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Original article

# A new method to calculate the beat-to-beat instability of QT duration in drug-induced long QT in anesthetized dogs

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**Abstract**

**Introduction:** Instability of QT duration is a marker to predict Torsade de Pointes (TdP) associated with both congenital and drug-induced long QT syndrome. We describe a new method for the quantification of instability of repolarization. **Methods:** Female, adult beagle dogs anesthetized with a potent morphinomimetic were treated with either solvent ($n=7$) or dofetilide ($n=7$). Poincaré plots with $QT_n$ versus $QT_{n+1}$ were constructed to visualize the beat-to-beat variation in QT intervals from the lead II ECG. Short-term instability (STI), long-term instability (LTI) and total instability (TI) were quantified by calculating the distances of 30 consecutive data-points from the $x$ and $y$-coordinate to the "centre of gravity" of the data cluster. Dofetilide at 0.0025 to 0.04 mg/kg i.v. (plasma concentrations of 4±0.6 to 41±2.7 ng/ml), dose-dependently prolonged QT and QTcV (at 0.04 mg/kg i.v.: QT: 280±ms versus 236±5 ms with solvent; $p<0.05$ and QTcV: 290±9 ms versus 252±4 ms with solvent; $p<0.05$). Concomitantly, the compound induced an increase in the instability parameters in a similar dose-dependent manner (at 0.04 mg/kg i.v.: TI: 6.8±0.9 ms versus 1.7±0.3 ms; $p<0.05$, LTI: 3.6±0.5 ms versus 1.0±0.2 ms; $p<0.05$ and STI: 4.2±0.6 ms versus 1.0±0.2 ms; $p<0.05$). The increases induced by dofetilide were associated with a high incidence of early afterdepolarizations (EADs) in the endocardial monophasic action potential (in 6 out of the 7 compound-treated animals versus 0 out of the 7 solvent animals; $p<0.05$). **Conclusion:** Quantification of beat-to-beat QT instability by our method clearly detects changes in short-term, long-term and total instability induced by dofetilide, already at pre-arrhythmic doses. Dofetilide administration to anesthetized dogs prolongs ventricular repolarization, concomitantly increases beat-to-beat QT instability and induces early after depolarizations (EADs). As such, the use of these parameters in this in vivo model shows clear potential for risk identification in cardiovascular safety assessment.


*Keywords:* Anesthetized; EAD; Dofetilide; Dog; Instability; LQT; Poincaré plot; QT; TdP; Variability

## 1. Introduction

In recent years, several drugs (including non-cardiovascular drugs) have been withdrawn from the market due to cardiovascular side effects associated with induced long QT and a ventricular tachyarrhythmia known as Torsade de Pointes (TdP) (Haverkamp, Breithardt, & Camm, 2000). As such, special attention to predicting this adverse effect early in the preclinical development is warranted (Fenichel et al., 2004). At a given QT interval, the severity of pro-arrhythmia varies from drug to drug and the risk of TdP with a given drug may not be linearly related to the dose or plasma level of the compound (Yap & Camm, 2003). The elucidation of the electrophysiological events that underlie TdP may provide a useful surrogate marker for the identification of drugs with the potential of causing this polymorphic ventricular arrhythmia (Belardinelli, Antzelevitch, & Vos, 2003). Current biomarkers associated with congenital and drug-induced TdP include: (1) prolongation of the ventricular action potential (Redfern et al., 2003); (2)

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generation of early afterdepolarizations (EADs) and triangulation of the action potential (Lu, Vlaminckx, Van Ammel, & De Clerck, 2002); (3) increased spatial (transmural dispersion; Antzelevitch & Shimizu, 2002; Yan, Kowey, & Lankipalli, 2002) and temporal dispersion of ventricular repolarization (instability; beat-to-beat variation; Hondeghem, Carlsson, & Kamen, 2001; Hondeghem, Dujardin, & De Clerck, 2001; Hondeghem & Hoffmann, 2003). Therefore, a rational preclinical testing strategy includes an integrated risk assessment at multiple levels of physiological complexity and no single test is adequate on its own to predict clinical risk. An efficient testing strategy includes early in vitro testing for effects on $I_{Kr}$ and action potential configuration as well as well-conducted in vivo assessment (Gralinski, 2000; De Clerck et al., 2002; and Guth, Germeyer, Kolb, & Markert, 2004).

The Poincaré plot, an emerging quantitative-visual technique, plots one beat against the next. The plot provides summary information as well as detailed beat-to-beat information, with simple visual interpretation of data. But obvious quantitative measures that characterize the features of the plot are lacking (Brennan, Palaniswami, & Kamen, 2001).

In this study, we provide a novel mathematical model for quantification of the beat-to-beat variability of the QT interval of the ECG. We name this parameter the index of QT instability and link it to the morphological properties of the plot (Brennan, Palaniswami, & Kamen, 2002), the width being a measure of the short-term instability (STI), the length a measure of the long-term instability (LTI) and a width- and length-dependent parameter as an indication for the total instability (TI). The newly developed parameters were challenged with a pharmacological compound, dofetilide (class III antiarrhythmic agent; $I_{Kr}$ blocker), known to cause prolongation of the QT interval (Ward & Gill, 1997; and Yan et al., 2002).

Furthermore, the parameters are compared with other methods of quantifying Poincaré plots that either describe the instability of heart rate (Kamen & Tonkin, 1995; and Tulppo, Makikallio, Takala, Seppanen, & Kuikuri, 1996) or ventricular repolarization (Thomsen et al., 2004).

## 2. Methods

This investigation was conducted in accordance with "The provision of the European Convention" on the protection of vertebrate animals, which are used for experimental and other scientific purposes, and with "the Appendices A and B", made at Strasbourg on March 18, 1986 (Belgian Act of October 18, 1991).

