structural level. In the majority of the animals, this ventricular remodeling creates a new steady state of compensated hypertrophy. A detrimental adaptation, however,

is the cardiac predisposition for drug induced TdP: the incidence increases from non-inducible (0%) in the unremodeled, anesthetized control (acute AVB) animal up to 70-80% in the CAVB dogs after administering specific class III anti-arrhythmic drugs, like dofetilide or d-sotalol.[5] Whereas intravenous administration of drugs is most frequently performed in anesthetized circumstances, oral prescriptions have most often been reported using awake CAVB animals.[4] In these conscious conditions, TdP incidence at comparable drug plasma concentrations seems much lower,[4-5] indicating that anesthesia may be an essential element for the creation of the pro-arrhythmogenic situation. In addition, the type of the anesthesia seems relevant. In canine hearts, it has been suggested that pentobarbital has a very low or even no pro-arrhythmogenic potential whereas thiopental, halothane and isoflurane, alone or in combination, are much more arrhythmogenic.[6-9] Forced by government regulations, our anesthetic regimen pentobarbital-halothane (P+H) has to be changed, preferably combining thiopental with isoflurane (T+I).



In this study, both aspects: being relevance and type of anesthesia were investigated in baseline and after dofetilide in dogs with remodeled hearts. To control rate changes due to dofetilide (and anesthesia), the recently described high septal paced CAVB dog was investigated first.[10] In the second part, the electrophysiological and arrhythmogenic consequences of the individual as well as the combined components of the anesthetic regimen were evaluated in regular, non-paced, CAVB animals.

## Methods

Animal handling was in accordance with the European Directive for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (86/609/EU) and the Dutch Law on Animal Experimentation. The Committee for Experiments on Animals of Utrecht University approved the experiments.

*Preparation:* In a preliminary experiment, adult (approximately 1.5 years) purpose bred dogs (20-25 kg, Marshall, North Rose, New York) of either sex received pre-medication consisting of methadone -vetranquil - atropine (10/10/0.5 mg i.m.). After 30 minutes, general anesthesia was induced with sodium pentobarbital (P: 25 mg/kg i.v.) and maintained by halothane (H:0.5% in $O_2/N_2O$ (1:2)). Six standard and four precordial ECG leads were continuously registered and stored on a hard disk. Complete AV-block was induced by applying radiofrequent currents to the proximal His bundle. In a subset of dogs, the thorax was opened through the fourth or fifth intercostal space. In these animals an electrogram (EGM) lead was screwed in the apex of the left ventricle (LV) to the subendocardium (BRC Medtronic, Maastricht, Netherlands) and tunneled to the neck. Also a screw-in pacing lead was inserted through a right-sided purse-string atriotomy into the interventricular septum, near

the His bundle and slightly distal to the ablation site.[10] An atrial pacing lead was placed in the auricle of the right atrium. The His-bundle lead and the atrial lead were connected to a DDDR pacemaker (Vitatron, Arnhem, Netherlands). The dogs were given a two-week recovery period from the open thorax surgery. During this period the dogs were paced at VDD mode (paced on atrial activation), effectively reversing the AV-nodal ablation. Pacemaker functioning was checked three times per week. After these two weeks, the pacemaker was programmed to VVI mode at the lowest captured ventricular rate, mean 52 ± 7 bpm in conscious state. Both in the paced (part #1) as in the regular, non-paced dogs, the heart was allowed to remodel during a period of ≥ 4 weeks.[5]

*Experiments part 1:* anesthetized versus conscious conditions: Ten paced dogs were tested twice (awake vs. anesthetized) at 1000 ms during the experiment in a random cross over design with a recovery period of 2 weeks in between. Baseline electrophysiological parameters were recorded for a period of 10 minutes before the specific $I_{kr}$-blocker dofetilide (25 µg/kg/5 min i.v.) was given to determine TdP susceptibility (see further). Ten ECG leads and the EGM recording were saved. During the anesthetized experiments, also 2 endocardial LV and right (RV) monophasic action potentials catheters (MAP) were placed against the endocardium of the free walls and stored. Five dogs received P+H (see above), while the other 5 animals received thiopental (15 mg/kg i.v.) and isoflurane (1.5%) for maintenance. When the dog showed the first TdP within 5 minutes of the injection, dofetilide was stopped.

*Experiments part 2:* Baseline comparison of the individual effects of anesthesia: In 6 regular CAVB dogs at IVR, we serially tested (3 experiments) the electrophysiological and arrhythmogenic consequences of the individual components of anesthesia. First, the effect of premedication on $QT_C$ was investigated (from 0 to 30 min). Secondly, T was compared to P alone (at t=35 min), and thirdly the combinations with the volatiles H and I were evaluated, as T+I, P+H and P+I (t=45 and 120 min after start of premedication). T+H was not investigated because governmental ruling forbids the use of halothane any further.

Finally, the dofetilide-induced arrhythmogenic outcome of the 3 anesthetized combinations was quantified retrospectively using 75 experiments in 60 regular CAVB dogs.

*Arrhythmia quantification:* A TdP was defined as a polymorphic ventricular tachycardia with typical twisting around the iso-electric line of more than 5 beats.[5] Inducibility was defined as a dog showing 3 or more TdP`s within 10 minutes after injection of dofetilide. If normal rhythm was not restored within 15 seconds of TdP, the dog was

electrically defibrillated. Number of TdPs, single and multiple ectopic beats (sEB, mEB), were quantified during 10 minutes comparing baseline with dofetilide.[5]

*Data analysis:* Applying a custom-made computer program (ECGview), QT interval (lead II), was measured offline at a resolution of 2 ms, from 5 consecutive beats. Durations of the MAP to 90% repolarization ($MAPD_{90}$) and the activation recovery interval (ARI)[11] were determined semi-automatically (ECG-auto, EMKA technologies, France) from 30 consecutive beats. Parameters were measured at baseline (t=0) and at specific moments after dofetilide: The first time point was directly prior to the first ectopic beat. If there was no ectopic activity, parameters were measured at 5 minutes after dofetilide injection. Beat-to-beat variability (BVR) was quantified as short-term variability (STV) describing the orthogonal distance to the line of identity of a Poincaré plot, $STV = \sum_{1..30} |D_n - D_{n-1}| / [30*\sqrt{2}]$, where D represents LV MAPD or duration of the activation recovery interval from the LV EGM.[12]

*Statistical analysis:* All data are presented as mean ± SD. Statistical significance of differences was evaluated by paired and unpaired two-sided Student t-test or a 2-way ANOVA analysis followed by post-hoc Bonferroni test. For paired inducibility a McNemar's and for unpaired a Chi-square test was used, while sEBs and mEBs were evaluated with a rank-sum test or Wilcoxon test. Regression analysis was used for correlation. One-way ANOVA followed by Bonferroni t-test was used for temporal measurements. A p-value $<0.05$ was considered statistically significant (Sigmastat version 3.11, Systat Software Inc.).



# Results

## Part 1: anesthetized versus conscious conditions

With the exception of one dog that showed 2 TdP after the full dose of dofetilide, no TdP arrhythmias were seen in the conscious dogs (inducibility 0/10). This in contrast to anaesthetized conditions in which reproducible TdP ($\geq$ 3 times) was seen in the majority of the dogs: 5/5 for T+I and 2/5 for P+H) . A representative example for the two experiments in a single dog using T+I is given in figure 1. Besides inducibility, pro-arrhythmic events after dofetilide were also quantified as number of TdP, sEBs and mEBs over a 10 min period, and duration of TdP (Table 1). Although all arrhythmic parameters were higher during anesthesia, only TdP incidence and number of TdP demonstrated significance. Also in this quantification, T+I showed a higher number of TdP than P+H (24±17 vs. 3.4±8).

Our anesthetic regimen predisposed by weakening repolarization strength: the QT interval was increased from 281±31 ms to 390±71 ms ($p<0.05$, Table 1 and Figure 2). After dofetilide, these values increase significantly to 329±52 ms and 532±93 ms

respectively ($p<0.05$), with the highest relative QT time increase in anesthetized conditions (17±8 vs. 36±24%, $p<0.05$). A more accentuated response was also obtained with $STV_{LV\ EGM}$ that showed no increase in conscious circumstances (Table 1), whereas a significant increase was seen when dofetilide caused TdP in anesthetized conditions. $STV_{LV\ EGM}$ correlated with $STV_{LV\ MAPD}$: $r=0.66$, $p<0.05$.

## Part 2: Electrocardiographic and arrhythmogenic consequences with the different components of anesthesia

Thirty minutes after the start of premedication, $QT_C$ increased from 278±15 (awake) to 314±20 ms, $p<0.05$. No arrhythmias were seen.

Directly after administration, thiopental transiently resulted in reproducible ventricular arrhythmias in 2/6 animals (TdP, Figure 3). This was never seen after pentobarbital, which was tested twice in the same dogs. The TdP occurrence was associated with a stronger effect of T on $QT_C$ compared to P (Figure 4, t=35 min). In fact, thiopental shortened RR (from 1390±144 to 955±50 ms, $p<0.05$) without sufficient shortening of the QT ($QT_C$: 449±26 ms, example in figure 3). The addition of isoflurane to T kept the RR and $QT_C$ at the same level for the 85 min observation period. The $QT_C$ increased after P+I (becoming significant at t=120 min, $QT_C$: 393+23 ms, Figure 4). Adding halothane to P (P+H) did not change the $QT_C$ (357±17 ms), leading to the rank order of $QT_C$ at t=120 min of: T+I > P+I > P+H, $p<0.05$).

In the retrospective study performed in regular CAVB dogs, TdP incidence (Figure 5a) was very high >80% and differed not between the anesthetic regimens, although the highest incidence was seen with T+I (94%). TdP occurred more frequently (19±14) after dofetilide in T+I than with the combinations of P (P+I: 7±6 and P+H 8±7 #TdP/10 min, Figure 5c) and this arrhythmogenic outcome required a smaller dose of dofetilide (Figure 5b) and therefore was faster in time: the first ectopic beat (Figure 5d) occurred earlier with T+I (2.3 min). P+I did not differ from P+H in any of the comparisons.

In the 16 experiments performed with T, one animal reproducibly showed TdP in baseline and was therefore excluded from the dofetilide test.

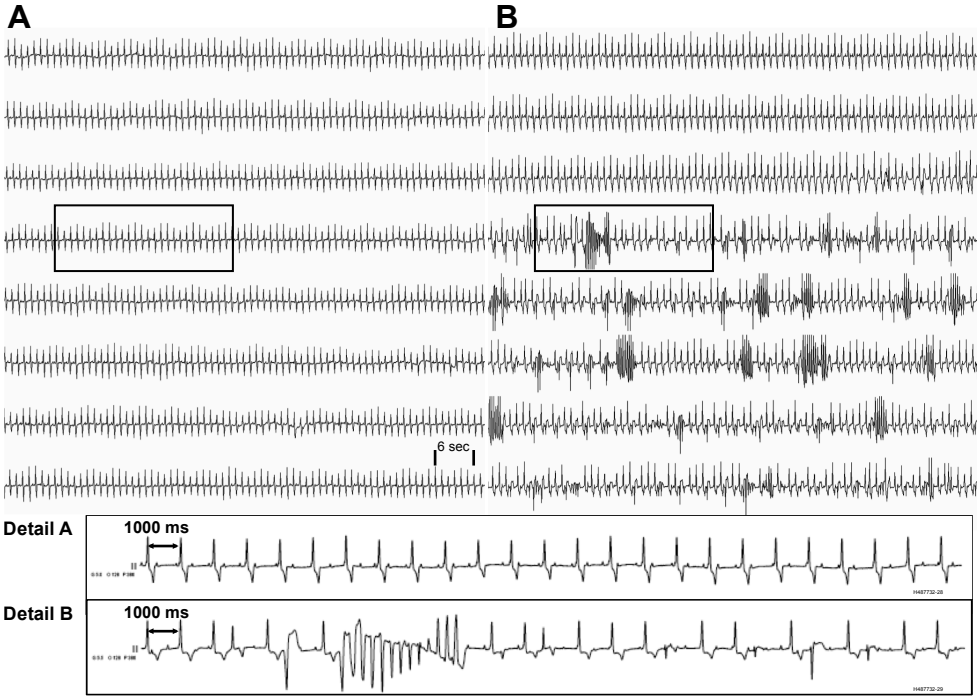


*Figure 1. Two 10 min ECG (lead II) recordings are shown in awake (A) and anesthetized circumstances (B) in a single dog paced from the high septum at 1000 ms. Dofetilide was started at the top of each figure. Please note that only under T+I anesthesia the dog responded with single and multiple ectopic beats, and repeated self-terminating TdP. This is better illustrated in the two enlargements below the continuous ECG tracing which were taken from the boxes.*

*Table 1: A comparison between awake and anesthetized experiments in the presence and absence of dofetilide.*

| | Conscious | | Anesthetized | |
|---|---|---|---|---|
| | **Baseline** | **Dofetilide** | **Baseline** | **Dofetilide** |
| **QT (ms)** | *281 ± 31* | *329 ± 52¶* | *390 ± 71** | *532 ± 93§†* |
| **LV MAPD (ms)** | - | - | *304 ± 45* | *371 ± 38†* |
| **STV LV MAPD (ms)** | - | - | *1.5 ± 0.6* | *2.5 ± 0.8†* |
| **STV EGM (ms)** | *1.1 ± 0.4* | *1.2 ± 0.3* | *1.6 ± 0.5* | *2.6 ± 0.7§†* |
| **TdP-incidence** | *0/10* | *0/10* | *0/10* | *7/10§†* |
| **Single Ectopic Beats** | *0* | *19.1 ± 36.9* | *0.9+1.7* | *48.8 ± 45.8* |
| **Multiple Ectopic Beats** | *0* | *4.9 ± 10.3* | *0* | *22.7 ± 27.9* |
| **Number of TdP`s** | *0* | *0.2 ± 0.6* | *0* | *12.7 ± 15.8§* |
| **TdP-duration (s)** | *0* | *1.8 ± 0.6* | *0* | *4.1 ± 2.3* |

**: p<0.05, Anesthesia vs. Conscious at baseline. §: p<0.05, Anesthesia vs. Conscious after dofetilide. †: p<0.05, Anesthesia after dofetilide vs. Anesthesia at baseline. ¶: p<0.05, Conscious after dofetilide vs. Conscious at baseline.*

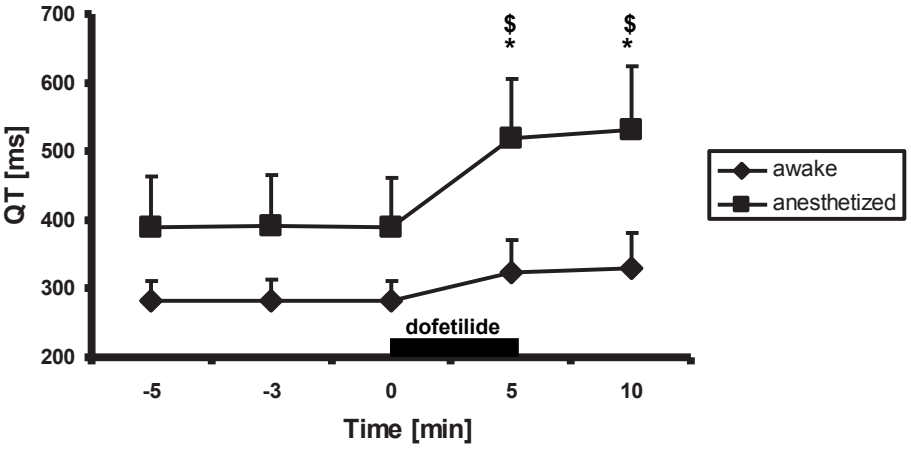


*Figure 2. The mean QT-time (y-axis) is shown at different time points: awake (lower line) and under anesthetized (upper line) conditions in the absence (left) and presence of dofetilide infusion (black line). The 10 dogs were tested serially and paced at 1000 ms. Anesthesia prolongs QT-time (* p< 0.05 vs. conscious). Dofetilide increases QT-duration significantly ($ p< 0.05 vs. baseline) under anesthesia.*

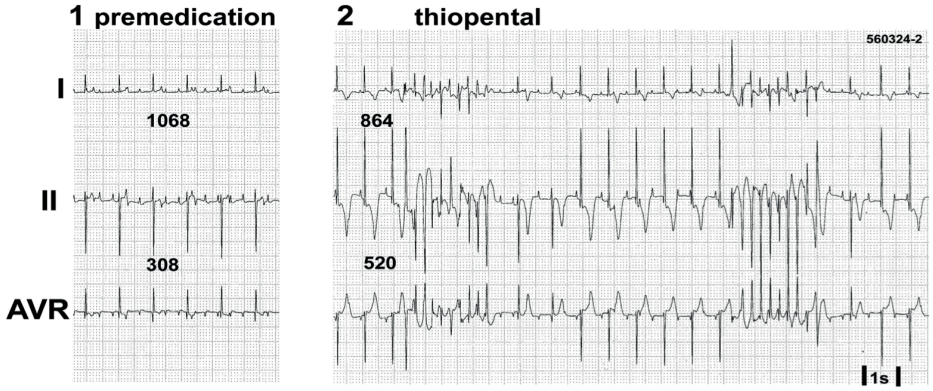


*Figure 3. Occurrence of TdP arrhythmias directly after thiopental.*
*Three ECG leads (I, II, AVR, paper speed 10 mm/s) are shown, representing premedication (left) and after thiopental (right). RR and QT are provided (in ms). Besides causing an increase in heart rate and QT-time, thiopental also reproducibly induced short lasting runs of TdP within 3 minutes after infusion. This was never observed with pentobarbital.*

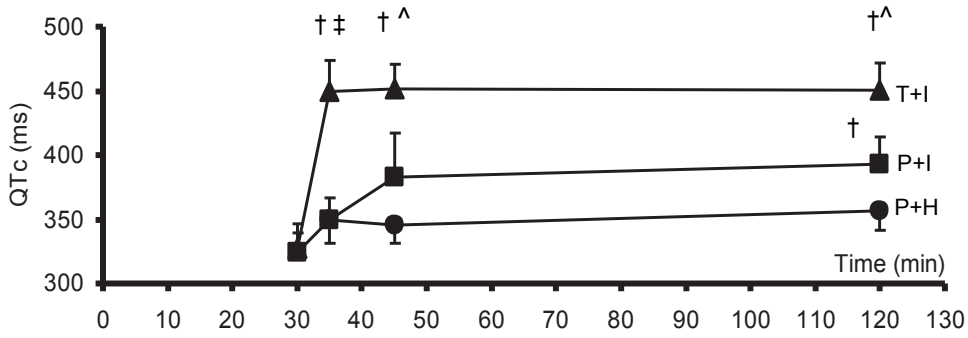


*Figure 4. Temporal electrophysiological effects of the 3 anesthetics combinations.*
*$QT_C$ changes at spontaneous idioventricular rhythm (ndogs=6) are depicted in time (min) for the 3 anesthesia combinations (T+I, triangles; P+I, squares; P+H, circles). t=0 is injection of premedication. t=30 reflects the time point at which either pentobarbital or thiopental are infused. At t=35, the volatile addition is started and quantified at t=45 and t=120. The latter is the end of the preparation.*
*^, p<0.05 vs P+H; †, p<0.05 vs premedication (t=30); ‡, p<0.05 vs pentobarbital.*



## Discussion

Thiopental anaesthesia causes arrhythmias sec whereas anaesthesia in general predisposes for drug-induced TdP in the CAVB dog. In combination with dofetilide, T+I has a more severe proarrhythmic outcome that occurs faster than with P+I or P+H,

### CAVB and high-septal paced model

By creating AV-block in the dog, the heart becomes bradycardic, the ventricular activation changes importantly and the AV-synchrony disappears. The location of the ventricular pacemaker is unpredictable and therefore differs between dogs. In addition, the pacemaker site may shift due to changes in autonomic tone that modify rate. In acute circumstances directly after AVB, this results in a decreased cardiac output, that normalizes (compensation) in time reaching steady state values around 4-6 weeks.
The CAVB dog model has been characterized extensively to elucidate the molecular and cellular basis underlying ventricular remodeling and susceptibility for ventricular arrhythmias.[5] Electrical adaptations consist of downregulation of major repolarizing ion-currents, including the rapid and slow component of the delayed

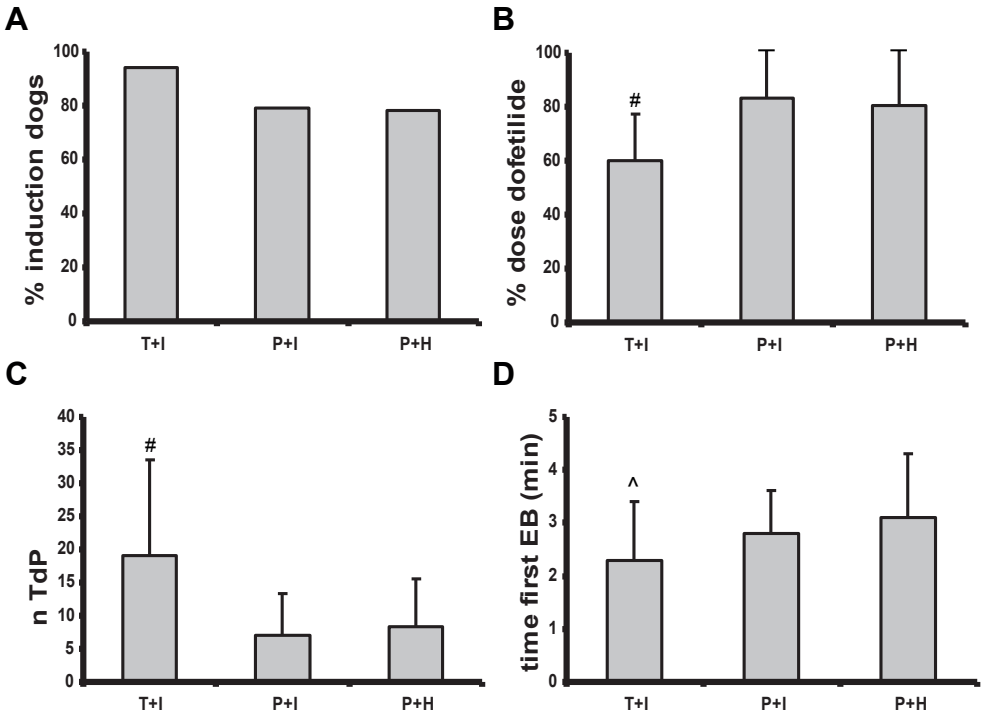


*Figure 5. Arrhythmogenic comparison of the 3 different combinations of anesthesia.*
*Three anesthesia regimens (T+I, P+I, and P+H) were compared in relation to %TdP induction (A), dose of dofetilide (B), number of TdP (C), and time to first Ectopic Beat (EB, panel D). T+I was faster, required less dofetilide (B) to become arrhythmogenic, measured as time to first EB (D) or time to TdP (not shown), and induced more TdP occurrences (C) within the 10 min counting period. There was no difference between P+I versus P+H. # p<0.05 vs. P+I and P+H, ^ p<0.05 vs. P+H.*

rectifier ($I_{Kr}$ and $I_{Ks}$, respectively). Upregulation is seen for the sodium-calcium exchange current, both in forward as reverse mode. These adaptations underlie the lengthening of the cellular and monophasic APD and the QT-interval. Moreover, because of regional differences in remodeling, there is accentuation of spatial dispersion of repolarization. Finally, the compromised control over repolarization is also seen in an increase in the baseline $STV_{LV\ MAPD}$ (temporal dispersion) which is clearly dependent on the arrhythmogenic phenotype: the highest values are seen in CAVB dogs that die suddenly.[13]

Recently, we have adapted the model to gain control over the ventricular activation sequence.[10] It was demonstrated that maintaining a normal high-septal initiated activation by pacing, decreased the severity of electrical remodeling, thereby reducing the pro-arrhythmic outcome: with P+H, TdP inducibility decreased from 7/9 to 4/9.[10] Because anesthesia slows the heart rate, and the focus of activation during IVR is not controlled; we decided to use this dog model with constant pacing

at 1000 ms (part 1) to have the opportunity to investigate the relevance of anesthesia under comparable conditions in part #1.

