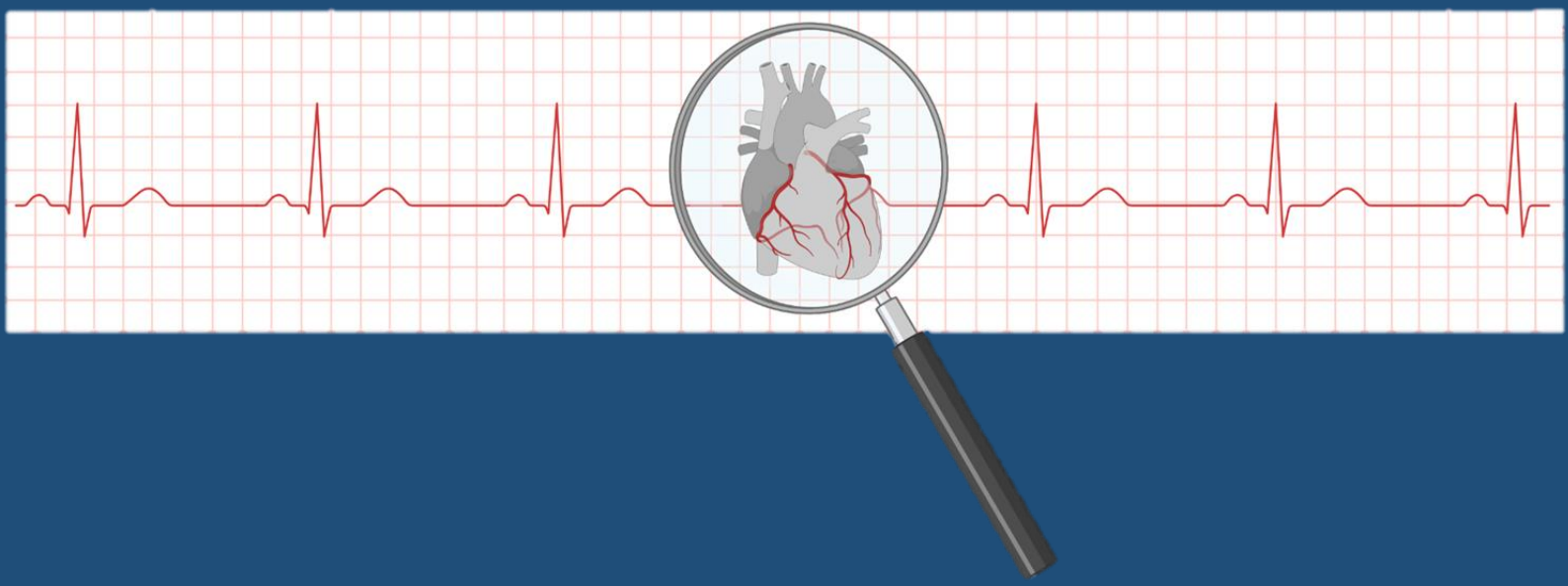
Diagnostic value of electrocardiography for coronary vasomotor dysfunction in patients with persistent angina with non-obstructive coronary artery disease



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Abstract

Introduction: Coronary vasomotor dysfunction (CVDys) comprises coronary vasospasm (CVS) and/or coronary microvascular dysfunction (CMD) and is highly prevalent in patients with angina and non-obstructive coronary artery disease (ANOCA). The current reference standard to diagnose CVDys is with invasive coronary function testing (CFT), which carries non-negligible risks and costs. A low-risk non-invasive diagnostic test would therefore be valuable to optimize patient selection for CFT. Current evidence suggests the presence of potential diagnostic markers for CVDys on the rest ECG. Therefore, this study was set up as a first exploration to investigate the potential of a 12-lead 10 seconds rest ECG as non-invasive diagnostic tool for CVDys in ANOCA patients.

Methods: We collected clinical data and 12-lead 10 seconds rest ECGs of 128 ANOCA patients who underwent CFT and participated in the NL-CFT registry. We compared heart axis and conduction times between patients without CVDys and patients with CVS, CMD and the combination of both. Furthermore, binary logistic regression was performed using univariable and multivariable analysis, with CVDys as binary endpoint and sex, heart axis, heart rate and the conduction times as possible predictors. In addition, we calculated the sensitivity, specificity and Area Under the Curve (AUC) and created ROC curves with 95% CI using bootstrapping for the QTc interval corrected with Bazett (QTcB) and Fridericia (QTcF).

Results: In total, we analyzed 23 patients without CVDys, 35 patients with CVS, 24 with CMD and 26 with CVS/CMD, of whom 95% were women. Heart axis, heart rate, PQ interval and QRS duration were comparable between the groups. A small prolongation of the QTcB and QTcF was observed in patients with CVDys compared to patients without CVDys. Univariable logistic regression analysis showed that the QTcB and QTcF were both associated with a diagnosis of CVDys (QTcB: OR (95% CI) = 1.03 (1.00-1.06), p=0.03; QTcF: OR = 1.04 (1.00-1.08), p=0.045) and were the only predictors that

remained significant during multivariable logistic regression. Use of the QTcB and QTcF interval resulted in similar AUCs (QTcB: AUC (95% CI) = 0.65 (0.52-0.77); QTcF: AUC (95% CI) = 0.65 (0.51-0.77)), but very low ability to discriminate between CVDys and non-CVDys.

Conclusions: The 12-lead 10-seconds rest ECG is not deemed suitable for the diagnostic evaluation of CVDys. We recommend focusing on ECG monitoring tools other than the 10-seconds rest ECG to further explore the value of ECG for non-invasive CVDys risk stratification.

