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Prognostic Significance of Different Ventricular Ectopic Burdens During Submaximal Exercise in Asymptomatic UK Biobank Subjects

Short title: Prognostic value of PVCs on submaximal exercise

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Abstract

Background

The consequences of exercise-induced premature ventricular contractions (PVCs) in asymptomatic individuals remain unclear. This study aimed to assess the association between PVC burdens during submaximal exercise and major adverse cardiovascular events (MI/HF/LTVA: myocardial infarction (MI), heart failure (HF), and life-threatening ventricular arrhythmia (LTVA)), and all-cause mortality. Additional endpoints were: MI, LTVA, HF and cardiovascular mortality.

Methods

A neural network was developed to count PVCs from ECGs recorded during exercise (6 min) and recovery (1 min) in 48,315 asymptomatic participants from UK Biobank. Associations were estimated using multivariable Cox proportional hazard models. Explorative studies were conducted in subgroups with CMR imaging data (N = 6290) and NT-proBNP levels (N = 4607) to examine whether PVC burden was associated with subclinical cardiomyopathy.

Results

Mean age was 56.8 (+/-8.2 years); 51.1% were female, and median follow-up was 12.6 years. Low PVC counts during exercise and recovery were both associated with MI/HF/LTVA risk, independently of clinical factors: adjusted hazard ratio [HR]: 1.2 (1-5 exercise PVCs, $p < 0.001$) and HR 1.3, (1-5 recovery PVCs, $p < 0.001$). Risks were higher with increasing PVC count: HR 1.8 (>20 exercise PVCs, $p<0.001$) and HR 1.6 (>5 recovery PVCs, $p < 0.001$). A similar trend was observed for all-cause mortality, although associations were only significant for high PVC burdens: HRs: 1.6 (>20 exercise PVCs, $p < 0.001$) and 1.5 (>5 recovery PVCs, $p < 0.001$). Complex PVCs rhythms were associated with higher risk compared to PVC count alone. PVCs were also associated with incident HF, LTVA, and cardiovascular mortality, but not MI. In the explorative studies, high PVC burden was associated with larger left-ventricular volumes, lower ejection fraction, and higher levels of NT-proBNP compared to participants without PVCs.

Conclusion

In this cohort of middle-aged and older adults, PVC count during submaximal exercise and recovery were both associated with MI/HF/LTVA, all-cause mortality, HF, LTVAs and cardiovascular mortality, independent of clinical and exercise test factors, indicating an incremental increase in risk as PVC count rises. Complex PVCs rhythms werere associated with higher risk compared to PVC count alone. Underlying mechanisms may include presence of subclinical cardiomyopathy.

Keywords: Electrocardiogram, exercise, ventricular ectopy, major adverse cardiovascular events, mortality

Clinical Perspectives

What is new?

- This study assessed the risk for a wide range of premature ventricular complex (PVC) counts, during submaximal exercise and recovery, in the largest population-based cohort study to date with exercise data, comprising 48,400 asymptomatic individuals with a mean age of 56.8 (+/-8.2 years) from the UK Biobank study.
- Results show incremental increases in risk between PVC burden during submaximal exercise and major adverse cardiovascular events, heart failure, and ventricular arrhythmias, independently of clinical risk factors, during a median follow-up period of over 10 years in this population.

What are the clinical implications?

- Widespread use of wearable cardiac rhythm monitors, many of which are used during exercise, has enabled detection of PVCs in asymptomatic individuals at larger scale and is expected to result in more consultation requests from concerned patients whose monitors detect PVCs.
- Consequently, clinicians need to be aware of the potential prognostic significance of high PVC burden during and after exercise in middle-aged and older adults without known cardiovascular disease.

Introduction

Exercise testing poses a dynamic physiological stress on the heart that may unmask underlying cardiac anomalies not evident at rest and is commonly ordered to guide risk assessment in low or intermediate risk individuals[1]. Premature ventricular complexes (PVCs) are commonly observed during exercise testing (prevalence ~7%)[2]. The prognostic implications of high PVC burden are well-recognised in patients with structural heart disease [3–7], however the implications for asymptomatic individuals remain incompletely understood. As highlighted recently, the increased availability of wearable ECG monitoring devices has significantly improved the identification of asymptomatic participants with PVCs, emphasising the need to better understand the association between PVCs and cardiovascular risk[8].

The prognostic implications of low and intermediate PVC counts are unknown, and little is known about the specific risks carried by different PVCs rhythms (e.g. couplets, triplets, and bigeminy). A very limited number of studies have investigated the prognostic implications of PVCs on exercise in asymptomatic individuals [8–14]. The latest evidence suggests that high-grade PVCs (frequent or complex PVCs) during *recovery after exercise* are associated with a 1.7 times higher long-term cardiovascular mortality risk, independent of established clinical risk factors, whereas no significant association was found for PVCs *during* exercise in 5,486 individuals of which 311 died due to cardiovascular disease[8]. However, the quality of the evidence is limited because study cohorts are small, usually not population based, and, as highlighted previously, there is lack of uniformity among definitions used to designate frequent PVCs[15]. In addition, little is known about the association of PVCs with other important cardiac outcomes including myocardial infarction, life threatening ventricular arrhythmia (LTVA), and heart failure (HF). We hypothesised that the risk associated with PVCs increases with frequency of PVCs and their pattern.

In the present study, we investigated PVCs observed during submaximal exercise in 48,502 asymptomatic individuals without known cardiovascular disease with over 10 years follow-up from the UK Biobank (UKB) study to address the following hypotheses: 1. PVC burden during submaximal exercise (N= 0,1-5, 6-10,11-20,>20 beats) and recovery (N = 0,1-5,>5) determine the clinical adjusted risk for major adverse cardiovascular events (MI/HF/LTVA) and all-cause mortality. 2. PVC burden is associated with myocardial infarction, LTVA, and heart failure (HF), and cardiovascular mortality. 3. Levels of risk differ according to the different PVC patterns.

Methods

UK Biobank

The UK Biobank is a large population cohort established between 2006 and 2010, with even numbers of men and women aged 40-69 years on recruitment from 21 assessment centres across England, Wales, and Scotland. The study has extensive baseline and follow-up clinical, biochemical, and outcome measures and approval from the North West Multi-Centre Research Ethics Committee. All participants provided informed consent at the time of enrolment for their data to be linked to the health record systems.

Exercise test

From the full UK Biobank cohort, 95,154 participants consented to participate in a submaximal exercise test between 2009 - 2013. The test used cycle ergometry on a stationary bike (eBike, Firmware v1.7) in conjunction with a single lead (lead I) electrocardiograph device (CAM-USB 6.5, Cardiosoft v6.51, sample rate 500Hz). The exercise protocol consisted of a 15s pre-test resting phase, followed by graded activity (6 min) and a recovery period with hands remaining on the handlebars whilst remaining still and silent (1 min, no cool-down period). According to protocol, participants were risk-stratified into groups and those who were allowed to exercise were categorized to perform a test at either 35% or 50% of the maximum predicted workload depending on their risk

profile[16]. A submaximal workload allowed testing to be conducted safely across participants with a wide fitness range, including those not normally considered for exercise testing. The predicted absolute maximum workload was calculated according to a formula, which includes age, height, weight, resting heart rate and gender. We excluded participants who prematurely terminated their test due to (chest) discomfort.

ECG analysis

As we did not had access to a method to detect and count premature ventricular contractions (PVCs), we developed, trained, and validated a deep-learning model to detect PVCs from raw single-lead ECG recordings from the exercise cohort in the UK Biobank study. ECGs were first band-pass filtered (0.5 – 45Hz), down sampled to 250Hz and then split in non-overlapping segments of 2048 time samples (~8.2s) for computational efficiency. A 1D convolutional neural network (CNN) was trained on the ECG segments to estimate the probabilities for each time sample in the segment that it belonged to (i) a normal (narrow) QRS complex, (ii) a PVC QRS morphology, and (iii) neither of both (Supplemental Figure 1). The network was implemented using the Keras framework with a TensorFlow (Google) backend and Python (v3.8.5)[17]. The main architecture was composed of two 1D convolutional layers connected to a drop out, dense and classification layer. Post processing was applied to re-join segments and to filter the estimated probabilities to improve localisation and classification of QRS complexes in the ECG.

The network was trained on 41,025 ECG segments from 723 participants who had at least 1 PVC during the exercise test identified following visual inspection of exercise ECGs from 1142 participants with abnormal RR intervals from data previously derived by our group [18]. The performance algorithm was tested in a dataset of 79,257 ECG segments from 1500 participants that were randomly selected from the remaining ECGs in UK Biobank from participants without cardiovascular disease (Figure 1). All ECGs in training and testing sets were manually reviewed by an expert (SVD) to localise all QRS complexes and to label them as normal beats or PVC. A PVC was de-

fined as a QRS-T complex with QRS duration ≥0.12 seconds not preceded by at least 0.12 seconds by a P wave.