### 2.1. Animals

Female, adult beagle dogs were used in this study, their bodyweight averaging 12.0 kg (range: 10.0–13.3 kg). All dogs were examined before use and found to be healthy and active. Food (but not water) was withheld for at least 12 h prior to anesthesia and cardiovascular experimentation.

### 2.2. Anesthesia and measured parameters

Briefly, under total intravenous anesthesia induced by 0.075 mg/kg lofentanil, 0.0015 mg/kg scopolamine (Janssen Pharmaceutica, JNJ PRD, Beerse Belgium) and 1.0 mg/kg succinylcholine (Myoplegine, Christiaens N.V. Brussel Belgium) and maintained by infusion of etomidate 1.5 mg/kg/h (Janssen Pharmaceutica, JNJ PRD, Beerse Belgium), the dogs were ventilated with 30% oxygen in pressurized air to normocapnia. The electrocardiogram (ECG lead II limb leads; Emka, France) and the monophasic action potential signal (MAP; bi-directional steerable diagnostic MAP catheter, placed at the endocardium of the right ventricle; EP Technologies, USA) were continuously monitored. An injection catheter was placed into the caudal vena cava and positioned close to the heart.

Calculation of the instability parameters was performed before and after administration of dofetilide or its solvent in a period where prolongation of the QT interval reached a steady state (approximately 5 min after administration). In the same periods, QT intervals (QT), QT intervals corrected for changes in heart rate according to Van de Water (QTcV; Van de Water, Verheyen, Xhonneux, & Reneman, 1989), RR intervals (RR) and monophasic action potential duration to 90% repolarization ($MAPD_{90}$) were automatically analyzed (Notocord-Hem 3.3, Croissy-sur-Seine, France) and represented as median values over 1 min. An EAD was defined as a small afterpotential that interrupts or delays the normal repolarization of the action potential (Zipes, 1991).

### 2.3. Study protocol

Dogs were assigned at random to a solvent group ($n=7$) or a dofetilide group ($n=7$). After a stabilization period, increasing doses of the compound or equivalent volumes of the solvent solutions were slowly (over approximately 10 s) injected intravenously at 30-min intervals. Dofetilide (solution of 0.1 mg/ml in saline) was administered in a dose range of 0.0025, 0.005, 0.01, 0.02 and 0.04 mg/kg i.v. (total dose=0.0775 mg/kg i.v.). Volumes of solvent ranged from 0.025 to 0.4 ml/kg i.v. (total volume=0.775 ml/kg i.v.). Arterial blood samples for the measurement of dofetilide plasma concentrations were taken in 4 experiments at 5 and 30 min after each injection of dofetilide. The heparinized blood was centrifuged within 10 min after sampling and the plasma stored at −20 °C until analysis by LC-MS/MS with a limit of quantification of 1 ng/ml.

### 2.4. Visual examination

Before performing the quantitative analysis of instability of the QT interval, visual examination of the ECG tracings was done. Special emphasis was placed on artifact detection and elimination, because these strongly affect instability analysis of the ECG signal. Starting from a total trend graph of all QT intervals (Fig. 1), an ECG

