

Hyperventilation-Induced Heart Rate Response as a Marker of Cardiovascular Risk - Large-Scale Validation from the Courtois Cardiovascular Signature Program

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Abstract

Hyperventilation-induced change in heart rate (Δ HR) is attenuated in cardiovascular patients, but validation in a large cohort was lacking. We analysed 3,134 participants from the Courtois Cardiovascular Signature Program (CCVSP), spanning five pre-specified cardiovascular risk categories from zero-risk healthy individuals to established atherosclerotic cardiac disease. Δ HR was defined as heart rate at end of 60-second paced hyperventilation minus resting baseline. Δ HR decreased monotonically across risk groups (Kruskal-Wallis $H = 145.2$, $p < 0.001$), from a median of 24.0 bpm [IQR 16.0–33.0] in Zero-Risk participants to 15.0 bpm [9.2–22.0] in those with atherosclerotic cardiac disease. After age adjustment, only the established cardiovascular and atherosclerotic disease groups remained significantly attenuated; intermediate risk groups did not. A logistic model incorporating age and Δ HR achieved an AUC of 0.659 [95% CI: 0.633–0.684], with LR + 2.17 and LR – 0.49, yielding a negative predictive value of 97.5% at 5% prevalence. An enhanced model adding history-based variables (sex, smoking, diabetes, hypertension, family history) achieved an AUC of 0.710 [95% CI: 0.686–0.735]. This study validates, in the largest prospective cohort to date, that hyperventilation-induced Δ HR is attenuated in cardiovascular disease. Age-adjusted Δ HR provides a clinically meaningful rule-out performance in low-prevalence settings, supporting its use as a simple, equipment-light pre-screening marker.

Introduction

Cardiovascular disease (CVD) is the leading cause of morbidity and mortality in developed countries, affecting approximately one-third of individuals over the age of 65. [1, 2] The development of simple, accessible pre-screening tools that can improve the selection of patients for advanced diagnostic testing therefore represents an important clinical priority. Current standard diagnostic pathways – including exercise or pharmacological stress testing, coronary CT angiography, and invasive angiography – are effective but resource-intensive, involve radiation exposure, contrast administration, or pharmacological agents, and are not suitable for broad initial screening. [3]

Heart rate (HR) is among the most accessible physiological measurements in clinical practice, and its responses to stress have long been recognized as markers of cardiovascular health. An inadequate chronotropic response to exercise has been observed in heart failure and coronary artery disease (CAD), and was shown to be independently associated with adverse outcomes. [4, 5] The hemodynamic response to hyperventilation offers an alternative, exercise-free physiological stimulus. Voluntary hyperventilation induces a decrease in blood carbon dioxide, which in turn activates the sympathetic nervous system and promotes vagal withdrawal, leading to a measurable increase in heart rate, comparable to pharmacological agents or physical exertion.[6] The magnitude of this response (expressed as Δ HR) may therefore reflect the integrity and responsiveness of cardiac autonomic regulation and coronary vasoreactivity.

Building on this premise, Hawkins et al. conducted a pooled retrospective analysis of 282 participants from nine studies and demonstrated that ΔHR during 60 seconds of paced hyperventilation was significantly and consistently attenuated in all cardiovascular patient groups, including CAD, heart failure, and angina with normal coronary arteries, as compared to age-matched healthy controls aged ≥ 55 years ($\Delta\text{HR } 7 \pm 7$ vs 15 ± 9 bpm, $p < 0.001$). [7] Preliminary cut-off values of 8.5 and 15.5 bpm yielded positive and negative predictive values of approximately 89% and 91%, respectively, in age-stratified analyses. Importantly, beta-blocker use did not significantly attenuate the response.

We aimed to validate the ΔHR –cardiovascular risk relationship using data from a large-scale cohort with a wide range of health and risk. The Courtois Cardiovascular Signature Program (CCVSP), enrolled participants prospectively at a single centre across five pre-defined cardiovascular risk categories. All participants underwent a standardized breathing-enhanced oxygenation-sensitive cardiovascular magnetic resonance (OS-CMR) scan as part of the base protocol.

Methods

Study Design and Setting

The Courtois Cardiovascular Signature Program (CCVSP) is a prospective, single-centre, cross-sectional cohort study designed to characterize cardiovascular phenotypes across a spectrum from phenotypically optimal health to established cardiovascular disease. All participants were enrolled and examined at the McGill University Health Centre, Montreal, Canada, under approval of the McGill University Health Centre Research Ethics Board and in accordance with the Declaration of Helsinki. All participants provided written informed consent, including for the secondary use of anonymized data for research purposes. The present analysis is a pre-specified secondary study of CCVSP data, directly referencing the retrospective pooled analysis by Hawkins et al. as the index publication.

Participants and Risk Group Classification

Participants were prospectively enrolled into one of five pre-defined cardiovascular risk categories based on standardized clinical assessment at the time of enrollment, incorporating the INTERHEART Risk Score and corroborating medical records: (1) Zero-Risk, individuals with no identifiable cardiovascular risk factors or known disease; (2) 1–2 Risk Factors, individuals with a limited burden of classical risk factors (hypertension, dyslipidemia, mild obesity, or tobacco use history) but no established cardiovascular diagnosis; (3) High-Risk, individuals with multiple or high-weight risk factors or a 10-year Framingham cardiovascular risk $> 20\%$, without an established diagnosis; (4) Cardiovascular Disease, individuals with documented non-atherosclerotic cardiovascular conditions, including heart failure or non-ischaemic cardiomyopathy; and (5) Atherosclerotic Cardiac Disease, individuals with confirmed atherosclerotic coronary or peripheral vascular disease. Standard MRI contraindications, inability to perform the breathing maneuver, acute coronary syndrome within the preceding four weeks, and the presence of an

implantable cardioverter-defibrillator or pacemaker constituted exclusion criteria. Participants were asked to abstain from caffeine for 12 hours and fast for 4 hours prior to the study. Vasoactive medications were not routinely withheld.

Breathing Maneuver and Measurement of Δ HR

All participants underwent the standardized breathing-enhanced OS-CMR protocol at rest, performed in a supine position during MRI scanning, as previously described. [8–10] Following a period of regular breathing, resting baseline heart rate (BL-HR) was recorded from the ECG trace integrated into the MRI system (MRI-compatible ECG monitor, 3–4 leads). Participants then performed paced hyperventilation for 60- seconds at a target rate of 30 breaths per minute, guided by pre-recorded voice commands. The maximal heart rate during the 60-second hyperventilation period (HV-HR) was recorded from the same ECG trace. Following hyperventilation, participants performed a maximal voluntary breath-hold in a comfortable end-exhalatory breathing position for the purpose of OS-CMR image acquisition, instructed to sustain the breath-hold for ≥ 40 seconds for the OS-CMR images. The hyperventilation-induced heart rate change was calculated as:

$$\Delta\text{HR} = \text{HV-HR} - \text{BL-HR} \text{ (bpm)}$$

as used by Hawkins et al. [7] The percentage change was additionally calculated as

$$\% \Delta\text{HR} = (\Delta\text{HR} / \text{BL-HR}) \times 100.$$

Data Quality Assessment and Exclusion Criteria

Prior to analysis, a systematic data quality review was applied to all CCVSP records. Records were excluded if: (1) BL-HR or HV-HR was absent (no value recorded), recorded as zero (encoded as missing data, negative, or if any heart rate was implausibly high (> 180 bpm)), all of which are consistent with erroneous data entries, missing-data encoding or sensor artefacts rather than physiological values. Records without a valid CCVSP risk-group classification were also excluded. A sensitivity analysis additionally excluding participants with a documented adverse breathing-maneuver event (early termination, symptomatic response, or incomplete maneuver as recorded in the CCVSP database) was performed to assess the impact of protocol non-adherence on study findings.