## Relevance of anesthesia

The fact that anesthesia plays an important role in the sensitivity of the CAVB dog model for drug induced TdP can be deduced from scarce literature comparing results between three labs: In awake conditions, it was shown that sotalol i.v. (4.5 mg/kg) induced TdP in 27% (3/11)[14] and in 25% (1/4) with 3 mg/kg d-sotalol p.o.[4] Under P+H anesthesia, 4 mg/kg i.v. d-sotalol induced TdP repetitively in 75% (6/8) of the CAVB dogs.[12] A similar high TdP incidence (4/4) was seen after 30 mg/kg sotalol p.o.[4] However, to the best of our knowledge, the relevance of anesthesia for drug induced TdP has never been directly compared.

Our results indicate that anesthesia has a dominant role by predisposing the CAVB animals for drug-induced TdP. Consciously, dofetilide did not induce TdP, whereas the majority of animals responded with TdP under anesthesia. When TdP did occur, the dosage of dofetilide was stopped. Therefore, in awake or P+H/P+I anesthesia, the dogs were resistant even with higher concentrations of dofetilide administered. Looking at the electrophysiological parameters, this predisposition can only be visualized by QT-times (Table 1). Anesthesia in general increases repolarization times considerably (Figure 2), suggesting that repolarization reserve is diminished thereby making the animal more vulnerable to other challenges.



## Type of anesthesia

During the last decades, hundreds of anesthetized CAVB dogs in IVR have been performed in our laboratory to screen drugs for proarrhythmic properties, using an anesthetic regimen which consisted of premedication (combination of methadone, acepromazine, and atropine), followed by pentobarbital, and eventually halothane (P+H).[3,5,10,12] New regulations forced us to consider replacing halothane with isoflurane and pentobarbital with thiopental. Their exact contribution for TdP risk assessment is not known, although there are literature data that indicate that thiopental, isoflurane and halothane are more arrhythmic than pentobarbital.[6-9]

When pro-arrhythmic drugs[6,8,9] are considered, confounding parameters were present prohibiting the effects of individual components: thiopental was always accompanied by inhalation anesthetics like isoflurane[9] and halothane[6] to maintain the anesthetic state, or preceded by propofol[8] to induce anesthesia. It is known that these drugs also possess electrophysiological effects (see further), thereby possibly influencing the outcome. Thus, our goal was to compare specific anesthetic regimens in the absence and presence of the repolarization prolonging drug dofetilide.

In this canine model with electrical remodeling, it is clear that thiopental administration is arrhythmogenic by itself (Figure 3). This observation in baseline confirms the data of an earlier study,[7] that stated that this anesthetic is very dangerous. In addition, T lengthens $QT_C$ most profoundly while it accelerates the heart rate. Pentobarbital shows no arrhythmias, while it prolongs $QT_C$ slightly (Figure 4).
When looking at the anesthetic regimens tested, the addition of I to T maintains the increased $QT_C$-times. This $QT_C$ lengthening was larger than the combinations with pentobarbital. P+I had stronger effects on $QT_C$ than P+H. Moreover, dofetilide accentuated this predisposition between T and P further: more arrhythmias that appeared earlier were seen after T+I, whereas there was no arrhythmic difference between P+H and P+I. Therefore, we will replace P+H for P+I in future experiments. The differences in TdP inducibility and in the speed of occurrence can be confounding when electrophysiological comparisons of the different anesthetic regimens are performed. This has to be taken into account for a proper analysis.

*Table 2: Blocking of ion currents. for 4 types of anesthesia.*

| | Ionic currents | | | | | | |
|---|---|---|---|---|---|---|---|
| **Anesthesia** | $I_K$ | $I_{Kr}$ | $I_{Ks}$ | $I_{to}$ | $I_{K1}$ | $I_{CaL}$ | $I_{Na}$ |
| **Pentobarbital** | | | ++[15] | | ++[15] | | +[16] |
| | | | | | | | *-10%* |
| **Thiopental** | +[17] | =[17] | ++[17] | =[19] | ++[18] | +[18-19] | |
| | *-20%* | | | | | *-10%* | |
| **Halothane** | | +[21] | ++[20] | +[24] | =[24] | +[22-24] | +[23] |
| | | *-14%* | | *-20%* | | *<40%* | *<24%* |
| **Isoflurane** | | +[25] | ++[25] | +[24] | =[24] | +[22-24] | +[23] |
| | | *-20%* | | *-25%* | | *<20%* | *<10%* |

*The electrophysiological effects of 4 anesthetics on cardiac potassium, calcium and sodium currents are summarized using data from the literature. The former have been divided in the delayed rectifier (IK), the transient outward current (Ito) and the inward rectifying current (IK1). In addition, IK is further divided in the rapid and slow component IKr and IKs, respectively. ++: strong blocking effect: an IC50 has been reported in the clinical range of the anesthethic; +: minor blocking effect: some block (relative value included) has been reported in the clinical range; = no blocking effect reported.*

## Electrophysiological actions of anesthetic regimens

It is known that single administration of all anesthetics used in this study will prolong repolarization seen as lengthening of cellular APD, MAPD or QT-time. In table 2, we have summarized the electrophysiological actions of the different anesthetics used on specific ion currents.[15-25] Unfortunately, not all information is available nor are their results concerning their combined use. It can be seen that all anesthetics block repolarizing currents at dosages that are relevant for their clinical use. Especially, $I_{Ks}$ is blocked by all anesthetics, whereas differential effects are seen for $I_{Kr}$, the inward rectifier ($I_{K1}$) and the transient outward current ($I_{to}$). This lengthening in repolarization time may be prevented when the drug is additionally blocking inward currents, either the L-type calcium current ($I_{CaL}$) or the sodium current ($I_{Na}$). In this way, a protective or even an anti-arrhythmic effect can be achieved. Protection is defined as lack of pro-arrhythmic effects despite block of outward currents, whereas some drugs even suppress or prevent TdP arrhythmias because of a more dominant role of the block of the inward currents. Example is the $I_{CaL}$-blocker verapamil that also is a potent $I_{Kr}$-blocker. However, no TdP arrhythmias have been reported, it is even known that verapamil is anti-arrhythmic against arrhythmias of the long QT syndrome.[26,27]

Most anesthetics do block inward currents (Table 2), although the potency is rather low and by that protection or even anti-arrhythmic actions may be dismissed. When looking at table 2, it is not evident why pentobarbital is less and thiopental more arrhythmogenic. However, this overview is limited to a selection of ion channels and does not take other relevant modulators of repolarization into account, like differential effects on autonomic nervous system, or cell-cell communication and its effect on cardiac conduction.

## Surrogate parameters for arrhythmic events

$QT_C$-time has been criticized as a prognostic marker for pro-arrhythmic events.[2] However, in this study QT-durations had a prognostic value: both the absolute value reached as well as the relative increase were higher under T+I anesthesia. Among the alternatives that have been suggested is beat-to-beat variability of repolarization. In this study, we replaced part of the BVR quantification from the $STV_{LV\ MAPD}$ with $STV_{LV\ EGM}$ in order to have information under conscious conditions. Baseline STV did not predict a lower repolarization reserve in anesthetized conditions in general. With dofetilide, there was an increase in STV only in anesthetized conditions, where arrhythmic events occurred.

# Conclusions

In the CAVB dog model, 1) thiopental anaesthesia causes arrhythmias in baseline, 2) anesthesia is an essential part of the enhanced susceptibility for drug induced TdP, and 3) T+I is the most "aggressive", both in time as in severity.

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## Chapter 5

# Short-term variability of repolarization predicts ventricular tachycardia and sudden cardiac death in patients with structural heart disease: a comparison to QT variability index

Peter Oosterhoff [*†], Larisa G Tereshchenko [‡], Marcel AG van der Heyden [†], Raja N Ghanem [§], Barry J Fetics [‡], Ronald D Berger [‡], Marc A Vos [†]

* Medtronic Bakken Research Center, Maastricht, The Netherlands, † Medical Physiology, University Medical Center Utrecht, The Netherlands, ‡ Division of Cardiology, Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, USA, § Medtronic Inc. Minneapolis, Minnesota, USA

*Submitted*

## Abstract

*Background:* Monitoring arrhythmic risk may improve management of patients with implantable cardioverter defibrillators (ICD) and prevent ICD shocks. Changes in repolarization duration between subsequent beats quantified as short-term variability (STV) is associated with ventricular arrhythmias in several animal models.

*Objective:* We evaluated STV of QT from right ventricular intracardiac ICD electrograms in patients with structural heart disease and compared its predictive value to QT variability index (QTVI).

*Methods:* In 233 patients, QT and RR intervals were determined from 60 beats. STV was calculated for QT and RR intervals ($STV_{QT}$, $STV_{RR}$) as $\Sigma_{1..60} |D_n - D_{n-1}| / (60*\sqrt{2})$, and $STV_{Ratio}$ as $STV_{QT}/STV_{RR}$. QTVI was derived from mean and standard deviation of QT and heart rate. Follow-up duration was 26±15 months. Predictive value was determined for sudden arrhythmic death (SAD) defined as sudden cardiac death or fast ventricular tachycardia/fibrillation [CL<240 ms].

*Results:* In univariate analysis $STV_{Ratio}$, but not $STV_{QT}$ or $STV_{RR}$, was predictive of SAD. Hazard ratios for highest quartile $STV_{Ratio}$ and QTVI were comparable ($STV_{Ratio}$: 1.9, 95% C.I. 1.1-3.3, p=0.038, QTVI: 2.2, 95% C.I. 1.2-3.8, p=0.010). In a multivariate model highest quartile $STV_{Ratio}$ was predictive of SAD after adjustment for NYHA-class, history of ischemia or previous arrhythmias and use of class-I antiarrhythmics (HR 1.8, 95% C.I. 1.0-3.4, p<0.050). A combined criterion of highest quartile for both $STV_{Ratio}$ and QTVI identified patients at highest risk (HR 2.4, 95% C.I. 1.3-4.3, p=0.005, PPV 38%, NPV 82%).

*Conclusion:* $STV_{Ratio}$ from ICD electrograms is predictive of SAD. Predictive value is similar for order-based $STV_{Ratio}$ and distribution-based QTVI, but the combination of both parameters can further improve results.


## Introduction

Sudden cardiac death remains a major cause of cardiovascular death. Implantation of an intracardiac defibrillator (ICD) can be effective in preventing arrhythmic death. The two major studies used to derive current guidelines, report a moderate absolute reduction in mortality of 5-7% over 2-5 years.[1, 2] Meanwhile, ICD shocks are known to cause severe anxiety and reduce quality of life.[3] Reports show that up to 47% of ICD shocks are delivered inappropriately for conducted supraventricular tachycardia.[4, 5] Based on results of the PREPARE study, in primary prevention where appropriate shocks are slightly less frequent, more conservative detection parameters were proposed that reduced the number of ICD shocks while maintaining the mortality

benefit (e.g. longer delay before shock or shock fast ventricular tachycardia only).[6] If accurate information would be available on the present likelihood of ventricular arrhythmia occurrence, this can be incorporated in the classification algorithms used by ICDs. We propose beat-to-beat variability of repolarization as a parameter of arrhythmic risk, which in the future may be monitored in implantable cardiac devices and used to guide therapy.

Changes in repolarization duration between consecutive beats can be quantified as short-term variability of repolarization duration ($STV_{QT}$), which has been put forward as a predictive electrophysiological marker of cardiac arrhythmias. The predictive value of $STV_{QT}$ has been shown in animals models[7-11] and recently, in 3 distinct patient populations.[12-14] High values of baseline $STV_{QT}$ are related to unstable repolarization, and are seen in patients with non-ischemic heart failure and patients susceptible to drug-induced arrhythmias. Variability of repolarization duration is expected to be influenced by beat-to-beat changes in cycle length. To correct for this physiological component of $STV_{QT}$, we introduce a cycle length corrected derivative ($STV_{Ratio}$) as the ratio between $STV_{QT}$ and $STV_{RR}$. As a first feasibility study into device based STV measurement, we evaluated the long-term predictive value of $STV_{QT}$ and $STV_{Ratio,}$ derived from ICD based intracardiac electrograms (EGM), for arrhythmic events in a patient population with structural heart disease. QT variability index (QTVI) as proposed by Berger et al.[15] is an alternative measure of repolarization variability, with proven long term predictive value for total mortality, sudden cardiac death (SCD) and arrhythmia in several patient populations.[15-19] QTVI is based on the variance and mean of QT and RR intervals. Therefore, the value of QTVI is not affected by the order in which the intervals were recorded. In the current study we compared predictive value for arrhythmic events of distribution based QTVI with the order based parameters $STV_{QT}$ and $STV_{Ratio}$.



## Methods

The study protocol was approved by the Johns Hopkins University and the Washington University Human Studies Committees. All patients gave written informed consent before entering the study.

*Study population:* All adult patients with ICD implanted for primary or secondary prevention of sudden cardiac death were eligible for the study, as previously described by Tereshchenko et al.[19] Patients who were pregnant or with known inherited channelopathies were excluded from the study, as were patients with concomitant non-cardiovascular disease and a life expectancy of less than one year.

*Intracardiac electrogram recording:* At study enrolment, a bipolar (tip to ring) right ventricular (RV) endocardial EGM simultaneously with surface ECG (Lead II) was recorded while the patient was at rest, using a Medtronic 2090 programmer and a portable data acquisition system. Beat-to-beat QT interval was measured offline as described by Berger et al.[15] For each recording a template beat was defined and start of QRS, J-point and end of the T-wave were selected manually. Software automatically detected the peak of the R-wave for all other beats and the QT interval was determined by stretching the JT segment to best fit the template beat. For each patient 60 normal sinus beats were analyzed. Recordings were excluded when there was more than 15% ectopic or noise distorted beats, or more than 5% atrial or ventricular pacing according to device stored data in the period preceding the baseline recording (range 7 to 90 days). Ectopic beats were detected automatically based on EGM signal morphology, and were excluded from analysis.
From the automatically determined QT and RR intervals $STV_{QT}$, $STV_{RR}$ and $STV_{Ratio}$ were calculated (Equation 1 and 2).

(1) $STV_{QT}$, $STV_{RR} = \Sigma_{1..60} | D_n - D_{n-1} | / (60*\sqrt{2})$, D being QT or RR interval

(2) $STV_{Ratio} = STV_{QT} / STV_{RR}$

QTVI was determined according to equation 3.

(3) $QTVI = LOG[(QT_{Var}/QT_{Mean}^2)/(HR_{Var}/HR_{Mean}^2)]$

*End points:* The primary endpoint was defined as sudden cardiac death or appropriate ICD shock for sustained fast ventricular tachycardia/fibrillation with a cycle length below 240ms (hereafter called sudden arrhythmic death). We did not include ICD therapy for slower ventricular tachycardia in the endpoint, as this tends to overestimate the frequency of prevented sudden cardiac death.[6, 20] Programming of the ICD device was left at the discretion of the attending electrophysiologist. Patients were followed up at the Washington University Arrhythmia Clinic and remotely via the Internet-based CareLink remote monitoring system. All events were reviewed by an ICD endpoint committee consisting of the attending electrophysiologist and 2 of the investigators (L.G.T. and R.D.B.), who adjudicated each ICD event. Device stored data was used to discriminate ventricular tachycardia from supraventricular tachycardia.[19] Cases of SCD were adjudicated based on information available in the medical records. Cases of SCD were included as endpoints in this analysis only if death was witnessed and described in medical records.

Statistical analysis: All statistics were computed using STATA 10 (StataCorp LP, College Station, TX). Results are presented as mean ± standard deviation (SD) for normally distributed variables and as median and interquartile range for non-normally distributed variables (STVQT, STVRR). Normally distributed continuous variables were compared using Student's t-test. Wilcoxon Rank-Sum test was applied to the skewed continuous variables STVQT and STVRR. Dichotomized variables were compared by Pearson's chi-square test. We specified the high-risk subgroups of patients by identifying separately those in the highest quartile of the STVQT, STVRatio and QTVI, and lowest quartile STVRR. Unadjusted and adjusted Kaplan-Meier survival analysis was used to compare the highest quartile of the tested predictors. The log-rank statistic was computed to test the equality of survival distributions.

# Results

## Patient population and endpoints

We analyzed data of 233 patients. From these 233 patients, 167 (72%) were male. Average age was 59±15 years. Ischemic cardiomyopathy with previous myocardial infarction was diagnosed in 145 (62%) of the patients. ICD implantation was indicated for primary prevention in 180 (77%) patients and for secondary prevention in 53 (23%). Over a follow up period of 26±15 months, 50 patients (21%) met the primary endpoint. Defibrillation for fast ventricular tachycardia/fibrillation was successful in 28 patients (56%), and cardiovascular death was determined in 22 patients (44%). Patients that met the study endpoint were more likely to have a history of revascularization procedures (PTCA), be in NYHA class III heart failure, suffer from diabetes mellitus, have a history of ventricular tachycardia or SCD events before ICD implantation (secondary prevention), or be using class I antiarrhythmics (Table 1).

## Repolarization variability

Patients with subsequent sudden arrhythmic death had higher $STV_{Ratio}$ at baseline (0.83 ±0.43 vs 0.66±0.41, endpoint and non-endpoint respectively, p=0.010), but no significant difference for uncorrected $STV_{QT}$ (16 (6-33) vs 14 (6-28) ms), endpoint and non-endpoint respectively, p=0.89). Also, average RR, $STV_{RR}$ and average QT were not different between the two groups. Similar to $STV_{Ratio}$, QTVI was lower in the non-endpoint group (-0.032±0.528 vs -0.272±0.550, p<0.01).