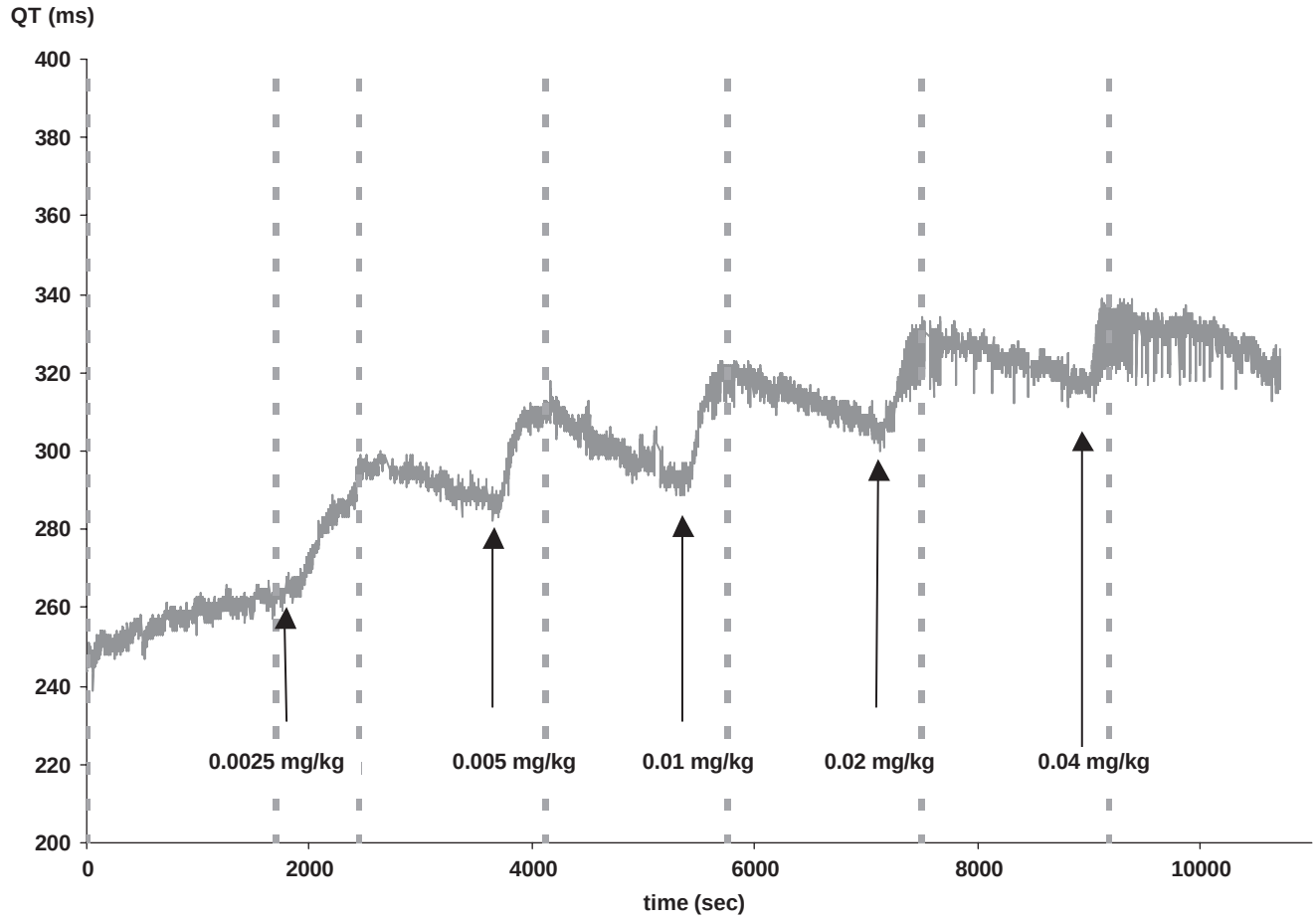



Fig. 1. A trend graph is recorded of the beat-to-beat QT intervals of one individual experiment. Dofetilide was administered at increasing doses of 0.0025 to 0.04 mg/kg at 30-min intervals. The moment of the administration is indicated with an arrow. The dotted lines are the exact places were the 30 beat tracings are selected. The width of the line of the QT intervals is a rough indication for the QT instability.

tracing of 30 qualified consecutive beats (no artifacts or ectopic beats) was chosen for further analysis, based on earlier experimental data. In these earlier experiments, the optimal length of the ECG tracings for the generation of Poincaré plots was evaluated. Tracings with beats less than 30 (20, 10 and 5) showed significantly different results, while tracings with more beats (40, 50 and 60) yielded similar results compared to tracings with 30 beats (data not shown). The $QT_{n+1}$ was plotted as a function of $QT_n$ in a Poincaré plot (Fig. 2).

### 2.5. Quantitative analysis

To quantify the plot, we first calculated the centre of gravity (cg) of the cluster of data points by means of the following equation:

$$\text{Centre of gravity(cg)}: \quad \mathrm{cg}(x) = \sum_{i=m}^{m+29} (\mathrm{QT}_i)/30$$
$$\mathrm{cg}(y) = \sum_{i=m+1}^{m+30} (\mathrm{QT}_i)/30$$

Afterwards, the distances of all 30 data points to the cg were calculated. The median of these distances is the total instability (TI) over the 30 points (Fig. 3):

$$\text{Total instability}: \quad \mathrm{TI}_n = \sqrt{\left((\mathrm{cg}(x) - \mathrm{QT}_n)^2 + (\mathrm{cg}(y) - \mathrm{QT}_{n+1})\right)^2}$$
$$\mathrm{TI} = M(\mathrm{TI}_n)$$

To measure the length and the width of the plot, we performed a rotation of $-45°$ around the origin and obtained following mathematical formulae:

$$\text{Rotated cg}(x): \quad \mathrm{Rcg}(x) = (\cos\theta \times \mathrm{cg}(x)) - (\sin\theta \times \mathrm{cg}(y))$$
$$\text{Rotated cg}(y): \quad \mathrm{Rcg}(y) = (\sin\theta \times \mathrm{cg}(x)) + (\cos\theta \times \mathrm{cg}(y)).$$

In the reference system of the new [Rcg($x$),Rcg($y$)], coordinates of all distances were measured to the $x$ coordinate of the cg and to the $y$ coordinate of the cg. The distance to the $x$ coordinate represents the length of the plot and reflects the long-term instability (LTI) and the distance to the y coordinate is the width of the plot and reflects the short-term instability (STI). After calculation of these distances for

