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Model Based Analysis of QT Variability Independent of Heart Rate and Respiration

By

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Contents

Abstract

Electrical instability in the ventricles of the heart predisposes patients to abnormal heart rhythms (ventricular arrhythmia) and sudden cardiac death (SCD). Current risk assessment strategies for identifying patients at high risk of SCD are mainly based on markers of structural dysfunction. However, most deaths occur in those deemed at low risk by traditional risk markers, stipulating the need for improvement of existing risk assessment strategies. Malignant neural modulation of the repolarisation process also contributes to arrhythmogenesis and increased risk of SCD. Improving the ability to sense instability in the repolarisation process and quantifying neural contribution to arrhythmia can improve the ability to predict the onset of ventricular arrhythmias and improve risk stratification strategies. The main aim of this thesis is to quantify repolarisation variability independent of heart rate and respiration, explore its prognostic value for improving risk prediction and examine its relationship to sympathetic neural activity.

Parametric power contribution analysis methods and autoregressive modelling are used to quantify repolarisation variability independent of heart rate and respiration. Temporal ECG features that best capture the influence of sympathetic activation on repolarisation independent of other factors are identified by comparing LF powers of several features following increase in sympathetic activation elicited by orthostatic stress. Measurements of QTV independent of heart rate and respiration from single leads (II and V5) are compared to that of multi-lead approaches in a group of coronary artery disease patients to investigate the suitability of single-lead ECG for the analysis of repolarisation variability independent of heart rate and respiration. The influence of sympathetic activation elicited by orthostatic stress on this fraction of repolarisation variability is investigated in healthy adults and adolescents. Finally, survival analysis is used to explore the prognostic value of this fraction in a large cohort of myocardial infarction patients. Using stepwise multivariable Cox regression analysis, a QTV risk score was developed, and its predictive value was investigated.

Results show that the variabilities of QTend and RTend increased with sympathetic activation elicited by tilt independent of heart rate and respiration. The improved model fit or signal-to-noise ratio in the multi-lead approaches did not lead to significant differences in QTV compared to single leads. Measurements of the intervention effect and pathophysiological group differences in QTV independent of heart rate and respiration were consistent in single and multi leads. Results also show that QTV independent of heart rate and respiration increased

with sympathetic activation elicited by tilt and exhibited a low frequency peak consistently. Finally, this fraction of QTV was elevated in non-surviving myocardial infarction patients and exhibited a clear LF peak indicating a rhythmical source. Cox proportional hazard model analysis shows that QTV independent of heart rate and respiration is predictive of mortality. A QTV risk score that includes QTV independent of heart rate and respiration is found to be predictive of mortality independent of traditional risk markers. Further the proposed QTV risk score was able to identify a subgroup of patients at higher risk of mortality within a group deemed as low risk using traditional risk markers.

In conclusion, results from this thesis show that QTV independent of heart rate and respiration originates from a rhythmical source, is increased by sympathetic activation, and might help improve stratification of patients at higher risk of mortality in combination with existing risk assessment strategies.

Thesis declaration

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name, in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission in my name, for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

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Fatima El-Hamad

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29/11/2022

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Thesis convention

The following conventions have been adopted in this Thesis:

1. Spelling: Australian English spelling conventions have been used as defined in Macquarie English dictionary.
2. Typesetting: this document was created using Microsoft Word 365.
3. Referencing: The Harvard style has been adopted for referencing.

Publications

Journal articles

EL-HAMAD, F., LAMBERT, E., ABBOTT, D. & BAUMERT, M. 2015. Relation between QT interval variability and muscle sympathetic nerve activity in normal subjects. *American Journal of Physiology: Heart and Circulation Physiology*, 309, H1218–24.

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List of figures

List of abbreviations and acronyms

APD	Action Potential Duration
CVD	Cardiovascular Disease
ECG	Electrocardiography
HF	High Frequency
HR	Heart Rate
HRV	Heart Rate Variability
ICD	Implantable Cardioverter Defibrillator
LF	Low Frequency
MI	Myocardial Infarction
MSNA	Muscle Sympathetic Nerve Activity
NE	Norepinephrine
QTV	QT Variability
QTVi	QT Variability index
RRV	RR Variability
RV	Repolarisation Variability
SCD	Sudden Cardiac Death
SDQT	Standard Deviation of QT
VF	Ventricular Fibrillation
VT	Ventricular Arrhythmia

Chapter 1

Introduction

This chapter describes the motivation for this thesis, defines the aims of the thesis, explains the thesis structure, and provides an overview of the forthcoming chapters.

1.1 Background and motivation

Cardiovascular disease (CVD) is the leading cause of death in the world, according to the World Health Organisation (2022), claiming 32% of all deaths worldwide. It encompasses a range of conditions that impact the heart function, affecting the cardiovascular structure such as blood vessels, heart valves and heart muscles and the electrical activity of the heart related to the triggering of abnormal heart rhythms (arrhythmias). The majority of deaths due to CVD are classified as sudden cardiac deaths (SCD), where the person dies within an hour of the onset of any symptoms in cases of witnessed death and within 24 hours from last seen in cases of unwitnessed deaths (Wong et al., 2019). While the prevalence of CVD deaths appears to be declining with improved access to health care and early detection, the incidents of SCD within this population are increasing (Adabag et al., 2010, Wong et al., 2019).

Indeed, SCD is a major health problem worldwide with a severe impact on families who experience this sudden loss. Incidents of SCD vary with age, peaking during infancy (under 6 months) and after the age of 45 (Zipes and Wellens, 1998, Adabag et al., 2010). While SCD incidents are rare in young individuals, the percentage of SCD out of all deaths in this group is high (Hayashi et al., 2015, Feng et al., 2017). As for sex differences, men have a higher incidence of SCD than women (Zipes and Wellens, 1998). The major clinical challenge in SCD prevention is that most SCDs occur in individuals without a previously diagnosed cardiac condition or in patients considered low risk by traditional risk markers (Zipes and Wellens, 1998, Sinner et al., 2020). This is demonstrated by the fact that SCD is the first manifestation of an underlying cardiovascular disease in nearly 50% of SCD incidents in women and in around 65% of SCD incidents in men (Hayashi et al., 2015). Hence, it is necessary to improve the identification of risk factors to assist in formulating strategies for risk prediction and subsequent prevention of SCD.

Generally, SCD is characterised by the presence of abnormal heart muscle tissue coupled with a transient trigger (Zipes and Wellens, 1998, Adabag et al., 2010). Electrical instability in the heart that results in arrhythmia is a major trigger predisposing individuals to SCD (Kuo et al., 1985, Hayashi et al., 2015), even in the absence of an evident structural heart disease. The two main arrhythmias responsible for SCD occur in the lower two chambers of the heart (ventricular arrhythmia), namely ventricular tachycardia (VT) and ventricular fibrillation (VF) (Schwartz et al., 1992, Liew, 2010, Wellens et al., 2014, Niemeijer et al., 2014). The normal heart rate for a healthy adult can range between 60 bpm and 100 bpm. In VT, an abnormal increase in heart rate (more than 100 bpm) is triggered by abnormal ventricular electrical

activity, in which case the heart contracts before the ventricles are adequately filled with blood, resulting in reduced blood supply for the rest of the body. Occasionally, VT can lead to the ventricles contracting in an uncoordinated manner causing them to quiver rather than contract (VF). This leaves the heart unable to supply the blood with blood circulation. An incident of VF is a medical emergency that can lead to cardiac arrest and subsequent SCD if not treated within minutes. Implantable cardioverter defibrillators (ICDs) are used for primary prevention of SCD. While both electrical instability and structural dysfunction contribute to VT and VF, current international guidelines for ICD implantation are predominantly based on measures of mechanical dysfunction of the left ventricles, mainly decrease in the ejection fraction (O'Gara et al., 2013, Priori et al., 2015, Bauer et al., 2008). Yet, most of SCDs occur in individuals with preserved left ventricular function (Buxton et al., 2007, Wellens et al., 2014). Therefore, improving the ability to predict or detect electrical instability is critical for improving risk assessment for SCD and ICD implantation (Sinner et al., 2020, Hamm et al., 2017, Isbister and Semsarian, 2019).

Electrocardiography is a commonly used tool that records the heart's electrical activity from the body surface, resulting in an electrocardiogram (ECG) signal. Many studies have explored the value of ECG-derived measures for assessing potential lethal electrical instability and improving risk stratification (Niemeijer et al., 2014, Wellens et al., 2014, Holkeri et al., 2020, Murugappan et al., 2021). More specifically, ECG-derived markers of ventricular repolarisation instability are associated with an increase in the risk of VT and SCD (Soliman et al., 2011, Rizas et al., 2016, Chen et al., 2011, Tomaselli et al., 1994, Atiga et al., 1998), and dispersion in repolarisation can precede ventricular arrhythmia in humans (Chen et al., 2011) and animals (Kuo et al., 1985). Thus, investigating ventricular repolarisation and its role in arrhythmogenesis is of great clinical importance.

The sympathetic arm of the autonomic nervous system also contributes to the initiation and progression of ventricular arrhythmia (Manolis et al., 2021, Franciosi et al., 2017, Schwartz et al., 1992). Increased sympathetic activity has been shown to precede the onset of lethal ventricular arrhythmia in animals (Zhou et al., 2008) and humans (Meredith et al., 1991, Cao et al., 2000). Interestingly, a considerable body of literature suggests that the dynamic changes in ventricular repolarisation duration (repolarisation variability, RV) reflect the influence of sympathetic activation on the ventricles (Berger et al., 1997, Porta et al., 2010). Hence, the analysis of RV and its relationship to sympathetic activation is key to enhancing our knowledge of the mechanisms underlying ventricular arrhythmia. One challenging aspect to investigating this relationship is that multiple factors contribute to RV concurrently. Hence, many widely

used measures of RV do not provide a quantitative distinction between RV due to sympathetic activation and that due to other factors. As targeted neural modulation has been found to be effective in managing ventricular arrhythmias, non-invasive quantification of neural regulation of the ventricles has become of pivotal importance and may improve early diagnosis of ventricular arrhythmia. Further, it will potentially help inform treatment choices and drug development by monitoring modulatory effects of the drug on neural regulation. Hence, a non-invasive quantification of sympathetic contribution to ventricular instability can assist in improving risk assessment for ICD implantation. It can also improve the ability of ICDs to detect electrical instability, which is vital considering that device dysfunction can contribute to ICD related mortality, and the occurrence of shock waves–even if appropriate–causes mental stress and affects the quality of a patient's life (Kossaify, 2020). In this thesis, we explore the relationship between the dynamicity of the repolarisation process and the sympathetic arm of the autonomic nervous system. Furthermore, we evaluate the prognostic value of RV in improving risk stratification beyond its well-established dependence on heart rate.

1.2 Thesis aims

The main objective of this thesis is to investigate RV independent of heart rate and respiration in different populations and elucidate the influence of enhanced sympathetic activity on that component of RV. From there, we aim to explore the clinical value of this fraction of RV for improving risk stratification. In this thesis we aim to:

1. Explore the utility of parametric decomposition of RV in quantifying repolarisation changes independent of heart rate in various populations,

2. Describe the influence of elevated sympathetic activity on RV independent of heart rate and respiration,

3. Identify ECG features that best measure the effect of elevated sympathetic activation on ventricular repolarisation,

4. Investigate the adequacy of single-lead ECG for analysis of RV independent of heart rate and respiration,

5. Investigate the relationship between RV independent of heart rate and respiration and a direct measure of sympathetic activity, namely muscle sympathetic nerve activity,

6. Explore the prognostic value of RV independent of heart rate and respiration for

improving risk stratification.

1.3 Thesis structure

This thesis consists of seven chapters and one appendix. Figure 1.1 depicts the thesis structure, and a detailed description of each chapter is provided below. Chapters 1 and 2 provide necessary background and limitations of current literature. Chapters 3 and 4 investigate technical aspects related to RV analysis, while chapters 5 and 6, and Appendix A investigate the physiological basis and prognostic value, respectively, of RV independent of heart rate and respiration.

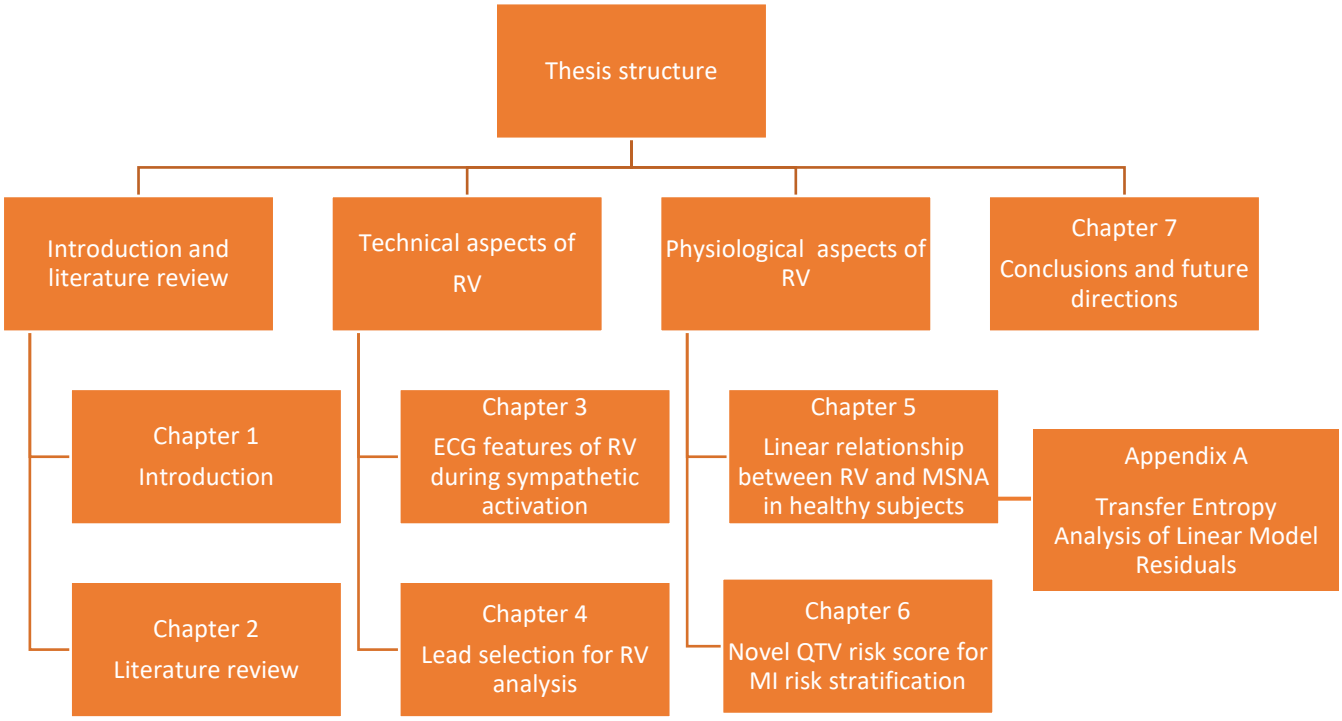

Figure 1-1 Thesis structure showing a brief description of each chapter.

Chapter 2 describes the physiological basis of the repolarisation process and factors contributing to the variation of its duration on a beat-to-beat basis. The chapter reviews current methods employed to quantify and disentangle the effect of these different factors on RV, focusing on the role of sympathetic activation. This chapter also discusses the clinical and prognostic value of RV, focusing on RV independent of heart rate fluctuations.

Chapter 3 aims to identify ECG-derived temporal RV measures that best reflect the influence of enhanced sympathetic activation on RV independent of heart rate.

Chapter 4 investigates the suitability of single-lead ECG for analysis of RV independent of heart rate compared to multi-lead ECG.

Chapter 5 aims to provide quantitative evidence of the associations, if any, between RV independent of heart rate and sympathetic activation in a population of healthy subjects under conditions of a heightened sympathetic drive.

Chapter 6 investigates the prognostic value of RV independent of heart rate in a large population of myocardial infarction patients. The chapter proposes a novel QTV risk score that improves risk stratification for subgroups of MI patients that would otherwise be deemed at low risk of mortality post MI using traditional risk markers.

Chapter 7 summarises the work carried out in this thesis and provides a perspective on future directions for the research topic.

Appendix A expands on the investigation in Chapter 5 by exploring the relationship between RV and sympathetic activity using nonlinear analysis methods. This chapter provides a novel methodology for disentangling linear and nonlinear interactions in a multivariate system.

1.4 Statement of original contribution

The four main chapters of this thesis, Chapters 3 to 6 and Appendix A, are all original peer-reviewed articles arising from work of this thesis. In Chapter 6, the author developed a novel risk score that improved risk stratification in MI patients. In Appendix A, the author presents a novel methodology for assessing linear and nonlinear relationships in a multivariate system. The formulation of the hypothesis, the development of suitable research methodology to test these hypotheses, and most statistical analyses were original and sole contributions of the author of this thesis.

Chapter 2

Literature review

Ventricular repolarisation is the process by which the cardiac cells in the ventricles of the heart recover and reset their electrochemical gradient leading to the initiative of ventricular muscle relaxation after a ventricular contraction. Repolarisation exhibits beat-to-beat fluctuations in its duration termed repolarisation variability (RV), which is the result of multiple physiological mechanisms both, intrinsic and extrinsic to the cardiac cells. This chapter briefly explains the repolarisation process as part of the heart's electrical activity, beat-to-beat fluctuations in repolarisation duration, factors driving this fluctuation and methods used to quantify and disentangle the effect of these different factors. This chapter also discusses the clinical and prognostic value of RV with a focus on RV independent of heart rate fluctuations.

2.1 The heart

2.1.1 Electroanatomical structures of the heart

The heart is responsible for delivering blood to the entire body. It consists of a mechanical and an electrical structure that work in synchrony to maintain this function. Figure 2-1 shows the features of the two structures. The mechanical structure mainly consists of four chambers; the right atria and ventricle, which are responsible for transporting blood from the body to the lungs through the pulmonary artery, while the left atria and ventricle transport oxygenated blood to the rest of the body through the aorta. Cardiac valves control the direction of blood flow within these chambers during cardiac contraction and relaxation. The walls of the cardiac chambers consist of many specialised cardiac cells called myocytes. Myocytes are connected in series and in parallel with one another thus allowing for a synchronised movement during contraction and relaxation of the heart muscle. Their cell membranes are fused in a way that allows fast diffusion of ions, facilitating the propagation of electrical potential from one cell to the next.

Contraction and relaxation of cardiac myocytes is controlled by the heart's specialised electrical

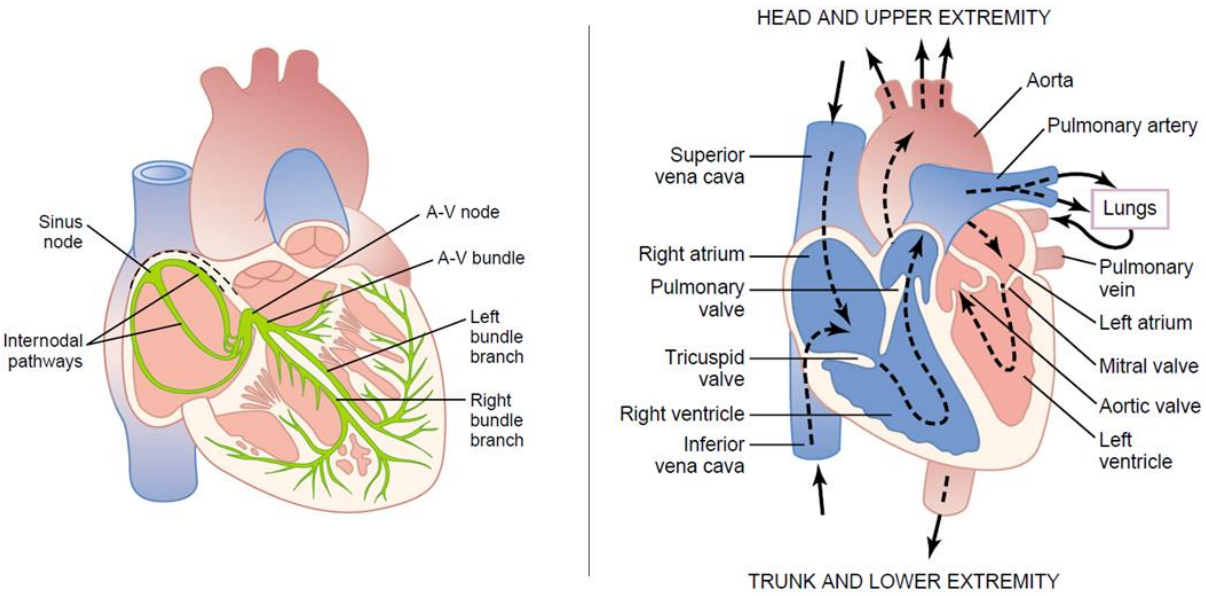

Figure 2-1 Electrical (left) and mechanical (right) structures of the heart (Hall, 2011).

structure (left panel in Figure 2-1), which maintains the heart's rhythmicity and propagates action potential throughout the cardiac muscle. The sinoatrial node is located in the wall of the left atria and generates intrinsic rhythmical electrical impulses that maintain a normal heart rhythm (sinus rhythm) at 70 to 80 beats per minute. Hence, the sinoatrial node is also called a pacemaker. The electrical impulse is carried on to the A-V node through the internodal pathways, which in turn conducts a delayed impulse to the ventricles through the A-V bundle.

The impulse is delayed by around 0.16 seconds (Hall, 2011) before reaching the ventricles, allowing for the filling of the atria before the excitation and consequent contraction of the ventricles. Finally, the left and right bundle branches are responsible for the rapid relay of the electrical impulse to the rest of the ventricles.

2.1.2 Action potential and muscle contraction

Figure 2-2 shows the four phases of the action potential of a single cardiac cell. The resting membrane potential of a cardiac cell is around -90mV. Once a stimulus from nerve cells or pacemaker cells reaches a cardiac cell, its membrane permeability for positive sodium ions increases. The influx of positive sodium ions causes the membrane potential to becomes less negative, causing the cell to depolarise (phase 0 in Figure 2-2). This action potential is then conducted to other connected cells in the cardiac muscle through their fused membranes,

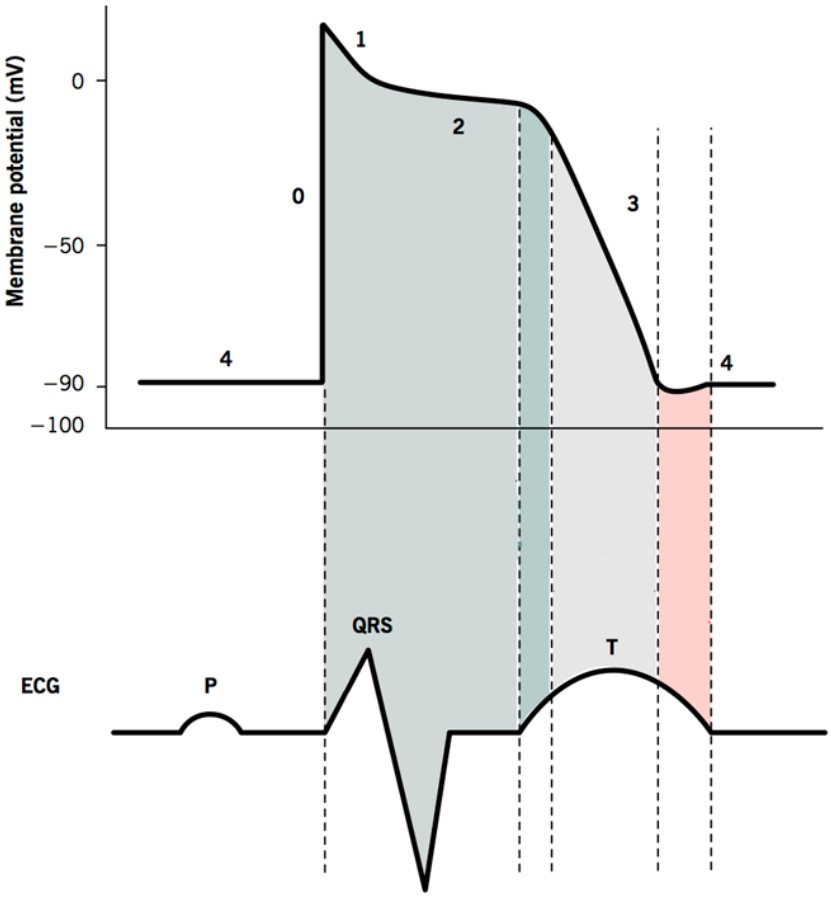

Figure 2-2. The phases of the cardiac action potential cycle and their corresponding ECG features (adapted from www.pathophys.org)

causing adjacent cells to depolarise. Depolarisation causes the sarcoplasmic reticulum to release large quantities of calcium ions, activating the contraction mechanism in the muscle fibre. The cell remains depolarised for around 0.2 seconds, causing a plateau in the action potential (phase 2 in Figure 2-2). Throughout most of the plateau phase, it is very difficult for

the cell to be re-excited by a stimulus, safeguarding the heart against erratic electrical excitation, which might cause abnormal rhythms.

Towards the end of the plateau phase, the sodium channels close and membrane permeability for potassium increases. The rapid outflow of positive potassium ions causes a rapid reduction in the cell's membrane potential returning it to its resting state at around -90 mV. This causes the cell to repolarise (phase 3 in Figure 2-2), ending the action potential. During repolarisation, calcium ions are pumped back into the sarcoplasmic reticulum, initiating cardiac muscle relaxation.

2.1.3 Spatial dispersion of repolarisation

The timing and duration of repolarisation has been found to vary between different ventricular cites and transmural layers; such as across the ventricular wall (Antzelevitch and Fish, 2001), between apex and base, between the posterior and anterior planes and between the left and right sides of the heart (Arteyeva, 2020). One reason which contributes to this heterogeneity is that in the ventricles, the repolarisation wave moves in the opposite direction of the depolarisation. Hence, regions which depolarise first are last to repolarise, resulting in a longer repolarisation duration compared to other regions (Arteyeva, 2020). Another factor is differences in ionic current expression between different cardiac cell types (Nerbonne and Kass, 2005). The intrinsic differences in repolarisation duration across the different ventricular cites is termed 'spatial repolarisation dispersion', which will possibly lead to lethal arrhythmia by forming re-entry circuits if it exceeds physiological levels.

2.1.4 Temporal variability of repolarisation

Action potential duration (APD) exhibits small beat-to-beat variations even within the same ventricular site. Beat-to-beat fluctuations in repolarisation variability has been linked to beat-to-beat fluctuations in APD in experimental models (Pueyo et al., 2011) and cardiac myocytes (Zaniboni et al., 2000, Szentandrássy et al., 2015). These intrinsic heterogeneities are essential for cardiac function under normal physiological conditions. Stochastic gating and distribution of ionic channels contributes to APD variability in isolated myocytes (Lemay et al., 2011, Pueyo et al., 2016), although this effect is masked at the tissue level leading to a decrease in APD variability compared to isolated myocytes (Pueyo et al., 2011). The fluctuation in the release of calcium from the sarcoplasmic reticulum also contributes to the increase in APD variability in calcium-overloaded cells (Johnson et al., 2013). Other extrinsic biological factors

that contribute to beat-to-beat repolarisation variability are discussed in detail in section 2.3.

2.2 Measurement of repolarisation variability

2.2.1 The electrocardiogram

Propagation of action potential from one cell to the next produces millions of electrical vectors. The sum of all these vectors can be recorded non-invasively using electrodes attached to the skin surface. The resulting signal is called an electrocardiogram (ECG). The 12-lead ECG recording system (and subsets of the system) is one of the most used lead configurations for recording the heart's electrical activity. Figure 2-3 shows how each of the 12 ECG leads views the heart. Lead I, II and III are the limb leads, while leads aVL, aVR and aVF are derived augmented leads. These six leads capture the electrical activity of the heart in the sagittal plane. Leads V1–V6 are precordial chest leads that capture the electrical activity of the heart in the transverse plane.

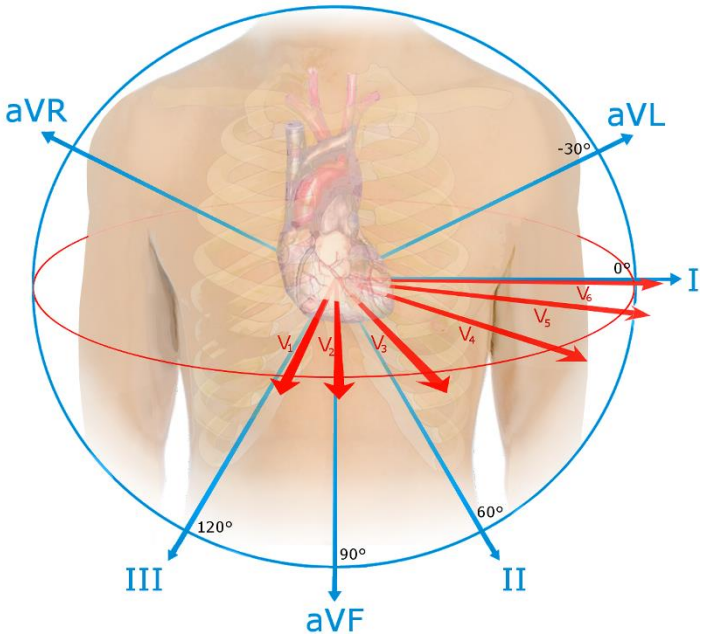

Figure 2-3. Twelve-lead ECG view of the heart. Leads I, II, III, aVL, aVR, and aVF view the heart electrical activity in the frontal plane (blue). Precordial leads V1-V6 view the heart in the horizontal plane (red) (www.wikipedia.org/wiki/electrocardiography).

Figure 2-4 shows a typical ECG waveform. Each heartbeat consists of multiple waves that reflect the heart's electrical activity during depolarisation and repolarisation at different locations in the heart. The morphology, timing, and durations of these waves provide valuable information on the function of the heart and its regulating mechanisms.

The P wave represents atrial depolarisation, while arterial repolarisation is masked by the QRS complex and cannot be seen on the ECG. The QRS complex reflects ventricular depolarisation, while the T wave reflects the rapid phase of ventricular repolarisation.

2.2.2 Temporal features extracted from the ECG signal

One of the main temporal features extracted from an ECG signal is the heart period, measured as the time interval between two consecutive R peaks (RR interval); which is the reciprocal of heart rate. Beat-to-beat fluctuations in RR interval are a measure of heart rate variability, which is thought to reflect the effect of the autonomic nervous system (ANS) on the sinoatrial node (Camm et al., 1996).