Importantly, the distribution of PVC count was highly skewed (Supplemental Figure 2) with excessive zero PVC counts in most participants (79.7% and 90.1% having 0 PVCs for exercise and recovery, respectively). We therefore evaluated the algorithm's performance to accurately predict 5 pre-defined categories (0, 1-5, 6-10, 11-20, and >20 PVCs), which were chosen based on clinical utility. Satisfactory performance was achieved: in the validation dataset, the overall accuracy was 98.7%, and sensitivity and positive predictive values ranged between 89.2 – 100.0% and 94.3 – 99.8%, respectively, across the PVC count categories (Supplemental Table 1). The algorithm was used to analyse all remaining ECGs in our dataset that were not part of training or validation set.

To compare our findings with the recent published work from Refaat et al, we reviewed ECGs with PVCs for high-grade PVCs using the same criteria: frequent (>10 per minute), multifocal, >2 consecutive PVCs in a row (including ventricular tachycardia), or R-on-T type [8]. R-on-T and multifocal PVCs were manually confirmed by reviewing all ECGs with short PVC coupling interval (<400 ms) or low correlation among PVC beats (correlation coefficient <0.75).

Ascertainment of study endpoints

All UKB participants consented to be followed-up through linkage to their health-related records and the UK death register [19]. Participants with known cardiovascular disease prior to the exercise test, identified using either health-recorded or self-reported in the baseline questionnaire, were excluded (codes are provided in Supplemental Table 2). The primary study endpoint was major adverse cardiovascular events (MI/HF/LTVA), defined as either hospitalisation or death due to myocardial infarction, HF, or LTVA. Cases were identified using relevant International Classification of Diseases 10th Revision (ICD10) or Operating procedures (OPSC4) codes in the health-related records or death register (Supplemental Table 3). The secondary endpoint was all-cause mortality. We also performed two additional analyses: 1) to evaluate the associations with myocardial infarction,

LTVA, and HF separately, and 2) the association with cardiovascular death. The follow-up period was determined by the first appearance of ICD10 or OPSC4 codes in either heath record or death register data. Participants who did not experience an event were censored at death or the end of the follow-up period (November 30th, 2022).

Statistical analysis

Due to its occasional nature, the distribution of PVC count is highly skewed, with a majority of participants having zero PVC count. We therefore categorized PVC count into frequency groups to capture the varying levels of PVC burden (0 PVCs, 1-5 PVCs, 6-10 PVCs, 11-20 PVCs, and >20 PVCs for exercise, and 0, 1-5, >5 PVCs for recovery). Using these systematic interval groupings, we aimed to create reasonably balanced risk groups while preserving the interpretability of the results. Descriptive statistics are presented as mean ± standard deviation (SD) for continuous variables or frequency (percentage) for categorical variables. Baseline clinical and exercise ECG characteristics of the groups with PVCs were statistically compared with the group with no PVCs. Bonferroni correction was applied for multiple testing (n=4 for exercise PVCs and n=2 for recovery PVCs). We used Wilcoxon rank-sum tests for continuous variables and the chi-square or Fisher exact test for categorical variables, whichever was appropriate. We constructed survival probability curves for MI/HF/LTVA and all-cause mortality stratified for PVC count. The association between PVC count and study endpoints was investigated using multivariable-adjusted Cox proportional hazards regression. A minimally adjusted analysis used gender, age, and the number of heart beats during exercise or recovery. By including the number of heart beats (either during exercise or recovery), we estimated the risk carried by PVCs independently from their frequency with respect to the total number of heart beats during exercise or recovery. In a second model we addressed potential sources of confounding by further adjusting for clinical variables (hypertension, type 2 diabetes, LDL-C and HDL-C, triglycerides, smoking, body mass index) and (exercise) ECG variables (QTc interval, QRS duration, ST-segment depression during exercise of >0.1mV, and heart rate increase

during exercise (for exercise PVCs) or decrease during recovery (for recovery PVCs). Hypertension was defined as systolic blood pressure of ≥140 mmHg or diastolic blood pressure of ≥90 mmHg measured on the day of assessment [20] or a previous a diagnosis of hypertension (details provided in Supplemental Table 4). We estimated 95% CIs for each group (including the reference (0 PVCs) group) that corresponded to the amount of information underlying each group[21]. Inspection of Schoenfeld residuals revealed a possible violation of the proportional hazards assumption by gender. To avoid potential bias, we stratified Cox models by gender.

Missing variables (smoking status (0.4%), low- and high-density lipoprotein cholesterol (LDL-C, HDL-C) (8.2 and 13.2%, respectively), triglycerides (8.2%), BMI (<0.01%), QRS duration (0.9%), QTc interval (4.6%), ST depression (0.02%)) were imputed using the multiple imputation by chained equations approach, with five imputed datasets and ten iterations [22]. For each variable we specified a predictive mean matching model, and we used all baseline variables to inform the imputation. Imputations were found acceptable by comparison of plots of the distribution of recorded and imputed values for all measurements. Cox regression models were estimated separately for each imputed dataset and then pooled together to obtain one overall set of estimates. A $P < 0.05$ was considered statistically significant. Statistical analyses were performed in R v4.0.2 [23].

Sensitivity analyses

We performed sensitivity analyses to examine the potential confounding influence of high PVC burden at rest and two major non-cardiovascular diseases (chronic obstructive pulmonary disease (COPD) and chronic kidney disease (CKD)). High PVC burden at rest was defined as ≥1 PVC recorded during the available 15s (pre-exercise) resting period. This information was added as a binary covariate to the fully-adjusted cox models. COPD was identified using recommended definitions published previously by UK Biobank[24]. CKD was defined as CKD stages 3-5 (estimated glomerular filtration rates (eGFR) < 60 ml/min/1.73m^2), analogous to previous work in UK Biobank[25]

where eGFR values were calculated using the CKD-EPI formula[26]. The contributing effect was then investigated by repeating the main analysis after excluding participants with prevalent COPD or CKD.

Explorative analysis - Association between PVC burden and markers of (subclinical) cardiomyopathy

In an explorative subgroup analysis, we examined whether subclinical cardiomyopathy could be a potential contributing factor to the associations between high PVC burden and cardiovascular outcomes. In one analysis, we evaluated the association between high PVC burden and functional and structural parameters derived from cardiovascular magnetic resonance (CMR) imaging. This study was conducted in a subgroup of participants who had imaging data derived during the UK Biobank imaging follow-up study. Prognostically important markers of cardiomyopathy (left-ventricular (LV) mass, ejection fraction, end-diastolic volume, stroke volume, and myocardial native T1) were derived using a fully automated quality-controlled image analysis pipeline previously developed and validated in UK Biobank[27,28]. In a second analysis, we evaluated the association with serum levels of N-terminal Pro-B-type natriuretic peptide (NT-proBNP, an established biomarker of heart failure and ventricular dysfunction) measured at baseline[29]. Measurements below the protein's lower limit of detection (LOD) were substituted by $LOD/\sqrt{2}$[30]. Models in both CMR and BNP analyses were adjusted using the same covariates as the cox regression analysis. In addition, results were also adjusted for the time interval between exercise test and imaging study for CMR parameters.

Results

Following exclusions for reasons highlighted in Figure 1, 48,315 participants without known cardiovascular disease and with complete ECGs were included in the study. The study population comprised a balanced set of middle-aged men and women (51.1% female, mean age 56.8 (+/- 8.2) years). Table 1 summarizes the number of participants in each PVC count category during exercise (A) and recovery (B). Participants with higher PVC counts were older, more likely to be male, hypertensive and have diabetes. They were also more likely to show ST-segment depression (>0.1mV). After a median follow-up period of 12.6 years (IQR: 3.5 months), there were 2175 (4.5%) MI/HF/LTVA events (hospitalisation or death) and 2441 (5.1%) participants died from any cause.

PVCs during exercise

The incidence of MI/HF/LTVA events and all-cause mortality were significantly higher in participants with PVCs during exercise and increased with PVC count. In the group without PVCs, the incidence was 4.0% compared to 5.8% in the group with 1-5 PVCs ($p < 0.001$). Among participants with PVCs, the incidence further increased to 10.2% (> 20 PVCs) and was significantly higher compared to the group with 1-5 PVCs ($p < 0.001$). A similar trend was observed for all-cause mortality: the incidence increased from 4.6% (0 PVCs) to 6.5% (1-5 PVCs, $p < 0.001$). Among participants with PVCs, the incidence further increased to 11.4% (> 20 PVCs, $p < 0.001$). The unadjusted Kaplan-Meier curves showed decreasing survival rates for both MI/HF/LTVA and all-cause mortality with increasing PVC counts ($p < 0.001$, Figure 2 and 3)).