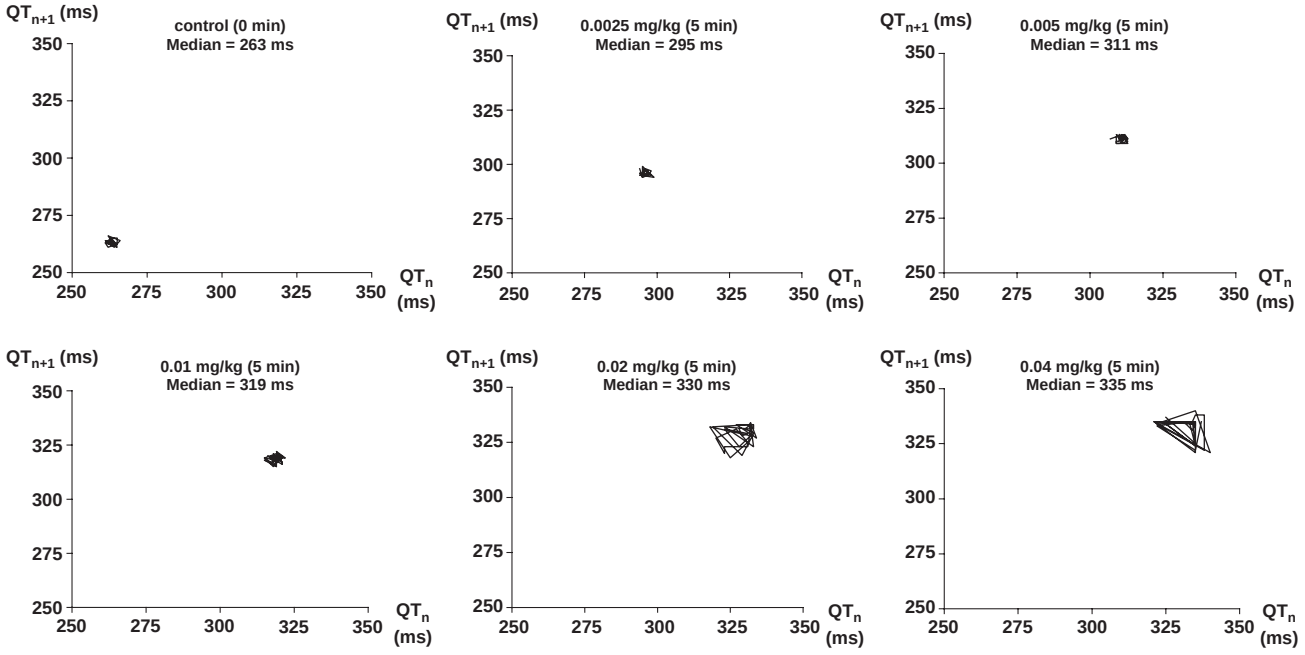



Fig. 2. Selected tracings are automatically visualized in Poincaré plots and manually checked for artifacts or ectopic beats. This example shows the dose-dependent effect of increasing doses of dofetilide in one anesthetized dog.

all data points, the median value is calculated for the instability parameters of the total tracing of 30 beats.

$$\text{Long}-\text{term instability}: \quad \begin{aligned} \text{LTI}_n &= \left|\text{Rcg}(x) - ((\cos\theta \times \text{QT}_{n+1}) - (\sin\theta \times \text{QT}_n))\right| \\ \text{LTI} &= M(\text{LTI}_n) \end{aligned}$$

$$\text{Short}-\text{term instability}: \quad \begin{aligned} \text{STI}_n &= \left|\text{Rcg}(y) - ((\sin\theta \times \text{QT}_{n+1}) + (\cos\theta \times \text{QT}_n))\right| \\ \text{STI} &= M(\text{STI}_n) \end{aligned}$$

These three instability parameters (TI, LTI and STI) were measured for all individual dogs.

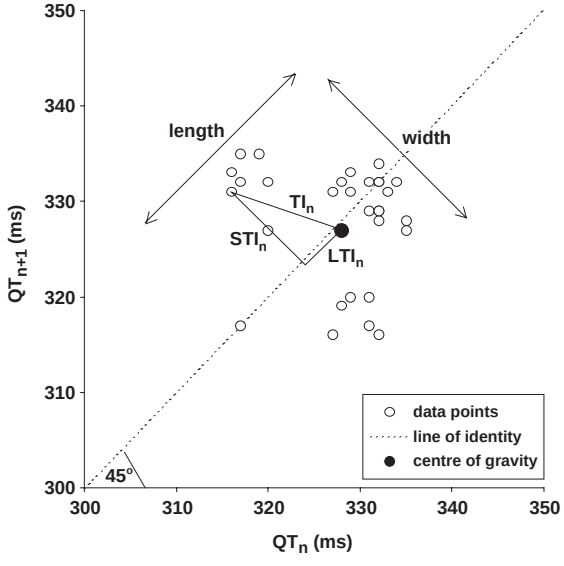



Fig. 3. Quantification of the instability visualised in the Poincaré plot is based on the distances of a cluster of 30 QT intervals to the centre of gravity. Only 26 data points are identifiable because 4 points are exactly the same. That is why visual examination alone is not always accurate. In this example, the centre of gravity is not exactly located on the line of identity.

Glossary

| | |
|---|---|
| $n$ | Number of the beat from 1 to 29 |
| $m$ | The first beat of a selected epoch |
| cg($x$) | x-coordinate of centre of gravity |
| cg($y$) | y-coordinate of centre of gravity |
| $M$ | Median over 30 beats |
| $\theta$ | $-\pi/4$ |

### 2.6. Comparison of methods

We compared our new method, in terms of theoretical results and the measured effects of dofetilide, with two commonly used methods: standard deviation of the successive differences (SDSD/SDQT; Kamen & Tonkin, 1995) and standard deviation around the line of identity (SD1/SD2; Tulppo, Makikallio, Takala, Seppanen, & Kuikuri, 1996), and with a recently developed method (STV/LTV; Thomsen et al., 2004) that shows a few similarities with our method.

### 2.7. Statistical analysis

All values are expressed as means ± standard error of the mean (S.E.M.). Statistical analysis was performed using one-way or two-way analysis according to the method of Wallenstein, Zucker, and Fleiss (1980). Fishers's probability test was used to evaluate the differences in the incidence of EADs between the groups. A $p$ value less than 0.05 was considered statistically significant. Fisher's probability test was used to evaluate the differences in the incidence of EADs between the two groups.

## 3. Results

Method 1 refers to our newly developed method, Method 2 to the calculation of SDQT, Method 3 to the calculation of SD2 and Method 4 to the calculation of STV/LTV.

### 3.1. Comparison of methods with theoretical results

#### 3.1.1. Example 1

A sequence with only short-term instability can be described as alternating between two values ($a$, $b$, $a$, $b$, $a$, $b$,...). The Poincaré plot of such a sequence ($a$=200 and $b$=230) is plotted in Fig. 4A. The length of this plot is zero, no long-term instability is present, so the width of the plot is totally responsible for the instability. Calculations of SDQT and SD2 gave a length of the plot of 7.6 ms and 5.5 ms, respectively. Both these methods implicate a width of zero. In contrast, our method (STI/LTI) as well as calculation of STV/LTV yielded a length of zero and a width of 10.6 ms.

#### 3.1.2. Example 2

This example used a constant increase in QT ($a$, $b$, $c$, $d$, $e$, $f$,...); therefore, it suggests a sequence with no short-term instability (Fig. 4B). With all methods, the calculations of the length of the plot indicated a value and again, Method 1 and Method 4 produced the same value. However, for calculation of the width of the plot, Method 4 gave a small value, in contrast to the other three methods that showed a width equal to zero. The difference in outcome is caused by the fact that the cg is not exactly on the line of identity, but 1 ms above this line.