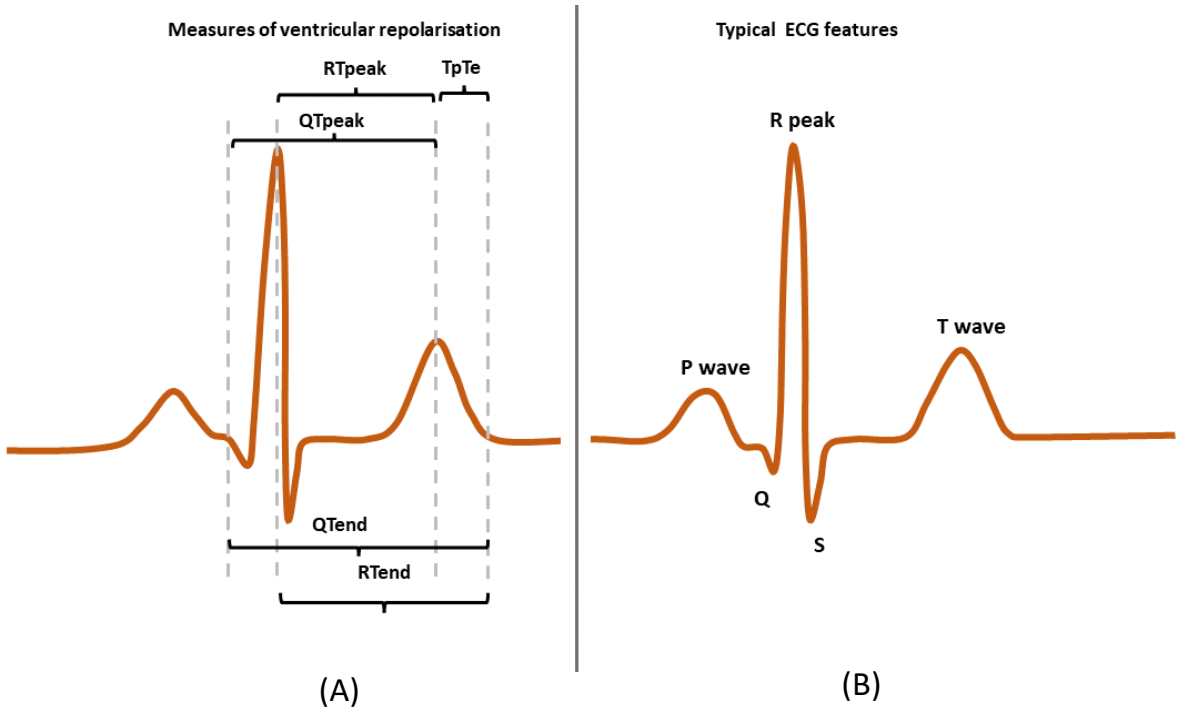

Figure 2-4. A typical heartbeat as measured by the ECG. Panel (A) shows measures of repolarisation variability and panel (B) depicts major morphological features of a typical heartbeat.

The interval between Q wave onset and T wave end (QTend) is the most widely used ECG measure for repolarisation duration (Baumert et al., 2016). From a technical standpoint, the R peak is easier to delineate compared to Q onset due to its high amplitude, and the T peak is generally better defined compared to T end for the same reason. Hence, R peak to T end (RTend), R peak to T peak (RTpeak), and Q onset to T peak (QTpeak) (indicated in Figure 2-4) intervals have been utilised as surrogates of QTend in a number of studies (Yeragani et al., 2007, Porta et al., 1998, Lombardi et al., 1996). However, it is unclear how the use of these different features influences the interpretation of repolarisation variability analysis results. For

example, Yeragani et al. (2007) found agreement between RTpeak and RTend in normal subjects and patients with anxiety but not in cardiovascular disease patients. Also, Tpeak to Tend (TpTe) interval has been shown to be independent of heart rate (Merri et al., 1989, Davey, 1999, Hevia et al., 2006, Hnatkova et al., 2017) and possibly reflects direct ANS influence on the ventricles (Piccirillo et al., 2012). Porta et al. (2010) found a stronger link between RTend variability and sympathetic drive, rather than with RTpeak. These findings suggest that sympathetic modulation of the ventricles can be present in the late part of repolarisation, hence, different measures of repolarisation can yield different results.

In chapter 3 we aim to identify repolarisation measures that best reflect the influence of increase in sympathetic activity the ventricles independent of RR. We compare results from different beat-to-beat features of ventricular repolarisation; namely Q-peak to T-end (QTend), Q-peak to T-peak (QTpeak), R-peak to T-end (RTend), R-peak to T-peak (RTpeak), and T-peak to T-end (TpTe); under conditions of heightened sympathetic activation.

2.2.3 Lead selection and repolarisation variability measurement

Single-lead ECG has been utilised in many studies investigating RV, possibly due to its reduced cost and simplified clinical setup. Lead II has been recommended for single-lead QT variability (QTV) analysis due to its high signal-to-noise ratio and relatively high T wave amplitude (Baumert et al., 2016, Avbelj et al., 2003, Hasan et al., 2012, Lester et al., 2019). Other studies recommend precordial leads V3-V6 as an alternative with sufficient T wave amplitude (Avbelj et al., 2003). Interestingly, in safety pharmacology, it has been shown that leads II and V3 are sufficient for investigating drug effect on the QT interval (Hamlin, 2008).

However, RV has been shown to be different between leads (Hasan et al., 2012, Yeragani et al., 2002), which renders direct comparison between results obtained from different leads quite difficult. These differences partially reflect intrinsic spatial repolarisation heterogeneity. Differences in T wave amplitude also contribute to the discrepancy in RV between leads, where higher T wave amplitude results in more accurate T end detection, leading to lower RV measurement (Cowan et al., 1988, Hasan et al., 2012, Schmidt et al., 2016). Furthermore, leads have different susceptibility to noise (Avbelj et al., 2003, Hasan et al., 2012), with precordial leads being more prone to movement noise due to chest movement with respiration.

Twelve-lead ECG provides a multi-angled view of the entire repolarisation process. Hence, one-dimensional representations of all 12 ECG leads possibly provides a more comprehensive

representation of the ventricular repolarisation process, reducing the influence of noise. Very few studies have investigated the utility of 12-lead ECG for QTV analysis. Brockway et al. (2018) compared Lead II to the spatial magnitude of the three orthogonal leads in a canine model. They found that Lead II and spatial magnitude leads results were in close agreement for QT interval analysis, but not for analysis of QT subintervals. In Chapter 4, we investigate the utility of different 12-lead ECG transformation methods for QTV analysis in comparison to the most used single leads.

2.2.4 Measures of temporal repolarisation variability

Several measures of temporal RV have been reported in the literature (Baumert et al., 2016). It is important to note that the focus of this thesis is on the analysis of QT interval variability, hence this section discusses the most common measures of QTV specifically. In this section, the scope is restricted to measures which are most relevant to the work done in this thesis. An extensive review of QTV measures reported in the literature can be found in the QT variability census paper by Baumert et al. (2016).

2.2.4.1 Time-domain measures of repolarisation variability

In the time domain, many studies report QT interval variance (Berger et al., 1997, Baumert et al., 2011b, Sacre et al., 2013, del Castillo et al., 2021) and standard deviation (SDQT) (Nayyar et al., 2013, Hasan et al., 2013, Li et al., 2019) as measurements of repolarisation variability. The QT variability index (QTVi) has been proposed as a time domain measure of repolarisation variability, which account for the dependence of QTV on HRV (Berger et al., 1997). Variable QTVi has been used as a marker for electrical disease in the ventricles (Berger, 2003), and as an index for disease progression in congestive heart failure (Piccirillo et al., 2009). Here, QTVi is defined as:

$$\mathrm{QTVi} = \log_{10}\left[\frac{\mathrm{QTV}/(\mathrm{QTm})^2}{\mathrm{RRV}/(\mathrm{RRm})^2}\right]. \qquad (1)$$

2.2.4.2 Frequency domain measures of repolarisation variability

2.2.4.2.1 Non-parametric frequency domain measures

In the frequency domain, commonly reported spectral measures include QTV power in the low frequency band (QTV LF) [LF: 0.04- 0.15Hz], which reflects in part QT adaptation to HR changes (Merri et al., 1993). Additionally, the high frequency power of QTV (QTV HF) [HF:

0.15–0.5Hz], which exhibits oscillations related to the respiratory rhythm (Merri et al., 1993, Emori and Ohe, 1999, Hanson et al., 2012). The normalised LF power of QTV (LF/LF+HF ratio) has also been used to measure the balance between both sympathetic and parasympathetic activity, assuming that both significantly affect ventricular repolarisation (Altuve et al., 2006).

2.2.4.2.2 Parametric frequency domain analysis

The cardiovascular system can be thought of as a complex multivariate feedback system, where multiple feedback and feed-forward mechanisms, external and internal to the system, regulate the level of critical biological signals. Beat-to-beat variations in these signals are thought to reflect the interaction of these regulating mechanisms. Recordings of cardiovascular signals (ECG derived beat-to-beat features and respiration in this thesis) can be represented as discrete time series, allowing the application of different time series analysis and modelling methods to these signals to explore their generating mechanisms.

One of the most promising techniques that has been applied to short-term analysis of repolarisation variability is a parametric spectral analysis using autoregressive linear time-invariant modelling. Linear time-invariant models are the most widely used class of linear parametric models in cardiovascular variability analysis (Lombardi et al., 1996, Almeida et al., 2003, Porta et al., 2009, Batzel et al., 2009, Baselli et al., 2011). Some argue that these methods oversimplify observed interactions by assuming linearity. However, linear models are considered sufficient for representing short-term system behaviour with small variation around the mean (Xiao et al., 2005, Cohen and Taylor, 2002, Porta et al., 2012b). Even in the presence of nonlinearities, linear parametric identification would, in the least, provide a partial description of the system. The major advantage of these parametric models in terms of cardiovascular variability modelling is their ability to related parameters to physiological mechanisms allowing for the development of models that give insight into the mechanisms and explain their function (Cohen and Taylor, 2002). The multivariate extension of these models facilitates the analysis of all the signals involved in the system, which provides a description for the system rather than the signals (Baselli et al., 2002). Another advantage of parametric methods is that they impose causality, allowing for the disentanglement of feedback and feed-forward mechanisms in closed-loop interactions. Furthermore, they facilitate the evaluation of both the direction and strength of causal interactions (Porta et al., 2012a).

In this thesis, the analysis is based on an autoregressive model with two external inputs (ARXX model) (Baselli et al., 1997). The general form of the model is defined as:

$$A_1(z)Y(i) = B_{11}(z)\,X_1(i) + B_{12}(z)\,X_2(i) + e_Y(i) \quad (2)$$

where

$$A_1(z) = 1 + a_{1,1}z^{-1} + \ldots + a_{1,n_{a1}}z^{-n_{a1}}, \quad (3)$$

$$B_{11}(z) = b_{11,0} + b_{11,1}z^{-1} + \ldots + b_{11,n_{b11}}z^{-n_{b11}}\ , \quad (4)$$

$$B_{12}(z) = b_{12,0} + b_{12,1}z^{-1} + \ldots + b_{12,n_{b12}}z^{-n_{b12}}\ . \quad (5)$$

Here, z^{-1} is the one-step delay operator in the z-domain, and i is the progressive beat number. Variable Y is the output, X_1 and X_2 are the inputs, and e_Y is a white noise source representing actual noise and rhythms originating from sources not accounted for in the model. Variables n_{a1}, n_{b11}, and n_{b12} are the model orders, while A_1, B_{11} and B_{12} are polynomials representing the transfer functions of the model transformed into the z-domain. They describe the effect of the samples of Y, X_1, and X_2 up to a delay of n_{a1}, n_{b11}, and n_{b12}, respectively, on the one step ahead prediction of Y. Equation (2) describes Y as a function of its past, and past and present values of $X_1$and X_2.

The inputs $X_1$and X_2 are modelled using one of two model structures. If the input X_2 also drives variability in X_1, then X_1 is modelled as an autoregressive process with X_2 as an external input (ARX model). In which case, the model would be defined as:

$$A_2(z)\mathrm{X}_1(i) = B_{21}(z)\mathrm{X}_2(i) + e_{X_1}(i) \quad (6)$$

where

$$A_2(z) = 1 + a_{2,1}z^{-1} + \ldots + a_{2,n_{a2}}z^{-n_{a2}}, \quad (7)$$

$$B_{21}(z) = b_{21,0} + b_{21,1}z^{-1} + \ldots + b_{21,n_{b21}}z^{-n_{b21}}\ . \quad (8)$$

If the inputs do not have a known causal relationship, then each input is modelled as a separate autoregressive processe with no inputs (AR models) defined as:

$$A_3(z)X_2(i) = e_{X_2}(i) \quad (9)$$

where

$$A_3(z) = 1 + a_{3,1}z^{-1} + \ldots + a_{3,n_{a3}}z^{-n_{a3}}. \quad (10)$$

The model in Equation 2 imposes the following assumption on the data (Korhonen et al., 1996):

1. Linearity of input/output relationship. As mentioned previously linear models at least provide a partial description of the system behaviour under small variation.

2. Inputs and output interact in an open-loop manner. For the analysis of repolarisation variability, the suitability of an open-loop model structure has been tested, reporting a limited relevance of the reverse causal pathway (Porta et al., 2017) (i.e. from RV to RRV).

3. The noise source is uncorrelated with the inputs. This assumption is verified by assessing the correlation between the model residuals and the whiteness of the resulting model noise sources (Porta et al., 1998). The advantage of imposing this assumption is that it allows the decomposition of the output into contributions by the different input sources.

4. Input and output signals are assumed to be stationary. To ensure stationarity, we used short-term recordings of 3–5 minutes. Also, we have used data segments from the later part of the recordings where possible to avoid any transient effect that would have been caused by intervention or change of posture during recording. Finally, we detrended the data using a time-varying finite-impulse response high-pass filter (Tarvainen et al., 2002).

After defining the model structure, the next step is to estimate the model parameters. Parameter estimation involves defining criteria for selecting the best set of parameters that fit the model to the data. In this thesis, the optimum model order is selected from a predefined range using the Akaike information criterion (AIC) (Akaike, 1974), which is defined as:

$$\mathrm{AIC} = 2k - 2\ln(\hat{L}), \quad (10)$$

where k is the number of estimated parameters (complexity), and $\hat{L}$ is the maximum value of the likelihood function for the model.

The AIC estimates the relative quality of each model in the predefined range of model orders. It provides a trade-off between the model's goodness of fit and its simplicity. The model order with the minimum value of AIC is selected as the best model representing the data.

For the estimation of model parameters Ljung (1999), in his gold standard book on system

identification, recommends the use of methods that minimises the prediction error. The least squares method and its variations remain the most widely used methods in the literature (Porta et al., 1998, Lombardi et al., 1996, Chaicharn et al., 2009) and hence have been employed in the thesis.

It is important to note that while this section describes the models using general input and output terms, model detail specific to each study, such as specific inputs and outputs, are described in more detail in each chapter.

2.3 Physiological mechanisms contributing to repolarisation variability

An increase in ventricular RV is associated with several pathological conditions. Analysis of the role of increase in RV in cardiac arrhythmias should start by identifying the mechanisms contributing to the increase in RV, as the choice of therapy is highly dependent on the source causing the arrhythmia (Pueyo et al., 2009). In addition to the intrinsic cellular mechanisms discussed in Section 2.1, other biological factors which contribute to short-term RV are discussed below.

2.3.1 Respiration

The main function of respiration is to provide the body with oxygen and remove carbon dioxide. The rate and force of this vital involuntary activity is controlled by the ANS, which adjusts respiration in response to the body's demand for gas exchange. Respiration exhibits rhythmic influences on beat-to-beat fluctuations in heart rate, termed respiratory sinus arrhythmia (RSA), mainly in the HF band (0.15 – 0.5 Hz) under normal physiological conditions. RSA contributes to RV indirectly through the dependence of the later on beat-to-beat fluctuations in heart rate (Larsen et al., 2010). Additionally, APD (Hanson et al., 2012, Hanson et al., 2014) and ECG-derived measures of repolarisation variability (Emori and Ohe, 1999) have been shown to exhibit cyclical fluctuations synchronous with respiration in the HF band, independent of heart rate changes. These influences can be mediated through the effect of respiration on both vagal and sympathetic activity (Hanson et al., 2012). Respiration also exhibits mechanical effects on RV due to changes in the electrical axis of the heart with chest movement (Noriega et al., 2012, Lombardi et al., 1996). Furthermore, mechanical changes in ventricular loading and filling, which occur with inspiration and expiration, have been shown to generate mechano-electrical feedback, which causes changes to APD (Taggart and Sutton, 2011). In this thesis, respiratory effects were accounted for by including beat-to-beat respiratory activity as an input to both,

repolarisation and heart rate variabilities in the multivariate models employed for the analysis.

2.3.2 Heart rate

The rate at which the heart pumps blood to the rest of the body is determined by the rate of self-stimulation of the sinoatrial node. The normal heart beats at an average range of 70-80 beats per minute. Heart rate exhibits beat-to-beat fluctuations around its mean value, termed heart rate variability (HRV). From the ECG signal, HRV is also measured as the beat-to-beat changes in the heart period (i.e. the distance between two consecutive R peaks, RR) variability (RRV). The clinical significance of HRV is that it is thought to reflect the net effect of both arms of the ANS on the sinoatrial node, affecting its firing rate in response to neural and mechanical changes in the heart (Camm et al., 1996).

Under normal physiological conditions, HRV is a major source of RV (Zaza et al., 1991, Lombardi et al., 1996). This influence liaises through the dependence of APD (and thus repolarisation) on cycle length (heart rate), where the APD has been shown to adapt to changes in cycle length (Zaza et al., 1991, Franz et al., 1988). This adaptation takes place in two different manners; an initial fast adaptation within the same heartbeat, and then a slow adaptation that can take up to 2-3 minutes for the APD to reach a new steady-state (Cabasson et al., 2012, Malik et al., 2008, Pueyo et al., 2004). Furthermore, repolarisation adaptation to an increase in heart rate is faster than its response to a decrease in heart rate (Lau et al., 1988), which has been termed 'QT/RR hysteresis'.

The static QT adaptation to changes in the RR interval has been studied extensively in the literature, with researchers proposing various correction formulas for the QT interval, such as Bazett (1920) and Fridericia (1920). However, it has been shown that this relationship is highly individual (Malik et al., 2008) and requires more complex models of adaptation due to the influence of the history of RR on QT rather than only the previous RR (Malik et al., 2008, Cabasson et al., 2012, Hnatkova et al., 2013). The dynamic changes in heart rate, HRV, have also been shown to contribute to QTV (Zareba and De Luna, 2005). The extensive studies on HRV led to the development of robust spectral and statistical analysis methods suited for all cardiovascular variability analysis. These methods were first applied for the analysis of RV by Nollo et al. (1992), who found prominent power peaks in QTV synchronous to those in RRV, specifically in the LF frequency band, reflecting, in part, QTV's fast adaptation to changes in RRV. Figure 2-5 shows a representative example of RRV and QTV oscillations in the LF and HF bands. Since then, the dynamic relationship between beat-to-beat changes in heart period

and QTV has been the focus of several interventional studies in normal subjects (Zhu et al., 2008, Porta et al., 2010, Baumert et al., 2013), and patients with cardiac disease (Zhu et al., 2008, Hintze et al., 2002). Variability of the RTpeak interval (a sub-interval of QT) has been shown to be completely abolished in the LF band after atrial pacing indicating that it is predominantly driven by RRV (Lombardi et al., 1996). LF power of RTend variability (a sub-interval of the QT interval) was found to be driven mainly by RRV (Porta et al., 1998). A large fraction of RT variability has been shown to be RR-dependent in young, healthy subjects (Zhu et al., 2008), while the coupling between RRV and QTV has been shown to be affected by age (Baumert et al., 2013).

On the other hand, several studies across a range of conditions, including healthy subjects (Almeida et al., 2003), patients post myocardial infarction (Zhu et al., 2008), heart failure patients (Nayyar et al., 2013) and patients with diabetes mellitus (Sacre et al., 2013) showed that an important part of QTV can not be solely attributed to RRV. Several of these studies proposed direct neural modulation of repolarisation as a possible mechanism contributing to RV independent of HR.

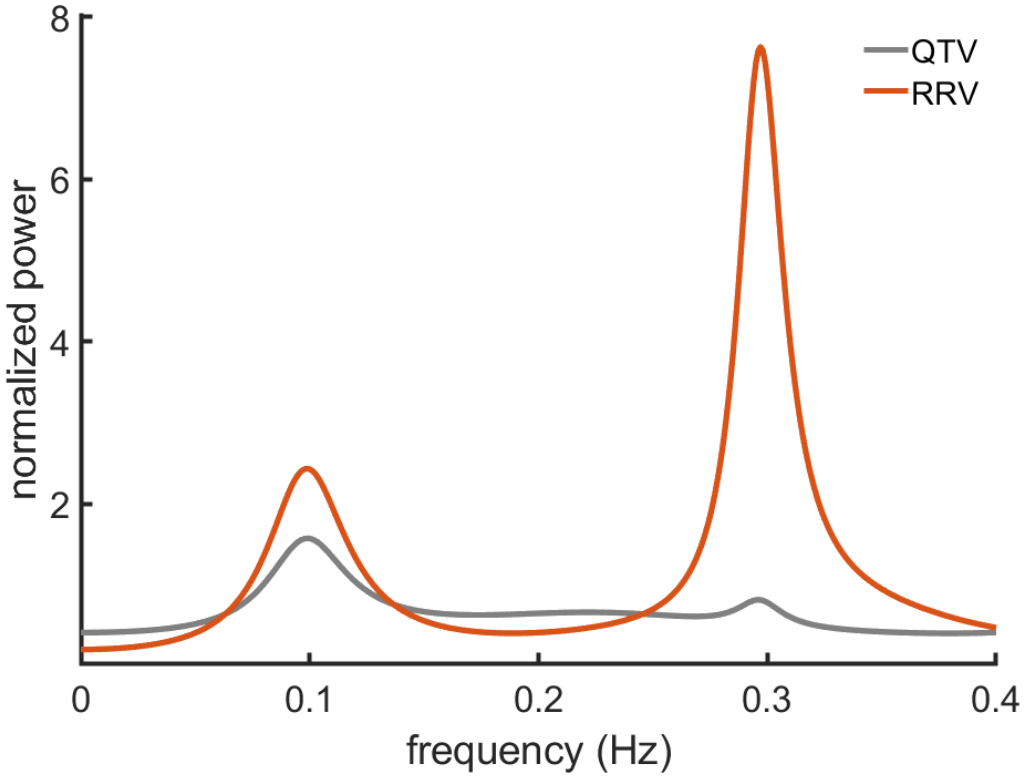

Figure 2-5 A representative example of normalised power spectrums of QTV (grey) and RRV (orange) for a healthy athlete in the supine position.

2.3.3 Autonomic nervous system

The autonomic nervous system (ANS) is the branch of the central nervous system that affects the activity of cardiac muscles, smooth muscles and glands (Strominger et al., 2012). It consists of two subsystems: the sympathetic and parasympathetic nervous systems. The sympathetic nervous system generally increases the rate of sinoatrial node firing, the rate of conduction, the level of excitability in all portions of the heart, and finally increases the force of contraction of both atrial and ventricular muscles. The parasympathetic nervous system has the opposite effect on the body, slowing down the heart rate and reducing muscle fibre excitability.

At a cellular level, norepinephrine released by sympathetic stimulation is thought to cause an increase in membrane permeability to sodium and calcium ions. In the sinoatrial node, this increases the resting membrane potential leading to an increase in the rate of self-excitation, resulting in increased heart rate. The increase in membrane porosity to calcium ions also contributes to the activation of contractile mechanisms in the muscle fibre, causing an increase in contractile forces of cardiac muscle. On the other hand, acetylcholine released by means of parasympathetic activation increases membrane permeability to potassium ions. This increases the negativity of cell membrane potential, requiring the cell to take a longer time to reach the threshold level of excitation, effectively reducing conduction time (Hall, 2011).

The atria are innervated by both sympathetic and parasympathetic afferents. Hence HRV is widely accepted as a marker for the balance of both branches of the autonomic nervous system (Malik et al., 1996). On the other hand, the ventricles are mainly innervated by sympathetic afferents (Hall, 2011), hence the possibility that RV can provide a measure for sympathetic activity motivated further research into neural modulation of RV. Sympathetic nerve activity is organised in a series of low frequency bursts and is thought to impose a low frequency oscillation contributing to the increase in RV (Porter et al., 2018). Excessive sympathetic activity can possibly initiate lethal ventricular arrhythmias in the compromised heart (Ng, 2016, Malpas, 2010). Hence, the evaluation of autonomic modulation of ventricular repolarisation might improve the understand the genesis of such arrhythmias (Coumel, 1993, Franciosi et al., 2017).

2.3.4 Other factors

Other biological factors that have been found to influence RV include circadian rhythm, where QTV has been shown to decrease during night-time (Bonnemeier et al., 2003). Age, where

QTV has been shown to be decreased in the older population (Baumert et al., 2013), albeit others have reported a decrease in younger subjects (Piccirillo et al., 2001). Sex, where QTV has been found generally increased in women compared to men (Nakagawa et al., 2005). An extensive review of these factors can be found in Baumert et al. (2016).

While the relationships between HRV, respiration and RV have been studied extensively in the literature, RV independent of heart rate has been less understood. The focus of this thesis is on the quantification and analysis of RV independent of heart rate and respiration. The thesis also explores the role of enhanced sympathetic activity in RV independent of heart rate and respiration and its prognostic value for risk stratification.

2.4 Approaches to the analysis of RV and sympathetic activity

Repolarisation variability appears to be correlated with abnormal increases in sympathetic activity found in specific pathophysiological conditions or elicited during well-defined experimental manoeuvres (Berger, 2009). Researchers have investigated the relationship between RV and sympathetic activity using one or a combination of the following approaches.

2.4.1 Direct measures of sympathetic activity

The most intuitive approach is to study the relationship between RV and a direct measure of sympathetic activity. Sympathetic nerve activity causes a release of norepinephrine (NE) at the nerve endings, and a fraction of NE spills over into the plasma, making it possible to measure. Hence, NE spillover has been used in the literature as a measure of sympathetic activity (Hasking et al., 1986, Ramchandra et al., 2018). Muscle sympathetic nerve activity (MSNA) measured in the peroneal muscle using microneurography is also a widely used technique to probe postganglionic sympathetic nerve activity (Kingwell et al., 1994).

Baumert et al. (2011b) found a correlation between normalised QT variability and cardiac norepinephrine spillover (but not with QTVi) recorded during resting in patients with hypertension, but not in normal subjects or patients with panic disorder and depression (Baumert et al., 2008). In another study, they found that NE spillover levels did not correlate with QTVi in both postural tachycardia syndrome and normal subjects following tilt, suggesting that QTVi is not indicative of sympathetic activity (Baumert et al., 2011a). Furthermore, QTVi did not correlate with MSNA following tilt in postural orthostatic tachycardia syndrome subjects and normal subjects (Baumert et al., 2011a).

2.4.2 Induced increase in sympathetic activity

Another approach to probing sympathetic influence on RV is investigating RV measures during an increase in sympathetic activity elicited by stress, such as head-up tilt (Baumert et al., 2011a), change in posture from supine to standing, pharmacological sympathetic activation (Yeragani et al., 2000); and mental stress (Hanson et al., 2014), or in pathophysiological conditions associated with elevated sympathetic activity (Piccirillo et al., 2009). Porta et al. (2007) observed an increased decoupling between RR and QT and RR and RT (Porta et al., 2009) with an increase in tilt angle during an orthostatic stress challenge independent of respiration. In another study, they found that squared coherence between RR and RT during tilt in the LF band remained constant, suggesting that sympathetic tone did not have a direct effect on RV (Porta et al., 2011). Both, QTVi and QTV increased significantly during standing compared to supine and following isoproterenol infusion (Yeragani et al., 2000). In patients with postural orthostatic tachycardia syndrome QTVi, but not QTV, was significantly increased compared to normal subjects following tilt (Baumert et al., 2011a). In a canine model of induced heart failure, Piccirillo et al. (2009) found an increased correlation between QTVi and integrated left stellate-ganglion nervous activity, but not before inducing heart failure. A decoupling between QTV and RRV has been observed with aging (Baumert et al., 2013) and between RRV and RTV in post-MI patients (Zhu et al., 2008).

Many of these studies consider QTVi or overall QTV in the analysis. However, it is important to note that there is disagreement on whether QTVi truly reflects RV (Hnatkova et al., 2013, Dobson et al., 2013). QTVi is the log ratio between the QT interval and RRV, each normalised by their squared mean. An increase in QTVi can indicate either an increase in QTV, a decrease in RRV or both (Dobson et al., 2013, Baumert et al., 2008). Furthermore, it has been shown that QTVi has poor intra-subject reproducibility and that it is preferable to use simpler measures of RV, especially for multivariable analysis (Andršová et al., 2020). Also, QTVi does not account for the dependence of the QT interval on past RR intervals excluding important information from the analysis (Baumert et al., 2008).

Additionally, as mentioned previously, sympathetic activity modulates both, ventricular repolarisation (Porta et al., 2010, Baumert et al., 2011a) and sinoatrial node activity (Malik et al., 1996). Hence, it is important to disentangle the direct influences of sympathetic activity on RV from those mediated through HRV. This can be achieved by focusing the analysis on repolarisation variability independent of heart rate and respiration.

2.4.3 Approaches to the analysis of repolarisation independent of heart rate and respiration

Atrial pacing has been employed in several studies to eliminate the effect of RRV on RV (Lombardi et al., 1996, Porter et al., 2018, Browne et al., 1982, Emori and Ohe, 1999, Nayyar et al., 2013). In patients with normal repolarisation, spectral peaks in RTpeak power spectrum synchronous to those in RR spectrum were abolished after atrial pacing, while autonomic blockade did not affect RTpeak power spectrum (Emori and Ohe, 1999). Lombardi et al. (1996) reported that LF power peaks in RV power spectrum were abolished after arterial pacing, leaving only a small HF component synchronous with the respiration frequency. However, in heart failure patients, QTV remained high during atrial pacing compared to patients with normal hearts (Nayyar et al., 2013). Furthermore, APD showed significant LF oscillations at a paced heart rate in heart failure patients undergoing mental stress (Hanson et al., 2014) and in those with increased sympathetic activation induced by forced expiration (Porter et al., 2018). The inconsistencies in these results can possibly be explained by the notion that an increase in RV is correlated with an abnormal increase in sympathetic tone rather than normal levels of sympathetic activation (Berger, 2009). Nonetheless, atrial pacing is an invasive procedure that can be performed only in patient groups such as those with implants or those undergoing catheter ablation. In addition, atrial pacing does not reflect changes in RV under normal physiological conditions.