Layman's summary

Patients with chest pain complaints are suspected of an obstruction in the vessels of the heart, also known as atherosclerosis. However, many of these patients do not have an obstruction. We call these patients the ANOCA patients. Nowadays, we know that the cause of symptoms in ANOCA patients may be due to a dysfunction of the vessels, called coronary vasomotor dysfunction (CVDys). CVDys is either squeezing of the vessels (abbreviated as CVS) and/or the inability of the small vessels to function properly (abbreviated as CMD). CVDys is diagnosed with coronary function testing (CFT), a test that takes place at the heart catheterization lab. However, this test carries non-negligible risks and costs and has a large burden on patients. Therefore, there is a need for a low-risk diagnostic tool for CVDys. The rest electrocardiogram (ECG) may be such a low-risk tool, as the electrical activity of the heart is measured for only ten seconds with electrodes placed on the body. There are studies that suggest that some markers (e.g. QTc interval prolongation) on the rest ECG differ in ANOCA patients with CVDys compared to patients without CVDys. As it is interesting to further test whether there are markers that can be used in diagnosis, we set up a study as a first exploration to investigate the potential of a rest ECG as diagnostic tool for CVDys in ANOCA patients.

We collected clinical characteristics (e.g. risk factors) and a rest ECG obtained on the day of and prior to CFT of 128 ANOCA patients that underwent CFT and participated in the NL-CFT registry. We compared several ECG parameters, such as heart axis and conduction times, between patients without CVDys and patients with CVS, patients with CMD and patients with a combination of both. Furthermore, we used different models to investigate which ECG parameters are linked to CVDys. For the ECG parameters linked to CVDys, we evaluated their ability to separate patients with CVDys from those without CVDys.

In total, we analyzed 23 patients without CVDys, 35 patients with CVS, 24 with CMD and 26 with CVS/CMD, of whom 95% were women. Most ECG parameters were comparable between the groups,

except for a small prolongation of the QT-interval corrected for heart rate in two different ways (i.e. QTcB and QTcF) in patients with CVDys compared to patients without CVDys. However, both QTcB and QTcF had very low ability to separate patients with CVDys from patients without CVDys. This study therefore shows that the rest ECG is not suitable for the diagnostic evaluation of CVDys. We recommend focusing on ECG monitoring tools other than the 10-seconds rest ECG to further explore the value of ECG for CVDys diagnosis in a low-risk manner.

Introduction

Patients with angina but no obstructive coronary artery disease (ANOCA) present a diagnostic challenge to physicians. Recent evidence indicates a high prevalence of coronary vasomotor dysfunction (CVDys) in these patients.[1] CVDys comprises coronary vasospasm (CVS) and/or coronary microvascular dysfunction (CMD).

Invasive coronary function testing (CFT) is currently the reference standard for CVDys diagnosis and is recommended in recent clinical guidelines to be considered in patients with ANOCA.[2] CFT involves the administration of intracoronary acetylcholine (Ach) or ergonovine (EG) for the detection of coronary spasm. To investigate the microvascular function, the coronary flow reserve and microvascular resistance are assessed in response to adenosine using a dedicated guidewire technique.[3] As a consequence of the recent guideline recommendations, CFTs are becoming more common in routine clinical care. However, these tests carry non-negligible risks and costs and its burden on patients is large. To optimize patient selection for CFT, a low-risk non-invasive diagnostic test would be valuable.

An essential and low-risk non-invasive tool to detect various cardiac diseases is the electrocardiogram (ECG). Signs of myocardial ischemia on the ECG recorded during angina episodes or invasive CFT are already used as criterium in the diagnosis of CVS.[4] However, the rest ECG may also be valuable as non-invasive diagnostic tool. For instance, in patients with heart failure with preserved ejection fraction, a cardiac disease linked to CVDys[5], changes in rest ECG were observed (i.e. QTc prolongation[6]). Moreover, evidence is available suggesting an association between QTc prolongation and CMD.[7–12] This implies the presence of potential diagnostic markers for CVDys on the rest ECG. Therefore, this study was set up as a first exploration to investigate the potential of a 12-lead 10 seconds rest ECG as non-invasive diagnostic tool for CVDys in ANOCA patients.

Methods

Study population

We analyzed clinical data and rest ECGs of 128 ANOCA patients who participated in the NL-CFT registry. The NL-CFT registry is a prospective observational registry of patients undergoing CFT in participating centers throughout The Netherlands.[4] We included patients without a history of a percutaneous coronary intervention or a coronary artery bypass graft who underwent complete CFT in the Radboud University Medical Center (Nijmegen, The Netherlands). To ensure similar power in each of the groups, we included a weighed sample of patients with no CVS/CMD (n=25), only CVS (n=41), only CMD (n=29), and the combination of CVS and CMD (n=33) based on CFT outcome. All patients provided informed consent.[4] We excluded patients 1) without a preprocedural ECG (n=12), 2) if their ECG did not show sinus rhythm (n=2) and 3) if the ECG showed a complete bundle branch block (n=6).

Data acquisition

The data consisted of clinical data collected in a web-based electronic data capture system (Castor EDC, The Netherlands) within the NL-CFT registry[4] and a 12-lead pre-procedural resting ECG measured on the day of CFT in PDF (Philips Pagewriter TC50) and raw-format (XML). The ECG was measured in clinical setting after hospital admission and prior to CFT.

ECG recordings analysis

The raw ECG recordings were analyzed using Python (version 3.8.10). The raw-format files were loaded into Python using the 'sierraecg' package (version 0.2.1). We filtered the ECG signals using a third-order bandpass Butterworth filter with a lower cut-off frequency of 0.67 Hz and higher cut-off frequency of 75 Hz. We marked the beginning and end of the P-wave, QRS complex and ST segment for all sinus rhythm beats per ECG blinded for CFT outcome. This strategy was used to calculate the

heart rate in beats per minute based on the median interval between the beginning of each QRS complex on the 10-seconds ECG. Furthermore, we calculated the median PQ-interval, QRS duration and QT-interval in milliseconds per ECG. The QT interval was corrected for heart rate using Bazett's formula (QTcB) and Fridericia's formula (QTcF). Additionally, we used the heart axis calculated by the ECG device as shown on the PDF.

