# Drug-Induced T-Wave Morphology Changes

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**PhD Thesis by**

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**SUMMARY**

The risk of life-threatening ventricular tachyarrhythmia such as Torsades de Pointes (TdP) is assessed in drug trials by measurement of QT prolongation on the ECG. However, the QT interval is not a strong biomarker for TdP risk. Especially, the sensitivity and specificity of the measurement is limited. Investigation of alternative biomarkers to assess the risk of drug-induced arrhythmia is therefore an active area of research. Much of the current research is based on the drug-induced changes in the morphology of the T-wave.

Drug-induced inhibition of the delayed rectifier potassium current ($I_{Kr}$) can cause delayed cardiac repolarization and lead to development of TdP. Inhibition of the potassium current appears as QT interval prolongation and changes in the morphology of the T-wave on the ECG. $I_{Kr}$ inhibiting drugs and their effect on ECG markers of abnormal repolarization therefore play a central role in this thesis.

In our first study, we used common classification methods to separate congenital and drug-induced $I_{Kr}$ inhibition from healthy controls who were not taking any medication during the trial period. We investigated separation of data by two electrocardiographic repolarization parameters: The heart rate-corrected QT interval (QTc) and the MCS, a composite score of T-wave morphology which measures flatness, asymmetry and the presence of notches on the ECG. Changes in the T-wave morphology were better at separating normal from abnormal repolarization compared to QTc. Also, nonlinear boundaries can provide better classifiers than linear boundaries.

In our second study we investigated the electrocardiographic T-wave peak-to-end interval ($T_{pe}$), a commonly used measurement, thought to represent transmural repolarization heterogeneity. Repolarization heterogeneities induced by torsadogenic drugs are thought to be responsible for inscription of electrocardiographic T-wave and the QT interval. However, there is no widely accepted approach which can be used to assess repolarization heterogeneity. The $T_{pe}$ interval was proposed to quantify transmural dispersion of repolarization (Tdr) in previous vitro experiments. However, there have been an increasing number of reports of inconsistencies about whether the $T_{pe}$ interval can be used to predict arrhythmia. It also remains controversial what the $T_{pe}$ measurements actually represents. However, it is important to quantify how the $T_{pe}$ interval is correlated with the whole heart repolarization time, represented by the QT interval. We therefore investigated whether the $T_{pe}$ interval is correlated with QT prolongation induced by two torsadogenic drugs. Despite significant QT prolongation with both $I_{Kr}$-inhibiting drugs, the $T_{pe}$ interval remained almost unchanged. Thus, at least, this study raises a doubt about usefulness of $T_{pe}$ as a biomarker for repolarization changes and torsadogenic potential in drug safety studies.

In our third study we propose a new method for assessing abnormal repolarization characteristics on the ECG. The T-wave is down-sampled to a minimal number of samples in such a way that reconstruction of the original T-wave is possible. Using a combination of 8 samples extracted from the down-sampled T-wave as features it was possible to separate normal from abnormal repolarization significantly better compared to QTc. In addition, this approach has the advantage, unlike the QT interval, of being robust to the T-wave end determination. In the down-sampled ECG representation of the T-wave, it is further indicated that $T_{pe}$ interval may shorten following $I_{Kr}$ inhibition and that the most prominent drug-induced repolarization changes occur on the ascending segment of the minimal T-wave representation.

Collectively, this work may lead to improved prediction and interpretation of ECG-related abnormal repolarization.

## DANSK RESUMÉ

Risikoen for livstruende ventrikulære takyarytmier såsom Torsade de Pointes (TdP) vurderes i lægemiddelstudier ved måling af QT forlængelse på EKG'et. QT intervallet er dog ikke en stærk biomarkør for TdP risiko. Især er sensitiviteten og specificiteten af målingen begrænset. Undersøgelse af alternative biomarkører til at vurdere risiko for lægemiddel-induceret arytmi er derfor et aktivt forskningsfelt. En stor del af den nuværende forskning er baseret på lægemiddel-inducerede ændringer i morfologien af T-bølgen.