Parametric autoregressive modelling, on the other hand, allows for the spectral decomposition of QTV into contributions of the different inputs. These methods have been utilised extensively for the analysis of HRV and baroreflex extensively (Baselli and Cerutti, 1985, Pagani et al., 1997, Chaicharn et al., 2009, Porta et al., 2012a). Porta et al. (1996) were the first to employ parametric modelling for quantifying RV independent of HRV without the need for atrial pacing, in addition to investigating the spectral components of this fraction of RV. The fraction of RV independent of RR variability has been of clinical interest as it reflects potential instability of the ventricular repolarisation process in heart disease (Couderc, 2009).

Using parametric autoregressive modelling, Almeida et al. (2003) reported finding more than 40% of QTV to be independent of RR in healthy subjects in the supine position. While Porta et al. (1998) found that RR-unrelated RV power was negligible (except for very LF band) during rest, tilt and controlled respiration in healthy subjects. However, in another study (Porta et al., 2010), they found that RT variability unrelated to RR and respiration increased with an increase in the magnitude of sympathetic activation stimulated by an increase in table tilt angles.

Thus, they proposed the utilisation of this fraction as an indirect measure of ventricular autonomic regulation. Zhu et al. (2008) reported an increase in RT variability independent of RR in patients with myocardial infarction. However, it is yet to be established whether this fraction of RV is directly correlated with sympathetic activation.

In chapter 5, we investigate the relationship between QTV independent of heart rate and MSNA in normal subjects during head-up tilt to provide quantitative evidence, if any, for the association between QTV independent of heart rate and respiration and sympathetic activity.

2.5 Clinical significance of QT variability

An increase in ventricular repolarisation is associated with several pathological conditions. The QTVi was increased during ischemic episodes in MI patients compared to non-ischemic episodes (Murabayashi et al., 2002). In heart failure patients and patients with spontaneous ventricular tachycardia, the SDQT was increased compared to subjects with normal hearts during atrial pacing (Nayyar et al., 2013). The SDQT and QTVi were both increased in congestive heart failure patients compared to normal subjects (Li et al., 2019). Normalised QTV and QTVi were increased in patients with essential hypertension compared to normotensive subjects (Baumert et al., 2011b). Both QTV and QTVi were increased in dilated cardiomyopathy patients compared to normal subjects (Berger et al., 1997). The SDQT measured in 12-lead ECG was found to be increased in MI patients compared to normal subjects in 6 leads, even after covarying for T wave amplitude (Hasan et al., 2013). Highly variable repolarisation has been shown to trigger malignant arrhythmias and subsequent SCD (Liew, 2010). Short-term QTV was also found to be increased in patients with impaired glucose intolerance compared to normal subjects (Orosz et al., 2017).

The prognostic value of different QTV measures has been established in several studies (Baumert et al., 2016, Wellens et al., 2014, del Castillo et al., 2021). For example, QTVi has been shown to improve risk stratification in patients with dilated cardiomyopathy (Fischer et al., 2015) and in chronic heart failure with slightly depressed ejection fraction (Piccirillo et al., 2007). The ratio between QTV and HRV has been shown to improve risk stratification in MI patients (Jensen et al., 2005, Milliez et al., 2005). In a large cohort of MI patients with severe left ventricular dysfunction, increased normalised QTV and QTVi were both independent predictors of VT/VF (Haigney et al., 2004). Also, RV, measured as short-term variability from monophasic APD in dogs with remodelled hearts, was shown to be predictive of pro arrhythmic drugs (Oosterhoff et al., 2007). Impaired rate-adaptation of QT to RR showed a strong

predictive value of SCD (Chevalier et al., 2003, Hintze et al., 2002).

Yet, the mechanisms that underpin excessive QTV and their direct contribution to risk stratification are not fully understood. Only a few studies have focused on the prognostic value of a specific aspect of QTV. In MI patients, Holter recordings showed a strong predictive value of attenuated rate-adaptation of the QT interval for SCD (Chevalier et al., 2003, Hintze et al., 2002). Interestingly, LF oscillations of QTV independent of heart rate, but not overall LF QTV, were found to be an independent predictor of coronary artery disease (del Castillo et al., 2021).

These findings demonstrate the importance of focusing the analysis on RV independent of heart for risk stratification. In chapter 6, we investigate the prognostic value of QTV independent of heart rate for risk stratification in a large cohort of MI patients.

Chapter 3

Repolarization variability independent of heart rate during sympathetic activation elicited by head-up tilt.

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Contribution to the Paper	Study design, data analysis, interpretation of data and results, preparation and revision of manuscript.		
Overall percentage (%)	80%		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
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Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

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ORIGINAL ARTICLE

Repolarization variability independent of heart rate during sympathetic activation elicited by head-up tilt

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Abstract

The fraction of repolarization variability independent of RR interval variability is of clinical interest. It has been linked to direct autonomic nervous system (ANS) regulation of the ventricles in healthy subjects and seems to reflect the instability of the ventricular repolarization process in heart disease. In this study, we sought to identify repolarization measures that best reflect the sympathetic influences on the ventricles independent of the RR interval. ECG was recorded in 46 young subjects during supine and then following 45 degrees head-up tilt. RR intervals and five repolarization features (QTend, QTpeak, RTend, RTpeak, and TpTe) were extracted from the ECG recordings. Repolarization variability was separated into RR-dependent and RR-independent variability using parametric spectral analysis. Results show that LF power of TpTe is independent of RR in both supine and tilt, while the LF power of QTend and RTend independent of RR and respiration increases following tilt. We conclude that TpTe is independent of RR and is highly affected by respiration. QTend and RTend LF power might reflect the sympathetic influences on the ventricles elicited by tilt.

Keywords Repolarization · Heart rate variability · QT interval · Tpeak-Tend · Sympathetic activation · Head-up tilt · Autonomic nervous system

1 Introduction

Dispersion in ventricular repolarization from ECG is associated with an increased risk of ventricular fibrillation and sudden cardiac death [1–8]. One of the main factors contributing to variability in repolarization is the relation between action potential duration and diastolic interval, leading to heart rate (HR) dependence of the QT interval [9]. The dynamic relationship between beat-to-beat changes in heart period (RR) and repolarization measured as QT interval has been the focus of several interventional studies in normal subjects [10–12] and patients with cardiac disease [13, 14]. It is important to disentangle the physiological response of ventricular repolarization to HR changes from repolarization variability attributable to other source and pathophysiological processes.

The fraction of repolarization variability independent of RR variability (RRV) is of clinical interest as it reflects a potential instability of the ventricular repolarization process in heart disease [15]. In normal subjects, repolarization variability has been linked to the direct autonomic nervous system (ANS) regulation of the ventricles [16, 17]. The relationship between QT variability (QTV) and some direct measures of sympathetic nerve activity has been explored in patients with hypertension [18], depression, and panic disorders [19].

Several temporal features from ECG are used to measure ventricular repolarization: The QTend interval represents the entire duration of action potentials of the ventricular myocardium [1]. The QTend interval is the most commonly used feature to represent the repolarization process in healthy subjects as well as patients with a range of cardiovascular diseases

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[9, 20–23]. However, studying the variabilities of subintervals within QTend may elucidate localized repolarization variability rather than overall ventricular variability [1]. The QRS complex represents ventricular depolarization, while the rest of the QT interval reflects repolarization. Because the R-peak is easier to delineate than the Q-wave, and likewise, the T-peak is better defined than T-wave offset, RTend and RTpeak intervals have been used in many studies as a surrogate of QTend [8, 10, 24]. It has been shown that there is a less significant relationship between RTpeak and RTend variability indices in a group of patients with a range of cardiovascular disease compared with normal controls or patients with anxiety, lending support to detailed repolarization analysis [8]. These differences might exist because the latter part of the T-wave (interval from the peak of the T-wave to the end of the T-wave—TpTe interval) has been found to be independent of HR in some studies [25–27] and possibly reflects direct ANS influence on the ventricles [28]. The entire duration of the T-wave (from T-onset to T-end) is thought to reflect temporal repolarization dispersion [29]; however, TpTe may be a better alternative for ease of detection [1, 30]. TpTe has also been shown useful for arrhythmic risk stratification [26, 31].

Here, we investigated the relationship between beat-to-beat features of ventricular repolarization (Q-peak to T-end (QTend), Q-peak to T-peak (QTpeak), R-peak to T-end (RTend), R-peak to T-peak (RTpeak), and T-peak to T-end (TpTe)) and RR intervals during rest and following sympathetic activation elicited by head-up tilt in young subjects. We sought to identify repolarization measures that best reflect the sympathetic influences on the ventricles independent of RR.

2 Methods

2.1 Subjects and study protocol

Forty-six young volunteers (30 females, 16 males) with a mean age of 17 years (SD = 2.7 years) and an average body mass index of 24 kg m^{-2} (range, 17–32 kg m^{-2}) participated in this study. All subjects were normotensive, with no overt cardiovascular, respiratory, or endocrine disease. Subjects were instructed not to use substances influencing the autonomic nervous system or cardiovascular system activity. Female subjects were examined in the proliferative phase (6th − 13th day) of the menstrual cycle. In compliance with Declaration of Helsinki for studies involving human subjects, all procedures were approved by Ethical Committee of the Jessenius Faculty of Medicine, Comenius University and all participants or their legal guardian (if the subject was aged below 18 years) signed written informed consent. In the original study [32], ECG, blood pressure, and respiratory signals were recorded during 5 different phases; however, for the purpose of this study, only the ECG signals (horizontal bipolar thoracic lead; CardioFax ECG-9620, NihonKohden, Japan) and the respiratory volume changes (RespiTrace 200; NIMS) from phase 1 and 2 were used for the analysis. The ECG signal was recorded at a sampling rate of 1000 Hz which is sufficient for a reliable measurement of QT variability [33]. In phase 1, subjects were recorded for 15 min in supine rest, while in phase 2 subjects were tilted to 45 degrees on a motor-driven tilt table for 8 min to evoke mild orthostatic stress. During the whole measurement, the volunteers were asked to avoid disturbing movements and speaking.

2.2 Data pre-processing

To avoid transient changes, the analysis of the ECG started 8 min after the beginning of phase 1 and 3 min after the change in position in phase 2. Heart period was measured as RR interval and QTend, QTpeak, RTend, RTpeak, and TpTe intervals were measured representing difference features of repolarization (Fig. 1). Measures were extracted on a beat-to-beat basis. The features were extracted on a beat to beat basis using a template matching, two-dimensional signal warping (2DSW) algorithm [34, 35]. Briefly, the algorithm creates a template beat, on which the QT interval is semi-automatically annotated and then morphed in both time and amplitude to match consecutive beats, thereby accounting for variations in the ECG waveform. Finally, the QT interval duration of each beat is obtained based on the scaled version of the annotated QT interval in the adapted template. The algorithm also incorporates rejection criteria for beats that are highly corrupted by noise. The length of the final extracted series was 295 beat on average. Three subjects were excluded from the analysis due to their high rate of rejected beats (> 5%). Beat-to-beat respiration was calculated by sampling the respiratory signal at the occurrence of the R-peak.

The extracted beat-to-beat series were detrended using a time-varying impulse response high-pass filter [36] with a cutoff frequency of 0.04 Hz and normalized to unit variance and zero mean for frequency domain analysis. Variance and mean values for each variable were computed before detrending.

2.3 Frequency domain analysis

In order to study the variability of the selected features in the frequency domain, we employed linear parametric autoregressive modeling and system identification. In these models, the transfer functions, which describe the relationships between inputs and outputs, are represented with a set of parameters that describe the system properties. These parameters are estimated using the least squares method (in the current study), then used to compute an estimate of the power spectrum of the input and output variables. In this particular analysis, a linear open loop autoregressive model (ARXX)

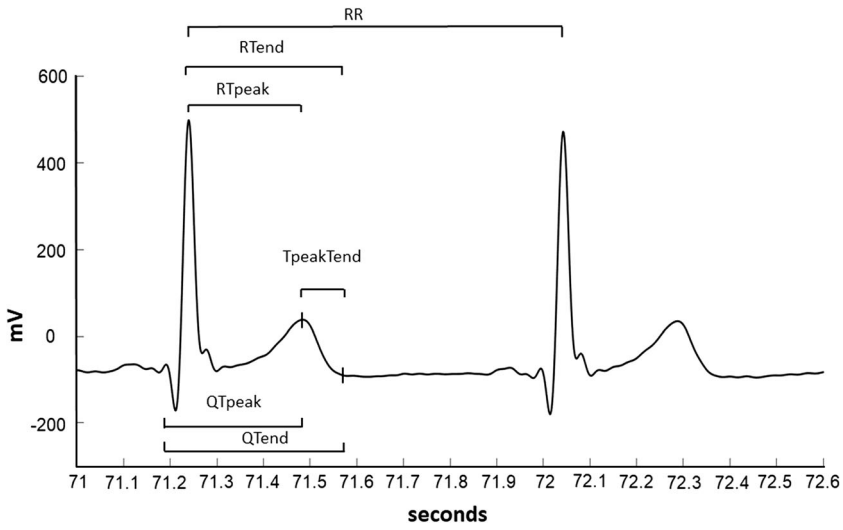

Fig. 1 Representative example of RR, QTend, QTpeak, RTend, RTpeak, and TpTe interval measurements from a single lead ECG recorded in the supine position

[37] with one output (repolarization variability (RV)) and two exogenous input (RR interval variability (RRV) and respiration variability (RespV)) was used to model the relationship between each repolarization feature, heart period, and respiration. The beat-to-beat time series of the respiratory cycles were included in the model to account for the effects of electrical axis movement induced by respiration with the change of posture. The suitability of an open loop model structure was recently tested [38] reporting a limited relevance of the reverse causal pathway (i.e., from RV to RRV). Figure 2 shows a schematic diagram of the used model.

A mathematical representation of the ARXX model is shown in Eq. 1.

$$\mathrm{RV}(i) = \sum_{n=1}^{p} a_n \times \mathrm{RV}(i-n) + \sum_{n=0}^{k} b_n \times \mathrm{RR}(i-n) + \sum_{n=0}^{r} c_n \times \mathrm{RespV}(i-n) + e_{\mathrm{RV}} \quad (1)$$

where a_n, b_n, and c_n are the estimated model parameters that describe the dependence of RV on its own past, on the present and past of RRV, and the present and past of RespV respectively. The variables p, k, and r are the model orders defining the number of previous intervals included in the model for each input. Also, i refers to the beat number in the discrete time series, while e_{RV} is a white noise source with zero mean and σ_{RV} variance. RR was modeled as an autoregressive process with respiration as a single input (ARX), while respiration was modeled as an autoregressive process (AR).

The main advantage of this model structure is its ability to separate repolarization variability into RR-dependent, respiration-dependent, and RR- and respiration-independent variabilities [39]. Model orders were selected from the range 4 to 12, minimizing the Akaike figure of merit [40]. The model parameters were estimated using the least squares method. Model goodness of fit (GOF) was computed to assess the ability of the models to capture the dynamics of the relationship. Low-frequency power (LF) (ms^2) and LF power independent of RR and respiration (ms^2) were computed for each model. The percentage of contribution from RR, respiration, and other sources to LF power was computed for each of the repolarization features [37].

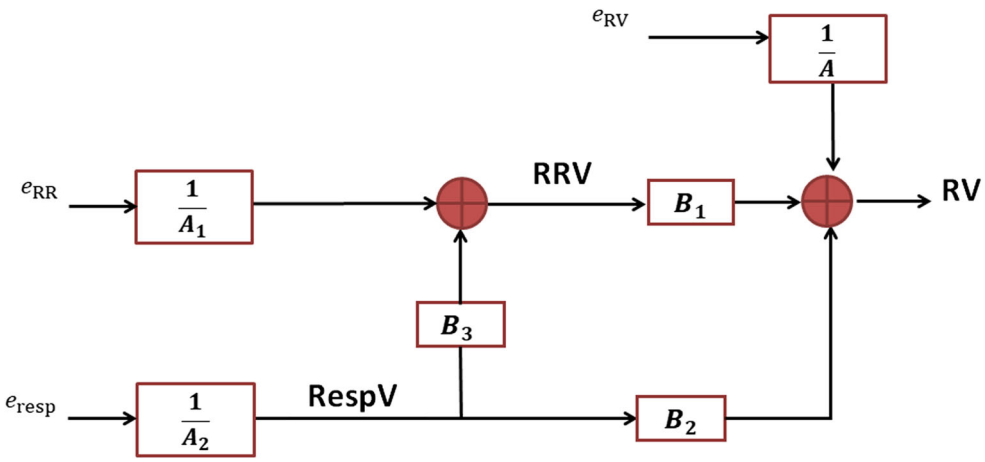

Fig. 2 Schematic diagram of the ARXX model. Repolarization variability (RV) is the output, while heart period variability (RRV) and respiration variability (RespV) are inputs to the model (ARXX). RRV was modeled as an autoregressive process with RespV as an input (ARX) to account for the common effect of respiratory sinus arrhythmia on both RRV and RV, while RespV was modeled as an autoregressive process (AR)

3 Statistics

GraphPad Prism 7 (GraphPad Software Inc.) was used for statistical analysis. The paired Wilcoxon test was used to test for differences between variables in the supine and head-up tilt positions. The Spearman correlation coefficient was used to test for associations between measures QTend LF power independent of RR and respiration and that of other repolarization features. The Spearman correlation coefficients were calculated between the intra-subject means in the complete population. LF band was defined as 0.04–0.2 Hz. Results are reported as median (25th percentile–75th percentile). A p value < 0.05 was considered statistically significant.

4 Results

At rest, the median ARXX model GOF was 76% (51–86%) on average for all repolarization features (except TpTe). However, following tilt, the median GOF decreased significantly to 64% (47–78%) ($p < 0.0001$), except for TpTe where the model goodness of fit was lowest at rest (22% [15–29%]) and did not change significantly with tilt.

Figures 3 and 4 summarize RR and repolarization, respectively, derived measures obtained from ECG recordings in the supine position versus subsequent head-up tilt. Mean RR and the mean of all repolarization features decreased significantly following tilt. RR variance and its LF and HF power decreased significantly following tilt, while the variance of all repolarization features increased significantly. LF power of all repolarization features increased significantly following tilt (except for QTpeak where $p = 0.08$). However, the HF power showed a trend of small decrease (except for TpTe) but did not reach statistical significance. LF power independent of RR and respiration increased significantly for QTend, RTend, and RTpeak, while it did not change for TpTe and QTpeak.

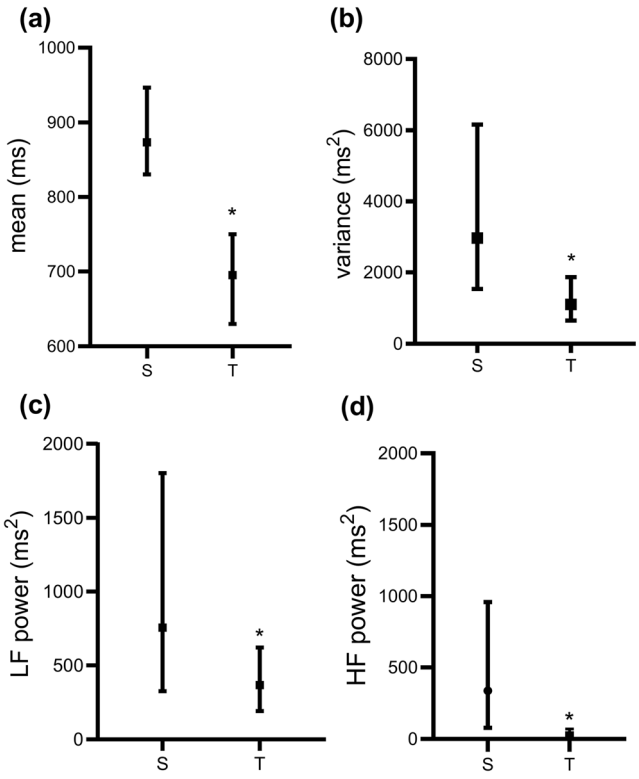

Fig. 3 The median and interquartile range of RR mean (**a**), RR variance (**b**), RR LF power (**c**), and RR HF power (**d**) in the supine position (S) and following head-up tilt (T). *$p < 0.0001$ using the paired Wilcoxon test for supine vs. tilt

4.1 Decomposition of RV LF power

Figure 5 summarizes the decomposition of LF power of all repolarization features into contributions by RR, respiration, and sources independent of RR and respiration. The percentage of RR contribution to LF power of all repolarization features was high (except for TpTe where RR contribution was only 4% [2–12%] in the supine position) and decreased significantly following tilt only for QTend and RTend variabilities.

Respiratory contribution increased significantly following tilt for all features, and the highest increase in that contribution was for TpTe variability (4% [1.5–10%] in supine vs 20% [7–41%] following tilt).

The percentage of LF power independent of RR and respiration showed a trend of increase in QTend and RTend LF powers but did not reach statistical significance (QTend, $p = 0.7$; RTend, $p = 0.3$). On the other hand, it constituted a large fraction of TpTe LF power but decreased significantly following tilt (86% [79–95%] vs 68% [40–83%]).

4.2 Association between QTend LF power independent of RR and respiration and that of other repolarization features

Table 1 shows the correlation between QTend LF power independent of RR and respiration and that of other repolarization features. This correlation is significant for all features in both positions. The correlation is highest and increases with RTend, while it seems to weaken following tilt for QTpeak and RTpeak and does not change for TpTe.

5 Discussion

The main findings of our study are as follows: (i) The beat-to-beat variations in late repolarization represented by TpTe variability is independent of RR variability, and its LF power is highly affected by respiration following tilt; (ii) although TpTe LF power independent of RR and respiration constituted a large fraction of TpTe LF power, this percentage decreased significantly following tilt indicating that TpTe independent

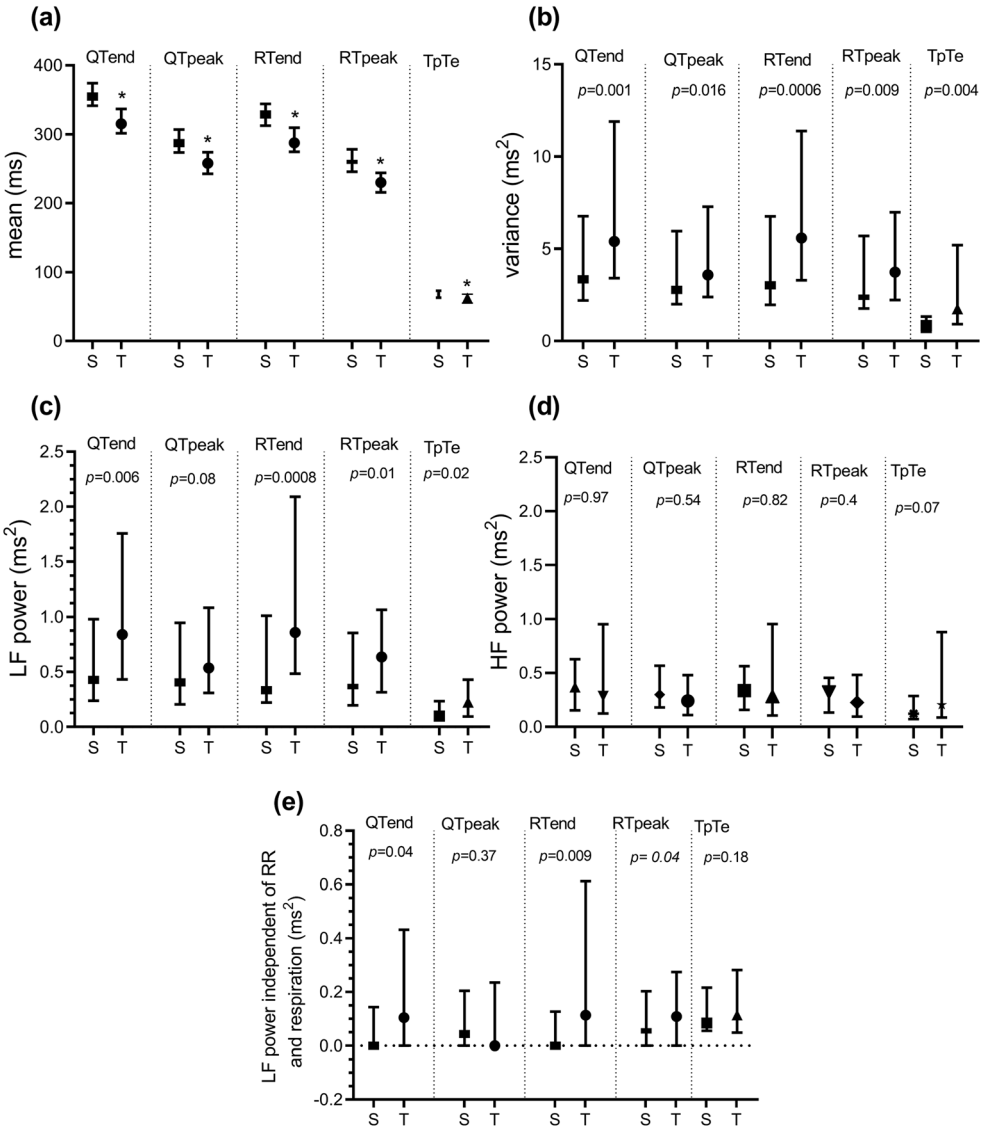

Fig. 4 Mean (**a**), variance (**b**), LF power (**c**), HF power (**d**), and LF power independent of RR and respiration (**e**) in the supine position (S) and during head-up tilt (T) for QTend, QTpeak, RTend, RTpeak, and TpTe. *$p <$ 0.0001 using the paired Wilcoxon test for supine vs. tilt

of RR and respiration does not reflect the increase sympathetic activity elicited by tilt; finally, (iii) QTend and RTend LF power independent of RR and respiration increase significantly with tilt, possibly reflecting changes in direct sympathetic control of the ventricles elicited by head-up tilt better than other repolarization features.

Quantification of repolarization duration variability independent of RR variability has become of clinical interest. It is widely believed that this fraction of repolarization variability reflects direct ANS influences on the ventricles in normal subjects, and potentially also in patients with structural heart disease in whom repolarization variability is typically increased. The non-invasive quantification of these direct influences can be of high importance in understanding susceptibility to arrhythmia. In this study, we sought to identify repolarization features and related indices that can better reflect the direct influence of ANS on the ventricles independent of that mediated through heart period, i.e., via sinoatrial node.

Mean values of all repolarization features significantly decreased with tilt, and their variance increased significantly, while mean RR and variance decreased significantly similar to findings in other studies [11, 41]. Exercise, vagal block, and pharmacological sympathetic stimulation caused changes in mean QT independent of RR [42]. The influence of sympathetic activation on RR is mediated through the release of epinephrine and norepinephrine which activates the β-adrenergic receptors leading to the increase in cardiac Ca^{2+} and hyperpolarizing-activated current, resulting in acceleration of the slow diastolic depolarization and a shortening of RR interval [43]. On the other hand, sympathetic activity influences ventricular repolarization directly through affecting L-type channel Ca^{2+} current and the slow delayed rectifier potassium current in ventricular

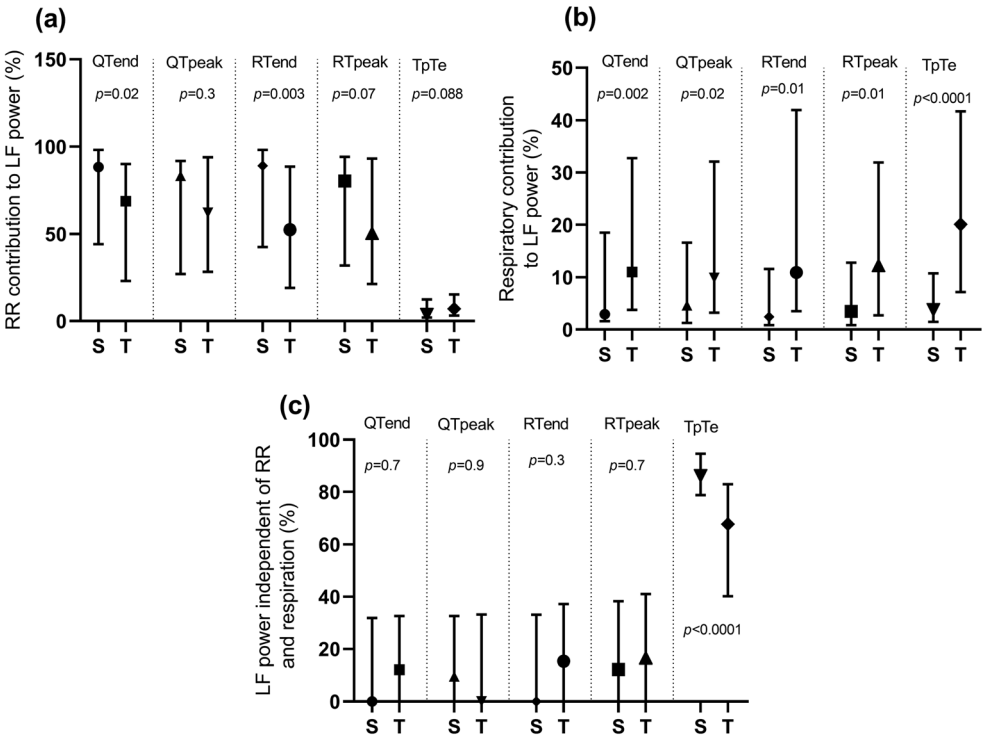

Fig. 5 Decomposition of RV LF power into power contributions by RR (**a**), respiration (**b**) and unknown sources (**c**) in the supine position (S) and during head-up tilt (T) for QTend, QTpeak, RTend, RTpeak, and TpTe. *$p <$ 0.0001 using the paired Wilcoxon test for supine vs. tilt

myocytes [44] shortening repolarization duration and increasing its heterogeneity.

The decrease in RR HF power and the trend of decrease in HF power of repolarization variables indicate a decreased parasympathetic activity following tilt [45]. Absolute LF power increased for all repolarization features following tilt (except for QTpeak). However, the decomposition of LF power into contributions by RR, respiration, and other sources unaccounted for by the model (possibly direct neural influences on the ventricles) can help elucidate the sources of this increase.