The highest quartile for $STV_{QT}$ and $STV_{Ratio}$ and the lowest quartile for $STV_{RR}$ were used to evaluate predictive value of both parameters (boundaries 30 ms, 0.88 and 55 ms respectively). Kaplan-Meier survival analysis showed $STV_{Ratio}$ was

predictive for the primary endpoint, while $STV_{QT}$ and $STV_{RR}$ were not (Figure 1). The hazard ratio for highest quartile $STV_{Ratio}$ was 1.9 (95% C.I. 1.1-3.3, p=0.038,). Highest quartile QTVI (QTVI>0.14) showed a hazard ratio slightly higher than $STV_{Ratio}$ (2.2, 95% C.I. 1.2-3.8, p=0.010, Figure 1a). In a multivariate model highest quartile $STV_{Ratio}$ remained predictive of SAD after adjustment for NYHA class, history of ischemia, ICD indication (primary/secondary prevention) and use of class I antiarrhythmics (HR 1.8, 95% C.I. 1.0-3.4, p<0.050). Highest quartile $STV_{Ratio}$ was associated with the use of beta-blockers, aldosterone antagonists and class III antiarrhythmics (Table 2). Figure 2 shows the relation between $STV_{Ratio}$ and QTVI, with a clustering of endpoint-positive patients at the higher values of $STV_{Ratio}$

*Table 1: Baseline clinical characteristics by endpoint*

| **Baseline Clinical Characteristics** | **No SAD (n = 183)** | **SAD (n = 50)** | **p** |
|---|---|---|---|
| *Age, mean±SD, years* | *59±15* | *60±15* | *0.500* |
| *Females, n (%)* | *51(28)* | *15(30)* | *0.767* |
| *African American, n (%)* | *35(19)* | *9(18)* | *0.158* |
| *QRS Width, mean±SD, ms* | *114±26* | *120±29* | *0.421* |
| *CHF NYHA class III, n (%)* | *19(10)* | *14(28)* | *0.010* |
| *Ischemic CM with MI history, n (%)* | *108(59)* | *37(74)* | *0.053* |
| *- CABG, n (%)* | *56(52)* | *14(38)* | *0.141* |
| *- PTCA, n (%)* | *45(42)* | *24(65)* | *0.015* |
| *Primary prevention of SCD, n (%)* | *147(80)* | *33(66)* | *0.032* |
| *Single-chamber ICD, n (%)* | *123(67)* | *34(68)* | *0.975* |
| *LVEF, mean±SD, %* | *33±12* | *32±11* | *0.620* |
| *Diabetes mellitus, n (%)* | *56(31)* | *24(48)* | *0.022* |
| *Hypertension, n (%)* | *134(73)* | *41(82)* | *0.203* |
| *ß-Blockers, n (%)* | *148(81)* | *43(86)* | *0.403* |
| *Aldosterone antagonists, n (%)* | *60(33)* | *19(38)* | *0.490* |
| *Class I antiarrhythmic medication, n (%)* | *1(1)* | *5(10)* | *0.000* |
| *Class III antiarrhythmic medication, n (%)* | *44(24)* | *18(36)* | *0.090* |

*SAD: Sudden arrhythmic death, CHF: congestive heart failure, NYHA: New York Heart Association, LVEF: Left ventricular ejection fraction, CABG: Coronary artery bypass graft, PTCA: percutaneous transluminal coronary angioplasty.*

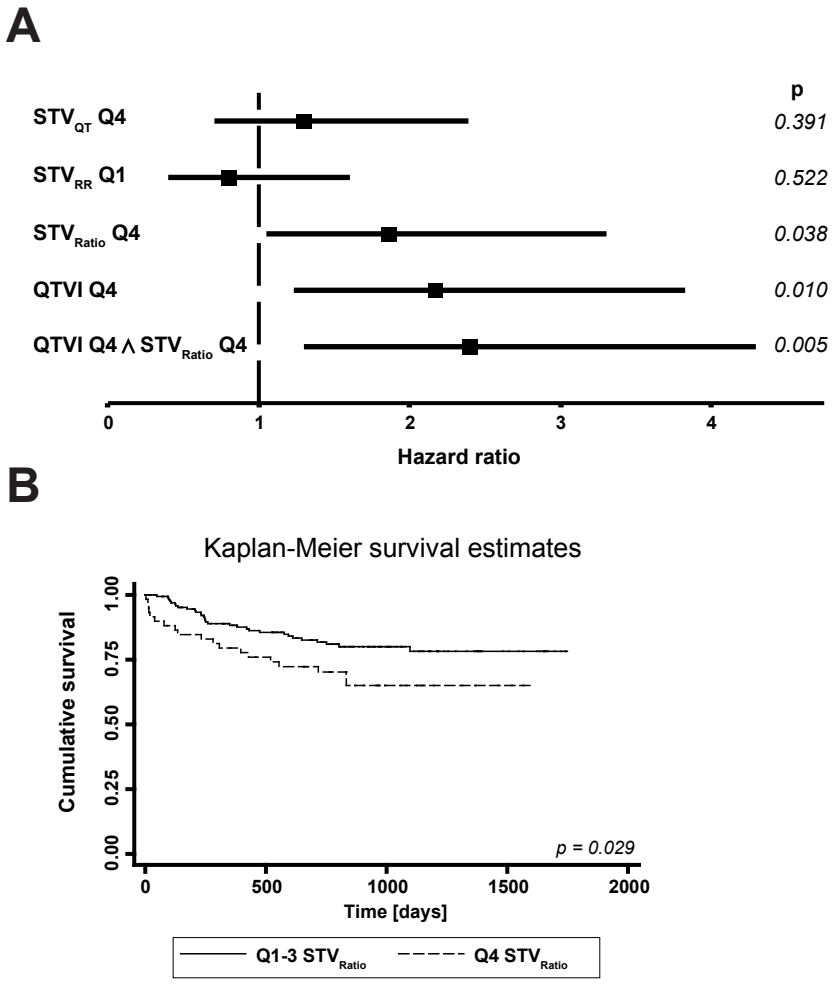


*Figure 1: Univariate predictive value for sudden arrhythmic death.*
*A. Univariate hazard ratios with 95% confidence interval and p-values for sudden arrhythmic death. Presented parameters are lowest quartile short-term variability of RR ($STV_{RR}$) and highest quartile $STV_{QT}$, cycle length corrected STV ($STV_{Ratio}$), and QT variability index (QTVI). The lower value represents the combination of highest quartile $STV_{Ratio}$ and QTVI.*
*B. Kaplan-Meier curves for freedom of events of sudden arrhythmic death in patients in the highest quartile and the lowest three quartiles of $STV_{Ratio}$.*

and QTVI. Correlation analysis revealed significant correlation between the two parameters ($r^2$=0.50, p<0.001). Kaplan-Meier analysis shows patients with highest quartile $STV_{Ratio}$ and QTVI have the worst prognosis, illustrating incremental value of combining the two parameters (Figure 3). The combination of highest quartile $STV_{Ratio}$ and highest quartile QTVI identified patients at highest risk for sudden arrhythmic death (HR 2.4, 95% C.I. 1.3-4.3, p=0.005, positive predictive value 38%, negative predictive value 82%, sensitivity 32%, specificity 86%; Figure 1a). Predictive value was preserved in a multivariate model after adjustment for NYHA class, history of ischemia, diabetes mellitus and use of class III antiarrhythmics (HR 1.9, 95% C.I. 1.1-3.5, p=0.035).

*Table 2: Baseline clinical characteristics for highest and lower three quartiles of $STV_{Ratio}$*

| Baseline clinical characteristics | $STV_{Ratio}$ Q1-3 (n=174) | $STV_{Ratio}$ Q4 (n=59) | p |
|---|---|---|---|
| *Age, Mean±SD,years* | *60±15* | *58±15* | *0.467* |
| *Females, n(%)* | *54(31)* | *12(20)* | *0.115* |
| *African American, n(%)* | *31(18)* | *13(22)* | *0.661* |
| *QRS Width, mean±SD, ms* | *115±26* | *117±28* | *0.856* |
| *CHF NYHA classIII, n(%)* | *24(14)* | *9(15)* | *0.080* |
| *Ischemic CM with MI history, n(%)* | *108(62)* | *37(63)* | *0.930* |
| *- CABG, n(%)* | *51(47)* | *19(51)* | *0.664* |
| *- PTCA, n(%)* | *52(48)* | *17(46)* | *0.817* |
| *Primary prevention of SCD, n(%)* | *138(79)* | *42(71)* | *0.198* |
| *Single-chamber ICD, n(%)* | *111(64)* | *46(78)* | *0.096* |
| *LVEF±SD, %* | *34±13* | *31±10* | *0.106* |
| *Diabetes mellitus, n(%)* | *57(33)* | *23(40)* | *0.384* |
| *Hypertension, n(%)* | *126(72)* | *49(83)* | *0.102* |
| *ß-blockers, n(%)* | *137(79)* | *54(92)* | *0.027* |
| *Aldosterone antagonists, n(%)* | *47(27)* | *27(46)* | *0.000* |
| *Class I antiarrhythmic medication, n(%)* | *6(3)* | *0(0)* | *0.148* |
| *Class III antiarrhythmic medication, n(%)* | *38(22)* | *24(41)* | *0.005* |

*SAD: Sudden arrhythmic death, CHF: congestive heart failure, NYHA: New York Heart Association, LVEF: Left ventricular ejection fraction, CABG: Coronary artery bypass graft, PTCA: percutaneous transluminal coronary angioplasty.*

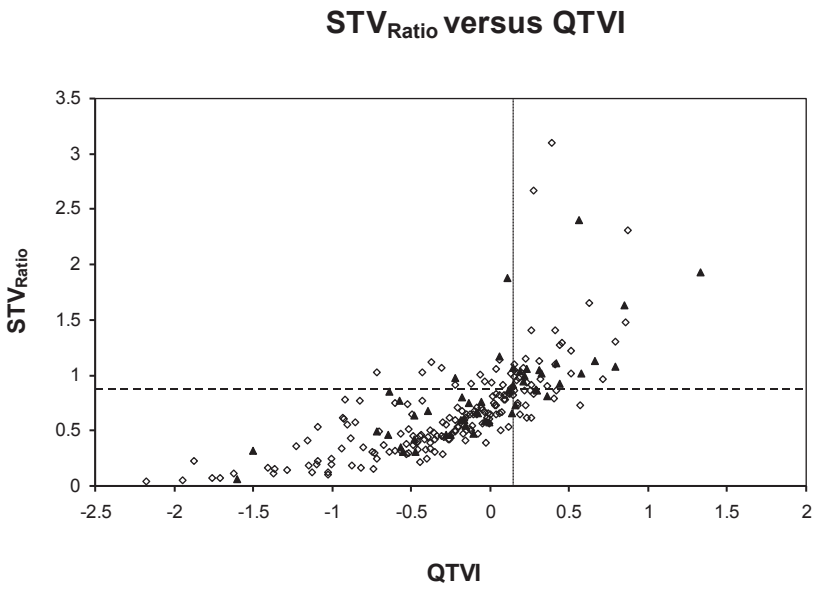


*Figure 2: $STV_{Ratio}$ plotted versus QTVI. Patients that reached the sudden arrhythmic death endpoint marked with closed triangles, non-endpoint marked with open diamonds. The dotted lines indicate the lower limit of the 4th quartile for $STV_{Ratio}$ and QTVI.*



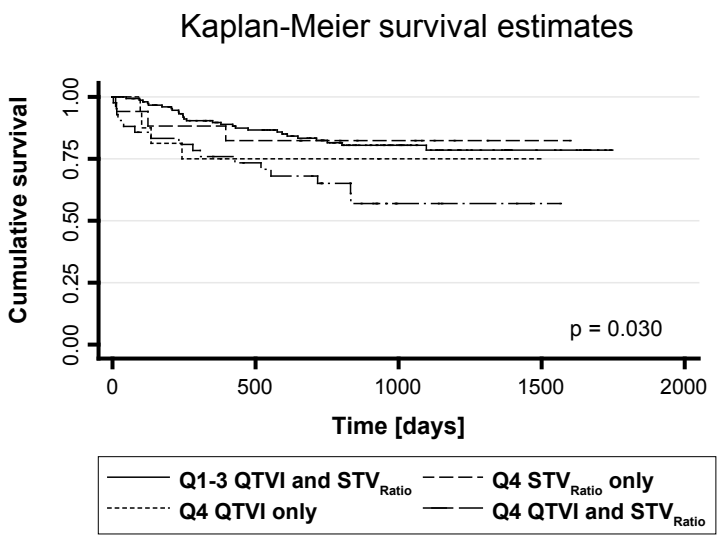


*Figure 3: Predictive value of combinations of $STV_{Ratio}$ and QTVI.*
*Kaplan-Meier curves for freedom of events of all combinations of 4th quartile and first three quartiles of $STV_{Ratio}$ and QTVI. Survival for sudden arrhythmic death was worst in patients in both 4th quartile $STV_{Ratio}$ and 4th quartile QTVI.*

# Discussion

In patients with structural heart disease, $STV_{Ratio}$ derived from the endocardial RV EGM is predictive of the endpoint sudden arrhythmic death, while $STV_{QT}$ and $STV_{RR}$, the components of $STV_{Ratio}$, are not. The predictive value $STV_{Ratio}$ was similar to the predictive value of QTVI. A combination of $STV_{Ratio}$ and QTVI led to a moderate improvement in results.

## Predictive value of STV

The predictive value of STV was first determined in animal models using left ventricular monophasic action potential duration (MAPD): Sudden increase in $STV_{MAPD}$ preceded drug-induced ventricular arrhythmia in dogs with remodeled hearts due to chronic atrioventricular block.[9] Similarly, in a methoxamine-sensitized anesthetized rabbit model, $STV_{QT}$ from surface ECG was increased before dofetilide-induced torsade de pointes arrhythmias, but returned to baseline values after arrhythmias were suppressed with AZD1305.[10] Lengyel et al. confirmed in awake dogs and anesthetized rabbits that $STV_{QT}$ has better predictive value than $QT_C$ for torsade de pointes arrhythmia after combined $I_{Ks}$ and $I_{Kr}$ block.[21] Furthermore, even before infusion of a proarrhythmic drug, chronic AV-block dogs with a predisposition for drug-induced arrhythmia show higher baseline $STV_{MAPD}$ values than their drug-resistant counterparts.[7] Recently Floré et al. reported elevated $STV_{QT}$ from surface ECG in a porcine model during acute ischemia and further increase in the first three weeks after MI, which was accompanied by ventricular remodeling.[22] The value of baseline STV in specific patient populations was determined by Hinterseer et al. for drug-induced long QT syndrome (LQTS),[12] symptomatic congenital LQTS,[13] and in patients with non-ischemic cardiomyopathy.[14] In the current study, as a next step to a clinical application, we investigated the use of $STV_{QT}$ and $STV_{Ratio}$ in a heterogeneous population with structural heart disease and an ICD, showing that $STV_{Ratio}$ can be used in a population representative for patients presented to a clinical electrophysiologist. The relation between RR variation and QT variation had already been noted by Hinterseer et al.,[12] but the positive relation between $STV_{QT}$ and $STV_{RR}$ was only present in controls and not in patients susceptible to drug-induced arrhythmia. However, this may be a feature of that specific substrate, while in the current study different mechanisms link arrhythmia to repolarization variability. In the current study, $STV_{QT}$ was not able to predict ventricular tachycardia, as opposed to the ECG studies performed by Hinterseer et al. Furthermore, the average levels and standard deviation of $STV_{QT}$ are two- to threefold higher. Differences in methodology may explain the different outcome: First, in the current study the QT

interval was recorded until the end of the U-wave, when present, while Hinterseer et al. attempted to exclude a possible U-wave. As a result the reported average QT interval is 90 to 100ms longer in the current study. The inclusion of the U-wave may increase the sensitivity for changes in repolarization, but at the expense of introducing a measurement error due to the low amplitude of the U-wave. Secondly, Hinterseer et al. report seeing no ectopic activity or recorded only during consecutive beats not containing ectopic activity. In the contrast, in our study ectopic beats were frequent, in some cases leading to exclusion of patients.[19] Even though ectopic beats were excluded from analysis and care was taken to measure QT during stable heart rate, there may have been more electrical and hemodynamic instability in the current study. However, visual inspection of QT traces in patients with high QT variability did not reveal any relation to ectopic activity.

The rationale for correcting $STV_{QT}$ for cycle length variations is twofold: (1) pronounced RR variation is expected to result in QT variation, without this necessarily being related to increased arrhythmic risk. The low heart rate variability caused by anesthesia in the animal model may have masked this effect in previous studies.[7,21] (2) Low heart rate variability itself is related to a worse outcome in various pathologies, especially ischemic heart disease.[23] A decrease in $STV_{RR}$ with unchanged $STV_{QT}$ will lead to an increase in $STV_{Ratio}$, and thereby to a higher perceived risk. The relation between heart rate, repolarization variability and arrhythmic risk is apparently that much intertwined that in this heterogeneous population only the combination of $STV_{RR}$ and $STV_{QT}$ in $STV_{Ratio}$ can predict arrhythmic risk, while separately $STV_{RR}$ and $STV_{QT}$ have no predictive value.



## Comparison to QTVI

STV is one of several repolarization based markers discussed in literature to predict arrhythmic outcome. We compared predictive value of $STV_{Ratio}$ and QTVI as proposed by Berger et al.,[15] but calculated over 60 beats and using QT intervals derived from EGM instead of ECG. In a previous publication on this patient population, using a shorter follow-up time and a different arrhythmic endpoint, EGM-derived QTVI showed the same predictive value as ECG-based QTVI.[19] Several studies have shown the long-term predictive value for total mortality, SCD or ventricular tachycardia of baseline QTVI in various patient populations.[16-18] In the current study $STV_{Ratio}$ and QTVI both were evaluated at resting heart rates, but while $STV_{Ratio}$ quantifies changes in repolarization and RR between consecutive beats, QTVI is based on the distribution (mean and variance) of QT and heart rate over a certain time interval. As a consequence $STV_{Ratio}$ attenuates slow variations (e.g. recovery from exercise), while emphasizing sudden changes in QT interval. This makes $STV_{Ratio}$ more suitable for

pre-clinical drug testing, where controlled QT prolongation is not always a sign of pro-arrhythmic potential[24]. On the other hand, QTVI will be more sensitive to slower changes in QT (e.g. resulting from breathing or variation in autonomic tone). Figure 4 illustrates how the order of recorded QT intervals influences $STV_{QT}$ (and its derivative $STV_{Ratio}$) and QT variance (which is used to calculate QTVI): re-ordering the same set of QT intervals will not change distribution-based parameters like QTVI, but can have a profound effect on $STV_{QT}$ and thereby on $STV_{Ratio}$. There may be incremental value in combining several parameters of repolarization variability, as is illustrated by the survival analysis for the combination of highest quartile QTVI and $STV_{Ratio}$ (Figure 3). The significance of various frequency components of QT variability during normal activity has not fully been determined. Further studies into the mechanisms linking repolarization variability to ventricular arrhythmia may help discern which type or pattern of repolarization variability is related to arrhythmic risk.

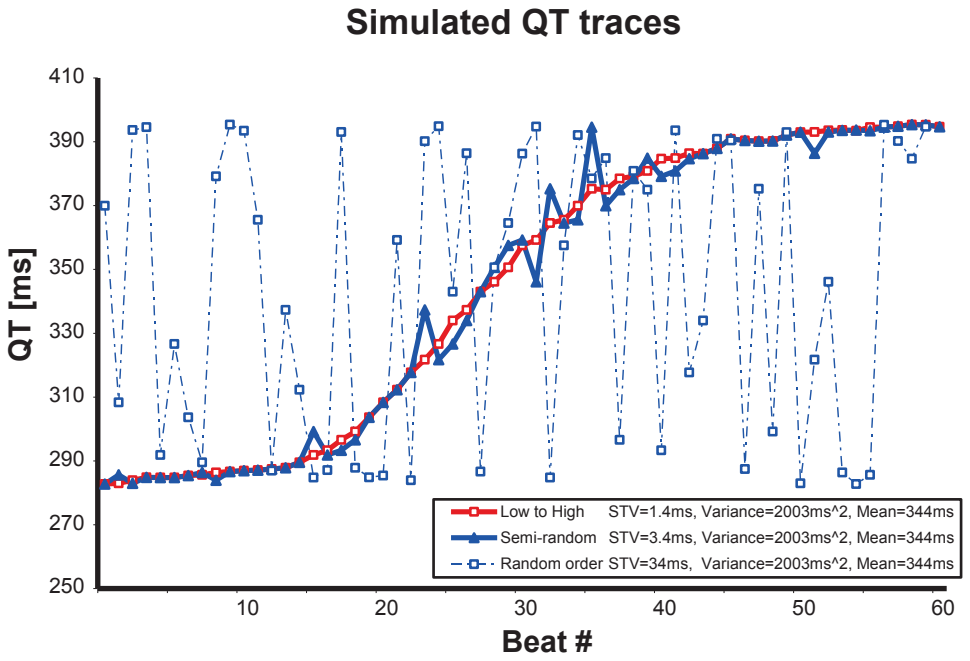


*Figure 4: Effect of beat order on $STV_{QT}$ and QT variance in simulated tracings. Placing the same simulated QT intervals in low to high, semi-random or random order affects $STV_{QT}$, but not QT mean and variance, which are used to compute QTVI.*

Increased repolarization variability in patients using class III antiarrhythmics was previously shown for QTVI,[25] where highest hazard ratio of QTVI for fast ventricular tachycardia was reported in this subgroup. Interestingly, the predictive value of QTVI was most prominent in patients receiving this drug for treatment of atrial fibrillation. This fits well with data recorded in dogs with chronic AV-block, where the increase in $STV_{MAPD}$ after pharmacological $I_{Kr}$-block was highest in animals with severely reduced repolarization reserve.[7]

## Future considerations

For continuous monitoring of repolarization variability, easy access to information on beat-to-beat repolarization duration is required. In most animal studies, repolarization was quantified using monophasic action potential catheters placed in the left ventricle (LV). Recently the use of the LV endocardial electrogram for $STV_{QT}$ measurement was validated.[26, 27] In the current study, we showed that even the RV endocardial EGM can be used for $STV_{QT}$ measurement. This opens perspectives for a clinical application, because the RV endocardium is the most widely-used position for ventricular pacing and defibrillation leads, making $STV_{QT}$ measurement feasible in the majority of implanted pacemakers and ICDs. The increased storage of diagnostic information in modern implantable devices, combined with the ability to upload this information to a central database through home monitoring systems, significantly adds to the potential of device based $STV_{QT}$ measurement. Because memory use and calculations increase power consumption and thereby decrease device longevity, we compared computational efficiency for calculations over *n* beats. Memory requirements are comparable for single calculations of $STV_{Ratio}$ and QTVI. Although a true variance calculation requires all *n* original QT or RR intervals, simplified running variance calculation are available that bring down the memory requirement to 4 variables for intermediate results at the expense of some rounding errors. For $STV_{Ratio}$ 2 running sums (delta QT and RR) would suffice. For a continuous update of $STV_{Ratio}$ or QTVI *2n* stored numbers are required. Calculation requires 2x2 additions per beat for $STV_{QT}$ and $STV_{RR}$ and one division for calculating $STV_{Ratio}$ itself. This should be within the capabilities of current devices without a notable sacrifice to longevity. The variance calculations (QT and RR) for QTVI require 2 multiplications and 3 to 5 additions per beat and 5 multiplications/divisions are required to calculate QTVI. Because computational complexity increases linearly with the number of digits for addition, but exponentially for multiplication/division, we conclude that in current devices continuous calculation of QTVI would not be possible without dedicated hardware. However, using median and percentile ranges instead of variance and mean may bring complexity down to acceptable levels.

We found hazard ratios for $STV_{Ratio}$ around 2, which is similar to values reported for QTVI for this study population. For long-term clinical decision making this will be too low. This is supported by the negative and positive predictive value for highest quartile $STV_{Ratio}$ over the total follow up time (82 and 32% respectively, sensitivity 38%, specificity 78%). In our opinion the real potential of $STV_{Ratio}$ lies in the ability to monitor risk continuously, capturing the proarrhythmic effects of progression of cardiac disease without requiring repeated in hospital testing (compared to e.g. ejection fraction measurement).

The limited data that is available on repolarization variability assessed from EGM suggests repolarization variability greatly increases just before a cardiac event,[28] but the vast amount of signals that is becoming available through the roll out of home monitoring systems is still unexplored. The dynamic changes in QT variability may prove to hold new information on development of arrhythmic substrates. The ability to uplink data for off line analysis on a daily basis can have a tremendous impact on patient management if we are able to recognize the signs. The detailed information on the risk profile of individual patient can be a first step to personalized medicine in cardiac devices. Implementation of real-time repolarization variability measurement in an implantable device may be used to improve ICD therapy. Including arrhythmic risk in ventricular tachycardia classification algorithms can reduce the number of inappropriate shocks, while preserving the reduction in mortality. Furthermore, animal studies have shown that increasing heart rate through pacing can prevent drug-induced arrhythmia which is reflected in lower values of $STV_{MAPD}$.[26] This may provide safe, asymptomatic prevention of arrhythmias in specific patient groups.