Lægemiddel-inhibering af den forsinkede genregulerende kalium strøm ($I_{Kr}$) kan forårsage forsinket repolarisering af hjertet og føre til udvikling af TdP. Inhibering af kaliumstrømmen forekommer som QT interval forlængelse på EKG'et og ændringer i morfologien af T-bølgen. $I_{Kr}$ inhiberende lægemidler og deres effekt på EKG markører for abnormal repolarisering spiller derfor en central rolle i denne afhandling.

I vores første studie brugte vi almindeligt anvendte klassifikationsmetoder til at separere medfødt og lægemiddel-induceret $I_{Kr}$ inhibering fra raske kontrolpersoner som ikke havde intaget lægemidler i forsøgsperioden. Vi undersøgte separering af data for to elektrokardiografiske repolariseringsparametre: Det pulskorrigerede QT interval (QTc) og MCS, en kompositscore for T-bølge morfologi som måler fladhed, asymmetri og tilstedeværelsen af indhak på EKG'et. Ændringer af T-bølge morfologien var bedre til at separere normal fra abnormal repolarisering sammenlignet med QTc. Desuden kan nonlineære grænser være bedre som klassifikationsmetode end lineære grænser.

I vores andet studie undersøgte vi det elektrokardiografiske interval fra T-bølgens top til enden af T-bølgen ($T_{pe}$), en almindelig anvendt måling som antageligt repræsenterer transmural repolariserings-heterogeneitet. Repolariserings-heterogeneitet som induceres af torsadogeniske lægemidler antages almindeligvis at være ansvarlig for inskription af den elektrokardiografiske T-bølge og QT intervallet. Der er dog ingen bredt accepteret tilgang som kan bruges til at vurdere repolariserings-heterogeneitet. $T_{pe}$ intervallet er blevet foreslået som en måde til at kvantificere transmural dispersion (Tdr) baseret i tidligere in vitro studier. Dog har der været et stigende antal rapporter med uoverensstemmelser om hvorvidt $T_{pe}$ intervallet kan bruges til at forudsige arytmi. Det forbliver også kontroversielt hvad $T_{pe}$ intervallet faktisk repræsenterer. Det er dog stadigvæk vigtigt at kvantificere hvordan $T_{pe}$ intervallet er korreleret med repolariseringsvarighed af hele hjertet, repræsenteret ved QT intervallet. Vi undersøgte hvorvidt $T_{pe}$ intervallet er korreleret med QT forlængelse induceret at to torsadogeniske lægemidler. På trods af signifikant QT forlængelse med begge $I_{Kr}$-inhiberende lægemidler, forblev $T_{pe}$ intervallet næsten uændret. Studiet rejser derfor tvivl om anvendeligheden af $T_{pe}$ som biomarkør for repolariseringsændringer og torsadogenisk potentiale i studier af lægemiddelsikkerhed.

I vores tredje studie foreslår vi en ny metode til at vurdere abnormal repolariseringskaraktaristika på EKG'et. T-bølgen nedsamples til et minimum antal samples på en sådan måde at rekonstruering af den originale T-bølge er mulig. Ved at bruge 8 samples udtrukket fra den nedsamplede T-bølge som features var det muligt at separere normal fra abnormal repolarisering signifikant bedre sammenlignet med QTc. Derudover har denne tilgang også den fordel, i modsætning til QT intervallet, at være robust overfor bestemmelse af slutningen på T-bølgen. På den nedsamplede EKG repræsentation af T-bølgen er det yderligere indikeret at $T_{pe}$ intervallet kan forkortes efter $I_{Kr}$ inhibering og at de mest udtalte lægemiddel-inducerede repolariseringsændringer forekommer på det ascenderende segment af den minimale T-bølge repræsentation.

Samlet set kan dette arbejde føre til forbedret forudsigelse og fortolkning af EKG-relateret abnormal repolarisering.

