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QT variability unrelated to RR variability during stress testing for identification of coronary artery disease

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Stress test electrocardiogram (ECG) analysis is widely used for coronary artery disease (CAD) diagnosis despite its limited accuracy. Alterations in autonomic modulation of cardiac electrical activity have been reported in CAD patients during acute ischemia. We hypothesized that those alterations could be reflected in changes in ventricular repolarization dynamics during stress testing that could be measured through QT interval variability (QTV). However, QTV is largely dependent on RR interval variability (RRV), which might hinder intrinsic ventricular repolarization dynamics. In this study, we investigated whether different markers accounting for low-frequency (LF) oscillations of QTV unrelated to RRV during stress testing could be used to separate patients with and without CAD. Power spectral density of QTV unrelated to RRV was obtained based on time-frequency coherence estimation. Instantaneous LF power of QTV and QTV unrelated to RRV were obtained. LF power of QTV unrelated to RRV normalized by LF power of QTV was also studied. Stress test ECG of 100 patients were analysed. Patients referred to coronary angiography were classified into non-CAD or CAD group. LF oscillations in QTV did not show significant differences between CAD and non-CAD groups. However, LF oscillations in QTV unrelated to RRV were significantly higher in the CAD group as

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compared with the non-CAD group when measured during the first phases of exercise and last phases of recovery. ROC analysis of these indices revealed area under the curve values ranging from 61 to 73%. Binomial logistic regression analysis revealed LF power of QTV unrelated to RRV, both during the first phase of exercise and last phase of recovery, as independent predictors of CAD. In conclusion, this study highlights the importance of removing the influence of RRV when measuring QTV during stress testing for CAD identification and supports the added value of LF oscillations of QTV unrelated to RRV to diagnose CAD from the first minutes of exercise.

This article is part of the theme issue 'Advanced computation in cardiovascular physiology: new challenges and opportunities'.

1. Introduction

Coronary artery disease (CAD) represents the first cause of death worldwide [1]. A literature review reported 5–18% prevalence of CAD worldwide [2]. It consists of progressive stenosis of coronary arteries, which reduces oxygen supply to cardiac cells and can lead to stroke and death. Stress testing is the most commonly used method for CAD diagnosis prior to coronary angiography due to its non-invasive nature and the fact of being non-expensive. Nevertheless, the accuracy of conventional stress test electrocardiogram (ECG), mainly based on the analysis of the ST segment, is limited, presenting low sensitivity and specificity [3–6]. In the meta-analysis by Gianrossi *et al.* [6], sensitivities ranging from 23 to 100% ($68 \pm 16\%$) and specificities from 17 to 100% ($77 \pm 17\%$) were reported. ECG markers quantifying information from other ECG waveforms and time intervals could increase the accuracy of stress testing and provide valuable prognostic information.

Altered autonomic function has been associated with CAD progression and increased mortality [7,8], probably due to increased sympathetic nervous system modulation, as suggested by heart rate variability (HRV) measurements [9–12]. These changes in autonomic modulation in CAD patients have effects not only at the level of the sinoatrial node activity, as reflected by HRV, but also at the level of ventricular repolarization activity. An increase in QT variability (QTV) compensated for HRV has been demonstrated in ambulatory ECG recordings of CAD patients during acute ischemia, which has been associated with greater sympathetic modulation [13]. Low-frequency (LF) oscillations in the ECG T wave vector, measured by the so-called periodic repolarization dynamics and postulated to be related to sympathetic LF oscillations, have been shown to predict mortality in CAD and after myocardial infarction [14–16]. Clinical, experimental and theoretical studies have provided insight into potential mechanisms underlying the relationship between enhanced LF repolarization variability and all-cause mortality, in general, and arrhythmic mortality in particular [17–19]. However, the value of LF oscillations of repolarization, quantified as independent of HRV, has not yet been demonstrated to diagnose CAD from stress test ECG. Different ECG markers have been proposed in the literature to non-invasively quantify repolarization variability, being beat-to-beat QT interval variability (QTV) the most widely studied, associated with sympathetic ventricular outflow and a marker of cardiovascular risk [20]. However, QTV largely depends on RR interval variability (RRV). The decomposition of QTV into fractions related and unrelated to RRV was already proposed in [21] using a parametric approach. The concept was later extended to deal with non-stationary signals using time-frequency representations [22], thus allowing the instantaneous decomposition of QTV into a component related to RRV and a component unrelated to RRV, the latter being suggested to represent intrinsic ventricular repolarization variability.