## Limitations

In the current study we used segments of 60 beats from recordings of several minutes. This enabled us to select ECG segments with good signal quality and low number of ectopic beats. However, for QTVI in prior studies a period of 5 minutes is often used. This may have reduced the accuracy of our QT variability measurements (QTVI and $STV_{QT}$). When used in an implantable device, QT variability can easily be calculated over longer periods if required.

## Conclusion

$STV_{Ratio}$ from intracardiac ICD electrograms, but not its components $STV_{QT}$ and $STV_{RR}$, is predictive of the study endpoint sudden arrhythmic death. For resting EGMs similar predictive value was seen for $STV_{Ratio}$ and QTVI. Combining both parameters of repolarization variability may improve predictive value. Further research is required to evaluate the clinical value of continuous monitoring of repolarization variability in implantable devices.

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## Chapter 6

# Beat-to-beat variability in preload unmasks reduced repolarization reserve in anesthetized dogs with chronic atrioventricular block

Peter Oosterhoff, Thom RG Stams, Albert Dunnink, Jet DM Beekman, Roel van der Nagel, Harold VM van Rijen, Marcel AG van der Heyden, Marc A Vos

Department of Medical Physiology, DH&L, University Medical Center Utrecht, The Netherlands

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## Abstract

*Introduction:* In patients and animal models reduced repolarization reserve predisposes to ventricular arrhythmia and has been associated with increased beat-to-beat variability of cardiac repolarization duration (BVR). In dogs with chronic AV-block (AVB) the increase in BVR that follows on a reduction of repolarization reserve through cardiac remodeling or pharamacological $I_{Kr}$-block, predicts susceptibility to drug-induced ventricular arrhythmias. The AVB dog is characterized by bradycardia, altered ventricular activation and dissociation of timing between atrial and ventricular contraction. We hypothesize that this increase in BVR becomes apparent as the result of an altered response to beat-to-beat changes in preload. *Methods*: In anesthetized mongrel dogs with AVB, left ventricular endocardial monophasic action potentials were recorded. BVR was quantified as short-term variability of action potential duration (STV). Variability of ventricular preload was controlled through stimulation protocols, resulting in either constant (150 ms) or variable PQ intervals (alternating between 150 and 350 ms), while maintaining ventricular bradycardia (60 bpm). Control of ventricular loading was verified using sonomicrometry. We determined STV before and after $I_{Kr}$-block at acute and chronic AVB (>3 weeks), both with constant and variable PQ.
*Results:* Electrical remodeling was confirmed by $QT_C$ lengthening (327±19 to 440±46 ms, $p<0.01$) and the occurrence of dofetilide-induced TdP arrhythmias: from 0% to 57% at acute and chronic AVB respectively. At constant PQ interval, STV did not increase after AVB (acute: 0.3±0.1 ms, chronic: 0.3±0.1 ms, NS). In contrast, at variable PQ, STV was significantly increased at chronic AVB (0.4±0.1 vs. 2.6±0.8 ms, $P<0.01$). At chronic AVB dofetilide-induced incidence of TdP arrhythmias was not affected by PQ variability (3/6 at constant versus 4/7 at variable PQ), but STV increased only at variable PQ. Inducible animals presented with a higher baseline STV at alternating PQ (2.7±0.4 ms versus 1.5±0.4 ms, $p<0.05$), but not at constant PQ (0.4±0.5 ms versus 0.3±0.3 ms, NS). *Conclusions*: In the chronic AVB dog, elevated BVR after proarrhythmic remodeling or pharmacological $I_{Kr}$-block depends on beat-to-beat changes in mechanical loading of the heart. Although variation of PQ interval itself augments the response of BVR to reduced repolarization reserve, it does not seem to attribute to TdP incidence provoked by dofetilide.

## Introduction

Symptomatic and sometimes even life threatening ventricular arrhythmias may present in both congenital and acquired forms of Long QT syndrome (LQTS). In LQTS patients increased temporal variability of repolarization is shown, which extent can be quantified in different ways.[1-5] The occurrence of repolarization variability is often explained by the concept of decreased repolarization reserve, meaning that the excess of repolarization capacity by for example the redundant action of repolarizing currents, becomes smaller.[6] Moreover, the positive correlation between repolarization instability and cardiac arrhythmias is also found in experimental cardiac arrhythmia models: In animal models sensitive to drug-induced torsade de pointes (TdP), beat-to-beat variability of repolarization duration (BVR) is used as a marker of proarrhythmic side effects in pre-clinical drug screening.[7-9] Finally, similar observations are made in isolated cardiomyocytes, where torsadogenic drugs induce beat-to-beat changes in transmembrane action potential duration (APD) as a precursor to early after-depolarizations (EAD).[10-12] However, since even moderate electrical coupling of cardiomyocytes is known to suppress both APD variability and EAD formation,[13] the still unknown underlying mechanisms linking repolarization variability and proarrhythmia may differ between isolated cells and the in-vivo situation.

In the chronic atrio-ventricular block (CAVB) dog model, volume overload after ablation of the AV node results in cardiac remodeling, including electrical remodeling affecting the repolarization reserve, which subsequently predisposes the animal to drug-induced arrhythmias. This proarrhythmic electrical remodeling is reflected in elevated baseline values of BVR, while TdP-inducing drugs that impair repolarization capacity even further yield an even stronger increase in BVR before arrhythmias become evident. As a consequence of variable atrial-ventricular contraction asynchrony in the CAVB dog, uncontrolled beat-to-beat changes in ventricular preload will occur. At close inspection of previous recordings from this model, we noted that patterns of repolarization variability often matched patterns of PQ-variation and mechanical ventilation. Therefore, we hypothesized that in this in-vivo model beat-to-beat variability in preload, as an external stimulus, is needed to provoke repolarization variability in a situation of decreased repolarization reserve. In the current study we investigated the role of beat-to-beat changes in preload both on the relation between BVR and repolarization reserve, and on the arrhythmic outcome. For this purpose, changes in preload were controlled through stimulation protocols applied to both the atria and the ventricles, resulting in maximal or almost

absent beat-to-beat changes in preload in hearts before and after cardiac remodeling. Finally, proarrhythmic response to pharmacological $I_{Kr}$-block was evaluated.

## Methods

*Animal handling:* Animal handling was in accordance with Dutch law on animal experiments and the European Directive for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (86/609/EU). All experiments were approved by the Animal experiment committee of University Medical Center Utrecht.
In total 24 experiments were performed in 11 anesthetized purpose bread mongrel dogs (16-24 kg, Marshall, New York, USA). All experiments were performed under complete anesthesia. Premedication consisted of an intramuscular injection with vetranquil, methadone and atropine (10 mg, 10 mg and 0.5 mg respectively). Complete anesthesia was induced by sodium pentobarbital (25 mg/kg i.v.) and maintained with isoflurane (1.5% in $O_2$ and $N_2O$, 1:2).

*Preparation:*In the first experiment a right thoracotomy was performed through the fourth or fifth intercostal space. A screw-in pacing electrode (5076, Medtronic Inc. Minneapolis, USA) was placed in the right atrium using a right-sided purse-string atriotomy. A custom made electrode with a 10 mm screw (Medtronic Bakken Research Center, Maastricht, The Netherlands) was placed transmurally in the left ventricular apex. The electrode was electrically isolated except for the most distal part, allowing sub-endocardial stimulation during experiments. In five animals two sonomicrometry crystals (Sonometrics, London, Canada) were placed in the left ventricular lateral wall, just above the apex, to record local long axis stretch. Because of the transient ischemia associated with placement of the crystals, these animals were excluded from further measurements in the first experiment.
After closing the thorax, radiofrequency ablation was used to ablate the proximal bundle of His.[14] After the initial experiments the dogs were returned to the stables at slow idioventricular rhythm to allow cardiac remodeling to complete (>3 weeks).

*Stimulation protocols:* An experimental external pacemaker (PK5, Vitatron, Arnhem, The Netherlands) was adapted to perform stimulation of atria and ventricles at different pacing rates, while maintaining the ventricular bradycardia required for TdP induction.[15] The stimulation sequence could be started at a preset AV interval. The top tracing of figure 1a demonstrates that when the paced VV interval is an exact multiple of the paced AA interval, each ventricular activation is preceded by the same PQ interval. At any other VV/AA ratio the PQ interval will vary from

beat-to-beat, resulting in variation in preload. To evaluate the effect of constant PQ we used a combination of a ventricular pacing rate between 60 and 70 bpm and a VV/AA-ratio of 2.0 or 3.0 that captured both the atria and the ventricles. A VV/AA-ratio of 2.5, at the same ventricular pacing rate, was used to provoke maximal beat-to-beat variation in PQ interval (alternating PQ), and thereby in preload (Figure 1a lower tracing). In all cases the starting AV-interval was set at 150 ms as echocardiography and pressure measurements showed this provided optimal atrial

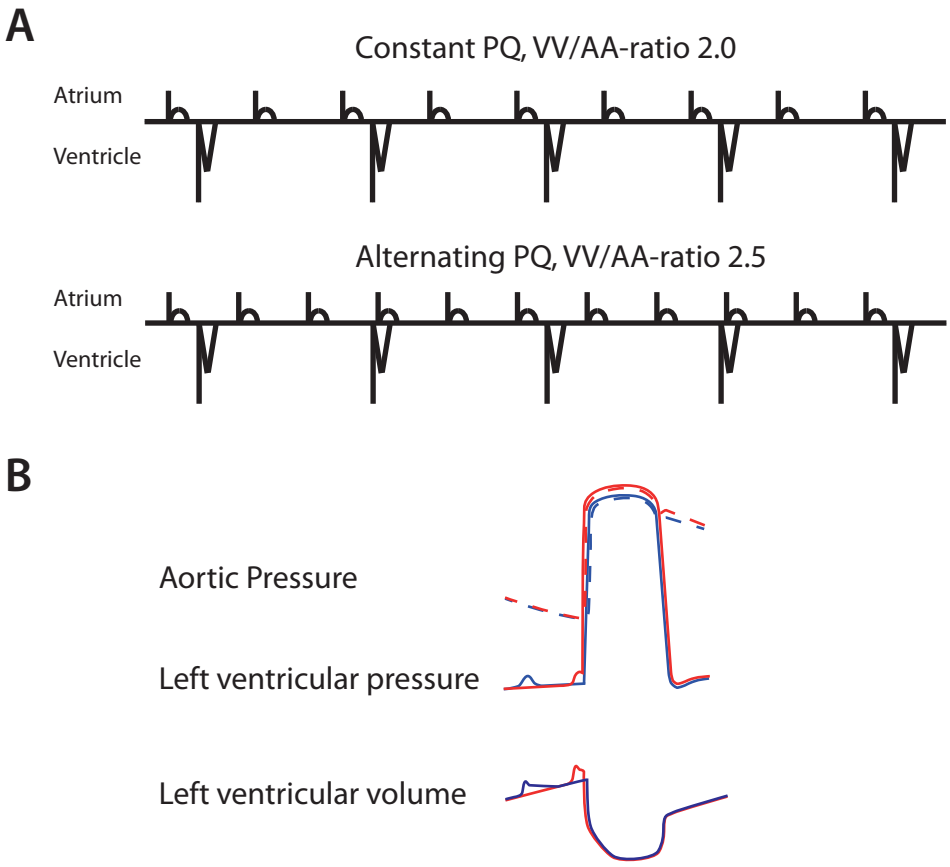


*Figure 1: A: Schematic ECG representation of the used pacing protocol. Stimulation of atria and ventricles at different heart rates allows control of atrioventricular timing, while preserving the ventricular bradycardia required for TdP induction. When the paced VV interval is an exact multiple of the AA interval, each ventricular activation is preceded by the same PQ interval (top tracing). A VV/AA-ratio of 2.5 results in alternation of the PQ interval (lower tracing). B : Diagram showing 1) the expected effect of an optimal short (150ms) PQ interval on left ventricular pressure, aortic pressure and left ventricular volume (red). 2) Same at a prolonged PQ interval (blue).*[30]

contribution to ventricular filling. Figure 1b demonstrates how a short PQ interval will result in higher ventricular end diastolic volume and pressure relative to beats with a longer PQ time. In a subset of animals we performed recordings at a range of VV/AA-ratios from 2.0 to 4.0 in 0.1 increments, to separate the effect of PP interval per se from the effect of VV/AA-ratio.

***Baseline recordings and arrhythmic challenge:*** Baseline BVR and response to pharmacological $I_{Kr}$-block was determined in anesthetized animals immediately after induction of AV-block (acute AV-block, AAVB) and after remodeling in separate experiments at 3 and 5 weeks after AV-block (chronic AV-block, CAVB).
A monophasic action potential catheter was placed at the left ventricular endocardial free wall, close to the pacing electrode. Monophasic action potential signals and 6-lead ECG was recorded with a sampling frequency of 2 kHz and 500 Hz respectively. Left ventricular pressure (LVP) was determined using a pig-tail catheter (Leycom, Zoetermeer, The Netherlands). Baseline recordings at paced rhythm were performed with constant PQ and alternating PQ. Ventilation frequency was set to one fifth of the pacing frequency. Inspiration volume was set to maintain an expired $CO_2$ concentration between 3.5 and 4.5%. Baseline recordings were repeated while mechanical ventilation was ceased for 30 s.
Repolarization reserve was probed by pharmacological $I_{Kr}$-block (dofetilide, 25 μg/kg/5 min i.v.). Experiments at AAVB were randomized to constant or alternating PQ pacing. At CAVB the dofetilide challenge was performed twice, once at constant and once at alternating PQ interval in random order in the 3 and 5 weeks experiments. ECG was monitored for induction of TdP, which was defined as a ventricular polymorphic tachyarrhythmia of at least 5 beats characterized by a twisting shape of QRS complexes and T waves around the isoelectric line. Dofetilide infusion was stopped after the first detected TdP episode. Ventricular arrhythmia lasting more than 10 s were terminated using electrical cardioversion. Levcromakalim (0.01 mg/kg/3 min, i.v.) was applied as antidote to suppress cardiac arrhythmias after the recordings were completed.

*Data analysis:* Monophasic action potential duration was measured at 80 and 90% of repolarization ($MAPD_{80}$ and $MAPD_{90}$ respectively) using custom built software (Matlab, Mathworks, Natick , USA). BVR was quantified as short-term variability (STV) of $MAPD_{80}$ over 30 beats: $STV=\sum_{1..30} | D_n-D_{n-1} | / (30*\sqrt{2})$, where D represents LV $MAPD_{80}$.[10] After dofetilide challenge STV was determined before induction of ectopic activity or at 10 minutes after dofetilide, whichever came first. QT intervals from ECG (lead II) were determined manually using onscreen calipers (AutoECG, EMKA,

Paris, France). ECG was monitored for short-coupled ectopic beats starting within the previous T-wave, multiple ectopic beats and TdP episodes until 20 minutes after start of dofetilide infusion. Animals were considered inducible when 3 or more TdP episodes occurred within the first 10 minutes after starts of dofetilide infusion.
The effect of atrial contractions on LVP, dP/dt and end diastolic pressure was evaluated in baseline recordings.

*Statistical analysis:* Data are expressed as mean ± standard deviation. Pairwise comparisons were performed using T-test for normal distributed variables and rank-sum test for non-normal distributed variables. The effect of dofetilide was evaluated using one-way or two way repeated measures ANOVA with post-hoc Bonferroni T-tests when appropriate. Incidence of arrhythmic events was tested using the Fisher exact test. A p-value of less than 0.05 was considered significant.

## Results

### BVR is sensitive to preload variation in the remodeled, but not in the normal heart

To confirm that electrical remodeling upon CAVB occurred, ventricular repolarization time at idioventricular rhythms was determined by ECG recordings. Prolongation of repolarization was observed (QT:354±33 to 473±50, $p<0.01$, $QT_c$: 327±19 to 440±46 ms, $p<0.01$) at similar heart rate (RR: 1312±319 to 1375±147, $p=0.73$) when comparing AAVB and CAVB respectively.
Next, measurements of LV MAPD at paced rhythm were performed at AAVB and CAVB. The effect of preload changes due to mechanical ventilation and PQ alternans were evaluated separately. Figure 2a shows that beat-to-beat changes in preload did not have any effect on average MAPD. BVR at AAVB, quantified as STV, was not affected by mechanical ventilation or PQ-alternans (Figure 2b). In contrast, at CAVB both mechanical ventilation and PQ-alternans resulted in elevated STV values. In the presence of beat-to-beat PQ variation there was a significant increase in STV at CAVB compared to AAVB. Figure 2c further illustrates the effect of the VV/AA ratio on STV; a range of AA intervals was evaluated at fixed VV interval. The figure shows local maxima for alternating PQ (ratios 2.5 and 3.5), local minima for constant PQ (ratios 2.0, 3.0 and 4.0), but no overall effect of increasing atrial frequency.
The observation that after remodeling variations in preload increased repolarization variation could be explained by mechanical variation occurring during the repolarization phase. To verify this assumption, we need to show that variation in preload results in mechanical variation during the repolarization phase, while

a constant PQ eliminates this mechanical variation. To this end, local stretch at the approximate position of the LV MAP catheter was evaluated using sonomicrometry. The most left plot of figure 3 shows that in the absence of preload changes the stretch pattern is constant from beat-to-beat (i.e. red and blue recordings coincide). Alternans of PQ interval results in reproducible, alternating stretch patterns, with increased stretch just before ventricular systole for beats with a short PQ interval (blue lines), and marked changes during isovolumic relaxation and early diastole

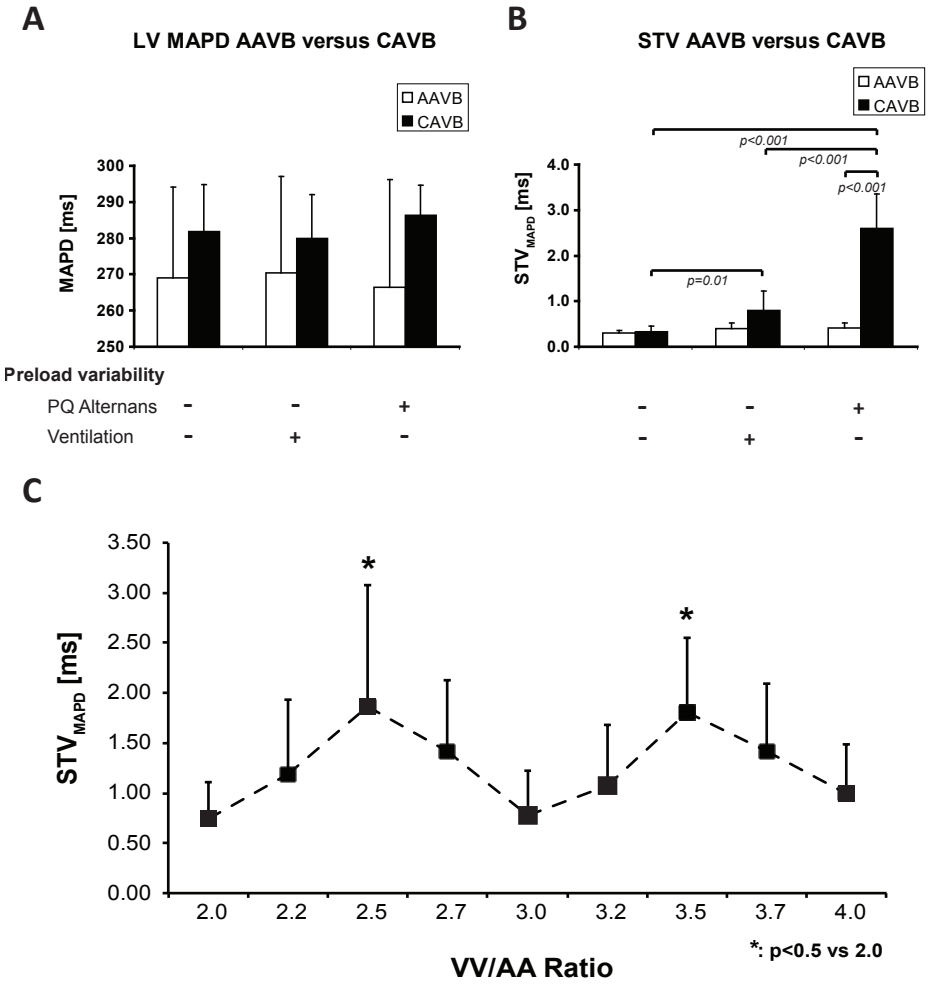


*Figure 2: The effect of beat-to-beat changes in preload caused by PQ variation and mechanical ventilation on (A) left ventricular monophasic action potential duration (LV MAPD) and (B) short-term variability of LV MAPD (STV) at acute and chronic AV-block (AAVB, CAVB). (C) Effect of the VV/AA ratio on STV by shortening AA, while maintaining a fixed VV interval. Local maxima for STV were seen at alternating PQ at ratios 2.5 and 3.5, local minima at 2.0, 3.0 and 4.0 where PQ was constant.*

(Figure 3 right plot). These changes coincided with repolarization (phase-3) or early diastole (phase-4) on LV MAP signals.

Measurement of LVP revealed no difference in instantaneous atrial contribution for long or short PQ intervals, or between AAVB and CAVB (Table 1). However, during alternating PQ, a short PQ interval resulted in higher end diastolic pressure, higher maximum LVP, and delayed relaxation. In CAVB a small but significant prolongation of contraction phase (time to $dP/dt_{min}$) was seen at short PQ intervals. Comparison of CAVB versus AAVB revealed increased $dP/dt_{max}$ at long PQ interval, with trends for short PQ interval.

Our data demonstrate that most prominent mechanical variation (i.e. alternating PQ and ventilation) is observed during the time of isovolumic relaxation and early diastole and coincides in time with ventricular repolarization.