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## INTRODUCTION

### -Developing Biomarkers (Clinical Problems and Needs)

From the early twentieth century, antiarrhythmic drugs have been introduced for treating arrhythmias. Although these agents are effective, there have been some reports on their side effects. Amongst the most worrying side effects is the unexpected occurrence of a life-threatening arrhythmia, later called "torsades de pointes" (TdP). The term TdP was first used by Francois Dessertenne [1], as a description of the electrocardiogram (ECG) tracing which had occurred in an elderly woman with high-grade atrioventricular block. The term describes a specific characteristic appearance of polymorphic ventricular tachycardia. Although the early reports mostly described proarrhythmia occurring during administration with antiarrhythmic agents, such as quinidine [2], TdP can also be seen in other settings, for instance in patients with one of the congenital long-QT syndromes (LQTS).

It was found that the risk of TdP is associated with repolarization abnormalities. Therefore, repolarization analysis using the surface electrocardiogram (ECG) has been used for research purposes and for the diagnostics of heart diseases and risk assessment. Generally, important features can be extracted from wave forms of ECGs. A number of those features have been obtained to provide information regarding abnormal and normal repolarization. New models and techniques for the analysis of abnormal repolarization on ECGs could also provide more accurate risk predictors for drugs, such as d,l-sotalol and sertindole [3] which have been shown to prolong repolarization, cardiac refractoriness and occasionally cause TdP.

There are numerous regulatory authorities around the world to ensure that drugs are safe. Among them, the Food and Drug Administration (FDA) is an important body pertaining to drugs. The relationship between the FDA and the ICH (International Conference on Harmonisatio), is also an important component to foster the advancement of the global harmonisation of regulatory requirements and practices. For example, their guidelines place stringent constraints on the allowable prolongation of the repolarization process. Especially, the "thorough QT/QTc study", a centre-piece of ICH E14 guidance, has been established to clinically assess a new drug's liability by identifying the repolarization prolongation. Therefore, as a part of the approval process for every new drug, before introducing it to the market, testing for cardiac safety using some risk predictors is required. ECG-based predictors can be used to assess the danger of the torsadegenic risk.

However, it's been several decades that in clinical ECG analysis, the only routinely used measure for cardiac safety, drug development and drug approval is the QT interval [4]. The QT interval can be measured by identifying the two critical points on the ECG: the initiation of the Q-wave and the termination of the T-wave. The measured QT interval, if prolonged because of the drug's effects, is essentially due to delayed termination of the T-wave [5]. If the prolongation is more than the threshold -defined from a particular guidance- it violates the regulatory concerns, presumingly indicating risk of TdP.

Prenylamine was the first drug withdrawn from the market due to cases of arrhythmia in 1988. Many more withdrawals, restrictions, and denied market authorizations have since followed for a range of drugs including terodiline, astemizole, cisapride, lidoflazine, droperidol, levomethadyl, grepafloxacin, mesoridazine, sparfloxacin, terfenadine, thioridazine, and sertindole. But the most used drugs that cause TdP are still QT-prolonging antiarrhythmics, such as quinidine, sotalol, dofetilide and ibutilide [6-9]; an up-to-date list is maintained at the www.torsades.org.

In recent years, the FDA has expanded its requirements for drug testing. In February 2002, the ICH S7B [10] guidance was proposed and although it has not been finalized, provided more specific

directions for cardiac safety testing of new drugs, both in vivo and vitro studies. The ICH S7B specifies that new drugs should be tested in three preclinical assays: the human ether-a-go-go–related gene channel assay to check for blockade of the $I_{Kr}$ channel, the action potential duration (APD) in vitro assay (typically cell culture) to check for significant APD prolongation, and through a series of in vivo ECG experiments with a detailed description of test methods. The guidance also suggests that if the tested drug is shown in these three preclinical assays to cause some blockade of the $I_{Kr}$ channel or prolongation of the APD, its potential clinical risks should be evaluated in carefully designed clinical trials.

In addition, the ICH E14 guideline (available at www.fda.gov/cder/guidance/) [11] provides recommendations for conducting clinical studies to assess the potential of a drug to delay cardiac repolarization. Especially, the "thorough QT/QTc study" determines whether the drug has a below threshold pharmacological effect on cardiac repolarization, (QTc prolongation), before it can be approved and marketed. These studies must have adequate sample sizes and should ensure frequent recording of ECGs because an adverse outcome of a study can be detrimental to the safety profile of the drug.