In this study, we tested the hypothesis that LF oscillations of QTV unrelated to RRV during stress testing can be used for CAD identification. We measured the magnitude of those oscillations during both the exercise and recovery phases and we compared them between patients with and without CAD. ROC analysis was performed to assess sensitivity and specificity of repolarization

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variability-based detection of CAD. Logistic regression analysis served to confirm variability markers evaluated at different phases during the stress testing as independent predictors of CAD.

2. Material and methods

(a) Study population

The ECG of a subgroup of 100 patients from those referred for stress testing at Tampere University Hospital was analysed. Continuous ECG was recorded at 500 Hz with the CardioSoft exercise ECG system (v. 4.14, GE Health care, Freiburg, Germany) using the Mason-Likar modification of the standard 12-lead system. The study protocol was approved by the Ethical Committee of the Hospital District of Pirkanmaa, Finland, and all patients gave informed consent prior to the interview and measurements as stipulated in the Declaration of Helsinki.

Patients underwent a maximal exercise test on a bicycle ergometer, starting at an initial workload of 20–30 W, which increased stepwise by 10–30 W each minute. An initial workload of 20 W was commonly used for females and males having poor fitness. The decision of workload increment was taken by physicians based on the patient's state of health and fitness in order to get a maximal effort in 12–15 min. Patients with positive stress testing underwent coronary angiography within 180 days of stress testing [23,24].

Patients were classified in the following groups. A low-risk group was defined based on detailed patient information and stress testing symptoms. Any patient undergoing angiography or reporting chest pain was excluded from this group. Patients undergoing angiography were classified as CAD0, CAD1 or CAD2 group, depending on whether they presented less than 50%, between 50 and 75%, or more than 75% of luminal narrowing of the diameter of at least one major epicardial coronary artery or main branches. For the analysis of this study, 25 patients randomly selected from each of these four groups were considered. A group denoted as CAD was defined by combining groups CAD1 and CAD2. A group denoted as non-CAD contained the patients in group CAD0. This classification is shown in table 1.

(b) ECG preprocessing and delineation

Baseline wander was removed using cubic splines interpolation, with knots taken 60 ms before QRS fiducial time point if the previous RR interval was above 430 and 55 ms otherwise.

To improve the delineation of the T wave end, which can be problematic in highly noisy scenarios, an optimum lead was selected for each subject based on the T wave noise level and signal-to-noise ratio (SNR). First, a T-wave window was defined from the QRS fiducial point plus 110 ms (or 100 ms if $RR < 720$ ms) to the QRS fiducial point plus 360 ms (or minimum between 360 ms and $\frac{2}{5}(\mathrm{RR}+200)$ value if $RR < 720$ ms). The T wave noise level was defined as the root mean squared error of the difference between the T wave and a lowpass filtered version of it with 25 Hz cut-off frequency. The SNR of each T wave was defined dividing the maximum amplitude within the T-wave window by the corresponding T wave noise level. The three ECG leads with the highest SNR were selected and the one out of these three ECG leads with the lowest T wave noise level was selected for further analysis.

A lowpass filter with a cut-off frequency of 25 Hz was applied to the selected ECG lead prior to delineation. ECG delineation was performed using a validated wavelet-based method [25], with some updates to account for the high levels of noise during stress testing, which can lead to extra variability in QT interval time series. In particular, all T waves were delineated as monophasic. The QT interval was measured from the onset of the QRS complex to the end of the T wave.

To avoid that arrhythmic episodes present along the ECG recordings could negatively influence the analysis, a set of rules on the RR interval time series was imposed following an approach similar to that described in [26]. In brief, the maximum difference between consecutive RR intervals was required to be lower than 150 ms in at least 75% of the beats and there were

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Table 1. Patient groups analysed in the study.

	angiography		
no angiography	<50%	50–75%	>75%
low-risk	CAD0	CAD1	CAD2
low-risk	non-CAD	CAD	

less than 5% of beats identified as ectopics. Only 20 s segments free of arrhythmic episodes were included in the study.

Outlier values in the time series of QT intervals (RR intervals, respectively) were identified by first applying a 30th order median filter over the times series of absolute differences between successive intervals. Outliers in the QT (RR, respectively) time series were identified as those for which the absolute difference was above five times the corresponding value in the median filtered series. Instantaneous variations of QT or RR values exceeding their adjacent values by more than 150 and 60 ms, respectively, were also considered as outliers. Those segments presenting gaps of non-valid interval measurements longer than 2 s or with more than 5% of outlier values were excluded from the analysis. In other cases, outlier values were replaced with the mean of their adjacent values. The obtained QT and RR interval time series were interpolated at a sampling rate of 4 Hz, thus leading to uniformly sampled time series.