In the CAVB dog model, induction of beat-to-beat changes in preload are a prerequisite to reveal decreased repolarization reserve as determined by an increase in STV. In the non-remodeled heart (AAVB) where repolarization capacity is not

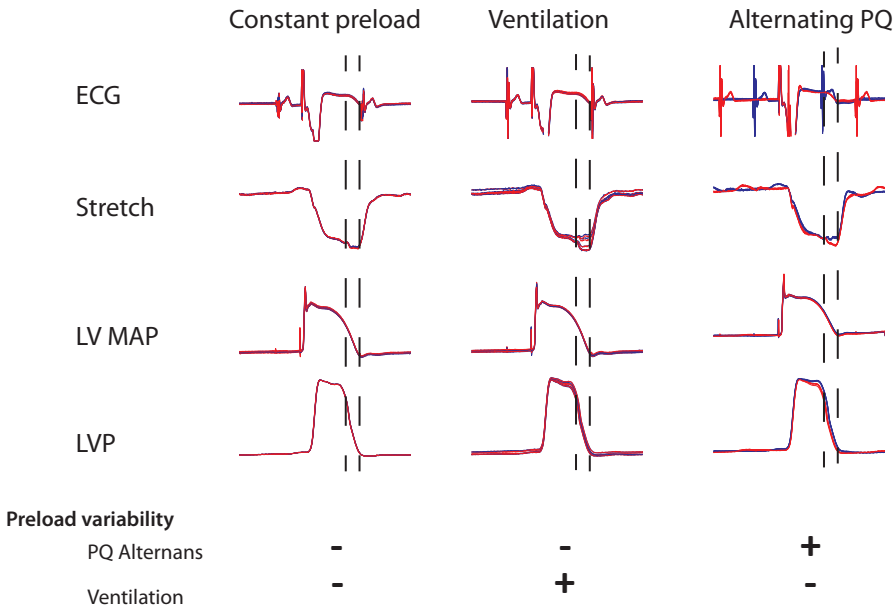


*Figure 3: Effect of variation in preload on local stretch. Overlay plot of ECG, LVP, LV MAP and apico-basal stretch from 10 consecutive beats, with odd beats in blue and even beats in red. At alternating PQ the blue/odd beats are preceded by a short PQ interval l(150ms). Ventilation affects stretch only during late systole, relaxation and early diastole. Alternating PQ interval produces reproducible changes to end diastolic stretch and stretch during late systole, relaxation phase and early diastole. Stretch and LV MAPD recorded at the left ventricular apical free wall. Dotted line indicate the isovolumic relaxation phase.*

*Table 1: Left ventricular pressure measurement.*

| **AAVB** | **Alt. PQ, Short 150 ms** | **Alt. PQ, Long >335 ms** | **Constant PQ 150 ms** |
|---|---|---|---|
| Atrial contribution [mmHg] | *1.5±1.0* | *1.4±0.7* | *1.6±1.1* |
| EDP [mmHg] | *16.5±2.1* | *15.3±2.1** | *14.5±3.6* |
| $LVP_{max}$ [mmHg] | *86.9±8.5* | *83.7±10.2** | *86.2±12.9* |
| Time $dP/dt_{max}$ [ms] | *50.8±3.4* | *53.5±7.8* | *47.7±13.9* |
| $dP/dt_{max}$ [mmHg/s] | *1213±432* | *1206±454* | *1226±516* |
| Time $dP/dt_{min}$ [ms] | *293±39* | *288±38** | *295±43* |
| $dP/dt_{min}$ [mmHg/s] | *-1200±320* | *-1118±305* | *-1172±373* |
| **CAVB** | | | |
| Atrial contribution [mmHg] | *1.9±0.7* | *1.6±0.5* | *1.5±0.5* |
| EDP [mmHg] | *13.2±5.3* | *11.4±4.8** | *12.6±5.4** |
| $LVP_{max}$ [mmHg] | *99±11* | *97±11** | *99±11* |
| Time $dP/dt_{max}$ [ms] | *45±9* | *48±8** | *47±7* |
| $dP/dt_{max}$ [mmHg/s] | *2016±771* | *2069±666‡* | *1984±798* |
| Time $dP/dt_{min}$ [ms] | *284±30* | *276±32** | *283±33* |
| $dP/dt_{min}$ [mmHg/s] | *-1463±187* | *-1468±211* | *-1545±267* |

** $p<0.05$ compared to Short PQ during alternating PQ. ‡ $p<0.05$ compared to acute AV-block. Atrial contribution defined as change in LVP over 150ms following an atrial stimulus. Time $dP/dt_{max,min}$ relative to start of systole.*

affected, repolarization is not sensitive to changes in preload and therefore STV remains low.

## Variability of preload and arrhythmic response to dofetilide

To impair repolarization reserve beyond the point in which remodeled hearts show TdP arrhythmias, we treated animals with the class III antiarrhythmic dofetilide. Consistent with previous results, dofetilide did not induce TdP arrhythmia at AAVB, before cardiac remodeling (0/5, 3 alternating and 2 constant PQ).[16] The full dose of dofetilide prolonged LV MAPD from 265±27 ms to 384±67 ms at 10 min ($p<0.05$)), with no significant change in STV (0.7±0.3 ms to 1.5±0.5, $p=0.10$).

At CAVB, dofetilide infusion could reproducibly induce TdP, irrespective of PQ variation: 3/6 at constant PQ and 4/7 at alternating PQ interval. Alternans in

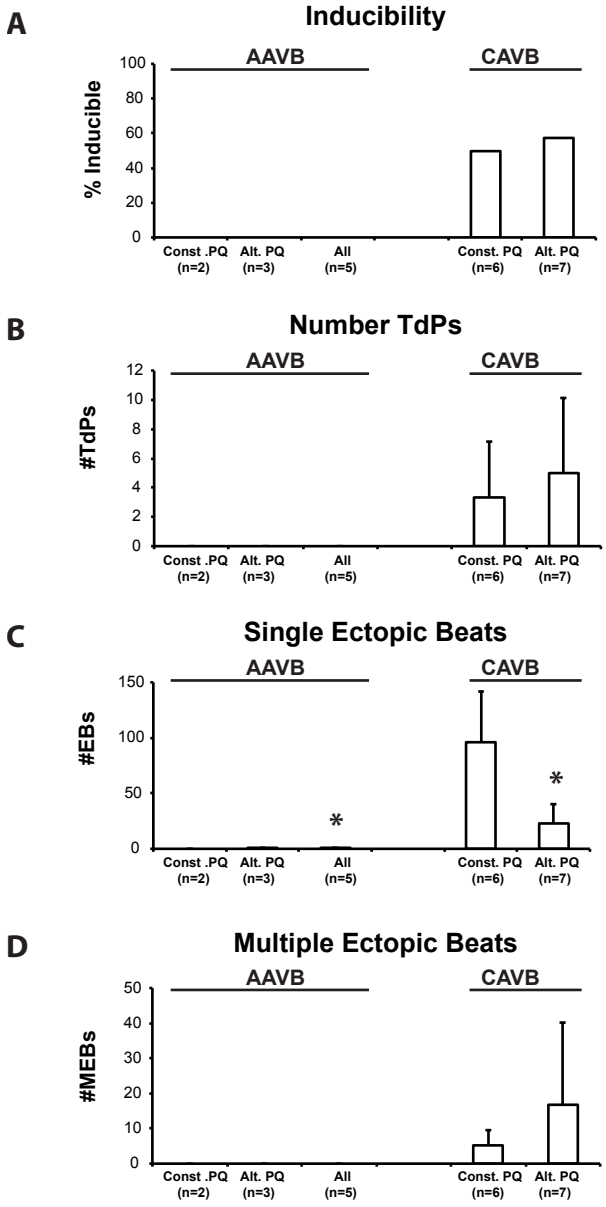


*Figure 4: Arrhythmic response to dofetilide over 10 minutes at acute and chronic AV-block (AAVB, CAVB), at constant and alternating PQ interval. *: p<0.05 versus CAVB constant PQ.*

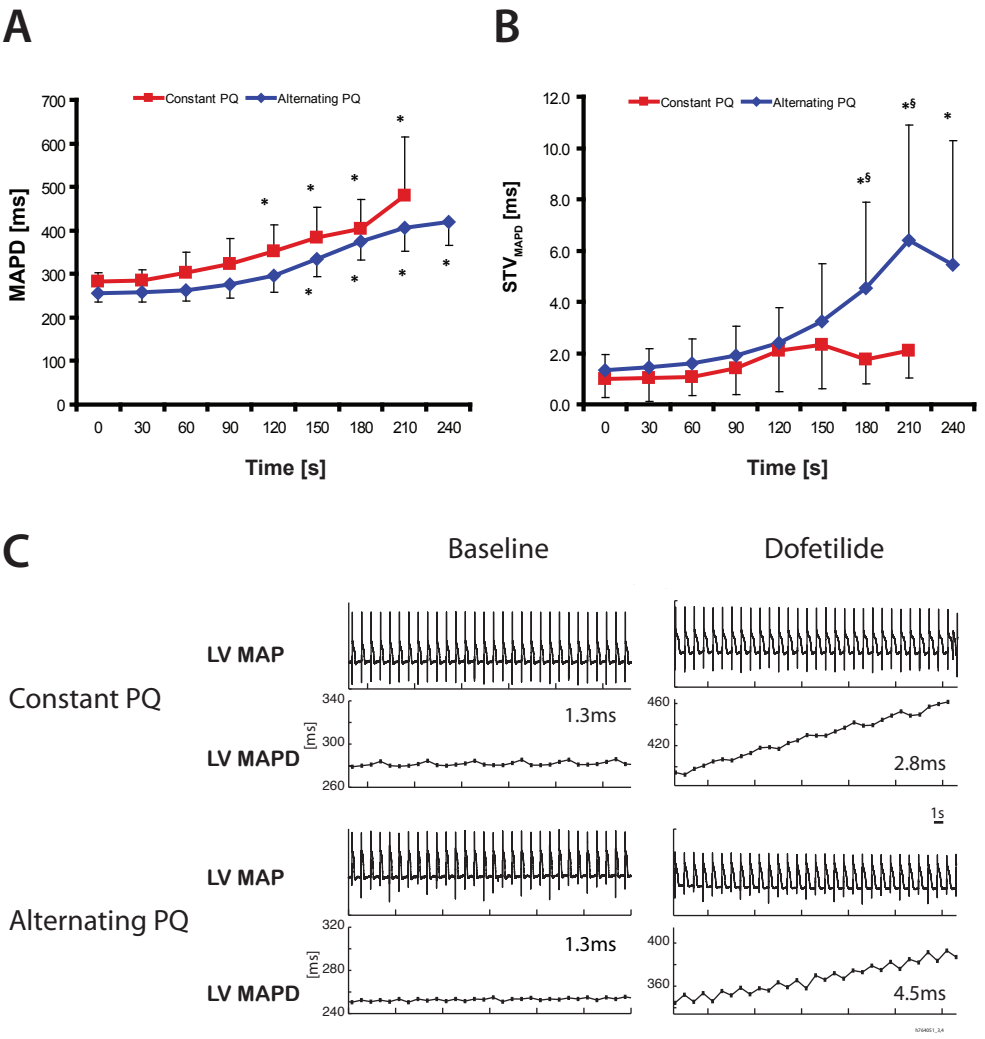


*Figure 5: Effect of dofetilide on LV MAPD and STV.A. After infusion of dofetilide LV MAPD increases equally for constant and alternating PQ. B. Only at alternating PQ increases STV significantly after dofetilide. C. Beat-to-beat traces of LV MAPD at baseline and after dofetilide. Top tracings recorded at baseline and at first ectopic beat 190s after start of dofetilide infusion with constant PQ. Periodic variation is visible corresponding to ventilation frequency. Bottom tracings recorded at the same time points in the same animal, but with alternating PQ. The effect of the PQ alternans on LV MAPD increases after dofetilide. TdP arrhythmias were induced in both experiments. Values of STV are printed in the graphs. *: p<0.05 versus baseline.§: p<0.05* versus constant PQ.

PQ did not affect number of multiple ectopic beats or number of TdP episodes, but reduced the number of single ectopic beats significantly(Figure 4). Dofetilide prolonged LV MAPD to similar extent for constant and alternating PQ (Figure 5a). Only at alternating PQ did STV increase significantly before TdP induction (Figure 5b). Figure 5c shows representative tracings of MAPD after dofetilide infusion. Both tracings were recorded in the same animal and TdP was induced at both constant and alternating PQ interval. At alternating PQ, MAPD variation was dominated by an alternating pattern, synchronous to PQ variation. At constant PQ, MAPD variation was determined by periodic patterns of 5 beats, consistent with the frequency of mechanical ventilation, and linear MAPD prolongation.

To determine the predictive value of baseline STV for TdP inducibility we compared STV values for inducible and non-inducible animals, at constant and alternating PQ. Inducible animals presented with a higher baseline STV at alternating PQ (2.7±0.4 ms versus 1.5±0.4 ms, $p<0.5$, inducible and non-inducible respectively), a difference that was absent when preload was constant (0.4±0.5 ms versus 0.3±0.3 ms, NS).

Again, but now following further perturbation of repolarization capacity that allows TdP arrhythmias, the reduction in repolarization reserve as measured by an increase in STV can only be probed upon mechanical variation in preload. The response of STV to PQ variation at baseline correlated with the outcome of the arrhythmic challenge. Furthermore, we demonstrate that in the dog CAVB model, with its AV dyssynchrony as an intrinsic property, the absence of mechanical variation as achieved by our constant PQ interval pacing does not preclude the occurrence of TdP arrhythmia.



## Discussion

The results of this study can be summarized as follows: 1) In anesthetized dogs, variation in preload increases STV only when ventricular remodeling (a reduced repolarization reserve) is present (CAVB), 2) this increased STV in baseline corresponds to an enhanced susceptibility to dofetilide-induced TdP with susceptible dogs showing a stronger increase after preload variation, and 3) the further reduction in repolarization strength by dofetilide is only seen in an increase in STV as preload variability is present. From this, it is concluded that STV is able to 1) quantify repolarization reserve in baseline and 2) predict the risk for repolarization dependent ventricular arrhythmias.

## Baseline STV and an increased risk for drug-induced TdP

Previous publications have shown the relation between ventricular remodeling, STV in baseline and the susceptibility to drug-induced arrhythmias in the anesthetized CAVB dog. The CAVB dog is characterized by a) an unpredictable location (focus) that is responsible for its idioventricular rhythm, b) AV-dyssychronization or alternating PQ, and c) a slowing and more irregular RR-interval after dofetilide. Thomsen et al. showed that in these CAVB dogs, the STV determined at baseline was increased in comparison to unremodeled hearts from dogs in sinus rhythm or in AAVB. This was presumably related to the presence of cardiac remodeling, which resulted among others in a reduced repolarization reserve. The latter was deduced from the fact that 1) electrical remodeling had taken place and 2) it was concomitant with the development of susceptibility to drug-induced arrhythmia.[16] The highest baseline STV values were measured in susceptible animals as compared to their drug resistant counterparts. Clinically, Hinterseer et al., confirmed this relation between baseline STV and arrhythmia susceptibility in patients with 1) a history of drug-induced TdP,[3] 2) inherited long QT, and 3) heart failure.

To quantify a reserve, it is mandatory to have a stimulus that can measure the remaining power or strength available in the heart. The trigger that enables quantification of repolarization reserve using STV, however, has not been determined.

## Baseline STV and quantification of repolarization reserve

In this study, variability in preload was identified as an external stimulus, which translates increased inherent lability into measureable MAPD variability. Variation of preload at a constant rate with controlled activation originating in the LV, as induced by PQ variation or mechanical ventilation, had no effect on repolarization variability at AAVB while similar preload changes increased STV in remodeled hearts at CAVB. This opens perspective to apply changes in preload to safely increase the predictive power of STV as an arrhythmic marker, both in humans as in animal models.

During the course of this study, a publication by van der Linde et al. sparked renewed interest in the relation between mechanical and electrical systole,[17] as seen in our figure 3. In normal hearts (i.e. cardiomyocytes), repolarization initiates mechanical relaxation. The electro-mechanical window (EMW), defined as the time between the end of the T-wave and the end of the LVP envelope, will therefore be positive in normal individuals, but a negative EMW is a sign of arrhythmic risk.[18] Van der Linde et al. proposes a negative EMW as a risk marker for drug-induced arrhythmia in animal models. When we evaluate EMW from our data, a negative trend during cardiac remodeling (56±38 ms at AAVB to -1±68 ms at CAVB, p=0.13) was noted. Dofetilide shortened the EMW at AAVB to -34±51 ms at

3 minutes ($p<0.001$) and -76±10 ms at 10 minutes ($p<0.001$). Despite the negative EMW no ventricular arrhythmias were seen and STV was not increased. However, the arrhythmic threshold for EMW may not have been reached: van der Linde et al., reports a 100% TdP induction by $I_{Ks}$-block and isoprenaline at -109 ms in the fentanyl/etomidate-anesthetized beagle, but complete suppression at -23 ms after atenolol.

## The increase in STV after dofetilide in CAVB dogs with PQ alternating circumstances

Our group and others showed in animal models that drugs that induce TdP will lead to increased STV values, irrespective of prolongation of QT or LV MAPD.[7, 8, 19, 20] Interventions that suppress or prevent TdP, also lead to a decrease in STV.[15, 21] A similar relation between repolarization variability and arrhythmogenicity is present in isolated cardiomyocytes, where STV of transmembrane action potential duration precedes pharmacological induction of early after-depolarizations (EAD).[10-12] However, evidence exists that electrical coupling of cells through connexins can suppress both STV and EAD formation.[13] Therefore, in the in-vivo situation, where cardiomyocytes are well coupled, the relation between unstable repolarization and variability of repolarization duration may be weakened.

In our hands, modulation of PQ interval is able to control STV after dofetilide has been administered (Figure 5). Constant PQ intervals maintain STV at a lower level, whereas alternating PQ intervals do increase STV. However, in these paced dogs, this has no consequence for TdP inducibility which is similar in both instances. The further increase in STV after dofetilide is therefore only seen when variation in preload is present. It is important to say that this is normally the case in our regular CAVB animals. At idioventricular rhytm variation in PQ-intervals is present, providing the trigger to the ventricles. However, controlling the pattern of variation by pacing may increase sensitivity and stability of STV over time. The association between STV and TdP can be explained by the response of STV to preload variability being augmented by the same two factors that together induce TdP arrhythmias in the CAVB dog: i.e. electrical remodeling in combination with pharmacological $I_{Kr}$-block.

Because our study was not designed to investigate EMW, we have limited data on EMW after dofetilide in CAVB. As compared to AAVB, there was a tendency to a more negative EMW seen at 3 minutes dofetilide (-150±60, n=2, $p=0.07$). We do wish to note that the time from Q-wave to the end of the LVP envelope was not decreased by remodeling (393±47 ms at AAVB to 396±53 ms at CAVB) or dofetilide (390±47 ms at AAVB 10 min, 394±83 ms at CAVB 3 min n=2, all NS). The reduction of EMW was

therefore fully attributable to QT prolongation associated with electrical remodeling and $I_{Kr}$-block.

## Mechanistic link between BVR and arrhythmia

Van der Linde et al. do not provide a mechanistic explanation for a negative EMW. If it would exist on a cellular level, this would result in a contraction duration that is not affected by prolongation of the transmembrane action potential. In patients with congenital long QT, Haugaa et al. provide intraventricular dyssynchrony in contraction patterns as an explanation: the largest dyssynchrony is seen in patients with a history of ventricular arrhythmia.[22] Because ventricular pressure during systole can only be maintained if a large amount of cardiomyocytes contract simultaneously, dyssynchrony of contraction during late diastole will result in premature decrease in LVP. The QT interval, on the other hand, is determined by the latest repolarizing cells and dispersed prolongation of repolarization will result in increased QT time, which has been reported after dofetilide.[23] Therefore, dispersed prolongation of repolarization, without affecting the relation between APD and contraction duration on a cellular level, will result in a more negative EMW.

Alternating PQ increased response of STV after dofetilide, but only in a setting of reduced repolarization reserve in CAVB. From the LVP and sonomicrometry data, it is clear that the mechanical effect of altered PQ interval and ventilation is most prominent in late systole, isovolumic relaxation and rapid filling during early diastole (Figure 3). With a negative EMW these mechanical alterations in time will overlap with late repolarization, allowing the first to influence the latter. Variation of PQ itself however, did not increase the arrhythmic response to dofetilide. From this we conclude that STV is not a prerequisite for arrhythmia, but probably is the result of the same electrophysiological changes that predispose to TdP. At the other hand, no change in STV despite PQ alternans and a drug still indicates that the drug is not reducing repolarization reserve and therefore can be considered safe. In the CAVB dog a negative EMW may facilitate (CAVB), but does not seem to be sufficient to increase STV and induce TdP (AAVB and dofetilide).

## Mechanism responsible for STV

Stretch activated current (SAC), especially in a context of reduced repolarizing current, may translate changes in ventricular volume to changes in action potential duration.[24] Furthermore, SAC is reported to be increased in cardiac hypertrophy.[25] Sudden increase in ventricular volume during late repolarization has been shown to trigger EAD-like depolarizations in cells that are not yet fully repolarized.[24]

Short coupled activation, whether originating from within the cell (EADs) or through artificial stimulation are known to trigger TdP arrhythmias when repolarization reserve is compromised.[26]

To possibly elucidate a role of SAC, we tested the effect of SAC-block by streptomycin (40 mg/kg/5 min i.v., target plasma level 200 μM) in CAVB dogs treated with dofetilide, and compared it with dofetilide without further treatment. To increase group size, 4 animals from a different study were included. In inducible animals, streptomycin was able to completely suppress TdP in the 10 minutes after infusion of streptomycin. In contrast, in untreated experiments, TdP remained present in 44% (4/9) of the animals at the same time interval. The maximum LVP, which was increased by dofetilide, returned to baseline values after streptomycin. This normalization is also seen after complete suppression of TdP arrhythmias by verapamil (0.3 mg/kg/5 min i.v.), which is known as an $I_{CaL}$-blocker. Whether the effect of streptomycin should be associated with $I_{CaL}$-block is unknown: The IC50 of streptomycin to block $I_{CaL}$ is reported at higher concentrations (2 mM) than used to block SAC (200 μM).[27, 28] However we cannot rule out that part of the anti-arrhythmic effect and LVP normalization is attributable to $I_{CaL}$-block.