On the other hand, the cost and time involved in running a thorough QT study is substantial. Specifically, although a "thorough QT/QTc study" requires between $1.5 and $5 million [12], the study has several notable limitations. The study results vary depending upon the methodological variability [13]. Also the list of QT-prolonging drugs includes several drugs that provide substantial health benefits. Therefore, it would be more costly (not only economically but also socially) if potentially beneficial compounds would be withdrawn early in the development.

As a result, some researches may criticize the fractious relationship between ICH E14 and ICH S7B, the high cost for obtaining the approval and the inefficiency at providing a fair distribution of the resources required for the drug development. Especially, the "thorough QT/QTc study" just uses the simple QT interval to assess the cardiac repolarization of the drug effect. The QT/QTc interval as a current biomarker has been shown to be unconvinced and inefficient in accurate prediction of cardiac toxicity [14]. Thereupon, more robust innovative methods for preclinical and clinical testing of drugs, resulting in lower costs and shorter time frames are needed.

One of the most exciting developments in our understanding of ECG-based biomarkers for drug screening, drug development and safety toxicology over the last several years has been the recognition of the contribution of the ion channel to the cellular electrical properties in a way that promotes the occurrence and maintenance of arrhythmia.



**- Underlying Electrophysiological Mechanisms (Cellular Level and Ion Channel Effects)**

At the cellular and tissue levels, ion channel studies for the prediction of cardiac toxicity can be performed early in the drug development process. It is now recognized that the analysis of ion channel current as the result of a combination of biophysical, biochemical, and biogenic properties is an important step in the evaluation of drugs. Similarly, evolution of knowledge about the complex cellular mechanism that modules ion channel functions, has added an important dimension to our understanding of TdP and indeed of generation of other arrhythmias.

According to recent experimental studies, the heterogeneity of the physiologic action potentials - with the longest action potentials in the mid-myocardium (the 'M-cell' layer), and the shorter action potential durations in the epicardium and endocardium- might be responsible for the occurrence of TdP [15]. Based on these experiments, the long time-course of M-cell repolarization determines QT prolongation and relevantly, the short time-course of epicardial repolarization coincides with the peak of the T-wave in an artificial ECG. Therefore, the intensive different repolarization properties of these cell types, as well as their interplay, appear to be responsible for the distinctive

changes in the electrocardiographic T-wave morphology. These may inscribe the determination of the height and width of the T-wave morphology and for example, whether it has a monophasic, bifid or notched appearance in conditions of drug-induced TdP.

In addition, the so-called early after depolarization (EAD) is known as a potential triggering factor of TdP and other arrhythmias [16]. It is generated with abnormal depolarization during phase 2 or phase 3 of an action potential. It is caused by an abortive action potential prolongation before normal repolarization is completed. It may appear as the distinctive morphologic changes in the trajectory of terminal repolarization on the surface ECGs.

From a cardiac electrophysiological point of view, QT prolongation may reflect an increase in action potential duration in at least some regions of the ventricle. An increased action potential duration, in turn, must reflect an increase in inward current or a decrease in outward current. Several ion channels are involved in the generation of the cardiac action potential. However, it has been shown that the vast majority of drugs associated with TdP act by reducing one specific potassium current, the so-called rapid component of the delayed rectifier, $I_{Kr}$. This ion channel is primarily formed by the KCNH2 (hERG) gene [17]. It is critical in correctly timing the repolarization of the cardiac membrane during the action potential.

Consequently, QT prolongation and T-wave changes can be the result of the interaction of drugs with the "hERG channel" which increases the risk of lethal arrhythmias. This is of particular interest because it was found that one of the most common forms of LQTS, LQT2, is caused by dysfunction of the hERG protein that forms the $I_{Kr}$ channel and $I_{Kr}$ inhibition is affected by a large number of cardiac and non-cardiac drugs as well [17]. Of note, the congenital long QT syndromes are in this way simpler than many drug-induced syndromes. Many drugs have effects on multiple channels, whereas each of the congenital syndromes is caused by dysfunction of a specific gene and thus a specific channel.