Statistical Analysis

All analyses were performed in Python (version 3.10; NumPy, SciPy, pandas). Non-normally distributed continuous variables are reported as median [interquartile range, IQR]; normally distributed variables as mean $\pm$ standard deviation (SD). Normality was assessed by the Shapiro-Wilk test. Statistical

significance was set at $p < 0.05$ (two-sided). Given the large sample size, effect size metrics are reported alongside p-values, as statistical significance alone does not imply clinical importance.

Differences in ΔHR across the five risk categories were assessed by Kruskal-Wallis test; pairwise Mann-Whitney U tests with Bonferroni correction (ten comparisons) were used to compare each group to the Zero-Risk reference. Spearman rank correlation (ρ) was used for associations between ordinal risk rank, age, and ΔHR . Cohen's d quantified effect size for the binary patient/non-patient comparison.

The relationship between age and ΔHR was characterized by Spearman rank correlation and linear regression, both across the full sample and within the Zero-Risk group. An analysis of covariance (ANCOVA) was performed with ΔHR as the dependent variable, cardiovascular risk group as the categorical predictor (Zero-Risk as reference), and age as a continuous covariate, to estimate the independent contributions of age and risk group to ΔHR variance. Partial Spearman correlation between risk rank and ΔHR , controlling for age, was computed by residualization.

A multivariable logistic regression model was fitted with binary patient/non-patient status (Patients: Cardiovascular Disease + Atherosclerotic Cardiac Disease; Non-Patients: all others) as the outcome and age and ΔHR as joint predictors. Coefficients were estimated by Newton-Raphson optimization applied to the log-likelihood. Model discrimination was assessed by AUC, with 95% confidence intervals from 2,000 bootstrap resamples. The optimal probability threshold was selected by maximizing Youden's J (sensitivity + specificity - 1). Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were computed at the optimal threshold, with bootstrap confidence intervals. Positive (LR+) and negative (LR-) likelihood ratios were derived from sensitivity and specificity and were used with Bayes' theorem to project expected PPV and NPV across clinically relevant prevalence levels (2%, 5%, 10%, 30%).

To evaluate whether ΔHR retains incremental value when combined with information readily available from history-taking and simple anthropometric assessment, an extended logistic regression model was constructed using age, sex, body mass index (BMI), smoking history (current or former vs. never), diabetes, hypertension, family history of cardiovascular disease, and ΔHR as joint predictors of patient/non-patient status (eight predictors in total). Because BMI requires height and weight measurement and contributed minimally to model discrimination (see Results), a reduced seven-variable model omitting BMI was subsequently fitted in the same participants. Both models were evaluated using the same discrimination (AUC, with 95% confidence intervals from 2,000 bootstrap resamples) and classification metrics (sensitivity, specificity, PPV, NPV, LR + and LR - at the Youden-optimal threshold) as the age + ΔHR model, and accuracy was additionally examined within each cardiovascular risk group and according to the presence or absence of a recorded adverse breathing-maneuver event.

Furthermore, the category-free net reclassification improvement (NRI) and the integrated discrimination improvement (IDI) were calculated to directly quantify the proportion of individuals correctly reassigned to higher or lower predicted risk and provide a more clinically informative assessment.

A three-zone gatekeeper framework was evaluated using the logistic model predicted probability. Three zones were defined: gate-out (probability below a lower threshold), gate-in (probability above an upper threshold), and an indeterminate zone requiring further investigation. Gatekeeper performance metrics were as follows: Disease miss rate (proportion of patients incorrectly assigned to gate-out), gate-in PPV, and proportion of the population requiring further investigation. Threshold pairs were evaluated across lower boundaries of 0.08 to 0.15 and upper boundaries of 0.20 to 0.30.

Subgroup analyses by age strata (35–55, 56–65, 66–85 years) and a sensitivity analysis excluding participants with adverse breathing events were also performed.

Results

Study Population

Of 3,485 CCVSP participants, 351 (10.1%) were excluded following the data quality review: 133 had no BL-HR or HV-HR value recorded; 42 had values consistent with missing-data encoding or sensor artefact, comprising 15 records with HV-HR or Δ HR entered as zero, 16 with BL-HR entered as zero, 9 with a negative BL-HR or HV-HR value, and 1 with HV-HR of 405 bpm; and 167 had no valid CCVSP risk-group classification. The final analytic sample comprised 3,134 participants (Table 1). Risk group distribution was: Zero-Risk n = 296, 1–2 Risk Factors n = 1,421, High-Risk n = 869, Cardiovascular Disease n = 266, and Atherosclerotic Cardiac Disease n = 282. In the binary classification, Patients numbered 548 (17.5%) and Non-Patients 2,586. The overall sample was 54.1% female (n = 1,697), with a mean age of 56.6 ± 10.3 years.

Age increased monotonically across risk groups (Kruskal-Wallis H = 202.3, p < 0.001), from 52.0 ± 10.3 years in the Zero-Risk group to 62.3 ± 9.4 years in the Atherosclerotic Cardiac Disease group. Resting BL-HR did not differ meaningfully across groups (range of means: 65.0–67.0 bpm), consistent with the observation of Hawkins et al. that resting heart rate is not a reliable discriminator of cardiovascular risk status. [7]

Among 236 participants with available breath-hold duration data, 220 (93.2%) achieved the protocol target of ≥ 40 seconds (mean 53.6 ± 9.2 s; range 9–66 s). A recorded adverse breathing maneuver event was present in 704 of 3,034 participants with available event data (23.2%), and an incomplete OS-CMR sequence was noted in 30.9% of participants with available completion status. These acquisition quality indicators are further addressed below.