After adjusting for age, gender, and the number of heartbeats during exercise, all PVC counts were significantly associated with MI/HF/LTVA and remained significant after further adjusting for clinical factors (Figure 4A, Supplemental Table 5). The risk increased with PVC count with HRs ranging from: 1.2 (95% CI: 1.1 – 1.3, $p < 0.001$) for 1-5 PVCs to 1.8 (95% CI: 1.4 – 2.2, $p < 0.001$) for >20 PVCs. For all-cause mortality, the strongest association was found for >20 PVCs (fully-

adjusted HR 1.6 (95% CI: 1.3 – 1.9, p < 0.001, Figure 4B). We also found a weak, but significant, association for 1-5 PVCs (fully-adjusted HR 1.1 (95% CI: 1.0 – 1.2, p = 0.022), but associations were not significant for the other (higher) PVC burdens (6-10 and 11-20 PVCs, Figure 4B, Supplemental Table 5). In the further adjusted models, myocardial ischemia (ST depression) was not significantly associated with MI/HF/LTVA nor all-cause mortality (HR: 1.2, (95% CI: 0.8-1.8, p = 0.443), and HR: 1.4 (95% CI: 1.0-1.9, p = 0.092), respectively).

PVCs during recovery from exercise

The MI/HF/LTVA incidence was significantly higher in the group with PVCs during recovery: 4.2% (0 PVCs) vs. 6.5% (1-5 PVCs, p < 0.001). Among participants with PVCs, the incidence was higher in those with >5 PVCs compared to 1-5 PVCs (8.7% (>5 PVCs), p = 0.040). A similar trend was observed for all-cause mortality: the incidence increased to 6.9% (1-5 PVCs, p < 0.001) and 10.9% (>5 PVCs p < 0.001).. The Kaplan-Meier curves showed increasing event rates for both MI/HF/LTVA and all-cause mortality with increasing PVC counts (p < 0.001, Figure 2 and 3)).

After adjusting for age, gender, and the number of heartbeats during recovery, PVC count was significantly associated with MI/HF/LTVA and results remained similar after further adjusting for clinical factors (adjusted HRs: 1.3 (95% CI: 1.1 – 1.4, p < 0.001, 1-5 PVCs; and 1.6 (95% CI: 1.2 – 2.0, p < 0.001) for >5 PVCs (Figure 4C, Supplemental Table 6). Only the highest PVC-count (>5 PVCs) was associated with all-cause mortality: HR: 1.5 (95% CI: 1.2-1.9, p < 0.001, Figure 4D).

Combining PVCs during exercise and recovery

A majority of the participants with repetitive PVCs during exercise also had PVCs during recovery: 435 (51%) from all participants with >20 PVCs during exercise had also >5 PVCs during recovery. This group comprised 61% of the participants with >5 PVCs during recovery. After adjusting for clinical risk factors, the combination of >20 PVCs during exercise *and* >5 PVCs during

recovery was associated with both MI/HF/LTVA (HR: 1.7, 95% CI: 1.2 – 2.3, p < 0.001) and all-cause mortality (HR: 1.6, 95% CI: 1.2 – 2.2, p < 0.001).

Association with additional study endpoints

The number of myocardial infarctions, LTVA, and HF events were 1378 (2.9%), 307 (0.6%), and 838 (1.7%), respectively. There were 502 (1.0%) cardiovascular deaths. PVCs during exercise and recovery were both associated with incident LTVAs, HF and cardiovascular mortality, but not with myocardial infarction (Supplemental Tables 7 and 8). The highest risk was observed for the highest PVC counts: For exercise, the fully adjusted HRs of >20 PVCs were: 3.6 (95% CI: 2.3 – 5.7, p < 0.001), 2.8 (95% CI: 2.1 – 3.7, p < 0.001), and 2.8 (95% CI: 1.9-4.0, p < 0.001), for LTVA, HF, and cardiovascular mortality, respectively. Similarly, the HRs of >5 recovery PVCs were: 2.9 (95% CI: 1.8-4.9, p < 0.001), 2.4 (95% CI: 1.7 – 3.4, p < 0.001), and 2.3 (95% CI: 1.5 – 3.5, p < 0.001) for LTVA, HF, and cardiovascular mortality, respectively.

Prognostic value of high-grade & complex PVC rhythms

To compare our data with the recently published work from Refaat et al [8], we identified high-grade PVCs in 1560 (3.2%) and 727 (1.5%) participants, during exercise and recovery respectively. High grade PVCs were associated with MI/HF/LTVA independent of clinical and ECG factors (HR 1.7 (1.4-2.0), p<0.001; HR 1.7 (1.3-2.1), p<0.001, for exercise and recovery, respectively, Supplemental Figure 3) and all-cause mortality (HR 1.3, 95% CI: 1.1-1.5, p = 0.003; HR 1.3, 95% CI 1.0-1.7, p=0.028). Both markers were also associated with cardiovascular mortality (HR 2.3, 95% CI 1.7-3.1, p<0.001, and HR 2.0, 95% CI 1.3- 3.2, p = 0.001, Supplemental Table 9). Complex PVC rhythms (couplets, triplets, and bigeminy) were all associated with higher risk for MI/HF/LTVA compared to PVC count alone with fully adjusted HRs ranging between 2.4 (single PVC couplet) and 7.1 (≥1 episode with ≥3 consecutive PVCs, Supplemental Figure 3).

Sensitivity analyses

Results obtained after adjusting for high resting PVC burden (N=1437 (3.0%)) or excluding participants with prevalent COPD (N=466; 1.0%) or CKD (N=1374; 3.1%) were similar compared to the main analyses for both exercise and recovery PVCs (Supplemental Table 10 and 11).

Exploring associations between exercise-induced PVC burden and markers of cardiac structure & function

CMR measurements were available for 6290 (13.0%) participants from our study cohort and taken on average 6.4 (± 2.3) years after the exercise test. The group with high PVC burden had larger LV end-diastolic and systolic volumes and lower ejection fractions compared to participants without PVCs (Supplemental Table 12). After adjusting for clinical and exercise ECG variables, high exercise PVC burden remained significantly associated with larger volumes (~14ml and ~11ml, $p < 0.001$, for end-diastolic and systolic volume, respectively) and lower ejection fraction (-3%, $p < 0.001$, Supplemental Table 13). Findings were similar for high PVC burden during recovery, although the effects were smaller (Supplemental Table 13). No significant associations were found for native myocardial T1 and LV mass and stroke volume. Serum levels of NT-proBNP measured at the time of the exercise test were available in 4607 (9.5%) participants and were higher in participants with high PVC burden compared to those without PVCs (Supplemental Table 14). Considering that 1 unit increase in Normalized Protein eXpression equates a doubling of protein concentration, high PVC burden was associated with a ~10% ($p = 0.008$) and ~13% ($p < 0.001$) increase in NT-proBNP protein concentration, for exercise and recovery respectively (Table 2).

Discussion

To our knowledge, this is the largest population-based cohort study to date to investigate the prognostic value of exercise-induced PVCs in asymptomatic individuals without known cardiovascular disease during submaximal exercise. The main important findings are: (1) Low PVC counts (1-5 PVC during exercise or recovery) were already significantly associated with MI/HF/LTVA, independent of clinical risk factors, (2) PVC count during submaximal exercise and recovery are *both* associated with MI/HF/LTVA, mortality, and cardiovascular death; (3) there is an incremental risk of MI/HF/LTVA for increasing PVC count; and (4) complex PVCs rhythms are associated with higher risk compared to PVC count alone. This has important implications in the assessment of asymptomatic individuals particularly with the advent of wearable ECG technologies worn by otherwise healthy individuals during daily activity and exercise prompting cardiological review, but keeping in mind that the study group had a mean age of 56.8 (+/-8.2 years) when the data on exercise was collected. Also, the exercise performed here was limited intensity.

Recently, Refaat et al [8] investigated the prognostic value of PVC during exercise vs. recovery in 5,486 asymptomatic patients not suspected of having heart disease from the Lipid Research Clinics study. Compared to previous studies, they used a more inclusive definition of "high-grade PVCs" (>10 PVCs over 60-second interval, >2 consecutive PVCs including ventricular tachycardia, R-on-T PVCs, or multiform PVCs). Their data showed that high-grade PVCs during recovery were significantly associated with higher risk of cardiovascular mortality (adjusted HR: 1.7 (1.1-2.6); p = 0.02), but no associations were found for high-grade PVCs during exercise. Furthermore, high-grade PVCs (neither during exercise nor recovery) were not associated with all-cause mortality after adjusting for clinical variables. Our data from >48,000 participants of UK Biobank only partially support these findings. In a submaximal exercise test, we found that high-grade PVCs during *exercise and recovery* were *both* associated with cardiovascular mortality, MI/HF/LTVA, and all-cause mortality, using the same definition for high-grade PVCs and included similar clinical and exercise parameters as covariates. It is possible that conflicting differences in baseline

characteristics between both populations e.g higher proportion of hyperlipidaemia patients and smokers compared to our cohort could explain these differences. Sensitivity analysis from Refaat et al [8] showed no link between PVC risk and hyperlipidemia, but other work investigating PVC load and aortic stiffness did find an association between the number of PVCs in 24h ambulatory monitoring [31]. Another important difference between our and Refaat's work is the exercise protocol. The activity performed by UK Biobank participants in this study was limited to either 35% or 50% of the maximum predicted workload, whereas participants in Refaat's tstudy[8] exercised at maximal Bruce protocol. Consequently, the etiology of PVCs observed in our and their study may be different. For example, plasma catecholamine levels have been linked with exercise intensity [32,33] and may mediate PVCs. It is possible that participants in Refaat et al have experienced higher catecholaminergic levels by exercising at maximum workload and that their exercise PVCs are more likely to reflect a physiological response to exercise compared to UK Biobank. However, this explanation may not be fully compatible with the incremental risk observed in this study, which suggests that the risk for MI/HF/LTVA increases with PVC count.