Although screening drugs for acute $I_{Kr}$ block has become standard in the pharmaceutical industry, it is important to recognize the possibility that other mechanisms may contribute to drug-induced QT prolongation and the torsadegenic risk. For example, unlike most other $I_{Kr}$-blocking drugs, DPI-201 as a positive inotropic agent, or ibutilide, as a "pure" Class III antiarrhythmic drug, thought to result in QT prolongation and causing torsades [18-21], have action on the enhancing inward current through sodium channels or calcium channels.

In addition, there is a newer recognition that drugs may also reduce KCNH2-mediated current not by channel block, but rather by reducing cell surface expression of functional channels, likely by reducing trafficking of mature channels to the cell surface. This has been reported for arsenic [22] and pentamidine [23], which are well recognized as causes of TdP. Thus, a drug that does not acutely block $I_{Kr}$ in heterologous expression systems may still be associated with QT prolongation during clinical therapy.

Therefore, biological studies of cellular and ion channels in understanding of drug suspected TdP are complex since cardiac repolarization cannot result solely from $I_{Kr}$, but rather from a complex interplay among this current and other repolarizing currents. This complex biology which has not been well understood is further regulated by the external environment, notably extracellular potassium, heart rate and adrenergic tone. Thus, identifying such compounds producing TdP has not yet been worked out completely.

Adding to the cellular and ion channel complexity, different parts of the heart, even from different layers of a particular part have different APDs and may respond differently to external factors such as by $I_{Kr}$-blocking drugs. This may make the repolarization process more heterogeneous. Particularly, our understanding about the functional and clinical importance of M cells remains controversial. Its electrophysiological properties may pay major role in determining QT interval and relevant for the configuration of the T-wave [24].

**- QT Interval (Limitations and Problems)**

The QT interval is a clinical parameter of particular interest in cardiology. It represents the duration of ventricular electrical systole, i.e., the time required for completion of both ventricular depolarization and repolarization. However, the relationship between duration of cellular action potentials as mentioned above and the QT interval measured at the body surface is complex [25]. Especially, there is still incomplete understanding of the recovery process and its projection on the peakedness of the T-wave form. Consequently, precise analysing of the ECG-based reporalisation is difficult from a practical point of view. Particularly, one of the most challenging problems in clinical studies is the inherent inaccurate measurement based on the termination of the T-wave. Usual difficulties occur when the T-wave is flat, bifid, biphasic, broad, notched or when it overlaps with a U-wave.

Second, significant variation both in the onset of the QRS complex and the end of the T-wave among some ECG leads provides less repeatable QT values depending on the leads selected for the measurement. Moreover, even in a normal ECG of good quality, there is relatively difficulty in the determination of the onset of the Q-wave. Thus, manual measurements of QT interval would potentially lead to considerable intraobserver and interobserver variations [26].

Third, technical factors such as paper speed and sensitivity influence QT measurements. The higher paper speed, the shorter interval values is likely to result, for example. Although the ICH E14 guideline as well as technical development has encouraged researchers to provide more developed automatic measurement methods, the above problems remain unsolved even in digital QT recordings and automatic measurement techniques.

Fourth, lack of enough information regarding the impact of various heart diseases and drugs on QT intervals may make it difficult to have a certain threshold for pharmacologic effect on cardiac repolarization. Unfortunately, the difficulties in measuring the QT interval, which may depend on personal experience, result in different interpretations from the same finding [27].

Another issue regarding accurate evaluation of the QT interval is that this measurement varies with heart rate (RR interval). The QT interval changes are inversely correlated to heart rate changes, even in the case of no drug intervention, heart rate variation itself causes QT interval changes.

Clinically, several heart rate corrected QT interval formulas, such as Bazett's, Fridericia's, etc. are usually used to evaluate QT prolongation due to changes in the duration of the RR interval. The goal of the correction is to obtain a corrected QT interval that is statistically independent of the RR interval. But, numerous correction formulas in the ECG literature reflect the variety of statistical models that could fit the data. Unfortunately, from the date of introducing the corrected-QT interval (QTc) in 1921 [28] until now, the problem has not been resolved. There is still a lack of a universally accepted formula for heart rate correction. The challenge still exists in choosing a suitable correction among various correction methods that result in different QTc values for a particular dataset [29].