Outcome assessment

All included patients underwent a CFT according to the NL-CFT protocol.[4] Patients were diagnosed with CVDys in case of CVS and/or CMD. CMD was defined as an abnormal coronary flow reserve (CFR ≤ 2.0) and/or abnormal index of microvascular resistance (IMR ≥ 25) as measured with the bolus thermodilution method. We defined CVS as the presence of epicardial and/or microvascular vasospasm during acetylcholine spasm provocation testing. To ensure that vasoreactivity is not influenced by medication when performing spasm provocation testing, patients temporarily stopped the intake of long-acting anti-anginal medication and other vasoactive substances 24–48 h before CFT.

Statistical analysis

Statistical analyses were performed using R (version 4.2.2). To assess differences in baseline characteristics between the groups, we compared the baseline characteristics between patients without CVDys, patients with only CVS, patients with only CMD and patients with a combination of both CVS and CMD. Continuous data are presented as mean with the standard deviation or median with interquartile range, where appropriate. Categorical data are presented as frequencies and proportions. To investigate which ECG parameters can be potential diagnostic markers for CVDys, we compared heart axis and conduction times between patients without CVDys and patients with CVS, CMD and the combination of both. Considering all ECG parameters were continuous with a normal distribution, we presented the results as mean with standard deviation. In addition, we used an

unpaired t-test to test for statistical significant differences (defined as $p<0.05$) in ECG parameter between patients without CVDys and patients with different CVDys endotypes. We performed a sub-analysis in women for the ECG parameters that showed of interest. Binary logistic regression was performed using univariable and multivariable analysis with CVDys as binary endpoint, to examine the relation between CVDys and sex, heart axis, heart rate, PQ time, QRS duration, QTcB and QTcF as predictors. The results of the regression analysis are presented as OR (95% CI). To assess which predictors were highly correlated, Pearson's correlation coefficients were calculated between the continuous predictors and the point-biserial correlation between the binary variable sex and the continuous predictors. We constructed four different full multivariable models based on the correlation coefficients, ensuring the absence of significantly correlated predictors within the same model, and used stepwise backward selection and the likelihood ratio test to refine the model until achieving optimal parsimony. We calculated the sensitivity, specificity and Area Under the Curve (AUC) for the predictors that showed of importance for CVDys diagnosis in the regression analysis to evaluate the diagnostic accuracy. Bootstrapping was employed to create ROC curves and estimate the 95% CI for the sensitivities, specificities and area under the curve using Python (version 3.8.10). A total of 1000 bootstrap samples were generated by resampling of the original dataset with replacement. In a sub-analysis, ROC curves were plotted to evaluate the discriminatory ability of the predictors for CMD diagnosis.

Results

Clinical characteristics

In total, we analyzed 108 patients of whom 95% were women and the mean age was 58 (± 8) years (Figure 1). We included 35 patients with CVS, 24 with CMD and 26 with a combination of both

outcomes (CVS/CMD). The group with a negative CFT result (CFT-) and therefore no CVDys were diagnosed as having no cardiac chest pain and consisted of 23 patients.

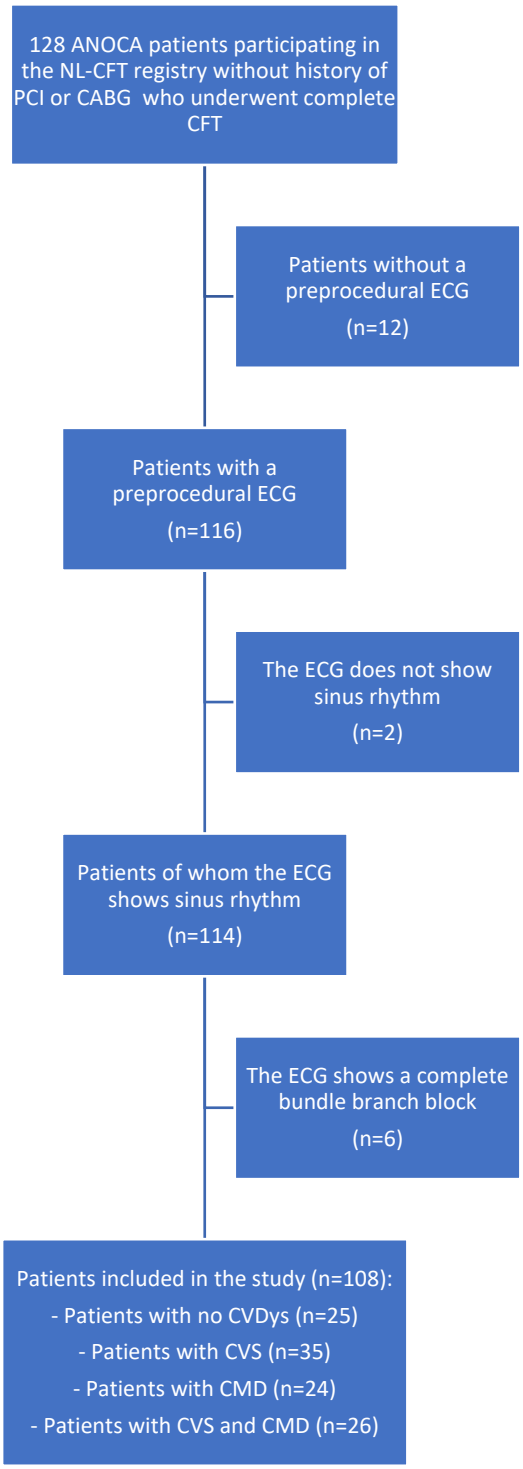


Figure 1: Study flow diagram

Table 1 shows the baseline characteristics of all patients, stratified by diagnosis following the CFT. Overall, CMD patients had a high cardiovascular risk profile compared to patients without CVDys, with a higher median BMI (28.0 vs 24.1, respectively) and higher prevalence of all cardiovascular risk factors (CMD vs CFT-; hypertension: 75% vs 26%, hypercholesteremia: 67% vs 48%, diabetes mellitus: 17% vs 4%, former/current smoker: 67% vs 48%, positive family history: 50% vs 26%). The prevalence of hypertension and a positive family history for cardiovascular disease was also higher in patients with CVS or the combined endotype compared to patients without CVDys (CVS vs CFT-: 51% vs 26% and 63% vs 26%, respectively; CVS/CMD vs CFT-: 54 vs 26% and 38% vs 26%, respectively).