## Limitations

Although in our study the variation in preload, as expressed in LVP, was similar in AAVB and CAVB we can not rule out that hemodynamic or structural changes at CAVB play a role in the altered response of repolarization. However, Donkers et al showed that end diastolic pressure and wall stress was elevated but constant from acute to 6 weeks AVB.[29] On the other hand, diastolic inner diameter of the left ventricle was increased in CAVB. Therefore, the definition of an equivalent variation in preload is not straightforward and factors other than repolarization reserve may play a role in the increased response of LV MAPD to PQ variation.

In CAVB we observed that beat-to-beat preload variations resulted in variation in local stretch during isovolumic relaxation and early diastole. Because of the small EMW in CAVB, the changes in stretch pattern coincided with the repolarization phase. The negative EMW at AAVB may provide an explanation why the mechanical changes did not affect repolarization However, sonomicrometry signals were not yet stable shortly after placement, at AAVB. Therefore, we cannot exclude the possibility that the variability in stretch patterns itself was a result of the remodeling process.

## Conclusions

In the anesthetized AV-block dog, at paced rhythm, STV of MAPD is a sensitive marker for proarrhythmic remodeling and proarrhythmia after dofetilide infusion. The underlying mechanism is an augmented response of repolarization to beat-to-beat changes in cardiac preload when repolarization reserve is reduced. For use of STV as an arrhythmic marker changes in preload may be a safe way to improve predictive value.

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## Chapter 7

# Automated analysis of beat-to-beat variability of repolarization from monophasic action potentials and electrograms

Peter Oosterhoff, Albert Dunnink, Joep N Maas, Roel van der Nagel, Jet DM Beekman, Thom RG Stams, Marcel AG van der Heyden, Marc A Vos

Medical Physiology, University Medical Center Utrecht, The Netherlands

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## Abstract

Short-term variability of repolarization duration (STV) is proposed as a predictive marker for arrhythmic risk. The beat-to-beat changes in repolarization duration that indicate an elevated risk can be within the range of 2-5ms. Thus measurement accuracy is vital to obtain sensitivity. We propose automatic methods for measurement of monophasic action potential duration (MAP, MAPD) and electrogram based activation recovery interval (ARI), optimized for beat-to-beat quantification. The algorithms were implemented in Matlab software. Results from the automated MAPD analysis closely match previously used manual and semi-automatic analysis, while the new methodology is better defined and standardized. Results from the automated ARI analysis correlate well with MAPD, both for instantaneous value and for STV. Finally, remaining user input for the analysis was further standardized to increase reproducibility. In conclusion, using the proposed methods, automated analysis of STV with high precision is feasible, highly reproducible, and objective.


## Introduction

Abnormal repolarization is related to cardiac arrhythmias in heart failure, congenital and acquired long QT syndrome. Analysis of temporal changes in cardiac repolarization parameters may assist in discovering factors that contribute to pro-arrhythmia and identify patients at increased risk. Variability of ventricular repolarization duration is used in clinical and pre-clinical research as a marker of arrhythmic risk. Berger et al proposed QT variability index as a cardiac risk marker.[1] Its predictive value for sudden cardiac death and arrhythmia was evaluated in several patient populations using surface ECG and recently electrograms from implanted ICDs.[1-4] Thomsen et al. proposed beat-to-beat variability of repolarization duration (BVR) quantified as short-term variability of monophasic action potential duration (MAPD, $STV_{MAPD}$) as a marker of reduced repolarization reserve in dogs with chronic atrioventricular block (CAVB).[5, 6] Recently this method was applied to patients with acquired or congenital long QT syndrome and patients with non-ischemic cardiomyopathy by Hinterseer et al. using manual QT measurements from surface ECG [7-9]. In this thesis we further extended this work to patients with structural heart disease (Chapter 5).

In CAVB dogs volume overload, altered ventricular activation and AV-dyssynchrony after creation of atrioventricular block could be important parameters attributing to cardiac remodeling and increased susceptibility for drug induced Torsade de Pointes arrhythmias (TdP). We present analysis methods for STV implemented in a software package, which address several challenges associated with BVR research:

1) For our pre-clinical research, we have used catheters to record monophasic action potentials (MAP) from endocardial tissue in anesthetized animals. The analysis methods proposed in literature are often theoretically correct in representing local trans-membrane action potential, but may be susceptible to small measurement errors that cloud variations in local repolarization.[10]
2) The use of MAP catheters prohibits the evaluation of STV in awake animals and is a major obstacle for application in a clinical setting. One could use surface ECG as an alternative, but this is prone to movement artifacts and can therefore only be used when the subject (animal or patient) is not moving. The use of chronic electrograms (EGM) may overcome these limitations, because the intracardiac recording is not affected by body movement. Furthermore, chronic EGMs can readily be obtained from implanted pacemakers, ICDs or telemetry devices. The activation recovery interval (ARI) from EGM has been proposed as a measure of repolarization duration that correlates well with local MAPD. However, a validation of EGM based STV has to be performed.
3) For practical reasons the operator of the software is not always blinded to details of the experiment. In some cases the signal itself will give away what drug was used or which procedure performed. To overcome a possible bias in analysis results we attempted to standardize the method of analysis.
4) Commercially available analysis software is often not fully configurable or uses undisclosed, proprietary analysis algorithms. Lack of understanding of the analysis methods may lead to low repeatability and large difference in results between operators and between analysis systems. Using manual on-screen caliper based methods to estimate MAP duration (MAPD) may limit detection of the typical 2-5 ms beat-to-beat changes that are relevant for BVR measurements.[10-12]



## Methods

For validation of the algorithm signals collected in previous studies were used. We refer to the original publications for details on study setup.[12, 13] In short: under anesthesia radiofrequency ablation of the atrioventricular node was performed in healthy mongrel dogs (Marshall, New York, USA). Animals were returned to the stables at spontaneous idioventricular rhythm (IVR).[12, 13] After at least three weeks of remodeling, under general anesthesia, left ventricular endocardial MAPs were recorded before and after challenge with a torsadogenic drug.
Signals were recorded with a PC-based data acquisition system (IOX, EMKA, Paris, France [13] or ScapSys, Maastricht University, Maastricht, The Netherlands;[12] digitized at 500Hz and 1kHz respectively). A DC-coupled amplifier was used to record the MAP signals. Chronic unipolar electrograms were recorded using an

implantable telemetry device, sampling at 800Hz (bandwidth 0.7-250Hz) and stored on an external Holter (Vitatron, Arnhem, The Netherlands).[12] We quantified BVR by calculating STV from LV MAPD and ARI over 30 beats, according to equation 1. This measure is based on Poincaré plots, where STV is defined as the average distance of all data points to the line of identity.[5] After an arrhythmic challenge STV was determined before induction of ectopic activity.

$$(1) \qquad STV_{MAPD}, STV_{ARI} = \sum_{1..30} | D_n - D_{n-1} | / (30*\sqrt{2}), \text{ D being MAPD or ARI}$$

The analysis algorithms presented here were implemented in MatLab (Mathworks, Natick, USA). After manual validation of software functioning according to algorithm specifications, MAPD results of the software implementation were compared to previously used methods. Results from EGM analysis were compared to automatically determined MAPD and $STV_{MAPD}$ at the same time point. Part of this validation has been published before.[12]

# Results and discussion

## Method of MAPD analysis

Several fiducial points have been proposed for MAPD analysis. Franz proposed the steepest upslope of the MAP as the start for MAPD measurement.[14] However, we found that residual biphasic far-field signals often contaminate the MAP signals, making it difficult to accurately detect the moment of steepest upstroke of the MAP. The fixed threshold method used by Lee et al. may require correction for the MAP amplitude to attain consistent beat-to-beat detection of the same phase of activation.[15] The method proposed in the current paper uses the maximum of the MAP as the start of the MAPD measurement. Although this will slightly underestimate MAPD, it may reduce random measurement errors, thereby improving beat-to-beat detection of small fluctuations in MAPD that make up $STV_{MAPD}$. The input of the user is limited to assigning a window that contains the MAP maximum, but no artifacts that are of larger amplitude (e.g. pacing spikes).

## Implementation of MAPD analysis

After the software has imported the data file, the user can select the channel containing the MAP signal (Figure 1). A threshold is applied to the derivative of a second channel to get a rough detection of all relevant beats that will be analyzed. Results can be validated by comparing detection markers to the original MAP signal, reviewing the resulting RR-intervals or inspecting the resulting overlay plot of all MAPs detected. The user can set a window relative to the moment of threshold

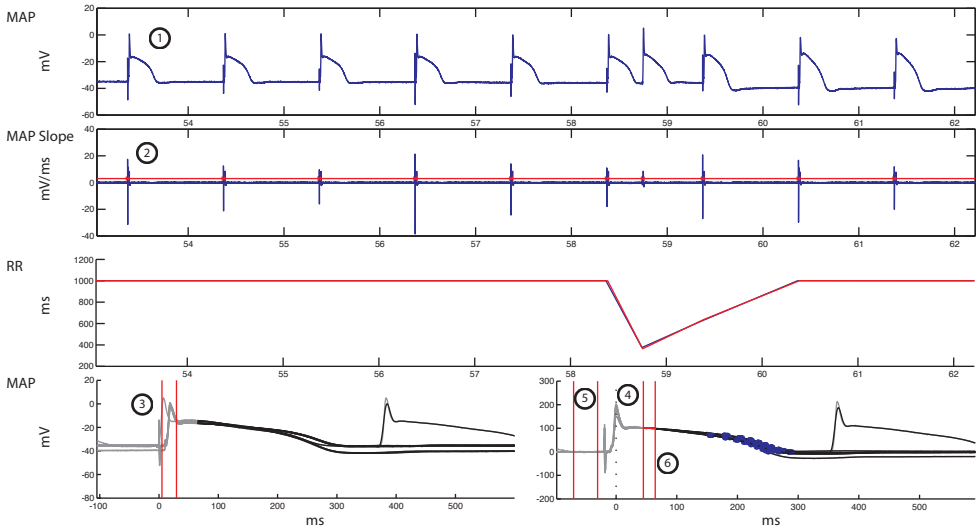


*Figure 1: Overview of the analysis of monophasic action potential (MAP) duration: 1) After file import the MAP signal is selected. 2) A rough beat detection is performed using a threshold on the slope of a recorded signal (red line). Markers indicate where the software detects a potential MAP. The RR plot can be used to detect missed, aberrant or spurious beats. 3) All detected beats are shown in an overlay plot, synchronized on the threshold detection of the slope signal. The user can define a window where the signal maximum is selected as the start of the MAP. Care should be taken to exclude pacing artifacts when these have larger amplitude than the MAP. Original signal is plotted in grey and the smoothed, up-sampled signal in black. 4) In a further analysis step a second overlay plot is created where all MAPs are synchronized on the start of MAP. 5) The user defines a window relative to the start of the MAP, which is used to calculate the DC-offset. Care is taken that this window does not include pacing artifacts or far field signal components. 6) The user defines a window relative to the start of the MAP, which is used to calculate the level of the MAP plateau (0% repolarization). Calculated MAP durations at several percentages of repolarization are plotted on the MAP signal (blue markers).*



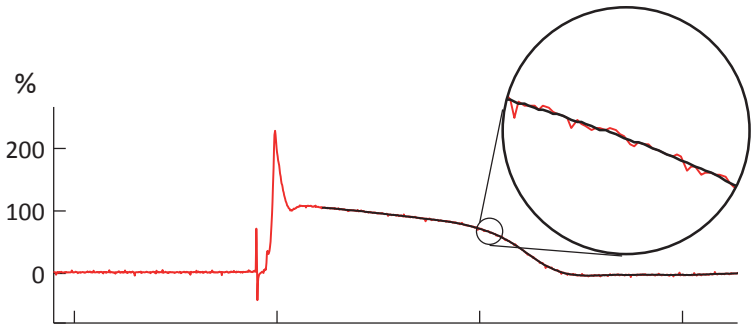


*Figure 2: Spline fitting is used to remove noise and, when necessary, increase time resolution of the MAP signal, without affecting the original morphology. Original signal can been seen in red, while the smoothed signal is illustrated in black.*

crossing; the maximum of the MAP signal within this window will be used as the start of the MAP. Relative to the start of the MAP, windows are defined to calculate DC-offset and plateau amplitude. Action potential durations at a configurable set of fractions of repolarization (e.g. $MAPD_{90}$) are calculated. To reduce errors introduced by noise and low sampling frequency, spline interpolation is used to smooth the MAP signal and improve time resolution through interpolation (Figure 2). This interpolated signal is used to determine the end of MAPD, since the repolarization phase contains primarily low frequency components. Detection of the local maximum of the activation phase of the MAP will not benefit from smoothing or interpolation. For the current validation the signals sampled at 500Hz/2 ms are interpolated to 2kHz/0.5ms.

Figure 3 shows the relation between manually determined MAPD (ECGView, Maastricht University, Maastricht, The Netherlands) versus automatically determined MAPD and $STV_{MAPD}$ at 90% repolarization.[13] This manual method was used in the original publications on $STV_{MAPD}$ in the CAVB model.[5, 11, 13, 16] Data was recorded in CAVB dogs at baseline and after a fast (5min) or slow (60min) infusion of enacadine (NS-7, See Detre et al for details[13]). The $R^2$ of 0.89 for LV MAPD confirms the close relation between manual and automatically measured MAPD. Correlation is comparable to results found by Franz et al.[10] For $STV_{MAPD}$ we find a reasonable $R^2$ of 0.57, considering the 8-bit signal resolution and inherent errors of manual measurement.

In more recent publications, a semi-automatic method was used (AutoECG, EMKA, Paris, France) that applies user-defined templates to match fiducial points for activation, isoelectric line and plateau, and calculate MAPD at 90% repolarization.[12, 17-19] Because of a high pass filter built into the software $MAPD_{90}$ was sometimes underestimated and more resembling $MAPD_{80}$. Analysis by multiple analysts showed lower inter- and intra-observer variability for STV from $MAPD_{80}$ compared to $MAPD_{90}$. Because substantial differences between STV calculated at 80% and 90% repolarization were confined to recordings that expressed high inter- and intra-observer variability for $MAPD_{90}$, it was decided to use $MAPD_{80}$ for STV calculation in future studies (chapter 6).

Figures 4A and B illustrate the close correlation between the template based $MAPD_{90}$ and automatically determined $MAPD_{80}$ including STV. The precision of the automatic method is further illustrated by the result in chapter 6 where in dogs at acute AV-block $STV_{MAPD}$ values were measured of 0.3±0.1ms, which is close to the theoretical lower limit of 0.1 ms at the sampling frequency of 2kHz used in that study.[12] Meanwhile, at CAVB the method was sensitive enough to detect an increase of 0.5 ms resulting from mechanical ventilation. We conclude that the automatic

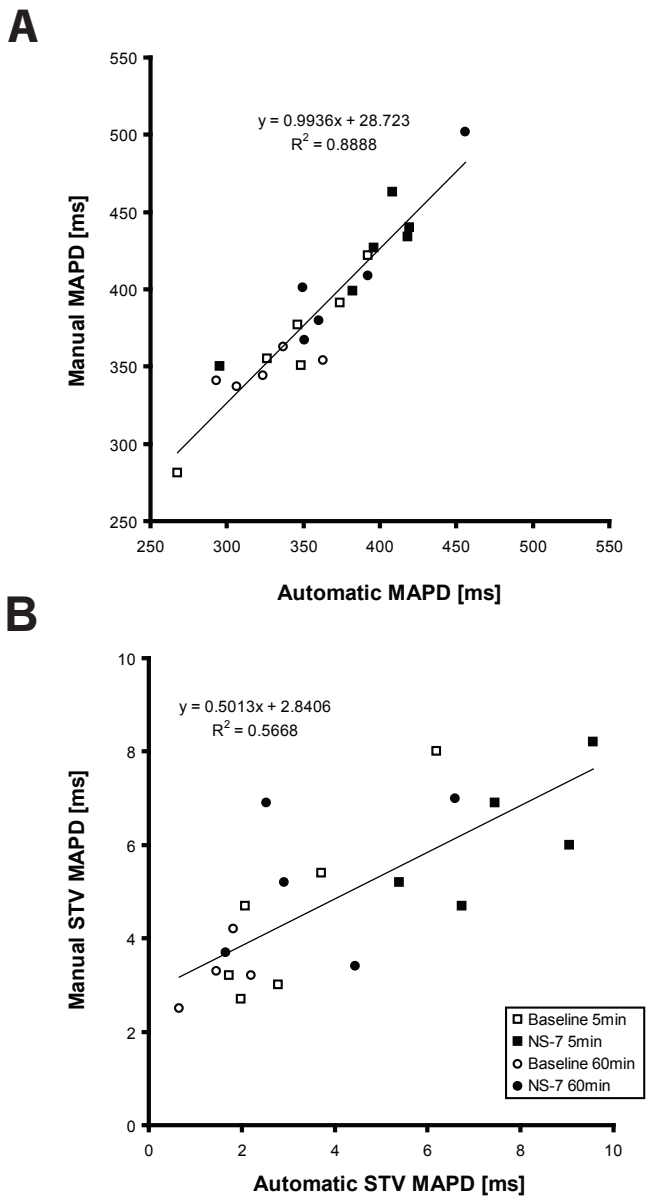


*Figure 3: Relation between manual and automatically determined MAPD and $STV_{MAPD}$. Values recorded at baseline and after fast (5min) or slow (60min) infusion of the experimental neuroprotective drug enecadine. Original MAP signals from Detre et al.*[13]

MAPD method provides high precision and objective, standardized measurements, while results are comparable to previously used methods.

## Method of ARI analysis

To allow for awake measurements and determine feasibility of STV measurement in pacemakers or ICDs, we incorporated calculation of activation recovery interval (ARI) from EGM.

We evaluated the unipolar EGM as an alternative source of information on BVR. Chronic EGM has certain advantages over the MAP, by not requiring acute placement of catheters, hence allowing awake measurements. Furthermore, the position of measurement is identical from experiment to experiment. However, the unipolar EGM is more susceptible to external interference (e.g. from 50 or 60 Hz electric line frequency). In the AV-block dog particularly, P-waves can coincide with the ventricular T-wave and hamper ARI measurement.

The ARI has been shown to correspond to the local MAPD.[20, 21] The steepest downslope of the unipolar EGM during the QRS is used as the moment of activation and start of the ARI. For bi-phasic and negative T-wave the steepest upslope of the unipolar EGM during the T-wave is used as moment of repolarization and end of the ARI (Figure 5a). However, controversy remains over the methodology of ARI measurement for strictly positive T-waves. Theoretical models suggest that also for positive T-waves the upstroke should be used as end of the ARI and this is supported by experimental data.[21-23] However, others have published better fit between MAPD and ARI when using the downslope of the T-wave for positive T-waves (Figure 5b),[20, 24] while difference in methodology of MAPD measurement can only in part explain these conflicting results. From a sub-analysis, we found ARIs calculated by the latter method better to fit our MAPD data. Our positive T-waves sometimes show a non-zero ST segment without a clear maximum, as figure 5b illustrates. Very much different from the positive T-waves Coronel et al. report, but not unlike T-waves reported by Haws et al. as seen in figure 1d of that publication.[23] Without going into the background of this phenomenon, for STV measurement a well-defined downslope that is influenced by remote repolarization is preferred over an upslope with local origin which timing cannot be determined accurately. There are situations where one is limited in placement of the EGM electrode. For many clinical applications electrode location is dictated by clinical practice; most pacemakers or ICDs often only have a ventricular electrode positioned on the right side of the heart, while in our hands BVR derived from the left ventricle holds more relevant information on repolarization reserve.[5] In these cases it may be beneficial to include the end of the T-wave for repolarization duration, as it contains the far-

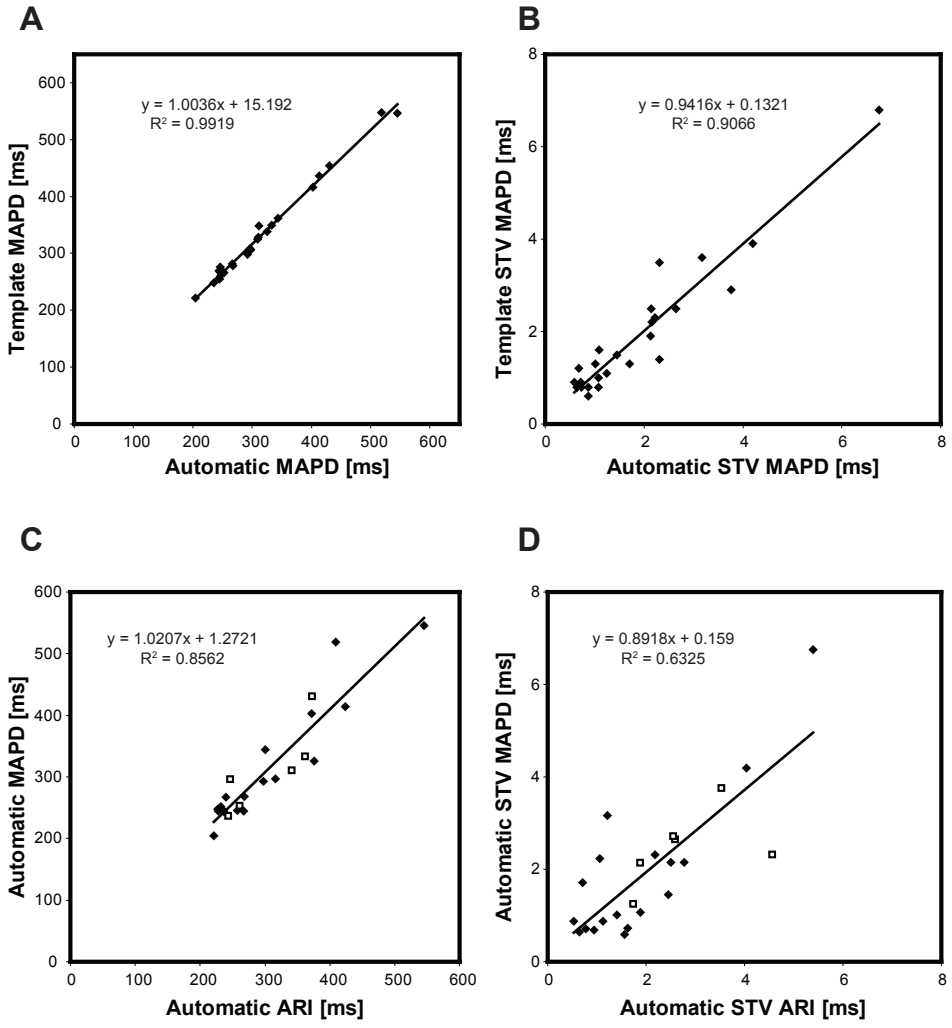


*Figure 4: Relation between template-based LV monophasic action potential duration (MAPD), automatic MAPD measurement and automatic activation-recovery interval (ARI). A: Scatter plot of template based $MAPD_{90}$ versus automatic $MAPD_{80}$ (see text). B. Scatterplot of STV of same. C. Scatter plot of automatic MAPD and ARI recorded at baseline and after dofetilide. Positive T-waves on the electrogram are represented by open markers, negative or biphasic T-waves by closed markers. D: Scatterplot of STV of same. Original data from Oosterhoff et al.*[12]

field signal from the later repolarizing left chamber.[21] Hence, we used the method proposed by Berger et al. to determine STV from right ventricular EGM in patients with structural heart disease in chapter 5.[1] This method incorporates the whole JT-segment, including a possible U-wave, in the calculation of repolarization duration.