To our knowledge, this is the first study to document an incremental risk for exercise-induced PVCs in asymptomatic individuals without cardiovascular disease. Uniquely in this study we did not use one cut-off threshold for PVC burden but evaluated the risk for a wide range of PVC counts, during exercise and recovery. Little is known about the risk associated with low and intermediate PVC counts. Two previous studies have investigated the prognostic value of infrequent PVCs: Jouven et al[9] observed infrequent PVCs (<10% of all ventricular depolarizations, no consecutive PVCs) in 8.5% and 7.3% of a middle-aged male population during exercise and recovery, respectively. They found that cardiovascular and total mortality rates were higher for infrequent PVCs with respect to participants without PVCs, however they only performed formal association studies for frequent PVCs (≥10% of all ventricular depolarizations). In the second study, Morshedi-Meibodi et al[13] investigated infrequent and frequent PVCs during submaximal exercise (< and ≥0.22/min of exercise) and found that both type of PVCs were significantly associated with

mortality in 2885 participants from the Framingham Offspring Study during 15 years of follow-up (adjusted HR: 1.9 (1.2 – 2.8), and 1.7, (1.2 - 2.5), for infrequent and frequent PVCs, respectively). In our cohort, this cut-off value translates to between 1 and 2 PVCs during exercise. Both 1-5 PVC counts were not associated with all-cause mortality in this work.

Pathophysiological Rationale

Previous studies in both patients and asymptomatic participants have suggested that PVCs during recovery carry a worse prognosis for (cardiovascular) mortality compared to PVCs during exercise[6,8]. This has fuelled the hypothesis that the prognostic value of exercise-induced PVCs might be linked to attenuated vagal reactivation during recovery, perhaps by reduced suppression of PVCs. Our data does support this hypothesis but indicates at least equal prognostic value for PVCs *during* submaximal exercise. PVCs during exercise may reflect increased norepinephrine levels during exercise in high-risk individuals, for example because they had to produce more effort during exercise compared to their healthier counterparts who may have more cardio-pulmonary reserve and skeletal muscle efficiency. The mechanisms of how PVCs during exercise and recovery are related to cardiovascular events remains to be further explored. Our explorative study suggested that high PVC burden are associated with higher BNP levels measured at the same day as the exercise test, and we also observed differences in cardiac function and structure during follow-up imaging, which may both support existence of subclinical cardiomyopathy. However, PVCs observed during submaximal exercise may have a different etiology compared to PVCs observed during maximal stress testing. The associations between PVC count and LTVA and HF implicates the existence of an arrhythmic substrate (i.e. fibrosis or ischemia), which enables re-entry and reduces cardiac function precipitating these events. The development of PVCs during exercise and recovery indicates the presence of this underlying substrate with PVCs arising due to the effects of subclinical ischemia (reflected by ST depression on exercise) and adrenergic stress causing triggered ectopic activity which could be due to early afterdeporisations typically occurring in conditions of catecholamines, tissue injury, altered electrolytes, hypoxia, or acidosis[34].

Increased PVCs during exercise may also reflect the presence of subclinical pathologic myocardial substrate or ischaemia. Although ST depression, was not associated with MI/HF/LTVA nor mortality, it carried a higher risk for LVTA and HF. Ischemia can promote optimal conditions for VAs & impair LV function through multiple mechanisms and it is well recognised to increase myocardial refractoriness to promote wavebreak and polymorphic VT[35,36]. However, the fact that single lead ST measurements with submaximal exercise could result in underestimation of ischaemia burden is a potential limitation in this study. Previously, using 2 bipolar leads (V5 and V5R) and *a maximal exercise* test frequent exercise-induced PVCs and ischemia were *independent predictors* of cardiovascular mortality with similar hazard ratios in asymptomatic men[9]. In that dataset, ischemia was only present in 6% of PVC positive patients and 2% of our series, indicating other pathophysiological factors are operative. Combined with the lack of evidence to support an association between PVC count and myocardial infarction, findings seem to suggest that exercise and recovery PVCs are not necessarily related to myocardial ischemia[9].

The relationship between PVCs recorded during rest and exercise remains to be further investigated. Beckerman et al. studied 5754 male veterans referred for exercise testing and found that a combination of exercise-induced ventricular arrhythmias and resting PVCs carried a higher risk than exercise-induced ventricular arrhythmias alone (HR 1.6 vs. HR 2.7)[37]. Although the authors did not precisely quantify the PVC rate at rest or during exercise as in our work, their findings may suggest complementarity mechanisms in modulating risk. Results from our sensitivity analyses showed that the risk associated with exercise-induced PVCs were independent from resting PVC burden, which may support this hypothesis. However, the resting ECGs (recorded pre-exercise) in this work were of very short duration (15s) and therefore likely to have provided very limited sensitivity to detect PVCs as their occurrence is highly variable.

Limitations

Our study benefited from several important advantages including access to the largest and most detailed dataset of PVC activity from a population-based cohort without known cardiovascular

disease, combined with long-term follow-up. Nevertheless, several limitations, apart from the limited sensitivity to detect myocardial ischemia need to be acknowledged. First, there is a "healthy volunteer" selection bias in the UK Biobank with the participants being older and healthier than the general population. Furthermore, although the codes used in this study to determine the presence or absence of clinical conditions were carefully selected, we could not rule out misclassification of diagnoses or inaccuracies related to incorrect diagnoses. Secondly, participants performed submaximal exercise only and the etiology of the PVCs may be different compared to other studies where exercise was performed at maximal workload. However, we could already observe different event rates between PVC burdens, which might indicate stronger association with greater intensity exercise. Third, as discussed above, signs of exercise-induced myocardial ischemia may have remained undetected as our data was limited to single lead ECG measurements. We therefore cannot fully rule out that PVCs were driven by ischemia. However, we believe this would be unlikely as it does not seem to be fully compatible with the observed lack of association between PVC burden and MI. Fourth, the study cohort is predominantly (>90%) of white British ancestry, which may limit the generalisability of our findings in under-represented ethnicities, although the study is not likely to be flawed by an important co-morbidity burden. Finally, cardiac imaging and NT-proBNP data were only available in small subset of our study cohort. Furthermore, imaging data was acquired several years after the exercise test and is therefore more likely to capture the effect of disease progression rather than the actual situation at the time of the test. Further evaluation is required to investigate the mechanisms underlying exercise-induced PVCs.

Clinical Perspectives

In the recent years there has been a widespread use of wearable cardiac rhythm monitors, many of which are used during (submaximal) exercise. This has enabled detection of PVCs in asymptomatic individuals at larger scale and is expected to result in more consultation requests from concerned patients whose monitors detect PVCs. High PVC counts during submaximal exercise and recovery predicts MI/HF/LTVA and all-cause mortality independent of standard CV

risk factors in this middle aged and older population. Clinicians need to be aware of the measurable prognostic significance of these PVCs to optimally assess and manage CV risk in these otherwise asymptomatic individuals to prevent life threatening events.

Conclusions

High PVC counts during exercise and recovery are associated with MI/HF/LTVA and all-cause mortality independent of standard cardiovascular risk factors. In addition, there is an incremental risk of MI/HF/LTVA for increasing PVC count, and complex PVCs have additional prognostic significance. This has major implications for risk stratification, screening and interpretation of wearable ECG rhythm readouts in the middle aged and older population and should be investigated in younger individuals.

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Conflict of interest

SEP provides Consultancy to Circle Cardiovascular Imaging Inc., Calgary, Alberta, Canada

Data availability statement

This research has been conducted using the UK Biobank Resource under application numbers 8256 and 2964. Anonymised data and materials generated in this work will be returned to UK Biobank and can be accessed upon request.