Patients with CVDys (i.e. for any of the three subtypes) were more often prescribed calcium channel blockers and nicorandil compared to patients without CVDys (CVS vs CFT-: 77% vs 57% and 11% vs 0%, respectively; CMD vs CFT-: 79% vs 57% and 13% vs 0%, respectively; CVS/CMD vs CFT-: 73% vs 57% and 23% vs 0%, respectively). Furthermore, patients with CVS less often used cholesterol lowering medications as compared to CFT- patients (34% vs 61%, respectively). Table 1 also shows that in total 6% of all patients did not use any cardiovascular medication. On the other hand, 75% of all patients were prescribed medications for at least two of the cardiovascular drug categories.

Table 1: Baseline characteristics stratified by CFT outcome.

	CFT- (n=23)	**CVS (n=35)**	**CMD (n=24)**	**CVS/CMD (n=26)**
Female sex (%)	91%	94%	96%	100%
Age [years] (mean ± SD)	57 ± 11	57 ± 8	59 ± 5	59 ± 8
BMI (median [Q1-Q3])	24.1 [21.8-27.4]	26.0 [23.5-28.5]	28.0 [26.5-30.4]	24.6 [22.8-26.7]
Risk factors				
Hypertension (%)	6 (26)	18 (51)	18 (75)	14 (54)
Hypercholesteremia (%)	11 (48)	14 (40)	16 (67)	13 (50)
Diabetes Mellitus (%)	1 (4)	3 (9)	4 (17)	1 (4)
Former/current smoker (%)	11 (48)	12 (34)	16 (67)	8 (31)
Positive family history (%)	6 (26)	22 (63)	12 (50)	10 (38)
Medical history				

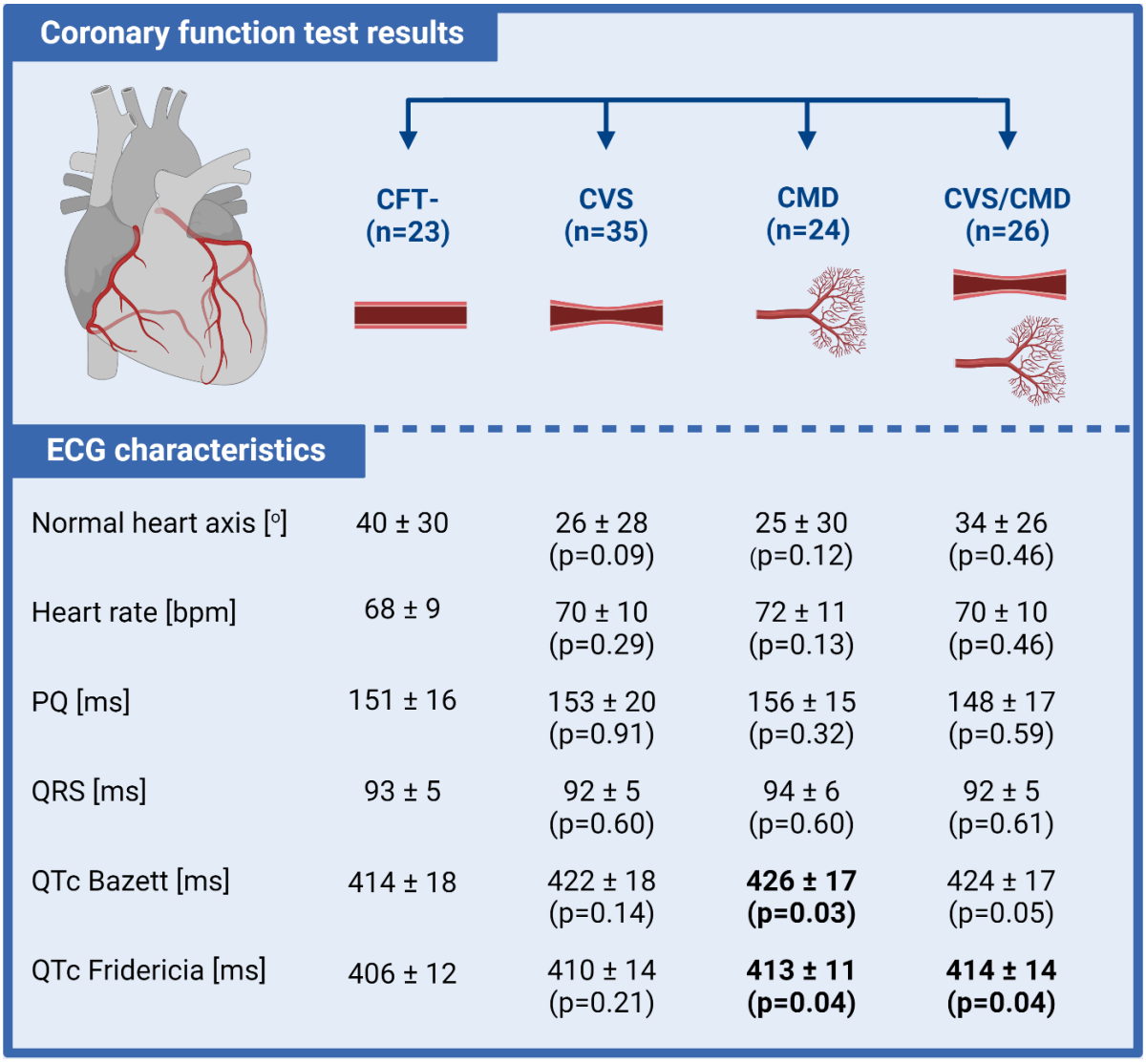
ACS (%)	3 (13)	5 (14)	3 (13)	3 (12)
Medication use				
Beta blockers (%)	6 (26)	8 (23)	7 (29)	5 (19)
Calcium channel blockers (%)	13 (57)	27 (77)	19 (79)	19 (73)
Long-acting nitrates (%)	5 (22)	5 (14)	7 (29)	6 (23)
Nicorandil (%)	0 (0)	4 (11)	3 (13)	6 (23)
Antiplatelets (%)	7 (30)	13 (37)	7 (20)	9 (35)
Anti-hypertensives (%)	7 (30)	14 (40)	11 (31)	12 (46)
Cholesterol lowering (%)	14 (61)	12 (34)	16 (67)	15 (58)
At least one drug per category				
0 Categories (%)	2 (9)	1 (3)	1 (4)	3 (12)
1 Category (%)	5 (22)	9 (26)	3 (13)	3 (12)
2 Categories (%)	6 (26)	6 (17)	8 (33)	3 (12)
3 Categories (%)	5 (22)	15 (43)	4 (17)	8 (31)
4 Categories (%)	5 (22)	3 (9)	4 (17)	6 (23)
>4 Categories (%)	0 (0)	1 (3)	4 (17)	3 (12)