Table 1. Baseline characteristics and Δ HR across cardiovascular risk categories (CCVSP, n = 3,134)

Risk Group	n	Age (years)	Female (%)	BL-HR (bpm)	HV-HR (bpm)	Δ HR (bpm)	CV (%)
Zero-Risk	296	52.0 $\pm$ 10.3	60	65.0 $\pm$ 9.9	90.2 $\pm$ 16.9	25.3 $\pm$ 12.2	48
1–2 Risk Factors	1,421	55.3 $\pm$ 10.0	59	66.5 $\pm$ 10.3	89.9 $\pm$ 15.6	23.7 $\pm$ 12.4	52
High-Risk	869	58.4 $\pm$ 9.5	54	67.0 $\pm$ 10.6	88.0 $\pm$ 15.3	21.6 $\pm$ 11.4	53
Cardiovascular Disease	266	57.7 $\pm$ 11.3	54	66.1 $\pm$ 10.8	84.9 $\pm$ 16.1	19.2 $\pm$ 11.2	58
Atherosclerotic Cardiac Disease	282	62.3 $\pm$ 9.4	23	65.8 $\pm$ 10.2	80.7 $\pm$ 14.8	16.2 $\pm$ 10.2	63
All Patients (CVD + Athero)	548	60.0 $\pm$ 10.5	38	66.0 $\pm$ 10.6	82.9 $\pm$ 15.6	17.7 $\pm$ 10.8	61
All Non-Patients	2,586	56.1 $\pm$ 10.1	58	66.3 $\pm$ 10.3	89.4 $\pm$ 15.6	23.2 $\pm$ 12.1	52

Values are mean $\pm$ SD. BL-HR = baseline (resting) heart rate; HV-HR = heart rate at end of 60-second hyperventilation; Δ HR = HV-HR – BL-HR; CV = coefficient of variation (SD/mean $\times$ 100%). Female sex expressed as %. Kruskal-Wallis $H = 145.2$, $p < 0.001$ for Δ HR across all five groups.

Δ HR Across Cardiovascular Risk Categories

The 60 seconds of paced hyperventilation induced a measurable increase in heart rate in all five risk groups, confirming that the hyperventilation stimulus is effective across the cardiovascular risk spectrum. Δ HR showed a statistically significant, monotonically decreasing gradient from the Zero-Risk group (median 24.0 bpm [IQR 16.0–33.0]) through to the Atherosclerotic Cardiac Disease group (median 15.0 bpm [9.2–22.0]; Kruskal-Wallis $H = 145.2$, $p < 0.001$; one-way ANOVA $F = 22.3$, $p < 0.001$). The gradient corresponds to a 37% reduction in median Δ HR from the healthiest to the most diseased group. These findings directly replicate the pattern reported by Hawkins et al. in a cohort more than ten times larger and drawn from a single standardized protocol.

Bonferroni-corrected pairwise comparisons revealed that the Zero-Risk group was statistically indistinguishable from the 1–2 Risk Factors group ($p = 0.90$) but differed significantly from all higher-risk groups (High-Risk: $p = 0.0002$; Cardiovascular Disease: $p < 0.001$; Atherosclerotic Cardiac Disease: $p < 0.001$). The Cardiovascular Disease and Atherosclerotic Cardiac Disease groups also differed from the High-Risk group ($p = 0.011$ and $p < 0.001$, respectively) and borderline from each other ($p = 0.049$). In the binary comparison, patients had significantly lower Δ HR than non-patients (mean 17.7 ± 10.8 vs 23.2 ± 12.1 bpm; Mann-Whitney $p < 0.001$; Cohen's $d = 0.469$).

Age as a Confounder of Δ HR

Age was significantly and inversely correlated with Δ HR across the full sample (Spearman $\rho = -0.277$, $p < 0.001$), confirming the age-dependent attenuation of the hyperventilation HR response observed by Hawkins et al. in healthy controls. In the ANCOVA, the age coefficient was $\beta = -0.295$ bpm/year (SE = 0.026, $p < 0.001$), corresponding to a decrease of approximately 3.0 bpm per decade. Age explained 3.88% of Δ HR variance uniquely, compared to 1.43% explained uniquely by cardiovascular risk group classification; the two predictors shared 1.30% of variance attributable to their collinearity.

After controlling for age, only the Cardiovascular Disease ($\beta = -4.06$ bpm, SE = 1.21, $p = 0.001$) and Atherosclerotic Cardiac Disease ($\beta = -5.82$ bpm, SE = 1.22, $p < 0.001$) groups retained a significantly lower Δ HR relative to the Zero-Risk reference. The 1–2 Risk Factors ($p = 0.91$) and High-Risk ($p = 0.32$) groups were no longer significantly attenuated after age adjustment, indicating that the lower observed Δ HR in these intermediate groups largely reflects their older age distribution rather than an independent disease-related suppression of the HR response. The partial Spearman correlation between cardiovascular risk rank and Δ HR, controlling for age, was $\rho = -0.138$ ($p < 0.001$), retaining 69% of the magnitude of the unadjusted correlation ($\rho = -0.199$). These findings extend the qualitative observation of Hawkins et al. by formally quantifying the relative contributions of age and disease status to Δ HR variance.

Within the Zero-Risk group alone ($n = 299$ after outlier removal), the association between age and Δ HR was weak (Pearson $r = -0.120$, $p = 0.039$; % Δ HR = $52.6 - 0.23 \times \text{age}$; $R^2 = 0.014$). This association was further attenuated and lost statistical significance when participants with recorded adverse events were additionally excluded ($n = 193$; $r = -0.108$, $p = 0.134$; $R^2 = 0.012$), suggesting that part of the residual age- Δ HR relationship in healthy individuals may reflect acquisition noise rather than true physiology. Age-stratified median Δ HR in Non-Patients was 25.0 bpm (ages 35–55), 21.0 bpm (ages 56–65), and 17.0 bpm (ages 66–85), compared to 19.0, 15.0, and 13.0 bpm in Patients across the same strata. This pronounced age-by-group interaction underscores that a single age-unadjusted Δ HR cut-off will systematically misclassify older Non-Patients as suspected disease.

Age-Adjusted Logistic Regression Model

The logistic regression model with age and Δ HR as joint predictors yielded the equation:

$$\log\text{-odds(Patient)} = -2.480 + 0.029 \times \text{Age} - 0.037 \times \Delta\text{HR}$$

The odds of patient status increased by 3.0% per additional year of age (OR = 1.030) and decreased by 3.7% per additional bpm of Δ HR (OR = 0.963). Model discrimination and diagnostic performance metrics are presented in Table 2. The logistic model achieved AUC = 0.659 [95% CI: 0.633–0.684], significantly outperforming Δ HR alone (AUC = 0.642) and exceeding the Δ HR-only Youden J statistic by 8.1%. A secondary model using % Δ HR achieved AUC = 0.651 [0.626–0.674], with overlapping confidence intervals relative to the absolute Δ HR model; the absolute Δ HR model was retained as the primary model based on marginally superior Youden J (0.239 vs 0.228) and simpler interpretability.

Table 2. Diagnostic performance of ΔHR-based models, without and with age (n = 3,134; Patient prevalence 17.5%)

Model	AUC [95% CI]	Threshold	Sens	Spec	PPV*	NPV*	J
ΔHR alone	0.642	≤ 15 bpm	48.9%	73.2%	26.3%	88.5%	0.221
Age + ΔHR (logistic)	0.659 [0.633–0.684]	p ≥ 0.165	69.9%	54.0%	24.4%	89.4%	0.239

AUC = area under the receiver operating characteristic curve; 95% CI from 2,000 bootstrap resamples. Youden's J = sensitivity + specificity – 1. PPV* and NPV* at cohort prevalence of 17.5%. Sens = sensitivity; Spec = specificity; J = Youden's J statistic.