References

1. Fihn SD, Gardin JM, Abrams J, Berra K, Blankenship JC, Dallas AP, Douglas PS, Foody JM, Gerber TC, Hinderliter AL, King SB, Kligfield PD, Krumholz HM, Kwong RYK, Lim MJ, Linderbaum JA, Mack MJ, Munger MA, Prager RL, Sabik JF, Shaw LJ, Sikkema JD, Smith CR, Smith SC, Spertus JA, Williams S V, Anderson JL, American College of Cardiology Foundation/American Heart Association Task Force. 2012 ACCF/AHA/ACP/AATS/PCNA/SCAI/STS guideline for the diagnosis and management of patients with stable ischemic heart disease: a report of the American College of Cardiology Foundation/American Heart Association task force on practice guidelines, and the. *Circulation* [Internet]. 2012;126:e354-471. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23166211
2. Verdile L, Maron BJ, Pelliccia A, Spataro A, Santini M, Biffi A. Clinical significance of exercise-induced ventricular tachyarrhythmias in trained athletes without cardiovascular abnormalities. *Heart Rhythm*. 2015;12:78–85.
3. Califf RM, McKinnis RA, McNeer JF, Harrell FE, Lee KL, Pryor DB, Waugh RA, Harris PJ, Rosati RA, Wagner GS. Prognostic value of ventricular arrhythmias associated with treadmill exercise testing in patients studied with cardiac catheterization for suspected ischemic heart disease. *J Am Coll Cardiol* [Internet]. 1983;2:1060–7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/6630778
4. Dewey FE, Kapoor JR, Williams RS, Lipinski MJ, Ashley EA, Hadley D, Myers J, Froelicher VF. Ventricular arrhythmias during clinical treadmill testing and prognosis. *Arch Intern Med* [Internet]. 2008;168:225–34. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18227372
5. Udall JA, Ellestad MH. Predictive implications of ventricular premature contractions associated with treadmill stress testing. *Circulation*. 1977;56:985–989.
6. Frolkis JP, Pothier CE, Blackstone EH, Lauer MS. Frequent ventricular ectopy after exercise as a predictor of death. *N Engl J Med* [Internet]. 2003;348:781–90. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12606732
7. O'Neill JO, Young JB, Pothier CE, Lauer MS. Severe frequent ventricular ectopy after exercise as a predictor of death in patients with heart failure. *J Am Coll Cardiol* [Internet]. 2004;44:820–6. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15312865
8. Refaat MM, Gharios C, Moorthy MV, Abdulhai F, Blumenthal RS, Jaffa MA, Mora S. Exercise-Induced Ventricular Ectopy and Cardiovascular Mortality in Asymptomatic Individuals. *J Am Coll Cardiol*. 2021;78:2267–2277.
9. Jouven X, Zureik M, Desnos M, Courbon D, Ducimetière P. Long-Term Outcome in Asymptomatic Men with Exercise-Induced Premature Ventricular Depolarizations. *New England Journal of Medicine* [Internet]. 2000;343:826–833. Available from: http://www.nejm.org/doi/abs/10.1056/NEJM200009213431201
10. Dzikowicz DJ, Carey MG. Exercise-Induced Premature Ventricular Contractions Are Associated With Myocardial Ischemia Among Asymptomatic Adult Male Firefighters: Implications for Enhanced Risk Stratification. *Biol Res Nurs* [Internet]. 2020;22:369–377. Available from: http://www.ncbi.nlm.nih.gov/pubmed/32342704
11. Mora S, Redberg RF, Cui Y, Whiteman MK, Flaws J a, Sharrett AR, Blumenthal RS. Ability of exercise testing to predict cardiovascular and all-cause death in asymptomatic women: a 20-year follow-up of the lipid research clinics prevalence study. *JAMA* [Internet]. 2003;290:1600–7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/14506119
12. Janette Busby M, Shefrin EA, Fleg JL. Prevalence and long-term significance of exercise-induced frequent or repetitive ventricular ectopic beats in apparently healthy volunteers. *J Am Coll Cardiol* [Internet]. 1989;14:1659–1665. Available from: http://dx.doi.org/10.1016/0735-1097(89)90012-0

13. Morshedi-Meibodi A, Evans JC, Levy D, Larson MG, Vasan RS. Clinical correlates and prognostic significance of exercise-induced ventricular premature beats in the community: The Framingham heart study. *Circulation*. 2004;109:2417–2422.
14. Marine JE, Shetty V, Chow G V, Wright JG, Gerstenblith G, Najjar SS, Lakatta EG, Fleg JL. Prevalence and prognostic significance of exercise-induced nonsustained ventricular tachycardia in asymptomatic volunteers: BLSA (Baltimore Longitudinal Study of Aging). *J Am Coll Cardiol* [Internet]. 2013;62:595–600. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23747767
15. Saha SA. Premature Ventricular Complexes During Exercise in Asymptomatic Adults: Do They Deserve a Closer Look? *J Am Coll Cardiol* [Internet]. 2021;78:2278–2280. Available from: https://doi.org/10.1016/j.jacc.2021.09.1365
16. UK Biobank. UK Biobank Cardio Assessment Manual [Internet]. 2011 [cited 2023 May 17]. Available from: https://biobank.ndph.ox.ac.uk/showcase/ukb/docs/Cardio.pdf
17. Van Rossum G, Drake FL. Python 3 Reference Manual. Scotts Valley, CA: CreateSpace; 2009.
18. Ramírez J, Duijvenboden S van, Ntalla I, Mifsud B, Warren HR, Tzanis E, Orini M, Tinker A, Lambiase PD, Munroe PB. Thirty loci identified for heart rate response to exercise and recovery implicate autonomic nervous system. *Nat Commun* [Internet]. 2018;9:1947. Available from: http://dx.doi.org/10.1038/s41467-018-04148-1
19. Bycroft C, Freeman C, Petkova D, Band G, Elliott LT, Sharp K, Motyer A, Vukcevic D, Delaneau O, O'Connell J, Cortes A, Welsh S, Young A, Effingham M, McVean G, Leslie S, Allen N, Donnelly P, Marchini J. The UK Biobank resource with deep phenotyping and genomic data. *Nature*. 2018;562:203–209.
20. Jones NR, McCormack T, Constanti M, McManus RJ. Diagnosis and management of hypertension in adults: NICE guideline update 2019. *British Journal of General Practice*. 2020;70:90–91.
21. Plummer M. Improved estimates of floating absolute risk. *Stat Med*. 2004;23:93–104.
22. Buuren S van, Groothuis-Oudshoorn K. mice : Multivariate Imputation by Chained Equations in R. *J Stat Softw* [Internet]. 2011;45. Available from: http://www.jstatsoft.org/v45/i03/
23. R Core Team. R: A Language and Environment for Statistical Computing [Internet]. 2022;Available from: https://www.r-project.org/
24. Zhang Q, Bush K, Nolan J, Schnier C, Sudlow C. Definitions of Chronic Obstructive Pulmonary Disease for UK Biobank Phase 1 Outcomes Adjudication [Internet]. 2018 [cited 2023 May 17]. Available from: https://biobank.ndph.ox.ac.uk/showcase/showcase/docs/alg_outcome_copd.pdf
25. Sullivan MK, Jani BD, McConnachie A, Hanlon P, McLoone P, Nicholl BI, Carrero JJ, Nitsch D, McAllister D, Mair FS, Mark PB. Hospitalisation events in people with chronic kidney disease as a component of multimorbidity: parallel cohort studies in research and routine care settings. *BMC Med*. 2021;19.
26. Levey AS, Stevens LA, Schmid CH, Zhang YL, Castro AF, Feldman HI, Kusek JW, Eggers P, Van Lente F, Greene T, Coresh J, CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration). A new equation to estimate glomerular filtration rate. *Ann Intern Med*. 2009;150:604–12.
27. Aung N, Vargas JD, Yang C, Cabrera CP, Warren HR, Fung K, Tzanis E, Barnes MR, Rotter JI, Taylor KD, Manichaikul AW, Lima JAC, Bluemke DA, Piechnik SK, Neubauer S, Munroe PB, Petersen SE. Genome-wide analysis of left ventricular image-derived phenotypes identifies fourteen loci associated with cardiac morphogenesis and heart failure development. *Circulation*. 2019;140:1318–1330.
28. Raisi-Estabragh Z, McCracken C, Hann E, Condurache D-G, Harvey NC, Munroe PB, Ferreira VM, Neubauer S, Piechnik SK, Petersen SE. Incident Clinical and Mortality Associations of Myocardial Native T1 in the UK Biobank. *JACC Cardiovasc Imaging*. 2022;