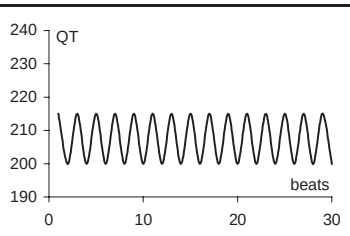
#### 3.1.3. Example 3

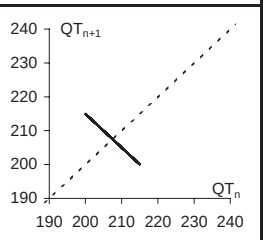
A sequence of a real experiment was plotted (Fig. 4C). This resulted in a Poincaré plot with a length and a width, or in other words, in a plot that represents a long-term and a short-term instability, respectively. In this situation, the total instability is influenced by the short-term and long-term fluctuations, but it is not just an addition of these two parameters. Although Method 1 and Method 4 are similar, an advantage of our method is that the total instability is calculated by taking the position of every point in the plot into account.

Another difference between our method and Method 4 is that we calculated median values, while in Method 4 mean values were calculated. The median results in a parameter that is not as sensitive for extremes. This is reflected by

**A**

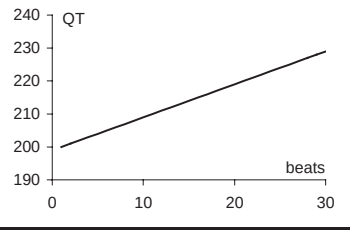
| Techniques | Parameters | Quantification of the shape: short-term width | long-term length | total both |
|---|---|---|---|---|
| Method 1 | STI / LTI / TI | 10.6 | 0.0 | 10.6 |
| Method 2 | SDSD / SDQT | 0.0 | 7.6 | - |
| Method 3 | SD1 / SD2 | 0.0 | 5.5 | - |
| Method 4 | STV / LTV | 10.6 | 0.0 | - |

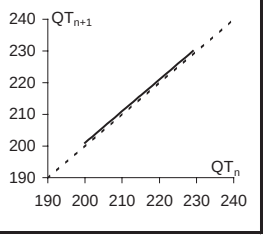



**B**

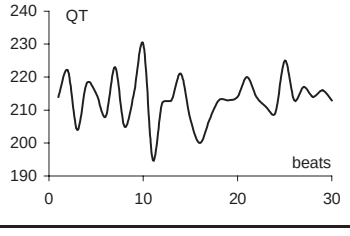
| Techniques | Parameters | Quantification of the shape: short-term width | long-term length | total both |
|---|---|---|---|---|
| Method 1 | STI / LTI / TI | 0.0 | 10.6 | 10.6 |
| Method 2 | SDSD / SDQT | 0.0 | 8.8 | - |
| Method 3 | SD1 / SD2 | 0.0 | 5.9 | - |
| Method 4 | STV / LTV | 0.7 | 10.6 | - |




**C**

| Techniques | Parameters | Quantification of the shape: short-term width | long-term length | total both |
|---|---|---|---|---|
| Method 1 | STI / LTI / TI | 5.1 | 3.9 | 7.9 |
| Method 2 | SDSD / SDQT | 7.4 | 7.3 | - |
| Method 3 | SD1 / SD2 | 5.3 | 3.0 | - |
| Method 4 | STV / LTV | 6.4 | 4.6 | - |



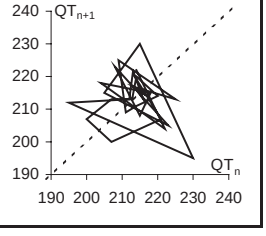




Fig. 4. Theoretical examples of extreme situations are shown and instability parameters are calculated with 4 different techniques. (A) An example with no long-term instability, (B) an example with no short-term instability and (C) an example of both, long-term and short-term instability and a total instability (influenced by long- and short-term instability).

Table 1A
Dose-dependent changes in the duration of the QT and QTcV interval, QT instability and occurrence of EADs after administration of various volumes of solvent ($n=7$) to anesthetized dogs

| Solvent ($n=7$) | | | | | | |
|---|---|---|---|---|---|---|
| | Baseline | 0.025 (ml/kg) | 0.05 (ml/kg) | 0.1 (ml/kg) | 0.2 (ml/kg) | 0.4 (ml/kg) |
| QT (ms) | 244±4 | 244±4 | 243±4 | 240±4 | 238±5 | 236±5 |
| QTcV (ms) | 263±4 | 261±4 | 256±4 | 252±4 | 247±5 | 245±5 |
| TI (ms) | 1.9±0.4 | 1.8±0.3 | 1.7±0.3 | 2.0±0.3 | 1.9±0.3 | 1.7±0.3 |
| STI (ms) | 1.0±0.2 | 0.9±0.2 | 0.9±0.3 | 0.9±0.3 | 0.8±0.2 | 1.0±0.2 |
| LTI (ms) | 1.1±0.2 | 1.1±0.3 | 1.0±0.2 | 1.3±0.2 | 1.0±0.3 | 1.1±0.2 |
| EAD ($n$/7) | 0/7 | 0/7 | 0/7 | 0/7 | 0/7 | 0/7 |

smaller values in our method compared to Method 4 (5.1 and 3.9 ms versus 6.4 and 4.6 ms, respectively).

### 3.2. Comparison of methods with pharmacological results

#### 3.2.1. Solvent experiments

None of the measured and calculated parameters (QT, QTcV, TI, STI and LTI) were influenced by increasing volumes of the solvent. All parameters were stable during the whole experiment (over approximately 3 h) and were not significantly different from the baseline values ($p>0.05$). A slight decrease was observed in QTcV duration over time. Consistent EADs were not found during the course of the solvent experiments (Table 1A and Fig. 5).