Usually these methods are population-based correction formulas assuming that the relationship between QT interval and heart rate is the same for all subjects. Thus, each correction model handles a particular set of QT/RR data from a population. Therefore, understanding the relationship and the limitations of each correction are critical. For example, the Bazett and Fridericia corrections are based on simple models and both formulas produce similar results when the range of heart rates is not high. But the higher range of heart rates causes more variation of QTcB. Thus, QTc deals with a limited range and the relationship between QT interval and heart rate could be subject-specific [29] depending on some variables such as age, gender, and drug regime. Although, subject-specific

corrections may be preferable to population-based correction formulas, crucially enough, this causes some problem in clinical trials. Especially, in studies with multiple ECG recordings per subject at baseline when subjects are resting and their heart rates do not have usually an adequate range of heart rates in the baseline data. In this case, a subject-specific correction perhaps with a narrow range of pretreatment heart rate is not the accurate correction and may lead to false conclusions in clinical studies.

Furthermore, even though there are QT-related exclusion criteria in accordance with the ICH E14 guidance regarding drug monitoring, other sets of challenges are faced: the severity of pro-arrhythmia at a given QT interval varies between drugs and between subjects; the extent of QT prolongation and TdP risk with a given drug may not be linearly related to the dose or plasma concentration of the drug; patient-metabolic risk factors of chronic diseases may play a role in this case such as blood pressure and diabetes. Consequently, the relation of the QT/QTc interval to the TdP risk is unclear.

Finally, an increasing number of clinical studies shows that there is not a simple relation between the degree of drug induced QT prolongation and the likelihood of TdP. The arrhythmia can occasionally develop without any substantial mark of QT interval prolongation. On the other hands, some drugs can cause substantial prolongation of QT interval but have rarely (amiodarone [30] or never (pentobarbital, verapamil) [31] been associated with torsades or other malignant tachyarrhythmias.

There are thus numerous experimental and clinical studies showing evidence that the QT interval is a poor surrogate marker of proarrhythmic susceptibility, and there are many shortcomings to the assumption of a simple QT proarrhythmia relationship [14, 25, 32, 33]. These may limit QT's clinical utility, creating a gray zone for diagnostic accuracy determined by both types of errors, the percentages of false-positive and false negative classification errors. A false positive in drug-quality control discards a drug that is actually well made, whereas a false negative stamps a dangerous drug as operational.

Therefore, QT remains a poor surrogate marker of proarrhythmic susceptibility. Particularly, the QT interval ignores the information in the shape of the curve (the T-wave morphology), relying instead upon only two points of the complex curve. Rather, in clinical research there is an ongoing search for other ECG-based markers of repolarization to examine the T-wave morphology changes.

**- Alternatives to QT Interval**

A number of repolarization-based parameters exploited from the 12 leads surface ECG have been suggested as alternatives or in addition to QT interval measurements. QT dispersion (QTD) as one of the newer ECG-based repolarization parameter might be the best known and most widely investigated. It is defined as the difference in duration between the longest and shortest QT intervals (maximum – minimum QT intervals) on a given set of leads. QTD has been considered as an indirect measure of spatial heterogeneity of repolarization reflecting regional variation in ventricular repolarizations. Since several experimental studies have demonstrated that heterogeneity in repolarization has been linked to the induction of ventricular fibrillation [34] and ventricular tachycardia [35], QTD might be useful in assessing drug efficacy and safety. In contrast, there are some reports [36, 37] indicating that heterogeneity of ventricular repolarization is not directly presented in QTD.

T-wave alternans has been also reported with congenital LQTS [38] and appears to be an important indicator because it is commonly observed just prior to episodes of TdP. But these approaches have also been proved to be the least successful in predicting the risk of drug-induced TdP [39].