ACS = Acute coronary syndrome; CFT- = Negative coronary function test result; CMD = Coronary microvascular dysfunction; CVS = Coronary vasospasm.

Diagnostic accuracy of electrocardiographic signals

Figure 2 shows a summary of the ECG characteristics. We observed a left anterior fascicular block in six patients (CFT-: n=2, CMD: n=3, CVS: n=1). Based on the ECGs showing normal heart axis (-30^{o} to 90^{o}, n=102), no significant differences in heart axis were observed between ANOCA patients with and without CVDys (CVS vs CFT-: 26^{o} $\pm$ 28^{o} vs 40^{o} $\pm$ 30^{o}, p=0.09; CMD vs CFT- 25^{o} $\pm$ 30^{o} vs 40^{o} $\pm$ 30^{o}, p=0.12; CVS/CMD vs CFT-: 34^{o} $\pm$ 26^{o} vs 40^{o} $\pm$ 30^{o}, p=0.46). Heart rate, PQ interval and QRS duration were comparable between all groups. The QTcB and QTcF interval were statistically significantly longer in CMD patients compared to patients without CVDys, although the difference was small (ΔQTcB= 12 ms, ΔQTcF = 7 ms). The QTcF interval was also statistically significantly longer in patients with the combined endotype (CVS/CMD) in comparison to patients without CVDys (ΔQTc = 8 ms). In women, the statistically significant difference in QTcF remained between patients without CVDys and patients with CMD (406 ± 12 ms vs 413 ± 11 ms, p=0.03, respectively) or CVS/CMD (406 ± 12 ms vs

414 ± 14 ms, p=0.03, respectively). The QTcB was no longer statistically significantly different between patients without CVDys and CMD (415 ± 18 ms vs 426 ± 18 ms, p=0.06, respectively).



ECG characteristics

	CFT- (n=23)	CVS (n=35)	CMD (n=24)	CVS/CMD (n=26)
Normal heart axis [°]	40 ± 30	26 ± 28 (p=0.09)	25 ± 30 (p=0.12)	34 ± 26 (p=0.46)
Heart rate [bpm]	68 ± 9	70 ± 10 (p=0.29)	72 ± 11 (p=0.13)	70 ± 10 (p=0.46)
PQ [ms]	151 ± 16	153 ± 20 (p=0.91)	156 ± 15 (p=0.32)	148 ± 17 (p=0.59)
QRS [ms]	93 ± 5	92 ± 5 (p=0.60)	94 ± 6 (p=0.60)	92 ± 5 (p=0.61)
QTc Bazett [ms]	414 ± 18	422 ± 18 (p=0.14)	**426 ± 17 (p=0.03)**	424 ± 17 (p=0.05)
QTc Fridericia [ms]	406 ± 12	410 ± 14 (p=0.21)	**413 ± 11 (p=0.04)**	**414 ± 14 (p=0.04)**

Figure 2: Overview of the ECG characteristics in ANOCA patients diagnosed with and without CVDys. P-values were determined with the unpaired t-test and relative to the CFT- group. CFT- = Negative coronary function test result; CMD = Coronary microvascular dysfunction; CVS = Coronary vasospasm.

Table 2 shows the results of the univariable logistic regression analysis. The QTcB and QTcF were both associated with a diagnosis of CVDys (QTcB: OR (95% CI) = 1.03 (1.00-1.06), p=0.03; QTcF: OR = 1.04 (1.00-1.08), p=0.045).

Table 2: Predictors of CVDys

	OR (95% CI)	**P-value**
Univariable logistic regression		
Sex (male)	0.38 (0.06-2.45)	0.311
Heart axis [^{o}]	0.99 (0.98-1.01)	0.266
Heart rate [bpm]	1.03 (0.98-1.08)	0.202
PQ time [ms]	1.00 (0.97-1.03)	0.943
QRS duration [ms]	0.99 (0.91-1.08)	0.824
QTc Bazett [ms]	1.03 (1.00-1.06)	0.030
QTc Fridericia [ms]	1.04 (1.00-1.08)	0.045

Correlation coefficients between the predictors are shown in Supplemental Table 1. Significant correlation existed for the heart rate and PQ time (R=-0.19, p=0.047), heart rate and QTcB (R=0.67, p<<0.05), PQ time and QRS duration (R=0.27, p=0.006) and QTcB and QTcF (R=0.83, p<<0.05). This resulted in the following predictors in the four full multivariable models: 1) Sex, heart axis, heart rate, QRS duration and QTcF, 2) Sex, heart axis, QRS duration and QTcB, 3) Sex, heart axis, PQ time and QTcF and 4) Sex, heart axis, PQ time and QTcB. Only the QTcF remained significant for model 1 and 3 and for model 2 and 4 only the QTcB remained significant.