(c) QT variability unrelated to RR variability

QT variability unrelated to RR variability was obtained using the methodology described in [22], based on time-frequency representations. First, QTV and RRV were obtained by highpass filtering QT and RR interval time series with a cut-off frequency of 0.03 Hz. Cohen's class distributions were used to obtain the time-frequency representations of QTV series, $S_{\mathrm{QTV}}(t,f)$, as well as the time-frequency coherence (TFC) between QTV and RRV, $\gamma_{\mathrm{QTV,RRV}}(t,f)$, with temporal and spectral resolutions of 11.7 s and 0.039 Hz, respectively. Based on TFC, the time-frequency spectrum of QTV was decomposed into two spectra, one representing QTV linearly related to RRV (QTVrRRV) and the other representing QTV unrelated to RRV (QTVuRRV) [22]:

$$S_{\mathrm{QTVuRRV}}(t,f) = (1 - |\gamma_{\mathrm{QTV,RRV}}(t,f)|^2) S_{\mathrm{QTV}}(t,f). \tag{2.1}$$

Since TFC estimators are known to be biased, the bias was estimated and corrected as described in [22].

The instantaneous power of LF oscillations for QTV and QTVuRRV series were calculated by integrating their time-frequency distributions, $S_{\mathrm{QTV}}(t,f)$ and $S_{\mathrm{QTVuRRV}}(t,f)$, respectively, in the 0.03–0.15 Hz band, and denoted as $P_{\mathrm{QTV}}(t)$ and $P_{\mathrm{QTVuRRV}}(t)$. The normalized LF power of QTVuRRV was estimated as

$$P_{\mathrm{QTVuRRVn}}(t) = \frac{P_{\mathrm{QTVuRRV}}(t)}{P_{\mathrm{QTV}}(t)}. \tag{2.2}$$

(d) Statistical analysis

The temporal evolution of $P_{\mathrm{QTV}}(t)$, $P_{\mathrm{QTVuRRVn}}(t)$ and $P_{\mathrm{QTVuRRVn}}(t)$ was studied in different time intervals based on maximum HR percentages. During the exercise phase, three intervals were defined where HR lay within 0–25% of ΔHR (E_{25}), 25–50% of ΔHR (E_{50}) and 50–75% of ΔHR (E_{75}). During the recovery phase, three other intervals were defined where HR lay within 75–50% of ΔHR (R_{75}), 50–25% of ΔHR (R_{50}) and 25–0% of ΔHR (R_{25}). In the above expressions, ΔHR was used to denote the maximum theoretical HR of each patient minus the mean HR obtained in a

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1 min window prior to the exercise onset (starting 30 s after the beginning of the recording). The maximum theoretical HR was calculated as:

$$\mathrm{HR}_{\max} = 211 - 0.64x_{\mathrm{age}}, \tag{2.3}$$

where x_{age} represents the age of the subject and $\mathrm{HR}_{\max}$ is expressed in beats per minute.

For each of these intervals, the 20 s segment with the highest HR was selected for analysis and the median value of $P_{\mathrm{QTV}}(t)$, $P_{\mathrm{QTVuRRV}}(t)$ and $P_{\mathrm{QTVuRRVn}}(t)$ was computed and denoted as P^I_{QTV}, P^I_{QTVuRRV} and P^I_{QTVuRRVn}, respectively, where I denotes the corresponding time interval E_{25}, E_{50}, E_{75}, R_{75}, R_{50} or R_{25}.

Unless otherwise specified, group comparisons were performed between non-CAD (CAD0) and CAD (CAD1 and CAD2) patients. For certain analyses, additional comparisons between low-risk versus CAD patients were conducted.

The Kolmogorov–Smirnov statistical test was used to test for normality distribution of sampled data, rejecting the hypothesis of normal distribution for all the analysed indices. Thus, the Mann–Whitney U test was used to compare the values of each analysed marker between patient groups. The χ^2 test was used to compare clinical categorical variables between patient groups. A p-value < 0.05 was used to determine statistical significance.

ROC analysis was performed for those markers presenting significant differences between groups to determine their sensitivity and specificity for CAD identification. Multi-variate binomial logistic regression was applied to investigate whether the markers were independent predictors of CAD.

3. Results

Table 2 summarizes the descriptive characteristics of the patient population. Patients in the CAD group were older than those in the non-CAD group. Cardiovascular medications like ACE inhibitors, beta-blockers and long-acting nitrate were more frequently taken by CAD patients.

Figure 1 presents representative examples of RR and QT interval time series of a patient in the non-CAD group and a patient in the CAD group. The lower panels in the figure show the corresponding instantaneous LF powers for QTV, QTVuRRV and QTVuRRVn along the stress test. While $P_{\mathrm{QTVuRRV}}(t)$ showed larger values in the CAD patient than in the non-CAD patient during the whole exercise and recovery phases, relevant differences in terms of $P_{\mathrm{QTV}}(t)$ and $P_{\mathrm{QTVuRRVn}}(t)$ were only found in some intervals along the stress test.