However, it is important to notice which methods of determining the termination of the T-wave are used in developing repolarization-based parameters from the ECG. As mentioned in the section above, precise measurements of the QT interval is challenging. This problem may still remain for measuring other repolarization-based parameters. Such measurements may thus be dependent on the methods available for detection of the T-end point. There are various methods such as: tangent method [40], template matching [41] or measurement to the maximum derivative of the T-wave down slope [42]. But recent work of computerized systems for ECG analysis [40], using the tangent method showed that this algorithm worked appropriately in ECG recordings that were of good quality and had a normal QRST morphology. Otherwise, a more robust method seems to be needed to enable analysis of less perfect ECG signals on a single-beat basis.

As the trailing edge of the T-wave appears to shift in parallel with varying QT interval, another repolarization-based parameter, deltaT50, was developed. It measures the temporal variability of the steep part of the trailing edge of the T-wave instead of the end of the T-wave. The measurement was applied to assess the beat-to-beat variability in cardiac repolarization time [43].

The $T_{pe}$ interval was also proposed from previous experimental studies [15] in ventricular wedge preparations obtained from the free wall of the canine left ventricle. These studies concluded with the theory that $T_{pe}$ might be an important index in quantifying transmural heterogeneity of repolarization. The theory was based conceptually on differences in the action potential duration of the 3 principal cell types that comprise the ventricular myocardium. In this case there are also some clinical studies supporting this theory. Some of these studies have shown that drug-related arrhythmic potential is also associated with marked increases in $T_{pe}$ [5], and thereby $T_{pe}$ might be a biomarker in identifying arrhythmia.

However, there are some controversies as to what the $T_{pe}$ interval actually represents in clinical settings [44]. These controversies in the literature have highlighted the following assumptions: First, if $T_{pe}$ expresses transmural repolarization heterogeneity and if $I_{Kr}$-blockers increase this heterogeneity, will the $T_{pe}$ intervals in subjects receiving $I_{Kr}$-blockers be longer than before receiving drugs? Second, if the proarrhythmic effects of these drugs are at least in part achieved by the increase of QT interval, will $T_{pe}$ correlate to the QT interval? These questions are discussed in-depth in the second article in this thesis.

**-Quantifying T-wave Manifestation**

Previous canine wedge-model experiments [15] by Antzelevitch et al. have shown that QT/QTc interval prolongation induced by $I_{Kr}$ inhibition is not solely an expression of abnormal repolarisation on ECGs. Indeed, the transmural dispersion of action potentials causes remarkable changes in T-wave morphology. Similarly, but in humans, examining the T-wave area-based in subjects receiving sotalol, several studies have confirmed the phenomenon on the surface ECG showing that drug induced shape changes occur across the entire repolarization segment [45]. Hence, T-wave morphology changes may play a more important role in differentiating between safe and unsafe drugs than the crude QT interval duration.

The particular interest is the ECG-analysed information from patients with congenital and acquired LQTS, potentially associated to TdP. The findings represent distinctive T-wave morphology patterns characterized by the appearance of flat, bifid, notched and low amplitude T-waves [46].

This phenomenon could be described by various degrees of heterogeneity which is dependent on the cells differential properties within the ventricular wall. As mentioned in previous sections, the unique electrophysiologic and pharmacologic profiles along epicardial, endocardial surfaces were demonstrated from a number of hearts of several species [15, 47]. Especially, these recent studies in

the cellular basis for the ECG-based repolarization manifestations of LQT2 have implicated predominant M cells located in the intramural layers of the ventricular myocardium associated with more proarrhythmic potential [47].

In addition, drug-induced $I_{Kr}$ inhibition can also cause triangulation of the action potential with loss of notch and-dome morphology replaced by a linear phase repolarization. Triangulation is reported to be a strong predictor of arrhythmia [33]. It may appear on the ECG as a widened, flattened or notched T-wave. Interestingly, triangulation may be accompanied by either shortening or lengthening of the cardiac action potential duration which indicates that drug-induced T-wave morphology changes may be independent of QT interval measurements.

In conclusion, drug-induced repolarization heterogeneity may manifest, not only as differences in action potential durations, but also as changes of action potential shapes. The interplay between the voltage gradients generated by three predominant ventricular cell types determined the height of the T-wave as well as the degree to which the ascending or descending limb of the T-wave was interrupted, giving rise to bifurcated T-waves and "apparent T-U complexes" as could be seen under LQT conditions [48].