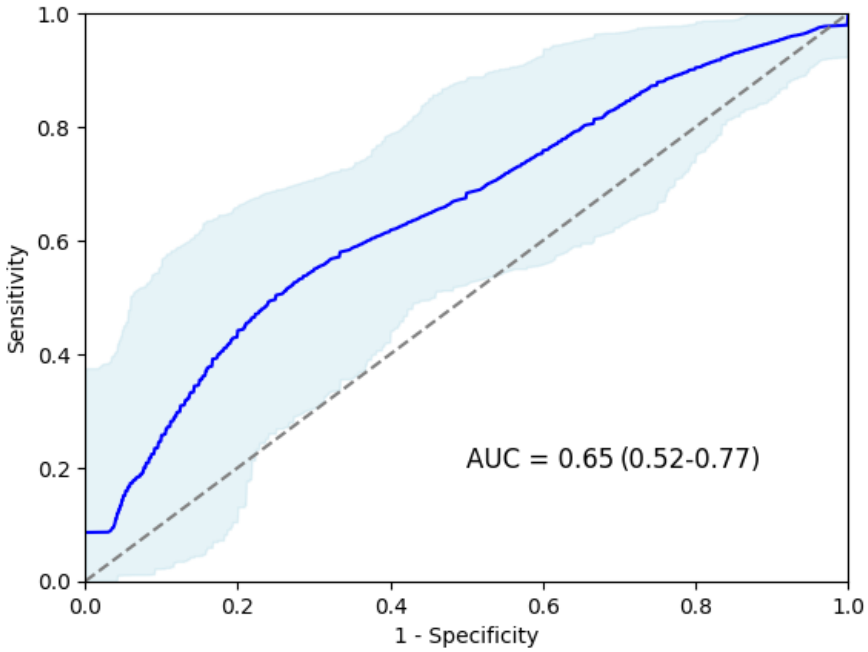
Figure 3 shows the receiver operating characteristic (ROC) curves and area under the curve (AUC) with 95% CI for CVDys diagnosis using QTcB (Figure 3A) and QTcF (Figure 3B) separately. In Table 3 the corresponding sensitivities and specificities with 95% CI can be observed. Use of the QTcB interval and QTcF interval resulted in similar AUCs (QTcB: AUC (95% CI) = 0.65 (0.52-0.77); QTcF: AUC (95% CI) = 0.65 (0.51-0.77)), but low ability to discriminate between CVDys and non-CVDys. To

distinguish patients with CMD from patients without CMD, the AUC remained the same as for CVDys diagnosis for both the QTcB interval (AUC (95% CI)= 0.65 (0.49-0.71)) and the QTcF interval (AUC (95% CI) = 0.65 (0.52-0.73)) (Figure 4).

Table 3: Sensitivity and specificity for CVDys diagnosis corresponding to different QTc thresholds calculated with the Bazett's formula and Fridericia's formula.

QTc	**Bazett's formula**		**Fridericia's formula**	
Threshold [ms]	**Sensitivity (95% CI)**	**Specificity (95% CI)**	**Sensitivity (95% CI)**	**Specificity (95% CI)**
>375	1.00 (1.00-1.00)	0.00 (0.00-0.00)	1.00 (1.00-1.00)	0.00 (0.00-0.00)
>385	0.99 (0.96-1.00)	0.04 (0.00-0.15)	0.99 (0.96-1.00)	0.04 (0.00-0.14)
>395	0.93 (0.87-0.98)	0.22 (0.05-0.40)	0.92 (0.86-0.98)	0.26 (0.09-0.44)
>405	0.87 (0.80-0.94)	0.30 (0.12-0.48)	0.68 (0.58-0.78)	0.48 (0.29-0.69)
>415	0.69 (0.60-0.79)	0.43 (0.23-0.65)	0.42 (0.32-0.53)	0.83 (0.65-0.96)
>425	0.47 (0.36-0.58)	0.78 (0.59-0.94)	0.21 (0.13-0.30)	0.91 (0.77-1.00)
>435	0.24 (0.15-0.33)	0.91 (0.77-1.00)	0.04 (0.00-0.08)	1.00 (1.00-1.00)
>445	0.11 (0.05-0.17)	0.91 (0.76-1.00)	0.00 (0.00-0.00)	1.00 (1.00-1.00)
>455	0.01 (0.00-0.04)	1.00 (1.00-1.00)	0.00 (0.00-0.00)	1.00 (1.00-1.00)
>465	0.00 (0.00-0.00)	1.00 (1.00-1.00)	0.00 (0.00-0.00)	1.00 (1.00-1.00)

A) QTc Bazett – ROC curve with 95% CI



B) QTc Fridericia – ROC curve with 95% CI

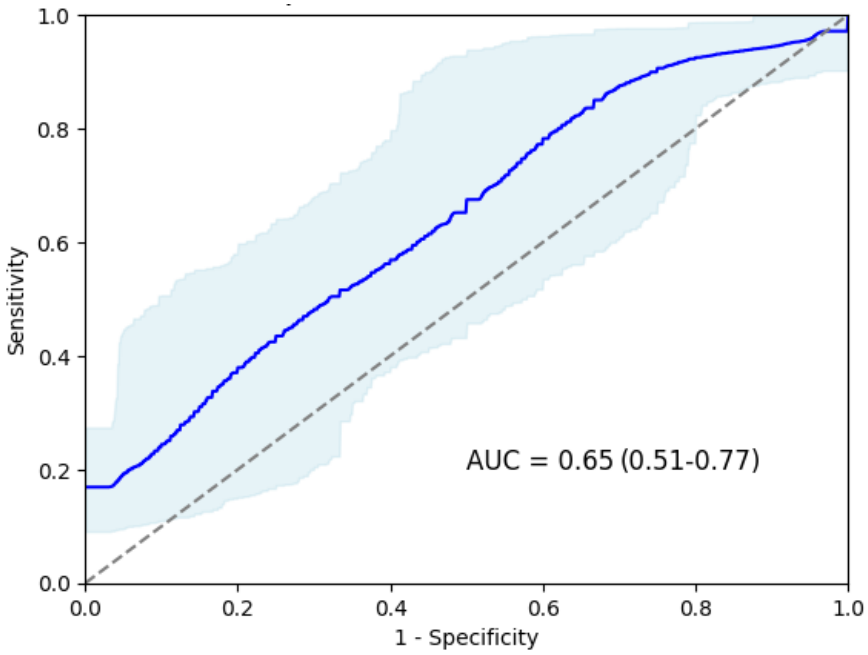
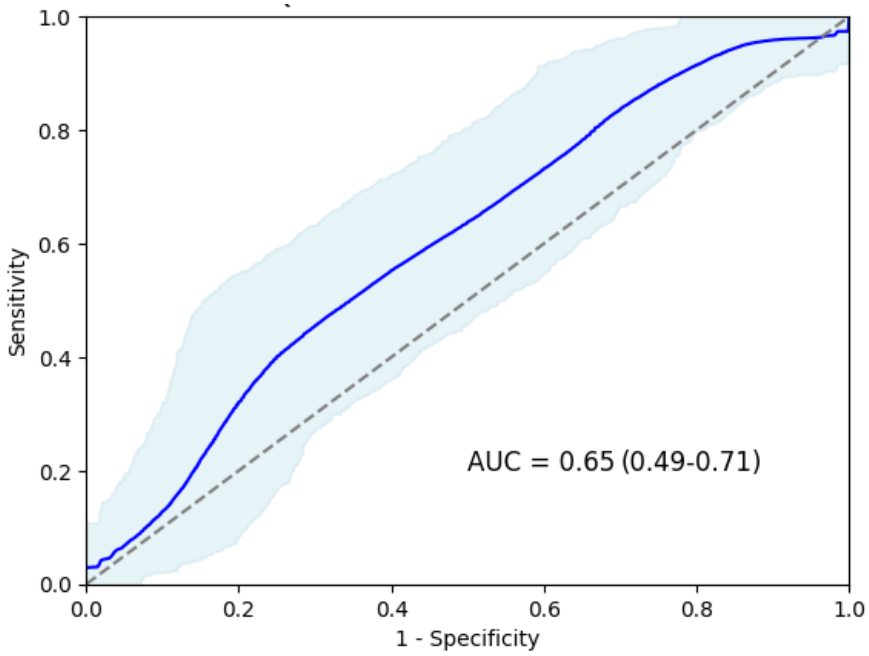