## Implementation of ARI analysis

After selecting the EGM channel, a first beat detection step is performed using a threshold detector, identical to the MAPD analysis. In the overlay plot the user can set a window relative to the moment of threshold crossing. The minimum of the slope of the EGM signal within this window will be used as the start of the ARI measurement. Relative to the start of the ARI, the user can set a window where the maximum or minimum slope of the EGM signal is used to calculate the ARI (Figure 5). For biphasic or negative T-waves maximum slope is used as the end of the ARI. For positive T-waves we use the minimum slope of the EGM as end of the ARI.[20] For the end of the ARI spline interpolation is used to calculate the signal derivative and increase time resolution, similar to the MAPD analysis.

The automatic ARI measurement has been validated recently in paced CAVB dogs. Both MAPs and EGMs were recorded at baseline and after dofetilide, both at a rate of 60 bpm and at 100 bpm. Figure 4 c and d show the scatter plots for automatically determined MAPD and ARI and for STV of both. Resulting $R^2$ was 0.86 for MAPD versus ARI, and 0.63 for STV. In figure 6, a tracing of MAPD and ARI is presented recorded after intravenous infusion of dofetilide. Both measures of repolarization duration show a similar pattern of prolongation and increase of STV before induction of ectopic activity and eventually TdP. The beat-to-beat differences in repolarization duration and level of STV may be related to the distance between the MAP and the EGM electrode, since a good MAP signal could not always be acquired at the site closest to the EGM electrode. It is also possible that the field-of-view of both methods is not identical, or ARI measurements are disturbed by ischemia, structural abnormalities or local dispersion of action potential morphology during dofetilide infusion.[25]

## Standardization of analysis

Although we have not shown a higher accuracy than manual measurement, in our experience an automated analysis results in shorter analysis time and a well-defined analysis process with shorter learning curves.

No results of the STV calculation are presented in the screen where the user controls and inspects the MAPD and ARI analysis. This allows the user to optimize and judge analysis of the separate beats without prior knowledge of the resulting STV values.

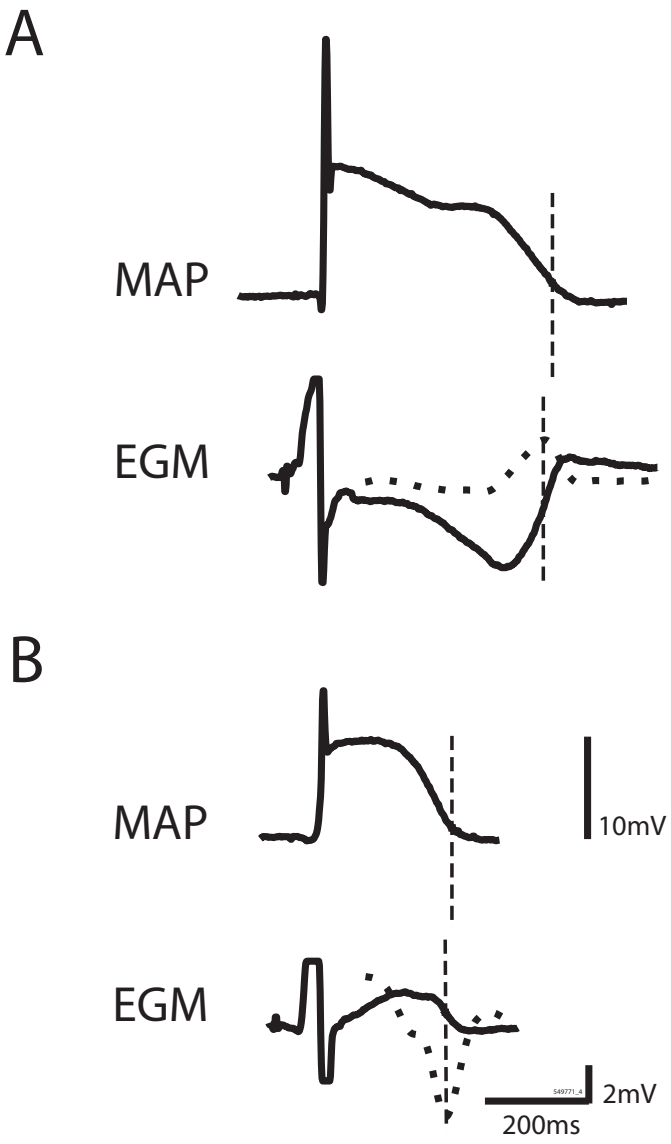


*Figure 5: Determining the moment of repolarization from electrograms (EGM). In each panel a monophasic action potential (MAP, top) and an EGM (bottom) is depicted with a vertical line at the moment of repolarization: for the MAP 90% repolarization is used, while for the activation-recovery interval EGM repolarization is determined by the signal derivative (dashed line), using the modified Wyatt methodology.[20] A: Example with a negative EGM T-wave morphology. B: Example with a positive EGM T-wave morphology. Figure from Oosterhoff et al.[12]*

In a different screen MAPD, ARI and STV results are presented as time series, together with all original and processed signals (e.g. ECG or smoothed MAP signals). Here the user can determine the timing of relevant events as induction of ectopic activity, which hampers STV calculation, or detect anomalous beats that were missed in previous analysis steps. Overlay plots with preceding beats can be generated to inspect beat-to-beat morphology changes and guide further analysis. Calipers allow for evaluation of up to three signals at the same time point (absolute time, time between cursors, local signal value) or over the same interval (minimum, maximum, average).
The result of the analysis together with all settings can be saved to a binary file for later reference or repeated analysis if analysis methods are modified. Selected analysis results can be exported to Excel format as beat-to-beat results or moving average for further processing and statistical analysis.

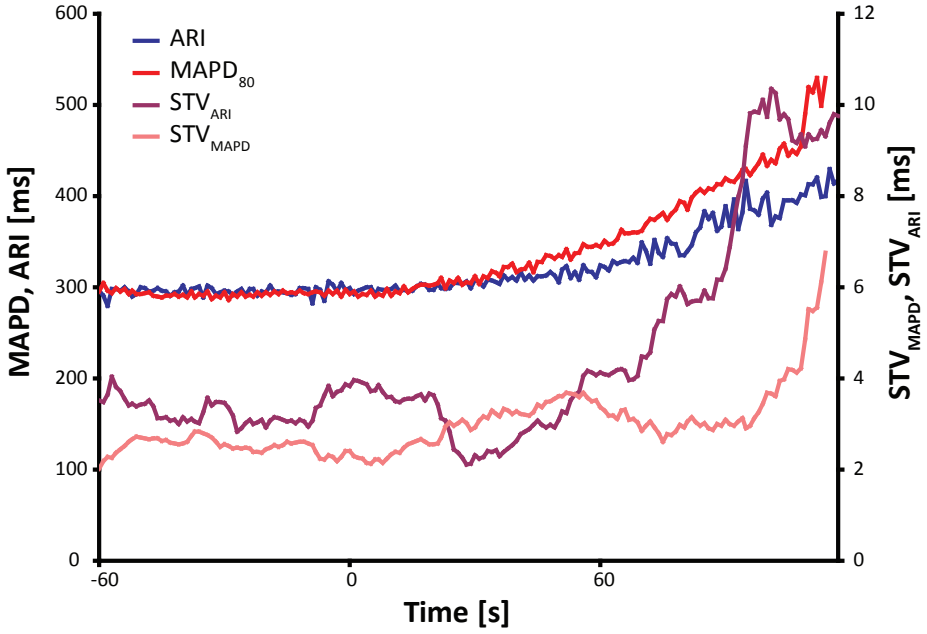


*Figure 6: Tracing of monophasic action potential duration (MAPD) and activation recovery interval (ARI) from EGM with short-term variability (STV) of both parameters after dofetilide infusion (25mg/kg/5min starting at t=0).*

### Transparency and flexibility of analysis

The analysis algorithms presented here were implemented in MatLab (Mathworks, Natick , USA), which allows code execution on Windows, OS X and Unix based platforms. An advantage of having access to the source code of the analysis software is the ability to modify and expand the derived parameters. Commercial software packages often limit the user in the types of analyses that can be performed, and details of the methods that are available are sometimes not disclosed. A better understanding of the methodology used improves repeatability of the analysis and shortens the learning curve.

## Conclusion

We have presented analysis methods for MAPD and ARI, implemented in Matlab software that can be used on multiple software platforms. It provides well-defined, objective methods for MAPD and ARI measurement optimized for STV measurement. Results correlate well with previously used manual and semi-automatic measurements.

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# Chapter 8

## General discussion

Peter Oosterhoff

## Introduction

In this thesis we have explored the clinical application of short-term variability of repolarization (STV) as a marker of arrhythmic risk. To 1) identify individuals at increased risk (risk stratification) and to 2) guide anti-arrhythmic therapy by monitoring risk in a device (risk monitoring). We found the chronic electrogram to be a reliable source of STV, which enables a monitoring in an implantable device (e.g. ICD)(chapter 3). An increase in heart rate through ventricular pacing was found to increase repolarization reserve, which was reflected in a reduction of STV, both at baseline and after dofetilide (chapter 3). For evaluation of an algorithm that would modulate heart rate guided by STV-based risk estimation, prolonged testing would be required, limiting us in the use of anesthetics. However, we found that anesthesia was a critical factor in our current method of arrhythmia induction by $I_{Kr}$-block (chapter 4). As a first step to STV monitoring in a clinical setting we showed long-term predictive value of STV derived from ICD electrograms for arrhythmic death in a patient population with structural heart disease (chapter 5). To further improve STV as a risk marker we investigated the mechanisms linking STV to arrhythmic risk (chapter 6). We found the beat-to-beat changes in preload in the chronic AV-block dog (CAVB), resulting from the asynchronous atrial and ventricular rhythm, to be critical for the response of STV to changes in repolarization reserve. This allows us to further improve sensitivity and specificity for use of STV as a marker of arrhythmic risk. In chapter 7 we presented the validation of the analysis algorithms we developed and used when performing the aforementioned studies.

## Aspects of the AV-block model

The animal model used in this thesis: the CAVB dog is presented in chapter 2. The development of compensatory hypertrophy, without symptoms of cardiac failure, and an increased susceptibility to polymorphic ventricular tachycardia linked to the occurence of early after-depolarizations, makes it a relevant model for prediction and prevention of repolarization related tachycardia in man. Cardiac arrhythmias are already seen in the pre-stages of heart failure, where symptoms are mild.[1] The mechanisms that play a role in arrhythmia are not yet fully understood, but like in the CAVB dog, early after-depolarizations are believed to play an important role in initiation and perpetuation of cardiac arrhythmias, especially in non-ischemic cardiomyopathies.[2, 3] The anesthetized CAVB dog shows a 70-80% incidence of dofetilide-induced torsade de pointes (TdP), which is reproducible in the same animal[4]. These features allowed us to evaluate preventive measures in repeated experiments. We have sought to control a number of parameters that take part in the arrhythmic test. Dofetilide is known to prolong the RR interval in the animal at

idioventricular rhythm.[5] Acute cardiac pacing during the experiment allowed us to control the activation pattern and ventricular rate, thereby isolating the preventive effect of an increased heart rate (chapter 3). Increasing heart rate through cardiac pacing, to improve a compromised repolarization reserve, is a therapy that could easily be implemented in an ICD providing there is real-time feedback on arrhythmic risk to indicate when intervention is appropriate.

We identified variation in cardiac pre-load, which was not controlled in previous studies, as an important determinant of short-term variability of repolarization (STV). Moreover, we found in the absence of preload variation, TdP was no longer preceded by an increase in STV, thereby prohibiting its use as a risk marker. Apparently coupled cells in the intact heart do not reveal lability of repolarization as variation in repolarization, unless an external stimulus is applied. The response to small beat-to-beat pre-load variations revealed a decrease in repolarization reserve. A systems approach may be warranted for a clinical application of repolarization variability as well; when monitoring STV in a heterogeneous patient population during daily activities, the heart receives many uncontrolled stimuli from its environment (e.g. posture, heart rate, level of activity). To interpret the observed response of repolarization, information is needed on the underlying stimulus. Savelieva et al. found an increased response of QT to atrial or ventricular ectopic beats in patients with VT but preserved ejection fraction (EF>40%).[6] By monitoring the environment of the heart (e.g. blood pressure or preceding ectopic beats), or by intervention (e.g. preload changing by pacing) the sensitivity for these specific stimuli can be determined; when the stimulus is known, one can more easily distinguish physiological from pathological response of repolarization.

## Use of the CAVB dog for risk monitoring research

Although the anesthetized CAVB dog is an appropriate model to study the mechanism of drug induced TdP, it may be less suitable for developing markers for continuous risk monitoring. The outcome of an arrhythmic challenge is typically an all-or-nothing response. We have identified electrical remodeling, anesthesia and pharmacological $I_{Kr}$-block as challenges that are required to reduce repolarization reserve to a critical level and induce TdP. Changing factors like the type of anesthesia can have a major impact on arrhythmia incidence (chapter 4), but in identical circumstances induction of TdP is highly reproducible.[4] While these aspects of the model make it an excellent tool for evaluation of pro- or anti-arrhythmic effects of various drugs or interventions, it may be less suited for elucidating the mechanisms linking STV and TdP; the events leading up to the arrhythmia follow each other in rapid succession. Initiation of ectopic activity often occurs within the first three

minutes, limiting the time available to determine STV and quantify the reduced repolarization reserve. We therefore have to choose between accuracy of STV, by using many beats for its calculation, or increased time resolution by reducing the number of beats used. However, the rapid induction of $I_{Kr}$-block is an essential part of the arrhythmic trigger, and prolonging the infusion time of dofetilide may eliminate TdP altogether.[7]

Proper evaluation of an algorithm for preventing arrhythmias by an increase in heart rate, triggered by a continuous risk marker, requires animal experiments with a prolonged period of varying degrees of arrhythmic risk, with multiple, non-life threatening arrhythmias. In this way the effect on number of arrhythmias, defibrillations and average heart rate can be compared to a control situation where the heart rate is left unchanged. Ideally the algorithm should result in a maximal reduction of arrhythmias, with a minimal increase in average heart rate. Therefore, awake experiments would be preferred, but this appears not compatible with our current method of arrhythmia induction using $I_{Kr}$-block, where anesthesia is a critical factor (chapter 4). Drugs that block other ion channels than $I_{Kr}$ should be considered. Recently Lengyel et al. was able to induce TdP in healthy, awake beagle dogs at sinus rhythm using a double hit of $I_{Ks}$- and $I_{Kr}$-block,[8] whereas the combination of $I_{Kr}$-block with isoproteronol also induced TdP under anesthesia[9] and awake (unpublished data). The arrhythmia was preceded by an increase in $STV_{QT}$ in inducible animals, which could not be explained by changes in RR variability. Considering the high RR variation in awake dogs, controlling heart rate by DDD pacing may further increase the sensitivity of $STV_{QT}$ in this model.

## Clinical use of STV

We presented STV data from right ventricular electrograms in patients with structural heart disease and an ICD (Chapter 5). Patients with baseline values for the ratio between $STV_{QT}$ and $STV_{RR}$ ($STV_{Ratio}$) in the highest quartile were almost 2 times as likely to experience fast ventricular tachycardia, ventricular fibrillation or sudden cardiac death in the two year follow up period. Surprisingly, $STV_{QT}$ was not predictive for arrhythmic death, different from the results published by Hinterseer et al.[10-12] Table 1 presents $STV_{QT}$, and $STV_{RR}$ for aforementioned studies. Note that $STV_{QT}$ are much higher in the ICD study, and $STV_{RR}$ in the Hinterseer studies was not different for patients and controls. However, in our study $STV_{RR}$ had an almost significant inverse relation to arrhythmic risk, which is consistent with studies showing low heart rate variability is a risk factor in heart failure patients.[13] This is confirmed by the superior predictive value of $STV_{Ratio}$. The lack of predictive value for $STV_{QT}$ in our ICD study may further be explained by the fact

that Hinterseer compared to matched healthy controls, while we compared ICD patients based on arrhythmic outcome. Furthermore, The higher values of $STV_{QT}$ may also be the result of the high-risk profile and heterogeneity in our population, the use of device based right ventricular EGM with limited resolution, or inclusion of the intracardiac U-wave in the QT interval. As mentioned in chapter 5, the high number of ectopic beats, although not included in the STV measurement itself, may also have lead to electrical or hemodynamic instability in the ICD population. Concluding, interpretation of $STV_{QT}$ in a heterogeneous ICD population may be less straightforward than results from carefully selected patient groups.

For a high-risk population already eligible for ICD implantation, a hazard ratio of around 2 is insufficient to withhold the device from the 'low-risk' group. In my opinion, the real advantage would be in continuous monitoring of STV by the ICD; while evaluation of most current risk markers (e.g. ejection fraction) is not performed on a regular basis, STV monitoring by the device can continue even in-between hospital visits. Home-monitoring systems can report changes in risk that warrant intervention to the treating physician, while an automatic increase in heart rate (chapter 3) may reduce a transient risk (e.g. resulting from hypokalemia). In primary prevention, using conservative settings for tachycardia detection until an increase in arrhythmic risk is detected can prevent inappropriate ICD therapy without reducing patient safety.[14] Swerdlow et al. found indications that repolarization variability on ICD electrograms is increased just before ventricular tachycardia treated by shock or anti tachycardia pacing.[15] However, diagnostic information in current ICDs is not sufficient to evaluate repolarization variability in the minutes preceding an arrhythmia. Considering the low incidence of ICD therapy in most patients, capturing the history of treated arrhythmias using a Holter will most likely not be feasible. The best option to determine the value of ICD based STV monitoring may

*Table 1: Baseline values of $STV_{QT}$ and $STV_{RR}$.*

| **Patient group** | **$STV_{QT}$** | | | **$STV_{RR}$** | | |
|---|---|---|---|---|---|---|
| | **Control** | **Patients** | **Change** | **Control** | **Patients** | **Change** |
| *Drug induced LQTS* | *3.6±1.3* | *8.1±3.7* | *+125%* | *19±16* | *15±11* | *-11%* |
| *Congenital LQTS* | *4.1+_1.6* | *6.4±3.2* | *+56%* | *22.0±16* | *24.5±18* | *+11%* |
| *Non-ischemic heart failure* | *4.1±2* | *7.8±3* | *+90%* | *16±12* | *13±9* | *-14%* |
| | **No SAD** | **SAD** | **Change** | **No SAD** | **SAD** | **Change** |
| *Structural heart disease* | *14(6-29)* | *16(6-33)* | *+14%* | *30(11-58)* | *20(6-51)* | *-33%* |

*Data presented as mean±standard deviation or median with interquartile range. SAD: Sudden Arrhythmic Death. Data from Hinterseer et al.[10-12] and chapter 5 respectively.*

be to include the measurement in current devices and, in patients populations with well defined etiology, compare values shortly before ICD therapy to variation in event free periods. Standardized modulation of paced AA, VV or AV interval may increase sensitivity of STV in a similar way as in the CAVB dog.

## Mechano-electrical interaction and early after-depolarizations in-vivo

It has been suggested that early after-depolarizations (EADs) in small patches of cell underlie repolarization variability in the intact heart.[16] Due to loading mismatch these local EADs would not result in triggered beats, but local MAP may be disturbed. In isolated cardiomyocytes pharmacological induction of EADs is preceded by instability of the action potential duration.[17-19] These EADs are thought to result from reactivation of L-type calcium current during the prolonged action potential or increased $I_{NCX}$ after spontaneous calcium release from the sarcoplasmic reticulum. Electrical coupling of cardiomyocytes has been shown to suppress both repolarization variability and EADs to some extent;[20] the chaotic inward currents that trigger EADS in isolated cells are shunted to neighboring cells. Therefore, in the intact heart additional or stronger triggers may be required to trigger after-depolarizations in multiple cells.

Computer modeling has been used to elucidate how the chaotic behavior of coupled unstable cells can synchronize to form propagated EADs and deteriorate to multifocal polymorphic tachycardia.[21] For an EAD to result in a PVC a minimal number of neighboring cells have to form an EAD simultaneously. The likelihood of this to happen depends on 1) the electrical coupling to neighboring cells, 2) the repolarization reserve of the cells, and 3) the synchronicity of the inward currents in neighboring cells during late repolarization;[22] This is where the negative EMW (chapter 6) comes into play: the stretch activated currents provoked by a sudden increase in ventricular volume during late repolarization can generate an inward current that can trigger a PVC, even in a healthy heart.[23] During a negative EMW isovolumic relaxation and the rapid filling phase will results in global or regional stretch that coincides with the final part of repolarization, leading to synchronous inward current in many cells. If these cells already have a reduced repolarization reserve and tend to express EAD-forming inward currents, the chance that a group of neighboring cells depolarizes synchronously is increased. However, this will not lead to a propagated PVC if the minimum number of cells is not reached. Still, it may be visible as a preload sensitive change in MAPD, since we have shown that preload modulates stretch during isovolumic relaxation and early diastole. This provides a possible explanation why STV depends on preload changes in our model, but is modulated by repolarization reserve. When repolarization reserve is further

compromised by dofetilide, the same mechanism of stretch induced inward current may lead to synchronous EAD formation in enough cells to trigger a propagated EAD. Hence the association between negative EMW and proarrhythmia (i.e. PVCs and TdP).