Figure 2 presents the distributions of P^I_{QTV}, P^I_{QTVuRRV} and P^I_{QTVuRRVn} for non-CAD and CAD groups during the six analysed intervals of the stress test as well as during baseline. Although P^I_{QTV} showed higher values in the CAD group than in the non-CAD group, except in E_{75}, differences were not statistically significant. P^I_{QTVuRRV} was significantly higher in the CAD group compared with the non-CAD group when measured during the first intervals of the exercise phase and the last intervals of the recovery phase, specifically during exercise intervals E_{25} and E_{50} and recovery intervals R_{50} and R_{25}. The normalized index P^I_{QTVuRRVn} was significantly higher in the CAD group with respect to the non-CAD group only at baseline and during the first recovery interval R_{75}. Additionally, the distributions of P^I_{QTVrRRV} and P^I_{RTV} are presented in the figure for the same time intervals. No significant differences in any of these two indices between non-CAD and CAD groups were found either at baseline, exercise or recovery phases of the stress test.

ROC curves are presented in figure 3 for P^I_{QTVuRRV} calculated during E_{25}, E_{50}, R_{50} and R_{25} as well as for P^I_{QTVuRRVn} calculated at baseline and at R_{75}. The area under the curve (AUC) is displayed in table 3 together with the associated sensitivity (Se) and specificity (Sp) values. The highest AUC value (71%) was obtained for normalized HRV-unrelated QTV at baseline, P^B_{QTVuRRVn}, with a sensitivity of 75% and a specificity of 72%. For non-normalized HRV-unrelated QTV, the highest AUC value was obtained for $P^{R_{25}}_{\mathrm{QTVuRRV}}$ (68%), with a sensitivity of 62% and a specificity of 74% for the optimal cut-off point (defined as the one minimizing the Euclidean

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Table 2. Population characteristics. BMI denotes body mass index, MI denotes myocardial infarction and ACE denotes angiotensin converting enzyme. Medications are provided for non-CAD and CAD groups. Superindex α indicates that median $\pm$ median absolute deviation (MAD) values are provided for the variable.

	non-CAD (n=25)	CAD ($n = 50$)
characteristic		
age (years)$^{\alpha}$	52.44 ± 8.31	59.26 ± 9.96*
gender (male/female)	16/9	36/14
BMI (kg $\cdot$ m^{-2})$^{\alpha}$	27.2 ± 5.58	26.32 ± 4.07
MI (patients)	5	16
chest pain (patients)	24	43
exercise length (min)$^{\alpha}$	6.68 ± 1.58	6.32 ± 2.17
medication		
ACE inhibitors	3	18*
beta-blockers	14	43*
calcium channel blockers	2	12
digitalis	0	0
glyceryl trinitrate	6	17
long-acting nitrate	2	20*
diuretics	2	7

*p-value < 0.05.

distance to the upper left corner of the ROC curve). A sensitivity of 90% was obtained for a specificity of 41% in the case of $P^{B}_{\mathrm{QTVuRRVn}}$ and a specificity of 26% in the case of $P^{R_{25}}_{\mathrm{QTVuRRV}}$.

Since CAD and non-CAD patients differed significantly in age, a correlation study was conducted between age and each of the analysed indices presenting significant differences between CAD and non-CAD patients. Only $P^{B}_{\mathrm{QTVuRRVn}}$ showed a moderate ($\rho = 0.34$), but significant, correlation with age and was therefore not considered in the subsequent regression analysis.

Results from the binomial logistic regression analysis are presented in table 4. When entering $P^{E_{25}}_{\mathrm{QTVuRRV}}$ and $P^{R_{75}}_{\mathrm{QTVuRRVn}}$ as covariates in the regression model, only $P^{E_{25}}_{\mathrm{QTVuRRV}}$ was found as an independent predictor of CAD (odds ratio = 1.16, $p = 0.02$). Similarly, when entering $P^{R_{25}}_{\mathrm{QTVuRRV}}$ and $P^{R_{75}}_{\mathrm{QTVuRRVn}}$ as covariates, only $P^{R_{25}}_{\mathrm{QTVuRRV}}$ independently predicted CAD (odds ratio = 1.11, $p = 0.04$). If entering a higher number of covariates representing P_{QTVuRRV} measured at other intervals during the stress test, no independent CAD predictors were found.