Figure 3: Receiver operating characteristic (ROC) curves and Area Under the Curve (AUC) with 95% CI for CVDys diagnosis of the QTc calculated with A) Bazett's formula, and B) Fridericia's formula.

A) QTc Bazett – ROC curve with 95% CI



B) QTc Fridericia – ROC curve with 95% CI

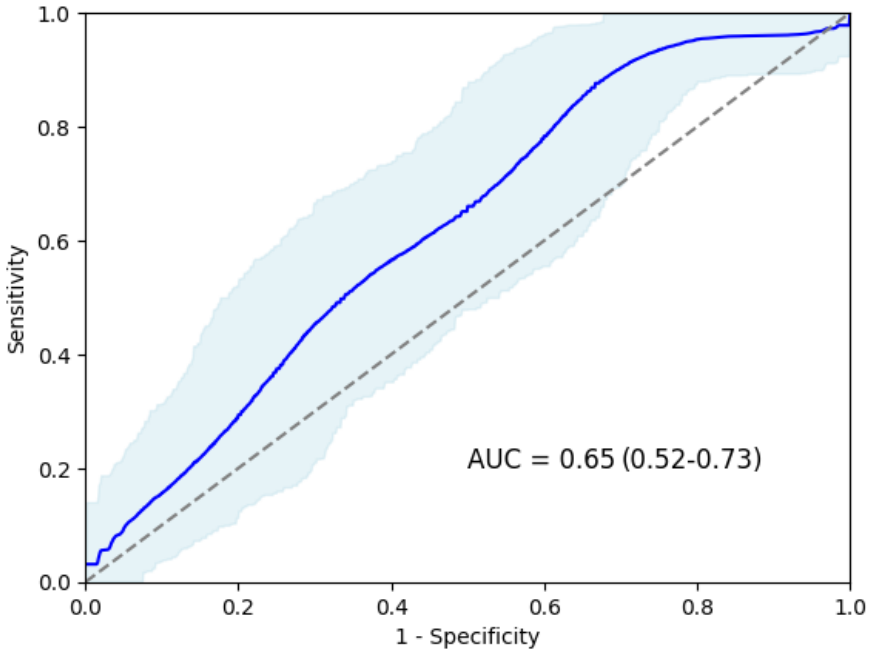


Figure 4: Receiver operating characteristic (ROC) curves and Area Under the Curve (AUC) with 95% CI for CMD diagnosis of the QTc calculated with A) Bazett's formula, and B) Fridericia's formula.

Discussion

This study served as an exploratory study investigating the potential of a 12-lead 10 seconds rest ECG as non-invasive risk tool for CVDys. Our findings suggest that the 12-lead 10-seconds rest ECG has very low diagnostic value for CVDys. The 12-lead ECGs were mostly comparable between patients with and without CVDys undergoing CFT, except for a slightly longer QTc interval in patients with CVDys compared to patients without CVDys. Although the regression analysis showed that the QTcB and QTcF were both associated with a diagnosis of CVDys, their ability to discriminate between patients with CVDys and those without CVDys in this study was very weak as indicated by the moderate to low AUC values and the 95% CI exceeding the 0.5 chance line for nearly every possible combination of sensitivity and specificity. The discriminatory ability of the QTcB and QTcF did not improve for distinguishing patients with CMD from those without CMD in comparison to patients with CVDys from patients without CVDys.

The observed QTc interval prolongation can be an effect of myocardial ischemia, but can also be influenced by other factors, such as diabetes mellitus. The longer QTc interval in patients with CVDys compared to patients without CVDys can also be an early sign of electrical remodeling as seen in patients with HFpEF.[5] However, the QTc interval in CVDys patients is smaller than seen in HFpEF patients[6] and well within normal values. To correct the QT interval for heart rate, we used two formulas (Bazett's and Fridericia's). Bazett's formula is the most frequently used formula in clinical practice but tends to under-correct the QTc interval at lower heart rates. As the observed heart rates in our cohort were generally low, we also applied Fridericia's formula which is more suitable for low heart rates.[13] The two formulas gave similar results and showed longer QTc intervals in patients with CVDys as compared to patients without CVDys, although the statistical significance varied between the groups.