Using power spectral decomposition, a number of studies report a large fraction of QTV independent of RRV [11, 46]. While a large fraction of the variability of all repolarization features is driven by RR variability in our study (except for TpTe), the percentage of RR contribution decreased for the LF powers of QTend, RTend, and RTpeak, reflecting a decoupling between these variabilities and RRV. Furthermore, the reduction in the goodness of fit for all repolarization features (except TpTe) indicates an increase in complexity of modeled relationships following tilt. The decoupling between repolarization variability and RR variability has been observed between QT [12], RTpeak [47], and RR with aging; between RR and QT [48] and RT [10] in healthy subjects with tilt; between RTpeak/RTend [49] and RR with tilt in normal subjects; and between RTpeak and RR in myocardial infarction patients [47]. In patients with heart failure, QTV remains high even after uncoupling the effect of RRV [50], while QTV independent of RRV was increased in post myocardial infarction patients compared with healthy

Table 1 Correlation between repolarization variability indices and analogous QTend indices

Variable	Supine	Tilt
LF power independent of RR and respiration (ms^2)	QTend LF power independent of RR and respiration	
QTpeak	0.61***	0.34*
	(0.38–0.77)	(0.04–0.58)
RTend	0.77***	0.87***
	(0.62–0.87)	(0.78–0.93)
RTpeak	0.62***	0.44**
	(0.39–0.77)	(0.16–0.65)
TpTe	0.58***	0.51**
	(0.34–0.74)	(0.17–0.67)

Results are presented as correlation coefficient (95% confidence interval). ***$p < 0.0001$, **$p < 0.01$, *$p < 0.05$

subjects [47]. This decoupling might be a result of differential neural regulation of the SA node and the ventricles triggered by sympathetic activation elicited by tilt [48].

Respiration can affect repolarization through respiratory sinus arrhythmia, and it may also introduce measurement artifacts due to changes in the electrical axis of the heart with chest movement [9, 51], which is even more pronounced with tilt. Respiration has also been shown to improve the model prediction and goodness of fit [49]. While respiratory contribution was relatively small (< 15% on average in both positions), it was increased significantly following tilt for all repolarization features. This might be due to the decrease in respiratory rate following tilt reported in our previous study [32].

Sympathetic nerve activity is organized in a series of low-frequency bursts, and it is thought to impose low-frequency oscillation further increasing repolarization variability [52]. We found that the percentage of LF power independent of RR and respiration (possibly reflecting a direct increase of sympathetic influences on the ventricles) showed a trend of increase for QTend and RTend, while their absolute LF power independent of RR and respiration increased significantly following tilt. These findings are in agreement with a reported increase in QTend variability independent of RR and MSNA variabilities following tilt [11] and gradual increase in the variance of RT independent of RR variance with an increase in tilt angle from 15 to 90 degrees [10]. These findings indicate that the LF power of QTend and RTend might best reflect the effect of the increase in direct neural influences on the ventricles following tilt.

Studies investigating the relationship between TpTe and RR have provided controversial results with some reporting TpTe independence from RR [25, 27, 53], while others found that TpTe is rate dependent [54]. The percentage of RR contribution to TpTe variability was negligible in our study and was not affected by tilt, indicating that TpTe variability is largely independent of heart rate variability. While Davey [25] reported the development of a weak correlation between mean values of TpTe and RR at peak exercise, our results show that TpTe variability remains independent of RR variability even under sympathetic activation elicited by tilt. Using 12 lead ECG, Mincholé et al. [54] found that TpTe adapts to heart rate changes faster than the QT interval. In our study, we use single lead ECG which might have limited our model's ability to capture TpTe rate adaptation.

Meanwhile, respiratory contribution to TpTe LF power increases and the percentage of TpTe LF power independent of RR and respiration decreases following tilt. This interesting finding indicates that TpTe does not seem to reflect an increase in neural influences on the ventricles following mild orthostatic stress (at 45 degrees). The projection of the T-wave loop into a specific lead is highly affected by changes in the heart position due to respiration. TpTe is also susceptible to measurement noise due to difficulty in detecting the T-peak in cases of flat T-wave or more complex morphologies [55].

Finally, the correlation between QTend LF power independent of RR and respirations and that of all other repolarization features was significant in both positions but seemed to decrease for features that do not account for late repolarization (TpTe). Aside from possibly reflecting direct neural influences, this fraction of repolarization variability independent of RR and respiration possibly reflects repolarization reserve. Repolarization reserve is enhanced by sympathetic activation under normal physiological conditions [56].

Our study has several limitations. The method of linear autoregressive modeling requires stationarity in the data, and, since cardiovascular signals are inherently non-stationary, we allowed the subjects resting time before recording, removed slow trends, and restricted our analysis to short-term variability. Furthermore, our analysis was limited to single lead ECG, while repolarization measurements are known to be lead dependent [20]. However, this study has been performed on data pooled from a previous study which only recorded single-lead ECG. Furthermore, single lead ECG was more suitable for this initial study due to the large number of variables tested. Further investigation using 12 lead ECG data would be of interest as it would allow us to explore some important parameters that correlate with sympathetic activity such as periodic repolarization dynamics [57] and also allow for a more robust removal of respiratory effects. Furthermore, during tilt, the heart position changes; hence, the projection of the cardiac vector on the ECG leads might change; this effect cannot be accounted for using single-lead ECG. However, with LF power independent of RR and respiration increasing only in QTend and RTend but not all repolarization features, it seems unlikely that this increase is merely due to postural change. Finally, our sample consisted of both females and males and we did not account for sex differences in repolarization while there is some evidence of small gender difference related to repolarization variability in the literature [9].

6 Conclusion

In conclusion, our study suggests that the variability of TpTe is independent of RR variability and its LF power is highly susceptible to respiratory artifacts. For QTend and RTend, LF power independent of RR and respiration increased following tilt, suggesting that this fraction of repolarization variability might be a good surrogate for sympathetic influences on the ventricles. It remains to be investigated whether QTend and RTend variabilities independent of RR and respiration are directly associated with absolute levels of sympathetic activity such as muscle sympathetic nerve activity, norepinephrine spillover, or iSGNA, or/and their magnitude of changes. QT variability has been shown predictive of sudden cardiac death [9, 58]. It is also worth investigating whether QTend and RTend variabilities independent of RR and respiration can be used for improved risk stratification in patient groups such as heart failure and myocardial infarction.

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Compliance with ethical standards

In compliance with Declaration of Helsinki for studies involving human subjects, all procedures were approved by Ethical Committee of the Jessenius Faculty of Medicine, Comenius University and all participants or their legal guardian (if the subject was aged below 18 years) signed written informed consent.

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Chapter 4

Comparison of single-lead and multi-lead ECG for QT variability assessment using autoregressive modelling

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PAPER

Comparison of single-lead and multi-lead ECG for QT variability assessment using autoregressive modelling

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Abstract

Objective. Beat-to-beat fluctuations in the QT interval—QT variability (QTV)—have been shown to vary amongst the different ECG leads. This study aims to compare the utility of single and multi-lead ECG to disentangle the mechanisms contributing to QTV. *Approach*. Twelve-lead ECG was analysed in 57 coronary artery disease patients before and after an elective percutaneous transluminal coronary angiography (PTCA) procedure. QT, RR and respiration time series were extracted. QTV was decomposed into contributions by heart rate, respiration and QTV independent of heart rate and respiration using parametric autoregressive modelling. Signal-to-noise ratio, model goodness-of-fit, mean QT, corrected QT, QT variability and RR variability were also computed. Results from two single leads (Lead II and V5) and three one-dimensional representations of 12-lead ECG (principal component analysis (PCA), vector magnitude (VM), and root mean square of the 8 independent leads of the standard 12 leads (RMS8)) were compared during resting conditions, before and after PTCA, and between patients with myocardial infarction and those without. *Main results*. At baseline, mean QT and corrected QT were significantly lower in VM and RMS8 compared to single leads. While overall QT variability was not different between the leads, QT independent of heart rate and respiration was significantly lower in VM and RMS8. Following PTCA, changes in these variables were similar in all leads. Differences between patients with MI and those without MI were consistent in all leads. *Significance*. Despite the differences in some QTV components amongst various leads, single-lead ECG could be sufficient for analyzing QTV in populations with pathological cardiovascular conditions compared to those without, or for quantification of intervention effects.

1. Introduction

An increase in repolarisation variability has been associated with sympathetic activation (Porta *et al* 2010, EL-Hamad *et al* 2015) and an increased risk of ventricular arrhythmias (Atiga *et al* 1998, Chen *et al* 2011). Beat-to-beat fluctuations in the QT interval—QT variability (QTV)—mainly reflect temporal changes in ventricular repolarisation.

Several technical aspects influence the measurement of QTV from the ECG recordings, including lead selection. QTV has been shown to differ between some ECG leads (Yeragani *et al* 2002, Hasan *et al* 2012), in part reflecting the dispersion of the repolarisation process across the different recording sites (Cowan *et al* 1988, Baumert *et al* 2016). Moreover, the projection of the repolarisation wave differs across leads due to changes in the heart's position in the thorax with respiration (Macfarlane *et al* 1998). Leads also have different susceptibility to noise (Avbelj *et al* 2003, Hasan *et al* 2012); for example, in a supine position, the precordial leads are more prone to movement noise (due to respiration) than the limb leads. Due to these differences, lead selection has been shown to influence the predictive accuracy of QT in different patient groups (Lund *et al* 2001), with the suitable lead to use varying between groups. QTV also depends on T wave amplitude, which influences the

accuracy of T end delineation (Cowan *et al* 1988, Hasan *et al* 2012). A higher T wave amplitude allows for a more accurate T end detection and results in lower QTV, whilst a lower T wave amplitude could lead to an artificial increase in QTV. T wave amplitude is also significantly different between ECG leads (Hasan *et al* 2012). A formula for T wave amplitude has been proposed by Schmidt *et al* (2016).

Few studies have explored lead selection for QTV analysis. Yeragani *et al* (2002) were the first to report a significant difference in QTV and QTV index when comparing leads V1 and V5 to V3, highlighting the importance of lead selection for QTV analysis. In a more comprehensive study, Avbelj *et al* (2003) compared QTV, T wave amplitude and signal-to-noise ratio (SNR) in 35-channel ECG. They concluded that QTV measures differed amongst leads and demonstrated an inverse relationship with T wave amplitude and SNR. These findings were substantiated by Hasan *et al* (2012) and Hasan *et al* (2013), who compared the standard deviation of the QT interval of all twelve standard ECG leads in healthy subjects and in patients with myocardial infarction (MI).

Lead II has been recommended for single lead QTV analysis due to its high SNR and relatively high T wave amplitude (Avbelj *et al* 2003, Hasan *et al* 2012, Baumert *et al* 2016, Lester *et al* 2019). Some studies recommend precordial leads V3-V6 as an alternative option with sufficient T wave amplitude (Avbelj *et al* 2003).

More recent approaches involve the transformation of multiple leads into a one-dimensional lead representative of the entire electrical process across the whole myocardium. Some of the most common transformations are root mean square, vector magnitude, and principal component analysis (Castells *et al* 2007, Hnatkova *et al* 2019). The advantage of these transformations is the significant reduction in noise and redundancy. In the context of ventricular repolarisation analysis, these approaches have mainly been employed to study QT interval, QT dispersion and JTpeak interval. To the best of our knowledge, no existing study has investigated the utility of these 12-lead transformation approaches specifically for QTV analysis.

This study aims to compare the utility of single-lead and multi-lead ECG analysis approaches specifically to disentangle the mechanisms contributing to repolarisation variability.

2. Methods

2.1. Study cohort and data acquisition

This study analyses standard 12-lead ECG data from the STAFF III Database (Martínez *et al* 2017) publicly available on PhysioNet (Goldberger *et al* 2000). The STAFF III database was acquired during 1995–96 at Charleston Area Medical Center (WV, USA). The study was approved by the investigational review board, and all patients provided informed consent (Pettersson *et al* 2000). One hundred and four coronary artery disease patients underwent elective percutaneous transluminal coronary angiography (PTCA) procedures. The average age of patients for the entire database was 61 $\pm$ 11 years, 39 were female, 95 were male and 69 had prior MI. Twelve-lead ECG recordings were obtained for these patients before the procedure (baseline), during balloon inflation, and after the procedure was complete (post-PTCA). For our study purposes, only baseline and post-PTCA ECG were included in the analysis. Baseline and post-PTCA ECG recordings were acquired for 5 min at rest in the supine position at the catheterization laboratory. ECG data acquisition was performed using custom-made equipment by Siemens–Elema AB (Solna, Sweden), with a sampling frequency of 1000 Hz and an amplitude resolution of 0.625 μV (Martínez *et al* 2017). Full details of the procedure for data collection can be found here (Pettersson *et al* 2000, Martínez *et al* 2017).

2.2. ECG leads selected for comparison

2.2.1. Single-lead ECG

ECG leads II and V5 were selected for single lead analysis, as they remain the most commonly used leads for both QT and QTV analysis (Avbelj *et al* 2003, Whyte *et al* 2005, Baumert *et al* 2016, Lambiase *et al* 2019). The wave vector axes of the QRS and T waves are directed inferolaterally towards Lead II. Moreover, U waves are not evident or as prominent as in the precordial leads (Lester *et al* 2019). Lead II and V5 have been shown to have high T wave amplitude (Avbelj *et al* 2003) and minimum interobserver variability (Whyte *et al* 2005).

2.2.2. Multi-lead ECG

Several transformations have been suggested to convert multiple-lead ECG into a one-dimensional lead representative of the entire electrical process across the whole myocardium (Hnatkova *et al* 2019). We consider the following one-dimensional representations:

(1) The orthogonal leads X, Y and Z were obtained from 12-lead ECG using the inverse Dower transform (Edenbrandt and Pahlm 1988). Then, the vector magnitude (VM) was obtained and used as a one-dimensional representation of the 12-lead ECG (Hnatkova *et al* 2019):

$$VM = \sqrt{X^2 + Y^2 + Z^2} \tag{1}$$

(2) The root mean square of the 8 independent leads of the standard 12 leads (RMS8) was used to represent the distribution of the ECG leads, excluding the potential bias that the derived leads might create towards the limb leads (Hnatkova *et al* 2019):

$$RMS8 = \sqrt{\sum_{L=V1-V6,\ I,\ II} L^2} \tag{2}$$

(3) Principal component analysis (PCA) has been used for data compression and noise reduction in multi-lead ECG (Castells *et al* 2007). The first two principal components account for most of the variability in the original 12 leads. Hence, the eight principal components were computed for each subject as detailed in Castells *et al* (2007). Then, either the first or second principal component was selected for the analysis based on a subjective assessment of T wave amplitude and morphology.

2.3. Data preprocessing

From the STAFF III database, only patients with baseline and post-PTCA recordings obtained in the catheterisation laboratory were included in the analysis. F.E. and M.B. performed the visual screening of ECGs. ECGs with flat T waves and excessive noise were excluded as they would introduce unrealistic QTV values. Medical screening of subjects was performed during the initial data collection process, and those with ventricular tachycardia or undergoing an emergency procedure were excluded. Short recordings (less than 200 beats) were also excluded to ensure sufficient data was available for model estimation and validation.

High-frequency noise and baseline wander were removed from the remaining ECG recordings using a Butterworth bandpass filter with a passband of 0.5Hz–40 Hz (Lenis *et al* 2017). Next, PCA, VM and RMS8 transformations were computed and leads II and V5 were extracted for each patient. An automated template-based ECG feature extraction algorithm proposed by Schmidt *et al* (2014) was used to obtain beat-to-beat values of the heart period (RR) and Q onset to T end (QT) interval from all selected leads. Beat-to-beat values of respiration were estimated directly from the ECG signal as the integral of the R peak $\pm$ 20 ms (Correa *et al* 2008).

The resulting RR series were validated against their respective ECG recordings to identify detection errors and check for the presence of ectopic beats. RR series consisting of more than 10% ectopic beats were excluded from further analysis, while those with less than 10% ectopic beats were interpolated using the spline interpolation method. Hence, the final number of patients included in the analysis was 57 with an average age of 60 $\pm$ 11.9 years; of which 22 were female and 35 were male. Eighteen patients had previous MI.

We then selected stationary segments of about 350 beats long from the resulting QT, RR and respiration time series. Data segments were detrended using a time-varying finite-impulse response high-pass filter with a corner frequency of 0.04 Hz (Tarvainen *et al* 2002) and normalized to zero mean and unit variance for autoregressive modelling. Similar ECG pre-processing has been applied previously (EL-Hamad *et al* 2020).

2.4. Model-based QTV analysis

A parametric linear autoregressive model with two external inputs (ARXX) (Baselli *et al* 1997) was used to decompose QTV into RR-dependent and respiration-dependent components and contributions unexplained by the model.

The z-domain representation of the model was defined as:

$$\mathrm{A_l}(z)\mathrm{QT(i)} = B_1(z)\mathrm{RR(i)} + B_2(z)\mathrm{respiration(i)} + e_{\mathrm{QT}}(\mathrm{i}), \tag{3}$$

with

$$A_1(z) = 1 + a_{1,1}z^{-1} + \ldots + a_{1,\,n_{a1}}z^{-n_{a1}}, \tag{4}$$

$$B_1(z) = b_{1,0} + b_{1,1}z^{-1} + \ldots + b_{1,n_{b1}}z^{-n_{b1}}, \tag{5}$$

$$B_2(z) = b_{2,0} + b_{2,1}z^{-1} + \ldots + b_{2,n_{b2}}z^{-n_{b2}} \tag{6}$$

where z^{-1} is the one-step delay operator in the z-domain and i is the progressive beat number. Variables A_1, B_1 and B_2 are polynomials of the model parameters that account for the dependence of QTV on: (i) up to n_{a1} samples of its past, (ii) up to n_{b1} samples of the present and past of RR and (iii) up to n_{b1} samples of the present and past of respiration. e_{QT} is a noise source that represents the effect of actual noise in addition to rhythms originating from sources not accounted for in the model.

RR was modelled as an autoregressive process with respiration as an external input, while respiration was modelled as a separate autoregressive process. Further details of the mathematical models and their parameters are described elsewhere (Baselli *et al* 1997, EL-Hamad *et al* 2015, EL-Hamad *et al* 2020).

Model orders were selected based on the Akaike information criterion (Akaike 1974) within the range of 4 to 12. Model parameters were allowed to have different orders to account for the different influences each input exhibits on the output over time. Model parameters were estimated using the least-squares method and models were validated using residual analysis (Porta *et al* 1998, EL-Hamad *et al* 2015).

We computed the following variables from the resulting models:

- SNR—Signal-to-noise ratio at baseline was computed as the ratio of median T wave amplitude power to the isoelectric line power representing noise (Hasan *et al* 2013); in dB.
- QTm, RRm—the average QT and RR intervals respectively; in ms.
- QTc—QT interval corrected for heart rate using the Bazett formula (Bazett 1920); in ms.
- QTV, RRV—the variance of beat-to-beat QT and RR intervals respectively; in ms^2.
- QTrr—the contribution of RRV to total QTV; in percent.
- QTrespiration—the contribution of respiration to total QTV; in percent.
- QTindependent—QTV independent from RR and respiration; in percent.
- GOF—Model goodness-of-fit measures the model's ability to explain the variability in the data; in normalized units. GOF ranges between 0 and 1, where a 1 indicates a perfect fit while a 0 indicates the inability of the model to explain the variability in the data.

2.5. Statistical analysis

GraphPad Prism 9 (GraphPad Software Inc.) software was used for statistical analysis. Data are presented as median (25th percentile–75th percentile). The Friedman test was used to test the differences in measured variables across all leads at baseline. Post-hoc analysis was performed using Dunn's multiple comparisons test. The Wilcoxon matched-pairs signed-rank test was used to compare variables at baseline and post-PTCA and to compare between the group with MI and without prior MI in each lead. Values of $p < 0.05$ were considered statistically significant.

3. Results

3.1. Lead comparison—baseline

Figure 1 shows that SNR was highest in the PCA lead (30.53 dB [25.21 dB—33.80 dB]) compared to all other leads (25.85 dB [22.50–29.85] in Lead II, 26.62 dB [23.11–30.28] in V5, 25.55 dB [19.84–28.73] in VM, and 24.52 dB [21.07–29.34] in RMS8). GOF was significantly higher in VM (0.35 [0.25–0.55]) and RMS8 (0.40 [0.20–0.60]) compared to other leads (0.2 [0.14–0.33] in Lead II, 0.24 [0.17–0.42] in V5 and 0.33 [0.19–0.49] in PCA). Table S1 in the supplementary material (available online at stacks.iop.org/PMEA/43/105002/mmedia) shows the median and interquartile range of all variables in all leads. Figure 2 compares variables in all leads at baseline.

In figure 2, QTm and QTc were both significantly lower in VM (QTm: 396.7 ms, QTc: 418.1 ms) and RMS8 (QTm: 397.8 ms, QTc: 420.6 ms) leads compared to all other leads, while QTV (7.4 ms^2 in Lead II, 8.8 ms^2 in V5, 5.83 ms^2 in PCA, 6.62 ms^2 in VM and 5.87 ms^2 in RMS8) and RRV (267.3 ms^2 in Lead II, 221.6 ms^2 in V5, 228.5 ms^2 in PCA, 231.3 ms^2 in VM and 257.9 ms^2 in RMS8) were not significantly different between the leads. QTindependent was significantly lower in VM and RMS8 (4%–14% lower than other leads), while QTrr was significantly higher in these two leads compared to all other leads (2%–12% higher than other leads). QTrespiration was not different amongst the leads. Absolute values of all variables can be found in table S1.

Figure 3 shows the median power spectrum of QT and all its components in each lead.

3.2. Lead comparison—baseline versus post-PTCA

Figure 4 compares variables in all leads at baseline (pre) and post-PTCA (post). In all leads, QTm and QTc were significantly increased post-PTCA (QTm increased by 22.4 ms in Lead II, 23.1 ms in V5, 17.2 ms in PCA, 18.1 ms in VM, and 17.4 ms in RMS8; QTc increased by 32.8 ms in Lead II, 19.5 ms in V5, 24.7 ms in PCA, 27.9 ms in VM, and 20.4 ms in RMS8), while RRm was significantly decreased post-PTCA by a median of 39.5 ms in all leads. RRV was significantly decreased in all leads (decreased by 202.76 ms^2 in Lead II, 158.24 ms^2

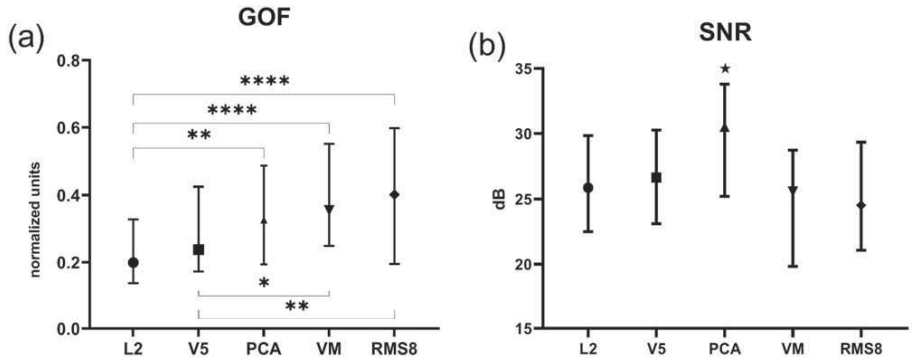

Figure 1. Comparison of (a) model goodness-of-fit (GOF) and (b) signal-to-noise ratio (SNR) in all leads at baseline. Values are expressed as median and interquartile ranges. Friedman test and post-hoc Dunn's multiple comparison test p values < 0.05 are considered significant. N = 57. * p $\leqslant 0.05$, ** p $\leqslant 0.01$, *** p $\leqslant 0.001$, **** p $\leqslant 0.0001$. ★ significantly different from all other groups.

in V5, 165.88 ms^2 in PCA, 166.65 ms^2 in VM and 188.88 ms^2 in RMS8) while QTV was significantly increased by 7.01 ms^2 only in Lead II. QTindependent was generally increased in all leads, but only reached statistical significance in Lead II and VM (increased by 5.64% in Lead II and 9.3% in VM). QTrr was decreased in all leads post-PTCA (decreased by 5% in Lead II, 3.84% in V5, 8.06% in PCA, 5.2% in VM and 4.27% in RMS8). QTrespiration was not changed post-PTCA in any of the leads. Absolute values of all variables can be found in table S1.

3.3. Subgroup analysis—MI versus no MI

When comparing patients with MI to those without MI at baseline (figure 5), QTm was slightly shorter in the MI group (shorter by a median of 25 ms in Lead II, 6.5 ms in V5, 10.2 ms in PCA, 10.6 ms in VM and 21.1 ms in RMS8) while QTc was not different between the two groups. These results were consistent across all leads. RRm was shorter in the MI group in all leads (except V5), but differences did not reach statistical significance (shorter by 8.2 ms in Lead II, 9.6 ms in PCA, 4.6 ms in VM and 12.8 ms in RMS8). Interestingly, QTV was not different between the two groups, while RRV was significantly reduced in the MI group in most leads (all except for RMS8). In all leads, QTindepedent showed a trend to increase in the MI group ($p > 0.05$), while QTrr tended to decrease in the MI group (p > 0.05). The absolute values of all variables can be found in table S2.

4. Discussion

The main findings of this study are (1) vector magnitude representation of 12-lead ECG produced models with a GOF 10% higher than single leads despite having an SNR similar to that of single leads; (2) vector magnitude representation of 12-lead ECG resulted in significantly different QTm, QTc and RRm, QTindependent and QTrr compared to single-lead ECG, while QT and RR variabilities were not different amongst the different leads; Finally, (3) all leads showed similar changes in all measured variables following PTCA, and differences between patients with prior MI and those without MI were consistent across all leads. These findings suggest that using single-lead ECG for QTV analysis might be sufficient for capturing intervention effects and pathophysiological differences, even though vector magnitude representation of 12-lead ECG seems to provide more information and improves modelling.

4.1. Lead comparison at baseline

Twelve-lead ECG provides a multi-angled view of the ventricular repolarisation process. Obtaining a one-dimensional representation of all 12 leads would facilitate direct comparison between multi-lead and single-lead analysis. Principal component analysis (PCA) has been applied to 12-lead ECG for data compression and noise reduction (Castells *et al* 2007). Vector magnitude (VM) of the orthogonal XYZ leads and the root-mean-square of the eight independent leads (RMS8) are also methods that have been applied to obtain a one-dimensional representation of 12-lead ECG (Hnatkova *et al* 2019).

In our study, PCA resulted in the best SNR yet its GOF was significantly less than VM and RMS8. This could be a consequence of only including either the first or second components in the analysis. The exclusion of the other principal components might have reduced not only noise content but also important information simultaneously. VM and RMS8 resulted in better model fits than single leads despite having similar SNR.

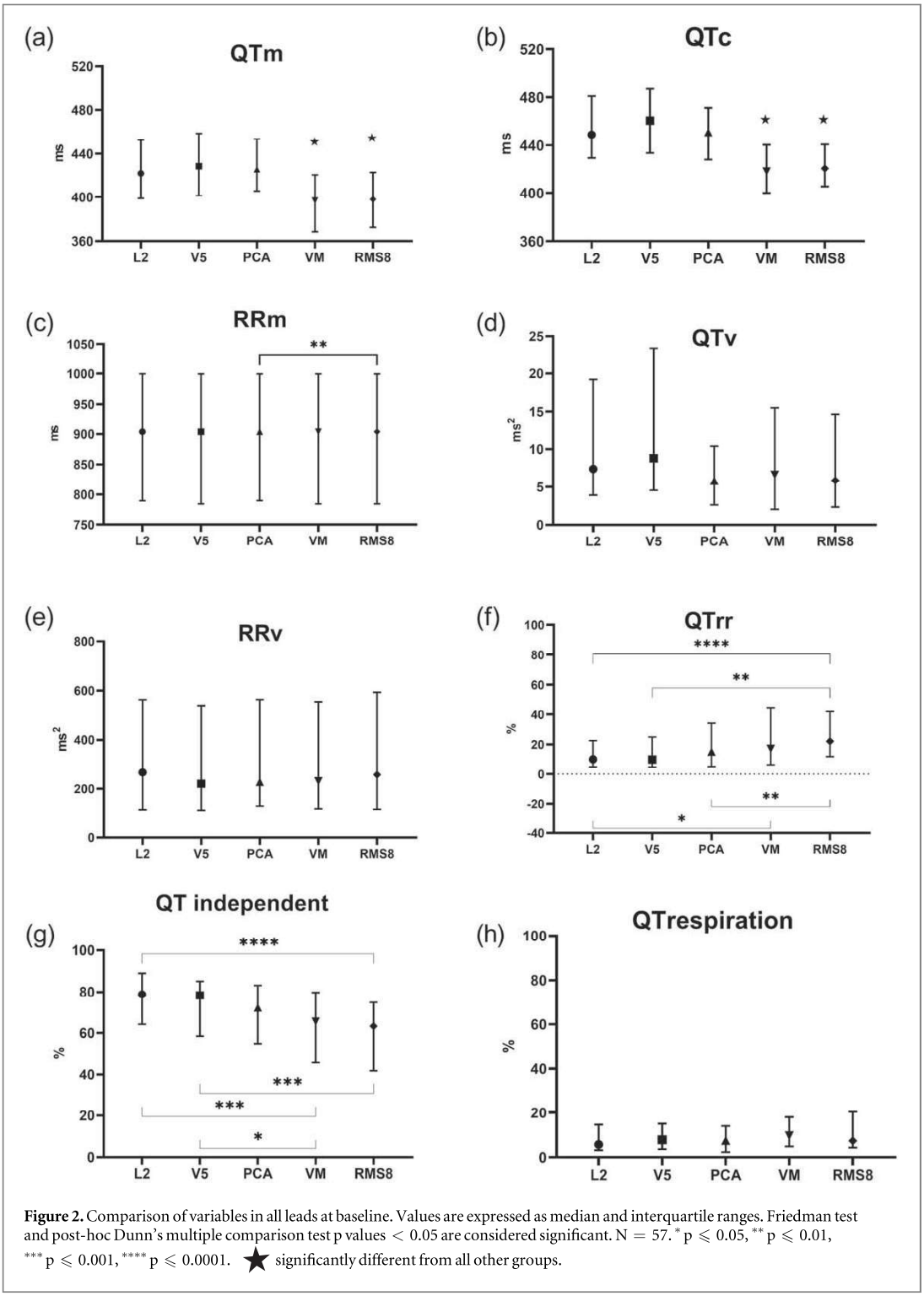

Figure 2. Comparison of variables in all leads at baseline. Values are expressed as median and interquartile ranges. Friedman test and post-hoc Dunn's multiple comparison test p values < 0.05 are considered significant. N = 57. * p $\leqslant$ 0.05, ** p $\leqslant$ 0.01, *** p $\leqslant$ 0.001, **** p $\leqslant$ 0.0001. ★ significantly different from all other groups.