However, prominently lacking are data regarding various degrees of heterogeneity that exists among the cells. This may exist as transmural gradients, locally across the ventricular wall or as global gradients. Moreover, the role of M-cells or their existence is unknown in humans. The functional M cells could also relatively contribute to the electrocardiographic T-wave and to our understanding of the mechanisms responsible for the T-wave under normal as well as pathophysiological conditions. However, although the magnitude and direction of ventricular gradients within the heart during the T-wave are not clearly understood, it is generally accepted that the T-wave on the surface ECG is generated by voltage gradients during the ventricular repolarization of the heart. Thereby, the T-wave is more likely to reflect the cardiac repolarization of the heterogeneous myocardium than QT interval.

Therefore, $I_{Kr}$-blocker-induced cardiac repolarization, for example, may potentially result with more changes in the T-wave morphology than in the QT prolongation though the underlying mechanisms are not well understood.

Importantly, quantifying T-wave manifestation in drug studies can play a principle role to gain more characteristics of cardiac repolarization than just measuring a simple duration determined by the QT interval.



**-T-wave Morphology Analyses**

Attempting to quantify the repolarization changes of drug-induced arrhythmia by other parameters than QT interval, a number of different T-wave morphology-based parameters have been proposed in the literature. The parameters can be based on: duration [45], amplitude [49, 50], T-wave area and symmetry [45, 50, 51]. Although these parameters have proven to be sensitive to drug effects, so far no robust, widely accepted method has been made available [52].

According to a comprehensive review of T-wave morphology based biomarkers by Brennan and Tarassenko [53], although the morphology of the T-wave could be of greater importance than QT interval for the assessment of the abnormal repolarization, the wide variety of morphology-based biomarkers necessitates the selection of a subset for further analysis. Indeed, repolarization is more complicated and contains more information than just to be expressed by assessment of readily apparent characteristics, for example, duration, area, amplitude, and slopes. A full description of T-wave morphology changes might be done by analysing the whole T-wave morphology elements.

Some mathematical approaches such as principal component analysis (PCA) [54] and Gaussian models [55] applied to analyses of the entire shape of the T-wave. These methods could fit the wave forms quite well and reduce the dimension of the data. However, they could not provide the physically interpretable framework of the T-wave morphology changes and also they are not robust to small changes in T-waves such as those caused by noise or inaccuracies in measuring the termination of the T-wave.

The sampling of the T-wave into a minimum number of discrete-time samples can be used as a full descriptor of T-wave shape characteristics. The method can also make the descriptor less dependent on other ECG features such as QT interval or heart rate. This may provide a new opportunity to better describe and understand repolarization abnormalities induced by the drug-related arrhythmic potential.



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**PURPOSE OF THIS STUDY**

The primary objective of the present study is to describe the development and the validation of a novel methodology to assess abnormal repolarization reflected in ECG-related repolarization. To this end, we apply the technique on digital ECGs recorded from subjects who are healthy or with a particular disease or/and medication, studied under standardized conditions.

Based on our previous experience, a combination of parametric cases of ECGs reflecting duration and amplitude of repolarization properties resulted in high efficiency detection and classification in the abnormal repolarization condition. Therefore, this work has further aimed to discover whether other kinds of ECG-related repolarization properties behind readily apparent characteristics as duration or peak parameters represent a more robust and interpretable manifestation of drug-induced repolarization abnormality. The results of the proposed method are to be compared to the usual parametric cases of the particular interest in cardiology. Especially the finding is comparable to the QT interval which is currently considered as the gold standard for assessment of abnormal repolarization.

It reminds us of the importance of robustness in ECG-based predictors. This property may greatly influence the validation of the results using electrocardiographic evaluation for drug safety. This approach may help to explain systematically the effects of different components of the T-wave and find more properties within the T-wave shape. The results may make better predictions and efficient interpretation about abnormal repolarization induced by the torsadogenic drug comparing to parametric cases like QT/QTc interval.

Evaluation and improvement of the methodology is ongoing for further research.