Additionally, the proposed indices were compared between the low-risk and CAD groups. P^{I}_{QTVuRRV} was significantly lower in the low-risk group when compared with the CAD group only in R_{25}. However, $P^{I}_{\mathrm{QTVuRRVn}}$ was significantly lower during R_{50} and R_{25}. In other intervals of the stress test, P^{I}_{QTV} and P^{I}_{QTVuRRV} showed high inter-individual variations in the low-risk group, larger than those observed in the non-CAD group, especially in E_{25}.

4. Discussion

The main results of this study can be summarized as: (1) The fraction of repolarization variability not related to HRV can be used to non-invasively diagnose CAD from stress test ECGs; (2) The capacity of HRV-unrelated repolarization variability for separation of CAD and non-CAD patients holds both when measured during the exercise and the recovery phases of the test; (3) In

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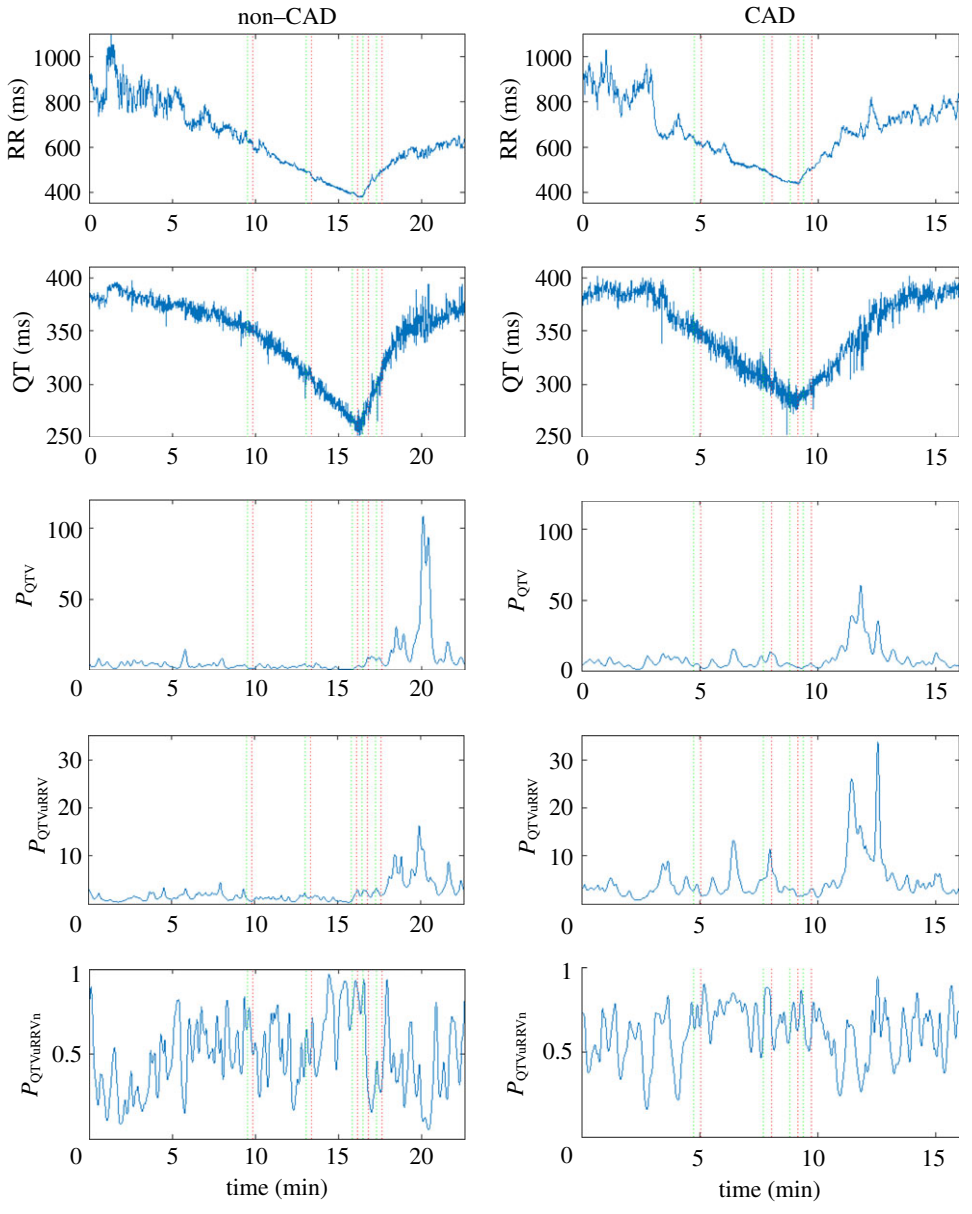

Figure 1. Example of RR and QT series, $P_{QTV}(t)$, $P_{QTVuRRV}(t)$ and $P_{QTVuRRVn}(t)$ for (left) non-CAD patient and (right) CAD patient. Green and red vertical lines denote the onset and end, respectively, of the 20 s segments used for analysis. (Online version in colour.)