Experiments in a perfused working heart setup may allow to further elucidate the mechanism of STV, EAD propagation and stretch activated currents. Attempts to reproduce TdP in susceptible CAVB hearts in Langendorff have failed (unpublished data). Apart from the absence of anesthetics or autonomic influence, the absence of mechanical loading of the non-pumping heart may explain the resistance to arrhythmia.

We have shown that in the CAVB dog, in the absence of preload variation, dofetilide did not induce an increase in STV before occurrence of ectopic activity. However, other in-vivo models have reported STV increase after $I_{Kr}$-block: the anesthetized methoxamine-sensitized rabbit,[24] the anesthetized dog with $I_{Ks}$-block and beta-adrenergic stimulation,[9] and the awake dog with $I_{Ks}$- and $I_{Kr}$-block.[8] Recently Jonsson et al. showed STV increase after $I_{Kr}$-block in a multicellular model of clusters of human embryonic stem cells;[25] although the low electrical coupling seen by others in embryonic stem cells may facilitate the induction of STV and EADs.[26] To our knowledge no attempt has been made in any of the in-vivo models to correlate repolarization variations to variation in any physiological parameter.

## Possible involvement of myocardial perfusion as a modulator

In symptomatic patients with congenital LQTS1 and 2, using tissue Doppler imaging, Haugaa et al. found heterogeneous contraction and delayed relaxation, concluding this would result in impaired diastolic function.[27] In a letter to the editor of the European Heart Journal Belardinelli et al. added a parallel between ventricular relaxation and drug induced prolongation of repolarization:[28] in an dog LQTS2 model he noted delayed relaxation after administration of clofilium, which may impair coronary flow by reduction of the diastolic interval. Coronary blood flow is minimal during systole when the heart wall is compressed. Instead it reaches a maximum just after relaxation of the ventricles, which generates a suction wave and continues during diastole.[29] Especially for the sub-endocardium a sufficiently long diastolic interval is vital for cardiac perfusion.[29] To explore the effect of dofetilide on cardiac perfusion we performed an open chest experiment where we recorded coronary flow through the left descending coronary artery during dofetilide infusion. After 90 seconds of dofetilide infusion we noted decreased flow during systole, consistent with a more forceful contraction or slower relaxation in at least part of the ventricular wall (Figure 1). At 120 seconds systolic flow remained depressed, while

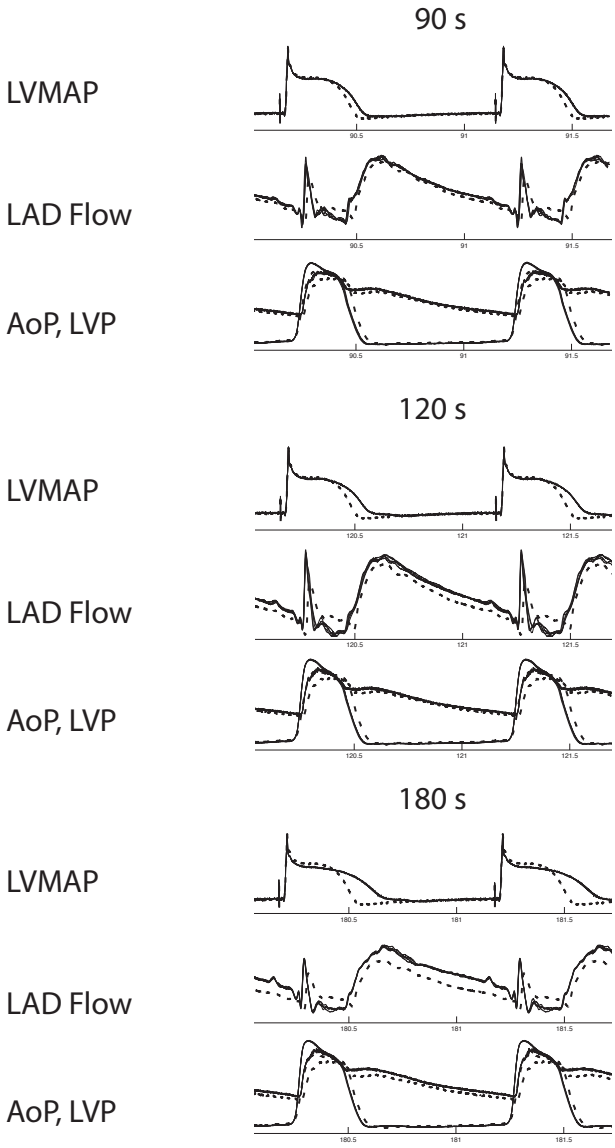


*Figure 1: Coronary flow pressure and monophasic action potential after dofetilide infusion at constant PQ. Overlay plot of 5 consecutive beats (solid lines), together with a tracing at baseline (dotted line). Numbers indicate time since start of dofetilide infusion. LV MAP: left ventricular monophasic action potential. LAD flow: flow through the left descending coronary artery. AoP: Aortic pressure. LVP: Left ventricular pressure.*

diastolic flow had increased, although diastolic aortic and left ventricular pressure was unchanged, suggesting an increased demand. At 180 seconds of dofetilide, with minimal change in diastolic pressures, diastolic and total flow kept increasing further when ectopic beats and TdP ensued. In the dynamic phase during the first minutes of dofetilide infusion, where the distribution of coronary flow has to adapt to the increased and still changing demand, local oxygen debt may occur. Since a high infusion rate is a prerequisite for TdP induction, oxygen debt during the initial phase may be a modulator of proarrhythmia. Note that our measurements gave no information on the distribution of flow between the sub-epicardium and sub-endocardium.

We also observed that coronary flow was just as reproducibly depending on preload as we noted for ventricular stretch and ventricular pressure (Figure 2; chapter 6). If coronary flow was close to critical it may be that small changes in flow affect electrophysiology. In fact, it has been shown that in a situation of reduced coronary perfusion any change in flow directly affects contraction duration and therefore presumably intracellular calcium transients.[30]

Intramural measurement of local $O_2$-tension and diffusion rate may clarify whether the change in oxygen demand and coronary perfusion contributes to drug induced TdP.

## Concluding remarks

In conclusion: In the CAVB dog, STV derived from LV MAP or LV EGM is predictive of dofetilide-induced TdP, and is reduced by antiarrhythmic increase of heart rate. We confirmed anesthesia to be a critical factor for proarrhythmia in this model. The predictive value of STV depends on an augmented response of repolarization to changes in ventricular preload. Further research is required into the mechanical contributions to STV and TdP in the CAVB dog. Monitoring of STV in ICDs is feasible, but continuous data must be collected to determine its value in risk monitoring.

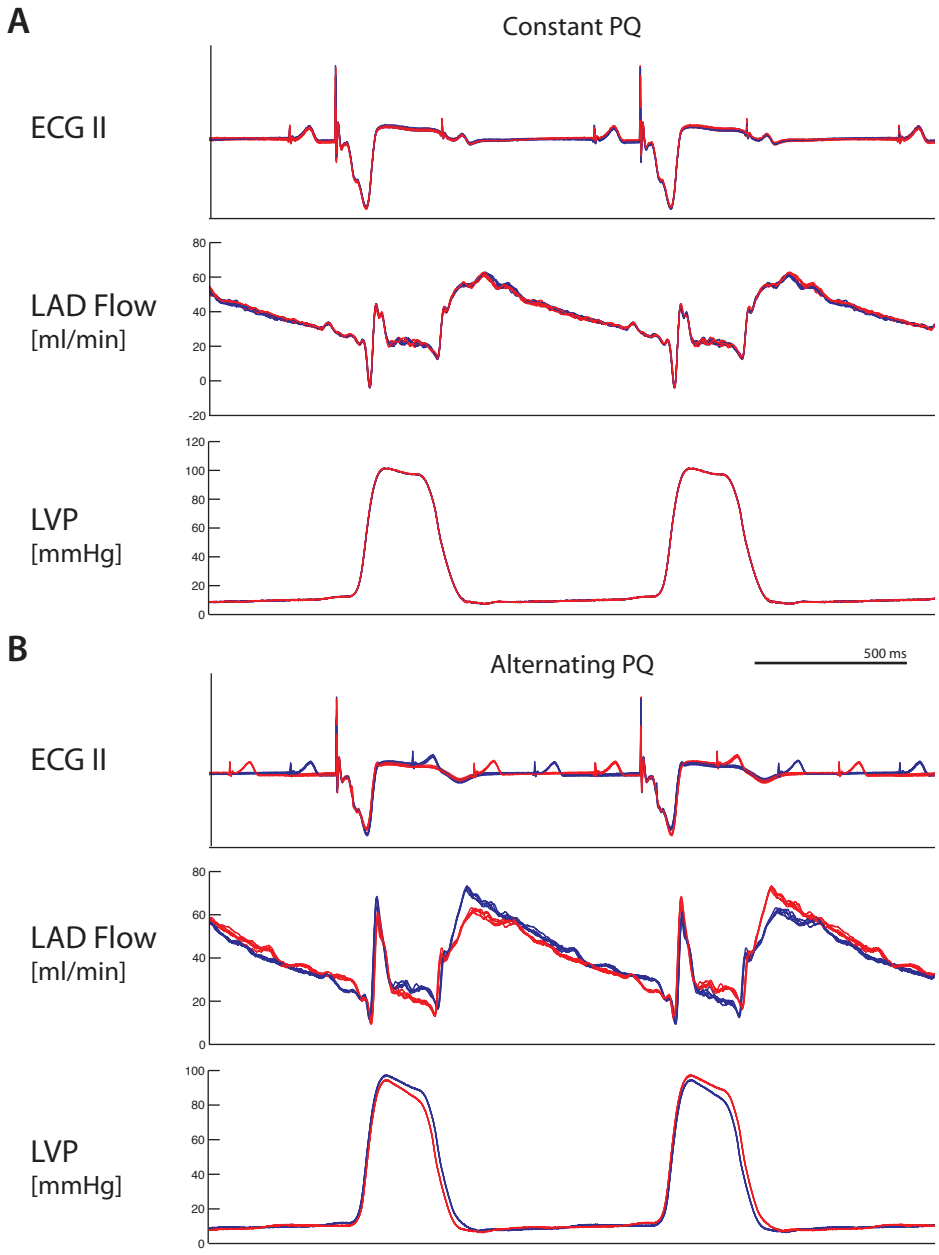


*Figure 2: Effect of PQ alternans on coronary flow at baseline.*
*Overlay plot of 20 beats, without mechanical ventilation of ECG lead II, Coronary flow through the LAD, left ventricular pressure (and LV monophasic action potential). A: Recorded at constant PQ interval.B: Recorded at alternating PQ interval*

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## Summary

The majority of deaths in the western world are of cardiovascular origin. Approximately 60% of these cardiovascular deaths are sudden, meaning death occurs within 1 hour after the onset of symptoms.
Implantation of an implantable cardioverter defibrillator (ICD) has been proven effective in preventing sudden cardiac death in patients at increased risk. An ICD can monitor the heart for rhythms that are excessively fast (tachycardia) or chaotic (fibrillation) and apply a high-energy shock to restore normal sinus rhythm. However, ICD shock is associated with serious discomfort
, while depression and anxiety have been reported in patients subjected to ICD therapy. This is especially troubling considering that up to 47% of the delivered ICD shocks are inappropriate. These false-positive detections of ventricular arrhythmia by the ICD are hard to avoid completely, considering the high cost of an undetected arrhythmia (false negative). If the dynamic changes in the risk profile could be monitored on a per-patient basis, less symptomatic measures could be taken to prevent cardiac arrhythmia and ICD shock (e.g. pharmacological interventions or changes in heart rate through pacing). Moreover, arrhythmia detection could be adapted in real-time to match the current risk.
Beat-to-beat variation in cardiac repolarization (the electrical recovery phase of the heart) may hold information on the electrical stability of the heart and its susceptibility to arrhythmias. Monitoring this instability in an ICD may help guide therapy and prevent ICD shocks.
In this thesis we investigated the use of short-term variability of repolarization duration (STV) for monitoring of arrhythmic risk in ICDs. For pre-clinical studies we used the dog with chronic atrio-ventricular block, as previous studies have shown that in this model STV is related to proarrhythmic remodeling of the heart, and predictive of drug-induced ventricular arrhythmia. We explored the steps to be taken to translate these results in animal experiments to a clinical application
An introduction to the research presented in this thesis has been provided in **Chapter 1**. **Chapter 2** summarizes result from previous studies into the relation between short-term variability of repolarization, electrical remodeling and drug-induced ventricular arrhythmia, in the anesthetized chronic atrio-ventricular block dog. Ventricular remodeling, after ablation of the atrio-ventricular node, is associated with increased baseline values of STV and increased susceptibility for drug-induced arrhythmia. Induction of torsade de pointes arrhythmia by various drugs in the remodeled heart is preceded by a further increase in STV. Baseline values of STV are higher in dogs susceptible to drug induced arrhythmias than their drug resistant

counterparts. Furthermore, the increase in STV by repolarization prolonging drugs is indicative for their potency to induce torsade.
Temporarily increasing heart rate through electrical stimulation is used in a clinical setting to suppress torsade de pointes, but prolonged increase of heart rate can promote heart failure. **Chapter 3** describes a proof-of-concept study into STV guided heart rate modulation to prevent dofetilide-induced arrhythmia, in anesthetized dogs with chronic atrio-ventricular block. As expected, an increase in heart rate suppressed the arrhythmia, and this decrease in arrhythmic risk was reflected in lower values of STV. Furthermore, it was shown that STV can be derived from a chronic electrogram, as is used in an ICD to interpret cardiac rhythm.
For evaluation of an algorithm that would modulate heart rate guided by STV-based risk estimation, prolonged testing would be required, limiting the use of anesthetics. However, anesthesia was found to be a critical factor in our current method of arrhythmia induction by dofetilide in our animal model, as is described in **chapter 4**. Furthermore, we found the type of anesthesia has a profound effect on the incidence of drug-induced torsade de pointes.
**Chapter** 5 ventures into the clinical domain: As a preliminary step towards risk monitoring in ICDs, STV was determined in patients with structural heart disease using ICD electrograms. The long-term predictive value of baseline STV for sudden arrhythmic death was determined. Patients in the highest quartile of STV showed an almost twofold higher risk for sudden arrhythmic death. Predictive value was compared to a different method of quantification of repolarization variability: QT variability index.
To further improve the predictive value of STV for ventricular arrhythmia, the mechanistic link between STV and proarrhythmia was investigated in the dog with atrio-ventricular block. **Chapter 6** describes how electrical remodeling and dofetilide only increase STV in a context of beat-to-beat changes in preload. This preload variability is a direct result of the dissociation between atrial and ventricular rhythms in this animal model. However, its importance for generation of STV had not been acknowledged previously. The response of repolarization (i.e. STV) to an external stimulus (i.e. variation in ventricular preload) unmasks the electrical lability of the heart. In a clinical setting, changes in preload or other known variation in the environment of the heart may be used to improve sensitivity of STV for arrhythmic risk.
A description and validation of the developed analysis methods for electrograms and monophasic action potentials is presented in **chapter 7**. These analysis methods allow objective, standardized and precise measurement of STV. The correlation with

previously used analysis methods and the relation between STV from electrograms and monophasic action potentials is confirmed.
Finally, in **chapter 8** results of the combined studies are placed in a broader perspective and future avenues of research are discussed.

## Samenvatting

Het hart is de pomp die ons leven lang, door ritmische samentrekking het bloed laat circuleren door ons lichaam. Na elke samentrekking vult het hart zich opnieuw met bloed. Een hartslag begint met een elektrische prikkel die ontstaat in de rechter boezem (of atrium). Dit veroorzaakt als eerste een samentrekking van beide boezems die een laatste beetje bloed in de al goeddeels gevulde hartkamers (of ventrikels) pompen. In de AV-knoop wordt de elektrische prikkel, met een kleine vertraging, doorgeleid naar de kamers, waarna ook deze samentrekken en het bloed via de longslagader (rechts) of de aorta (links) het hart verlaat. Na enkele tienden van een seconde herstellen de hartspiercellen zich van de elektrische activatie; dit heet de repolarisatiefase. Vervolgens ontspant het hart, waardoor het zich opnieuw met bloed vult en de cyclus zich kan herhalen. Zowel de elektrische activatie als de repolarisatie zijn waar te nemen via metingen op de huid (hartfilmpje) of via elektroden in het hart (electrogram zoals wordt gemeten door een pacemaker of implanteerbare defibrillator).
Afwijkingen in het elektrisch functioneren van hartspiercellen kunnen leiden tot levensbedreigende hartritmestoornissen, die de pompfunctie van het hart verstoren. Deze afwijkingen kunnen veroorzaakt worden door een aangeboren genetisch defect, maar zijn ook vaak het gevolg van hartfalen, bijvoorbeeld na een infarct. De uitdaging voor de cardioloog is het vroegtijdig herkennen van een verhoogd risico op hartritmestoornissen en, indien nodig, het instellen van een behandeling. Dat kan een ingreep zijn die het risico vermindert (bijv. medicatie of ablatie) of een hartritmestoornis kan beëindigen, zoals een (implanteerbare) defibrillator. Vanwege de kosten en mogelijke bijwerkingen van een medische behandeling is het van het grootste belang om te weten welke patiënt een verhoogd risico loopt (risicostratificatie) en hoe dit risico zich ontwikkelt (risicomonitoring).
Onderzoek in honden waarbij de elektrische verbinding tussen boezems en kamers (de AV-knoop) was verbroken, gaf aanwijzingen dat een verhoogd risico op een specifiek type hartritmestoornissen, torsade de pointes (TdP), resulteerde in kleine verschillen in de repolarisatie van opeenvolgende hartslagen. In een samenwerking tussen Bakken Research Center in Maastricht en de vakgroep Medische Fysiologie van het UMC Utrecht is onderzoek gedaan naar mogelijke toepassing van deze bevindingen in een implanteerbare defibrillator. Het project werd financieel ondersteund door NWO Casimir en heeft uiteindelijk geresulteerd in dit proefschrift.
**Hoofdstuk 1** geeft een inleiding op de achtergrond en doelen van het onderzoeksproject.

In **hoofdstuk 2** wordt het hondenmodel met chronisch atrioventricular block geïntroduceerd. Het buiten werking stellen van de AV-knoop vertraagt de hartslag, verandert het patroon van activatie van de kamers en verbreekt de relatie tussen samentrekking van de boezems en de kamers. Deze veranderingen verminderen de pompfunctie van het hart. Dit veroorzaakt aanpassingen in de hartspiercellen die het hart tevens gevoeliger maken voor TdP hartritmestoornissen. Het verhoogde risico wordt gereflecteerd in een verhoogde slag-op-slag variatie in de duur van de repolarisatie: de tijd van elektrische activatie tot het einde van de repolarisatie. Dit wordt gekwantificeerd als 'Short-term variability' of STV. Met behulp van een katheter wordt de repolarisatieduur in het linker ventrikel gemeten om zo een waarde voor STV te bepalen. Na een aantal weken kan, onder anesthesie, TdP opgewekt worden na het toedienen van een medicament. De hartritmestoornis wordt voorafgegaan door een verdere stijging van STV. Medicamenten die geen TdP veroorzaken laten ook geen stijging van STV zien.
In **hoofdstuk 3** wordt aangetoond dat TdP voorkomen kan worden door het verhogen van de hartfrequentie door elektrische stimulatie (zoals ook in een pacemaker wordt toegepast). Dit verlaagde risico is tevens zichtbaar in een lagere STV. Tevens tonen we aan dat een elektrode zoals gebruikt met een implanteerbare defibrillator, geschikt is om STV te meten.
De rol van anesthesie voor het opwekken van TdP wordt onderzocht in **hoofdstuk 4**. Anesthesie blijkt een kritische factor in de gebruikte methode waarmee TdP wordt opgewekt. Dit betekent dat de experimenten in deze vorm niet kunnen worden uitgevoerd in wakkere honden.
Als een verkennende stap naar het meten van STV in patiënten met een implanteerbare defibrillator, is onderzocht of aan de hand van STV waarden voorspelt kan worden bij welke patiënten, over een termijn van enkele jaren, zich een levensbedreigende hartritmestoornis zal voordoen. In **hoofdstuk 5** rapporteren we een twee maal hoger risico in patiënten met de hoogste STV waarden (top 25%). Tevens wordt STV vergeleken met een alternatieve maat voor variabiliteit van repolarisatieduur: QT-variabiliteitsindex.
Om de voorspellende waarde van STV verder te verbeteren is onderzoek gedaan naar het mechanisme dat STV aan hartritmestoornissen verbindt in de hond met AV-blok (**hoofdstuk 6**). De variatie in repolarisatieduur (STV) in de hond blijkt een reactie op de variatie in vulling van de kamers door de dissociatie van boezemcontractie en ventriculaire contractie; de repolarisatieduur van een elektrisch instabiel hart reageert sterk op kleine vullingsverschillen, terwijl de repolarisatie van een gezond hart daar ongevoelig voor is. Het opleggen van kleine externe veranderingen kan de gevoeligheid van STV mogelijk verhogen. Hoewel de relatie tussen STV

en aritmische activiteit in het laboratorium ook in losse hartspiercellen aanwezig is, heeft STV in het intacte hart een oorzaak die niet aanwezig is in losse cellen (namelijk vulling).
Aangezien STV bestaat uit zeer kleine variaties in repolarisatieduur van maar enkele procenten, is het essentieel om nauwkeurig te meten. **Hoofdstuk 7** presenteert de gebruikte methoden voor signaalanalyse en de bijbehorende validatie. Ook wordt een vergelijking gemaakt met de in het verleden toegepaste analysemethoden.
Ten slotte plaatst **hoofdstuk 8** de resultaten van de uitgevoerde studies in een bredere context en worden mogelijke onderwerpen voor toekomstig onderzoek aangedragen.

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Peter (2) Oosterhoff
Zutphen, 2011

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