Prevalence-Adjusted Performance and Likelihood Ratios

Because PPV and NPV are strongly prevalence-dependent, we calculated likelihood ratios (LR + and LR – as a more generalizable representation of test performance. The logistic model yielded LR + = 2.17 and LR –=0.49. Table 3 projects the expected PPV and NPV across four clinically distinct prevalence scenarios using Bayes' theorem. At a prevalence of 2–5%, representative of wellness or primary care screening, the model provides excellent as a rule-out tool with a NPV of 97.5–99.0%. At higher prevalence levels typical of cardiology referral settings, the rule-in performance remains limited (PPV 48.2% at 30% prevalence), and the rule-out value diminishes accordingly.

Table 3. Projected PPV and NPV of the age + ΔHR logistic model across clinically relevant prevalence levels

Clinical setting	Prevalence	PPV (gate-in)	NPV (gate-out)	LR+	LR–	Context
Wellness / primary care	2%	4.2%	99.0%	2.17	0.49	Excellent rule-out
General population screening	5%	10.3%	97.5%	2.17	0.49	Good rule-out
Cardiology / symptomatic	30%	48.2%	82.8%	2.17	0.49	Moderate rule-out
Post-positive stress test†	~ 69%	83.1%	47.6%	2.17	0.49	Limited utility

LR + = positive likelihood ratio; LR – = negative likelihood ratio. Both are fixed at LR + = 2.17 and LR –=0.49, derived from sensitivity (69.9%) and specificity (54.0%) at the optimal Youden threshold. PPV and NPV computed by Bayes' theorem. †Representative of post-positive stress test referral population (Hawkins et al. 2019).

Enhanced Risk Model Combining Δ HR with History-Based and Anthropometric Predictors

To evaluate the incremental value of Δ HR beyond traditional risk factors, we first fitted a baseline clinical model comprising age, sex, BMI, smoking history, diabetes, hypertension, and family history, without Δ HR, resulting in an AUC of 0.688 [95% CI 0.664–0.716] ($n = 3,121$). Adding Δ HR to this baseline model yielded a seven-variable model (BMI removed; see below) with an AUC of 0.710 [95% CI 0.689–0.737], an improvement of 0.022 over baseline features alone, and 0.051 over Δ HR alone (AUC = 0.642). The full eight-variable model including BMI ($n = 3,121$) achieved AUC = 0.707 [95% CI 0.682–0.732], virtually identical. Male sex (OR = 2.06), diabetes (OR = 2.06), and hypertension (OR = 1.61) were the strongest additional contributors to risk, while ever-smoking (OR = 1.32) and family history (OR = 1.26) contributed more modestly; Δ HR remained an independent predictor in the fully adjusted model (OR = 0.970 per bpm), confirming that its physiological signal is not captured by conventional risk-factor data alone.

BMI contributed negligibly to discrimination (OR = 0.991 per kg/m^2 , with a 95% confidence interval overlapping unity) once the other history-based variables were accounted for. Removing BMI from the model, i.e. a seven-variable model of age, sex, smoking history, diabetes, hypertension, family history, and Δ HR ($n = 3,129$), left discrimination essentially unchanged (AUC = 0.710 [95% CI 0.686–0.735]) and additionally permitted retention of participants lacking height or weight measurements. At the Youden-optimal predicted probability threshold (≥ 0.173), the seven-variable model achieved a sensitivity of 65.0%, a specificity of 67.0%, with a PPV of 29.3%, and an NPV of 90.1% (LR+ = 1.97, LR– = 0.52; patient prevalence 17.4%).

Classification accuracy of the seven-variable model varied substantially across risk groups, reflecting the prevalence-dependence of accuracy at a fixed threshold: 91.2% in the Zero-Risk group, 77.1% in the 1–2 Risk Factors group, 42.3% in the High-Risk group, 49.6% in the Cardiovascular Disease group, and 79.8% in the Atherosclerotic Cardiac Disease group. Performance was similar in participants with a recorded adverse breathing-maneuver event (AUC = 0.657, accuracy 71.8%) and those without (AUC = 0.722, accuracy 65.3%), indicating that the enhanced model's performance is robust to acquisition-related variability in Δ HR.

Net Reclassification Improvement by Adding Δ HR to a Composite Clinical Model

The incremental reclassification from adding Δ HR to the full baseline clinical history model, i.e. the 6-variable model of age, sex, smoking history, diabetes, hypertension, and family history, was calculated as NRI = + 0.300 [+ 0.213, + 0.391].

Three-Zone Gatekeeper Analysis

The three-zone gatekeeper framework using the logistic model predicted probability was evaluated across multiple threshold configurations (Table 4). At the primary scenario of probability < 0.10 (gate-out,

reassure without further investigation) and probability > 0.25 (gate-in, refer directly for further evaluation), 18.5% of the sample was assigned to the gate-out zone with a disease miss rate of 9.9% (54 of 548 patients), 17.6% to the gate-in zone (PPV 31.5%), and 63.9% to the indeterminate zone. More conservative gate-out thresholds (probability < 0.08) reduced the miss rate to 4.0%, but gated out only 10.3% of the population, limiting practical impact. More permissive thresholds (probability < 0.15) gated out 42.2% of participants but entailed a miss rate of 24.5%, which is operationally unacceptable for a screening test. No threshold combination simultaneously achieved a miss rate < 10% and a gate-out fraction > 25%.

Table 4. Three-zone gatekeeper performance across selected probability threshold pairs

Thresholds (prob.)	Gate-out %	Miss rate	Gate-in %	PPV gate-in	LR–	Indeterminate zone
< 0.08 / > 0.30	10.3%	4.0%	7.2%	41.4%	0.47	82.4%
< 0.10 / > 0.25	18.5%	9.9%	17.6%	31.5%	0.49	63.9%
< 0.12 / > 0.25	27.8%	14.8%	17.6%	31.5%	0.51	54.6%
< 0.15 / > 0.25	42.2%	24.5%	17.6%	31.5%	0.56	40.1%

Gate-out = participants with predicted probability below lower threshold; these individuals would be reassured without further investigation. Miss rate = proportion of all Patients incorrectly assigned to the gate-out zone. Gate-in = participants above upper threshold; these would proceed directly to further evaluation. Indeterminate zone = participants between thresholds; these would require additional investigation (e.g., OS-CMR or stress imaging). Bold row = primary evaluated scenario. LR– = negative likelihood ratio of the gate-out assignment.

Age-stratified analysis of the primary gatekeeper scenario (probability < 0.10 / >0.25) showed a marked age effect on gatekeeper efficiency. Among participants aged 35–55 years, 37.4% were assigned to the gate-out zone; among those aged 56–65 years, only 7.0% were gated out; and among those aged 66–85 years, less than 0.2% were gated out. This pattern reflects the model's expected behaviour: younger individuals with high ΔHR are most reliably assigned to the low-risk gate-out zone, while the model appropriately withholds a low-risk classification from older individuals, in whom reduced ΔHR is physiologically expected regardless of disease status.

Data Quality and Acquisition Variability

Within-group coefficients of variation for ΔHR ranged from 48% (Zero-Risk) to 63% (Atherosclerotic Cardiac Disease), reflecting substantial individual-level variability. Systematic contributors to this variability include a 23.2% adverse breathing maneuver event rate, a 6.8% rate of breath-hold duration below 40 seconds among participants with available data, and a 30.9% incomplete OS-CMR sequence rate. The underlying causes for the breadth of these acquisition quality indicators are unclear but

underline the importance of a tightly standardized protocol for minimizing within-group variance and improve discriminative performance beyond the AUC values reported here.