QTV independent of RR and respiration was lower, and RRV contribution to QTV was higher in VM and RMS8. This indicates that the improvement in GOF for these leads is not necessarily attributed to the reduction in noise with the VM and RMS8 methods but may provide a more complete view of repolarisation variability, especially that driven by heart rate. Others have compared multiple one-dimensional representations of 12-lead ECG for JT interval analysis and found that the vector magnitude of the orthogonal XYZ leads was most suitable for the analysis (Hnatkova *et al* 2019). In a canine model, VM was also found to result in improved measurements of the JTp and TpTe intervals compared to Lead II (Brockway *et al* 2018).

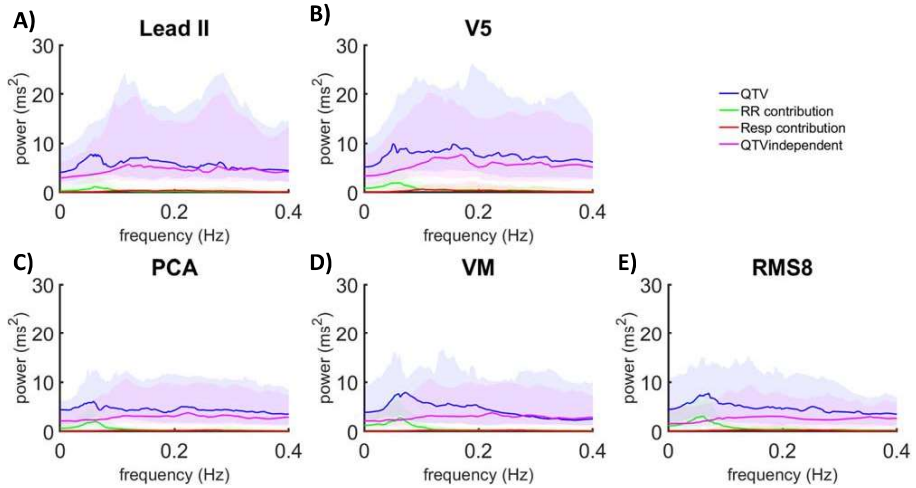

Figure 3. Decomposed power spectrum of QTV in (A) Lead II, (B) V5, (C) PCA, (D) VM and (E) RMS8. Total QT power is blue, RR contribution is green, Respiratory contribution is red, and QT independent is magenta. Powers are shown as median (line) and interquartile range (shaded area).

Interestingly, there were no differences in QTV between any of the leads in this study. Hasan *et al* found differences in the standard deviation of QT interval between some ECG leads, with the most prominent deviations being in Lead III, aVL and V_1 compared to all other leads (Hasan *et al* 2012). However, in agreement with our findings they did not find differences between Lead II and V_5.

4.2. Lead comparison: post-PTCA

Several studies have explored the influence of PTCA on QT and RR intervals. We observed an increase in QT interval following PTCA, consistent with other results (Michelucci *et al* 1996, Bonnemeier *et al* 2001). In contrast, others reported a reduction in QT interval (Giedrimiene *et al* 2002). RR interval was decreased in our study, similar to another study (Giedrimiene *et al* 2002), although others have reported it to increase (Bonnemeier *et al* 2001). Similarly, RRV was decreased in our study, while others reported an increase in RRV post-PTCA (Aydinlar *et al* 2009). As for QT variability, Bonnemeier *et al* reported a decrease in the standard deviation of the QT interval (Bonnemeier *et al* 2001), while we observed an increase in QTV only in Lead II. These discrepancies could be owing to differences in the inclusion criteria for patient groups, as MI patients were excluded from some studies but included in others. In addition, the time window within which the post-PTCA ECG was recorded varies between studies, while it has been shown that QTc and QT show a biphasic profile where they increase right after PTCA but decrease after the first hour post-PTCA (Bonnemeier *et al* 2001).

Nonetheless, the important finding here is that despite the differences in the measurement of variables in VM/RMS8 compared to single leads at baseline, all leads captured similar changes in all measured variables (except for QTV in Lead II) following PTCA. This demonstrates that single-lead ECG could be sufficient for capturing QT dynamics to evaluate the effect of PTCA.

4.3. Lead comparison: MI versus no MI

QTV has been shown to be increased in MI patients (Hasan *et al* 2013) and in coronary artery disease patients without prior MI (Vrtovec *et al* 2000) compared to healthy subjects. QTV has also been shown to be increased in coronary artery disease patients without MI compared to those with old MI (Yao *et al* 2019). We performed an additional subgroup analysis, comparing patients with prior MI to those without prior MI at baseline to explore how the different leads detected differences between these groups. We found that all leads also show similar differences between the two groups.

These observations indicate that single-lead ECG could be sufficient to investigate pathophysiological differences in cases where 12-lead ECG is not available or practical to record. Similar conclusions have been reached in safety pharmacology where a single lead (V3 or Lead II) is sufficient for detecting liabilities (Hamlin 2008) and in a canine model where measurements of the QT interval in Lead II were in good agreement with those of VM (Brockway *et al* 2018).

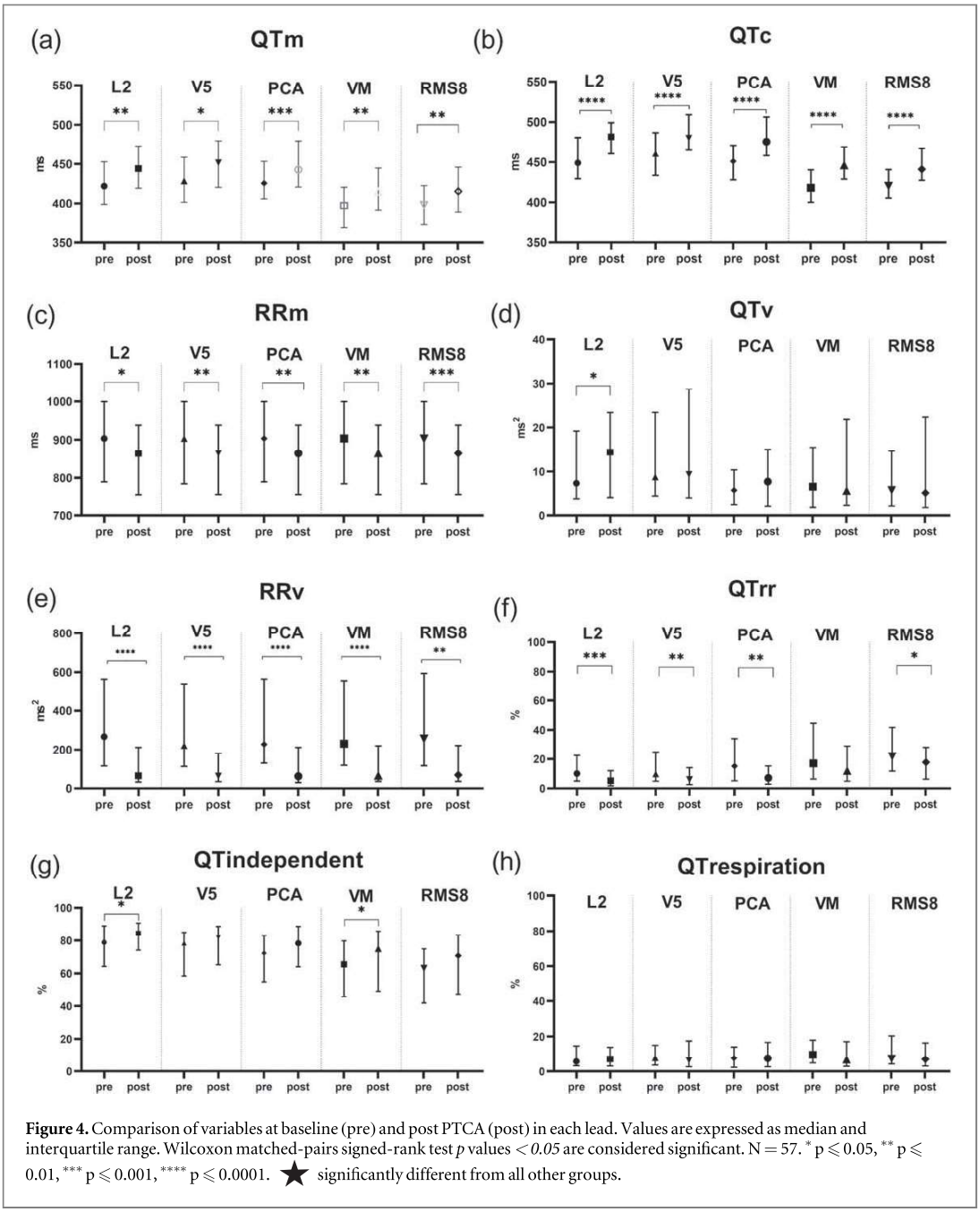

Figure 4. Comparison of variables at baseline (pre) and post PTCA (post) in each lead. Values are expressed as median and interquartile range. Wilcoxon matched-pairs signed-rank test *p* values < 0.05 are considered significant. N = 57. * p $\leqslant$ 0.05, ** p $\leqslant$ 0.01, *** p $\leqslant$ 0.001, **** p $\leqslant$ 0.0001. ★ significantly different from all other groups.

4.3.1. Our study has several limitations

PTCA was performed in one of the three main arteries. While ischemia-induced ventricular repolarization changes are dependent on the occlusion site we did not account for the possible influence this could have had on the analysis. While combining multiple leads into a single lead may be disadvantageous for characterizing coronary artery disease, it may hold value for studying global temporal repolarisation lability by means of QTV. The data included coronary artery disease patients only; hence these results might not apply to other patient groups. Some evidence shows a sex effect on QTV (Baumert *et al* 2016), yet our patient group had more males than females. Nonetheless, some studies have shown that coronary heart disease is more prevalent in men of older age (Leening *et al* 2014), and hence the percentage of women in our data seems reasonable especially as we are not testing for sex differences in our analysis. We employed a linear time-invariant model for QTV analysis, which does not account for QT-RR hysteresis. Finally, in our preprocessing step of data segments, heartbeats in recordings with less than 10% rejected beats were interpolated.

In conclusion, despite the differences in QTV amongst different leads, single-lead ECG could be sufficient to explore intervention effects or pathophysiological differences between groups. While 12-lead ECG recordings

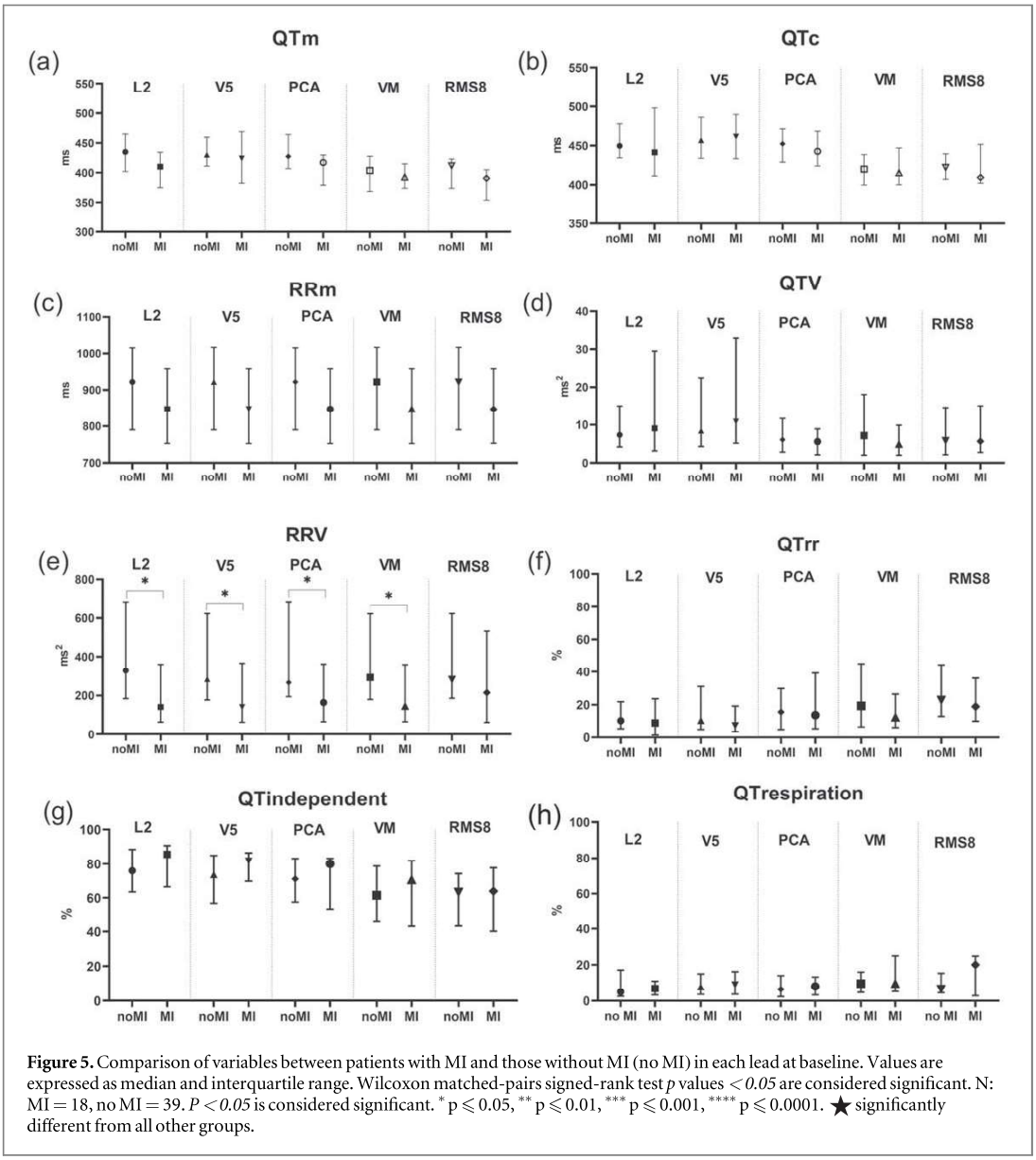

Figure 5. Comparison of variables between patients with MI and those without MI (no MI) in each lead at baseline. Values are expressed as median and interquartile range. Wilcoxon matched-pairs signed-rank test p values < 0.05 are considered significant. N: MI = 18, no MI = 39. $P < 0.05$ is considered significant. * p ⩽ 0.05, ** p ⩽ 0.01, *** p ⩽ 0.001, **** p ⩽ 0.0001. ★ significantly different from all other groups.

are common, often preclinical studies require single-lead ECG, possibly because it is easier to apply and more practical to record. Furthermore, many studies perform retrospective analyses of valuable existing datasets containing only single-lead ECG, eliminating the possibility of lead selection. Future work should validate results using simulated 12-lead ECG with well-defined noise levels and predefined QT-RR relationship. This analysis should also be validated on normal subjects to establish whether a single lead is sufficient irrespective of the presence of a pathophysiological condition.

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Supplementary data

Table S1: Median [25th – 75th percentile] values of variables at baseline (pre) and post-PTCA (post). ***** indicates significant difference compared to pre-PTCA (pre).

Variables	**Lead II**		**V5**		**PCA**		**VM**		**RMS8**	
	Pre	**Post**	**Pre**	**Post**	**Pre**	**Post**	**Pre**	**Post**	**Pre**	**Post**
QTm (ms)	421.8 [398.9-452.6]	444.2***** [419-473.3]	428.5 [401.4-458.7]	451.6***** [420.4-479.9]	425.6 [405.6-453.5]	442.8***** [420.6-479.6]	396.7 [368.5-420.4]	414.8***** [390.4-444.8]	397.8 [372.5-422.6]	415.2***** [387.9-446.1]
QTc (ms)	449.1 [429.4-481]	481.9***** [460.6-499.3]	460.7 [433.7-486.9]	480.2***** [465.1-509.0]	451.2 [428-471.2]	475.9***** [458.2-506.2]	418.1 [400-440.4]	446***** [428.9-469.2]	420.6 [405.5-440.7]	441***** [427.4-467.1]
RRm (ms)	903.7 [789.5-999.8]	864.2***** [756.3-938.3]	903.7 [784.4-999.7]	864.2***** [756.4-938.9]	903.7 [789.6-999.7]	864.2***** [756.4-938.9]	903.7 [784.4-999.7]	864.2***** [756.4-938.9]	903.7 [784.4-999.7]	864.2***** [756.4-938.9]
QTV (ms^2)	7.40 [3.9-19.22]	14.41***** [4.2-23.38]	8.80 [4.56-23.4]	9.38 [4.11-28.66]	5.83 [2.64-10.38]	7.77 [2.3 – 15.02]	6.62 [2.03-15.50]	5.7 [2.49-21.84]	5.87 [2.34-14.66]	5.24 [1.99 – 22.32]
RRV (ms^2)	267.3 [115.1-563.8]	64.54***** [32.5-212.1]	221.6 [112.9-539.7]	63.36***** [34.5-181.1]	228.5 [130.2-564.3]	62.62***** [29.77-211.7]	231.3 [119.2-555.4]	64.65***** [35.4-220.6]	257.9 [116.9-593.7]	69.02***** [35.4-221.5]

QTrr (%)	10.11 [5.03-22.53]	5.18***** [1.85-12.08]	9.89 [4.92-24.96]	6.06***** [2.59 – 14.13]	15.21 [5.21-34.14]	7.15***** [2.89 – 15.26]	17.09 [6.45-44.67]	11.89 [4.91 – 29.16]	22.1 [11.86-41.80]	17.83***** [6.33 – 28.22]
QTrespiration (%)	5.70 [3.19-14.62]	7.18 [3.05-13.89]	7.83 [3.58-15.04]	6.39 [2.66-17.47]	7.61 [2.38-14]	7.61 [2.70-16.75]	9.61 [4.86-17.97]	6.72 [2.93-17.12]	7.38 [4.34-20.36]	7.03 [3.07 - 16.32]
QTindependent (%)	78.81 [64.29-89.02]	84.45***** [74.14-90.71]	78.38 [58.46-85.10]	81.95 [65.30-88.67]	72.33 [54.83-83.13]	78.33 [64.06 – 88.68]	65.55 [45.77-79.75]	74.85***** [49.18 – 85.81]	63.34 [41.78-74.89]	70.80 [47.39 – 83.37]

Table S2: Median [25th – 75th percentile] values of all variables at baseline for patients with MI and those without MI. * indicates significantly different to patients without MI (no MI).

Variables	**Lead II**		**V5**		**PCA**		**VM**		**RMS8**	
	No MI	**MI**	**No MI**	**MI**	**No MI**	**MI**	**No MI**	**MI**	**No MI**	**MI**
QTm (ms)	435 [401.8-464.9]	410 [375.1-434.2]	430.3 [411.0-459.6]	423.8 [382.4-468.9]	427.3 [406.7-464]	417.1 [379.1-429.6]	403.5 [368.5-427.6]	392.9 [373.9-414.8]	411.7 [374-423]	390.6 [353.7-404.7]
QTc (ms)	449.8 [434.4-478]	441.6 [411.3-498.2]	457.1 [433.7-486.2]	461.4 [433.3-489.8]	452.4 [428.9-471.5]	442.8 [423.9-468.6]	419.9 [399.8-438.6]	415.3 [400.01-447.1]	422.2 [407.1-439.5]	409.4 [402-451.6]

RRm (ms)	922.5 [790.2-1016]	846.8 [753.1-958.6]	922.4 [790.2-1017]	846.8 [753-958.6]	922.4 [790.2-1015]	846.8 [752.8-958.6]	922.2 [790.2-1017]	846.8 [752.8-958.6]	922.4 [790.2-1017]	846.9 [753.4-958.6]
QTV (ms^2)	7.338 [4.185-15.01]	9.015 [3.141-29.50]	8.466 [4.320-22.43]	10.77 [5.186-32.97]	6.076 [2.797-11.58]	5.609 [2.098-8.915]	7.155 [2.006-18.05]	4.886 [2.006-9.869]	5.870 [2.138-14.56]	5.673 [2.757-15.02]
RRV (ms^2)	327.0 [183.8-684.9]	140.7 [63.97-359.8]	283.4 [177.0-622.6]	140.1 [63.59-365.6]	265.7 [193.9-685.5]	163.7 [65.84-361.1]	292.0 [179.7-621.7]	145.4 [65.94-358.7]	281.2 [185.7-622.2]	214.3 [62.97-532.3]
QTrr (%)	10.36 [5.404-21.79]	8.994 [1.404-23.61]	10.63 [5.061-31.10]	7.351 [3.617-19.20]	15.64 [4.964-29.89]	13.74 [5.476-39.31]	19.32 [6.582-44.96]	12.45 [6.253-26.49]	22.86 [12.90-44.31]	18.89 [10.03-36.05]
QTrespiration (%)	4.89 [2.60-17.17]	6.82 [9.32-11.0]	7.83 [5.55-14.99]	8.89 [3.73-16.26]	6.47 [2.32-14.01]	8.04 [3.31-13.27]	9.61 [4.72-16.03]	9.67 [5.27-25.02]	6.59 [4.53-15.42]	20.09 [2.86-24.87]
QTindependent (%)	75.85 [63.50-88.41]	85.56 [66.44-90.60]	73.65 [56.78-84.91]	81.63 [69.82-86.41]	71.10 [57.50-83.09]	79.96 [53.38-83.21]	61.48 [46.36-78.70]	70.61 [43.09-81.86]	63.34 [43.27-74.22]	63.93 [40.13-77.69]

Chapter 5

Relation between QT interval variability and muscle sympathetic nerve activity in normal subjects

EL-HAMAD, F., LAMBERT, E., ABBOTT, D. & BAUMERT, M. 2015. Relation between QT interval variability and muscle sympathetic nerve activity in normal subjects. *American Journal of Physiology: Heart and Circulation Physiology*, 309, H1218–24.

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Contribution to the Paper	Study design, data analysis, interpretation of data and results, preparation and revision of manuscript.		
Overall percentage (%)	80%		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
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Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

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Relation between QT interval variability and muscle sympathetic nerve activity in normal subjects

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El-Hamad F, Lambert E, Abbott D, Baumert M. Relation between QT interval variability and muscle sympathetic nerve activity in normal subjects. *Am J Physiol Heart Circ Physiol* 309: H1218–H1224, 2015. First published August 14, 2015; doi:10.1152/ajpheart.00230.2015.—Beat-to-beat variability of the QT interval (QTV) is sought to provide an indirect noninvasive measure of sympathetic nerve activity, but a formal quantification of this relationship has not been provided. In this study we used power contribution analysis to study the relationship between QTV and muscle sympathetic nerve activity (MSNA). ECG and MSNA were recorded in 10 healthy subjects in the supine position and after 40° head-up tilt. Power spectrum analysis was performed using a linear autoregressive model with two external inputs: heart period (RR interval) variability (RRV) and MSNA. Total and low-frequency power of QTV was decomposed into contributions by RRV, MSNA, and sources independent of RRV and MSNA. Results show that the percentage of MSNA power contribution to QT is very small and does not change with tilt. RRV power contribution to QT power is notable and decreases with tilt, while the greatest percentage of QTV is independent of RRV and MSNA in the supine position and after 40° head-up tilt. In conclusion, beat-to-beat QTV in normal subjects does not appear to be significantly affected by the rhythmic modulations in MSNA following low to moderate orthostatic stimulation. Therefore, MSNA oscillations may not represent a useful surrogate for cardiac sympathetic nerve activity at moderate levels of activation, or, alternatively, sympathetic influences on QTV are complex and not quantifiable with linear shift-invariant autoregressive models.

QT interval variability; muscle sympathetic nerve activity

NEW & NOTEWORTHY

QT interval variability in normal subjects does not appear to be significantly affected by the rhythmic modulations in MSNA following low to moderate orthostatic stimulation.

INCREASED SYMPATHETIC NERVOUS system activity contributes to the development and progression of cardiovascular disease, such as heart failure (17), and may even trigger ventricular arrhythmias (22). Therefore, quantification of the level of sympathetic outflow directed at the heart may be of diagnostic interest in clinical, as well as nonclinical, contexts (8, 15, 29).

Elevated beat-to-beat variability in ventricular repolarization duration, measured as changes in QT interval [QT variability (QTV)] on body surface ECG has been attributed to increased sympathetic outflow to the ventricles (32, 46). Although it is widely believed that QTV is predominantly driven by heart period (RR interval) variability (RRV) under normal conditions during rest (34), several studies across a range of conditions, including healthy subjects (2), patients post-myocardial infarction (50), heart failure patients (25), and patients with diabetes mellitus (39), showed that a large part of QTV could not be solely attributed to RRV. An increased decoupling between RRV and repolarization variability was reported in healthy subjects following head-up tilt, a maneuver well known to increase sympathetic nerve activity (12), and QTV unrelated to RR and respiration (35) was shown to increase under similar experimental conditions. Correlations between QTV and cardiac norepinephrine spillover, a direct measure of sympathetic activity, recorded during rest were observed in patients with hypertension (6), but not in normal subjects or patients with panic disorder and depression (5). Also, QTV has been found to increase significantly during standing and isoproterenol infusion (46). Collectively, these findings suggest that heightened sympathetic neural modulation of the ventricular myocardium augments repolarization variability independent of RRV. Thus, measurement of QTV may allow evaluation of the effects of sympathetic activity on the ventricles and, thereby, yield a noninvasive index for quantification of sympathetic activity levels.

Muscle sympathetic nerve activity (MSNA) measured in the peroneal muscle using microneurography is a widely used technique to probe postganglionic sympathetic nerve activity (18). As the analysis of MSNA has been proven useful for investigating the autonomic regulation of sinoatrial node activity in conjunction with RRV (18, 24, 28), it may help quantify the relationship between QTV and sympathetic modulation of the ventricles.

The aim of this study was to investigate the extent to which QTV can be used to measure sympathetic activity. To address this question, we used an autoregressive model and power contribution analysis to study the relationship between QTV and MSNA in normal subjects following head-up tilt.

METHODS

Subjects and experimental protocol. We studied 10 healthy subjects (2 men and 8 women; 25.1 ± 5.9 yr of age, 23.5 ± 3.1 kg/m^2 body mass index) selected from a previously published study (19). After giving written informed consent, subjects were tested in the morning, with caffeine and alcohol consumption restricted ≥12 h before the study was initiated. After instrumentation, subjects rested for 30 min before the actual recording started. The experimental protocol was approved by the Alfred Hospital Ethics Review Committee.

ECG lead III, arterial blood pressure, and MSNA were recorded simultaneously for 10 min in the supine position in each subject and then for 10 min in the head-up tilt position at 20°, 30°, and 40° using PowerLab (ADInstruments, Bella Vista, NSW, Australia). Blood pressure was monitored through percutaneous cannulation of the radial artery. MSNA was recorded using microneurography, as described previously (19). Briefly, a tungsten microelectrode was in-

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serted into the common peroneal nerve to record nerve traffic from multiple postganglionic fibers. The MSNA signal was integrated using a resistor-capacitor circuit to obtain mean voltage of the multiunit nerve recording.

Data preprocessing. Recordings were visually inspected to identify artifact-free segments. Beat-to-beat series of the heart period (RR interval) were defined as the temporal distance between two consecutive QRS complexes. Beat-to-beat QT interval time series were obtained based on a recently proposed two-dimensional signal-warping algorithm (41). Briefly, the algorithm creates a template beat on which the QT interval is semiautomatically annotated and then morphed in both time and amplitude to match consecutive beats, thereby accounting for variations in the ECG waveform. Finally, the QT interval duration of each beat is obtained based on the scaled version of the annotated QT interval in the adapted template. The algorithm also incorporates rejection criteria for beats that are highly corrupted by noise.

Beat-to-beat systolic arterial pressure (SAP) and diastolic arterial pressure were defined as the local maximum and minimum, respectively, of the blood pressure signal enclosed by consecutive R peaks. Beat-to-beat values of MSNA were obtained by calculating the time average of the integrated MSNA signal between two consecutive diastolic arterial pressure time points.

For each subject, data segments of ~200 beats (~3 min) were selected for the analysis from both supine and 40° head-up-tilt positions. To simplify the analysis, intermediate tilt angles were not considered.

Beat-to-beat series of RR, QT, MSNA, and SAP were detrended using the smoothness priors method (43), comprising a time-varying finite-impulse response high-pass filter. A cutoff frequency of 0.04 Hz was chosen by setting the smoothing parameter to 22. The beat-to-beat series were normalized to zero mean and unit variance for frequency domain analysis.

Time domain analysis of QTV. Time domain analysis of RR, QT, MSNA, and SAP time series was performed to confirm the increase in variances expected with head-up tilt due to the increase in sympathetic activity. Mean RR interval (RRm), RR interval variance (RRv), mean QT interval (QTm), QT interval variance (QTv), MSNA variance (MSNAv), SAP variance (SAPv), and QT variability index (QTVi), given by

$$\mathrm{QTVi} = \log_{10}\left[\frac{\mathrm{QT}/(\mathrm{QTm})^2}{\mathrm{RRv}/(\mathrm{RRm})^2}\right] \quad (1)$$

were calculated for each subject in supine and 40° tilt positions.

Frequency domain analysis. Power contribution analysis was performed using a linear autoregressive model with two external inputs (ARXX) (3). We assumed an open-loop structure, where QT is affected independently by RR and MSNA while QT does not exhibit an influence on MSNA and RR. The model assumes that the rhythm sources generating the three signals are uncorrelated. Thus the autoregressive model was defined as

$$A_1(z)\mathrm{QT}(i) = B_1(z)\mathrm{RR}(i) + B_2(z)\mathrm{MSNA}(i) + e_{\mathrm{QT}}(i) \quad (2)$$

where

$$A_1(z) = 1 + a_{1,1}z^{-1} + \ldots + a_{1,n_{a1}}z^{-n_{a1}} \quad (3)$$

$$B_1(z) = b_{1,0} + b_{1,1}z^{-1} + \ldots + b_{1,n_{b1}}z^{-n_{b1}} \quad (4)$$

$$B_2(z) = b_{2,0} + b_{2,1}z^{-1} + \ldots + b_{2,n_{b2}}z^{-n_{b2}} \quad (5)$$

where A_1, B_1, and B_2 are polynomials of the parameters in the z domain that describe the effect of the samples of QT, RR, and MSNA up to a delay of n_{a1}, n_{b1}, and n_{b2}, respectively, on the one-step-ahead prediction of QT. *Equation 2* describes QT as a function of its own past, past and present values of RR and MSNA, and a noise source that represents actual noise and rhythms originating from sources not included in the model.