#### 3.2.2. Dofetilide experiments

Solvent did not induce clinically relevant or statistically significant changes in the parameters. Dofetilide dose-dependently increased QT, QTcV and QT instability. Maximal QT and QTcV prolongation was +12% versus baseline at 0.01 mg/kg i.v. ($p<0.05$ versus baseline and versus solvent; mean plasma concentration: $C_{mean}=11$ ng/ml). TI and STI achieved their maximum at 0.04 mg/kg i.v. (6.8 versus 1.4 ms=+385% and 4.2 versus 0.7 ms=+500% versus baseline, respectively; $p<0.05$ versus baseline and versus solvent; $C_{mean}=40$ ng/ml) and LTI at 0.02 mg/kg i.v. (3.7 versus 0.7 ms=+428% [bl1] versus baseline; $p<0.05$ versus baseline and versus solvent; $C_{mean}=18$ ng/ml). EADs developed after low clinically relevant doses of dofetilide (0.005 and 0.01 mg/kg i.v.) in 43% of the experiments (3 out of 7 dogs). At higher doses (0.02 and 0.04 mg/kg i.v.), EADs developed in 86% of the animals (6 out of the 7 dogs; $p<0.05$ versus solvent) (Table 1B and Fig. 5).

As calculated with the various methods, dofetilide at 0.04 mg/kg i.v., induced variable increases in short-term instability versus baseline, depending on the method (STI: +481% from a baseline value of 0.7 ms, SDQT: +400%

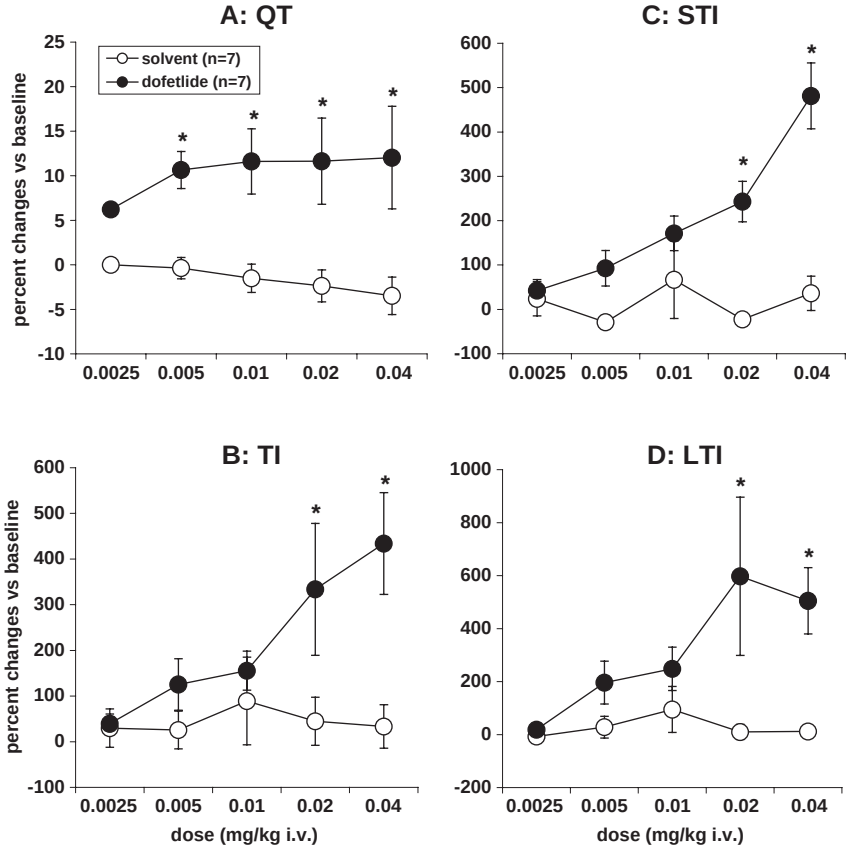



Fig. 5. QT-interval (A), total instability (B), short-term instability (C) and long-term instability (D) increased dose-dependently after administration of dofetilide in anaesthetised dogs. Each value is calculated as percentage change versus baseline at a period where the prolongation of the QT interval had reached a plateau. *: $p<0.05$ versus solvent group based on absolute values.

Table 1B
Dose-dependent changes in the duration of the QT and QTcV interval, QT instability and occurrence of EADs after administration of various doses of dofetilide ($n=7$) to anesthetized dogs

| Dofetilide ($n=7$) | | | | | | |
|---|---|---|---|---|---|---|
| | Baseline | 0.0025 (mg/kg) | 0.005 (mg/kg) | 0.01 (mg/kg) | 0.02 (mg/kg) | 0.04 (mg/kg) |
| QT (ms) | 250±6 | 266±6[a] | 277±8[a,b] | 279±11[a,b] | 279±13[a,b] | 280±15[a,b] |
| QTcV (ms) | 260±3 | 272±3 | 284±7[a,b] | 290±9[a,b] | 289±10[a,b] | 287±9[a,b] |
| TI (ms) | 1.4±0.1 | 1.8±0.1 | 2.8±0.5 | 3.3±0.5[a] | 5.2±1.1[a,b] | 6.8±0.9[a,b] |
| STI (ms) | 0.7±0.0 | 1.0±0.2 | 1.4±0.3 | 1.9±0.3[a] | 2.4±0.3[a,b] | 4.2±0.6[a,b] |
| LTI (ms) | 0.7±0.1 | 0.7±0.0 | 1.6±0.3 | 2.0±0.3 | 3.7±1.2[b] | 3.6±0.5[a,b] |
| EAD ($n$/7) | 0/7 | 1/7 | 3/7 | 3/7 | 6/7[c] | 6/7[c] |

QTcV: QT corrected according to Van de Water, TI: Total Instability, STI: short-term instability, LTI: Long-term instability, EAD: early afterdepolarization. All values are mean±S.E.M.