Discussion

The main finding of this study is that, in a prospectively recruited single-centre cohort of 3,134 participants spanning the full cardiovascular risk spectrum, the hyperventilation-induced change in heart rate is significantly attenuated in established cardiovascular disease relative to individuals without cardiovascular disease. This directly and independently replicates the core observation of Hawkins et al. [7] in a sample more than ten times larger and acquired under a standardized single-centre protocol, and extends it by formally quantifying age confounding and deriving an age-adjusted logistic model with likelihood ratios enabling prevalence-independent clinical interpretation.

A central finding beyond the primary replication is that, after adjustment for age, the Δ HR attenuation is specifically associated with established cardiovascular disease and not with cardiovascular risk factor burden per se. The 1–2 Risk Factors and High-Risk groups, which showed lower Δ HR than the Zero-Risk group in unadjusted analyses, were statistically indistinguishable from Zero-Risk individuals after accounting for their older age distribution. This confirms that Δ HR is not a marker of subclinical risk factor accumulation but of established disease and its associated autonomic and vascular impairment. Patients with CAD have well-documented reductions in vagal tone and heart rate variability, [11] and the attenuated Δ HR in this context likely reflects impaired parasympathetic reactivity to the hypocapnic stimulus of hyperventilation. The same mechanism likely underlies the attenuation seen in heart failure, in which heightened sympathetic activity and reduced parasympathetic reserve are hallmarks of the condition. [12]

The modest increment in AUC from adding Δ HR to the baseline clinical model (0.688→0.710; Δ AUC = + 0.022) should be interpreted in the context of the well-documented insensitivity of the c-statistic to incremental biomarker value. The AUC integrates discriminative performance across all possible thresholds, including a clinically irrelevant majority, thereby diluting the contribution of markers that meaningfully reclassify individuals near decision-relevant boundaries. [13] Category-free net reclassification improvement (NRI) and integrated discrimination improvement (IDI) directly quantify the proportion of individuals correctly reassigned to higher or lower predicted risk and provide a more clinically informative assessment. [14] Adding Δ HR to the baseline clinical model (age, sex, smoking history, diabetes, hypertension, and family history) yielded an NRI of + 0.300 [95% CI + 0.213, + 0.391], reflecting net upward reclassification of 24.5% of patients and net downward reclassification of 5.5% of non-patients. The corresponding IDI was + 0.015 [95% CI + 0.010, + 0.020], indicating a consistent shift in model-estimated probabilities toward confirmed patients and away from non-patients. These figures are comparable to those reported for several biomarkers now incorporated into clinical practice guidelines: High-sensitivity C-reactive protein added to the Framingham Risk Score improves AUC by only 0.006–0.012 in large prospective cohorts; coronary artery calcium scoring added to Framingham improves AUC by 0.02–0.04 with an NRI of approximately 0.25; [15] and the ankle-brachial index improves AUC by

approximately 0.014. The NRI of + 0.300 observed here therefore exceeds published hsCRP benchmarks and is comparable to that of CAC scoring, despite requiring no laboratory test, no imaging, and no radiation exposure. Within the enhanced model, Δ HR remained an independent predictor after full adjustment for conventional risk factors (OR = 0.970 per bpm, $p < 0.001$), confirming that the hyperventilation heart-rate response captures autonomic and vascular risk information not encoded in questionnaire-based history data.

A related and practically important observation is that the age- Δ HR relationship in healthy (Zero-Risk) individuals was weaker than expected from the larger literature and was no longer statistically significant when participants with protocol adverse events were excluded. This finding suggests that part of the apparent age-related Δ HR decline in healthy individuals in the CVCSP reflects acquisition heterogeneity rather than a purely physiological ageing effect, likely, at least in part, because participants who have difficulty completing the maneuver may tend to be older and therefore have lower HV-HR values. This interpretation is supported by the high within-group variability (CV 48–63%) and the 23.2% adverse event rate, further underscoring the importance of rigorous protocol standardization for the clinical deployment of Δ HR as a marker.

The absolute Δ HR values in the CVCSP differ from those reported by Hawkins et al. The median Δ HR in the Atherosclerotic Cardiac Disease group was 15.0 bpm in the CVCSP, compared to a mean of 7.6 bpm in the CAD group of Hawkins et al. Several factors may account for this discrepancy: The CVCSP enrolled participants from a community and outpatient population across a broad cardiovascular risk spectrum, whereas the Hawkins et al. cohort was heavily enriched with patients referred for cardiac magnetic resonance imaging, representing a clinical sample with more severe or symptomatic disease. Additionally, the Hawkins et al. analysis explicitly used healthy controls aged ≥ 55 years as the comparator, while the CVCSP Zero-Risk group spans a wider age range. Medication effects, scanner differences, and technologist experience in the pooled analysis may also contribute. These differences affirm the recommendation of Hawkins et al. that absolute cut-off values must be validated and calibrated for each specific clinical context and support the use of likelihood ratios, which are independent of the prevalence in the derivation cohort, as the primary vehicle for clinical translation.

Placing these findings in the context of other screening and diagnostic tools clarifies the potential role of Δ HR in various clinical settings. A meta-analysis of exercise stress electrocardiography for obstructive CAD reported a pooled sensitivity of 68% and a specificity of 77%, corresponding to $LR+ = 3.0$ and $LR- = 0.42$.^[13] The resting 12-lead ECG has generally lower diagnostic performance, with $LR+$ values in the range of 1.5–3.0 and $LR-$ typically above 0.6 for most ST-T abnormalities.^[14] The age + Δ HR model in the present study yielded $LR+ = 2.17$ and $LR- = 0.49$ (Fig. 3), which is directionally similar to exercise stress ECG, but derived from a two-minute breathing protocol requiring no treadmill, no pharmacological stress, and no specialist facility. As a gatekeeper, Δ HR can be positioned as a first-step physiological filter applied before the referral decision for any resource-intensive investigation.

Coronary artery calcium (CAC) scoring provides superior rule-out performance when the score is zero (LR = 0.10–0.15 for obstructive CAD), though its utility diminishes substantially with age.[15, 16] The age + Δ HR model cannot match a CAC score of zero for rule-out, and no claim is made to that effect. The relevant comparison is not head-to-head accuracy but position in the diagnostic cascade: CAC requires a CT scanner, a radiation dose of at least 1 mSv, a trained radiologist, and associated cost, making population-wide application impractical. Δ HR requires only a device capable of recording heart rate, and as such already available on most consumer wearables, and two minutes of a standardised breathing protocol executable in any clinical or non-clinical setting. Its function is not to diagnose disease but to identify the subpopulation that can be safely reassured without proceeding to formal imaging, and to flag the remainder for appropriate escalation. From a statistical standpoint, an NRI of + 0.300 for Δ HR compares favourably with the NRI of approximately 0.25 reported for CAC scoring added to conventional risk factors [22], and with those of other established cardiovascular risk markers [23, 24] (Fig. 4), with a fraction of costs for equipment and testing.