29. Sun BB, Chiou J, Traylor M, Benner C, Hsu Y-H, Richardson TG, Surendran P, Mahajan A, Robins C, Vasquez-Grinnell SG, Hou L, Kvikstad EM, Burren OS, Cule M, Davitte J, Ferber KL, Gillies CE, Hedman ÅK, Hu S, Lin T, Mikkilineni R, Pendergrass RK, Pickering C, Prins B, Raj A, Robinson J, Sethi A, Ward LD, Welsh S, Willis CM, Genetics AH, Initiative AG, Team BB, Squibb BM, Genetics GH, Sciences GG, Biology PI, Sciences PA of JD, Center RG, Burkitt-Gray L, Black MH, Fauman EB, Howson JMM, Kang HM, McCarthy MI, Melamud E, Nioi P, Petrovski S, Scott RA, Smith EN, Szalma S, Waterworth DM, Mitnaul LJ, Szustakowski JD, Gibson BW, Miller MR, Whelan CD. Genetic regulation of the human plasma proteome in 54,306 UK Biobank participants. *bioRxiv* [Internet]. 2022;2022.06.17.496443. Available from: http://biorxiv.org/content/early/2022/06/18/2022.06.17.496443.abstract
30. Patel H, Ashton NJ, Dobson RJB, Andersson LM, Yilmaz A, Blennow K, Gisslen M, Zetterberg H. Proteomic blood profiling in mild, severe and critical COVID-19 patients. *Sci Rep*. 2021;11.
31. Chen J-Y, Tsai W-C, Lee Y-L, Lee C-H, Tsai L-M, Chao T-H, Li Y-H, Chen J-H, Lin L-J. Association of premature ventricular complexes with central aortic pressure indices and pulse wave velocity. *Am Heart J* [Internet]. 2008;155:500.e1–6. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18294486
32. Fleg JL, Tzankoff SP, Lakatta EG. Age-related augmentation of plasma catecholamines during dynamic exercise in healthy males. *J Appl Physiol (1985)* [Internet]. 1985;59:1033–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/4055584
33. Dimsdale JE, Hartley LH, Guiney T, Ruskin JN, Greenblatt D. Postexercise peril. Plasma catecholamines and exercise. *JAMA* [Internet]. 1984;251:630–2. Available from: http://www.ncbi.nlm.nih.gov/pubmed/6690837
34. Antzelevitch C, Burashnikov A. Overview of Basic Mechanisms of Cardiac Arrhythmia. *Card Electrophysiol Clin*. 2011;3:23–45.
35. Sutton PMI, Taggart P, Opthof T, Coronel R, Trimlett R, Pugsley W, Kallis P. Repolarisation and refractoriness during early ischaemia in humans [Internet]. 2000. Available from: www.heartjnl.com
36. Cherry EM, Fenton FH, Gilmour RF. Mechanisms of ventricular arrhythmias: A dynamical systems-based perspective. Am J Physiol Heart Circ Physiol. 2012;302.
37. Beckerman J, Mathur A, Stahr S, Myers J, Chun S, Froelicher V. Exercise-Induced Ventricular Arrhythmias and Cardiovascular Death. 2005.

Table 1A: Baseline Clinical and Stress Test Characteristics During Exercise

		PVCs during exercise				
	Full Cohort (N = 48,315)	**0 (N = 38,502 (79.7%))**	**1-5 (N = 7561 (15.6%))**	**6-10 (N = 832 (1.7%))**	**11-20 (N = 573 (1.2%))**	**>20 (N = 847 (1.8%))**
Clinical Variables						
Men, %	48.9	48.4	49.0	55.2**	57.6**	59.6**
Age, y	56.8 (8.2)	56.1 (8.2)	58.9 (7.7)**	60.6 (7.0)**	60.4 (7.4)**	61.0 (7.0)**
White ancestry, %	92.3	91.8	93.5**	95.4**	95.1*	95.3**
Type 2 Diabetes, %	4.0	3.9	4.1	5.6	4.9	6.0**
Hypertension, %	51.0	49.1	56.7**	62.5**	64.4**	65.8**
Beta-blocker usage, %	3.5	3.3	4.2**	3.4	4.5	4.4
Smoking, %	8.2	8.2	8.1	6.2	7.2	7.7
LDL cholesterol, mmol/L	3.6 (0.8)	3.6 (0.8)	3.6 (0.8)*	3.6 (0.8)	3.6 (0.8)	3.5 (0.8)
HDL cholesterol, mmol/L	1.5 (0.4)	1.5 (0.4)	1.5 (0.4)**	1.5 (0.4)	1.5 (0.4)	1.5 (0.4)
Triglycerides, mmol/L	1.7 (1.0)	1.7 (1.0)	1.7 (0.9)**	1.8 (1.0)	1.7 (0.9)	1.7 (0.9)
Body Mass index	27.4 (4.3)	27.4 (4.3)	27.1 (4.3)**	27.4 (4.1)	27.5 (4.0)	27.8 (4.3)**
Framingham Risk Score, %	14.1 (10.1)	13.5 (9.7)	15.8 (11.0)**	18.2 (11.2)**	18.5 (11.4)**	19.1 (11.6)**
Exercise test variables						
Achieved Maximum Workload, Watt	85.4 (23.2)	86.0 (23.3)	83.0 (22.9)**	82.5 (22.2)**	84.1 (22.3)	84.5 (22.1)
Heart rate increase during exercise, bpm	42.2 (12.0)	42.4 (11.9)	41.9 (12.5)**	39.7 (12.4)**	40.8 (12.5)**	40.5 (12.4)**
ST-segment depression >0.1mV, %	1.2	1.1	1.8**	1.3	1.4	2.2**
QRS duration, ms	81.6 (9.6)	81.3 (9.4)	82.6 (10.5)**	82.7 (10.1)**	83.1 (10.8)**	84.4 (10.6)**
QTc interval, ms	396.4 (23.2)	395.7 (22.8)	398.3 (23.6)**	401.8 (24.7)**	401.0 (24.5)**	404.9 (28.8)**
Number of QRS complexes during exercise, N	576.2 (62.9)	576.8 (62.7)	573.3 (64.3)**	570.6 (64.0)*	575.0 (60.2)	578.3 (61.7)

Continuous variables are presented as mean (SD); categorical variables are presented as %. HDL = high-density lipoprotein; LDL = low-density lipoprotein; PVC = premature ventricular contractions. Values indicated with * are significantly different from the 0 PVC group after Bonferroni correction for multiple testing (n=4). * Bonferroni corrected P-value < 0.05 ($p < 0.05 / 4$), ** Bonferroni corrected P-value < 0.01 ($p < 0.01 / 4$)

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Table 1B: Baseline Clinical and Stress Test Characteristics During Recovery

	Full Cohort (N = 48,315)	PVCs during Recovery		
		0 (N = 43,511 (90.1%))	1-5 (N = 4095 (8.5%))	>5 (N = 709 (1.5%))
Clinical Variables				
Men, %	48.9	48.7	50.5	51.9
Age, y	56.8 (8.2)	56.5 (8.2)	59.3 (7.7)**	60.5 (7.5)**
White ancestry, %	92.3	92.0	94.8**	94.1
Type 2 Diabetes, %	4.0	3.9	5.2**	4.2
Hypertension, %	51.0	50.1	57.9**	64.6**
Beta-blocker usage, %	3.5	3.5	3.5	3.9
Smoking, %	8.2	8.2	7.5	7.5
LDL cholesterol, mmol/L	3.6 (0.8)	3.6 (0.8)	3.6 (0.8)	3.6 (0.8)
HDL cholesterol, mmol/L	1.5 (0.4)	1.5 (0.4)	1.5 (0.4)**	1.5 (0.4)
Triglycerides, mmol/L	1.7 (1.0)	1.7 (1.0)	1.7 (1.0)**	1.7 (0.9)
Body Mass index	27.4 (4.3)	27.4 (4.3)	27.2 (4.2)**	27.3 (4.1)
Framingham Risk Score, %	14.1 (10.1)	13.8 (9.9)	16.5 (11.4)**	17.6 (10.7)**
Exercise test variables				
Achieved Maximum Workload, Watt	85.4 (23.2)	85.7 (23.3)	83.2 (22.6)**	82.1 (22.5)**
Heart rate recovery, bpm	29.6 (10.0)	29.7 (9.9)	28.4 (10.4)**	27.1 (11.1)**
ST-segment depression >0.1mV, %	1.2	1.2	1.5	2.8**
QRS duration, ms	81.6 (9.6)	81.4 (9.5)	82.8 (10.6)**	84.3 (11.0)**
QTc interval, ms	396.4 (23.2)	396.0 (22.9)	399.8 (24.1)**	406.1 (28.9)**
Number of QRS complexes during recovery, N	89.2 (13.1)	89.1 (13.1)	89.2 (13.0)	90.9 (12.2)**

Continuous variables are presented as mean (SD); categorical variables are presented as %. HDL = high-density lipoprotein; LDL = low-density lipoprotein; PVC = premature ventricular contractions. Values indicated with * are significantly different from the 0 PVC group after Bonferroni correction for multiple testing (n=5). * Bonferroni corrected P-value < 0.05 ($p < 0.05 / 2$), ** Bonferroni corrected P-value < 0.01 ($p < 0.01 / 5$)

Table 2: Multivariable regression results for the association between high PVC burden and serum NT-proBNP levels

	Minimally adjusted			**Further adjusted for clinical factors**		
	beta	**95% CI**	**p value**	**beta**	**95% CI**	**p value**
High PVC burden during exercise (> 20 PVCs)						
NT-proBNP level (NPX units)	0.13	0.05 to 0.20	<0.001	0.10	0.03 to 0.17	0.008
High PVC burden during recovery (> 5 PVCs)						
NT-proBNP level (NPX units)	0.18	0.09 to 0.28	<0.001	0.16	0.06 to 0.25	0.001

The Normalized Protein eXpression (NPX) is a relative quantification unit where a difference of 1 NPX equates to a doubling of protein concentration. High PVC burden (>20 exercise PVCs or > 5 recovery PVCs) was compared with having no PVCs (during exercise or recovery). Associations were tested in a minimally adjusted model, which accounted for age (at time of exercise study), gender, no of beats (either during exercise or recovery), and time-interval between exercise and imaging study; and a further adjusted model, which accounted for the same variables as the minimally adjusted model + clinical variables (diabetes, hypertension, beta-blocker medication, smoking, LDL-C and HDL-C, triglycerides, body mass index, QRS duration, QTc interval, ST depression (>0.1 mV), and heart rate exercise (for exercise PVCs) or heart rate recovery (for recovery PVCs).