The absence of differences in the PQ time and QRS duration and the longer QTc interval in patients with CMD compared to patients without CVDys are in line with previously published studies.[7–12,14,15] However, none of the previously published studies reported diagnostic accuracy measures. This is the first study examining cardiac rest electrophysiology and reporting on diagnostic accuracy measures of ANOCA patients without CVDys and ANOCA patients with different CVDys endotypes simultaneously. We explored the potential of a 12-lead 10 seconds rest ECG as non-invasive risk tool for CVDys and although we observed QTc interval prolongation in patients with CVDys compared to patients without CVDys our findings underscore a very low diagnostic value of the 10 seconds rest ECG.

Our study had some limitations. In daily practice most patients undergoing CFT have CVS.[1] Hence, oversampling was necessary to ensure that we would have a similar number of patients in all groups to be able to explore ECG differences. As a results, the positive and negative predictive value were not calculated, as these diagnostic performance measures depend on the disease prevalence. Considering the exploratory nature of this study this is not a concern. The positive and negative predictive value become more relevant in subsequent phases, where understanding the likelihood of true positive and true negative results given the prevalence of the disease becomes crucial for clinical implementation and guiding of patient care.[16] Contemplating that our results suggest a very low diagnostic value of the 10 seconds rest ECG for CVDys, further exploration of these diagnostic accuracy measures is probably unnecessary.

Noticeably, the number of males in our study was very low at 5%. It is however known that sex differences in the ECG exist between men and women. For instance, women generally exhibit a slightly higher resting heart rate and a longer (corrected) QT interval compared to men.[17,18] It is therefore uncertain whether our results are generalizable to men with ANOCA. Furthermore, current studies indicate that ANOCA is more common in women than in men, but the number of men

referred for and undergoing coronary function tests is even lower, suggesting a potential selection bias in referrals.[9] The intended target population for employing ECG as a non-invasive risk tool for CVDys therefore probably differs in male-female ratio from the population undergoing CFT. However, the results of our sub-analysis in women suggest that there is minimal disparity in the QTc between men and women with CVDys and without CVDys.

The standardized CFT protocol used by all participating centers of the NL-CFT registry dictates that long-acting anti-anginal medication and other vasoactive substances must be stopped temporarily 24 to 48 hours before CFT to ensure that vasoreactivity is not influenced by medication when performing spasm provocation testing.[4] Therefore, differences in anti-anginal medication use likely did not influence our results.

Patients with CVDys suffer from frequent angina episodes, which can have altered their cardiac electrophysiology in rest. On the other hand, it is possible that patients had complaints while the 12-lead rest ECG was made. It is unknown whether the observed differences in QTc interval are an effect of altered rest electrophysiology in patients with CVDys or from angina episodes during ECG acquisition, which could impact the diagnostic value of a rest ECG. For future research, it is therefore important to ascertain whether patients experience symptoms during ECG acquisition.

This study focused on conduction times in sinus rhythm ECGs. However, other electrophysiology features not covered by the conduction times might be altered in patients with CVDys. Furthermore, ECG differences can be left undetected due to the short 10-seconds measuring interval. Therefore, it can be of interest to investigate variability of ECG features over time. In addition, electrophysiologic differences can occur at other instants than in rest. For example, latent ECG abnormalities that are not evident during rest can be disclosed during exercise or angina episodes.[19] Other non-invasive ECG

monitoring tools, such as Holter devices, might therefore be worthwhile to explore in future studies focusing on non-invasive CVDys risk stratification.

Conclusion

The 12-lead 10-seconds rest ECG is not deemed suitable for the diagnostic evaluation of CVDys. Heart axis and conduction times were comparable between patients with and without CVDys undergoing CFT. Patients with CVDys had a slightly longer QTc interval compared to patients without CVDys undergoing CFT. We recommend focusing on ECG monitoring tools other than the 10-seconds rest ECG to further explore the value of ECG for non-invasive CVDys risk stratification.

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Supplemental Material

Supplemental Table 1

Supplemental Table 1: Correlation coefficients between predictors.

	Sex	Heart axis [°]	Heart rate [bpm]	PQ time [ms]	QRS duration [ms]	QTc Bazett [ms]	QTc Fridericia [ms]
Sex	R=1.00 (p<<0.05)	R=0.14 (p=0.15)	R=0.10 (p=0.31)	R=-0.06 (p=0.53)	R=-0.01 (p=0.93)	R=0.13 (p=0.18	R=0.10 (p=0.33)
Heart axis [°]	R=0.14 (p=0.15)	R=1.00 (p<<0.05)	R=0.12 (p=0.23)	R=-0.14 (p=0.15)	R=-0.06 (p=0.51)	R=-0.02 (p=0.83)	R=-0.11 (p=0.26)
Heart rate [bpm]	R=0.10 (p=0.31)	R=0.12 (p=0.23)	R=1.00 (p<<0.05)	R=-0.19 (p=0.047)	R=-0.05 (p=0.63)	R=0.67 (p<<0.05)	R=0.14 (p=0.14)
PQ time [ms]	R=-0.06 (p=0.53)	R=-0.14 (p=0.15)	R=-0.19 (p=0.047)	R=1.00 (p<<0.05)	R=0.27 (p=0.006)	R=-0.02 (p=0.87)	R=0.13 (p=0.18)
QRS duration [ms]	R=-0.01 (p=0.93)	R=-0.06 (p=0.51)	R=-0.05 (p=0.63)	R=0.27 (p=0.006)	R=1.00 (p<<0.05)	R=0.03 (p=0.77)	R=0.08 (p=0.38)
QTc Bazett [ms]	R=0.13 (p=0.18)	R=-0.02 (p=0.83)	R=0.67 (p<<0.05)	R=-0.02 (p=0.87)	R=0.03 (p=0.77)	R=1.00 (p<<0.05)	R=0.83 (p<<0.05)
QTc Fridericia [ms]	R=0.10 (p=0.33)	R=-0.11 (p=0.26)	R=0.14 (p=0.14)	R=0.13 (p=0.18)	R=0.08 (p=0.38)	R=0.83 (p<<0.05)	R=1.00 (p<<0.05)