The three-zone gatekeeper analysis operationalises this role directly. At the primary threshold configuration (predicted probability < 0.10 / >0.25), 18.5% of participants were safely classified as low-risk, with a disease miss rate of 9.9% and an expected NPV of 97.5 to 99.0% across clinically realistic prevalence levels of 2 to 5%. In an applied wellness-screening context serving 10,000 individuals per year, this would correspond to approximately 1,850 individuals who could be reassured and diverted from further cardiac workup annually, which represents a meaningful reduction in downstream investigation burden regardless of the specific follow-on test. A further 17.6% of participants exceeded the upper threshold and could be prioritised for expedited evaluation. The majority (63.9%) fell into the indeterminate zone, reflecting the inherent limits of any single physiological marker and appropriately directing most individuals toward standard clinical assessment. This distribution reinforces the intended role of Δ HR not as a binary diagnostic arbiter but as an upstream triage layer that modulates the conventional diagnostic cascade. Applied in wellness and prevention clinics, corporate health programmes, insurance-related health assessments, or primary care settings where stress imaging is not immediately available, a brief, radiation-free, equipment-light physiological pre-screen of this kind could improve the efficiency and equity of the referral pathway for cardiac investigation. This is specifically useful for prevention strategies involving remote monitoring with wearable sensors such as watches, bands, and rings.

Sex differences in heart rate response to physiological stress are well documented. Age-predicted maximum heart rate formulas derived from predominantly male samples systematically overestimate peak heart rate in women [16, 17], and the AHA recommends sex-specific thresholds for chronotropic incompetence [18]. In the present cohort, however, sex did not materially confound the Δ HR–disease association: although men were over-represented in the Atherosclerotic Cardiac Disease group (76.6% vs. 39–46% in other groups; $\chi^2=127.0$, $p < 0.001$), Δ HR differed only marginally by sex within risk groups, and adjusting the age + Δ HR model for sex left the Δ HR odds ratio essentially unchanged (0.963 vs. 0.964 per bpm). A formal sex $\times$ risk-group interaction test was of borderline significance ($p = 0.048$). While sex does not appear to confound the present results, sex-specific reference ranges or

normalization of Δ HR merit further evaluation. Notably, the hyperventilation-based Δ HR paradigm removes physical exertion and functional capacity as confounders, which may make it particularly well suited to sex-equitable cardiovascular screening; whether this translates into improved diagnostic performance for women warrants dedicated prospective evaluation.

Several limitations of this study warrant acknowledgment. The cross-sectional design precludes assessment of longitudinal change or clinical outcomes. 5- and 10-year follow-up data will be available in the future. Medication data, including beta-blockers, were not systematically available across the CCVSP, preventing formal adjustment for pharmacological HR suppression. Of note, however, Hawkins et al. did not observe a significant beta-blocker effect on Δ HR. [7] Risk group classification was based on clinical assessment and risk scoring rather than imaging-confirmed diagnoses, which may introduce misclassification. All AUC estimates are in-sample and have not been externally validated. Finally, the age-stratified gatekeeper analysis highlights that age-specific probability thresholds may be necessary for optimal clinical deployment.

Conclusions

The Courtois Cardiovascular Signature Program independently validates, in the largest prospective single-centre cohort to date, that the hyperventilation-induced change in heart rate is significantly attenuated in established cardiovascular disease. Age is the dominant confounder and its formal integration into a logistic model is necessary for accurate interpretation. The resulting age-adjusted model provides clinically useful negative predictive performance, particularly as a rule-out tool in low-prevalence settings and comparable in scope to exercise stress electrocardiography. These results support Δ HR as a simple, equipment-light first-step physiological marker for cardiovascular risk stratification.

Declarations

Competing Interests

Matthias G. Friedrich is co-founder and shareholder of AiVALON Health Technologies Inc., a company that develops software for a smartphone-based measurement of heart rate changes during breathing maneuvers. The other authors do not report any conflicts related to this study.

SUPPLEMENTARY MATERIAL

Examples of risk classification in 3 individuals with different a priori risk based on age + Δ HR

Author Contribution

M.G.F. conceived the study, performed the analysis, and wrote the manuscript. M.L. performed the data acquisition. A.S., G.T. and N.G. contributed to interpretation of results and critical revision of the manuscript. All authors reviewed and approved the final version.

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Data Availability

The datasets generated and analysed during the current study are available on reasonable request.