On the other hand, RR and MSNA were modeled as separate autoregressive processes

$$A_2(z)\mathrm{RR}(i) = e_{\mathrm{RR}}(i) \quad (6)$$

$$A_3(z)\mathrm{MSNA}(i) = e_{\mathrm{MSNA}}(i) \quad (7)$$

$$A_2(z) = 1 + a_{2,1}z^{-1} + \ldots + a_{2,n_{a2}}z^{-n_{a2}} \quad (8)$$

$$A_3(z) = 1 + a_{3,1}z^{-1} + \ldots + a_{3,n_{a3}}z^{-n_{a3}} \quad (9)$$

where n_{a2} and n_{a3} are the AR model orders. *Equations 6* and *7* describe MSNA and RR as a function of their own past and a noise source. Variables e_{QT}, e_{RR}, and e_{MSNA} are white noise sources with zero mean and σ^2_{QT}, σ^2_{RR}, and σ^2_{MSNA} variance, respectively.

The goodness of fit was calculated to quantify the ability of the ARXX structure to represent the measured data (37). Model parameters were estimated using the least-squares method. The uncorrelation between noise sources was verified based on residual analysis. Frequency domain representations of the models were obtained by Fourier transform, where the power of QT can be represented through individual contributions (34)

$$P(f)_{\mathrm{QT}} = \left|\frac{B_1(f)}{A_1(f)\times A_2(f)}\right| \times \sigma^2_{\mathrm{RR}} + \left|\frac{B_2(f)}{A_1(f)\times A_3(f)}\right|^2 \times \sigma^2_{\mathrm{MSNA}} + \left|\frac{1}{A_1(f)}\right|^2 \times \sigma^2_{\mathrm{QT}} \quad (10)$$

where the first term is the power contribution from RR, the second term is the power contribution from MSNA, and the third term is the power independent of RR and MSNA that is attributed to noise and other sources not accounted for in the model. Here, $A_1(f)$, $A_2(f)$, $A_3(f)$, $B_1(f)$, and $B_2(f)$ are the Fourier transform polynomials of the model parameters. Both RR and MSNA total powers were computed as follows

$$P(f)_{\mathrm{RR}} = \left|\frac{1}{A_2(f)}\right|^2 \times \sigma^2_{\mathrm{RR}} \quad (11)$$

$$P(f)_{\mathrm{MSNA}} = \left|\frac{1}{A_3(f)}\right|^2 \times \sigma^2_{\mathrm{RR}} \quad (12)$$

SAP was modeled as an independent AR process similar to that adopted for RR and MSNA.

The squared coherence function was calculated to measure the linear relationship between QT and MSNA as a function of frequency (24) as

$$k^2(f) = \frac{|P_{\mathrm{QT-MSNA}}(f)|^2}{P(f)_{\mathrm{QT}} \times P(f)_{\mathrm{MSNA}}} \quad (13)$$

To estimate the power contained in the low-frequency (LF) and high-frequency (HF) bands, each partial spectrum was decomposed into the spectral components of each pole, as described elsewhere (3). The power of the spectral component contributed by a pole was considered LF power if its central frequency fell in the frequency range 0.05–0.2 Hz and HF if its central frequency was in the range 0.2–0.4 Hz.

For model order selection and estimation, data segments were divided into two-thirds estimation data and one-third validation data. Model orders were selected from the range 4–12, minimizing the Akaike figure of merit (1). The model parameters were estimated using the least-squares method. Correlations within and across regression residuals were deemed negligible if no more than three points were outside the 95% confidence interval of the cross-correlation and autocorrelation plots.

Surrogate analysis. The method of surrogate data testing described by Faes et al. (10) was applied to test whether MSNA total and LF contributions are significant and to define the threshold for zero coherence. For each subject, 10 pairs of QT and MSNA surrogate

Table 1. *Time domain measures in the supine position and following 40° head-up tilt*

Variable	Position		*P* Value
	Supine	40° tilt	
RRm, ms	991 (904 to 1,177)	765 (743 to 858)	0.0020
RRv, ms^2	2,039 (1,813 to 4,805)	955 (382 to 1,743)	0.027
QTm, ms	368 (347 to 398)	338 (328 to 361)	0.0020
QTv, ms^2	2.6 (1.9 to 3.6)	3.9 (3.2 to 9.6)	0.0020
QTVi	−2.2 (−2.4 to −1.8)	−1.4 (−1.7 to −1.3)	0.0098
MSNAv, AU^2	0.00064 (0.00008 to 1.81)	0.002 (0.0003 to 3.8)	0.0488
SAPv, mmHg^2	5.2 (3.5 to 14.0)	6.9 (4.5 to 18.3)	>0.05

Values are medians (25th percentile–75th percentile). RRm, mean heart period; RRv, heart period variance; QTm, mean QT interval; QTv, QT interval variance; QTVi, QT variability index; MSNAv, muscle sympathetic nerve activity variance; SAPv, systolic arterial pressure variance; AU, arbitrary units.

series were generated by feeding the QT and MSNA models with 10 realizations of independent Gaussian white noise processes. MSNA total and LF power contributions and the maximum coherence between QT and MSNA in LF were computed for the generated surrogate series, resulting in 100 surrogate values for each variable across all subjects.

Statistical analysis. GraphPad Prism 6 software was used for statistical analysis. Data are presented as median (25th percentile–75th percentile). The paired two-tailed Wilcoxon test was used to test the change in time domain measures and frequency domain power estimates from the supine position to 40° head-up tilt. The Mann-Whitney test was performed to test whether MSNA power contributions estimated from measured data were significantly different from those obtained from the surrogate data. As proposed by Faes et al. (10), the threshold for zero coherence was set at the 95th percentile of the coherence sampling distribution computed from the surrogate data. $P < 0.05$ was considered statistically significant.

RESULTS

Time domain analysis. The time domain analysis results are summarized in Table 1. RRm, QTm, and RRv decreased significantly following 40° head-up tilt, while QTv, QTVi, and MSNAv increased significantly. However, SAP power did not change with tilt.

Frequency domain analysis. The goodness of fit of the ARXX model was 51% (41-56%) in the supine position and 41% (23–46%) following 40° head-up tilt. This reduction in goodness of fit was not statistically significant ($P > 0.05$).

Figure 1 shows the LF power of QTV and MSNA in the supine position and following 40° tilt. While LF power of QTV, measured in absolute units, increased significantly following 40° head-up tilt ($P = 0.037$), LF power of MSNA, measured in normalized units [LF/(LF + HF)], was not statistically significant between the two measurement conditions ($P > 0.05$). Furthermore, LF power of SAP was not affected by the tilt condition [0.12 (0.09–0.37) mmHg^2 and 0.24 (0.13–0.49) mmHg^2 in supine and standing positions, respectively, $P > 0.05$].

Figure 2 shows individual power contributions of RR and MSNA to QTV, as well as unexplained variance during the supine measurement and following 40° head-up tilt. In the supine position, RRV contributions to total and LF power of QTV were, on median, 38.8% and 45.5%, respectively. This contribution decreased significantly to ~20% of the total and LF power of QTV after head-up tilt ($P = 0.0195$, and $P = 0.0059$, respectively). The MSNA contribution to total and LF powers of QTV measured in the supine position during rest was, on average, 2.1% and 2.2%, respectively. Upon 40° head-up tilt, MSNA contributions tended to increase to 4.8% and 3.0% of the total and LF power of QTV; however, this increase was not statistically significant ($P > 0.05$). Surrogate data testing demonstrated that MSNA total and LF power contributions were significantly different from independent random processes ($P < 0.0001$ in the supine position and $P < 0.03$ following 40° head-up tilt).

In the supine position, total and LF power of QTV independent of RR and MSNA were ~59% and 54%, respectively, and increased significantly to ~74% and 76% following head-up tilt ($P < 0.02$).

Maximum coherence between LF oscillations in MSNA and QTV across all subjects was 0.05 (0.02–0.07) in the supine position, which tended to increase to 0.11 (0.04–0.17) after 40° head-up tilt ($P = 0.08$). For the surrogate data method, the threshold for zero coherence in the LF band was set at 0.5 in the supine position and 0.36 following 40° head-up tilt; hence, coherence values were not significant.

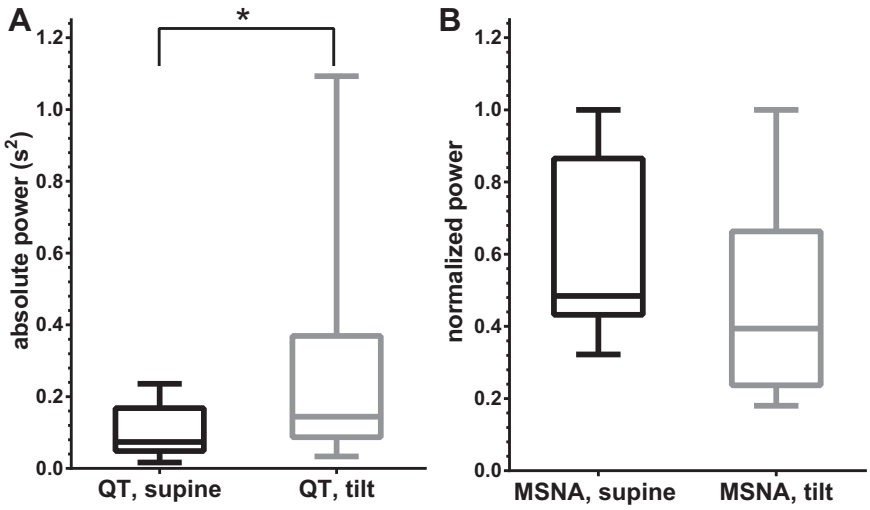

Fig. 1. Change in low-frequency (LF) power of QT (*A*) and muscle sympathetic nerve activity (MSNA; *B*) from the supine position to 40° head-up tilt. Bar plots represent median values and interquartile ranges. *Significant difference between supine and tilt.

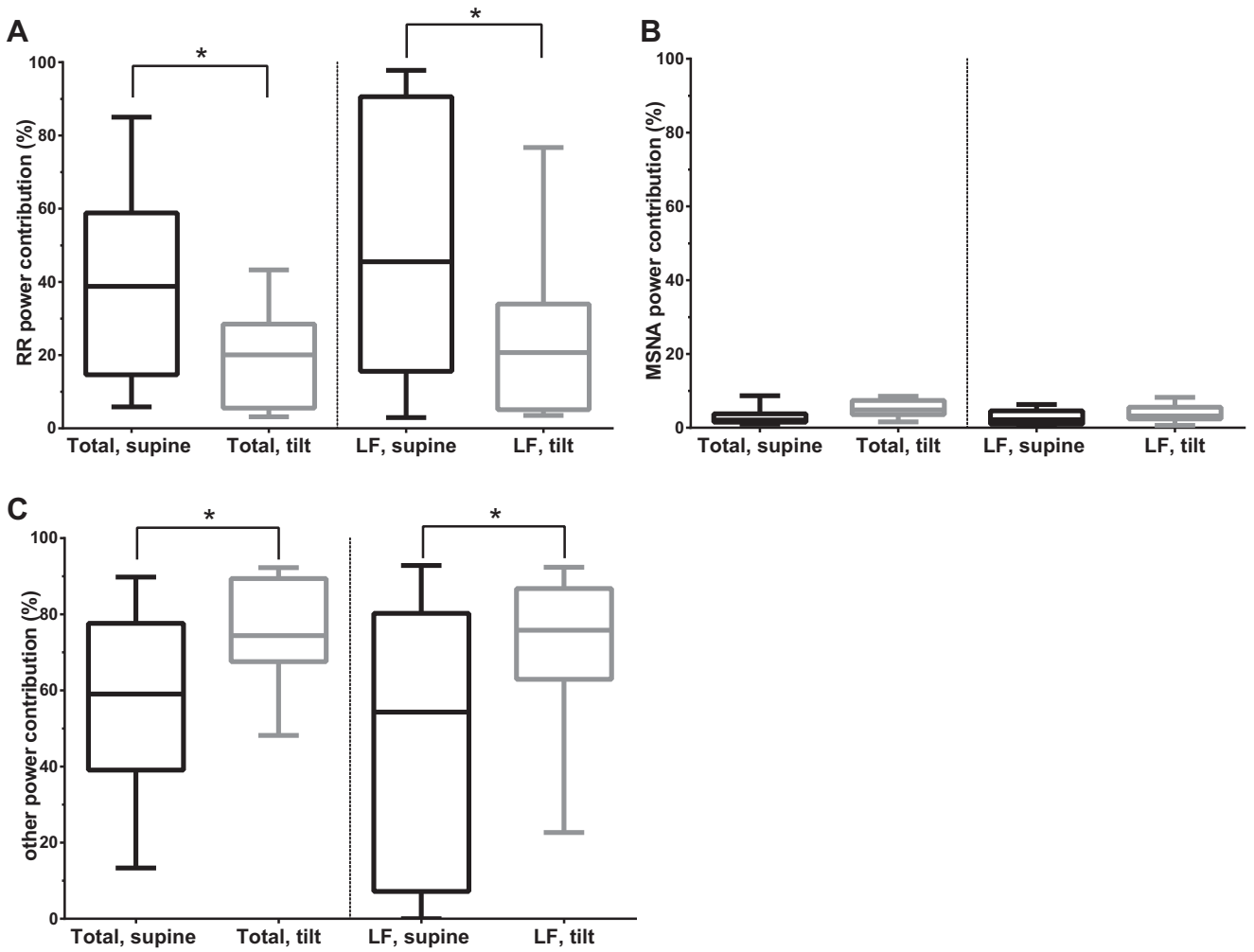

Fig. 2. Power contribution analysis results. *A*: heart period (RR interval) variability (RRV) contribution to QT interval variability (QTV). *Left*: RR contribution to total QT power; *right*: RR contribution to power in the LF band of QTV. *B*: MSNA contribution to QT power. *Left*: MSNA contribution to total QT power; *right*: MSNA contribution to power in the LF band of QT. *C*: QT power independent of RR and MSNA contribution. *Left*: total QT power independent of RR and MSNA. Bar plots represent median values and interquartile ranges. *Significant difference between supine and tilt.

DISCUSSION

The main finding of this study is that the oscillatory modulations in MSNA do not contribute significantly to QTV measured at rest or following moderate orthostatic stress.

The sympathetic nervous system influences the ventricular repolarization process. Within ventricular myocytes, the sympathetic nervous system can principally act on L-type calcium channels and the slowly activating delayed rectifier potassium current (I_{Ks}). The former affects myocardial contractility, while the latter affects the repolarization process. β-Adrenoceptor stimulation during I_{Ks} blockade was shown to increase variability in the cellular repolarization duration of canine myocytes (16). At the tissue level, transmural differences in action potential duration affect the T-wave morphology in body surface ECG (11). This may be altered during periods of sympathetic activation (21). Heterogeneous distribution of β-adrenoceptors, regional arborization of sympathetic nerves (48), and differential cardiac sympathetic control (49) may contribute to spatial dispersion in action potential duration across the ventricles during periods of high sympathetic activity.

Since the result of these effects can be observed on the averaged steady-state QT interval of the surface ECG (7), it has been suggested that the beat-to-beat variability of the QT interval carries information on sympathetic nerve activity. In normal subjects, studies have repeatedly shown increases in QTV in response to (graded) head-up tilt, the seated position, or the standing position (14, 31, 35, 46) suggestive of a sympathetic modulation of QTV. Increased QTV in response to sympathetic activation induced by acute hypoxia provides further evidence for the relationship between sympathetic nervous system activity and QTV in normal subjects (45). Spectral analysis of QTV in normal subjects during interview stress and physical exercise, both of which increased sympathetic activity, demonstrated increased LF oscillations in QTV (27). A subsequent study, in which QTV spectra were estimated during a mental stress test while the atria were paced at a constant rate to exclude heart rate variability-driven QTV, confirmed the increase in LF power during stress and suggests a direct, rate-independent influence of the sympathetic nervous system on QTV (26). Pharmacological β-adrenoceptor activation showed consistently an increase in QTV (25, 42, 45, 46).

Pharmacological β-adrenoceptor blockade, on the other hand, showed no effect on QTV when measured during resting conditions (25, 30) but a reduction in QTV when the effect of RRV was removed through constant atrial pacing (23). Correlations between QTV and cardiac norepinephrine spillover, a direct measure of sympathetic activity, recorded during resting were observed in patients with hypertension (6).

To provide quantitative evidence for the association between QTV and sympathetic activity, we studied the relationship between oscillations in QTV and MSNA, the latter being used as a marker of generalized sympathetic activity, following head-up tilt. Note that RRV was accounted for in the analysis, since its contribution to QTV is well established (34).

Consistent with results reported by others who studied QTV-involving protocols that induce of sympathetic activation, mean heart period, mean QT interval, and heart rate variability decreased significantly, while MSNAv, QTVi, and total QTv increased, after subjects were tilted from the supine position to 40° tilt. The LF power of QTV, measured in units of absolute power, also increased significantly with tilt. However, normalized LF power of MSNA, i.e., the relative amount of LF oscillations in MSNA, did not change with 40° tilt, in contrast to the previously reported increase in sympathetic burst rate (4) and the increase in normalized LF power of MSNA observed by Furlan et al. (13) following 70° head-up tilt. Furthermore, LF power in SAP did not increase following 40° tilt, contrary to findings of Pagani et al. (28), indicating that the orthostatic stimulus was insufficient in eliciting a significant sympathetic vasomotor response. However, since we observed an increase in QTv paralleled by a decrease in RRv (along with a total MSNAv increase), the tilt angle appears sufficient to elicit a sympathetic cardiac response.

Although beat-to-beat changes in the QT interval of normal subjects are believed to be mainly driven by changes in heart period (34), our results show that RRV contributed $<50\%$ to QTV even at rest, when the sympathetic drive is low. Consistent with results reported in the literature, the RRV contribution to both total QTV and LF power of QT decreased with tilt, indicating a decrease in the coupling/correlation between RRV and QTV (33, 38). This suggests that other factors, such as sympathetic activity, play a role in generating QT oscillations during orthostatic stress. Surrogate data testing demonstrated that although the percentage of MSNA power contribution to total and LF power of QT was small, it was not negligible in our data. Yet these contributions did not increase significantly with tilt. In addition, the coherence between LF oscillations in MSNA and QTV was not significant in both supine and tilt conditions, further emphasizing the weak correlation between the two variables.

While we did not find a notable change in the power contribution from MSNA to QTV, others have reported a correlation between LF oscillations in MSNA and RR when blood pressure changes were induced pharmacologically (28, 40), suggesting that MSNA may be used as a surrogate for sympathetic activity directed at the heart. Because the correlation between RR and MSNA is partly mediated by the cardiac baroreflex and sympathovagal outflow to the sinoatrial node, no conclusions may be drawn regarding the level of sympathetic modulation of the ventricles. Furthermore, the correlation between SAP and MSNA is stronger than that reported between RR and MSNA (28), which appears to be confirmed by the lack of change in LF power of both variables in our data.

The QTV power independent of RR and MSNA represented $>50\%$ of the total and LF power of QTV and increased significantly with tilt. While a fraction of this power might be attributed to the increase in measurement noise induced by muscle activity, this seems unlikely to account for $>50\%$ of QTV, especially in the supine position when body movement is at a minimum. Therefore, the possibility remains that other variables, including cardiac sympathetic activity, play a significant role in generating QTV.

Our study may suggest that MSNA oscillations measured in the efferent nerve fiber of the peroneal muscle do not provide a useful surrogate index of oscillations in sympathetic nerve activity directed to the heart, in particular to the ventricular myocardium. Possibly, orthostatic stress might cause an organ-specific sympathetic response, targeting the heart differently from skeletal muscles, as reported during isometric handgrip and mental stress maneuvers (44), in particular when the stimulus is only of moderate strength. In addition, MSNA itself responds inconsistently to different acute stressors (9). However, other measures of MSNA, such as single-unit muscle sympathetic nerve firing rate, have been associated with cardiac norepinephrine spillover in patients with hypertension and major depressive and panic disorders (20). Therefore, LF MSNA oscillations might not be associated with cardiac sympathetic activity, while other measures of MSNA might still be useful in this regard.

Our study has several limitations. In the experimental setup, ECG was recorded using lead III, which is not the optimum choice for capturing the cardiac repolarization process. Cardiovascular variables, as well as MSNA, are inherently nonstationary processes; however, to obtain quasi-stationary data, we removed slow trends and allowed the subjects to rest for 30 min before the actual recording started. A potentially relevant variable that affects QTV is respiration, which was not included in the model in favor of low complexity, yielding robustness in parameter estimation. However, additional modeling carried out with respiration as another exogenous input (data not shown) suggested that QT power independent of RR, MSNA, and respiration still represents almost half of QT power. In line with our observation, a previous study demonstrated a negligible effect of respiration on QTV that did not change with tilt (35).

In conclusion, beat-to-beat QTV in normal subjects does not appear to be significantly affected by the rhythmic modulations in MSNA during rest or moderate orthostatic stress. We suggest that MSNA may not be a useful surrogate for cardiac sympathetic activity if the levels are expected to be low and that sympathetic influences on QTV are complex and not quantifiable with linear shift-invariant autoregressive models.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

F.E.-H., E.A.L., and M.B. developed the concept and designed the research; F.E.-H. analyzed the data; F.E.-H., E.A.L., and M.B. interpreted the results of the experiments; F.E.-H. prepared the figures; F.E.-H. drafted the manuscript; F.E.-H., E.A.L., D.A., and M.B. edited and revised the manuscript; F.E.-H., E.A.L., D.A., and M.B. approved the final version of the manuscript; E.A.L. performed the experiments.

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Supplementary data

Figure S5-1 shows the decomposed QTV power spectrum in the supine position and following tilt. The figure demonstrates that QTV independent of heart rate and respiration (black line) exhibits a LF peak at around 0.1 Hz following tilt (panel B), which is absent in the supine position (panel A). This indicates that QTV independent of heart rate and respiration originates, at least in part, from rhythmical sources. Figure S5-1 is a supplementary figure that has been created for the purpose of this thesis and is not included in the original journal paper which composes Chapter 5.

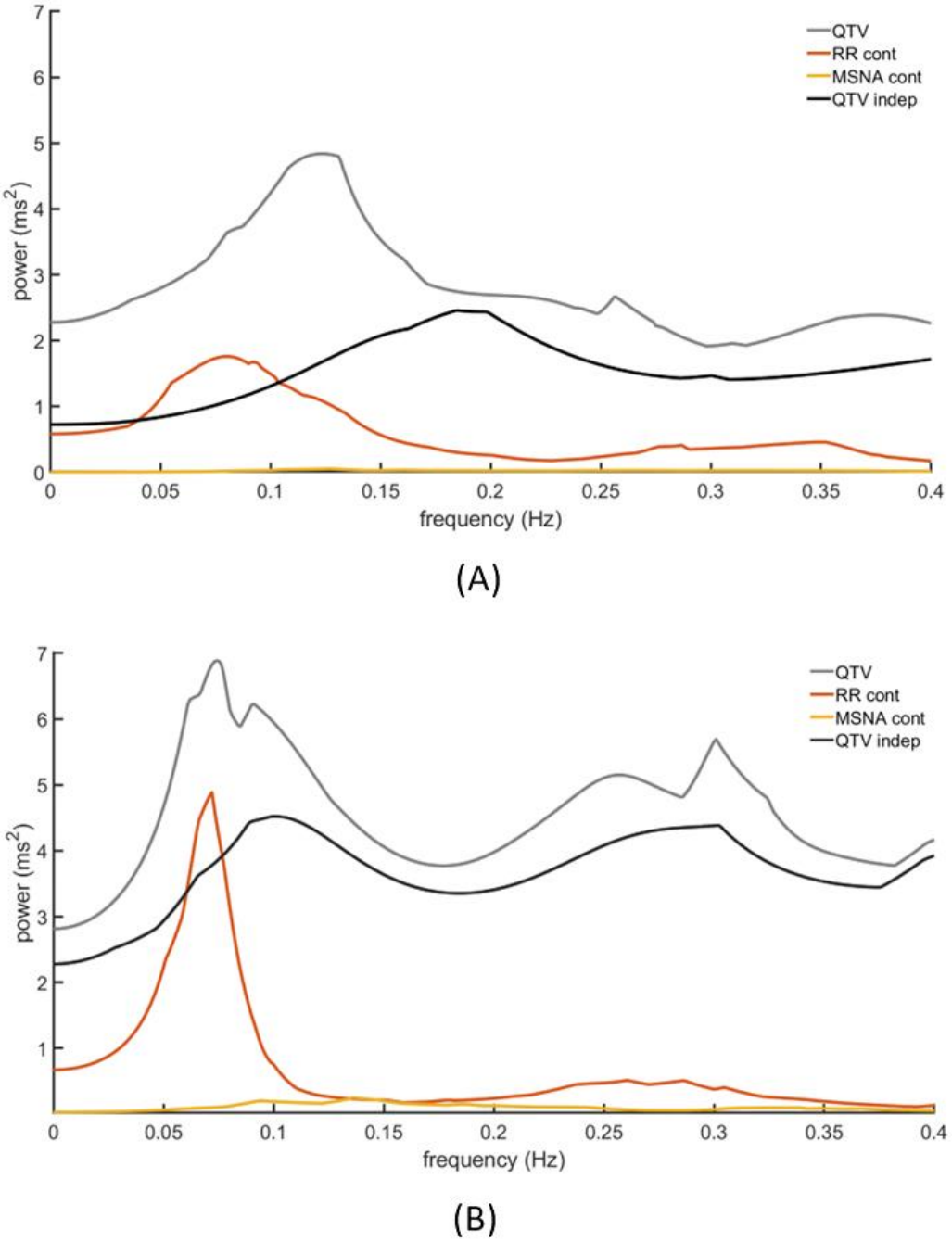

Figure S5-1. Median decomposition of QTV power spectrum (grey) into contributions by RR (orange), MSNA (yellow) and QTV independent (black) in the supine position (A) and following tilt (B). The figure shows a clear LF peak in QTV independent following tilt.

Chapter 6

Augmented oscillations in QT interval duration predict mortality post myocardial infarction independent of heart rate

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By signing the Statement of Authorship, each author certifies that:

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Augmented Oscillations in QT Interval Duration Predict Mortality Post Myocardial Infarction Independent of Heart Rate

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Objective: This study seeks to decompose QT variability (QTV) into physiological sources and assess their role for risk stratification in patients post myocardial infarction (MI). We hypothesize that the magnitude of QTV that cannot be explained by heart rate or respiration carries important prognostic information.

Background: Elevated beat-to-beat QTV is predictive of cardiac mortality, but the underlying mechanisms, and hence its interpretation, remain opaque.

Methods: We decomposed the QTV of 895 patients post MI into contributions by heart rate, respiration, and unexplained sources.

Results: Cox proportional hazard analysis demonstrates that augmented oscillations in QTV and their level of dissociation from heart rate are associated with a higher 5-year mortality rate (18.4% vs. 4.7%, $p < 0.0001$). In patients with left ventricular ejection fraction (LVEF) > 35%, a higher QTV risk score was associated with a significantly higher 5-year mortality rate (16% vs. 4%, $p < 0.0001$). In patients with a GRACE score $\geq$ 120, a higher QTV risk score was associated with a significantly higher 5-year mortality (25% vs. 11%, $p < 0.001$).

Conclusion: Augmented oscillations in QTV and discordance from heart rate, possibly indicative of excessive sympathetic outflow to the ventricular myocardium, predict high risk in patients post MI independent from established risk markers.

Clinical Trial Registration: www.ClinicalTrials.gov, identifier NCT00196274.

Keywords: repolarization variability, risk stratification, sudden death, myocardial infarction, autoregressive model, cardiovascular disease

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INTRODUCTION

Myocardial infarction (MI) is the most common cause of sudden cardiac death (SCD); over 80% of fatal arrhythmias are caused by structural coronary arterial abnormalities and their consequences (Huikuri et al., 2001). Prediction of SCD currently poses a clinical challenge and identification of high-risk patients needs to be considerably improved (Myerburg, 2001).

Autonomic dysfunction and electrical instability are key factors predisposing MI patients to ventricular tachycardia and ventricular fibrillation and consequent SCD (Schwartz et al., 1992; Liew, 2010; Wellens et al., 2014). Indices of ventricular repolarization lability, such as QT variability (QTV), have attracted considerable interest in the area of risk stratification as they allow for a non-invasive investigation of the autonomic nervous system influence on the ventricular myocardium and the lability of the ventricular repolarization process (Myerburg, 2001; Wellens et al., 2014). Measures of QTV (QTVI, SDQT) are increased post MI and their prognostic value has been demonstrated in several studies (Wellens et al., 2014; Baumert et al., 2016a). QTV was increased in six out of the 12-lead ECG recording of patients with recent MI even after covarying for low T wave amplitude (Hasan et al., 2013) and in post MI patients with reduced LVEF compared to those with preserved LVEF and those with uncomplicated coronary heart disease (Sosnowski et al., 2002). QTV was also increased in coronary disease patients with old MI when compared to those without MI (Yao et al., 2019). QTVI has been shown to be predictive of SCD and all-cause mortality in patients with LVEF between 35% and 40% (Piccirillo et al., 2007). In 24-h Holter recordings of patients with acute MI, QT/RR variability ratio was found to be predictive of all-cause mortality independent of other established risk markers (Jensen et al., 2005).

Beat-to-beat fluctuations in the QT interval (representing the variability of the repolarization process) are the result of a complex process that involves multiple physiological mechanisms. The rate adaptation of the QT interval constitutes a large fraction of QTV in the normal heart (El-Hamad et al., 2015; Baumert et al., 2016b). Respiration also influences QTV indirectly through respiratory sinus arrhythmia (Sutherland et al., 1983; Baumert et al., 2016b). Furthermore, ventricular repolarization is directly influenced by the autonomic nervous system (Merri et al., 1993) via the dense innervation of sympathetic nerves into the ventricular myocardium, contributing to rate-independent QTV (Hall, 2011). Hence, in the compromised heart, excessive sympathetic outflow might initiate lethal ventricular arrhythmias (Ng, 2016).