[a] $p<0.05$ (analysis based on a Dunnett's test: comparison between solvent and dofetilide treatment).

[b] $p<0.05$ (analysis based on a repeated measurements ANOVA model followed by a Newman–Keuls multiple comparisons test: effect after dose compared to baseline).

[c] $p<0.05$ (Fisher's probability test was used to evaluate the differences in the incidence of EADs between the two groups).

from a baseline value of 1.2 ms, SD1: +400% from a baseline value of 1.4 ms and STV: +375% from a baseline value of 0.9 ms; Table 2).

## 4. Discussion

This study describes a new method to quantify instability in the repolarization time of the heart. The instability parameters, including TI, STI and LTI (total, short-term and long-term instability, respectively), are essentially calculated as distances to the centre of gravity, derived from beat to beat Poincaré plots of QT values of the ECG. Constructing Poincaré plots is a dynamic method that reveals the complexity of beat-to-beat behavior of intervals. Variability in RR intervals is determined by complex interactions between sympathetic and parasympathetic influences that have been quantified originally by spectral power analysis with two main frequency components, a high-frequency (HF) and a low-frequency (LF) component (Akselrod et al., 1981). Later on, others have demonstrated that the length and width of a Poincaré plot are a weighted combination of low- and high-frequency power (Brennan, Palaniswami, & Kamen, 2002), which can also be used for calculating instability of QT intervals.

### 4.1. QT instability

Before 1996, a lot of different terms have been used to describe fluctuations in cardiac cycles. At that time, appropriate standards were developed to measure fluctuations in heart rate (Task force of the European society of cardiology and North American society of pacing and electrophysiology, 1996). Heart rate variability (HRV) has become the conventionally accepted term to describe variations of both instantaneous heart rate and RR intervals. However, fluctuation in ventricular repolarization of the heart measured by QT intervals and action potential (AP) durations is not categorized with equivalent consistency. Variability and instability are terms that are both regularly

Table 2
Dose-dependent increases of QT instability after dofetilide (0.0025–0.04 mg/kg), calculated with the various methods

| Dofetilide ($n=7$) | | | | | | | |
|---|---|---|---|---|---|---|---|
| Instability | Parameter | Baseline | 0.0025 (mg/kg) | 0.005 (mg/kg) | 0.01 (mg/kg) | 0.02 (mg/kg) | 0.04 (mg/kg) |
| Total | TI (ms)[a] | 1.4±0.1 | 1.8±0.1 | 2.8±0.5 | 3.3±0.5 | 5.2±1.1 | 6.8±0.9 |
| Short | STI (ms)[a] | 0.7±0.0 | 1.0±0.2 | 1.4±0.3 | 1.9±0.3 | 2.4±0.3 | 4.2±0.6 |
| Short | SDSD (ms)[b] | 1.2±0.1 | 1.4±0.2 | 1.8±0.3 | 2.6±0.5 | 2.9±0.3 | 5.4±1.0 |
| Short | SD1 (ms)[c] | 0.8±0.1 | 1.0±0.1 | 1.3±0.2 | 1.8±0.3 | 2.1±0.2 | 3.8±0.7 |
| Short | STV (ms)[d] | 0.9±0.1 | 1.2±0.2 | 1.6±0.2 | 2.2±0.4 | 2.9±0.5 | 4.2±0.5 |
| Long | LTI (ms)[a] | 0.7±0.1 | 0.7±0.0 | 1.6±0.3 | 2.0±0.3 | 3.7±1.2 | 3.6±0.5 |
| Long | SDQT (ms)[b] | 1.3±0.1 | 1.6±0.2 | 2.2±0.3 | 2.9±0.4 | 3.6±0.4 | 6.0±0.9 |
| Long | SD2 (ms)[c] | 1.4±0.0 | 1.5±0.1 | 1.9±0.1 | 2.0±0.2 | 2.5±0.2 | 2.9±0.2 |
| Long | LTV (ms)[d] | 1.0±0.1 | 1.2±0.1 | 1.8±0.3 | 2.3±0.3 | 3.0±0.3 | 4.9±0.8 |

TI: total instability, STI: short-term instability, SDSD: standard deviation of successive differences of the QT intervals, SD1: standard deviation of the QT intervals scaled by $1/\sqrt{2}$, STV: short-term variability, LTI: long-term instability, SDQT: standard deviation of the QT intervals, SD2: standard deviation around the line of identity and LTV: long-term variability.

[a] Present study.

[b] Kamen & Tonkin, 1995.

[c] Tulppo et al., 1996.

[d] Thomsen et al., 2004.

used to indicate fluctuations (Brennan et al., 2001, 2002; Kamen & Tonkin, 1995; Tulppo et al., 1996). Increased variability in ventricular repolarization time has been associated with enhanced risk of life-threatening arrhythmias (Berger et al., 1997; Bilchick et al., 2004). In contrast, an increase in HR variability is associated with a reduced risk of life-threatening arrhythmias (Pumprla, Howorka, Groves, Chester, & Nolan, 2002).

The dispersion of points perpendicular to the line of identity (the width) reflects the level of short-term instability. This parameter can be derived by two commonly used methods: the standard deviation of the successive differences (SDSD) and the standard deviation around the line of identity (SD1; ellipse fitting technique). Both methods ignore the powerful beat-to-beat structure displayed by the plot, as shown in Example 1. On the contrary, our method, as well as Method 4, is able to measure the width of all shapes a plot can have, even a shape without a length (without long-term instability). Since Poincaré plots sometimes have their centre of gravity not exactly on the line of identity, a small error has crept into the measurement of the width of the plot according to Method 4 (STV).