ROC analysis, normalized and non-normalized HRV-unrelated repolarization variability offers acceptable accuracy, with 72% sensitivity corresponding to 67% specificity for the best performing variable during the stress test; (4) In multi-variate regression, HRV-unrelated repolarization variability, measured either during exercise or recovery, is able to predict CAD independently of other variables with capacity for CAD and non-CAD separation based on normalized QT variability.

In previous studies, different ECG markers, based on HRV and repolarization variability, have been associated with increased mortality in CAD and after myocardial infarction [8–10,14–16,20]. Some of these indices have been already investigated for CAD diagnosis based on stress test

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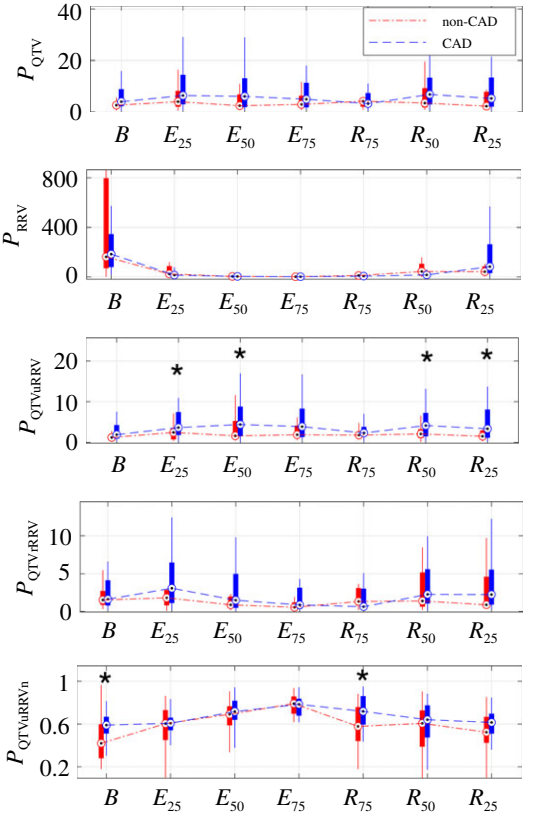

Figure 2. Distribution of P^{I}_{QTV},P^{I}_{RRV}, $P^{I}_{QTVuRRV}$, $P^{I}_{QTVrRRV}$ and $P^{I}_{QTVuRRVn}$ at baseline, during exercise and during recovery for non-CAD and CAD groups. * denotes significant differences between groups ($p < 0.05$). (Online version in colour.)

ECG. In particular, HRV indices have shown contradictory results, with [27,28] reporting accuracy values ranging from 75 to 96% in the classification of low-risk versus CAD patients and [26] concluding that HRV indices are inadequate for CAD diagnosis.

Regarding ECG repolarization, instability markers measuring microvolt T wave alternans have been shown to take higher values in CAD patients than in healthy subjects and also in CAD patients with significant stenosis than in patients with no major stenosis [29,30]. These results were confirmed in subsequent studies, which additionally revealed greater accuracy of T wave alternans when compared with conventional ST segment analysis for CAD detection [31]. Other repolarization markers, like the QT interval, HR-corrected QT interval (QTc) and spatial QT dispersion have been explored prior to, during and after stress testing in a large cohort of patients undergoing coronary angiography [32]. QTc interval and QT dispersion during recovery were significantly higher in the critical CAD group with respect to the non-critical CAD group. These results on increased dispersion are in accordance with our results, as they are all indicative of higher repolarization lability during recovery from exercise in CAD patients with respect to non-CAD patients, even if the markers measured in [32] are intended to measure spatial repolarization heterogeneities whereas our markers quantify temporal repolarization variability. In [32], ROC analysis based on QTc and QT dispersion revealed slightly better performance than in our study, with 90% sensitivity and 53% specificity. However, the critical CAD group in that study may include patients with more severe forms of myocardial ischemia than our CAD group.