In the context of MI, few studies report on the rate-independent component of repolarization variability. Zhu et al. (2008) found that repolarization variability independent of heart rate was increased in MI patients compared to age-matched healthy subjects, while the rate-dependent component was not different between the two groups. Others found no difference in the percentage of RR-dependent RT interval variability between MI patients and age-matched healthy subjects (Lombardi et al., 1998). Comparing MI patients with reduced LVEF to those with preserved LVEF and to another group with coronary artery disease but no MI, Sosnowski et al. (2002) reported an increase in overall RT variability and specifically an increase in its HF power, suggesting an important role for respiration. They also observed an uncorrelation between RR and RT interval variability in MI patients with depressed LVEF compared to the other two groups.

There are only few reports on QTV's individual components in patients post MI, and none on their individual role in risk stratification post MI. In this study, we hypothesize that the component of QTV that cannot be explained by heart rate or respiration, possibly reflecting sympathetic influences on the ventricles, carries important prognostic information. To test this hypothesis, we decomposed QTV into three components (hart rate dependent, respiratory dependent, and independent component) and assessed their individual roles in risk stratification in patients post MI.

MATERIALS AND METHODS

Study Cohort

We analyzed high-resolution ECG recorded previously for cardiac risk stratification post MI (Barthel et al., 2013). A total of 941 MI survivors were enrolled between March 2000 and May 2005. Acute MI was diagnosed based on at least two of the following findings: typical chest pain lasting $\geq$ 20 min, creatine kinase above twice the upper normal limit of the respective laboratory, and admission ST-segment elevation $\geq$ 0.1 mV in at least two contiguous limb leads or $\geq$ 0.2 mV in at least two contiguous precordial leads (Bernard et al., 1979). Eligible patients had survived acute MI less than four weeks before recruitment, they were aged 80 years or less, had sinus rhythm, and did not meet the criteria for secondary prophylactic implantation of implantable cardioverter defibrillator before hospital discharge. A total of 32 patients did not pass the initial screening due to atrial fibrillation (Sinnecker et al., 2015).

The main outcome measure was total mortality during a follow-up period of 5 years (once every 6 months), where the last follow-up was performed in May 2010. The study was conducted at the hospital of the Technische Universität München, the German Heart Centre, and the Klinikum Rechts der Isar, both in Munich, Germany. The study protocol was approved by the local ethics committee, and written consent from patients was obtained.

Measurements

ECG (Porti System, TMS, Netherlands) and thoracic respiratory signals (piezoelectric thoracic sensor; Pro-Tech, United States) were recorded at 1.6 kHz for 30 min in each patient within 2 weeks of index MI. Patients were studied in the morning in the supine position without interruption of their normal medication regimen. The GRACE score, including the age of the patient, a history of past heart failure, a history of past MI, serum creatinine at admission, the cardiac biomarker status at admission, systolic blood pressure at admission, the pulse at admission, ST-deviation at admission, and in-hospital percutaneous coronary intervention, was selected for predicting the long-term prognosis (Eagle et al., 2004). Details of other recorded signals can be found elsewhere (Barthel et al., 2013).

Data Pre-processing

All ECG signals were visually inspected for signal quality; recordings with poorly defined T wave were excluded from the analysis based on subjective assessment. Beat-to-beat heart period (RR) and beat-to-beat Q peak to T end interval (QT) were obtained from all ECGs. We used an automated template-based

algorithm that tracks QT changes beat by beat with high accuracy and robust to noise (Schmidt et al., 2014). The QT interval was automatically determined on the template beat using a slope method and manually adjusted if necessary.

The thoracic respiratory signal was sampled at the occurrence of the R-peak in the ECG signal to obtain beat-to-beat values of respiration. All beat-to-beat series (RR, QT, respiration) were visually inspected to select stationary segments of around 350 consecutive beats. All selected RR segments were then visually validated against the ECG to identify QRS detection errors and irregular heart rhythms. Recordings with RR segments consisting of more than 10% ventricular or supraventricular ectopic beats (EBs) were excluded from the analysis, while ectopic beats were mathematically interpolated in recordings with less than 10% EBs using the spline interpolation method. QT, RR and respiration time series were detrended using the smoothness priors method (Tarvainen et al., 2002) comprising a time-varying finite-impulse response high-pass filter with a corner frequency of 0.04Hz. The detrended data segments were normalized to zero mean and unit variance for power spectral analysis.

Model-Based QTV Analysis

Power contribution analysis was performed to decompose QTV into heart period dependent, respiratory dependent and unexplained contributions.

We employed a linear autoregressive model with two external inputs (ARXX) to decompose QT variability (Baselli et al., 1997). We chose an open-loop structure, where the rhythm sources generating the three signals are assumed uncorrelated, which allows the decomposition of QTV power into contributions by the different inputs. Thus, the autoregressive model was defined as:

$$A_1(z)\ QT(i) = B_1(z)\ RR(i) + B_2(z)\ Resp(i) + e_{QT}(i) \quad (1)$$

Equation (1) describes QTV as a function of its own past, past and present values of heart period (RR) and respiration (Resp) and a noise source that represents actual noise and rhythms originating from sources not accounted for in the model. Heart period was modeled as autoregressive model with respiration as an external input, while respiration was modeled as a separate autoregressive process. Details of the mathematical models and their parameters are described elsewhere (Baselli et al., 1997; El-Hamad et al., 2015).

The Akaike information criterion (Akaike, 1974) was used to select the model order of the multivariate autoregressive process from the range 6 to 12. Model parameters were estimated using the least squares method. Resulting models were validated by assessing the correlation between model residuals and whiteness of model noise sources (Porta et al., 1998; El-Hamad et al., 2015).

The following variables were computed:

- QT_{mean}–average QT interval (in ms).
- QT_c–rate-corrected average QT interval, using Bazett's formula (in ms).
- QTV_{total}–variance of beat-to-beat QT intervals (in ms^2).
- $QTV_{respiration}$–variance of beat-to-beat QT intervals related to respiration (in %).
- QTV_{RR}–variance of beat-to-beat QT intervals related to heart period (in %).
- $QTV_{unexplained}$–variance of beat-to-beat QT intervals independent from heart period and respiration (in %).

Statistics

Univariate Cox regression analysis was performed on each demographic variable to assess its predictive value for mortality; expressed as hazard ratios (HR) with 95% confidence intervals (CI). To test whether the characteristics of patients who were excluded from analysis were significantly different from those in the final study cohort, we compared both groups using the two-sample Wilcoxon test and the chi-squared test for continuous and categorical variables, respectively.

The predictive value of the different QT variability measures was assessed using univariate Cox regression analysis. The significant predictors resulting from the univariate analysis were then included in a multivariable Cox regression analysis with the stepAIC method (Hothorn and Lausen, 2003). The linear predictor model resulting from the multivariate cox regression analysis is termed 'QTV risk score' which includes QTV measures which were found to contribute independently to risk prediction. The optimal cut-off for the QTV risk score was calculated by the Maximally Selected Rank Statistics (Hothorn and Lausen, 2003; Lausen et al., 2004). We chose the maximum of the log-rank statistics with at least 90% of the observations in group 1 and no more than 90% of the observations in group 1 as constraints. To assess the additive predictive value of the QTV risk score beyond established clinical risk markers, we performed multivariable Cox regression analysis using the QTV risk score as well as a model consisting of the following clinical predictor variables: GRACE score, LVEF, presence of diabetes, chronic obstructive pulmonary disease and expiration-triggered respiratory sinus arrhythmia. Kaplan–Meier survival curves were computed for the QTV risk score and for subgroup analysis based on LVEF $\leq$ 35% and GRACE $\geq$ 120 points. A p-value < 0.05 was considered significant. All statistics were performed with R (R Core Team, 2014).

RESULTS

Data Pre-processing Results

Out of 941 recordings, we excluded 20 from the analysis due to poor signal quality. After manually selecting stationary segments of QT, RR and respiration, another 26 recordings were excluded due to the high percentage of irregular heartbeats (more than 10% ventricular or supraventricular ectopic beats; or bigeminus rhythm). Of the 895 patients included in the analysis, 62 died during the follow-up period. **Table 1** shows the clinical characteristics and demographics for subjects included in the final analysis compared to those excluded from the analysis. Mortality was higher in the group of excluded patients (10 deaths). On average, those patients were significantly older, had a higher GRACE score and had a lower estimated glomerular filtration rate (**Table 1**).

TABLE 1 | Patient demographics and hazard ratios of the entire study population as well as the comparison of characteristics of included versus excluded patients.

Variables	Study population (*n* = 941)	Hazard Ratio* (99% CI)	*p*-value	Included patients (*n* = 895)	Excluded patients (*n* = 46)	Included versus excluded (*p*-value)
Age (years), median (IQR)	60.9 (51.6–68.8)	1.09 (1.06–1.12)	<0.001	60.7 (51.5–68.6)	66.6 (58.9–73.1)	0.004
Females, *n* (%)	182 (19.3)	1.22 (0.67–2.21)	0.52	173 (19.3)	9 (19.6)	1.00
Diabetes mellitus, *n* (%)	184 (19.6)	2.72 (1.63–4.53)	<0.001	176 (19.7)	8 (17.4)	0.85
History of previous MI, *n* (%)	90 (9.6)	3.34 (1.87–5.97)	<0.001	84 (9.4)	6 (13)	0.57
Hypertension, *n* (%)	682 (72.5)	1.58 (0.84–2.96)	0.16	652 (72.8)	30 (65.2)	0.34
Smoking, *n* (%)	488 (51.9)	0.88 (0.54–1.45)	0.62	461 (51.5)	27 (58.7)	0.42
COPD, *n* (%)	39 (4.1)	3.85 (1.83–8.08)	<0.001	37 (4.1)	2 (4.3)	1
CK max (U/l), median (IQR)	1302 (646–2460)	1 (1–1)	0.89	1316 (648–2475)	1106 (599–2040)	0.64
LVEF (%), median (IQR)	53 (45–60)	0.95 (0.94–0.97)	<0.001	53 (45–60)	51.5 (38–58.75)	0.23
MI localization						
Anterior, *n* (%)	391 (41.5)	1.08 (0.65–1.78)	0.77	375 (41.9)	16 (34.8)	0.42
Posterior, *n* (%)	435 (46.2)	0.79 (0.47–1.31)	0.36	409 (45.7)	26 (56.5)	0.2
Lateral, *n* (%)	102 (10.8)	1.62 (0.83–3.18)	0.17	98 (10.9)	4 (8.7)	0.81
Unclassified, *n* (%)	12 (1.3)	0 (0–Inf)	0.996	12 (1.3)	0 (0 0)	0.91
BMI (kg/m^2), median (IQR)	26.6 (24.5–29.1)	1.02 (0.96–1.09)	0.52	26.6 (24.5–29.0)	25.1 (24.1–28.7)	0.26
Serum creatinine (mg/dL), median (IQR)	1.1 (0.9–1.3)	1.77 (1.46–2.15)	<0.001	1.1 (0.9–1.3)	1.1 (1–1.3)	0.29
Cardiogenic shock/CPR, *n* (%)	41 (4.4)	0.81 (0.2–3.32)	0.77	36 (4)	5 (10.9)	0.07
Intervention						
PCI, *n* (%)	878 (93.3)	0.65 (0.28–1.51)	0.31	835 (93.3)	43 (93.5)	1
Thrombolysis, *n* (%)	14 (1.5)	0 (0–Inf)	0.996	14 (1.6)	0 (0 0)	0.82
CABG, *n* (%)	6 (0.6)	3.36 (0.47–24.22)	0.23	5 (0.6)	1 (2.2)	0.7
No revascularization possible, *n* (%)	43 (4.6)	1.91 (0.77–4.77)	0.16	41 (4.6)	2 (4.3)	1
Aspirin, *n* (%)	913 (97)	0.56 (0.18–1.78)	0.33	869 (97.1)	44 (95.7)	0.91
Clopidogrel, *n* (%)	920 (97.8)	0.4 (0.13–1.29)	0.13	876 (97.9)	44 (95.7)	0.63
Beta-blockers, *n* (%)	897 (95.3)	0.67 (0.24–1.84)	0.43	854 (95.4)	43 (93.5)	0.8
ACE-inhibitors, *n* (%)	885 (94)	0.56 (0.24–1.31)	0.18	842 (94.1)	43 (93.5)	1
Statins, *n* (%)	879 (93.4)	0.52 (0.24–1.14)	0.1	837 (93.5)	42 (91.3)	0.78
Diuretics, *n* (%)	415 (44.1)	2.11 (1.26–3.51)	0.004	388 (43.4)	27 (58.7)	0.06
GRACE score	110.2 (93.4–25.8)	1.04 (1.03–1.05)	<0.001	109.3 (92.5–125.4)	121.7 (109.9–136.0)	0.001
eGFR Counahan Barratt	71.9 (62.4–82.7)	0.97 (0.95–0.98)	<0.001	72.2 (62.4–83.1)	67.6 (54.7–75.4)	0.02

*ACE, angiotensin-converting enzyme; BMI, body mass index; CABG, coronary artery bypass graft; CK, serum creatine kinase; COPD, chronic obstructive pulmonary disease; CPR, cardiopulmonary resuscitation; IQR, inter-quartile range; LVEF, left ventricular ejection fraction; MI, myocardial infarction; PCI, percutaneous coronary intervention. *Hazard ratio refers to risk of total mortality.*

Model-Based QTV Analysis Results

The average QT interval and rate-corrected QT interval were 425.7 ± 44.8 ms and 432.6 ± 39.7 ms, respectively. The variance of beat-to-beat fluctuations in QT interval was 10.4 ± 24.7 ms^2 on average.

Figure 1 shows the relative contribution of RR and respiration to total QTV in survivors, and non-survivors averaged across all patients. While the absolute value of the RR and respiration contribution is higher for non-survivors due to the overall increase in QT power in that group, the relative contribution of RR and respiration to QTV is decreased in non-survivors as demonstrated by the pie charts. The QT power independent of RR and respiration is increased and exhibits a clear peak in the low-frequency band.

Figure 2 shows the Kaplan–Meier curves for QTV and its individual components. An increase in QTV and QTV independent of RR and respiration was associated with increased risk of mortality (QTV:16.8% vs. 5.5%, $p < 0.001$; QTV independent:15.2% vs. 5.5%, $p < 0.001$). On the other hand, the 5-year mortality rate was higher for patients with reduced RR and respiratory contributions to QTV (13.5% vs. 4.8%, $p < 0.001$; 11% vs. 5.7%, $p = 0.003$, respectively).

Cox Regression Analysis

In univariate Cox regression analysis (**Table 2**), all QT variables were significantly associated with mortality except for $QTV_{respiration}$. Multivariable stepwise Cox regression analysis of QT variables identified the increase in QTV_{total} and $QTV_{unexplained}$ as significant predictors of mortality in addition to QT_c prolongation (**Table 2**). A linear predictor score (termed QTV risk score) was calculated from a linear combination of these significant predictors.

Analysis of deviance demonstrated the added value of QTV_{total} and $QTV_{unexplained}$ to the model when compared to a model with QT_c alone ($\chi^2 = 12.2$, $p = 0.002$). Using stepwise multivariable cox regression, we explored whether the

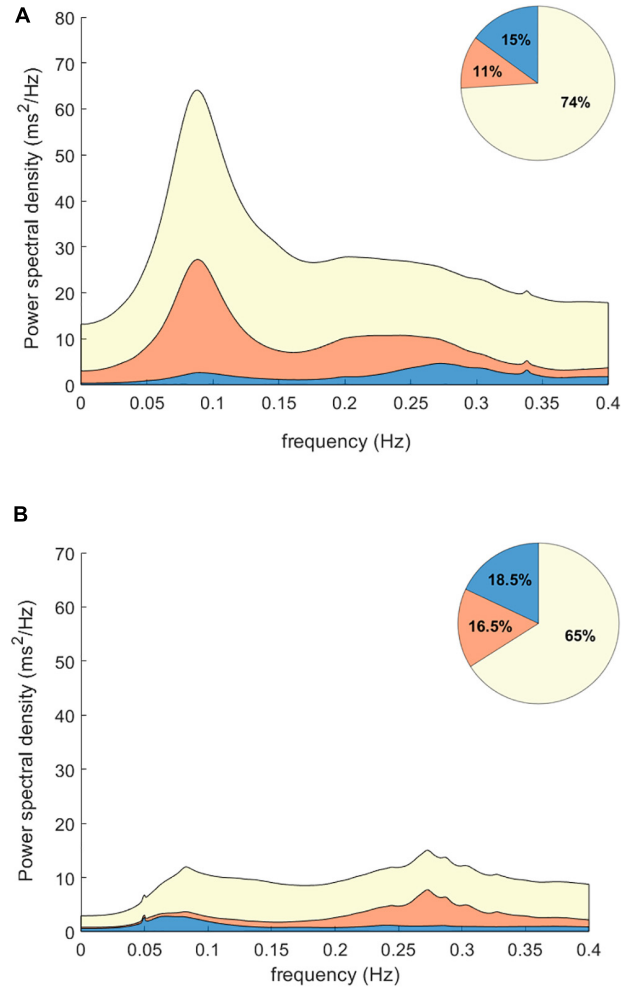

FIGURE 1 | Power contributions to QT interval variability. Plots show the spectrograms averaged for non-survivors **(A)** and survivors **(B)** post MI. The relative contribution of RR interval variability is shown in blue; the contribution of respiration is shown in orange. While the absolute value of the RR (blue) and respiration (orange) contributions are higher for non-survivors due to the overall increase in QTV in that group, the relative contribution of RR and respiration to QT power is decreased in non-survivors as shown in the pie charts. Notably, the QT power independent of RR and respiration (yellow) is increased and exhibits a clear peak in the low-frequency band.

QTV risk score adds predictive value to a model consisting of the following clinical predictor variables (model details can be found in **Table 3**): GRACE score, LVEF, presence of diabetes, chronic obstructive pulmonary disease and expiration-triggered respiratory sinus arrhythmia. Here, the QTV risk score added significant predictive value with an HR = 1.73 (1.23–2.51), $p = 0.002$ (**Table 4**).

Kaplan–Meier curves of the dichotomized QTV risk score are shown in **Figure 3**. The 5-year mortality rate for patients with a high QTV risk score was significantly higher compared to those with a lower QTV risk score (18.4% vs. 4.7%, $p = 2.92 \times 10^{-9}$).

The cohort was divided into subgroups based on LVEF. In the group with LVEF > 35%, the 5-year mortality risk was significantly higher for patients who had a high QTV risk score (16% vs. 4%, $p = 1.19 \times 10^{-7}$). The QTV risk score was also marginally predictive of mortality in patients with LVEF ≤ 35% (**Figure 4**).

In a subgroup of patients with GRACE score ≥ 120, patients with a higher QTV risk score had a significantly higher 5-year mortality risk compared to those with lower QTV risk score in the same group (25% vs. 11%, $p = 0.0009$) (**Figure 5**).

DISCUSSION

Our study demonstrates that heart rate-independent mechanisms play a dominant role in creating excessive QTV observed in MI patients with increased mortality risk. The novel QTV risk score yields improved risk stratification within a subgroup of MI patients who already display traditional risk markers (GRACE score ≥ 120). It can also identify vulnerable patients in patient groups with normal to moderate LVEF (LVEF > 35%).

Repolarization lability can trigger malignant arrhythmias and subsequent SCD in patients post MI (Liew, 2010). QTV was found to be elevated during ischemic episodes compared to non-ischemic episodes (Murabayashi et al., 2002). We found that QTV is elevated in non-surviving MI patients when compared to those who survived.

Previously, simple QTV metrics were combined with conventional risk markers to identify MI patients at high risk of mortality (Jensen et al., 2005; Piccirillo et al., 2007). Here, we sought to decompose QTV into its physiological sources and assessed their individual role in risk stratification. We found that the relative contribution of RR interval variability to QTV was reduced in non-survivors, indicating attenuated rate-adaptation of the QT interval. This attenuation of the QT-RR relationship has also been found in MI patients with reduced LVEF when compared to those with coronary artery disease but no MI (Sosnowski et al., 2002). Others found no difference in the RR dependent component of QTV when comparing MI patients to healthy subjects (Lombardi et al., 1998; Zhu et al., 2008). These aggregate results suggest that the attenuation in QT-RR relationship is only prevalent in high-risk patients rather than in all patients post MI. Possibly, the attenuation is linked to increased sympathetic activation (Zaza et al., 1991; Porta et al., 2010; El-Hamad et al., 2015). Holter recordings showed substantial predictive value of attenuated rate-adaptation of the QT interval for SCD in MI patients (Hintze et al., 2002; Chevalier et al., 2003).

In our study, the respiratory contribution to QTV did not play a significant role in predicting patients at risk in the univariate model (**Table 2**). Hence, we considered a simplified model without respiration. It's predictive performance was comparable to the original model (data not shown), suggesting that recording of respiration is not necessary. Contrary to our findings, others have reported an increase in high frequency of RT interval variability in patients with reduced LVEF compared to those with

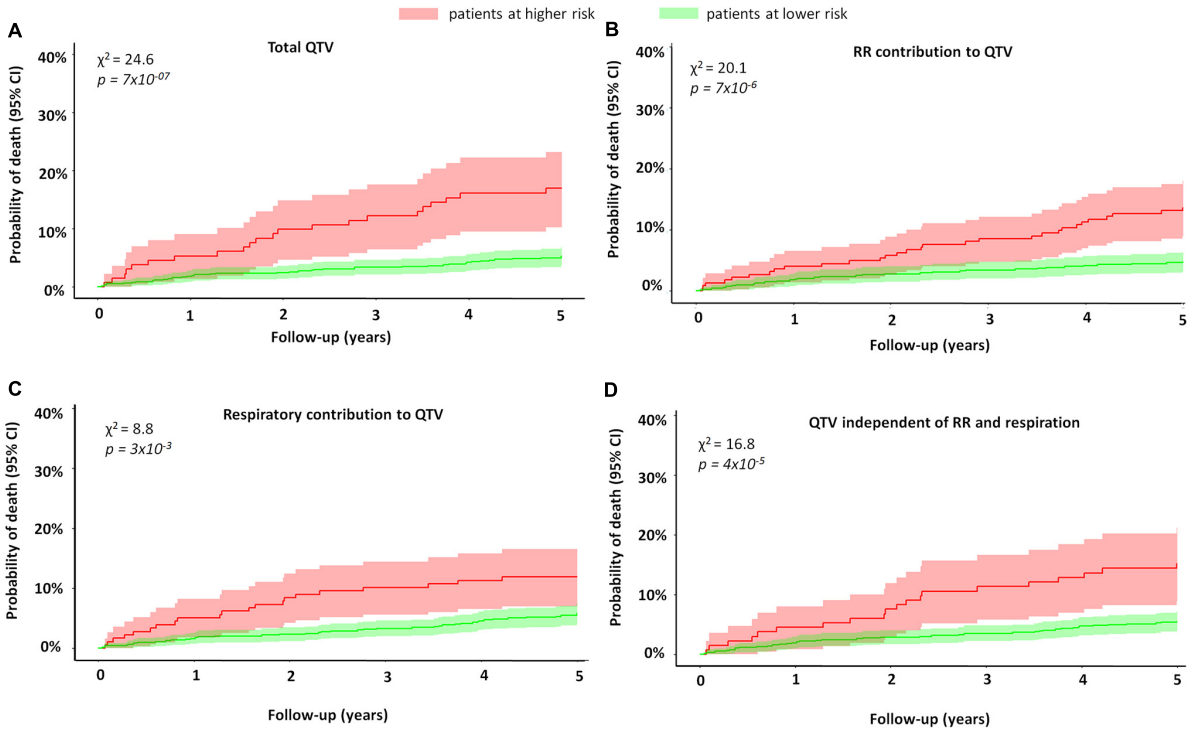

FIGURE 2 | Mortality of MI patients stratified by QTV and its components. The figure shows the Kaplan–Meier curves of MI patients stratified by **(A)** total QT variability, **(B)** percentage of RR contribution to QTV, **(C)** percentage of respiratory contribution to QTV, and **(D)** percentage of QTV independent of RR and respiration. The red curve represents patients at higher risk, while the green curve represents those with lower risk. Patients with higher QTV had a higher risk of mortality compared to those with lower QTV (16.8% vs. 5.5%, $p < 0.001$). Patients with higher QTV independent of RR and respiration also had a higher 5-year mortality rate (15.2% vs. 5.5%, $p < 0.001$). Finally, the 5-year mortality rate was higher for patients with reduced RR and respiratory contributions to QTV (13.5% vs. 4.8%; 11% vs. 5.7%, respectively). The figure demonstrates the univariate predictive value of QTV and its components is risk stratification.

preserved LVEF or with coronary artery disease but no MI (Sosnowski et al., 2002).

The portion of QTV that cannot be explained by rate-adaptation or respiration-related mechanisms was increased in patients who died during the follow-up period (**Table 2**). In healthy subjects, the unexplained fraction of QTV has been previously shown to correlate with an increase in sympathetic tone during tilt (Porta et al., 2010; El-Hamad et al., 2015) and is augmented in post-MI patients compared to healthy subjects (Zhu et al., 2008).

The pathophysiological mechanisms that increase overall QTV and QTV independent of heart period and respiration and contribute to mortality risk are not fully understood. By design, our study does not facilitate the direct interpretation of the QTV component independent of heart rate and respiration. However, the spectral component of QTV that is independent of RR and respiration exhibits a clear peak in the low-frequency (LF; 0.04-0.15 Hz) region that is elevated in the non-survivors, as shown in **Figure 1**. It is well-known that sympathetic nerve activity oscillates at the LF rhythm, creating Traube–Hering–Mayer waves in arterial blood pressure that coincide with oscillations in RR variability. Sympathetic nerve activity modulates the relationship between

TABLE 2 | Univariate and multivariable Cox regression analysis of QT variables.

Variable	Univariate		Multivariable (All variables in)	
	Hazard ratio (95%)	*p*-value	Hazard ratio (95%)	*p*-value
QT_c (ms)	1.01 (1.01–1.02)	<0.001	1.010 (1.01–1.03)	0.001
QTV_{total} (ms^2)	1.01 (1.006–1.014)	<0.001	1.005 (1.00–1.01)	0.08
$QTV_{respiration}$ (%)	0.99 (0.97–1.005)	0.168	-	-
QTV_{RR} (%)	0.96 (0.94–0.99)	0.004	-	-
$QTV_{unexplained}$ (%)	1.03 (1.01–1.045)	0.001	1.024 (1.01–1.04)	0.004

TABLE 3 | Multivariable Cox regression analysis of clinical risk markers.

Variable	Hazard ratio (±SE)	*p*-value
GRACE score	1.04 (±0.006)	<0.001
ETA	0.79 (±0.04)	<0.001
LVEF	0.97 (±0.01)	0.007
Diabetes	1.72 (±0.27)	0.047
COPD	2.19 (±0.39)	0.044

GRACE, global registry of acute coronary event; ETA, expiration triggered arrhythmia; LVEE, left ventricular ejection fraction; COPD, chronic obstructive pulmonary disease.

TABLE 4 | Hazard ratios and 95% confidence intervals of the clinical and QTV models in the multivariable cox regression analysis.

Variables	Hazard ratio[a] (95% CI)	*p*-value
Clinical model[b]	2.45(2.2–3.3)	<0.001
QTV risk score[c]	1.73 (1.23–2.51)	0.002

[a] *Hazard ratio refers to the risk of total mortality.*
[b] *Model details in* ***Table 3****.*
[c] *Model details in* ***Table 2****.*

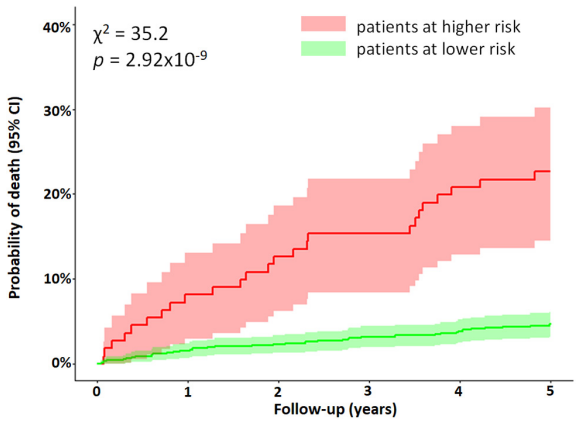

FIGURE 3 | Mortality of MI patients stratified by the QTV risk score. Kaplan–Meier curves were obtained from the dichotomized multivariable Cox regression model. The red curve represents patients above the cut off value (0.626), while the green curve represents all other patients. Difference between the two curves was tested using the log-rank test ($\chi^2 = 35.2$, $p = 2.92 \times 10^{-9}$). The figure demonstrates the predictive value of an increase in QTV risk score for identifying patients with a higher risk of mortality post MI.

repolarization and cycle length in the LF band (Zaza et al., 1991). It also contributes directly to the magnitude of QTV by affecting L-type channel Ca^{2+} current and the slow delayed rectifier potassium current in ventricular myocytes. Nerve sprouting (Zhou et al., 2004) and sympathetic hyperinnervation following acute MI can create electrical instability, where augmented sympathetic activity can lead to malignant ventricular arrhythmias (Cao et al., 2000). Other studies suggest that systemic inflammation post-MI could contribute at least in part to the increase in QTV (Tseng et al., 2012). Since the severity and prognosis of myocardial infarction are highly dependent on the size and location of the infarcted tissue, affecting the extent of spatial repolarization heterogeneity (Hiromoto et al., 2006), QTV independent of heart rate and respiration may also be affected by the characteristics of the damaged substrate.

Markers of an abnormal substrate or structural heart disease are some of the most common risk factors for SCD, and reduced LVEF is currently considered the most important marker for risk stratification (Wellens et al., 2014). Current international guidelines for implantable cardioverter defibrillator implantation for primary prevention are predominantly based on LVEF (O'Gara et al., 2013; Priori et al., 2015). However, the majority of SCDs occur in patients with preserved LVEF (LVEF > 30%) (Buxton et al., 2007; Wellens et al., 2014). Therefore, the identification of high-risk patients in this cohort is critical.