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Figures

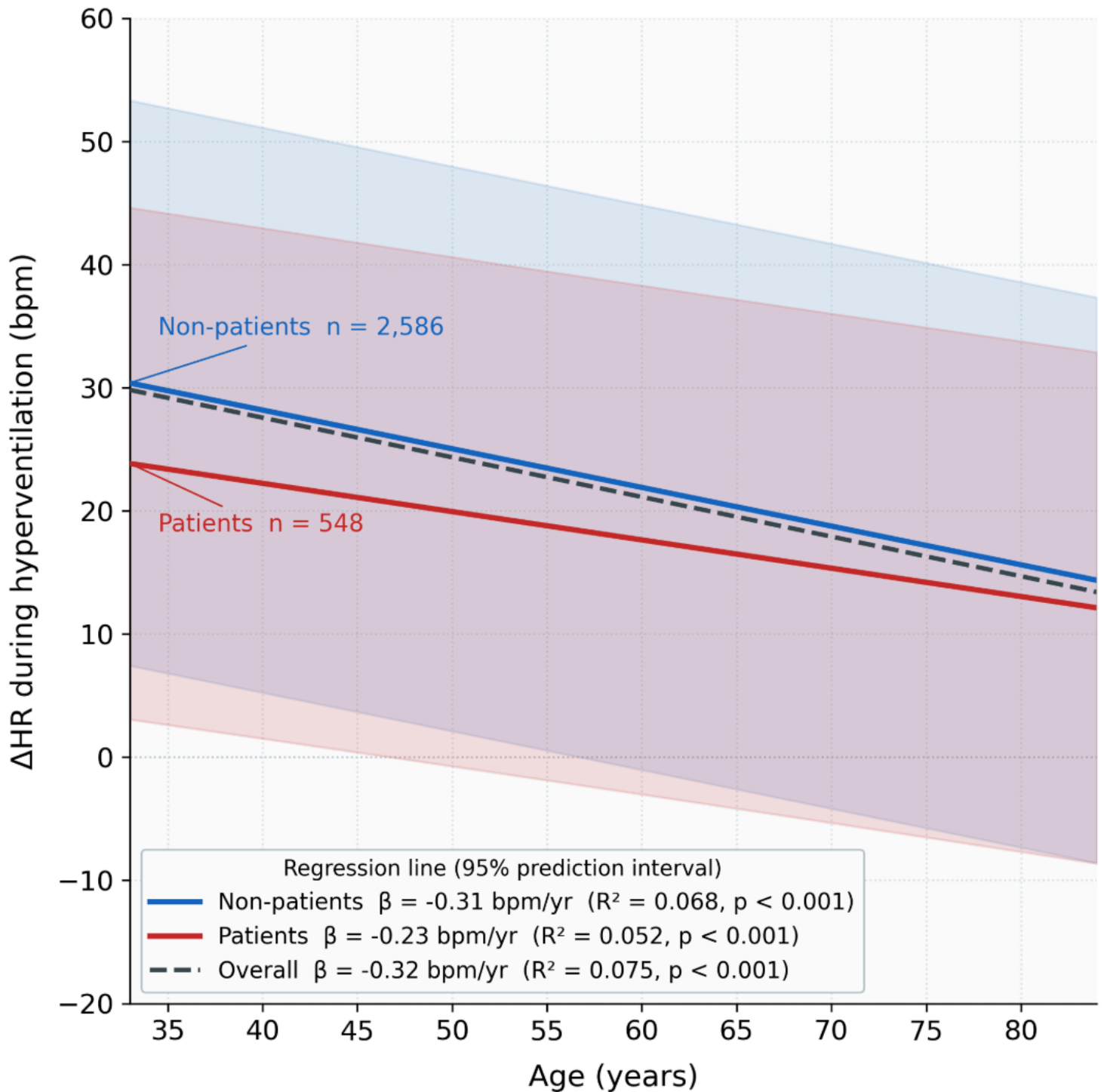


Figure 1

Relationship between age and Δ HR by patient status ($n=3,134$). Regression lines with 95% prediction intervals are shown separately for Non-Patients (blue; $n=2,586$; $\beta=-0.314$ bpm/yr) and Patients (CVD/ACD; red; $n=548$; $\beta=-0.230$ bpm/yr). The dashed line represents the overall regression across all participants ($\beta=-0.322$ bpm/yr). Shaded areas indicate 95% prediction intervals. Patients show a consistently lower Δ HR across all ages, with parallel but attenuated age-related decline. Spearman $\rho=-0.277$, $p<0.001$ (overall).

ROC Curves: Incremental Value of Δ HR

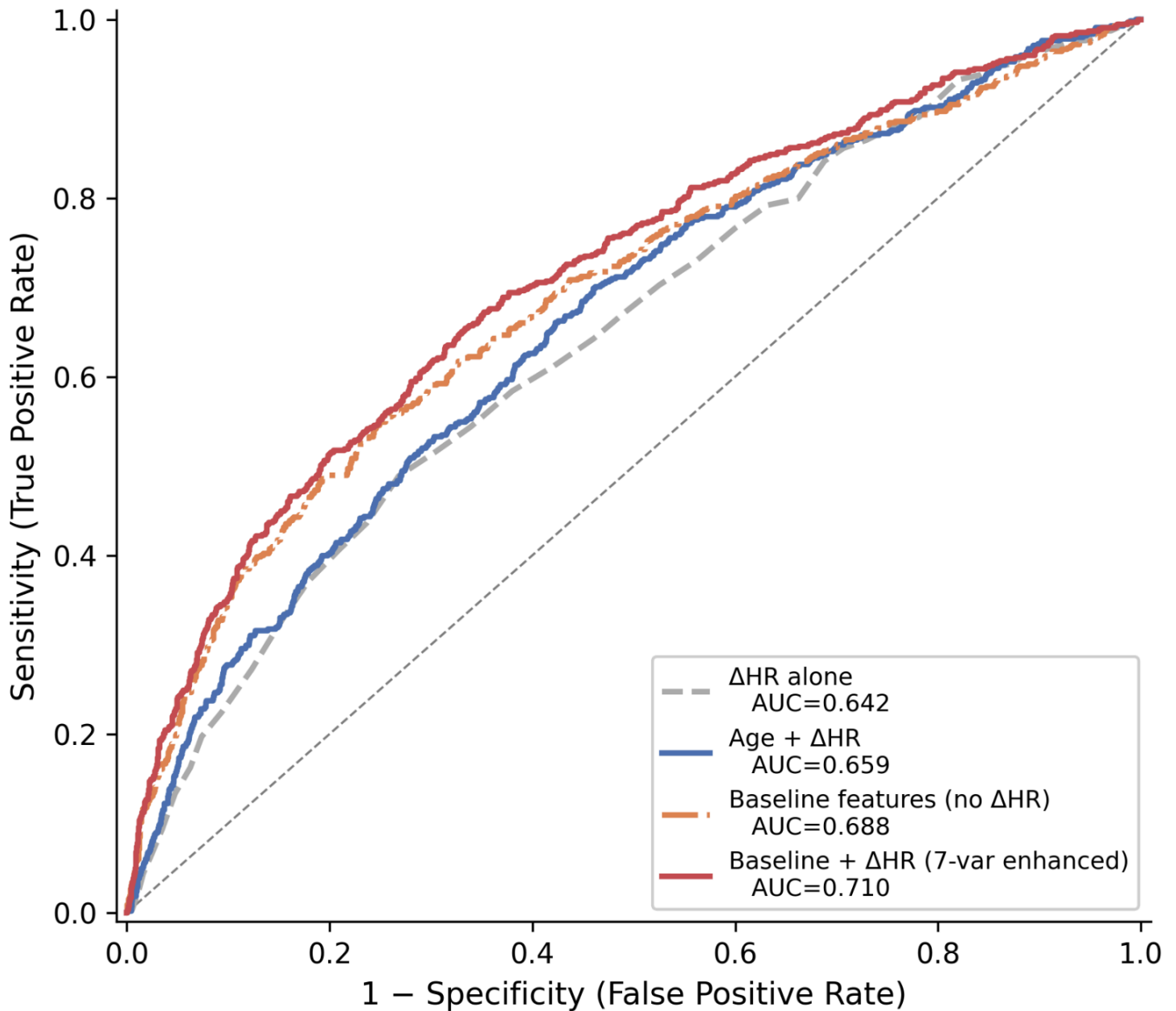


Figure 2

Receiver operating characteristic (ROC) curves demonstrating the incremental diagnostic value of Δ HR. Four models are shown: Δ HR alone (AUC=0.642; grey dashed), Age + Δ HR (AUC=0.659; blue), Baseline clinical features without Δ HR – age, sex, BMI, smoking, diabetes, hypertension, family history – (AUC=0.688; orange dash-dot), and the enhanced 7-variable model combining Δ HR with baseline features (AUC=0.710; red). Adding Δ HR to the baseline clinical model improved AUC by 0.022 (0.688→0.710), demonstrating independent discriminative value of the hyperventilation response beyond traditional risk factors.