Figure legends

Figure 1: Study exclusion diagram. History of cardiovascular (CV) disease was identified using self-reported and hospital episode data (Supplemental Table 1). ECG = electrocardiogram, AF = atrial fibrillation.

Figure 2: Cumulative Incidence Curves of MI/HF/LTVA Events According to Different PVC Counts.

Figure 3: Cumulative Incidence Curves of All-Cause Mortality Events According to Different PVC Counts.

Figure 4: Adjusted Hazard Ratios of MI/HF/LTVA Events and All-cause mortality According to PVC count. Hazard ratios were adjusted for clinical variables: age, gender, diabetes, hypertension, beta-blocker medication, smoking, LDL-C and HDL-C, triglycerides, body mass index, QRS duration, QTc interval, ST depression (>0.1 mV), and no. of heartbeats during exercise and heart rate exercise (for exercise PVCs), or no. of heartbeats during recovery and heart rate recovery (for recovery PVCs). The reference category is 0 PVCs. Sizes of the boxes are proportional to the inverse of the variance of the log-transformed hazard ratios. Vertical lines represent 95% confidence intervals. Numbers shown above and below each box are the hazard ratio and number of events, respectively.

Figure 1: Study exclusion diagram

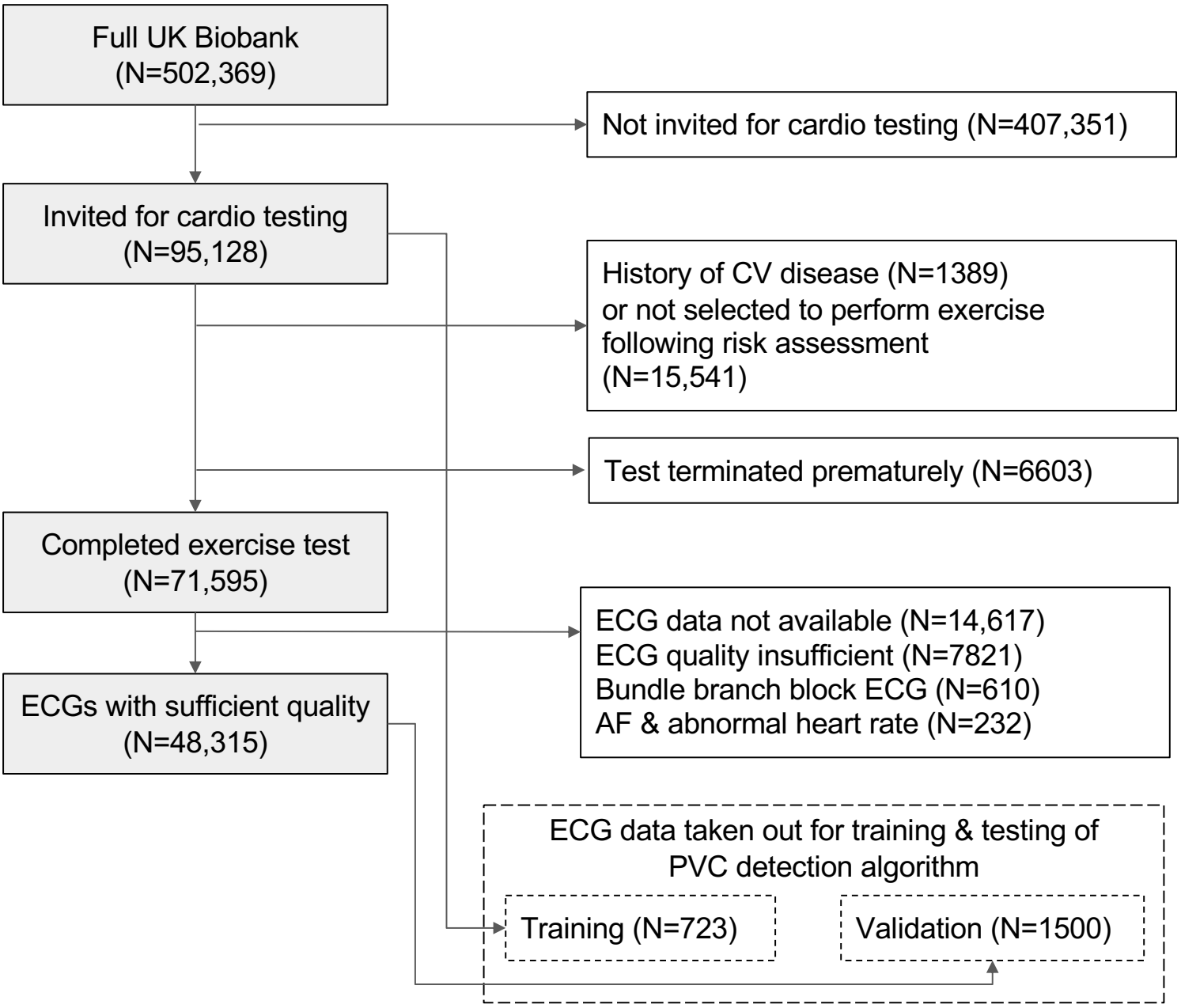


Figure 2: Cumulative Incidence Curves of MI/HF/LTVA Events According to Different PVC Counts