In previous studies, beat-to-beat QTV, as an indicator of temporal repolarization instability, has been shown to present larger values in CAD patients than in non-CAD patients and healthy controls [33,34]. Nevertheless, in the comparison with healthy controls, the significance of the

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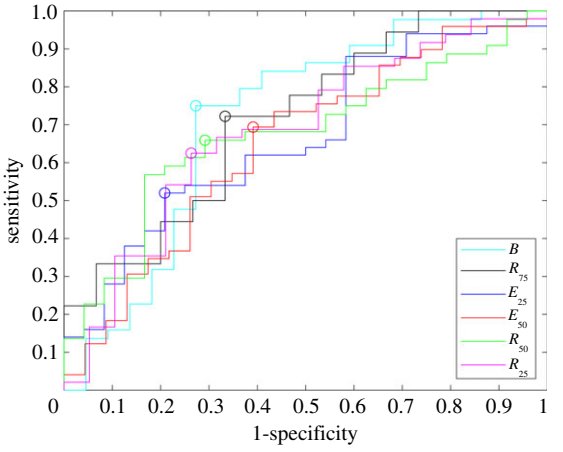

Figure 3. ROC analysis for $P^{I}_{QTVuRRV}$ in E_{25} (blue), E_{50} (red), R_{50} (green) and R_{25} (magenta). (Online version in colour.)

Table 3. AUC, sensitivity and specificity for $P^{I}_{QTVuRRVn}$ in B and R_{75} and $P^{I}_{QTVuRRV}$ in E_{25}, E_{50}, R_{50} and R_{25}.

	AUC (%)	Se (%)	Sp (%)
B	71	75	72
		80	59
		90	41
R_{75}	71	72	67
		80	47
		90	33
E_{25}	66	52	79
		80	42
		90	29
E_{50}	65	69	61
		80	35
		90	22
R_{50}	67	66	71
		80	33
		90	13
R_{25}	68	62	74
		80	42
		90	26

QTV increase in CAD patients only held when compensating for HRV by quantification of the so-called QTV index (QTVI). To the best of our knowledge, there is no study investigating QTV, or the fraction of it not related to HRV, for CAD diagnosis using stress test ECG recordings. On the basis of the value of LF oscillations of repolarization as a prognostic marker in CAD patients and patients after myocardial infarction, we aimed at determining the value of the LF power of QTV and of its fraction not linearly related to HRV for CAD diagnosis from stress test ECG. In particular, we focused our study on the comparison between CAD and non-CAD groups to

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Table 4. Binomial logistic regression analysis to identify independent CAD predictors.

	covariate	odds ratio	95% CI for odds ratio	*p*-value
model 1				
	$P^{E_{25}}_{\mathrm{QTVuRRV}}$	1.16	1.02–1.32	0.02
	$P^{R_{75}}_{\mathrm{QTVuRRVn}}$	0.70	0.20–2.36	0.56
model 2				
	$P^{R_{25}}_{\mathrm{QTVuRRV}}$	1.11	1.01–1.23	0.04
	$P^{R_{75}}_{\mathrm{QTVuRRVn}}$	1.01	0.33–3.13	0.98

improve the specificity of stress testing. Although we lacked a gold standard reference in patients of the low-risk group who did not undergo a coronary angiography, in some of our analysis, we included the separation between low-risk group and CAD groups for comparison purposes.

We observed that LF oscillations of QTV were generally higher in the CAD group compared with the non-CAD group, but no significant differences were found for any of the studied intervals. However, when linear influences from RRV were removed from QTV, significantly higher LF power of QTVuRRV was observed in the CAD group with respect to the non-CAD group, both during the exercise and recovery phases. These results are in line with previous findings regarding the need to compensate QTV for the effects of HRV to get more meaningful information for patient separation.

When we analysed LF power of QTV, QTVuRRV and QTVuRRVn at baseline, prior to the beginning of the stress test, we noticed that P_{QTV} and P_{QTVuRRV} were not significantly different between non-CAD and CAD groups. However, P_{QTVuRRVn} was significantly higher in CAD than non-CAD patients. Nevertheless, this variable $P^{B}_{\mathrm{QTVuRRVn}}$ was significantly correlated with age. Thus, we did not include it in our regression analysis, which we restricted to variables being significantly different between non-CAD and CAD groups and which could provide information not correlated to that of age, like $P^{E_{25}}_{\mathrm{QTVuRRV}}$, $P^{R_{25}}_{\mathrm{QTVuRRV}}$ and $P^{R_{75}}_{\mathrm{QTVuRRVn}}$ measured during the exercise and recovery phases of the stress test.

Since the proportion of patients taking β-blockers was considerably higher in the CAD group than in the non-CAD group, an additional analysis was conducted comparing the power of the LF component of QTV unrelated to RRV in CAD and non-CAD patients under β-blocker medication. Similar trends as those reported in figure 2 were observed, with lower values for non-CAD patients. Additionally, no differences were observed between the distributions of LF power of QTV unrelated to RRV in non-CAD patients with and without treatment with β-blockers.

When low-risk patients were included in the analysis, no significant differences between low-risk and CAD groups were found during the exercise phase, but only during the recovery phase. It should be noted that while exercise length was similar in the non-CAD and CAD groups, it was significantly higher in some patients of the low-risk group. This might explain the higher inter-individual variations in the low-risk group and the absence of significant differences between low-risk and CAD groups during exercise.