The dispersion of points along the line of identity (the length) reflects the level of long-term instability. The most commonly used method measures the standard deviation of the intervals. This is not the real length of the plot, but a reflection of the length on the $x$-axis. Our method calculates the length of the plot along the line of identity exactly; when the plot has no length, we measure exactly zero.

The length of the Poincaré plot is described by many authors as a reflection of the total instability (Brennan et al., 2002; Otzenberger et al., 1998; Tulppo et al., 1996). These interpretations are not fully correct, because in these studies the length of the plot is the standard deviation of intervals with a reflection on the $x$-axis. Total instability is influenced not only by the length, but also by the width of the plot. In a plot with no length (Fig. 4, Example 1), the standard deviation of the intervals produced a value (7.6 ms), caused by the width of the plot, which truly is a reflection of the short-term instability. If one measures, for each data point, the distance to the centre of gravity of the plot, one obtains a parameter that reflects the shape of the plot, which is defined by length and width. The latter is really a reflection of the total instability.

A difference between our method and Method 4 is that we calculate median values, opposed to mean values with the other one. Calculating the median values of all distances is a way to get rid of the influences of extreme values. This is reflected by smaller values in our method compared to the other.

### 4.2. Inhibition of $I_{Kr}$ channels and drug-induced long QT

Dofetilide is a potent $I_{Kr}$-blocking agent that prolongs the ventricular repolarization. In this study, we demonstrate that the compound not only prolongs the QT interval, but also increases the fluctuation of the QT interval. Indeed, dofetilide (0.0025–0.04 mg/kg i.v.; $C_{mean}$=4 to 40 ng/ml) dose-dependently increases the QT instability (Tables 1A and B). The plasma concentrations of dofetilide cover and exceed the therapeutic levels of the compound (Allen, Nichols, & Oliver, 2000). At low doses (0.0025 mg/kg i.v.; $C_{mean} \leq 4$ ng/ml), the compound prolongs QT and QTcV intervals. EADs occur in only one out of the 7 dogs, without significant increases in QT instability. At high doses (0.01–0.04 mg/kg i.v.; $C_{mean} \geq 10$ ng/ml), dofetilide significantly increases TI, STI and LTI. QT and QTcV prolongation seem to reach a plateau value, and the effects are associated with a high incidence in EADs (6 out of 7 dogs). These observations are in line with those of others who showed that the prolongation of QT interval induced by another $I_{Kr}$ blocking agent (d-sotalol) is achieved at low doses, while higher doses are required to induce an increase in QT instability (Safer & Gintant, 2003; Thomsen et al., 2004).

The dose-dependent effects of dofetilide on QT instability, calculated with different methods, are presented in Table 2. Only with our method can increases in TI by the compound (from 1.4 to 6.8 ms, maximally, after a cumulative total dose of 0.0775 mg/kg i.v.) be demonstrated. Increases in STI and LTI by dofetilide are obtained by all methods, albeit to a variable degree. These data indicate an advantage for our method: only with the present algorithm total instability based on beat-to-beat QT interval can be reliably detected. Whether the method improves the prediction of the liability of a NME for TdP has to be elucidated in further research. More importantly, the instability together with other markers (such as QT prolongation, spatial and temporal dispersion, reverse use, etc.) and adverse risk factors (gender, heart disease, etc.) should all be taken into account to determine the final torsadogenic potential of a QTc prolonging compound.

### 4.3. APD-instability

Calculation of instability by measuring distances to the centre of gravity of a cluster of data points can also be used for other parameters, such as heart rate (HR) and action potential duration (APD). We continuously register RR-intervals and monophasic action potential durations to 90% repolarization ($MAPD_{90}$). Measuring $MAPD_{90}$-instability using a MAP catheter placed endocardially in the right ventricle with an in vivo protocol lasting 3 to 4 h is not reliable. Repositioning of the catheter is often needed in the course of longer-lasting experiments. These interventions with the catheter influence the morphology of the signal and therefore also the calculations of $MAPD_{90}$-instability.

#### 4.3.1. Limitations

The small sample size used in the study may be the cause of some findings not reaching statistical significance. Seven animals is a minimum, although we tried to limit variation and to enhance sensitivity by performing the experiments

with adult animals of female gender only (Abi-Gerges et al., 2004). Furthermore, in our preliminary study, we exclusively looked to time domain variables of HRV, because these are readily accessible. Frequency domain variables may have provided additional information (Akselrod et al., 1981).

This report presents a new method for the calculation of instability of repolarization. Various markers are known to interfere in drug-induced arrhythmia. It is our intention to elucidate possible correlations between instability of repolarization and other biomarkers for polymorphic ventricular arrhythmias such as TdPs in future studies. Further experimental work with carefully designed studies is required to establish the individual predictive value in assessing the proarrhythmic potential of drugs in humans because QT interval prolongation is an imperfect biomarker (Kinter, Seigl, & Bass, 2004; Valentin, Hoffmann, De Clerck, Hammond, & Hondeghem, 2004).

Our limited data suggest that our method for detecting increases in QT instability in drug-induced long QT is superior to other methods. However, we could not perform statistical analysis on the differences between these methods due to the limited number of experiments with only one single compound tested. Therefore, further studies in this respect are needed to determine the extent of increases in instability in drug-induced long QT and the different sensitivity of the existing methods.

### 4.4. Conclusions

Constructing Poincaré plots by beat-to-beat QT values is a commonly used visual technique. Measuring length and width of these plots by calculating the distances to the $y$- and $x$-coordinate, respectively, of the centre of gravity to the data points is a valuable measurement for long-term and short-term instability. Total instability is introduced as the distance of all data points to the centre of gravity. This is a parameter constituted by the interaction of length (long-term) and width (short-term) of the Poincaré plot.

Administration of low doses of dofetilide to anesthetized dogs increases the duration of the QT and QTcV interval of the ECG, increases QT instability at higher doses and concomitantly induces EADs in the monophasic action potential.

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