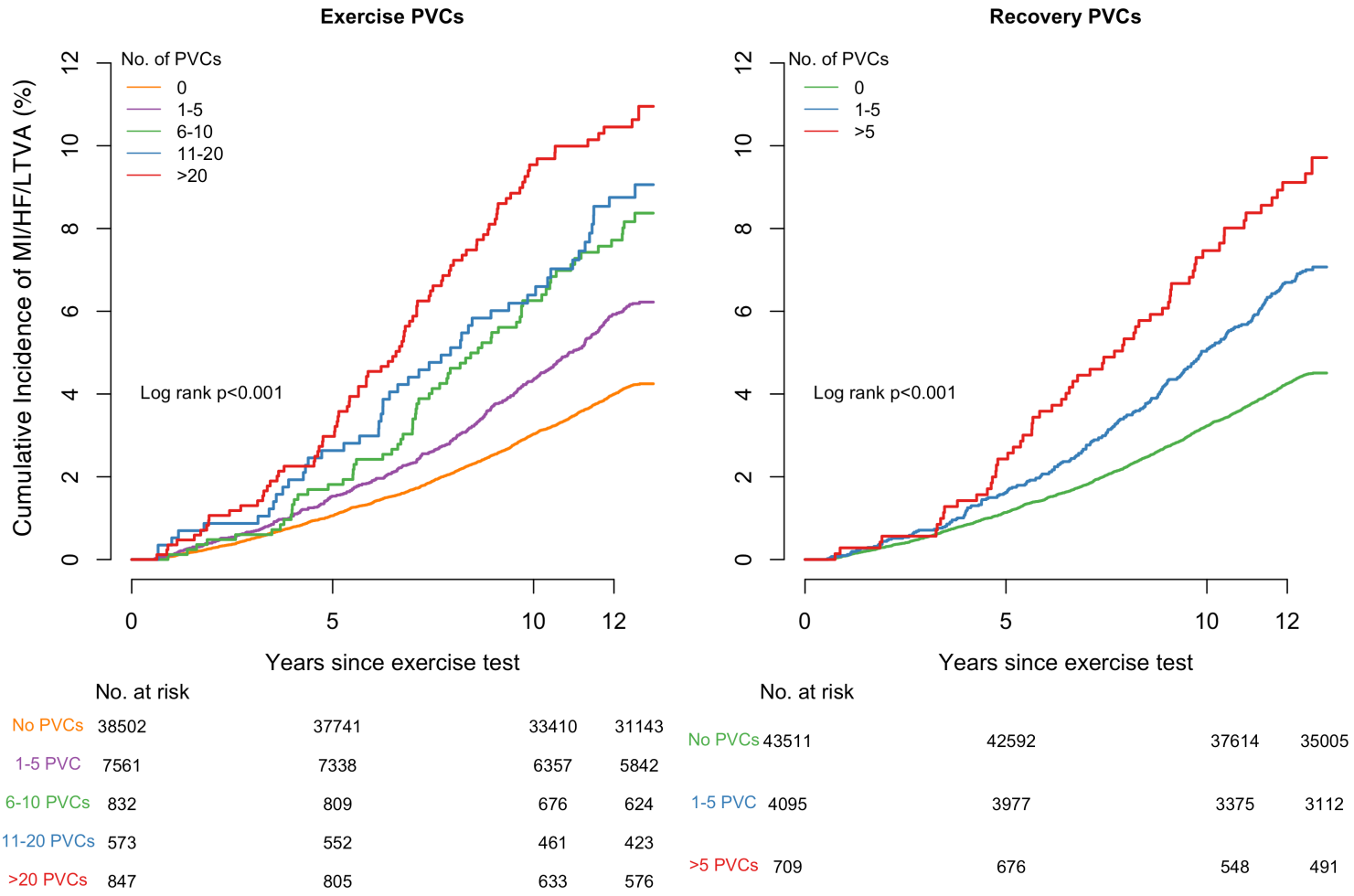
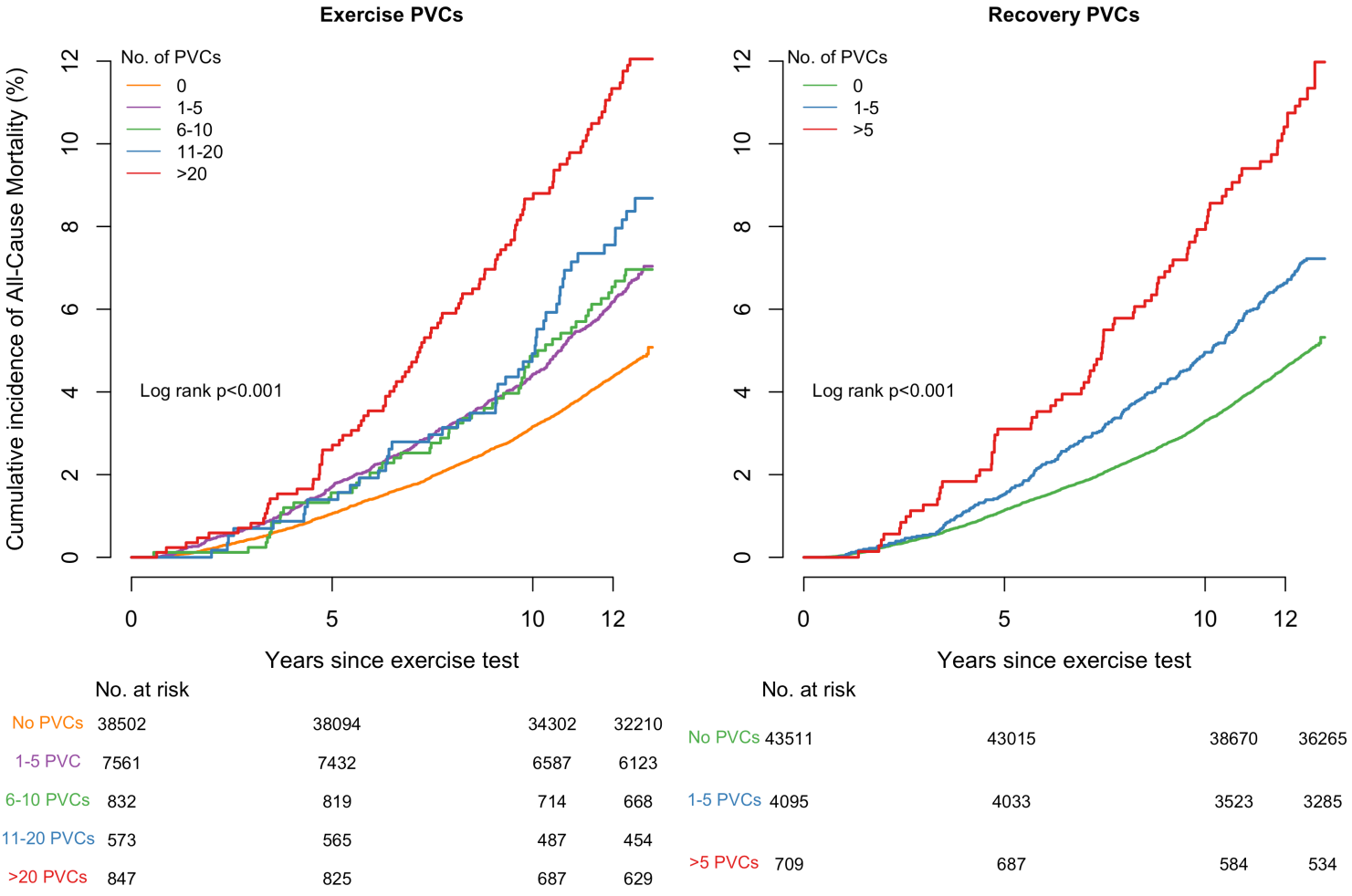
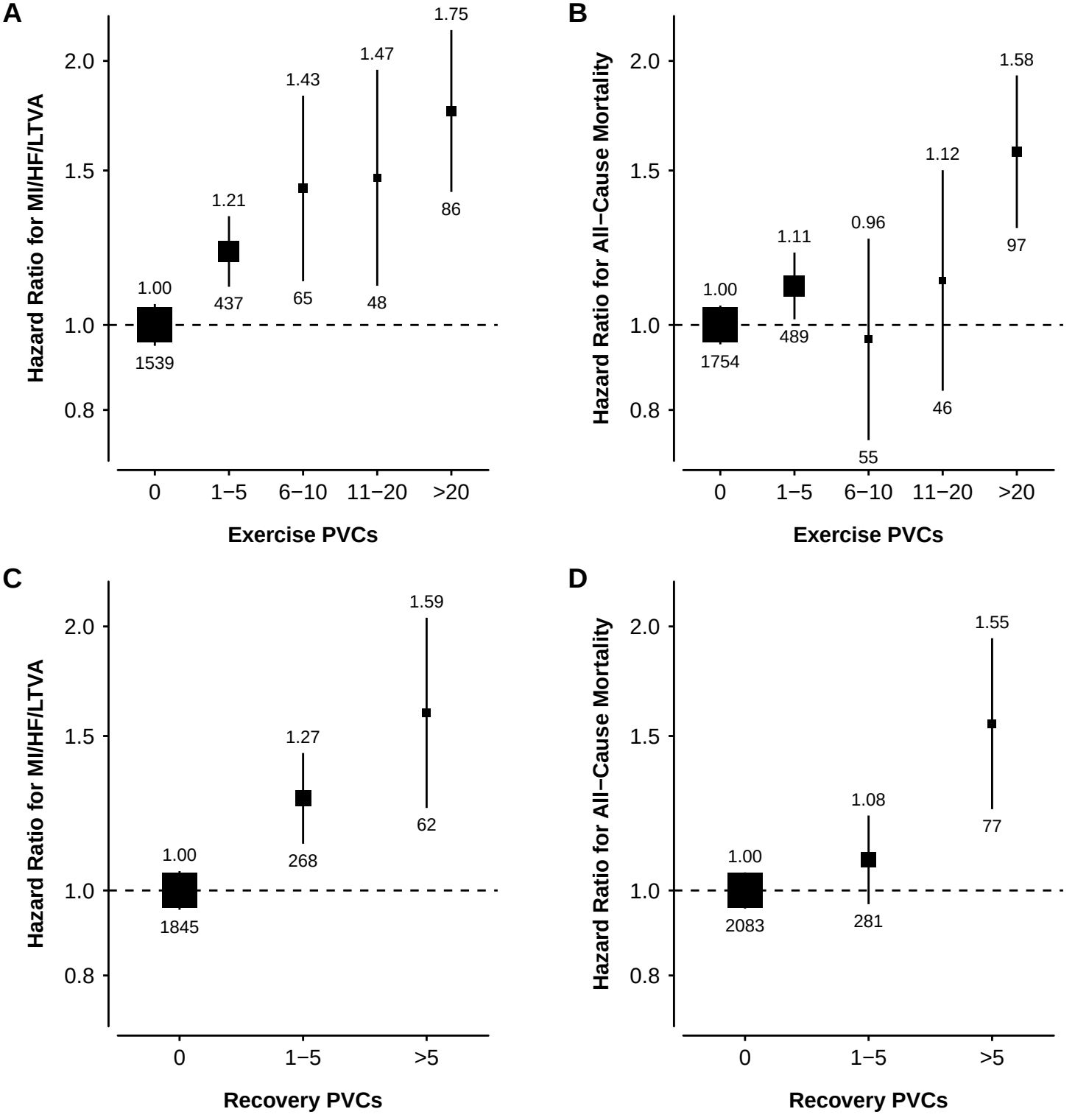


Figure 3: Cumulative Incidence Curves of All-Cause Mortality According to Different PVC Counts




A
Hazard Ratio for MI/HF/LTVA
2.0
1.5
1.0
0.8
1.00
1539
1.21
437
1.43
65
1.47
48
1.75
86
0
1−5
6−10
11−20
>20
Exercise PVCs
B
Hazard Ratio for All−Cause Mortality
2.0
1.5
1.0
0.8
1.00
1754
1.11
489
0.96
55
1.12
46
1.58
97
0
1−5
6−10
11−20
>20
Exercise PVCs
C
Hazard Ratio for MI/HF/LTVA
2.0
1.5
1.0
0.8
1.00
1845
1.27
268
1.59
62
0
1−5
>5
Recovery PVCs
D
Hazard Ratio for All−Cause Mortality
2.0
1.5
1.0
0.8
1.00
2083
1.08
281
1.55
77
0
1−5
>5
Recovery PVCs