Binomial logistic regression analysis revealed that the magnitude of LF oscillations in QTV unrelated to RRV, measured in the first exercise interval ($P^{E_{25}}_{\mathrm{QTVuRRV}}$) and the last recovery interval ($P^{R_{25}}_{\mathrm{QTVuRRV}}$) had capacity for CAD prediction. Our analysis showed that when each of them was separately entered into a model together with a marker measuring normalized QTV unrelated to HRV, which was also able to separate CAD and non-CAD groups, each of them were independent predictors of CAD. Interestingly, the marker $P^{E_{25}}_{\mathrm{QTVuRRV}}$ can be obtained from the first minutes of exercise, which can represent an advantage over other previously proposed markers which require evaluation either at peak exercise or during the recovery phase [32].

In this study, decomposition of repolarization variability into a component related to RRV and a component unrelated to RRV has been accomplished using a time-varying non-parametric

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approach, due to the highly non-stationary nature of cardiac variability series during exercise testing. In stationary situations, previous works studying the interactions between repolarization variability and RRV used parametric approaches mainly based on autoregressive modelling [21,35,36]. Model-based approaches allow for the study of QTV and RRV in a closed-loop system, assessing both the strength and direction of the interactions. For instance, in [37], using a network physiology approach, it has been shown that there exists a weak but significant influence of QT on heart period, which is not affected by graded-tilting sympathetic activation. The strength of the causal link from heart period to QT is strong and decreases with tilting angle, which is in agreement with our observed increase in the normalized LF power of QTV unrelated to HRV as exercise intensity increases.

QTV is known to be influenced by respiration, mainly due to its effect on T-wave end delineation, and should be considered as a confounder when studying ventricular repolarization dynamics [35]. In our study, respiratory rate was above LF band in all subjects and stress testing phases, including the resting phase [28], thus preventing any confounding effect of respiration on the analysed indices.

5. Study limitations and future research

Twenty-five patients were included in each of the patient groups (low-risk, CAD0, CAD1 and CAD2) analysed in this study. Thus, the comparison of non-CAD and CAD groups involved 25 and 50 patients, respectively. Future studies investigating a larger number of patients could improve the statistical power of our proposed methods for CAD detection. In particular, if Bonferroni correction had been applied, the comparisons performed in this study would not have reached statistical significance. Further studies in larger populations could confirm the value of the results presented here.

The QT interval was delineated by identifying an optimum lead in terms of noise level and SNR. Further studies could explore multi-lead delineation strategies to attenuate the impact of noise on ECG delineation, particularly in highly noisy recordings as those acquired during stress test.

The use of certain medications differed between patients in the non-CAD and CAD groups. We investigated the effect of β-blockers on our results, but not of other medications. As future research, the potential effects of such medications on QTV and HRV could be investigated. These effects could be taken into account in the interpretation of CAD and non-CAD separation based on repolarization variability analysis.

The QTV variables identified in this study as being able to separate non-CAD and CAD groups should be analysed in other independent populations. Future studies addressing that analysis could verify whether the performance of our proposed variables in this study is generalized to other datasets.

6. Conclusion

The potential value of LF oscillations of QTV unrelated to RRV during stress testing for CAD identification has been investigated in this study. Results show that only when QTV unrelated to RRV is studied, LF oscillations derived from both the exercise and recovery phases are significantly different in CAD with respect to non-CAD patients, allowing for CAD identification with 90% sensitivity and 40% specificity. These indices are also independent predictors of CAD in multi-variate regression. This study highlights the importance of removing the influence of RRV when measuring QTV during stress testing for CAD identification and supports the added value of LF oscillations of QTV unrelated to RRV to diagnose CAD from the first minutes of exercise.

Ethics. The study protocol was approved by the Ethical Committee of the Hospital District of Pirkanmaa, Finland, and all patients gave informed consent prior to the interview and measurements as stipulated in the Declaration of Helsinki.

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Data accessibility. The authors confirm that the data supporting the findings of this study are available within its electronic supplementary material.

Authors' contributions. M.G.d.C. carried out data and statistical analysis and drafted the manuscript. D.H. participated in data and statistical analysis and helped draft the manuscript. M.O. participated in the design of the study and in data analysis and revised the manuscript critically for important intellectual content. J.V. participated in the design of the study and acquisition of data and revised the manuscript critically for important intellectual content. P.L. participated in the design of the study and critically revised the manuscript. R.B. and E.P. conceived and coordinated the study and critically reviewed the manuscript. All authors gave final approval for publication and agree to be held accountable for the work performed therein.

Competing interests. We declare we have no competing interests.

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