Several markers of electrical instability and autonomic tone, such as heart rate variability, heart rate turbulence, T wave alternans, and QTV index, have been associated with increased risk of SCD in post MI patients with preserved LVEF (Piccirillo et al., 2007; Gatzoulis et al., 2019). In this group of patients, we found that a higher QTV risk score is associated with a four times higher risk of mortality compared to those with lower values of QTV risk score. Gatzoulis et al. (2019) reported that patients with preserved LVEF who display at least one ECG-derived non-invasive risk factor were at higher risk of SCD. As QTV reflects

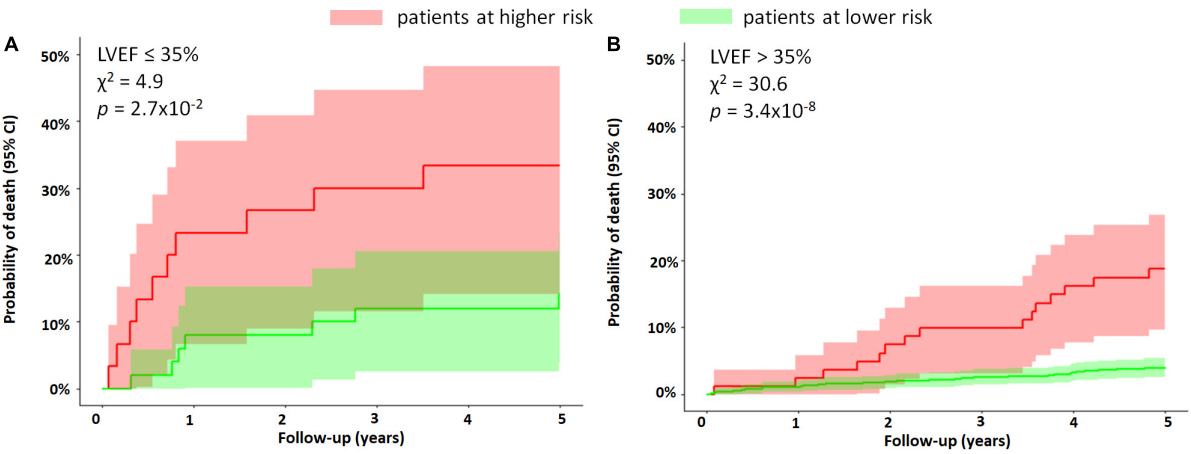

FIGURE 4 | Subgroup analysis based on LVEF. The figure shows the Kaplan–Meier curves of MI patients stratified by the QTV risk score for subgroups with **(A)** LVEF ≤ 35% and **(B)** LVEF > 35%. The 5-year mortality rate was significantly higher in patients with LVEF > 35% who had a high QTV risk score (16% vs. 4%, $p = 1.19 \times 10^{-7}$). The QTV risk score was only marginally predictive of mortality in patients with LVEF ≤ 35%. The figure demonstrates the ability of the QTV risk score to further stratify a subgroup at high risk in the group with LVEF > 35%.

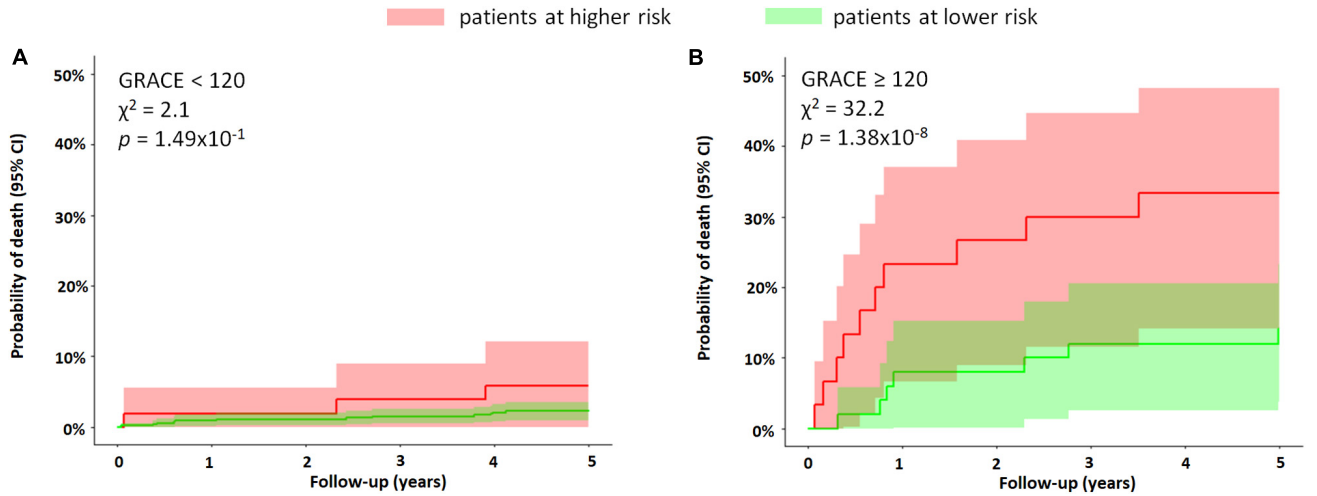

FIGURE 5 | Subgroup analysis based on GRACE score. The figure shows the Kaplan–Meier curves of MI patients stratified by the QTV risk score for subgroups with **(A)** GRACE score < 120 and **(B)** GRACE score ≥ 120. The QTV risk score was significantly predictive of mortality only in patients with a score ≥ 120, where the 5-year mortality rate was 25% versus 11%, p = 0.0009. The figure demonstrates the ability of the QTV risk score to further stratify a subgroup with a higher risk in the high-risk cohort (GRACE ≥ 120).

lability of the ventricular repolarization process, a higher QTV risk score could identify patients more susceptible to ventricular arrhythmia.

The QTV risk score was only marginally predictive in the group with reduced LVEF. This could be explained by the fact that patients with reduced LVEF have a higher competing risk of death from heart failure than other modes of death when compared to patients with preserved LVEF (Hall et al., 2018). The GRACE score is a hospital discharge risk score shown to be predictive of mortality in patients with acute coronary syndrome. It combines multiple prognostic factors such as age, history of heart failure and MI, and heart rate (Tang et al., 2007). Patients with a GRACE score ≥ 120 are considered to be at high risk. In our study, the QTV risk score was able to identify a subgroup of patients at higher risk within the subgroup of high-risk patients identified by a GRACE score ≥ 120.

Our study has several limitations. Current guidelines recommend using 12-lead ECG screening for specific high-risk patients (Priori et al., 2015); we used single-lead ECG in favor of lower complexity and cost. By choosing the lead with the largest T wave and the lowest noise, we obtained a reasonably good approximation of the global repolarization duration. However, potentially valuable information on the repolarization dispersion across leads could not be assessed. Although we used high-resolution ECG, conventional ECG sampled at > 300 Hz is sufficient for QTV analysis (Baumert et al., 2016a). Patients were studied at rest, and while autonomous nervous system provocation tests tend to increase the predictive power of ECG markers, their practical use in patients is limited due to the complexity of tests (Wellens et al., 2014). Stationary segments of heart period, QT interval and respiratory time series were present in all recordings as judged by visual assessment. Fully automated methods should be considered in the future for a more rigorous assessment. The 5-year mortality rate in our cohort was low, and hence, the identified groups with increased mortality may be small. This may have impacted our analysis by possibly reducing its statistical power and broadening the confidence intervals of the Kaplan–Meier curves, as seen in **Figures 3–5**. Accurate identification of specific patients at risk based on QTV alone might be challenging. Patients who were not in sinus rhythm were excluded from the analysis, which introduced a mortality bias. We did not systematically measure serum creatine kinase-muscle/brain and troponin levels. Since the study enrollment, improved treatments for MI have become clinically available (e.g., improved stents and antithrombotic drugs) that may have affected our findings. Our follow-up protocol did not include data collection on change in patient characteristics and therapy adherence. The primary study endpoint was all-cause mortality. Future studies should validate our findings on data from a different cohort stratifying for cardiovascular mortality, in particular, SCD.

CONCLUSION

In conclusion, the QTV risk score might help stratify high-risk patients that already display traditional risk markers and identify patients at higher risk of mortality, albeit their preserved LVEF. The implementation of risk stratification strategies to predict risk in patients with preserved LVEF requires the use of a combination of several risk markers. New stratification strategies which can be translated into daily clinical practice are necessary to stratify patients with moderate and normal LVEF yet are at higher risk of SCD. The QTV risk score is a non-invasive risk marker that can be easily incorporated into daily clinical practice. However, the prognostic value of the QTV risk score needs to be prospectively validated on other cohorts, and further investigation into its utility for specifically predicting SCD is required.

DATA AVAILABILITY STATEMENT

The data presented in this article are not readily available. Data access requests should be emailed to author Georg Schmidt directly.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the ethics committees at the hospital of the Technische Universität München, the German Heart Centre, and the Klinikum Rechts der Isar, both in Munich, Germany. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

FE-H was responsible for the conception, modeling, analysis, and interpretation of data; for drafting of the manuscript and revising it critically for important intellectual content; and for the final approval of the manuscript submitted. SB was responsible for the processing and analysis of data; for revising the manuscript critically for important intellectual content; and for the final approval of the manuscript submitted. AM was responsible for the analysis and interpretation of data; for drafting of the manuscript; and for the final approval of the manuscript submitted. AS was responsible for the conception, design, and analysis of the data; for revising the manuscript critically for important intellectual content; and for the final approval of the manuscript submitted. GS was responsible for the conception, design, analysis, and interpretation of data; for revising the manuscript critically for important intellectual content; and for the final approval of the manuscript submitted. MB was responsible for the conception, design, and interpretation of data; for drafting of the manuscript and revising it critically for important intellectual content; and for the final approval of the manuscript submitted. All authors contributed to the article and approved the submitted version.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Chapter 7

Conclusions and future directions

This chapter summarises the work conducted in this thesis, highlights key contributions this thesis makes to the field and discusses possible directions for further research into repolarisation variability analysis.

Electrical instability in the repolarisation processes of the ventricles and malignant neural modulation can predisposes patients to lethal ventricular arrhythmia, possibly leading to SCD. However, most SCDs occur in patients deemed at low risk by traditional risk markers. Therefore, improving the ability to sense the instability in the repolarisation process and quantifying malignant neural modulation of the repolarisation process are necessary to improve risk assessment. This thesis proposes signal processing approaches for quantification of repolarisation variability independent of heart rate and respiration. It also proposes a novel repolarisation variability risk score that improves risk stratification in MI patients independent of well-established risk markers. This chapter summarises the work conducted in this thesis, highlights key findings that this thesis makes to the field and discusses possible directions for further research into repolarisation variability analysis.

7.1 Thesis summary

Electrical instability in repolarisation and malignant neural modulation predisposes patients to ventricular arrhythmias. ECG is the primary tool for diagnosing a ventricular arrhythmia, and several ECG features have been used in the literature to measure repolarisation dynamics. However, it is unclear which feature best captures the dynamics independent of heart rate and respiration and the sympathetic influences on the repolarisation process. Late repolarisation has been shown to be independent of heart rate, and speculations have been made regarding it reflecting neural influences. Therefore, to determine which measures to use in our analysis, in Chapter 3 we investigated the influence of sympathetic activation on repolarisation variability independent of heart rate and respiration measured from five frequently used repolarisation measures derived from the ECG signal, including late repolarisation. Our study confirmed that late repolarisation is independent of heart rate but that it is mainly influenced by respiration rather than sympathetic activation. It is possible that changes in the heart's position with respiration influence the projection of T-wave loop on the measurement lead. These findings indicate that late repolarisation alone should not be used as a measure of sympathetic activity, as previously proposed in the literature, as it appears highly prone to respiratory artifacts. The low-frequency power of QTend and RTend were found to increase with sympathetic activation independent of heart rate and respiration. The QT interval is the most frequently used interval for temporal repolarisation variability analysis of the two intervals. It has been reviewed thoroughly in a recent position statement and consensus guide endorsed by the European Heart Rhythm Association and the ESC Working Group on Cardiac Cellular Electrophysiology (Baumert et al. 2016). Based on our findings, we used the QTend interval for our analysis in

Chapters 4, 5, 6 and Appendix A making our results directly comparable to the majority of relevant studies in the literature.

However, QT variability has been shown to differ between leads (Hasan 2014), partially reflecting the physiological heterogeneity in the repolarisation process. Twelve-lead ECG provides a multiangled view of repolarisation. Yet, many studies employ a single ECG lead for QTV analysis as it is easier to apply, and often, preclinical trials only require recording a single lead. Hence, a question arises on whether single-lead ECG is sufficient for the analysis of QTV. Studies comparing single-lead and multi-lead ECG for QTV analysis are lacking, and there is no existing literature on the utility of one-dimensional representations of 12-lead ECG for QTV analysis. Hence, in Chapter 4**,** we compare QTV analysis results from single-lead ECG to that of multi-lead ECG in patients with cardiovascular disease undergoing a medical intervention. We found that the improved GOF or SNR in the multi-leads did not significantly differ in QTV compared to single leads. Furthermore, measurement of the intervention effect and pathophysiological group differences was consistent in all leads, for all components of QTV, including that independent of heart rate and respiration. These findings indicate that a single lead is sufficient for quantifying QTV components and studying group differences and intervention effects, simplifying study design and setup for clinical trials. Hence, the suitability of using single-lead ECG databases in the analysis undertaken in Chapters 3, 5 and 6 has been established.

Since we found that QTV increases following tilt independent of heart rate and respiration, we sought to investigate whether there is a direct relationship between QT independent of heart rate and a more direct measure of sympathetic activity. In Chapter 5, we studied the relationship between QT independent of heart rate and respiration and muscle sympathetic nerve activity following head-up tilt in a group of healthy subjects. We found that QT independent of HR and resp was not associated with rhythmical modulation of MSNA neither at rest nor following tilt. We suggested that MSNA may not be a useful surrogate for cardiac sympathetic activity, but that conjecture is inconclusive since it can possibly be the case that MSNA influences on QTV are complex and not quantifiable with the linear models employed in our analysis.

Hence, in Appendix A, we explore whether the relationship between QTV and MSNA is indeed nonlinear. We propose a novel method for detecting concurrent linear and nonlinear interactions and verifying its utility on simulated data. The proposed method is then applied to MSNA and QT variability analysis conducted in Chapter 4. We found no information transfer between MSNA and QTV beyond that captured by the linear model. This indicates that a linear

model was suitable for modelling QT and MSNA interactions, suggesting that MSNA may not be a suitable surrogate for cardiac sympathetic activity. We concluded that low-frequency oscillations of MSNA should not be used as a surrogate for cardiac sympathetic control at the ventricular level, as it appears to exhibit an organ-specific response to sympathetic activation. Other more direct measures of cardiac sympathetic activity should be employed in future analysis. As mentioned previously, improving the ability to detect electrical instability in the ventricles and quantifying the role of the autonomic nervous system is imperative to improving the assessment of patients at risk of SCD. While the prognostic value of QTV has been established in the literature, the mechanisms that underpin excessive QTV and their direct contribution to risk assessment are unclear. Throughout this work, we have demonstrated the utility of the parametric decomposition of QTV in quantifying repolarisation changes independent of heart rate and respiration in healthy subjects and cardiovascular disease patients. We have also consistently found QTV independent of heart rate and respiration to be increased following sympathetic activation and to exhibit an LF oscillation that cannot be attributed solely to noise or measurement artifacts.

The question about the clinical significance of QTV independent of heart rate and respiration arises. Thus, in Chapter 6, we explore the prognostic value of QTV independent of heart and respiration in a large dataset of myocardial infarction patients. We found that QTV, independent of heart rate and respiration, was increased in non-surviving patients and exhibited prominent LF oscillations. Importantly, QTV independent of heart rate and respiration was predictive of mortality in MI patients, independent of other measures of the QT interval and QTV. Hence, we developed a novel QTV risk score and demonstrated its prognostic value independent of well-established clinical risk assessment strategies. The QTV risk score improved risk stratification by identifying a subgroup of patients at high mortality risk despite being classified as low risk by traditional risk markers. The QTV risk score is a non-invasive risk marker that can be easily incorporated into daily clinical practice.

7.2 Future directions

In this section, we propose directions for future research that would extend the work done in this thesis.

1. Future work should validate the adequacy of single-lead for QTV analysis using simulated 12-lead ECG with well-defined noise levels and a predefined QT-RR relationship. This analysis should also be validated on normal subjects to establish whether a single-lead analysis is adequate irrespective of the presence of a pathophysiological condition.

2. While we have found that QTend and RTend are the most appropriate measures that reflect the influence of sympathetic activation in single-lead ECG, further investigation using 12-lead ECG would be of interest as it would allow us to explore a number of important parameters that correlate with sympathetic activity, such as periodic repolarization dynamics and also allow for a more robust removal of respiratory effects.
3. Future studies exploring the relationship between QTV and sympathetic activation should investigate whether QTV independent of RR and respiration is directly associated with absolute levels or magnitude of change in more direct measures of cardiac sympathetic activity such as norepinephrine spillover measured in humans or integrated left stellate ganglion nerve activity recorded in animals.
4. The proposed QTV risk score should be validated in different cohorts stratifying for SCD. Its prognostic value for improving the prediction of ventricular arrhythmia should also be investigated.
5. While we have shown that a linear approximation of RV variability is adequate for capturing short-term dynamics in healthy subjects, nonlinear aspects of RV variability can still provide complementary information. It would be interesting to investigate the utility of the methods proposed in Appendix A for combining linear and nonlinear analysis in pathophysiological conditions and cardiovascular mechanisms known to exhibit nonlinear dynamics.

7.3 Summary of original contributions

A summary of the key contributions of this thesis is provided below.

- This thesis demonstrates that while late repolarization should be included in measuring repolarisation variability when probing sympathetic activation, it should not be used as a measure of cardiac sympathetic activity, as it is more prone to respiratory artifacts.
- While a correlation has been reported in the literature between MSNA and heart rate, this thesis shows that there is no correlation between MSNA and ventricular repolarisation, suggesting that MSNA could not be adequate for probing cardiac sympathetic activity at the ventricular level.
- This thesis suggests that single-lead ECG is adequate for assessing pathological group differences and intervention effects on QTV analysis.
- This thesis proposes a novel QTV risk score that improves risk stratification in MI patients independent of well-established risk markers.

Appendix A

Transfer entropy analysis of linear model residuals

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Contribution to the Paper	Study design, data analysis, interpretation of data and results, preparation and revision of manuscript.		
Overall percentage (%)	90%		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	4/11/2022

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Mathias Baumert		
Contribution to the Paper	Study design, interpretation of data and results, revision of manuscript.		
Signature		Date	31/10/22

Transfer Entropy Analysis of Linear Model Residuals

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Abstract

Several cardiovascular mechanisms exhibit both linear and nonlinear interactions. A linear approximation of these interactions cannot capture the nonlinear dynamics rendering results incomplete, while nonlinear methods generally do not distinguish between linear and nonlinear relationships. In this study, we investigated whether nonlinear analysis (transfer entropy analysis) of a linear model's residuals can provide this distinction. The linear dynamics in the data were captured using a linear autoregressive model. Transfer entropy analysis was applied to the model residuals to investigate whether they hold information regarding nonlinear dynamics. This procedure was tested on simulated data and then applied to real data to study the relationship between muscle sympathetic nerve activity (MSNA) and repolarization variability (QT). Results from simulated data show that the procedure was able to separate linear and nonlinear dynamics. While analysis of real data showed that a linear model was adequate for quantifying QT-MSNA relationship since no information transfer was detected in the residuals.

Keywords *linear, nonlinear, cardiovascular, transfer entropy.*

1 Introduction

The cardiovascular system exhibits complex interactions between cardiac, vascular and neural mechanisms, which can be represented by a class of linear parametric multivariate models [1]. The residuals of these models are usually attributed to noise, nonlinearities or other mechanisms unaccounted for by the model [2, 3]. While the linear approximation of these mechanisms is widely accepted and used by many researchers in the field, this notion has not been investigated thoroughly against the null hypothesis of a nonlinear relationship [1]. This might render results related to the variability unexplained by the model inconclusive. Indeed, employing linear models in the presence of both linear and nonlinear system characteristics means that any nonlinear interactions are overlooked and remain confined in the model residuals [4]. Nonlinear methods have also been employed for the analysis of cardiovascular variability [1, 5-7]. However these methods generally cannot separate linear and nonlinear interactions. It has been suggested that analysis of a linear model's residuals might be fruitful for exploring nonlinear aspects of the system under investigation [4]. We hypothesize that, using a nonlinear method, the analysis of a linear model's residuals can separate nonlinear from linear interactions in systems where both type of interactions are present. In this paper, the proposed analysis was restricted to an open-loop autoregressive model with two external drivers (ARXX). Analysis was carried out on both, simulated and real data comprising beat-to-beat values of heart period (RR), muscle sympathetic nerve activity (MSNA) and ventricular repolarization duration of the heart (QT). Changes in beat-to-beat QT have been suggested to reflect changes in the level of sympathetic outflow to the heart [8, 9]. MSNA is an invasive measure of postganglionic sympathetic nerve activity measured in the peroneal nerve [10]. To investigate the extent to which repolarization variability can be used as a noninvasive measure of sympathetic activity, we studied the relationship between beat-to-beat changes in QT and MSNA. Sympathetic activity and RR are thought to exhibit influences on QT in a feed forward fashion, hence an open-loop ARXX model was selected for this specific study.

2 Materials and Methods

To test our hypothesis, a linear autoregressive model with two external inputs (ARXX) [11] combined with standard system identification procedures was employed to quantify linear relationships within bivariate data. Subsequently, information domain analysis based on transfer entropy [5] was used to investigate the presence of any remaining, nonlinear relationships in the model residuals.

Linear model residuals

A parametric linear autoregressive model with two external inputs defined as

$$A_Y(z)\,Y(n) = B_1(z)\,X(n) + B_2(z)\,Z(n) + w_Y(n), \quad (1)$$

was used to represent the data. In Eq. 1, Y is the target variable, and X and Z are external input variables which are not modulated by Y. Also, A_Y, B_1, and B_2 are polynomials of the model parameters in the z-domain, w_Y is a white Gaussian noise source, and n is the sample number. X and Z were modeled as independent autoregressive processes. Details of the model structure are described in [11], while details of the adopted model

estimation and validation procedures are described in [2]. After model estimation and validation, the estimated model parameters were used to filter out the variance of the target variable Y which is not explained by the model; i.e. model residuals [3].

Nonlinear analysis of the residuals

To test whether the residuals hold any information regarding nonlinear dynamics from the inputs to the output, a binning estimator with non-uniform embedding was used to estimate the information transfer from X to the residuals of Y ($TE_{X->Y}$) in the case of simulated data, and the conditional information transfer from Z to the residuals of Y given X ($TE_{Z->Y|X}$) for both simulated and real data [5]. The same analysis was carried out on the original data to compare the detection of information transfer with and without the linear component.

Simulated data

In order to test the ability of the proposed procedure to distinguish linear from nonlinear interactions, we used simulated data generated from a non-linear autoregressive model with two external inputs defined as in Eq. 2-4. The simulation model was adapted from [12].

$$Y(n) = 0.707\,Y(n-1) - 0.5\,X^2(n-2) + 0.3536\,Z(n-1) + w_Y(n), \quad (2)$$

$$X(n) = 0.3536\,X(n-1) - 0.2025\,X(n-2) + w_X(n), \quad (3)$$

$$Z(n) = 0.707\,Z(n-1) - 0.707\,Z(n-2) + w_Z(n). \quad (4)$$

Eq. 2 shows that Y is a combination of a linear function of its own past and Z, and a nonlinear function of X. On the other hand, X and Z are represented by two independent linear autoregressive models. Also, $w_Y, w_X, and\ w_Z$ are uncorrelated Gaussian white noises with zero mean and unit variance.

Real data

We studied 10 healthy subjects selected from a previously published study [13]. Continuous signals of lead III ECG, arterial blood pressure and muscle sympathetic nerve activity (MSNA) were recorded simultaneously for 10 minutes in supine and 40 degrees tilt in each subject with a sampling frequency of 1600 Hz. Beat-to-beat series of the heart period (RR) and repolarization duration series, measured as the temporal distance between the onset of the Q-wave and the end of the T-wave (QT) were obtained using an algorithm recently proposed in [14]. MSNA was used as a surrogate for sympathetic activity. Beat-to-beat MSNA series were obtained by calculating the time average of the integrated MSNA signal between two consecutive diastolic points to compute the average MSNA per beat. For each subject, data segments of around 200 beats were selected for the analysis. Data were detrended using the smoothness priors method described in [15], then normalized to zero mean and unit standard deviation.

Statistics

Paired t-test was used to test whether the estimated transfer entropies computed from simulated data were different to those computed from their residuals. Wilcoxon sign rank test was used to test for differences in the transfer entropy estimated in supine and tilt conditions, using both real data and its residuals. Values of $p < 0.05$ were considered statistically significant.

3 Results

Simulated data

The linear model fit to the simulated data was 58% ± 4.0% (mean ± SD). Table 1 shows that the estimates of the parameters related to the linear components (A_Y and B_2) were acceptably close to their simulated values, while the parameter related to the nonlinear component (B_1) were poorly estimated.

Parameter	Simulated	Estimated (mean)
$A_Y(z)$	$0.707z^{-1}$	$0.719\ z^{-1}$
$B_1(z)$	$-0.5z^{-2}$	$-0.015\ z^{-2}$
$B_2(z)$	$0.3536z^{-1}$	$0.285\ z^{-1}$

Table 1: ARXX model simualted and estimated parameters.

Fig. 1 shows the results of the transfer entropy estimated using both; simulated data and the residuals resulting from fitting the simulate data to the linear ARXX model. $TE_{Z->Y|X}$ was significantly reduced in the residuals compared to the data ($p < 0.0001$), while the estimated $TE_{X->Y}$ remained unchanged ($p > 0.3$).

Cardiovascular data

The linear model fit to the cardiovascular data was on average 51% in supine and 41% following tilt. Fig. 2 shows the transfer entropy from MSNA to QT given RR ($TE_{MSNA->QT|RR}$) estimated using both; real data and the residuals resulting from fitting the real data to the linear ARXX model. $TE_{MSNA->QT|RR}$ demostrated a slight insignificant increase using real data ($p > 0.5$), while the information transfer was negligible (median = 0.027) in the residuals in the supine position, and not detected (median = 0) following tilt. $TE_{MSNA->QT|RR}$ computed from the residuals was decreased compared to real data in both supine and tilt. However this decrease was not statisticall significant ($p > 0.1$ for both supine and tilt conditions).

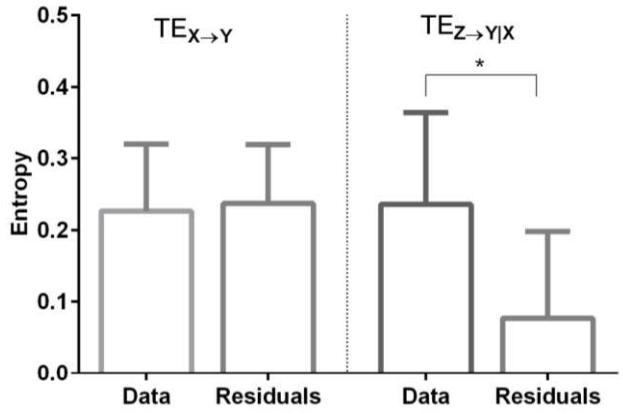

Figure 1: Information transfer from the inputs to Y estimated from simulated data and residuals. (A) information trasfer from X to Y ($TE_{X->Y}$), and (B) conditional information transfer from Z to Y given X ($TE_{Z->Y|X}$). Values shown are mean ± std. * indicates a signifcant difference.

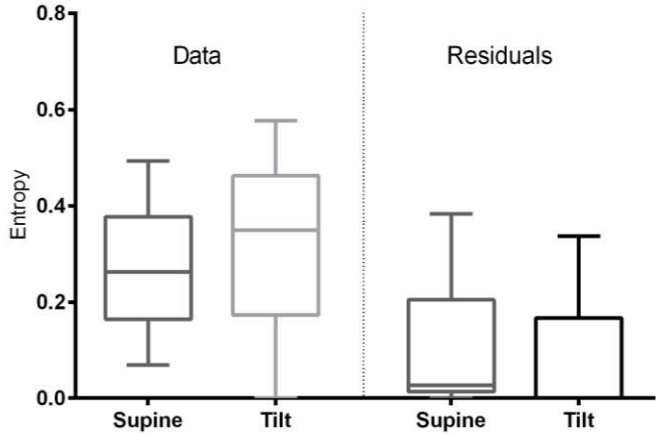

Figure 2: Conditional information transfer from MSNA to QT give RR ($TE_{MSNA->QT|RR}$) estimated from real data and residuals. Values shown are median (25th – 75th percentiles).

4 Conclusions

The cardiovascular system consists of complex interactions between different subsystems involving both linear and nonlinear dynamics. Linear estimations of these interactions render result inconclusive as nonlinearities are confined in the residuals [4], while nonlinear estimations do not distinguish linear from nonlinear dynamics.

Simulated data

In this paper we used simulated data to demonstrate that analysis of a linear model's residuals can distinguish linear from nonlinear dynamics driving the system. The simulated model involves both linear and nonlinear dynamics. The good model fit and the estimation of the linear parameters show that the linear model was able to capture the linear dynamics adequately rendering the model residuals a reliable estimation of the true variance which cannot be explained by a linear model. Then, the transfer entropy was computed using both simulated data and residuals. $TE_{X->Y}$ was detected in both data and residuals, while $TE_{Z->Y|X}$ was only detected in the data. This shows that the procedure was able to separate and identify both; the linear component from Z to Y was filtered and captured by linear model, while the nonlinear component from X to Y remained in the residuals and was clearly identifiable using the transfer entropy method.

Real data

The proposed procedure was applied to real data consisting of QT, RR and MSNA. In a previous study [2], we used the same linear model to study the relationship between MSNA and QT variability in an attempt to investigate the extent to which QT variability can be used as a measure of sympathetic activity. Power contribution analysis showed that MSNA contribution to QT was small but not negligible. We concluded that MSNA might not exhibit influences on QT variability, or that these influences might be complex and not quantifiable using a linear model. To test whether MSNA exhibits nonlinear influences which might have been confined in the model residuals, we computed $TE_{MSNA->QT|RR}$ from both real data and the residual s (Fig. 2). $TE_{MSNA->QT|RR}$ was detected in the data but not the residuals, which supports the notion that MSNA does not exhibit nonlinear influences on QT variability at moderate levels of sympathetic activation. Furthermore, the reduction in the transfer entropies estimated from the residuals compared to real data, despite being statistically insignificant, support the notion that the model in our previous study has captured the MSNA-QT dynamics adequately.

In conclusion, we have displayed how analysis of a linear model's residuals (using an ARXX model as an example) can distinguish linear from nonlinear dynamics. However, the clinical merit of this separation is yet to be established with further testing on larger datasets investigating cardiovascular mechanisms which are known to exhibit nonlinear dynamics; such as baroreflex [16, 17], and cardiac repolarization lability [18-20].

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