Diagnostic Performance: Δ HR + Age vs. ECG-Based Tests for Obstructive CAD

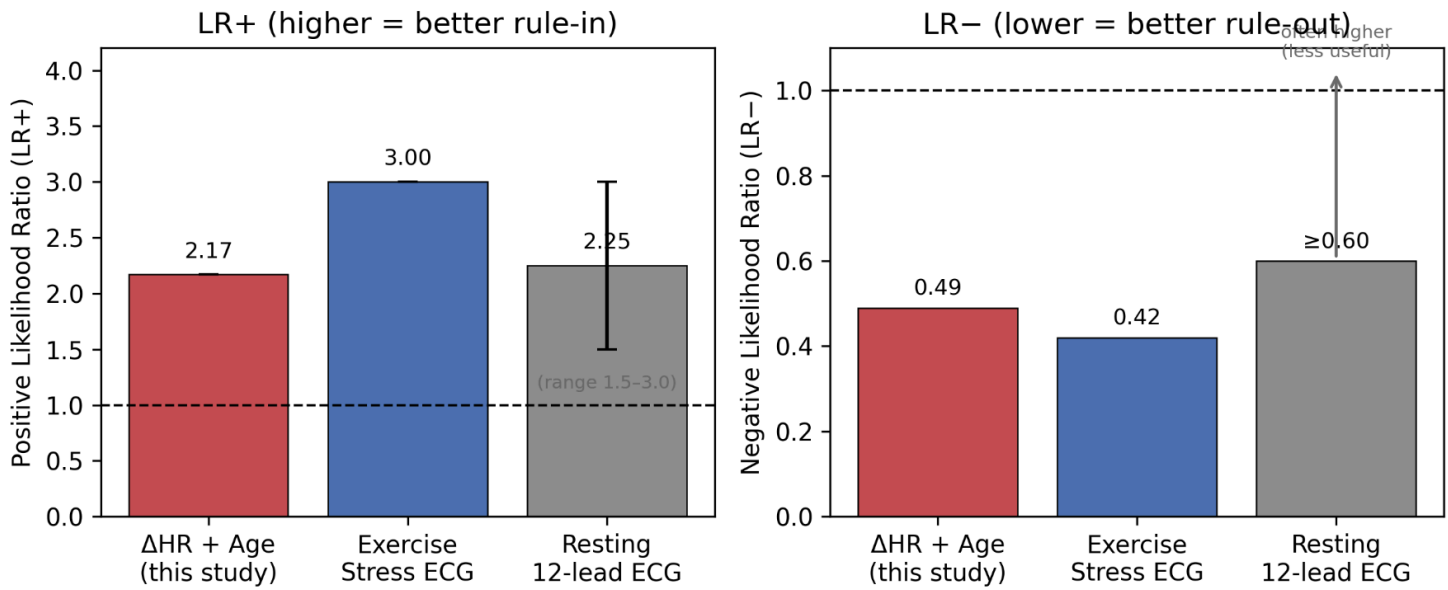
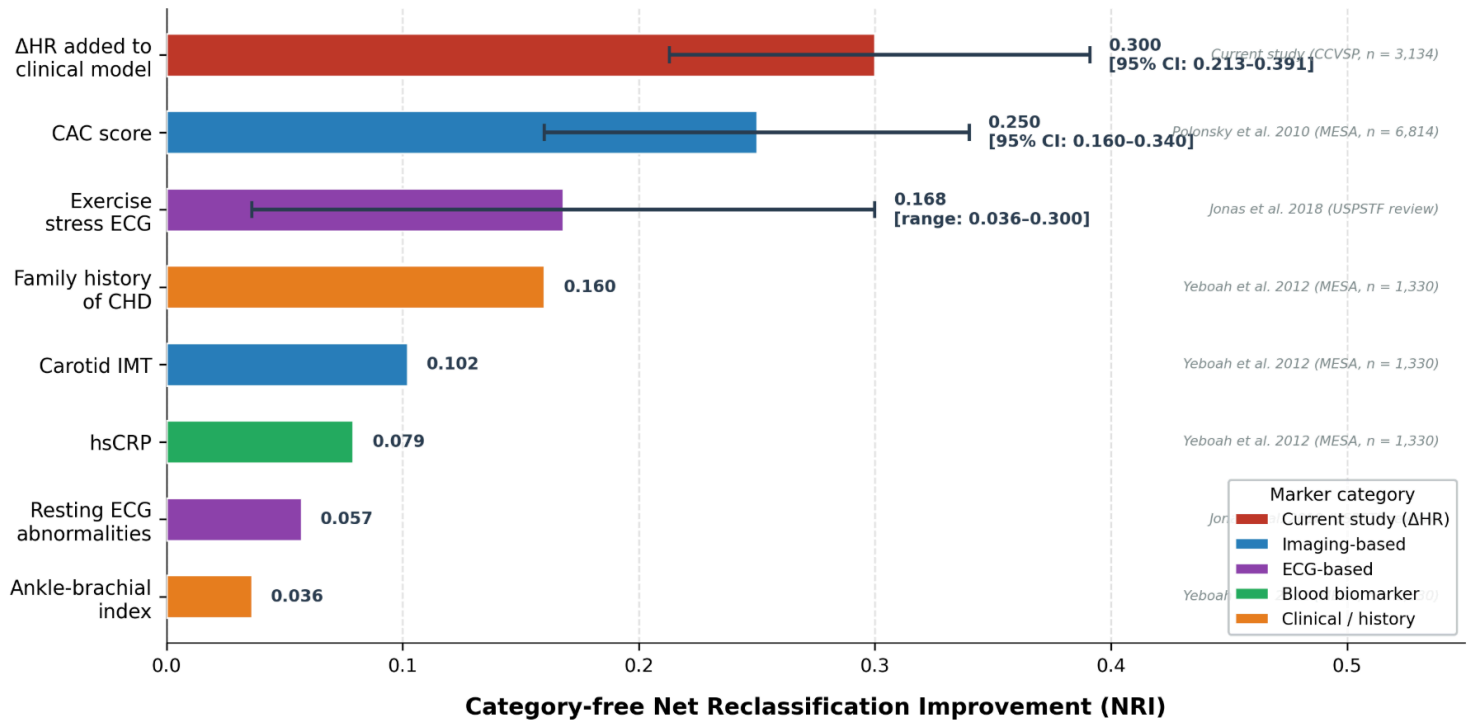


Figure 3

Comparison of likelihood ratios (LR+ and LR-) for the age + Δ HR model versus exercise stress ECG and resting 12-lead ECG for detection of obstructive coronary artery disease. Reference line at LR=1 indicates no diagnostic value; LR+ values further above 1 indicate stronger rule-in performance, and LR- values further below 1 indicate stronger rule-out performance.

Incremental Reclassification of Cardiovascular Risk Markers Added to Framingham / Clinical Risk Models



NRI = category-free net reclassification improvement. Solid error bars = 95% CI; dashed error bars = cross-study range (exercise stress ECG). All values reflect NRI when the marker is added to a baseline model of conventional risk factors (Framingham Risk Score or equivalent). Exercise stress ECG value is the mean of the range (0.036-0.300) reported across studies in the USPSTF 2018 systematic review (Jonas et al.). MESA values (Yeboah et al. 2012) are for intermediate-risk participants; CCVSP and Polonsky values are for the full study cohort.

Figure 4

Comparison of category-free net reclassification improvement (NRI) for cardiovascular risk markers added to the Framingham Risk Score. Δ HR is from the current study; coronary artery calcium (CAC) from Polonsky et al. [22]; family history, carotid intima-media thickness (cIMT), high-sensitivity C-reactive protein (hsCRP), and ankle-brachial index (ABI) from Yeboah et al. [23]; exercise stress ECG and resting ECG from Jonas et al. [24]. Error bars represent 95% confidence intervals where available; dashed lines for exercise stress ECG indicate the cross-study range reported in the systematic review.